The optimization of iron chelation with deferiprone

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List of Abbreviations

ATP	adenosine triphosphate
AUC	area-under-the-concentration-time-curve
AUC_{0-6}	six-hour area-under-the-concentration-time-curve
AUC_{0-12}	12-hour area-under-the-concentration-time-curve
AUC_{0-24}	24-hour area-under-the-concentration-time-curve
CD-36	cluster determinant-36
C_{max}	maximum concentration
Cl_{app}	apparent clearance
^{51}Cr	chromium isotope
DFO	desferrioxamine
DNA	deoxyribonucleic acid
^{59}Fe	iron isotope
FO	ferrioxamine
g/kg	gram per kilogram body weight
Hb	hemoglobin
HbF	fetal hemoglobin
HbS	sickle hemoglobin
HBT	homozygous beta-thalassemia
HPLC	high-performance liquid chromatography
ICAM-1	intercellular adhesion molecule-1
IgE	immunoglobulin E
IP	intraperitoneal
IQ	intelligence quotient
L1	deferiprone
LD_{50}	50% lethal dose
LD_{100}	100% lethal dose
L/kg	litre per kilogram body weight
L/h/kg	litre per hour per kilogram body weight
MEMS	medical event monitoring system
mg·min/L	milligram-minute per litre
mg/kg	milligram per kilogram body weight
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NTBI	non transferrin-bound iron
$\cdot O_2^-$	superoxide free radical
·OH	hydroxyl free radical
q6h	every six hours
q12h	every twelve hours
rpm	revolutions per minute
SD	standard deviation
SLE	systemic lupus erythematosus
SQUID	super-conducting quantum interference device
$t_{1/2}$	elimination half-life
UIE	urinary iron excretion
V_d	volume of distribution

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Frank Fotios Fassos **Doctor of Philosophy, 1997**

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The optimization of iron chelation with deferiprone

Abstract

Deferiprone is an orally active iron-chelating agent that reduces the body iron burden of chronically transfused, iron-overloaded homozygous beta-thalassemia patients. However, it is unclear how to optimize iron chelation with this drug.

I hypothesize that iron chelation by deferiprone can be optimized by matching the mode of administration of deferiprone to the kinetic profile of iron. Hence in patients with chronic iron-overload [thalassemia and sickle cell disease], greater iron chelation will occur when deferiprone is administered more frequently and in smaller doses than it will when the same daily dose of the drug is administered less frequently and in greater amounts. Conversely, in acute iron intoxication, large amounts of the drug will be more effective in chelating iron than smaller, multiple doses.

Greater urinary iron excretion (following transfusion) was induced in the homozygous beta-thalassemia patients, when deferiprone was administered every six hours compared to every twelve hours. In the same group of patients (prior to transfusion), similar quantities of urinary iron excretion resulted with the two regimens despite a decrease in the systemic deferiprone area-under-the-curve when the drug was administered every six hours. A significant quantity of fecal iron was excreted by sickle cell disease patients on the deferiprone regimen, and fecal iron excretion varied from 3 to 33% between patients.

Deferiprone did have any measurable antiplasmodial effect in asymptomatic malaria

patients.

Rats receiving a toxic iron meal had a 58% mortality, whereas those animals that received the iron meal followed by IP injections of deferiprone solution had a mortality of only 15%, an increase in urinary iron excretion. The decrease in body iron load was reflected in the iron stains of the gastrointestinal organs. The administration of a single IP injection of deferiprone solution induced greater urinary iron excretion than did [same-dose] four IP injections of deferiprone.

Optimal iron chelation by deferiprone is dependent on the kinetic profile of the iron. In the chronically-transfused patients, sustained levels of deferiprone are necessary to optimally chelate the sustained levels of non-transferrin-bound iron that are present in the serum. Conversely, in acute iron intoxication, a large administration of deferiprone is needed to chelate the circulating iron and prevent its entry into cells.

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1.0 Literature Review

1.0.0 Introduction

Iron is an essential component of a number of human physiological compounds including hemoglobin, myoglobin, heme and the non-heme enzymes, ferritin and transferrin. On average, the total body iron concentration in men and women is 50 mg/kg and 40 mg/kg, respectively (Brittenham, 1991). Humans are unique in their ability to conserve iron, because only 0.05% of all body iron is lost on a daily basis. The remainder is held in balance by an efficient recycling mechanism.

There are, however, inherited hemoglobinopathies, such as homozygous beta thalassemia and sickle cell disease, that require iron chelation therapy to reduce the iron body burden that occurs secondary to chronic transfusion therapy. In cases of chronic iron overload, the transferrin capacity in the blood is saturated and a toxic non-transferrin-bound iron (NTBI) fraction is formed (Hershko et al., 1978). By the catalytic activity of the NTBI, free radical species are thought to be generated. These free radicals non-specifically abstract electrons from cell membranes and proteins. In more acute cases of iron overload, such as accidental iron poisoning or suicide attempt, the toxic effects of the elemental iron are two-fold. The first insult occurs at the gastrointestinal lumen where severe hemorrhaging and fluid loss into the intestines create a condition known as "third spacing." The second destructive effect of free elemental iron in acute iron intoxication occurs at the cellular level, where iron concentrates in mitochondria and destroys the mitochondrial matrix necessary for sustaining energy generation (Wilzleben, 1966).

Although the clinical conditions mentioned here refer only to cases of excessive levels of body iron, malaria is an iron-related medical condition that involves normoferremic individuals. In this tropical parasitic infestation the parasites completely depend on hemoglobin iron for survival (Gordeuk et al., 1992).

In the past, all of these iron-related conditions have been treated effectively with desferrioxamine, a naturally occurring hexadentate iron chelator (Keberle, 1964). Though

proven efficacious, desferrioxamine therapy is plagued by poor compliance because of its cumbersome mode of parenteral administration, its toxicity with chronic use in high doses, and its high cost (Hershko and Weatherall, 1988).

As a result, there has been an intense effort to find an alternative iron chelator. Deferiprone is an orally active iron chelator that is currently at the stage of randomized human clinical trials (Olivieri et al., 1995). Deferiprone has been shown to be efficacious in reducing the total body iron burden in chronically transfused patients with thalassemia. However, inducing optimal iron chelation using deferiprone has not yet been studied.

1.1 Thalassemia

The term thalassemia is derived from the Greek word 'thalassa' (the sea), and was coined by Whipple and Bradford in 1932. According to the historical accounts of Weatherall and Clegg (1981), Whipple adopted the word to associate the disease with the Mediterranean, because most of the known cases at the time were patients of Mediterranean descent. However, subsequent population studies by a number of investigators showed that the thalassemia syndromes were not confined to the Mediterranean. The historical distribution of thalassemia up to the late 1950s has been reviewed by Chernoff (1959).

In order to understand not only the disease state but also the implications of the disease and its therapies, a brief discussion of normal hemoglobin and hemoglobin function will follow.

1.1.1 Hemoglobin

Hemoglobin functions as a carrier protein to transport oxygen from the lungs to the body tissues. The hemoglobin (Hb) molecule consists of four globin proteins (two alpha chains and two non-alpha chains), each of which contains a covalently bound iron-containing heme group.

All of the alpha (α) globin and alpha-like globin genes are on chromosome 16 of the human genome (Deisseroth et al., 1977). There are only three functional genes in the alpha cluster; these are the zeta (embryonic) and the two alpha (adult) genes.

All of the non-alpha globin genes are located on chromosome 11 (Deisseroth et al., 1978). There are five functional non-alpha gene sequences - epsilon (embryonic), G-gamma and A-gamma (fetal), and the delta and beta (adult) sequences. Except for ten amino acid residues, the delta globin chain is nearly identical to the beta-globin sequence (Efstratiadis et al., 1980). However, the promoter region of the delta gene is flawed (Humphries et al., 1982), resulting in a reduction in the transcription of the delta gene. Furthermore, Ross and Pizzarro (1983) showed that the delta messenger RNA transcript is less stable than that of the beta messenger RNA.

The different types of hemoglobin have different affinities for oxygen. Although embryonic hemoglobin has the highest affinity for oxygen, the affinity of fetal hemoglobin is lower and that of adult hemoglobin has the lowest of the three affinities for oxygen (Weatherall and Clegg, 1981).

1.1.2 Erythropoiesis

Erythropoiesis refers to the production of red blood cells. The demand for erythropoietic activity is regulated by the amount of oxygen delivered to the tissues and by the erythropoietin-stimulating factor, a hormone that is produced primarily in the kidneys of healthy humans.

1.1.3 Homozygous beta-thalassemia

The thalassemia syndromes are a group of inherited hemoglobin gene disorders, exhibiting an autosomal recessive inheritance pattern (Chini and Valeri, 1949). The thalassemia syndromes are the most common single-gene disorders in the world (Weatherall and Clegg, 1981).

Patients with thalassemia have an imbalance in the globin ratio resulting from a decrease or a complete lack of synthesis of one or more of the hemoglobin protein chains (Nienhuis and Wolfe, 1987). When an imbalance in the globin chain ratio occurs, the excess chains cannot create stable tetramers with their complements and therefore aggregate with other chains (Fessas, 1963). These inclusion bodies cause irreversible membrane damage to the erythrocyte membranes. In particular, when alpha chains form tetramers in patients with homozygous beta-thalassemia, the insoluble aggregate precipitates almost instantly, causing irreversible damage to the developing erythrocyte and hemolysis (Nienhuis and Wolfe, 1987). Oxygen transport throughout the body is compromised, resulting in a compensatory increase in erythropoietin output and a marked increase in erythropoiesis that is ineffective (Sturgeon and Finch, 1957). Historically, in untransfused patients, the marrow expands into the cavities of the long bones (Cooley et al., 1927), seriously affecting the growth and development of the bones and resulting in the characteristic deformations seen in thalassemia patients in the pre-transfusion era. In particular, the bones of the cranium and face expand and the long bones become brittle (Cooley and Lee, 1925). Autopsies of thalassemia patients showed overexpansion of the hematopoietic tissue throughout the skeletal system and hematopoietic activity in the viscera (Cooley and Lee, 1925; Cooley et al., 1927; Whipple and Bradford, 1932).

For the remainder of the thesis, the term 'thalassemia' will be synonymous with homozygous beta-thalassemia, unless otherwise stated. Other types of thalassemias are thoroughly discussed by Weatherall and Clegg (1981) and Nienhuis and Wolfe (1987).

The first clinical description of thalassemia was by Dr. Thomas B. Cooley in Detroit, Michigan (Cooley and Lee, 1925), and not in the Mediterranean area as one would expect. Consequently, the condition he described also became known as 'Cooley's anemia'. In his classic paper, Cooley presented a case report of four Greek and Italian children who presented clinically with hepatosplenomegaly, discoloured skin and sclerae, anemia, enlarged craniofacial bones, and deformities of the long bones (Cooley and Lee,

1925). The high frequency of the defective beta-globin gene in the Mediterranean population prevented the Mediterranean medical community from taking notice of the phenotypic result as a medical condition (Weatherall and Clegg, 1981).

1.1.4 Malaria and the origin of thalassemia

In Mediterranean countries, homozygous beta-thalassemia has a gene frequency of 15-20% (Weatherall and Clegg, 1981). One hypothesis as to why the gene frequencies of the thalassemia syndromes are so high is, that malaria may have been a powerful selective factor (Nienhuis and Wolfe, 1987). Cerebral malaria from *Plasmodium falciparum* may be fatal to children with normal hemoglobin as passive immunity from a previously infected mother disappears soon after birth (Nienhuis and Wolfe, 1987). Conversely, children who are heterozygous thalassemics are less vulnerable to malarial infections. Plasmodial survival in the erythrocytes of individuals with thalassemia trait appears to be reduced, giving those with the thalassemia trait a selective advantage for survival over individuals with normal adult hemoglobin.

The subject of malaria is discussed in greater detail in section 1.3.

1.1.5 Pathophysiology of thalassemia

Little was known about the hemolytic mechanisms that occur in thalassemia until 1957, when Sturgeon and Finch published the first results of an erythrokinetic study performed on thalassemia patients. The finding of overactivity of an ineffective erythropoiesis was later confirmed by Bannerman et al. (1959). It was discovered that the expansion of the bone marrow was associated with massive erythropoietic activity with the end result of the production of defective erythrocytes that were destroyed prematurely.

Intracellular inclusion bodies from the aggregation of excess alpha-globin chains in homozygous beta-thalassemia patients precipitate and create lesions on the surfaces of the erythrocytes (Rachmilewitz et al., 1985), compromising their function. Thalassemic

erythrocytes also lack flexibility and possess intracellular concentrations of calcium and potassium that are higher and lower than in normal erythrocytes, respectively. This reduction in intracellular potassium dehydrates the erythrocyte and increases its viscosity. Furthermore, membrane lipids show evidence of oxidation and the surface concentration of sialic acid residues is significantly lower than normal. The decrease in the number of sialic acid residues, the deposition of intracellular inclusion bodies, and the increase in viscosity may contribute to increased destruction of erythrocytes by the reticuloendothelial system and explain the decrease in survival of the erythrocytes in nonsplenectomized thalassemia patients (Nienhuis and Wolfe, 1987).

1.1.6 Treating the thalassemia patient

1.1.6.1 Transfusion

Before the institution of chronic transfusion therapy, patients afflicted with thalassemia major developed severe complications, such as congestive heart failure, infections, and bone fractures, and eventually died. Initially red blood cell transfusions were given only to patients with the most severe forms of thalassemia and only when they could not function any more. Beard et al. (1967) showed that routine red blood cell transfusions to patients with severe homozygous beta-thalassemia resulted in clinical improvement of their condition. Children maintaining a hemoglobin level of 85 g/L or higher on a chronic transfusion program developed fewer infections, and the heart, liver, and spleen of these patients were not as enlarged as those of non-transfused patients (Modell, 1976; Modell, 1977).

In the Hemoglobinopathies Clinics at The Hospital for Sick Children and The Toronto Hospital, patients are maintained on regimens of hemoglobin levels above 100 g/L with monthly transfusions of washed red blood cells. Red blood cells are washed prior to transfusion to remove white blood cells and prevent hypersensitivity reactions. Furthermore, transfusion units of washed red blood cells are also genotype-matched to the

genotype of the recipient in order to prevent sensitization and subsequent antibody production of these patients (Weatherall and Clegg, 1981).

1.1.6.2 Iron Overload

The physical effects of anemia and of ineffective bone marrow activity are suppressed by routine red blood cell transfusions (Wolman, 1964). However, the initial benefits of transfusions are compromised by the consequences of iron overload in the second decade of life, resulting from iron deposition in the organs of patients secondary to chronic transfusion therapy (Fink, 1964).

Transferrin is a transport glycoprotein with a molecular weight of approximately 80 kDa and possesses two iron-binding sites. These high-affinity iron-binding sites neutralize the reactivity of elemental iron molecules and make transferrin transport throughout the body safe without the generation of free radicals (Hershko and Peto, 1987). Normally, transferrin carries iron safely from the liver, where red blood cells are broken down, to the bone marrow, where the iron is utilized for new red blood cell formation. In thalassemia patients, transferrin becomes saturated with iron following the breakdown of transfused red blood cells (Hershko and Peto, 1987). Once transferrin levels are saturated in chronically transfused patients, the excess iron is termed 'non transferrin-bound iron' (NTBI; Hershko et al., 1978a; Batey et al., 1980; Anuwatanakulchai et al., 1984; Wang et al., 1986; Grootveld et al., 1989). The NTBI fraction is toxic to a variety of cells because it catalyzes the formation of free radicals (Gutteridge et al., 1985).

The catalytic action of iron on hydrogen peroxide, for example, has been known for over a century (Fenton, 1894). In Fenton's study, tartaric acid was oxidized to dihydroxymaleic acid in the presence of ferrous iron (Fe^{2+}) and hydrogen peroxide (H_2O_2) (together, these species are 'Fenton reagents' (Goldstein et al., 1993; Sutton and Winterbourn, 1989). Any reaction that includes Fe^{2+} and hydrogen peroxide is referred to as a Fenton reaction (Reaction 1). In 1934, Haber and Weiss suggested that in the

presence of the Fenton reagents, Fe^{2+} and H_2O_2, a hydroxyl free radical (denoted as $\cdot OH$) will be generated. Free radicals are short-lived, highly reactive intermediate chemical species that possess one or more unpaired electrons and are a part of normal metabolism. These species can, however, have devastating effects on an organism, because of their ability to non-selectively abstract single electrons from electron-rich sources in their environment, such as proteins (Dean et al., 1991), single and double-stranded DNA in plasmids (Toyokuni and Sagripanti, 1993), and the membranes of red blood cells (Stocks and Dormandy, 1971; Hershko, 1989a), hepatic lysosomes (Seymour and Peters, 1978), hepatic mitochondria (Hanstein et al., 1975), and cardiac myocytes (Scott et al., 1985; Arroyo et al., 1987; Barrington, 1990).

$$Fe^{2+} + H_2O_2 + H^+ \text{---> } Fe^{3+} + H_2O + \cdot OH \quad (1)$$

$$Fe^{3+} + \cdot O_2^- \text{ ---> } Fe^{2+} + O_2 \quad (2)$$

$$H_2O_2 + \cdot O_2^- \text{ ---> } O_2 + OH^- + \cdot OH \quad (3)$$

Reaction 3 normally proceeds *in vivo*, at a very slow rate. Yet in the presence of iron, the rate increases (Halliwell et al., 1985) and drives the reaction forward to produce free radicals.

So toxic are the effects of 'free' iron that all somatic cells are adapted to synthesize ferritin subunits in the presence of excessive iron levels (Harrison, 1977). Ferritin is a soluble protein shell consisting of 24 heavy (21 000 daltons) and light (19 000) protein subunits, with the inner cavity having the capacity to store up to 4500 atoms of iron in the form of ferric oxyhydroxide (Munro, 1993). Normally, large numbers of ferritin subunit messenger RNA (mRNA) sequences lie dormant in the cellular cytoplasm. These dormant mRNAs possess an untranslated region upstream from the reading frame of the ferritin gene. This unstranslated gene sequence -- iron-regulatory element -- is a binding site for the iron-regulatory element-binding protein (Aziz and Munro, 1987). In the absence of cytoplasmic iron, the binding protein binds to the regulatory element and

suppresses the translation of ferritin. Conversely, the binding protein can interact with increased levels of intracellular iron as the need arises to expose the regulatory element and allow the initiation of translation (Aziz and Munro, 1986).

Despite the inherent safety features of the available ferritin mRNA sequences, ready to be translated, there is excessive iron and the presence of insoluble hemosiderin. As a result of the accumulated iron, organopathies arise in the heart (Engle, 1964; Buja and Roberts, 1971; Wonke et al., 1989a) and liver (Barry et al., 1974; deVirgiliis et al., 1981; Weintraub et al., 1985), as well as in endocrine glands (Flynn et al., 1976; Cohen, 1987; de Sanctis et al., 1989).

Iron overload can affect the sexual maturation of patients (Cohen, 1987), and studies are currently underway at the hemoglobinopathies clinics at our hospitals to assess the fertility of each patient.

1.1.6.3 Iron chelation therapy

An increased iron burden necessitates iron chelation therapy. The clinical removal of iron from iron-loaded organs is achieved with the use of chemicals known as chelators. To date, the only clinically effective iron chelator available for reducing body iron stores is desferrioxamine (DFO) (Modell, 1977; Weiner et al., 1978; Wolfe et al., 1985; Pippard, 1987; Cohen, 1987; Olivieri et al., 1992). Compliance with DFO therapy, however, continues to be a problem, primarily because of its cumbersome mode of parenteral administration. As a result, there has been a concerted search for an orally effective iron-chelating agent. So far only the orally active iron chelator deferiprone (L1; 1,2-dimethyl-3-hydroxypyrid-4-one) has reached the stage of randomized clinical trials (Olivieri et al., 1990a; 1992b; 1995).

Desferrioxamine and deferiprone are discussed in greater detail in sections 1.5 and 1.7, respectively.

1.1.6.4 Assessment of total iron burden

Excessive iron stores play a significant role in the development of organopathies in patients with homozygous beta-thalassemia. Thus, it is important to know the magnitude of a patient's iron load, so as to determine any progress or changes in their chelation therapy.

There are a number of techniques that are used to quantitate body iron stores.

Serum ferritin levels are often used as an indirect measure of body iron load, since ferritin is the predominant protein that stores iron in humans (Borgna-Pignatti and Castriota-Scanderbeg, 1991). However, studies have shown that ferritin results are poor predictors of body iron stores (Brittenham et al., 1993). Liver biopsies allow a direct and accurate measure of liver iron stores and are currently used in the hemoglobinopathies clinics at The Hospital for Sick Children and The Toronto Hospital. Despite the accuracy of liver biopsies in dictating hepatic iron concentrations, such biopsies are painful, invasive, and can be associated with complications such as bleeding. The major forms of storage iron, ferritin (Munro, 1993) and hemosiderin (Weir et al., 1984), are known to be paramagnetic (Brittenham et al., 1980). Paramagnetic substances become magnetic in the presence of a magnetic field and lose their magnetism when the external field is extinguished. Thus, paramagnetic properties can be exploited and the magnetic field measured with a suitable detection system (Brittenham et al., 1980).

Magnetic resonance imaging (MRI) has been an effective tool for investigating the iron stores in transfusion-dependent thalassemia patients. A number of studies of MRI liver scans of iron-loaded patients show a decrease in the MRI signal. As the iron burden increases, the signal is further diminished and the image is darker for the organ (Stark et al., 1985; Brown et al., 1985; Murphy and Bernardino, 1986; Hernandez et al., 1988; Gomori et al., 1991). Similar findings were found when pituitary (Fujisawa et al., 1988) and cardiac MR images (Olivieri et al., 1992b) were examined. The advantages of the MRI

scans are that increases or decreases in organ iron content can be quantitated quickly and easily.

A super conducting quantum interference device (SQUID) is currently being used to assess liver iron in iron-overloaded patients. The SQUID induces a magnetic field over the liver region. The paramagnetic properties of the stored iron in the liver, respond positively in the presence of the induced magnetic field and give off their own magnetic field that is detected by the SQUID. The change in magnetic flux is translated into the concentration of hepatic iron by the SQUID computer (Farrell et al., 1980; Farrell et al., 1981). The SQUID offers the advantages of non-invasive measurement of liver iron concentrations in addition to its nearly linear relationship with the magnetic measurement (Brittenham et al., 1982; Brittenham et al., 1993).

1.1.6.5 New treatments

At the time of birth, homozygous beta-thalassemia babies appear normal. It is not until the bone marrow suppresses the synthesis of gamma-globin chain proteins and activates the beta-globin genes, that problems of anemia arise. If gamma-globin synthesis could be reactivated to sustain hemoglobin levels at a point where transfusions were no longer needed, the patient could lead a normal life. A number of articles suggest that this may be possible. In one study, the newborn offspring of diabetic women were discovered to possess unusually high levels of butyric acid and a profound delay in the gamma-to-beta switching mechanism (Perrine et al., 1985). The investigators hypothesized that butyric acid was responsible for the delay in the switching mechanism. The hypothesis was later supported by the findings of Perrine et al. (1987), Constantoulakis et al. (1989), and Perrine et al. (1993). More recently, a young girl with hemoglobin Lepore at the Hospital for Sick Children was treated with continuous intravenous butyrate therapy, that resulted in a significant rise in fetal hemoglobin production. This therapy was also attempted in other

patients, only to yield the rather disappointing findings that butyrate does not work as well in all patients (Sher et al., 1995).

One of the most ambitious hopes for an effective treatment of patients with thalassemia is the development of genetic therapy (Stammatoyannopoulos, 1992).

1.2 Sickle Cell Disease

1.2.1 Hemoglobin S

When a beta-gene mutation results in the substitution of a valine residue in the 6-position of the beta-globin chain in the place of a glutamic acid residue, the resulting beta-like globin protein combines with normal alpha-globin proteins to form sickle hemoglobin or hemoglobin S (Ingram, 1957).

Patients who are homozygous for hemoglobin S are said to have sickle cell disease, whereas those who are heterozygous for hemoglobin S have the sickle cell trait. Discussion in this thesis will pertain only to patients with sickle cell disease.

1.2.2 Sickle Cell Disease

Sickle cell disease is inherited as an autosomal dominant trait (Serjeant, 1985). Patients suffering from the more serious cases of sickle cell disease can develop painful episodes known as sickle cell crises, of which there are three types: vaso-occlusive, sequestration, and aplastic (Diggs, 1965).

Vaso-occlusive crises most often involve the skeleton, the central nervous system, the penis, and internal organs such as the liver (Sheehy, 1977), the spleen and the lungs (Barrett-Connor, 1971). The circulation to these organs can be blocked following intravascular sickling, resulting in acute pain.

Patients with sickle cell disease commonly experience bone crises. These crises are the result of bone marrow infarcts (Charache and Page, 1967; Milner et al., 1982).

Elbow, knee and shoulder joints can be affected (Konotey-Ahulu, 1974). Other abnormalities in the skeletal system may result from marrow expansion (Reynolds, 1977).

The central nervous system is also vulnerable to insults in a sickle crisis, with results that can be devastating and lifelong. Abnormal physical and neurological signs can occur and may include seizures, coma, speech defects, and decreased IQ scores (Portnoy and Herion, 1972; Powars et al., 1978).

Male sickle cell patients may suffer from sickle crises that afflict the penis. This condition is known as priapism and is characterized by painful, sustained erections (Campbell and Cummings, 1969; Emond et al., 1980). Priapism is treated with red blood cell transfusions (Seeler, 1973) or by exchange transfusions (Rifkind et al., 1979). Priapism may lead to sexual dysfunction (Emond et al., 1980).

Many sickle cell patients are at high risk for pulmonary infarcts (Diggs, 1969), infections and pneumonia (Barrett-Connor, 1973). In addition, respiratory syndromes in sickle cell patients may precipitate a sickling crisis brought about by hypoxia; this may result in more serious complications such as a stroke. Thomas et al. (1982) showed that sickle cell patients over ten years of age died more often from acute respiratory failure than from any other cause.

The second type of sickle cell crisis is termed acute splenic sequestration crisis. This type of crisis is caused by the sudden trapping and collection of erythrocytes in the spleen (Seeler, 1972). The sudden onset of weakness, enlargement of the abdomen and shock are characteristic signs for the precipitation of an acute sequestration crisis. The crisis is usually corrected after a red blood cell transfusion. So serious are these events, that in the past some authors had suggested splenectomy in patients after one (Jenkins et al., 1960) or two episodes (Seeler and Shwiaki, 1972) to prevent cardiovascular collapse in the future.

The third type of sickle cell crisis is an aplastic crisis. This can occur due to recurrent infections (Singer et al., 1950; Leikin, 1957). Although erythrocytes in sickle

cell disease patients have a shorter survival, the bone marrow compensates by increasing its output of red cells (Platt and Nathan, 1987). Aplastic episodes are usually of short duration and resolve spontaneously. Some patients require transfusion when the bone marrow is severely suppressed (Platt and Nathan, 1987).

Death of pediatric sickle cell patients most commonly results from infection (Seeler, 1972; Overturf et al., 1977). The high incidence of infections leading to death are believed to be a result of defective functions of the spleen and the white blood cells (Boggs et al., 1973), and of low levels of opsonins (Winkelstein and Drachman, 1968). Some sickle cell patients develop 'functional asplenia' starting in the first year of life (Pearson et al., 1969). This condition prevents the spleen from filtering the blood of particles and predisposes patients to pneumococcal infections (Seeler et al., 1972; Rogers et al., 1978). Antibody synthesis is also impaired, so that if pneumococcal vaccine is administered intravenously to sickle cell disease patients, an immune response fails to develop (Schwartz and Pearson, 1972). Yet a sufficient immune response does develop, when the same vaccine is administered intramuscularly (Schwartz and Pearson, 1972).

Other organ systems are affected chronically by the sickling trait. The heart, for example, often exhibits valvular disease (Falk and Hood, 1982). Erythrocytes are particularly susceptible to sickling in the hypertonic environment of the kidney medulla (Perillie and Epstein, 1963; van Eps et al., 1970). Intrahepatic sickling (Buchanan and Glader, 1977), infarcts, and viral hepatitis may follow multiple transfusions (Alli and Lewis, 1969). In addition, sickle cell patients may develop hearing loss, believed to result from hair cell destruction in the cochlea during sickling episodes (Morganstein and Manace, 1969). Retinopathies may result from intravascular sickling in retinal arterioles.

1.2.3 Malaria and Sickle Cell Disease

The incidence of sickle cell disease in Africa, India, and the Middle East is similar to that of malaria (Wiesenfeld, 1967). There have been a number of suggestions as to why malaria may be responsible for the high prevalence of sickle cell disease in tropical regions of the world. One suggestion is that the *Plasmodium* parasites cannot metabolize HbS as well as normal HbA (Allison, 1954). Another possibility is that parasitized red blood cells sickle more readily than non-parasitized ones. These infected erythrocytes are sequestered in the spleen (Luzzatto et al., 1970) and are exposed there to very low oxygen tensions, resulting in a decrease in intracellular potassium (Roth et al., 1978) and pH. These conditions have been shown to create a very unfavorable environment for parasitic growth *in vitro* (Pasvol et al., 1978). The lack of the Duffy antigen -- normally used by *Plasmodium vivax* to penetrate cells -- in the red blood cells of West-Africans may prevent the parasites from entering erythrocytes (Miller et al., 1975; Mason et al., 1977).

The subject of malaria is discussed in greater detail in section 1.3.

1.2.4 Pathophysiology

In a number of *in vitro* studies the effects of various environmental factors on the sickling process have been examined.

Hahn and Gillespie (1927) first described the importance of oxygen tension to the sickling process. In the same paper, the authors reported that acidification can also induce sickling. Later, Harris et al. (1950) showed an inverse relationship between the number of erythrocytes that sickled with decreasing oxygen tension in sickle cell disease patients. As deoxygenation of the blood proceeds, polymerization of the HbS tetramers results, together with a profound change in the shape of the erythrocyte. As the tetramers polymerize further, the erythrocytes appear leaf-shaped but may regain their original shape if adequately oxygenated (reversibly sickled cells), whereas others remain "irreversibly sickled" (Dobler and Bertles, 1968).

Once irreversibly sickled, the polymers formed in erythrocytes damage the cell membrane, affect homeostasis and predispose erythrocytes to be attacked by the reticuloendothelial system. Irreversibly sickle-prone cells retain sodium and calcium (Eaton et al., 1973; Palek et al., 1977). The sodium retention results from a decrease in the ability of the sodium pumps to extrude sodium ions (Clark et al., 1978) and although the calcium pump remains functional, (Litosch and Lee, 1980), the pumps are oriented in such a way that the intracellular calcium concentration increases (Bookchin et al., 1983).

Damage to the irreversibly sickled cells is also the result of HbS binding to the inner erythrocytic membrane (Bank et al., 1974; Fischer et al., 1975). Damage to the cytoskeleton may also play a significant role in the deformation of the red blood cell (Clark et al., 1980).

The external membrane of the erythrocyte itself has clusters of negative surface charges which may make irreversibly sickled erythrocytes adhere to the endothelium (Hebbel et al., 1980). Furthermore, the orientation of phosphatidyl serine and phosphatidyl ethanolamine undergoes a change during sickling, that has been shown to enhance coagulation (Chiu et al., 1981) and may predispose patients to vascular occlusion.

1.2.5 Treatment of Sickle Cell Disease.

Crises can usually be treated at home with increased fluid intake and the self-administration of analgesics (Platt and Nathan, 1987). More severe crises are treated in hospital with continuous oxygen and the administration of narcotic analgesics, such as morphine or meperidine (Szeto et al., 1977; Vichinsky et al., 1982).

Transfusions are effective in solving some sickle cell crises. In aplastic and sequestration crises, the red blood cell mass can be restored with a red blood cell transfusion (Platt and Nathan, 1987). In a serious vaso-occlusive crisis, such as one that affects the central nervous system, an exchange transfusion is warranted to reduce rapidly the Hb S content of the blood (Charache, 1974; Rifkind et al., 1979; Walker et al., 1983).

Although transfusions have been instrumental in reducing and reversing the clinical manifestations of sickle cell disease, chronic transfusion therapy results in iron-overload, necessitating iron chelation therapy with desferrioxamine (see section 1.5).

Finally, the maintenance of proper immunization schedules are important in sickle cell disease patients because many patients are splenectomized and may be predisposed to infections (Boggs et al., 1973; Ammann et al., 1977).

1.2.6 Future treatments.

Since sickle cell disease is a hemoglobinopathy of the beta-globin chains, many of the future therapies of the beta-hemoglobinopathies, such as beta-globin gene therapy (Stamatoyannopoulos, 1992) and the stimulation of hemoglobin F synthesis with investigational drugs such as arginine butyrate (Perrine et al., 1993; Sher et al., 1995), may also be used in patients with sickle cell disease .

1.3 Malaria

1.3.1 Background

Malaria is believed to be one of the oldest diseases afflicting man (Knell, 1991). Reports of human malaria symptoms of fever, enlarged spleen, headaches and chills date back to 2700 BC China (Russell et al., 1963; Wernsdorfer and McGregor, 1988).

Originally, malaria was believed to be caused by the foul smell of swamp air, hence the Italian derivation of 'bad air' (mal-aria). Malaria is now known to be a vector-borne, tropical parasitic infection affecting several species of animals, including various birds, rodents, amphibians, reptiles, and mammals (Russell et al., 1963). Discussion will be restricted to human malaria.

All human malaria infections are characterized by progressive illness and intermittent fever. *Plasmodium vivax* and *Plasmodium ovale* produce a high fever every 2 days (tertian) and *Plasmodium malariae* a fever every 3 days (quartan), yet are rarely fatal (Knell, 1991). Infection with the fourth species, *Plasmodium falciparum*, is a potentially

serious condition that can lead to cerebral malaria, coma, and death (Aikawa, 1988; Gordeuk et al., 1992a). Malaria parasites are transmitted to humans by the female *Anopheles* mosquito during blood feedings (Waterhouse and Riggenbach, 1967; Knell, 1991). The *Anopheles gambiae* mosquito is the most important vector for the transmission of malaria in Africa (Knell, 1991). The life cycle of the *Anopheles* mosquito will not be discussed here; this subject is discussed in greater depth in texts by Wernsdorfer and McGregor (1988) and Knell (1991).

1.3.2 Pathogenesis

The life cycle of malaria parasites consists of three stages: the sporozoite, the extraerythrocytic, and the erythrocytic stages (Sinden and Smalley, 1979).

The spread of malaria parasites begins when the mosquito bites an infected human and ingests male and female gametocytes enclosed in erythrocytes in the blood meal (Campbell et al., 1982; Knell, 1991). Following entry into the stomach of the mosquito, the male and female gametocytes are released from the erythrocytes and mature into gametes. The male gametes undergo a process of exflagellation, in which up to eight nucleated 'flagella' (ie. spermatozoa) form and erupt from the gamete (Carter and Nijhout, 1977; Sinden and Smalley, 1979; Gerber et al., 1981). Following the fertilization of a female gamete by one of the 'flagellae' (Aikawa et al., 1984), a zygote is formed (Bannister and Sinden, 1982; Aikawa et al., 1984; Wernsdorfer and McGregor, 1988). This is the only time that sexual reproduction takes place in the life cycle of the malaria parasite. The zygote elongates into a motile form called an ookinete (Alger, 1968; Yoeli and Upmanis, 1968; Weiss and Vanderberg, 1977; Bannister and Sinden, 1982), which may invade the wall and ultimately the basement membrane of the mosquito stomach, where it will grow into an oocyst (Schneider, 1968; Canning and Sinden, 1973; Bannister and Sinden, 1982; Knell, 1991).

Following growth in the stomach wall of the mosquito, the oocyst grows into the body cavity of the mosquito and continues to do so for about a week. Then, internal cell divisions (Canning and Sinden, 1973) take place which produce hundreds of sporozoites (Hollingdale et al., 1981; Bannister and Sinden, 1982) for the next stage of growth (Wernsdorfer and McGregor, 1988; Knell, 1991). Within two weeks of the formation of the oocyst, the membrane ruptures, releasing hundreds of sporozoites that penetrate the salivary glands and accumulate in the salivary ducts (Sterling et al., 1973).

The next time that the infected mosquito bites a human for another blood meal, sporozoites will be injected into the human blood stream (Garnham et al., 1961; Garnham et al., 1961). Then the motile sporozoites will first reach and enter the Kupffer cells and then into the hepatocytes (Shin, 1982; Knell, 1991). This stage is known as hepatic schizogeny. A trophozoite develops and enlarges, and divides asexually into a multinucleated hepatic schizont that produces merozoites (Meis et al., 1983; Mazier et al., 1984a; Mazier et al., 1984b). The hepatic schizonts eventually exceed the size of the liver cell, causing the hepatocyte to burst and release thousands of invasive merozoites (Knell, 1991). Merozoites, in turn, infect erythrocytes in the last phase of asexual development (Ladda et al., 1969; Dvorak et al., 1975). An interesting note is that not all sporozoites create a hepatic schizont, but some of the sporozoites of *Plasmodium vivax* will lie dormant as hypnozoites for many months or even years after they have entered the hepatocytes (Krotoski et al., 1982a; Krotoski et al., 1982b; Krotoski et al., 1982c).

The final stage of asexual growth in the life cycle of *Plasmodium* parasites occurs when merozoites penetrate the erythrocytes of the host (Ladda et al., 1969; Dvorak et al., 1975). For this step, the merozoites must first bind to surface receptors of the erythrocytes (Breuer et al., 1983; Hadley and Miller, 1988; Sherwood et al., 1989; Kain et al., 1993). In some African blacks, the absence of the 'Duffy antigen' from the surface of the erythrocytes may be responsible for protecting them from infections with *vivax* malaria (Miller et al., 1975; Mason et al., 1977). Genetically diminished amounts of surface

glycoproteins, such as glycophorin A and B, on erythrocytes have also been shown to prevent merozoites from invading erythrocytes (Pasvol et al., 1982a), suggesting that surface glycophorin proteins may act as potential receptors for malaria parasites (Pasvol et al., 1982b).

Once inside the erythrocytes, the merozoites develop into trophozoites (Knell, 1991), which feed on hemoglobin, collecting the iron-heme complex in vacuoles (Dvorak et al., 1975; Wernsdorfer and McGregor, 1988). Some authors have suggested that falciparum-infected erythrocytes possess a transferrin receptor, allowing the parasite to take up iron from transferrin (Rodriguez and Jungery, 1986). As more merozoites develop within the infected erythrocytes, they are liberated from the parent schizont, infecting other erythrocytes in the process (Wernsdorfer and McGregor, 1988). Before rupturing and releasing the newly-formed merozoites, the infected erythrocytes accumulate 'electron-dense material' beneath the erythrocytic membrane and develop surface 'knobs', seen especially in erythrocytes infected with *falciparum* malaria (Aikawa et al., 1986). These knobs make the infected erythrocyte particularly susceptible to adhesion to the vascular endothelium (Udeinya et al., 1981; Gordeuk et al., 1992a).

As the erythrocytic stage of development continues, some of the merozoites undergo gametocytogenesis instead of becoming a trophozoite (Smalley, 1976; Sinden and Smalley, 1979; Kaushal et al., 1980; Sinden, 1982) and become male and female gametocytes. When the gametocytes are ingested by another mosquito, the entire infection process is repeated in another victim (Aikawa et al., 1984; Wernsdorfer and McGregor, 1988; Knell, 1991).

Further information on pathogenesis can be found in the following review articles: Aikawa (1971), Bray and Garnham (1982) and Howard and Gilladoga (1989).

1.3.3 Pathophysiology:

In severe falciparum malaria, multi-organ dysfunction occurs. Pulmonary edema, metabolic acidosis and acute renal failure may result in the death of the patient. Pulmonary edema may complicate falciparum malaria (Spitz, 1946) and co-exist with other symptoms of organ dysfunction (Brooks et al., 1968) causing death in approximately 80% of patients despite treatment. Metabolic acidosis is another ominous symptom. Increased vascular lactic acid levels reflect the severity of the parasitic infestation, that may outstrip the buffering capacity of blood bicarbonate (White et al., 1985). Kidney failure associated with tubular necrosis, is one of the most common causes of death in adult patients with falciparum malaria (Sitprija, 1988). Large-scale intravascular hemolysis of parasitized erythrocytes may result in characteristic 'blackwater' urine (Blackie, 1944). Kidney failure can aggravate the metabolic acidosis because excess hydrogen ions cannot be excreted.

Lactic acidosis, hypoglycemia, and cerebral malaria are responsible for death in children. Hypoglycemia follows cases of serious lactic acidosis (Fisher, 1983; Taylor et al., 1988; Molyneux et al., 1989) and is believed to be a result of quinine therapy (White et al., 1983; Das et al., 1988), increased glucose consumption (Jensen et al., 1983), and impaired gluconeogenesis. Gluconeogenesis may be impaired because elevated levels of tumour necrosis factor (Evans et al., 1989; Grau et al., 1989; Kwiatowski et al., 1990). Cerebral malaria is the most severe form of falciparum malaria (Oo et al., 1987) and patients can develop coma, seizures, and other neurological symptoms of encephalopathy (Daroff et al., 1967; Phillips and Solomon, 1990) despite the absence of cerebral edema (Looareesuwan et al., 1983; Warrell et al., 1986). Nearly half of the children who develop cerebral malaria eventually die from it due to complications (Phillips and Solomon, 1990).

Blood flow to the brain is reduced, particularly in the microvasculature (White et al., 1985). A number of factors contribute to this blockage, such as the formation of 'rosettes' (Carlson and Wahlgren, 1992), which occurs when parasitized and non-parasitized erythrocytes adhere to each other and form aggregates (David et al., 1988). It

has been suggested that the rosettes slow down the blood flow in blood vessels, which may help the rosettes adhere to the vascular endothelium (Kaul et al., 1991). It is unknown how exactly this cytoadherence occurs. Parasitized erythrocytes develop 'knobs' on the surfaces of their membranes (MacPherson et al., 1985; Oo et al., 1987), which may make the infected erythrocytes and rosettes particularly susceptible to adhesion to the vascular endothelium (Udeinya et al., 1981; Gordeuk et al., 1992).

A number of specific interactions between erythrocyte receptors and endothelial glycoproteins -- thrombospondin, CD36 and ICAM-1 -- occur in patients with malaria.

Thrombospondin has been shown to be specifically involved in the adhesion of parasitized erythrocytes to the vascular endothelial surface (Rock et al., 1988). In 1990, Biggs et al. showed that falciparum parasites, that did not induce the formation of knobs, also did not bind to thrombospondin.

The endothelial surface glycoprotein CD36 may also be intimately involved in the cytoadherence of parasitized erythrocytes to the vascular endothelium (Barnwell et al., 1985; Sherwood et al., 1987; Ockenhouse et al., 1991). Monoclonal antibodies directed against CD36 were shown to interfere with cytoadherence of parasitized erythrocytes *in vitro* (Barnwell et al., 1985; Barnwell et al., 1989).

The third surface endothelial glycoprotein, intercellular adhesion molecule-1 (ICAM-1), also plays a role in increasing the binding of parasitized erythrocytes to CD36 in melanoma cell lines, with only slight binding in the absence of CD36 (Berendt et al., 1989; Tandon et al., 1989; Ockenhouse et al., 1991).

Together, these glycoproteins may play an important role in the cytoadherence of parasitized erythrocytes to the vascular endothelium, a phenomenon that may be responsible for the signs and symptoms of cerebral malaria.

The following reviews summarize some of the aspects that were mentioned: Aikawa (1988), Aikawa et al. (1990), White and Ho (1992).

1.3.4 Treatment

A variety of antimalarial preparations is advocated for prophylaxis and is essential for treatment, but there has also been a resurgence of malaria and an alarming development of resistance by the parasites to standard antimalarial medication (Guha et al., 1979; Pettersson et al., 1981; Phillips and Solomon, 1990). Chloroquine-resistant strains of *Plasmodium falciparum* are now common throughout the Tropics (Fogh et al., 1979; Wyler, 1992). Other parasiticidal drugs such as mefloquine (Trenholme et al., 1975; Jiang et al., 1982) and sulfadoxine-pyrimethamine (Fansidar) (Doberstyn et al., 1976; Doberstyn et al., 1979; Strickland et al., 1986) are reported to be effective against chloroquine-resistant falciparum malaria, however, reports of pyrimethamine-resistance have also been documented (Rumans et al., 1979; Hurwitz et al., 1981). Primaquine is an invaluable medication for destroying *vivax* hypnozoites that lie dormant within hepatocytes. Primaquine has the added ability of rendering gametocytes sterile, thus preventing sexual reproduction once the parasites are taken up by the mosquitoes (Peters, 1982).

Cases of malaria in which quinine therapy has failed due to resistance have been reported as far back as the late 1960s (McNamara et al., 1967). Although quinine is said to be one of the most effective antimalarial agents, resistance to it and other parasiticidal agents have staggering health implications for those in endemic malaria zones.

More recently, a different approach to antimalarial therapy has been taken. Rather than attempt to administer antimalarial medication directed specifically against the *falciparum* parasite, some scientists have found that chelating iron may be a more suitable approach to treatment. Several studies have shown that desferrioxamine inhibited the growth of *Plasmodium falciparum in vitro* (Raventos-Suarez et al., 1982; Fritsch et al., 1987; Whitehead and Peto, 1990). Gordeuk et al. (1990) administered DFO to comatose children with cerebral malaria and showed a reduction in the duration of coma. Gordeuk et al. (1992b) administered 100 mg/kg/day DFO to asymptomatic patients with *Plasmodium falciparum* in a small randomized trial and showed a ten-fold increase in the clearance of

parasites in treated patients. Unlike normal erythrocytes, parasitized erythrocytes are penetrated by DFO (Scott et al., 1990; Gordeuk et al., 1992a). The same authors performed a randomized trial in comatose Zambian patients by comparing the recoveries of patients following the administration of 100 mg/kg/day DFO and standard antimalarial therapy to those who received only standard therapy (Gordeuk et al., 1992a). Patients on DFO chelation therapy recovered more quickly than those on standard therapy. It is believed that DFO chelates iron from the parasites (Scott et al., 1990).

While these results are promising, the price of DFO is prohibitive for Third World nations and therefore the drug is not likely to be used on a large scale. As a result, studies with cost-effective, orally active iron chelators have been undertaken to investigate their potential in the treatment of malaria. Hydroxypyridones received particular attention as potential agents for the treatment of malaria, following positive *in vitro* studies (Heppner et al., 1988; Hershko et al., 1991; Herhsko et al., 1992; Lytton et al., 1993).

The following review articles deal with malaria pharmacotherapy: Hall (1976), Wyler (1983a), Wyler (1983b).

1.4 Acute iron intoxication

Acute iron intoxication is a condition with serious consequences. Most of the exposures reported by Poison Control Centres across America involve children under the age of six years (Litovitz, 1993) after accidental ingestion of large amounts of iron found in easily accessible over-the-counter multi-vitamin preparations. Common amounts of elemental iron normally range from 10 to 105 mg per tablet (Banner and Tong, 1986). Some of the brightly coloured, sweet-tasting children's preparations resemble candy, making them very appealing. Cartoon-type vitamins can range in elemental iron content of 12 to 18 mg per tablet (Banner and Tong, 1986). Most parents neglect precautions necessary to prevent children from reaching the seemingly innocuous vitamins, resulting in accidental overdoses (Krenzelok and Hoff, 1979).

1.4.1 The adverse effects of acute iron intoxication

The pathophysiology of acute iron intoxication was originally described to occur in four stages (Covey, 1954).

The first stage of iron poisoning is dominated by symptoms related to the corrosive effect that iron has on the gastrointestinal tract (Jacobs et al., 1965). The stage corresponds to approximately the first 6 hours following the ingestion of the iron tablets. Classical symptoms include nausea, vomiting, diarrhea, and abdominal pain (Spencer, 1951). The irritation of gastric mucosa by iron may progress to hemorrhagic gastritis and perforation (Proudfoot et al., 1986). While these are considered local effects, they can contribute to hypovolemia, secondary to "third spacing" (ie. the loss of vascular fluid into the small intestine), resulting in a rise in hematocrit and viscosity (Whitten, 1968). Hypotension and hypovolemia lead to tissue hypoperfusion (Spencer, 1951) and result in progressive cellular hypoxia, an increase in anaerobic respiration, and a subsequent rise in lactic acid levels (Vernon et al., 1989).

The second stage consists of a deceptively quiescent period. Most experts believe that this period begins within 6-24 hours following the ingestion of iron and represents the transition period between the subsiding of the gastrointestinal symptoms and the development of systemic toxicity (Jacobs et al., 1965). During this stage, the possibility exists that the attending physician misinterprets the patient's decreasing gastrointestinal complaints as a recovery (Spencer, 1951). Of particular concern are the often deceptively low levels of serum iron, which occur because of the rapid clearance of iron from the plasma into the cells (Reismann et al., 1955).

The third stage can begin within 12-48 hours following the ingestion and is characterized by acute circulatory shock and multi-organ dysfunction (Wilzleben, 1966; Tenenbein, 1988). Acidosis at this stage is the direct result of cellular toxicity by the iron (Robotham et al., 1974). The excess iron is concentrated in the mitochondrial cristae, the location of the electron transport system (Ganote and Nahara, 1973). The mitochondria are

especially prone to damage from free radicals generated by iron because of the high concentration of polyunsaturated lipids in the mitochondrial membrane (McGray et al., 1972). Electrons from the electron transport chain may be consumed by the iron and used for oxidation-reduction reactions, resulting in the generation of Haber-Weiss free radicals (Robotham et al., 1974). The steric configuration of the mitochondrial matrix may also become altered, which may disrupt the electron transport system and oxidative phosphorylation and cause a reduction in cellular ATP. Since protons are no longer consumed during oxidative phosphorylation, systemic acidosis results.

Patients, who survive stage 3, may encounter sequelae including hepatic necrosis, gastric scarring, and central nervous system dysfunction in stage 4 (Covey et al., 1954; Gandhi and Robarts, 1962; Tenenbein et al., 1990).

1.4.2 Managing acute iron intoxication

All cases of acute iron intoxication are treated according to three underlying considerations: 1) to prevent any further iron absorption, 2) to enhance elimination of the element and to 3) reverse the pathophysiological manifestations of iron (Engle et al., 1987).

1.4.2.1 Preventing iron absorption

Besides preventing the intoxication, the most important technique in reducing the morbidity and mortality associated with iron overdose is gastrointestinal decontamination (Goulding and Volans, 1977). Decontaminating the gastrointestinal tract of iron can be done by physically removing the gastric contents, by using surgical intervention (Venturelli et al., 1982; Tenenbein et al., 1991), by using iron binders (Neuvonen, 1982), or by intestinal cleansing (Tenenbein, 1987; Tenenbein, 1988).

Activated charcoal is used to reduce the absorption of excessive quantities of compounds. Activated charcoal contains a fine network of pores which adsorbs a variety of organic materials in its enlarged surface area (Neuvonen, 1982). Though effective when

given after lethal doses of chloroquine, phenobarbital, or other organic compounds, activated charcoal does not adsorb acids, bases or small ionized molecules including ferrous sulphate and lithium (Corby et al., 1970; Greensher et al., 1979). In fact, some studies have concluded that iron absorption is not prevented after administering activated charcoal (Henretig and Temple, 1984).

Inducing emesis or performing gastric lavage in an acutely iron-intoxicated individual are two of the most important methods of removing gastric contents (Heyndrickx et al., 1970; Goulding and Volans, 1977). Whereas patients may or may not have vomited when presenting in the emergency department, physicians may at that time choose one of two widely accepted emetics, ipecac syrup (Jonas and Smyth, 1975) or apomorphine (Jaeger and deCastro, 1976; deCastro et al., 1978).

Ipecac acts both locally and centrally by stimulating the chemoreceptor trigger zone in the floor of the fourth ventricle in the brain (Stewart, 1983). This activates the vomiting center in the reticular formation to create the coordinated motion in the stomach and intestine associated with vomiting. In children, most authors report emesis within 20 minutes (range 14 to 37 minutes) (Dean and Krenzelok, 1984). This delayed emetic response is the major disadvantage of using ipecac syrup for gastric evacuation because absorption continues during this period.

Apomorphine, an analogue of morphine, elicits a centrally mediated response (deCastro et al., 1978). It is occasionally used in a hospital setting. Like morphine, apomorphine maintains its ability to depress the central nervous system and respiratory functions and is readily reversible by the administration of naloxone (Hanson, 1967; deCastro et al., 1978). Emesis by apomorphine routinely occurs within four to seven minutes of admistration.

When neither ipecac syrup nor apomorphine are indicated, gastric decontamination may be accomplished by gastric lavage (Heyndrickx et al., 1970). Gastric lavage with normal saline is recommended over lavage with other solutions to minimize the

possibility of electrolyte imbalance. Death has resulted in patients following gastric lavage with hypertonic saline (Carter and Fotheringham, 1971), whereas hyponatremia and hypokalemia occurred in patients lavaged with water (Peterson, 1979). Sodium bicarbonate and phosphate solutions have also been used as intragastric complexing agents during gastric lavage (Dean and Krenzelok, 1987). This type of lavage has been carried out in the belief that the bicarbonate and phosphate ions will react with the ingested iron to form an insoluble carbonate or phosphate complex (Czajka et al., 1981), which will interfere significantly with iron absorption. In the best scenario examined (sodium bicarbonate: iron ratio, 10:1, time of measurement of 60 minutes), 83.6% of the iron remained soluble. Clinically, this is not encouraging. As the pH of the simulated gastric mixture increased, the solubility of the complexed reactants decreased to less than 20%. Thus, acidic conditions present in the stomach make it unlikely to expect that the iron and the bicarbonate or phosphate complexes will remain insoluble.

Furthermore, if the stomach is constantly being lavaged, it is perhaps unimportant whether the material is soluble or not. The use of the phosphate lavage appears to be contraindicated, as repeated doses of phosphate solutions have been shown to produce hyperphosphatemia, hypernatremia and hypotension (Bachrach et al., 1979).

Magnesium hydroxide has been effective in reducing the solubility of iron (Chadwick and Corby, 1982). Studies with milk of magnesia have shown a significant reduction in iron absorption in animals (Corby and McMullen, 1985).

There have been conflicting opinions about the use of desferrioxamine (DFO) as an oral intragastric iron chelator. DFO chelates iron efficiently in a 1:1 molar ratio, creating the soluble iron complex, ferrioxamine (FO). Ferrioxamine is positively charged and is poorly absorbed (Keberle, 1964). In this study, half of the rats received 50 mg/kg of radiolabelled ferric chloride and absorbed 19% of the iron whereas rats receiving DFO in addition to the iron did not absorb any iron. These findings were later supported by those of Moeschlin and Schnider (1963) after showing that 70% of guinea pigs treated with oral

DFO immediately upon receiving iron survived an LD_{100} dose of iron. In another study, four dogs were gavaged with ferrioxamine corresponding to 225 mg/kg of elemental iron (Whitten et al., 1965). All of the FO-treated dogs died with symptoms of shock. In that study, FO did not prevent the absorption of iron and the iron-drug complex precipitated toxicity.

The ability of oral DFO to chelate iron and prevent gastric absorption has also been studied more recently by Dean et al. (1988). In these studies, 60 mg/kg of elemental iron was administered to pigs along with an estimated 1 g/kg DFO. The DFO-treated animals exhibited a marked reduction in serum iron and significantly lower iron levels at six and eight hours following iron ingestion, but there was little reduction in the areas-under-the-curve. Furthermore, the DFO-treated animals developed vomiting and diarrhea and had FO in the urine, that suggested enhanced iron absorption.

In 1992, Jackson et al., did a crossover study to examine the effect of orally administered DFO on iron absorption in humans. In the first month of experiment, each of the seven male subjects received 5 mg/kg elemental iron as ferrous sulphate. Three months later, the same procedure was performed with the addition of equimolar concentrations of DFO orally. There was no difference between the two treatment groups in the maximum serum iron concentration and the serum iron area-under-the-curve.

From the studies presented, there is no evidence to suggest that, given orally, DFO significantly reduces iron absorption. The conflicting reports of Whitten et al. (1965) and Moeschlin and Schnider (1963) make it difficult to conclude whether or not oral DFO enhances the survival of animals treated acutely with excess iron. It also appears that aside from the work of Keberle (1964), all other studies suggest that FO is readily absorbed from the gastrointestinal tract and that FO itself may be toxic.

There are still other methods of reducing iron absorption that may be used if the ones mentioned have failed. Some of these include whole bowel irrigation and surgical gastrotomy. Results from whole bowel irrigation using polyethylene glycol solution have

been solely from case studies (Tenenbein et al., 1987; Tenenbein et al., 1988). As a final resort of gastric decontamination, gastrotomy may be performed to remove a solid mass of iron tablets that cannot be removed using traditional methods (Tenenbein et al., 1991).

1.4.2.2 Elimination of iron

Chelation therapy with desferrioxamine (DFO) has proven to be both efficacious and life-saving in cases of acute iron intoxication. DFO complexes with ferric iron to create a soluble ferrioxamine chelate, that is excreted by the kidneys (Proudfoot et al., 1986; Engle et al., 1987). The positive charge of the ferrioxamine complex restricts its distribution to the extracellular space (Keberle, 1964), which may prevent toxic intracellular accumulation of iron. Furthermore, once inside the cells, DFO can chelate cytoplasmic and excess mitochondrial iron (Moeschlin and Schnider, 1963) and may minimize mitochondrial damage (Wills, 1969; Engle et al., 1987; Schauben et al., 1990).

DFO is not absorbed orally and must be administered parenterally: intravenous, subcutaneous or intramuscular injection (Bentur et al., 1991). At this rate, DFO-bolus-related hypotension (Butterworth and Scott, 1965) is not seen (Peck et al., 1982; Henretig et al., 1983) and the intravenous lines can also be used for fluid administration. Once a patient stabilizes, the infusion rate of DFO should be decreased to 6 mg/kg/hour (Henretig et al., 1983).

The severity of iron poisoning makes it ethically difficult to perform controlled clinical trials and investigate new therapies. For this reason, after many case studies, one can generalize about therapeutic regimens. For example, the clinical histories and outcomes of 172 children treated with intravenous DFO for an iron overdose were presented by Westlin (1966). Mortality was 1.4%, compared to a pre-DFO era mortality of 45% (Aldrich, 1958). Since other new medical procedures had also been introduced, it was difficult to determine the effect that DFO alone had on mortality. However, if one considers the destructive effect or iron on mitochondria, it makes sense to remove as much

iron as possible. Many patients have survived lethal doses of iron overdose because of the administration of DFO (Peck et al., 1982; Henretig et al., 1983).

Though proven to be effective in acute iron intoxication, desferrioxamine exhibits toxic effect when used in high doses. One of these is an acute respiratory distress syndrome (Tenenbein et al., 1992). Other toxicities associated with desferrioxamine are listed in section 1.5.4.

1.5 Desferrioxamine

1.5.1 Pharmacology

Numerous compounds were isolated from *Streptomyces pilosus* during the early 1960s. One of these was a crystalline compound, named desferrioxamine B (desferrioxamine; DFO [Fig. 1]). This straight-chain compound, containing three hydroxamic acids, chelates ferric iron (Fe^{3+}) ions in a 1:1 molar ratio (Keberle, 1964; Westlin, 1971) and creates a stable complex, ferrioxamine B (ferrioxamine), with an affinity constant of 10^{31} (Keberle, 1964). The affinity constants of DFO for other metals were much lower than that of Fe^{3+}, ranging from affinity constants of 10^{1} for strontium to 10^{14} for cupric ions. DFO has an affinity constant of only 10^{10} for ferrous ions (Fe^{2+}). Not only do these findings demonstrate the selectivity of DFO for Fe^{3+} over Fe^{2+}, but more importantly they show the specificity of DFO for chelating ferric ions over other metal ions.

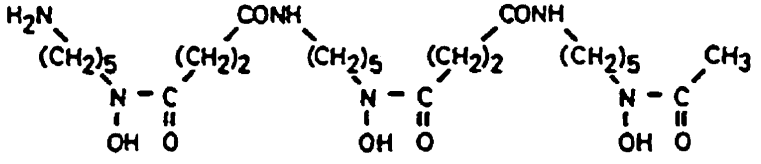


Figure 1: Structure of desferrioxamine (Keberle, 1964)

1.5.2 Pharmacokinetics

Desferrioxamine is positively charged and poorly absorbed orally (Keberle, 1964; Westlin, 1971), necessitating parenteral administration. Moreover, it has a short elimination half-life ($t_{1/2}$) of ten to thirty minutes (Propper and Nathan, 1982) and five to ten minutes (Summers et al., 1979). Summers et al. (1979) also showed that the $t_{1/2}$ between iron-loaded and non-iron-loaded individuals did not differ. Although the findings of Alain et al. (1987) showed $t_{1/2}$ values of DFO in healthy and iron-loaded individuals of 6.1 hours and 5.6 hours, respectively, these findings have not been duplicated.

The volume of distribution (V_d) of DFO was calculated to be 60% of bodyweight in nephrectomized dogs. This value corresponds to the total body water space (Peter et al., 1966). DFO exhibits a first order decrease in plasma concentrations. Thus, the findings of Alain et al. (1987) suggest that there is no difference between the volumes of distribution between healthy (2.59 L/kg) and iron-loaded (1.92 L/kg) patients.

Alain et al. (1987) also showed similar clearances in normal (0.296 L/h/kg) and iron-loaded (0.234 L/h/kg) individuals and calculated the renal clearance to be 33% of total body clearance. Bentur et al. (1990a), however, showed that iron-loaded children exhibited a clearance of 1.8-2.4 L/h/kg.

Ferrioxamine (Figure 2), has a volume of distribution (V_d) of 19% of body weight (Keberle, 1964; Peters et al., 1966; Summers et al., 1979). Ferrioxamine remains confined to the extracellular space and is rapidly excreted in the urine (Keberle, 1964; Peters et al., 1966), with a $t_{1/2}$ of 5.9 hours and 4.6 hours (Alain et al., 1987) and a clearance of 516 L/h/kg and 1716 L/h/kg, in normal and iron-overloaded individuals, respectively.

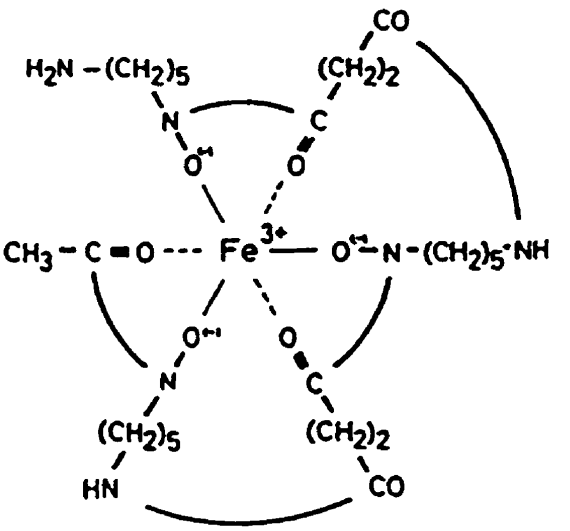


Figure 2: Structure of ferrioxamine (Keberle, 1964)

1.5.3. Efficacy and Effectiveness

Transfusion-dependent thalassemia patients acquire large deposits of iron in the heart (Engles, 1964), liver (Brittenham et al., 1993), and endocrine glands (Flynn et al., 1976). Of particular importance is the iron-loading of the myocardium, which, if left untreated with chelating agents, will lead to cardiac failure. This is the major cause of death in adult thalassemia patients (Weatherall and Clegg, 1981).

The results of the ground-breaking study by Sephton-Smith (1962) showed that the administration of DFO caused an increase in urinary iron excretion (UIE) in homozygous beta-thalassemia patients, and the value of DFO became apparent. Later, Sephton-Smith (1964) performed similar experiments examining the potential of DFO at inducing UIE given by different routes -- intramuscular, intravenous, oral, and subcutaneous. Except for the oral route, the others resulted in an increase in UIE, with the intravenous administration producing the greatest amount. The authors went on to forecast that patients would someday administer the drug to themselves.

These pioneering findings set the stage in 1966 for a long-term controlled clinical trial at the Hospital for Sick Children in London (Barry et al., 1974, Letsky, 1976).

Patients receiving either intramuscular DFO injections six days per week or intravenous DFO with a transfusion regimen, exhibited a dramatic decrease in hepatic iron concentration but no change in hepatic fibrosis (Barry et al., 1974). Patients who did not receive chelation therapy developed an increase in liver iron concentration and progressive hepatic fibrosis. Furthermore, puberty was delayed in only one out of the four chelated children, as opposed to four out of five of the unchelated children. The growth rates of the chelated children were also greater than those of the unchelated children. The effectiveness of intramuscular DFO at inducing UIE was also confirmed by Seshardi et al. (1974a). The treatment with DFO was so effective that in 1982, Modell et al. published the findings that treating iron-loaded patients with at least 3 g DFO per week improved the survival. During the course of the clinical trial by Modell and Beck (1974), in London's Hospital for Sick Children, UIE was shown to double on average when thalassemia patients received oral vitamin C (200 mg per day) along with their intramuscular DFO injections. Modell and Beck (1974) also confirmed the earlier findings of Sephton-Smith (1964), that DFO caused greater UIE when administered intravenously, than intramuscularly. Propper et al. (1976) re-examined the routes of administration of DFO and treated 10 patients with continuous intravenous DFO of the same DFO dose as the intramuscular injection. Significantly greater UIE with the continuous intravenous administration was found. This confirmed that although DFO is an effective drug, its potential is diminished because of the way the drug is administered. Graziano et al. (1978) enrolled ten iron-loaded patients in a study in which DFO was administered either subcutaneously or intravenously. From the results, the investigators found that the subcutaneous route of DFO was approximately 80% as effective as the intravenous route at inducing UIE. More recently, Olivieri et al. (1992a) showed that a decrease in liver enzymes and an increase in UIE over a 24-hour period could be achieved with continuous intravenous ambulatory DFO.

In numerous studies the effects of subcutaneous administration of DFO to iron-loaded thalassemia patients were examined. Hussain et al. (1976) showed that there is an

increase in the UIE of patients who receive DFO subcutaneously compared to intramuscular administration. Increases in UIE of 101% and 128%, respectively, occurred in subcutaneously treated thalassemia patients before and after transfusions. The authors suggested the use of portable pumps to administer DFO subcutaneously for 24 hours a day. Hussain et al. (1977) later showed that 12-hour subcutaneous infusions would be more acceptable to the patients than continuous 24-hour delivery, and that 12-hour infusions still resulted in acceptable UIE. Continuous 24-hour subcutaneous DFO administration was found to be 90% as effective at inducing comparable levels of UIE as intravenous administration (Propper et al., 1977).

Other studies presented the benefits of 12-hour subcutaneous infusions with DFO (Pippard et al., 1978a,b) at inducing greater UIE than intramuscular injections. Long-term subcutaneous DFO treatments in iron-loaded patients have been effective in reducing serum ferritin, hepatic iron concentration and liver enzyme levels (Hoffbrand et al., 1979; Maurer et al., 1988) and preventing the development of cardiac disease (Wolfe et al., 1985).

In most studies the emphasis is on reporting UIE data. The 24-hour urine specimens are relatively easy to collect and provide a good indication of iron excretion. Cumming et al. (1969) suggested that excretion of approximately one third of total body iron occurs in the stool. Later, Pippard et al. (1982) examined the effect of subcutaneous DFO infusions on both urinary and fecal iron excretion at various hemoglobin levels. These authors showed an inverse relationship between UIE produced by subcutaneous DFO and hemoglobin levels, and a direct relationship between DFO-induced fecal iron excretion and hemoglobin levels. Not only do these findings reveal the drawbacks of relying solely on the results of UIE, but they also suggest that perhaps the stored liver iron is in a more accessible form in preparation for erythropoiesis when hemoglobin levels are low (Pippard et al., 1982).

1.5.4. Toxicity

Despite the proven efficacy of desferrioxamine, a number of toxicities are associated with its use (Hershko and Weatherall, 1988; Porter and Huehns, 1989; Bentur et al., 1991).

One of the most immediate toxic effects evident with bolus DFO infusion is hypotension. Sudden hypotension and reflex tachycardia were reported in patients given DFO intravenously over 15 minutes for acute iron intoxication (Whitten et al., 1965; Westlin, 1966). It was postulated that a histamine-mediated mechanism may be responsible for venous dilation and poor venous return, leading to a decrease in cardiac output and subsequent hypotension (Whitten et al., 1965). Despite this plausible hypothesis, pre-treatment with antihistamine did not prevent the hypotension from occurring (Whitten et al., 1965).

In several case reports reduced visual acuity has been reported in patients treated with DFO. Davies et al. (1983) reported DFO-induced visual disturbances in four patients that were on high-dose DFO therapy. The DFO doses ranged from 75 to 235 mg/kg/day in one patient to 165 to 220 mg/kg/day in another. Of these, only the two patients treated with the highest dose of DFO developed progressive blurring of vision and retinopathy. The authors suggested that the chelation of other metal ions be investigated in progressive or acute ocular toxicity. These investigators felt that ocular toxicity was more likely the direct toxicity of DFO than to depletion of trace metals. The possibility that ocular abnormalities during high-dose DFO therapy develops because of the excretion of chelated trace metals, was examined despite the suggestion by Simon et al. (1983) that toxicity occurs too suddenly to be due to the removal of trace elements.

Acute loss of vision and neuropathy were also observed in patients on chronic DFO therapy (Borgna-Pignatti et al., 1984; Lakhanpal et al., 1984; Blake et al., 1985; Olivieri et al., 1986), and also in a patient after a single 'challenge' dose of DFO (Bene et

al., 1989) given to diagnose increased aluminum accumulation in patients with renal failure.

In a study by DeVirgiliis et al. (1988a), homozygous beta-thalassemia patients received intravenous infusions of 200 to 450 mg/kg/day DFO once a month, in addition to their daily subcutaneous therapy of 40 to 60 mg/kg/day DFO. Not only did the high-dose DFO therapy produce reversible retinal toxicity, but balance studies also showed that the fecal loss of iron, copper and zinc was higher than the urinary excretion of these elements. These results agree with the findings of Pippard et al. (1982), that fecal iron excretion was greater with high-dose DFO therapy than was urinary iron excretion when the hemoglobin levels were at their peak. Once again, the sole use of urine as an indicator of total body metal ion excretion with DFO may be misleading; and although urinary zinc and copper excretion may not be detected in the urine, depletion of these elements may occur when not measured in the feces.

Besides neurotoxicity involving the optic tract, there have also been a number of studies reporting patients treated with DFO who subsequently developed auditory neurotoxicity.

Desferrioxamine is commonly used to remove accumulated aluminum from bones of nephrectomized patients or patients with chronic or end-stage renal disease (Guerin et al., 1985). In one case report, a 26-year-old woman complained of hearing loss after six months of DFO therapy, following bilateral nephrectomy. Five weeks after DFO treatment ceased, her audiogram had returned to normal. Similar findings were cited by Cases et al. (1988) for one patient who received a total dose of 38 g of DFO in end-stage renal disease. Sensorineuronal hearing loss was confirmed in 22 of 89 patients in a Toronto cohort of chronically iron-overloaded patients (Olivieri et al., 1986). All these patients recovered completely after DFO was stopped, except for six patients who required hearing aids. Although audiosensory toxicity and high DFO doses were correlated, many of the patients from the Toronto cohort were only receiving between 40 and 80 mg/kg/day

DFO. While a cause-effect relationship was not seen in all patients, such a relationship was 'strongly suggested' when the drug was withdrawn (Olivieri et al., 1986). These findings were supported by further patterns of deterioration and improvement in audiograms of a patient following the administration and cessation, respectively, of DFO (Gallant et al., 1987). Bentur et al. (1990a) performed pharmacokinetic studies on the patients in the cohort of Olivieri et al., 1986. Patients who had experienced neurotoxicity had received significantly more DFO than had asymptomatic patients.

Later, Porter et al. (1989a) suggested that the mechanisms for auditory and visual toxicity are different because the former has been documented in patients on low-dose DFO therapy, whereas the latter occurred with substantially higher doses. Although higher doses of DFO may be needed to penetrate the blood-retinal barrier, DFO may reach the cochlea and accumulate there. The authors recommended that audiograms be done frequently and that patients report any auditory disturbances.

Further toxic effects seen in iron-loaded patients on DFO therapy are bacterial infections. *Yersinia enterocolitica,* for example, poses a hazard to iron-overloaded patients receiving DFO chelation therapy. Since most bacteria require iron for growth (Weinberg, 1978) and secrete high-affinity siderophores (iron carriers) into their environment, they take up chelated iron complexes via surface receptors. While the iron requirement of *Yersinia* is particularly high, these organisms do not secrete a siderophore, yet possess surface receptors for ferrioxamine (Perry and Brubaker, 1979). *Yersinia* are rod-shaped, gram-negative enterobacteria (Bouza et al., 1980), that are not pathogenic most of the time. The presence of DFO dramatically enhances their virulence as shown in the experiments with mice (Robbins-Browne and Prpic, 1985), receiving intraperitoneal injections of virulent *Yersinia enterocolitica.* The LD_{50} dose of the bacteria was found to be decreased by a factor of 10 and by 100 000 when the mice received iron-dextran and iron-dextran with DFO, respectively.

Therefore, iron-loaded patients may be susceptible to systemic yersiniosis because of excess iron stores and the use of DFO. Thalassemia patients have developed systemic yersiniosis while on DFO, and recovered following antibiotic therapy (Butzler et al. 1978) or following an ileostomy with antibiotic therapy (Gallant et al., 1986).

Acute renal insufficiency is yet another toxicity that has also been demonstrated with the use of intravenous DFO (Batey et al., 1979). Three patients treated with DFO -- two for thalassemia and a two-year-old girl for acute iron intoxication -- have developed acute renal changes (Koren et al., 1989). Two of these three patients had dramatic rises in serum creatinine levels and decreased creatinine clearance; the third had moderate increases in creatinine. A short time after discontinuing the drug, serum creatinine levels normalized. Similar changes in creatinine clearance followed the administration and removal of intravenous DFO, respectively, in eight mongrel dogs (Koren et al., 1989). This nephrotoxicity may be due to an acute decrease in renal perfusion with unchanged systemic blood pressure or to interference with tubular reabsorption of salts.

In thalassemia patients, desferrioxamine has also been shown to interfere *in vitro* with collagen synthesis and fibroblast proliferation (Hunt et al., 1979). Until recently, aggressive DFO chelation therapy was the standard procedure and was initiated early in life to improve growth and sexual maturation. DeVirgiliis et al. (1988b) challenged this approach to chelation therapy in young chronically-transfused patients. A cohort of thalassemia patients were divided into three groups (those who received subcutaneous DFO therapy before the age of three, those receiving subcutaneous DFO therapy starting at three years of age and those that received intramuscular DFO at age three). The patients' height and longitudinal growth rate were charted. Children who received DFO before the age of three had a significant reduction in height and exhibited metaphyseal changes on X-ray films. Whether DFO was directly toxic to the growth plate or whether the drug chelated metals for critical enzyme activity is uncertain. Olivieri et al. (1992c) reported similar findings in their patients. Later, Hartkamp et al. (1993) reported thalassemic patients

treated with DFO suffering from spinal deformities. However, the changes in the spinal column were substantially different from bone deformities observed from compensatory bone marrow expansion.

Pulmonary toxicity has also been linked to DFO use in four out of eight chronically-transfused thalassemia patients (Freedman et al., 1990). These four patients developed a pulmonary syndrome with hypoxemia, tachypnea and a diffuse interstitial pulmonary pattern on X-ray films. The lung biopsy specimens indicated inflammation, interstitial fibrosis and alveolar damage.

Although rare, anaphylactic reactions have been seen in patients on DFO therapy (Freedman et al., 1989) and desensitization to the drug is possible (Miller et al., 1981; Davies et al., 1983).

1.6 The search for an alternative iron chelator

1.6.1 The ideal iron chelator

Lack of compliance or refusal of subcutaneous or central intravenous DFO therapy results in progressive organopathies related to iron overload, ending with cardiac failure. The prohibitive cost of DFO restricts its use in developing countries by the many patients who require it. As a result, there has been a concerted effort to develop a cheaper, orally active alternative iron chelating agent.

Several criteria define the ideal iron chelator (Porter et al., 1989b). A chelator should:

1. be absorbed orally;
2. have a high affinity for Fe^{3+} and be selective for iron over other metals;
3. be accessible to all tissue iron pools;
4. be excreted in the urine or the feces;
5. not redistribute to other body sites;
6. not be toxic;
7. not be metabolized;
8. induce a negative net iron balance;
9. not encourage bacterial proliferation; and
10. be cost-effective.

1.7 Deferiprone

1.7.1 Chemistry

Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one; CP20; L1 [Fig. 3]) is a bidentate chelator belonging to the class of drugs, the hydroxypyridones (Kontoghiorghes, 1985). As a bidentate chelator, deferiprone is less efficient than desferrioxamine because three deferiprone molecules are required to chelate and effectively neutralize all six of the catalytic coordination sites on Fe^{3+} ions (Fig. 4). The binding constant of the deferiprone-

iron complex is 10^{36} (Motekaitis and Martell, 1991) and the partition coefficient of deferiprone was 0.19 (Kontoghiorghes and Sheppard, 1987) and 0.21 (Hershko et al., 1991; 1992). Deferiprone has a solubility of 16 to 18 mg/ml in saline (Kontoghiorghes et al., 1990) and 10 mg/ml in distilled water.

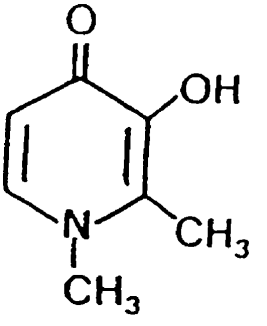


Fig. 3: Structure of deferiprone [Kontoghiorghes et al., 1989a]

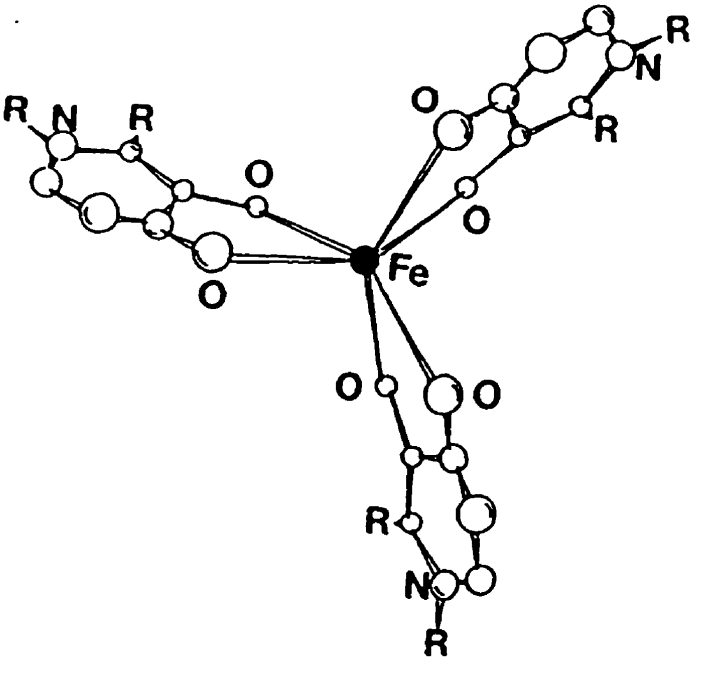


Figure 4: Structure of deferiprone-iron complex (3:1 ratio) [Kontoghiorghes et al., 1989a]

1.7.2 Pharmacokinetics

Deferiprone is absorbed orally and is measurable in serum (Kontoghiorghes et al., 1990b; Matsui et al., 1991; al-Refaie et al., 1995). The primary metabolite of deferiprone is deferiprone-glucuronide (Kontoghiorghes et al., 1990c; [Fig. 5]). Glucuronide formation implies that deferiprone must enter hepatocytes to be metabolized. Deferiprone can be measured by the HPLC method of Klein et al. (1991), described in greater detail in the Materials and Methods section (see Chapter 3).

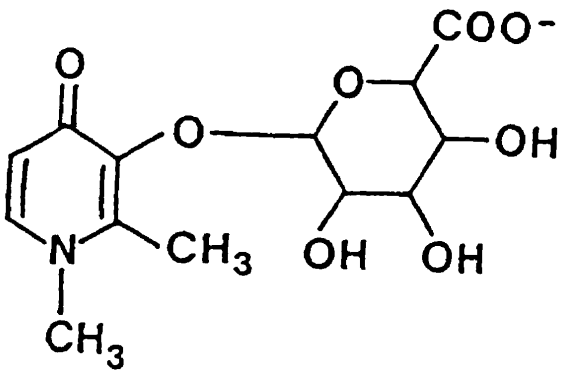


Fig. 5: Structure of deferiprone-glucuronide [Kontoghiorghes, 1990c]

Oral absorption is rapid (Kontoghiorghes et al., 1990b; Matsui et al., 1991; Al-Refaie, 1995). Matsui et al. (1991) also found that, although food significantly reduces the peak (C_{max}) of serum deferiprone levels, it does not interfere with the size of the area-under-the curve.

The findings of Kontoghiorghes et al. (1990b) showed an elimination half-life of approximately 77 ± 35 min (range, 47 to 134 minutes) and do not agree with those of Matsui et al. (1991), who calculated the $t_{1/2}$ to be three to four hours. Kontoghiorghes et al. (1990b) also suggested that a multiple dosing regimen be instituted to provide a continuous supply of deferiprone. Kontoghiorghes et al. (1990b) administered 3 g of

deferiprone as a single dose to his patients, regardless of their body mass. These patients received total calculated doses ranging from 35 mg/kg/day to 71 mg/kg/day. As a result, the C_{max} deferiprone concentrations from the patients of Kontoghiorghes et al. (1990b) could not be compared to the mean C_{max} concentrations (17.5 ± 2.1 μg/ml) of the patients of Matsui et al. (1991), who had received 25 mg/kg every eight hours (ie. total daily dose of 75 mg/kg/day). A comparison of those early studies also showed the importance of administering a defined dose of deferiprone based on each patient's body mass (ie. mg/kg/day).

Although a number of the pharmacokinetic parameters have been studied, there has been no examination to determine how often deferiprone should be administered to provide maximal urinary iron excretion.

1.7.3 Efficacy and Effectiveness

After the orally active hydroxypyridones became available, they received a great deal of attention not only because of their ability to induce iron excretion but also because of their apparent lack of toxicity (Kontoghiorghes, 1985; Kontoghiorghes, 1986a).

In early studies, the intragastric administration of 200 mg/kg deferiprone induced iron excretion in iron-loaded mice and rats at levels comparable to those induced by the same dose of DFO (Kontoghiorghes, 1985). Studies performed using N-alkyl substitutions yielded similar findings (Kontoghiorghes, 1986a). The N-alkyl-substituted hydroxypyridones that were shown to have partition coefficients between 0.2 and 3.2 induced the greatest iron excretion and were postulated to be beneficial in the treatment of iron-overloaded patients (Kontoghiorghes, 1986a). Although hydroxypyridone compounds with partition coefficients above 0.3 were found to be effective, hydroxypyridone compounds with partition coefficients above 1.0 were toxic (Porter et al., 1990).

Iron excretion was not based on partition coefficients alone. Kontoghiorghes (1986b) also showed that deferiprone-induced iron excretion was dose-dependent. Gyparaki et al. (1987) confirmed these findings for both urinary and total body iron excretion in iron-overloaded mice that received intraperitoneal injections of deferiprone.

Hydroxypyridone compounds with low partition coefficients exhibit low toxicity, those with higher partition coefficients were shown to produce higher fecal iron excretion. Gyparaki et al. (1986) demonstrated that N-alkyl-substituted, 2-methyl-3-hydroxypyrid-4-one molecules with partition coefficients close to 1.0 and similar partition coefficients of the free and the complexed forms of the drug (ie. the CP22 molecule: $-(CH_2)_2CH_3$) resulted not only in greater urinary iron excretion in iron-overloaded mice, but also in a two-fold increase in fecal iron excretion, compared to deferiprone-induced iron excretion. The investigators attributed this change in fecal iron excretion to the increase in partition of the drug molecule into iron-overloaded hepatocytes. The study also showed that as the partition coefficient of these drugs increased, the mortality of animals increased as well. Studies by Gyparaki et al. (1987) and Porter et al. (1990) supported these findings, which are believed to be caused by the lipophilic drugs redistributing iron to intracellular compartments and resulting in cellular toxicity.

Further work in rabbits showed that doses of deferiprone of 200 mg/kg, given intragastrically or intramuscularly resulted in increases in both urinary and fecal iron excretion similar to those following subcutaneous desferrioxamine (Kontoghiorghes and Hoffbrand, 1986). However, intramuscular doses were more effective at inducing urinary iron excretion than intragastric doses. Whereas urinary iron excretion increased with the administration of deferiprone, the urinary excretion of zinc, copper, magnesium, and calcium were not affected (Kontoghiorghes and Hoffbrand. 1986).

The ability of deferiprone to chelate iron in iron-overloaded rats was investigated by Kontoghiorghes et al., (1987a). Iron-dextran-treatment of iron-overloaded and non-iron-overloaded rats receiving intravenous injections of ^{59}Fe-ferritin two hours prior to

chelation to allow the ^{59}Fe-ferritin to enter the hepatocytes. Intraperitoneal injections of deferiprone caused approximately 40% of the ^{59}Fe to be excreted in the bile and stool (Kontoghiorghes et al., 1987a). The oral and intramuscular administration of deferiprone gave similar results. The findings, that deferiprone given *in vivo* preferentially chelated iron from ferritin, supported the previous *in vitro* work of Kontoghiorghes et al. (1987b).

Kontoghiorghes et al. (1987c) then published their findings on the efficacy of deferiprone in removing iron from three chronically-transfused myelodysplastic patients. Dose-dependent increases in urinary iron occurred in all patients without any increases in urinary zinc, calcium or magnesium excretion. Urinary iron excretion was comparable to the excretion following standard subcutaneous DFO therapy (Kontoghiorghes et al., 1987c). With the success of this preliminary study, the potential of deferiprone became apparent. Kontoghiorghes et al. (1987d) then published another study with a larger sample of eight chronically-transfused, myelodysplastic or thalassemic patients treated with oral deferiprone therapy. Oral deferiprone induced urinary iron excretion in all patients, with higher excretion levels in the more heavily iron-burdened patients with thalassemia. The authors showed that deferiprone induced a net negative iron balance in iron-overloaded patients, when administered in doses comparable to those of standard subcutaneous DFO therapy.

Kontoghiorghes et al. (1990a) later reported dose-dependent (confirmed by Agarwal et al., 1990; Olivieri et al., 1990a) and frequency-dependent increases in urinary iron excretion of thirteen iron-overloaded patients on long-term deferiprone therapy. Urinary iron excretion did not plateau and multiple doses of deferiprone (ie. 3 g oral deferiprone twice daily in twelve of the patients) was adequate to induce a negative net iron balance. The investigators showed that the major route of elimination of iron by deferiprone was the kidney, since increases in fecal iron were not noted, even at higher deferiprone doses (Kontoghiorghes et al., 1990a). These findings agreed with those of Venkataram and Rahman (1990). That deferiprone was at least as effective at inducing

urinary iron excretion as desferrioxamine was confirmed by Tondury et al. (1990) and later in total iron balance studies by Olivieri et al. (1990a; 1990b). Fecal iron excretion in six patients undergoing balance studies (Olivieri et al., 1990b) accounted for 15% of total body iron excretion, with a range of 0-28%, but urinary iron excretion appeared to be the major route of excretion in patients undergoing deferiprone chelation therapy.

Although in short-term studies, deferiprone appeared to be as effective as DFO at inducing urinary iron excretion, there had been little investigation as to whether the liver iron concentrations were reduced. One of the first histopathological studies of iron-loaded mice following 60 days of deferiprone treatment, were those done by Porter et al. (1991). Only the liver sections showed abnormalities related to the experimental treatment; the other organs did not show any abnormal changes (Porter et al., 1990). Compared to controls, all of the hydroxypyridones decreased hepatic iron accumulation, the investigators found that deferiprone was as effective as DFO in decreasing the hepatic iron concentrations. The authors also confirmed their previous findings that the liver is the major organ for chelation therapy with hydroxypyridones (Porter et al.,1988).

In clinical trials, no direct evidence was demonstrated that hepatic iron concentrations decreased in humans until Olivieri et al. (1992b) showed first that deferiprone decreases the total body iron content in humans. Normalized serum ferritin levels and dramatic reductions in both heart and liver iron concentrations in a 29-year-old man with thalassemia intermedia were reported following nine months of deferiprone therapy. The iron concentration in the liver was measured in a biopsy specimen and magnetic resonance imaging was used in assessing both hepatic and cardiac iron content both before and after nine months of deferiprone therapy. Shortly thereafter, al-Refaie et al. (1992) and Agarwal et al. (1992) reported similar reductions in serum ferritin levels in 11 and then in 52 transfusion-dependent homozygous beta-thalassemia patients, on deferiprone therapy.

More recently, Olivieri et al. (1995) in a long-term assessment of patients on deferiprone therapy, reported decreases in serum ferritin levels and hepatic iron concentrations treated for several years suggesting that deferiprone is efficacious in reducing body iron stores and for treating patients with thalassemia major.

In order to determine the true potential of a drug, compliance to therapy is essential. Compliance during chronic drug therapy decreases with time (Stewart and Cluff, 1972).

The Medical Event Monitoring System (MEMS) was designed in order to address the issue of compliance (Cramer et al., 1989). The MEMS device consists of a plastic opaque white pill jar and a large lid with an electronic microchip embedded inside the lid, along with a 6-month battery pack. Every time a patient removes the lid, the event is registered in the microchip and the compliance of the patient can be determined by the physician (or the company) using a software package provided by the company. Despite the proven efficacy of chronic deferiprone therapy in thalassemia patients, MEMS devices were used to determine compliance of patients, because diaries kept by patients were found to be unreliable (Olivieri et al., 1991a).

It is essential for patients to take all of their deferiprone medication because the formation of the 3:1 (deferiprone:iron) complex is a step-wise reaction and requires three molecules of deferiprone to neutralize all six of the coordination sites of iron. The argument for compliance is to ensure 'completeness' of deferiprone-iron complex formation; otherwise, there is a concern that 2:1 and 1:1 complexes may be formed and harmful free radical reactions may be generated in serum or tissues.

1.7.4 Toxicity

Pre-clinical animal studies showed evidence of toxicity with high doses of deferiprone much as changes in hematological indices. Following the administration of 200 mg/kg deferiprone solution intraperitoneally to iron-overloaded and non-iron-

overloaded mice for a period of 60 days, Porter et al. (1989c) found that the non-iron-overloaded mice had lower hemoglobin levels, leucocyte and platelet counts and an increase in mean corpuscular volume. These changes did not develop in the iron-overloaded mice. The authors proposed that these results may be caused by the N-demethylation of deferiprone, resulting in the formation of other types of metabolites. Shortly thereafter, Kontoghiorghes et al. (1989b) reported that when non-iron-overloaded male rats were gavaged with 200 mg/kg/day deferiprone for 90 days and 200 mg/kg twice daily for an additional 60 days, similar changes to those reported by Porter et al. (1989c) were noted, except for decreases in platelet counts. Changes in hematological parameters were not evident following a regimen of 60 mg/kg/day deferiprone for 36 days (Kontoghiorghes et al., 1989b). In other studies no changes were noted in any of the hematological parameters following either high-dose acute studies (200 mg/kg/day deferiprone for 30 days) (Kontoghiorghes, 1986a) or low-dose chronic studies (60 mg/kg/day deferiprone for 130 days) (Kontoghiorghes and Hoffbrand, 1989).

Kontoghiorghes (1986b) did not report any ill side-effects or unusual postmortem findings following either the acute administration of 300 mg/kg/day oral deferiprone for 24 days or intraperitoneally for one month. However, hypersalivation was noted later by Kontoghiorghes et al. (1987a) in iron-loaded rats two hours after receiving 200 mg/kg/day deferiprone injections in the third and sixth weeks of the experiments. Berdoukas et al. (1993) reported atrophy of the thymus in rats following the administration of 100 mg/kg/day or more of deferiprone.

One study reported what was believed to be a synergistic effect between deferiprone and barbiturates leading to unexpected death in rats (Huehns et al., 1988).

The LD_{50} of a single dose of oral deferiprone in iron non-overloaded rats was found to be in excess of 1 to 2 g/kg (Kontoghiorghes et al., 1989b), while the LD_{50} of a single intraperitoneal deferiprone dose was 600 to 700 mg/kg (Kontoghiorghes et al., 1990c). Despite the proven efficacy of deferiprone (Kontoghiorghes et al. 1987c,d;

Kontoghiorghes et al., 1990; Olivieri et al., 1992b; Olivieri et al., 1995), a number of toxic effects have also been reported in patients during the course of deferiprone trials around the world. One of the most serious of these is agranulocytosis. The first reported case of agranulocytosis occurred in a patient with Blackfan-Diamond anemia (Hoffbrand et al., 1989; Bartlett et al., 1990), who received 3 g of oral deferiprone daily and developed borderline neutropenia after five months of therapy. Following a 20-day period without deferiprone, the drug was reinstated for three months, during which the patient again developed borderline neutropenia. Six weeks following the re-development of borderline neutropenia on 3 g of oral deferiprone twice daily , the patient developed a sore throat and fever. Two days later the patient was admitted to the hospital with shock and septicemia. Pancytopenia had developed necessitating several platelet transfusions. After 36 days, neutrophil counts had become normal. There was no evidence of drug-related antibodies. Bartlett et al. (1990) discussed this case in greater detail because of the increase in the number of clinical trials being initiated with deferiprone around the world. Other investigators have also documented cases of agranulocytosis (Agarwal et al., 1992; al-Refaie et al., 1992). To date there have been a total of eleven reported cases of agranulocytosis worldwide in the medical literature (Olivieri et al., 1995), necessitating the repeated monitoring of neutrophil counts of patients on deferiprone therapy. Current prospective trials are underway to determine the incidence of neutropenia or agranulocytosis (Olivieri et al., 1995).

Despite the findings that deferiprone is efficacious and safe for use in patients with rheumatoid arthritis (Vreugdenhil et al., 1989), arthropathy (Berdoukas et al., 1993), joint pain (Bartlett et al., 1990; Berkovitch et al., 1994) and muscle pain (Bartlett et al., 1990) have been documented in patients on deferiprone therapy. As a result, patients treated with deferiprone at The Hospital for Sick Children and The Toronto Hospital undergo annual rheumatological assessments.

Deferiprone has also been reported to induce embryotoxicity and teratogenicity (Berdoukas et al., 1993) and, thus, should not be used in pregnancy, until more studies are conducted.

Although there have never been any deaths as a direct result of deferiprone therapy, there have been four patients who had at one time received deferiprone and died long after the cessation of this therapy. None of the patients had any evidence of neutropenia (Agarwal et al., 1992). The first patient died of disseminated varicella, the second of pneumococcal meningitis, the third succumbed to a severe gastrointestinal infection, and the fourth died of cardiac failure from iron overload (Agarwal et al., 1992). Investigators reporting on the death of the fourth patient originally made the claim that the patient had died of drug-induced systemic lupus erythematosus (SLE), although at the time of death, the patient had not taken deferiprone for 65 days (Mehta et al., 1991). In the cohort of patients of Olivieri et al. (1991b), anti-nuclear antibodies and rheumatoid factor were reported in some thalassemia patients. The authors considered the presence of these immunological parameters in the patient who reportedly had died from deferiprone-induced SLE (Mehta et al., 1991), not enough evidence to support the claim that deferiprone was in fact responsible for the death.

In summary, to date there have been no cases of patients who have died as a direct result of deferiprone therapy.

2.0 Summary of Objectives, Hypotheses and Aims

Overall Objective:

To optimize iron chelation with deferiprone thereby assuring maximal iron excretion.

Overall Hypothesis:

Iron chelation by deferiprone can be optimized by matching the mode of administration of deferiprone to the kinetic profile of iron. The way in which deferiprone is administered in chronic iron overload will differ from that in acute iron intoxication.

Conceptual Model:

In chronic iron overload, greater iron chelation will be induced with deferiprone when the drug is administered frequently in smaller doses, to achieve a more sustained systemic exposure. Conversely, in acute iron intoxication greater iron chelation will be achieved when deferiprone is administered in larger, less frequent doses.

2.1 Specific Aims and Hypotheses:

A) In Hemoglobinopathies:

1) To study the effects of two deferiprone dose schedules (q6h compared to q12h) on urinary iron excretion.

Hypothesis: Smaller, more frequent doses of deferiprone will produce greater urinary iron excretion than an equal daily dose of deferiprone given in larger, less frequent doses.

2) To assess the effect of hemoglobin concentrations on urinary iron excretion.

Hypothesis: Hemoglobin concentrations affect urinary iron excretion by changing the systemic AUC of deferiprone.

3) To assess the relative contribution of fecal iron excretion to the total chelated iron, eliminated from the body, with DFO and deferiprone.

Hypothesis: Potentially significant amounts of chelatable iron can be excreted in the stool after chelation with deferiprone.

B) In Malaria:

4) To study the pharmacokinetics of deferiprone in asymptomatic malaria patients, with normal iron stores.

Hypothesis: Deferiprone will produce a plasmodicidal effect in asymptomatic malaria volunteers, if the AUC reaches levels achieved *in vitro*.

5) To evaluate whether the pharmacokinetics of deferiprone can predict the plasmodicidal efficacy/failure.

Hypothesis: A greater systemic exposure of deferiprone in humans is required to produce plasmodicidal activity.

C) In Experimental Acute Iron Overload:

6) To evaluate the efficacy of deferiprone in preventing deaths from acute iron overload in a rat model.

Hypothesis: Mortality will be reduced in acutely iron-intoxicated rats following treatment with deferiprone.

7) To evaluate the pharmacokinetics of deferiprone in the treatment of acute iron intoxication.

Hypothesis: Systemically administered deferiprone will chelate toxic doses of orally administered iron and the chelate will be excreted in the urine.

8) To study the effects of dosing regimens (multiple compared to single injections) on urinary iron excretion in acutely iron-intoxicated rats.

Hypothesis: In acutely iron-intoxicated rats, a single injection of deferiprone will induce greater urinary iron excretion than multiple injections of a similar total dose.

3.0 Study of optimal deferiprone dose schedule in thalassemia

3.1 Introduction

The administration of DFO to iron-overloaded thalassemia patients reduces hepatic iron stores (Barry et al., 1974; Cohen et al., 1984), and the incidence of mortality (Modell et al., 1982; Olivieri et al., 1994) and of cardiac disease (Wolfe et al., 1985). The rapid administration of DFO can cause hypotension (Whitten et al., 1965). In addition, the drug has a short elimination half-life (Summers et al., 1979) and must be administered subcutaneously for 8 to 12 hours nightly to induce a negative net iron balance (Propper et al., 1977; Modell et al., 1982). Some drugs induce their optimal effect by being administered as a bolus to produce a gradient into a desired compartment. Lavoie and Bergeron (1985) for example, showed that antibiotics administered as a bolus penetrated infected and sterile fibrin clots more readily than when given by continuous infusion. The optimal dosing frequency for the orally active iron-chelating agent deferiprone, has not been examined systematically.

3.2 Objectives

1. To study the effects of two deferiprone dose schedules (q6h compared to q12h) on urinary iron excretion.
2. To assess the effect of hemoglobin concentrations on urinary iron excretion.

3.3 Hypotheses

1. Smaller, more frequent doses of deferiprone will produce greater urinary iron excretion than an equal daily dose of deferiprone given in larger, less frequent doses.
2. Hemoglobin concentrations affect urinary iron excretion by changing the systemic AUC of deferiprone.

3.4 Patients and Methods

3.4.1 Experimental procedure of the post-transfusion study

Thirteen homozygous beta-thalassemia (HBT) patients, who had been receiving 75 to 100 mg/kg/day deferiprone divided into three daily doses, gave informed consent to participate in this study. For the purpose of this study, all participants received 75 mg/kg/day deferiprone for three days divided in equal doses every six hours or every twelve hours, immediately following their monthly transfusion; they then received the other regimen the following month (Fig. 6). The data of three participants were excluded because of incomplete 24-hour urine collections evidenced by inconsistent urinary creatinine excretion (Henry et al., 1974; Scully et al., 1986). The data for the ten eligible deferiprone-treated HBT patients are presented, had a mean age ± SD of 20.9 ± 4.7 years (range 13 to 27 years).

Each patient, serving as his or her own control, was studied for three consecutive days with one regimen in the first month and with the other regimen in the second month. Six patients began the study receiving deferiprone every twelve hours and seven patients began by receiving the drug every six hours. Once steady state was achieved on the new regimen, two consecutive 24-hour urine collections were obtained for each of the two regimens (Matsui et al., 1991). Compliance was ensured by reminder phone calls to each patient at the times of every dose for each three-day study. During the time between the two studies, patients resumed their normal eight-hourly dosing regimen.

3.4.2 Experimental procedure of the pre-transfusion study

The same thirteen homozygous beta-thalassemia patients who had participated in the post-transfusion study, received 75 mg/kg/day deferiprone for three days in equally divided doses every six hours or every twelve hours, immediately prior to their monthly

Post-Transfusion Regimen

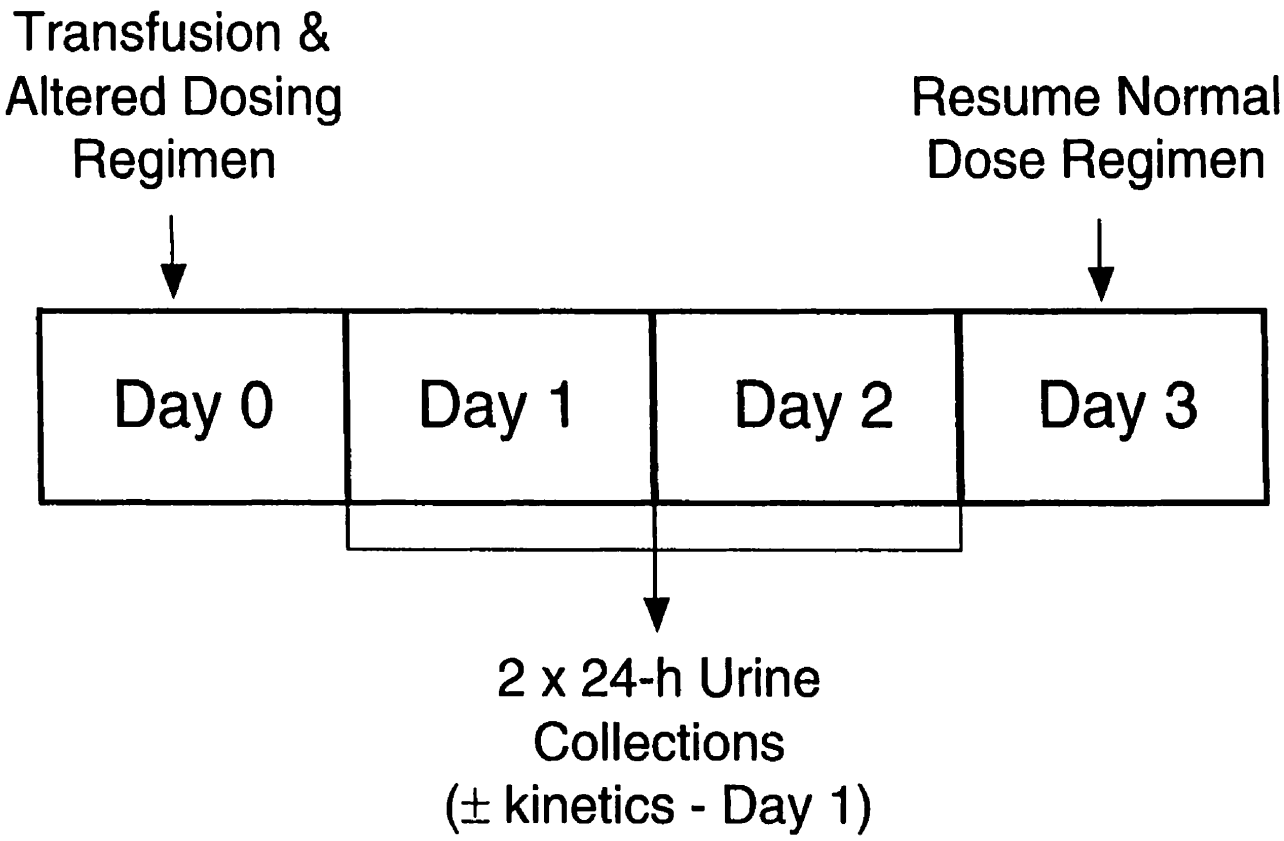


Fig 6: Diagrammatic representation of post-transfusion regimen of HBT patients receiving 75 mg/kg/day deferiprone given in divided doses q6h or q12h

blood transfusion, and then received the other regimen, the following month (Fig. 7).

The data of three participants were excluded because of inconsistent urinary creatinine excretion (Henry et al., 1974; Scully et al., 1986).

Each patient, serving as his or her own control, was studied for three consecutive days with one regimen in the first month and with the other regimen in the second month. Seven patients began the study receiving deferiprone every twelve hours and six patients receiving it every six hours. Patients achieved steady state on the new regimen and then completed two consecutive 24-hour urine collections for each of the two regimens (Matsui et al., 1991). Compliance was once again ensured by reminder phone calls.

3.4.3 Deferiprone synthesis and encapsulation

Deferiprone was synthesized by Dr. R. McClelland at the Department of Chemistry, The University of Toronto, Toronto, Canada according to previously published methods (Kontoghiorghes and Sheppard, 1989). Deferiprone was encapsulated in 300 mg quantities by the Pharmacy Department at The Hospital for Sick Children, Toronto.

3.4.4 Assays

Urinary iron concentrations were measured by atomic absorption spectrophotometry with a Varian Spectra AA-10 atomic absorption spectrophotometer. Complete blood counts were done in our institution.

To study the steady state pharmacokinetics of deferiprone when patients received deferiprone every six hours or every twelve hours, blood samples were drawn one day after the altered-dose regimen had commenced, ensuring at least five half-lives of two to three hours, so that a steady state was assured. Samples were drawn through an indwelling venous catheter placed in the antecubital fossa before deferiprone administration (trough level), and at timed intervals after deferiprone ingestion (15, 30, 45, 60, 90, 120,

Pre-Transfusion Regimen

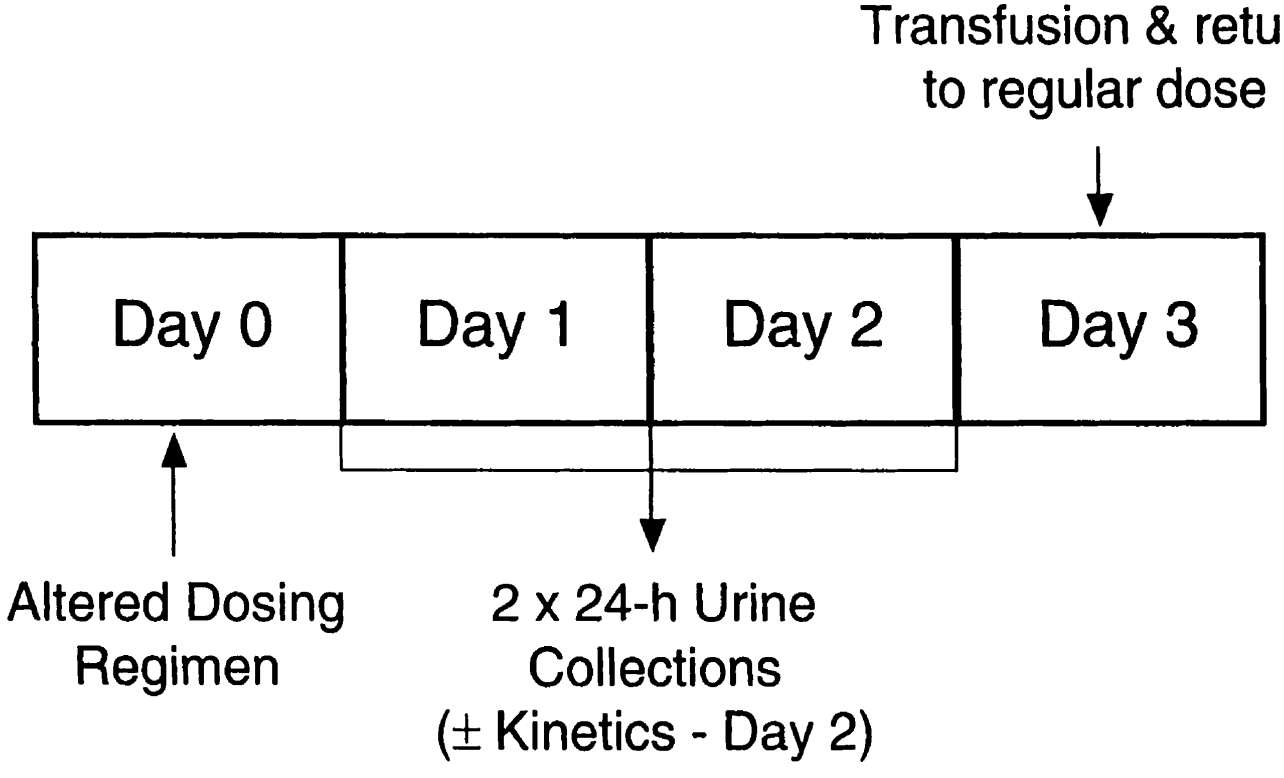


Fig 7: Diagrammatic representation of pre-transfusion regimen of HBT patients receiving 75 mg/kg/day deferiprone given in divided doses q6h or q12h

180, 240, 300, and 360 minutes). Patients were instructed to fast on the morning of the pharmacokinetic study and to arrive at the hospital for their morning dose. Fluids were not restricted and patients were allowed to eat two hours after the oral dose was administered. Separated plasma and serum samples were collected and frozen at -20°C until analyzed for deferiprone concentrations by high-performance liquid chromatography (HPLC [Klein et al., 1991]). Previous unpublished data from experiments performed in our laboratory, have shown that similar HPLC results are obtained whether plasma or serum samples are used for measuring deferiprone and deferiprone-glucuronide. The predominant deferiprone metabolite, deferiprone-glucuronide, was measured by incubating aliquots of plasma or serum with glucuronidase ((Sigma) 750 units/0.5ml) overnight at 37°C and then measuring deferiprone concentrations. Preliminary experiments have shown that overnight incubation with glucuronidase at 37°C leads to complete hydrolysis of the glucuronide conjugate. The total deferiprone (unmetabolized deferiprone and deferiprone released by glucuronidase treatment) measured was subtracted from the deferiprone released from the glucuronide form to determine the amount of metabolite produced.

3.4.5 Pharmacokinetic and statistical methods

The 24-hour area-under-the-plasma-concentration-time curves (AUC_{0-24}) and elimination half-life ($t_{1/2}$) of deferiprone were calculated using a noncompartmental model of the ADAPT program (D'Argenio and Schumitzky, 1979) on a Macintosh computer. The 24-hour systemic exposure at steady state (AUC_{0-24}), was calculated as $4*AUC_{0-6}$ whenever deferiprone was administered every 6 hours and $2*AUC_{0-12}$ whenever deferiprone was administered every twelve hours. AUC_{0-6} and AUC_{0-12} were calculated by using the elimination constant generated from the curve fitting. Initial estimates were calculated using the Niazi program on a Hewlett-Packard calculator. The apparent clearance (Cl_{app}) was calculated as the ratio between the oral dose and resultant AUC_{0-24}

and the apparent volume fo distribution (V_d) was calculated as $1.44*t_{1/2}*Cl_{app}$. The two-tailed paired Student t-test was used to compare the pharmacokinetic values of $t_{1/2}$, deferiprone AUC_{0-24}, deferiprone-glucuronide AUC_{0-24}, and of hemoglobin levels and urinary iron excretion data between the two regimens. Data are expressed as mean ± SD.

3.5 Results

3.5.1 Post-transfusion study

The post-transfusion data for the six-hourly regimen and the twelve-hourly regimens are presented in Table 1. The ten eligible patients had a mean age of 20.9 ± 4.7 years (range 13 to 27).

The mean UIE of patients, to whom deferiprone was administered every six hours, after the monthly transfusion, was 0.59 ± 0.29 mg/kg/day, and was significantly greater than the UIE of the same patients when deferiprone was administered every twelve hours (0.40 ± 0.26 mg/kg/day) (p = 0.0129).

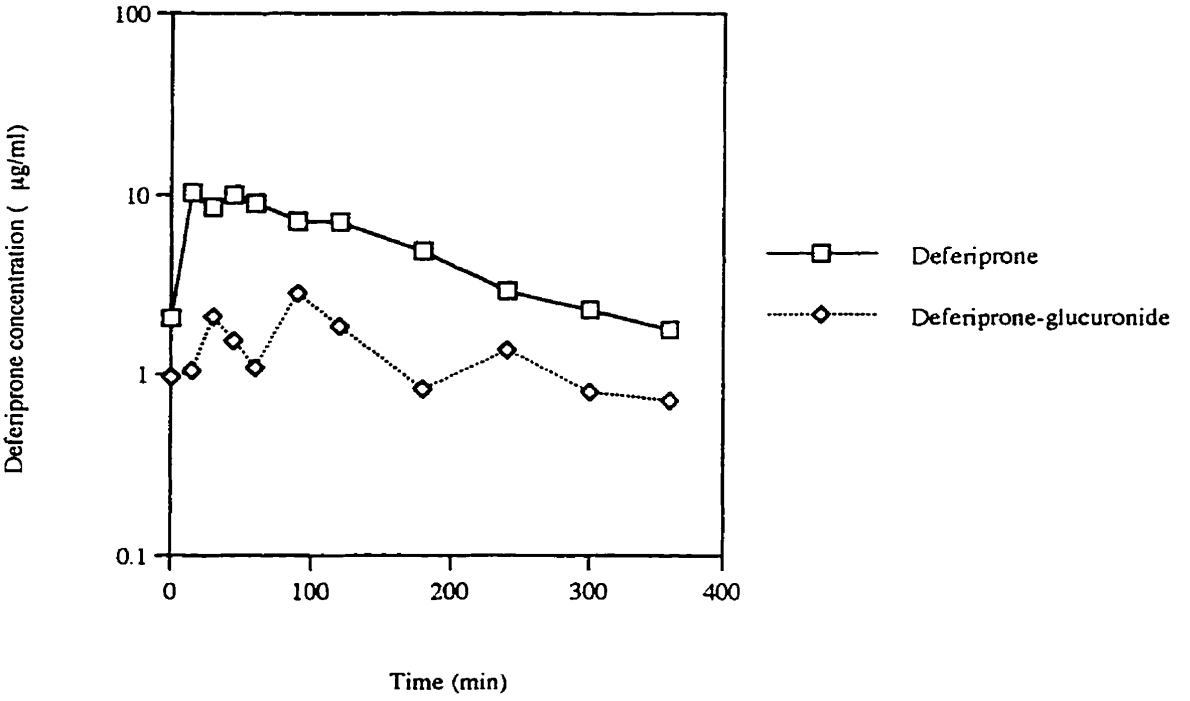
For the six patients in the pharamcokinetics study, the calculated mean total deferiprone AUC_{0-24} of the q6h regimen (7371 ± 2683 mg·min/L) was similar to that achieved with the q12h regimen (7228 ± 1499 mg·min/L) (p = 0.91). The observed mean peak plasma deferiprone concentration was 13.0 ± 7.6 μg/ml with the q6h regimen, and 30.6 ± 11.1 μg/ml with the q12h regimen (p = 0.0067). The mean elimination $t_{1/2}$ of deferiprone was 108.7 ± 36.3 min with the q6h regimen and 100.9 ± 29.0 min during the q12h regimen (p = 0.37). AUC_{0-24} of the glucuronide metabolite was 2160 ± 1387 mg·min/L with the q6h regimen and 3270 ± 2591 mg·min/L with the q12h regimen (p = 0.28). Total deferiprone (free deferiprone and deferiprone complexed to iron) and deferiprone-glucuronide mean concentrations from patients are presented in Figures 8 and 9, respectively.

Mean hemoglobin concentrations were similar when deferiprone was administered

Table 1: Kinetic (n=6) and laboratory (n=10) results (mean ± SD) of homozygous beta-thalassemia patients receiving 75 mg/kg/day deferiprone every 6 hours, or every 12 hours, following transfusion.

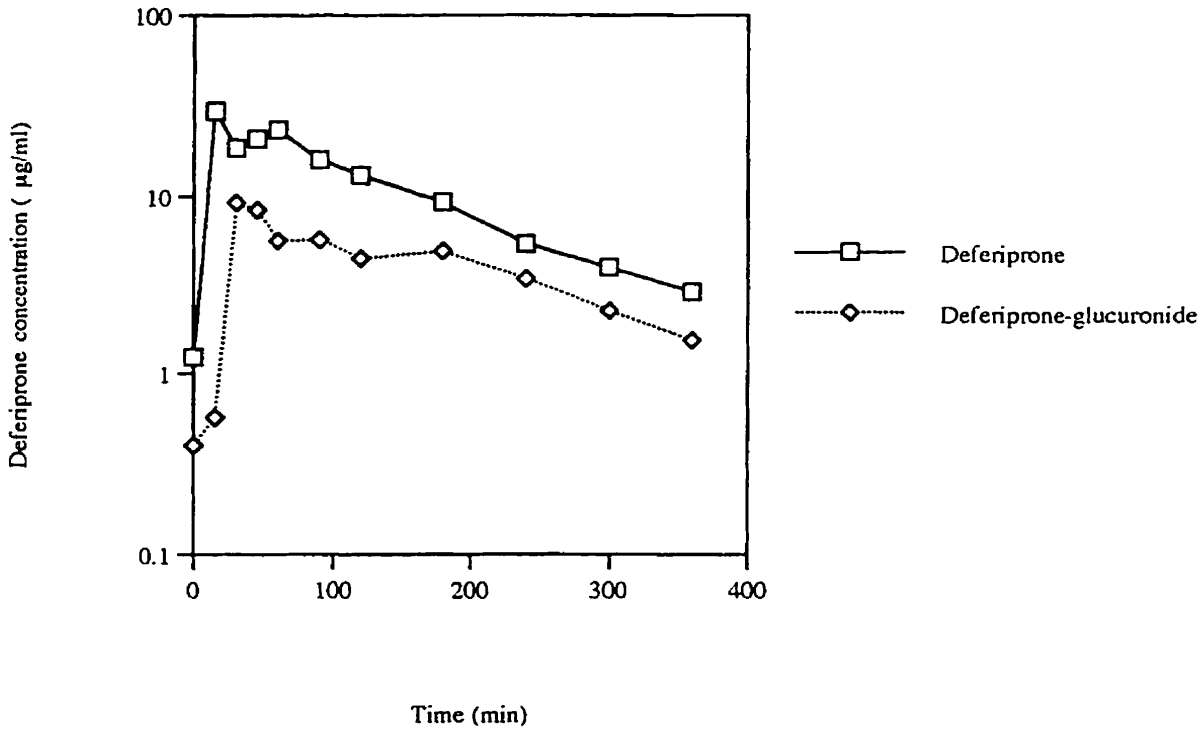
Parameters	q6h regimen	q12h regimen	p-value
Deferiprone			
24h AUC (mg·min/L)	7371 ± 2683	7228 ± 1499	0.91
Elimination Half-life (min)	108.7 ± 36.3	100.9 ± 29.0	0.37
Apparent Clearance (L/min/kg)	0.012 ± 0.005	0.011 ± 0.002	0.70
Apparent Volume of Distribution (L/kg)	1.8 ± 0.6	1.5 ± 0.4	0.51
C_{max} (μg/ml)	13.0 ± 7.6	30.6 ± 11.1	0.0067
Time to peak (min)	52.5 ± 36.4	40.0 ± 18.2	0.50
Metabolite			
24h AUC (mg·min/L)	2160 ± 1387	3270 ± 2591	0.28
C_{max} (μg/ml)	3.7 ± 2.4	14.5 ± 13.5	0.10
Time to peak (min)	45.0 ± 30.0	52.5 ± 29.6	0.65
Biochemistry			
Urinary iron excretion (mg/kg)	0.59 ± 0.29	0.40 ± 0.26	0.0129
Hematology			
Hemoglobin (g/L)	138.8 ± 12.5	139.0 ± 11.6	0.94

Figure 8: Mean serum deferiprone and deferiprone-glucuronide concentrations of patients receiving oral deferiprone 75 mg/kg/day every 6 hours, following transfusion.



Note: Standard deviations for deferiprone concentrations are listed in Appendix I

Figure 9: Mean serum deferiprone and deferiprone-glucuronide concentrations of patients receiving oral deferiprone 75 mg/kg/day every 12 hours, following transfusion.



Note: Standard deviations for deferiprone concentrations are listed in Appendix I

every six hours or every twelve hours [138.8 ±12.5 g/L and 139.0 ± 11.6 g/L] (p = 0.94).

3.5.2 Pre-transfusion study

All of the pre-transfusion data (mean ± SD) for the q6h and the q12h regimens are presented in Table 2. The ten eligible patients had a mean age of 20.9 ± 4.7 years (range 13 to 27).

The mean urinary iron excretion of patients receiving deferiprone every six hours was 0.56 ± 0.45 mg/kg/day and similar to that of patients receiving deferiprone every twelve hours (0.48 ± 0.52 mg/kg/day) (p = 0.79).

In the six patients who took part in the pharmacokinetic study, the calculated total deferiprone AUC_{0-24} for those patients receiving deferiprone q6h (6767 ± 1601 mg·min/L), was significantly lower than the AUC_{0-24} achieved by the patients who received deferiprone q12h (8256 ± 1235 mg·min/L) (p = 0.03). The observed mean peak deferiprone concentration during q6h administration was 10.6 ± 1.5 μg/ml, but 34.6 ± 7.4 μg/ml when deferiprone was administered q12h (p = 0.0002). The elimination $t_{1/2}$ of deferiprone was 108.2 ± 33.4 min when adminstered q6h and 111.8 ± 29.5 min (p = 0.59) when administered q12h. The AUC_{0-24} of the glucuronide metabolite was 1845 ± 954 mg·min/L when deferiprone was administered q6h and 2337 ± 1784 mg·min/L when deferiprone was administered q12h (p = 0.29). The mean total deferiprone (free and bound to iron) and deferiprone-glucuronide concentrations from patietns are presented, respectively, in Figures 10 and 11.

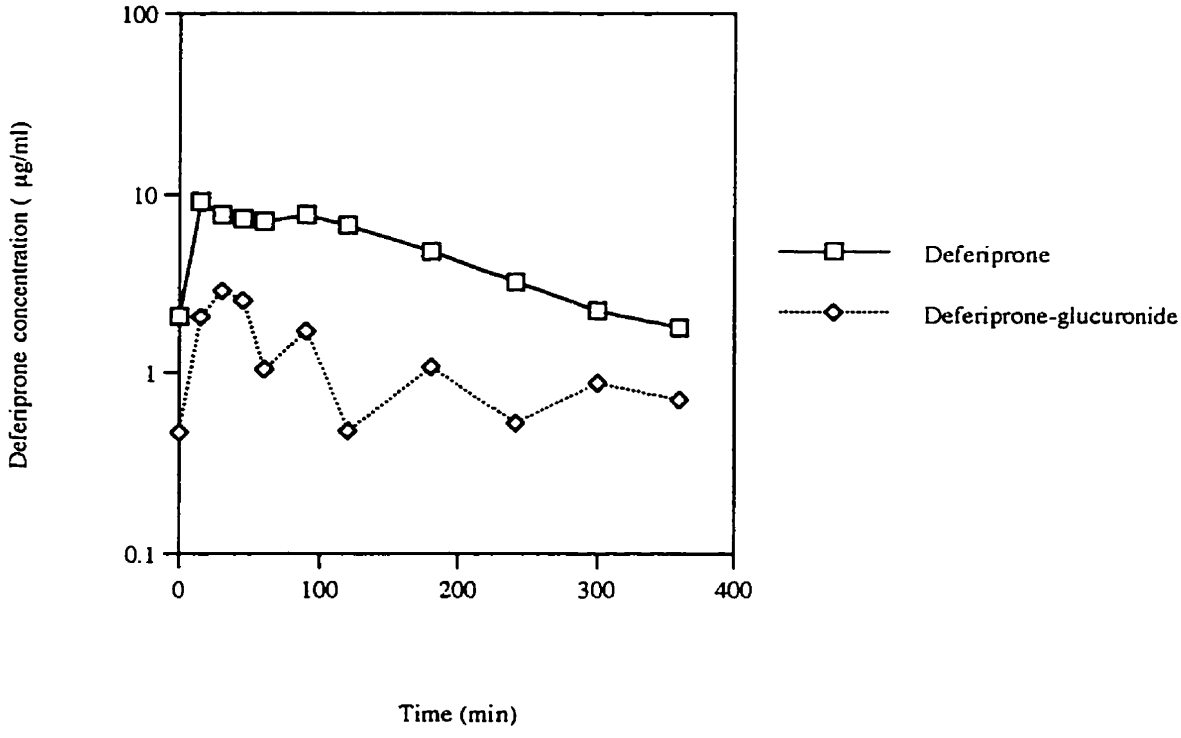
Urinary iron excretion and deferiprone AUC_{0-24} of the four regimens are compared in Table 3.

Mean hemoglobin concentrations were similar when deferiprone was administered every six hours or every twelve hours, 101.5 ± 4.8 g/L and 102.6 ± 5.0 g/L (p = 0.66).

Table 2: Kinetic (n=6) and laboratory (n=10) results (mean ± SD) of homozygous beta-thalassemia patients receiving 75 mg/kg/day deferiprone every 6 hours, or every 12 hours, before transfusion.

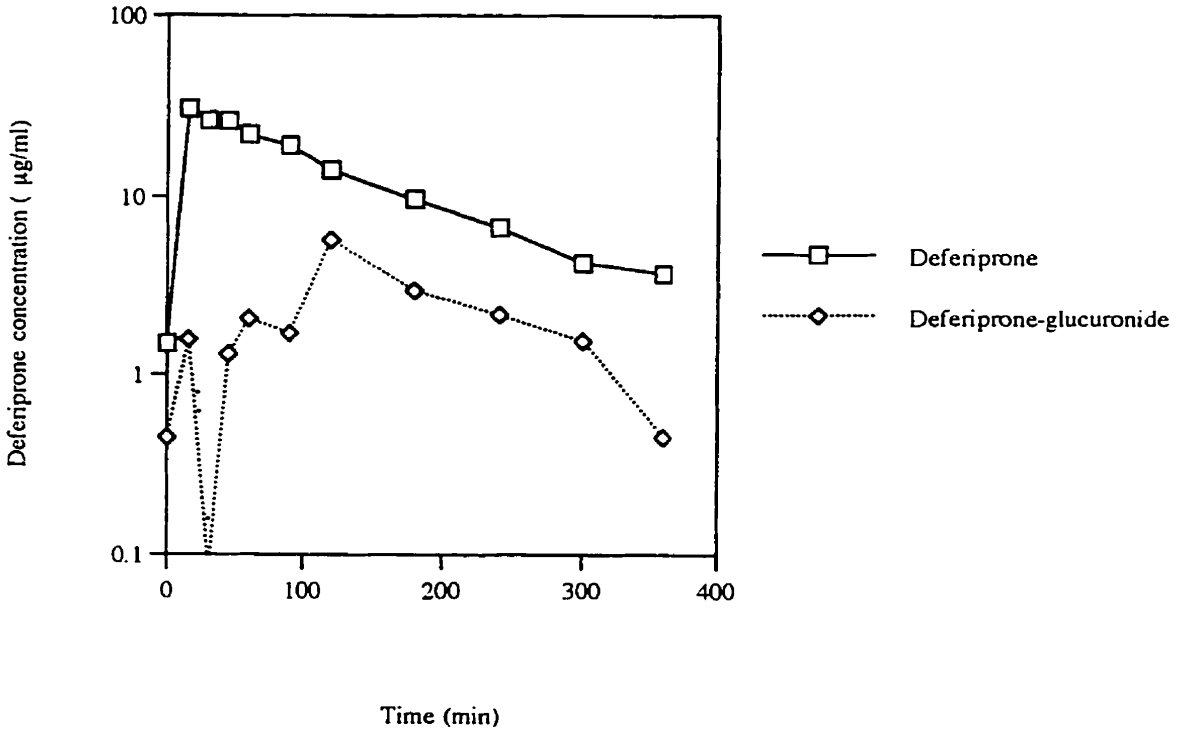
Parameters	q6h regimen	q12h regimen	p-value
Deferiprone			
24h AUC (mg·min/L)	6767 ± 1601	8256 ± 1235	0.03
Elimination Half-life (min)	108.2 ± 33.4	111.8 ± 29.5	0.59
Apparent Clearance (L/min/kg)	0.011 ± 0.003	0.010 ± 0.001	0.11
Apparent Volume of Distribution (L/kg)	1.8 ± 0.5	1.5 ± 0.4	0.22
C_{max} (μg/ml)	10.6 ± 1.5	34.6 ± 7.4	0.0002
Time to peak (min)	62.5 ± 63.9	32.5 ± 17.5	0.20
Metabolite			
24h AUC (mg·min/L)	1845 ± 954	2337 ± 1784	0.29
C_{max} (μg/ml)	4.1 ± 2.8	7.5 ± 6.1	0.25
Time to peak (min)	145.0 ± 149.0	97.5 ± 51.7	0.51
Biochemistry			
Urinary iron excretion (mg/kg)	0.56 ± 0.45	0.48 ± 0.52	0.79
Hematology			
Hemoglobin (g/L)	101.5 ± 4.8	102.6 ± 5.0	0.66

Figure 10: Mean serum deferiprone and deferiprone-glucuronide concentrations of patients receiving oral deferiprone 75 mg/kg/day every 6 hours, before transfusion.



Note: Standard deviations for deferiprone concentrations are listed in Appendix I

Figure 11: Mean serum deferiprone and deferiprone-glucuronide concentrations of patients receiving oral deferiprone 75 mg/kg/day every 12 hours, before transfusion.



Note: Standard deviations for deferiprone concentrations are listed in Appendix I

Table 3. Summary of urinary iron excretion (n=10) and total deferiprone 24-hour AUC of thalassemia patients (n=6) receiving deferiprone every 6 hours or every 12 hours, before or after transfusion.

Deferiprone regimen	**Urinary iron excretion (mg/kg/day)**		**p-value**
	PRE	POST	
Q6H	0.56 ± 0.45	0.59 ± 0.29	0.75
Q12H	0.48 ± 0.52	0.40 ± 0.26	0.40
p-value	0.79	0.01	
Deferiprone regimen	**Total deferiprone AUC_{0-24} (mg·min/L)**		**p-value**
	PRE	POST	
Q6H	6767 ± 1601	7371 ± 2683	0.60
Q12H	8256 ± 1235	7228 ± 1499	0.01
p-value	0.03	0.91	

3.6 Discussion

This study was initiated to determine whether deferiprone, a new orally active iron-chelating agent, induces greater urinary iron excretion when the drug is administered to iron-overloaded patients more frequently in smaller [sustained] doses or less frequently in higher [gradient] doses. When patients had high hemoglobin concentrations and received 75 mg/kg/day deferiprone, the frequency of deferiprone administration significantly affected the urinary iron excretion, but not the AUC. The results suggest that given similar systemic exposures to deferiprone there is greater iron chelation with more frequent deferiprone administration. It may be that deferiprone is chelating circulating NTBI as the deferiprone enters the systemic circulation. Since the NTBI is a small fraction, the deferiprone-iron complex is complete because of the presence of excess quantities of deferiprone. As the NTBI fraction is replenished from intracellular iron pools (Hershko, 1978; Wixon et al., 1980), deferiprone chelates the free iron and the complex is excreted in the urine. It may also be that the intracellular iron pools are readily accessible to the sustained concentrations of deferiprone and gradients are not required to penetrate the tissues.

A previous study by Pippard et al. (1982) showed that when DFO was administered to iron-overloaded patients, fecal iron excretion varied directly and urinary iron excretion varied inversely with hemoglobin concentrations. The authors also suggested that the increase in urinary iron excretion, documented following DFO administration, may occur because the storage iron is being mobilized or becoming "more accessible", for erythropoiesis when hemoglobin concentrations are low. Kontoghiorghes et al. (1987), showed findings that hemosiderin releases iron faster than ferritin -- findings that are contrary to those of O'Connell et al. (1989). Kontoghiorghes et al. (1987) documented lower pH values when hemosiderin was exposed to high concentrations of iron chelators. The authors proposed that protons released from the chelators in solution

were responsible for this reduction in pH. This reduction was not seen when ferritin iron was being exposed to the chelators because of the buffering capacity of the ferritin shell. Thus one hypothesis that may explain how this increase in "accessibility" described by Pippard et al. (1982), may be manifested by a reduction in the pH inside siderosomes. If there is a mechanism for the pH reduction, it is unclear at this time.

To determine whether the concentration of hemoglobin affects urinary iron excretion in thalassemia patients treated with deferiprone, my study was conducted not only after transfusion, but also immediately before transfusion. In the second study when hemoglobin concentrations were low, the frequency of deferiprone administration produced urinary iron excretion that was similar between the two regimens. But the deferiprone $AUC_{0\text{-}24}$ was signficantly lower when the drug was administered more rather than less frequently.

The present findings imply that deferiprone-induced urinary iron excretion may depend not only on the frequency of drug administration but may also be dependent on the systemic $AUC_{0\text{-}24}$ of deferiprone. The finding that the lower $AUC_{0\text{-}24}$ of deferiprone [when the drug was administered with greater frequency prior to transfusion] resulted in similar urinary iron excretion as the higher $AUC_{0\text{-}24}$ [when the drug was administered less often before transfusion], agrees with the findings from the previous experiment, when hemoglobin concentrations were higher; namely, that the frequent administration of deferiprone is more efficient at chelating iron than are the large gradient exposures to deferiprone. Furthermore, when the urinary iron excretion data from the same regimen (both before and after transfusion) are compared, there is no difference in the urinary iron excretion between the two regimens (Table 3). The data suggest that urinary iron excretion may not be dependent on hemoglobin concentrations. The data also appear to contradict the findings of Pippard et al. (1982), that urinary iron excretion varies inversely with hemoglobin concentrations.

The following model was formulated to integrate the observations that were documented in my studies into a unified model for a mode of iron chelation with deferiprone.

1) When hemoglobin concentrations are low, prior to a transfusion, storage iron may be "more accessible" for chelation, previously documented as an increase in erythropoietic activity. The sustained low levels of deferiprone may result in greater fecal iron excretion during a first pass through the liver. Furthermore, at low hemoglobin concentrations charged complexes may be less likely to diffuse into the systemic circulation. As the incomplete deferiprone-iron complexes enter the biliary system, the net result may be a lower systemic deferiprone $AUC_{0\text{-}24}$, and greater fecal iron excretion. Alternatively, the higher concentrations from the less frequently administered regimen, may result in the greater formation of the complete deferiprone-iron complex, which may facilitate the diffusion of the complex into the systemic circulation.

2) When hemoglobin concentrations are high, immediately following a transfusion, the chelatable iron pool may be substantially smaller because whatever mechanism mobilized the iron prior to transfusion has now been reversed. Hence the deferiprone may not chelate iron during a first pass and may not enter the biliary system. If the deferiprone remains in the circulation, it could explain why the $AUC_{0\text{-}24}$ between the two regimens is similar.

A total body iron balance study is needed to prove this theory, especially the presence of chelated iron in the bile. This forms the next experimental component of my thesis.

In summary, these studies show that hemoglobin concentrations may affect both the pharmacokinetics and the pharmacodynamics of deferiprone. Despite the lower $AUC_{0\text{-}24}$ of deferiprone when the drug is administered frequently, the administration of deferiprone in high, less frequent doses, may be as effective at excreting a similar amount of iron in the

urine as smaller, more frequent doses.

The above findings have a direct impact on the quantity of iron excreted from iron-overloaded patients. The findings that administering deferiprone more frequently induces greater iron excretion than administering the drug less frequently in higher doses, may lead to the development of a sustained-release preparation of deferiprone. This will allow deferiprone to be administered in such a manner that iron is continuously and effectively chelated.

3.7 Conclusions

1. Smaller, more frequent doses of the same total dose of deferiprone produce greater UIE than larger, less frequent doses of the same total dose of deferiprone (when hemoglobin concentrations are high), in iron-overloaded thalassemia patients.
2. A difference in the systemic exposure of deferiprone occured when hemoglobin concentrations were low, while UIE remains similar regardless of the frequency of drug administration.

4.0 Total Iron Balance Study with Deferiprone

4.1 Introduction

Sickle cell disease patients require chelation therapy with desferrioxamine to reduce transfusion-acquired iron stores. Desferrioxamine is efficacious at reducing liver iron stores (Cohen et al., 1984), preventing cardiac disease (Wolfe et al., 1985), and prolonging survival (Ehlers et al., 1991). Despite its proven efficacy in removing excess body iron via the urinary and biliary routes (Pippard et al., 1982), compliance to DFO is erratic because of the drug's cumbersome, parenteral mode of administration.

The orally active iron-chelating agent deferiprone also has proven efficacy in reducing tissue iron stores (Olivieri et al., 1995). Yet, the contribution of fecal iron excretion by deferiprone has not been assessed.

4.2 Objective

1. To assess the relative contribution of fecal iron excretion to the total chelated iron, eliminated from the body, with DFO and deferiprone.

4.3 Hypothesis

1. Potentially significant amounts of chelatable iron can be excreted in the stool after chelation with deferiprone.

4.4 Patients and Methods

4.4.1 Patients

Four chronically transfused female patients with homozygous sickle cell disease, with a mean age of 15.2 ± 2.7 years (range, 11 to 17 years) were enrolled in this study because of their lack of compliance on subcutaneous DFO therapy. All patients (or parents of patients) signed informed consent forms following study approval by the Human Subjects' Review Committee at The Hospital for Sick Children, Toronto, Canada.

4.4.2 Experimental Procedure

The total iron balance study consisted of a 45-day hospital admission (Fig. 12) to the Clinical Investigation Unit at The Hospital for Sick Children. For this part of the study all patients were maintained on a low iron diet. Iron balance was determined for three periods: 1) baseline asssessment, no chelator, 2) with 75 mg/kg/day oral deferiprone divided into three equal doses, and 3) with 12-hour subcutaneous infusions of 50 mg/kg/day DFO. Each of the three phases was preceded by an oral dose of 10 μCi of ^{51}Cr chloride in 25 ml of apple juice. A gamma counter was used to measure the ^{51}Cr excretion in the patients' stool collections. ^{51}Cr is a gastrointestinal transit marker (Woolf et al., 1983). Thus when 95% of the radioactive chromium had been retrieved from the stool, subsequent urine and stool specimens obtained during each of the three periods were collected in acid-washed containers and the iron was measured. Each baseline measurement consisted of three 24-hour urine collections and one 72-hour fecal iron measurement. A total of nine 24-hour urine specimens and three 72-hour fecal specimens were collected for each of the chelator regimens.

All patients were maintained at hemoglobin concentrations greater than 100 g/L and HbS levels below 20% (data not shown).

Each patient underwent pre-study and then weekly laboratory measurements of hemoglobin, platelet and white blood cell counts, aspartate and alanine transaminases, electrolytes, urea, creatinine, total protein, albumin, and copper, zinc, calcium, magnesium and phosphate were carried out (data not shown).

4.4.3 Assays

Iron was measured in urine by atomic absorption spectrophotometry with a Varian Spectra AA-100 atomic absorption spectrophotometer. Fecal iron was also measured using atomic absorption after several acid digestions. Following low heat digestion with 15 ml of concentrated nitric acid and repeat digestions in 15 ml of 1:1 nitric acid:hydrogen peroxide

Balance Study

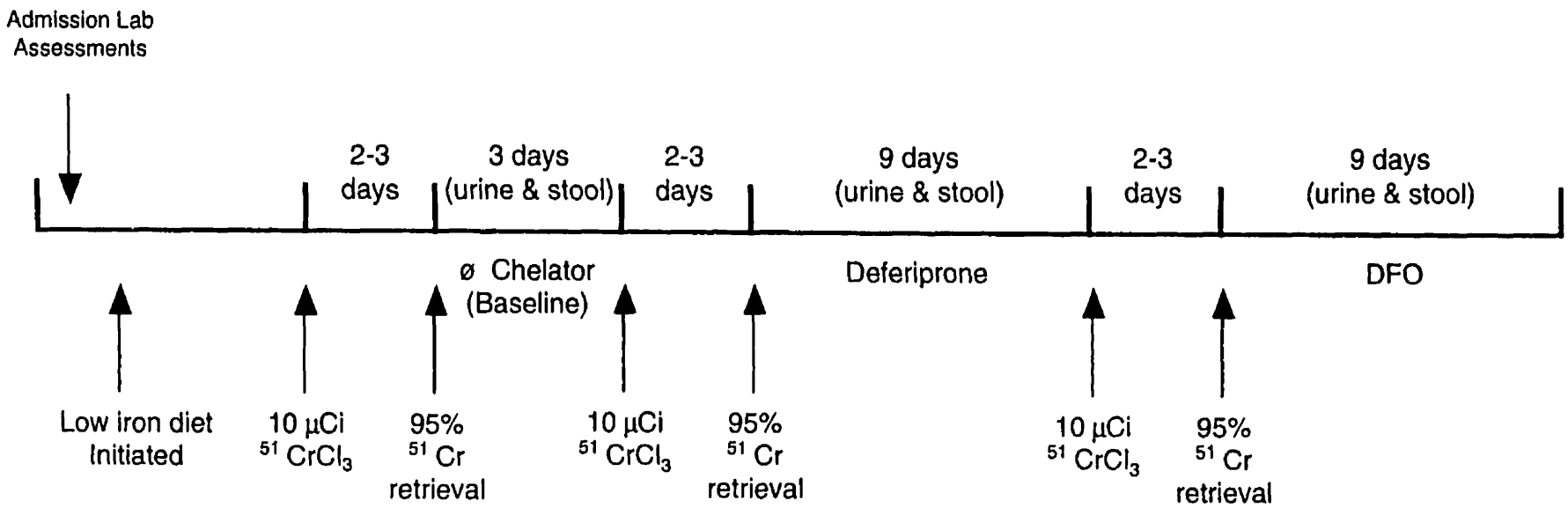


Fig 12: Diagrammatic representation of total iron balance study performed in four sickle cell disease patients receiving 75 mg/kg/day oral deferiprone or 50 mg/kg/day subcutaneous desferrioxamine

solution, the final concentrate was diluted in 25 ml deionized water and then the iron was measured by atomic absorption spectrophotometry.

4.4.4 Statistical analysis

Iron excretion was compared among treatment schedules or groups using the paired Student t-test. All results are expressed as mean ± SD.

4.5 Results

4.5.1 Total iron balance results

The data of the total iron balance study in patients with sickle cell disease are presented in Table 4.

Patients on the iron balance study showed mean urinary iron excretions of 0.38 ± 0.14 mg/kg/day on 50 mg/kg/day subcutaneous DFO and 0.41 ± 0.23 mg/kg/day on 75 mg/kg/day deferiprone ($p = 0.73$).

There was a significantly lower stool iron excretion with deferiprone (0.12 ± 0.08 mg/kg/day) when compared to stool iron excretion with subcutaneous DFO (0.50 ± 0.16 mg/kg/day) ($p = 0.007$). Despite this difference, deferiprone still effected a mean negative iron balance in the four patients (0.53 ± 0.17 mg/kg/day).

Mean deferiprone-induced stool iron excretion ranged from 3% to 33% of total deferiprone-induced iron excretion per day.

4.6 Discussion

In this study, the urinary and fecal iron excretion induced by desferrioxamine and then by deferiprone were measured and compared in four iron-overloaded sickle cell disease patients. Deferiprone induced similar urinary iron excretion to desferrioxamine. Also 3 to 33% of the total amount of iron excreted and induced by deferiprone, was via the biliary route. These results are consistent with previous findings (Olivieri et al., 1990; al-Refaie et al., 1992). My findings suggest that deferiprone may have potential therapeutic

Table 4: Results of urinary and fecal iron excretion results in sickle cell disease patients undergoing total iron balance with 75 mg/kg/day oral deferiprone or 50 mg/kg/day subcutaneous desferrioxamine.

	50 mg/kg/day desferrioxamine	**75 mg/kg/day deferiprone**	**p-value**
Urinary iron excretion (mg/kg/day)	0.38 ± 0.14	0.41 ± 0.23	0.734
Fecal iron excretion (mg/kg/day)	0.50 ± 0.16	0.12 ± 0.08	0.007
% fecal iron excretion (% of total)	59 ± 20 (32-77)	23 ± 14 (3-33)	0.012
Total iron excretion	0.88 ± 0.05	0.53 ± 0.17	0.031

application in iron-overloaded sickle cell disease patients. Furthermore, these results also support my hypothesis that biliary excretion of the chelated iron may account for the decrease in the systemic 24-hour AUC of deferiprone that was previously observed in the thalassemia patients, when the drug was administered q6h and hemoglobin concentrations were low.

One possible explanation as to why the fecal iron excretion induced by deferiprone is only 25% of the amount excreted by DFO is that unlike deferiprone, ferrioxamine may have been actively transported into the bile as previously shown (Meyer-Brunot and Keberle, 1968). Complete deferiprone-iron complexes are neutral and can diffuse across the hepatocyte membrane into the circulation and be excreted by the kidney. Incomplete deferiprone-iron complexes would be positively charged and may not be excreted as rapidly as ferrioxamine would be. Alternatively, slow intracellular removal may predispose the incomplete complexes to competition by iron-dependent enzymes or transferrin, resulting in the dissociation of the complex and subsequent elimination or metabolism of the parent deferiprone molecule. This slow intracellular removal may also explain the wide range of deferiprone-induced fecal iron excretion among the sickle cell patients. Alternatively, differences in either body iron load between patients or in storage iron pools, namely ferritin and hemosiderin, may also play a role in the amount of fecal iron excretion. *In vitro* studies have shown that the release of iron from ferritin is greater than from hemosiderin (O'Connell et al., 1989). Thus greater fecal iron excretion may result in patients with greater ferritin stores than with hemosiderin stores.

4.7 Conclusion

1. The deferiprone-iron complex is excreted into the bile. Variable fecal iron excretion occurs in chronically-transfused iron-overloaded sickle cell disease patients following treatment with deferiprone.

5.0 Malaria plasmodicidal study

5.1 Introduction

Malaria is a parasitic infestation endemic to the tropics (Knell, 1991). This disease is a major global health concern because of the resurgence of multidrug-resistant strains of *Plasmodium falciparum* (Rumans et al., 1979; Pettersson et al., 1981; Wyler, 1992).

A number of plasmodial enzymes require iron for survival (Cory et al., 1981). One would expect that chelating the iron from the parasites would inhibit the growth of the plasmodial parasites. This has been shown to be the case with DFO in both *in vitro* (Raventos-Suarez et al., 1982; Fritsch et al., 1987) and *in vivo* studies (Pollack et al., 1987) as well as in human trials (Traore et al., 1991; Gordeuk et al., 1992a,b).

Though effective at inhibiting plasmodial growth, DFO penetrates parasitized erythrocytes slowly (Fritsch and Jung, 1986). Uptake of DFO by the parasitized erythrocytes is essential for inducing an antimalarial effect (Scott et al., 1990). Although DFO is hydrophilic and positively charged, the drug is believed to penetrate parasitized erythrocytes by a parasitiphorous duct (Loyevsky et al., 1993; Loyevsky and Cabantchik, 1994).

In vitro studies have shown that sustained exposure to deferiprone has an antiplasmodial effect (Heppner et al., 1988). However the potential antiplasmodial efficacy in humans has not yet been evaluated.

5.2 Objectives

1. To study the pharmacokinetics of deferiprone in asymptomatic malaria patients with normal iron stores.
2. To evaluate whether the pharmacokinetics of deferiprone can predict the plasmodicidal efficacy/failure.

5.3 Hypotheses

1. Deferiprone will produce a plasmodicidal effect in asymptomatic malaria volunteers, if the AUC reaches levels achieved *in vitro*.
2. A greater systemic exposure of deferiprone in humans is required to produce plasmodicidal activity.

5.4 Patients and Methods

5.4.1 Patients

The study was performed at the Macha Mission Hospital in Zambia from September to December 1993. Ethics committees from each of the University of Zambia School of Medicine, the College of Medicine at Pennsylvania State University, and The [Toronto] Hospital for Sick Children approved the study.

Only asymptomatic adult male volunteers were candidates for this study. A thick blood smear was obtained from each volunteer, examined for the presence of asexual forms of *Plasmodium falciparum* in peripheral blood and stained with Giemsa. Volunteers who were positive for asexual merozoites and agreed to participate in the study gave written informed consent, underwent a physical examination, and a medical history was taken. A total of 31 otherwise healthy men were enrolled in the study. The study was divided into three sections: 1) pharmacokinetics, 2) placebo or 75 mg/kg/day deferiprone and 3) placebo or 100 mg/kg/day deferiprone.

5.4.2 Deferiprone synthesis and encapsulation

Deferiprone was synthesized using the methods of Kontoghiorghes and Sheppard, (1989) and encapsulated in 300 mg aliquots by Novopharm Ltd (Toronto, Canada). Placebo capsules containing starch were prepared by The Hospital for Sick Children, department of Pharmacy.

5.4.3 Pharmacokinetics

One of the six volunteers enrolled in the pharmacokinetic study was excluded from the study one day later because of low parasite counts. The other five volunteers received 75 mg/kg/day oral deferiprone in equally divided eight-hourly doses for a period of 72 hours. Parasite and blood counts were performed every eight and 24 hours, respectively.

Patients reached steady-state with deferiprone after 24 hours on deferiprone; subsequent timed venous blood samples were taken in heparinized vacutainers (every 30 minutes for the first two hours and then hourly for the remaining six hours). Plasma samples were stored at -70°C and within two months, were analyzed by myself for deferiprone concentrations at The Hospital for Sick Children according to the method of Klein et al., 1991. Elimination half-life and AUC_{0-24} were calculated using ADAPT for Macintosh (D'Argenio and Schumitzky, 1979).

5.4.4 Placebo-controlled crossover study with deferiprone (75 & 100 mg/kg/day)

Of the thirteen volunteers who enrolled for the placebo or 75 mg/kg/day deferiprone trial, one was removed after 24 hours, following a low parasite count; the other twelve completed the nine-day study. Every volunteer received each of the placebo and the deferiprone dose in a randomized, double-blind, crossover manner (Fig. 13). Six volunteers received deferiprone first and seven started with placebo. Either placebo or drug was administered for three days, followed by a washout period of three days of neither drug nor placebo, followed by another three days of the drug or placebo. All patients were monitored closely as inpatients. Thick blood smears were obtained from fingerpricks every four hours in the first 24 hours and eight-hourly for the remainder of the drug or placebo regimen. During the three-day period between the two regimens, patients underwent daily fingerpricks to obtain thick blood smears.

Malaria Study

- 75 mg/kg/d or placebo

3 - Day Treatment*	3 - Day Washout	3 - Day Treatment*

- 100 mg/kg/d or placebo

4 - Day Treatment*	1 - Day Washout	4 - Day Treatment*

*Treatment - deferiprone or placebo

Fig 13: Diagrammatic representation of randomized, double-blind, placebo-controlled, crossover trial in asymptomatic malaria patients receiving either deferiprone (75 or 100 mg/kg/day) or placebo

Of the twelve volunteers who were enrolled in the nine-day study of placebo or 100 mg/kg/day oral deferiprone, two discontinued the study on days three and seven for personal reasons. Once again, volunteers received placebo or 100 mg/kg/day deferiprone in a randomized, double-blind, crossover manner. Rather than three days of trial, patients received either placebo or drug for a four-day period, followed by one day of neither drug nor placebo, followed by a four-day course of either drug or placebo. All volunteers in the 100 mg/kg/day deferiprone trial were followed up in a manner similar to those volunteers in the 75 mg/kg/day deferiprone trial, except that blood smears were obtained every six hours in the 100 mg/kg/day deferiprone trial for the duration of the nine days.

5.5 Results

5.5.1 Malaria plasmodicidal results

The pharmacokinetic results calculated for the five volunteers are presented in Table 5. The mean peak deferiprone concentration was 15.2 ± 3.5 μg/ml, an elimination half-life of 92.4 ± 6.5 min, and a 24-hour area-under-the-curve of 7746 ± 1521 mg·min/L. The calculated 48-hour AUC of the in vivo exposure was 39% of the AUC of previous *in vitro* studies (Heppner et al., 1988) (Table 5; Fig. 14).

The mean deferiprone and metabolite concentrations of the malaria patients enrolled in the pharmacokinetic arm of the study are depicted graphically in Figure 15.

There were no significant differences in the parasite counts between patients who received either placebo, 75 mg/kg/day (Fig. 16), or 100 mg/kg/day deferiprone (Fig. 17).

There were no differences in the side effects reported by patients between those volunteers on placebo or deferiprone (data not shown).

5.6 Discussion

In the present study the administration of deferiprone to asymptomatic parasitemic male Zambian volunteers has no measurable effect on malarial parasite counts. Peak

Table 5. Kinetic parameters of volunteers (n=5) with asymptomatic malaria receiving 75 mg/kg/day deferiprone every 8 hours.

Kinetic parameters	**Results**
Plasma Deferiprone	
24h AUC (mg·min/L)	7746 ± 1521
Elimination Half-life ($t_{1/2}$) (min)	92.4 ± 6.5
Apparent Clearance (L/min/kg)	0.010 ± 0.002
Apparent Volume of Distribution (L/kg)	1.34 ± 0.27
Renal Clearance (L/min)	0.038 ± 0.023
C_{max} (μg/ml)	15.2 ± 3.5
(μmol/L)	108.9 ± 24.9
C_{min} (μg/ml)	0.7 ± 0.3
(μmol/L)	5.0 ± 2.0
Time to deferiprone peak (min)	84.0 ± 32.9
Plasma deferiprone-glucuronide	
24h AUC (mg·min/L)	1088 ± 638
C_{max} (μg/ml)	2.7 ± 2.4
Renal Clearance (L/min)	0.022 ± 0.015
Time to peak (min)	180.0 ± 103.9

Figure 14: Relative 48-hour deferiprone area-under-the-curve in failing *in vivo* and successful *in vitro* malaria studies.

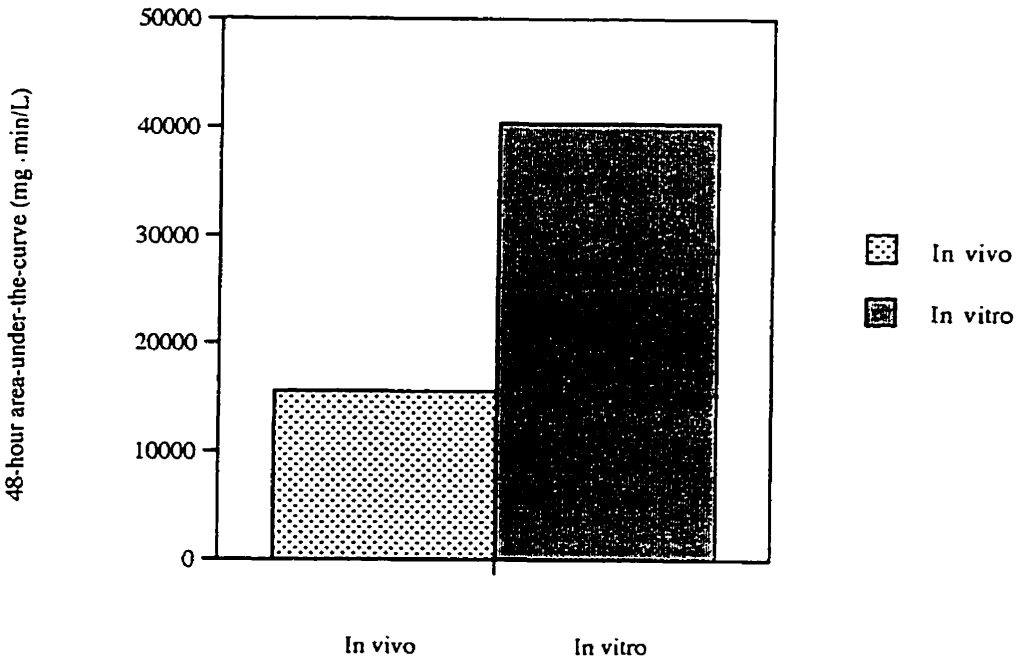
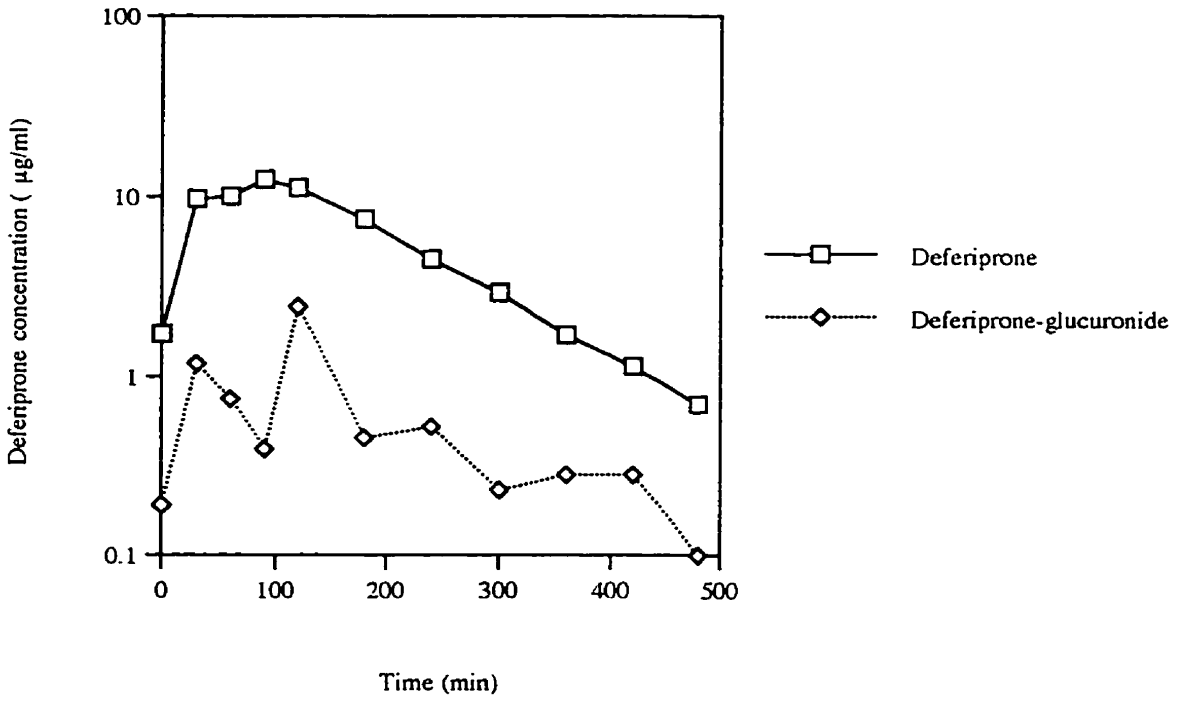


Figure 15: Mean serum deferiprone and deferiprone-glucuronide levels in volunteers with asymptomatic malaria receiving 75 mg/kg/day deferiprone in 8-hourly divided doses.



Note: Standard deviations for deferiprone concentrations are listed in Appendix II

Figure16: Mean parasite counts of volunteers randomized to receive either placebo or 75 mg/kg/day deferiprone.

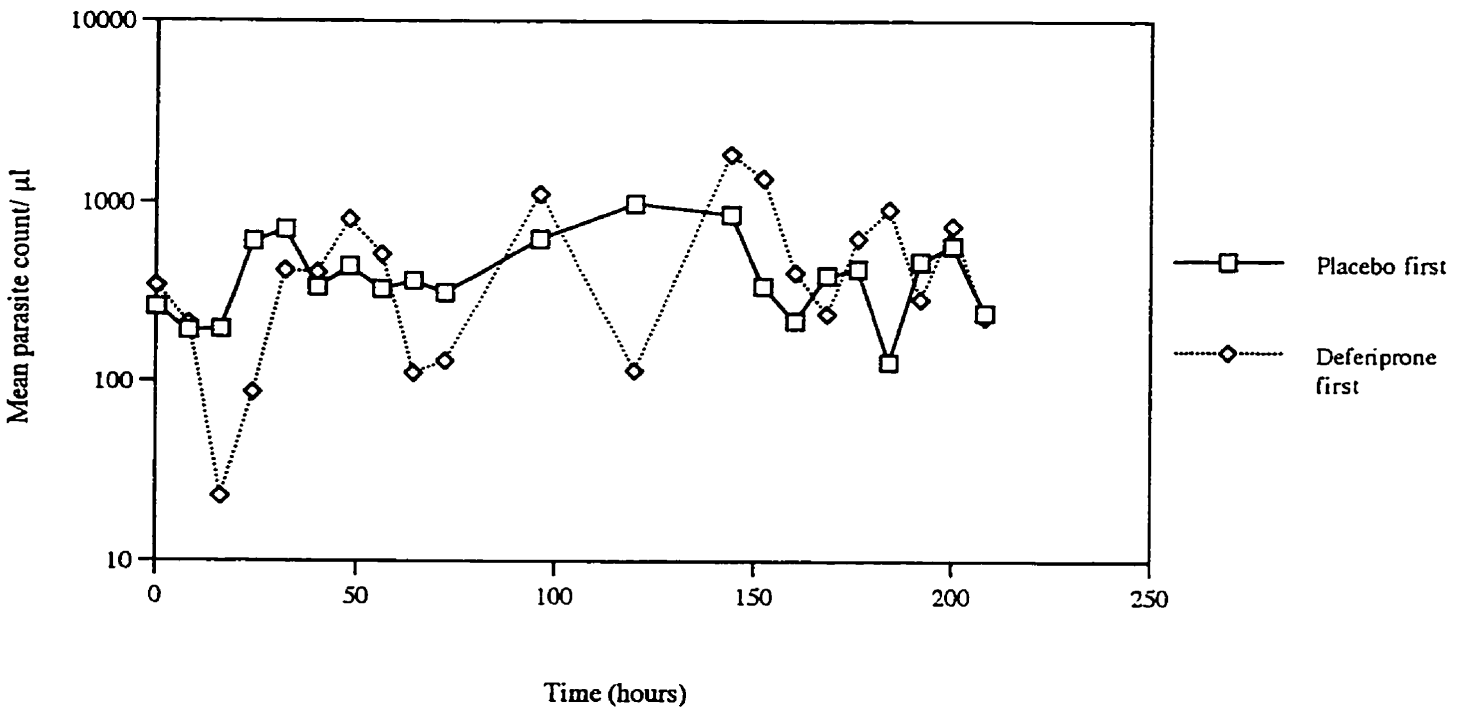
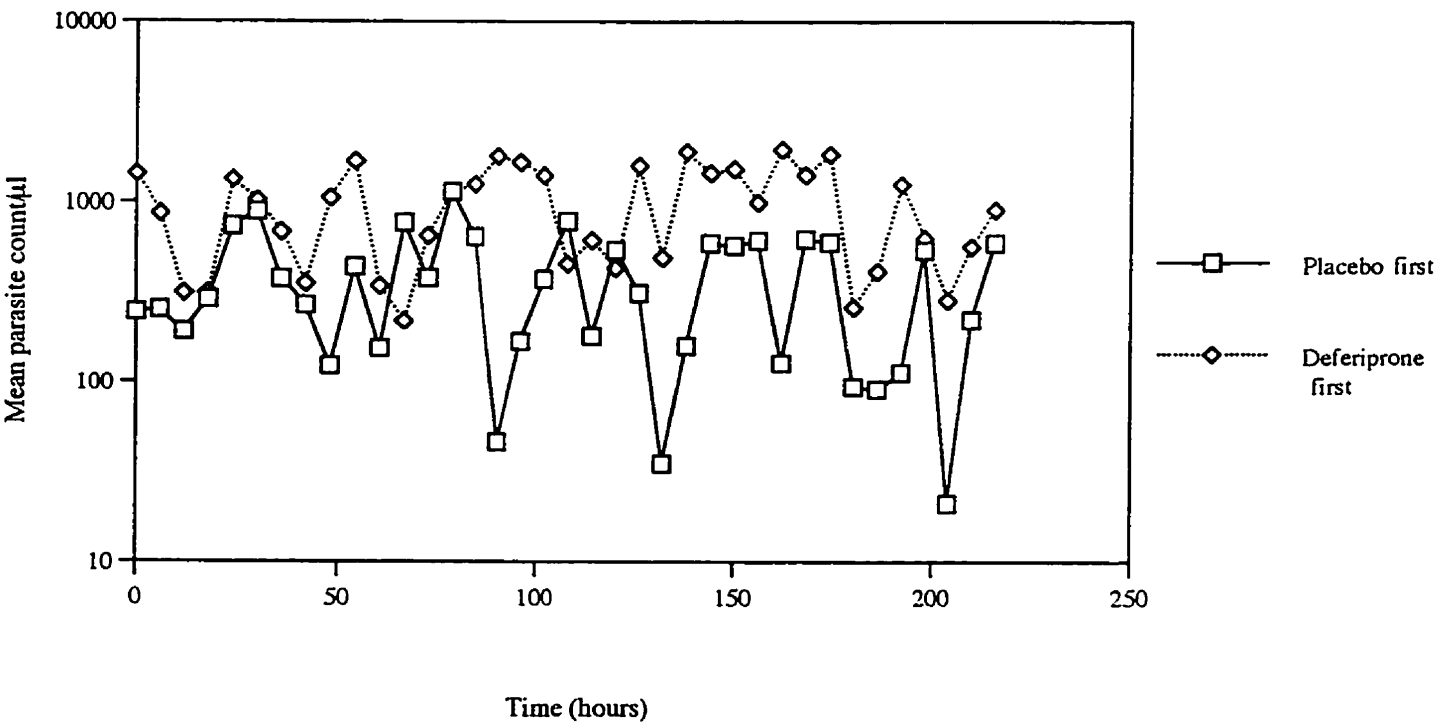


Figure 17: Mean parasite counts of volunteers randomized to receive either placebo or 100 mg/kg/day deferiprone.



serum deferiprone concentrations reached 108 μmol/L in the pharmacokinetic study; concentrations which are known to inhibit parasite growth *in vitro* (Heppner et al., 1988). Although the peak deferiprone concentrations in this trial were similar to those concentrations that effected antiplasmodial activity *in vitro*, the concentrations were maintained in the antiplasmodial concentration range *in vivo*, for only brief periods of time. Furthermore, the 48-hour AUC of the *in vivo* exposure was only 39% of the *in vitro* exposure (Table 5; Fig. 14). Sustained exposure to the peak concentration attained *in vivo* may have induced an antiplasmodial effect. Thus, to induce a plasmodicidal effect in human volunteers, a more sustained exposure of nearly 200 mg/kg/day may be required. Even doses of 75 mg/kg/day of oral deferiprone have been associated with agranulocytosis (Hoffbrand et al., 1989; Berdoukas et al., 1993). Thus a dose of 200 mg/kg/day deferiprone would expose non-iron-overloaded patients to toxic reactions.

In vitro studies have shown that as partition coefficient increases, the antimalarial activity of the iron chelator increases (Hershko et al., 1991). Deferiprone is not as efficient as DFO at chelating iron because deferiprone is a bidentate chelator and requires three moles of drug for neutralizing one mole of ferric iron. Furthermore, deferiprone has a relatively low partition coefficient of 0.21 (Hershko et al., 1991; 1992).

A number of factors may play a role in the lack of plasmodicidal activity of deferiprone. Firstly, its relatively low partition coefficient may make it difficult for it to access the iron pools in the parasitic cytosol. Furthermore, the intermittent dosing schedule may stifle any plasmodicidal activity of whatever deferiprone does enter the parasite, because the deferiprone is being eliminated.

5.7 Conclusions

1. Deferiprone did not induce a plasmodicidal effect in asymptomatic malaria patients. The pharmacokinetics of deferiprone predict a lack of plasmodicidal effect when

used at the clinically recommended dose.

2. A greater systemic exposure of deferiprone in humans would likely be required to produce plasmodicidal activity.

6.0 Acute iron intoxication study

6.1 Introduction

Severe acute iron intoxication has been associated with serious morbidity and mortality (Robotham and Lietman, 1980; Schauben et al., 1990). Following acute iron intoxication, large quantities of iron enter the circulation and are cleared quickly into the tissue cells (Reissmann et al., 1955). Once inside the cells, the mitochondria are particularly vulnerable to attack by free iron because of an abundance of polyunsaturated fatty acids in the mitochondrial membrane (McGray et al., 1972). Disruption of the mitochondrial membrane can change their configuration and impair the function of the electron transport chain needed for aerobic respiration (Robotham and Lietman, 1980).

Free iron is also a known cardiac toxin (Tenenbein et al., 1988). Exposure of myocardial strips to free iron results in dose-dependent decreases in contractility and increases the rate and irregularity of the beats (Artman et al., 1984a;b). Similar effects were documented by Moreb et al., (1988) and by Link et al. (1985). Increases in malonyldialdehyde, a marker of lipid peroxidation, have also been documented. These increases were later shown to be reversed by exposure to DFO (Link et al., 1985).

The administration of DFO is essential to reduce the consequences of acute iron intoxication. However, DFO is not always available in primary care facilities and valuable time is lost during transport of severely intoxicated patients to a tertiary care facility.

The use of deferiprone in chronic studies with transfusion-dependent iron-overloaded thalassemia patients has shown deferiprone to be efficacious in reducing body iron stores (Olivieri et al., 1995). The potential therapeutic application of deferiprone in reducing mortality and morbidity following acute iron intoxication has not been examined.

6.2 Objectives

1. To evaluate the efficacy of deferiprone in preventing deaths from acute iron

overload in a rat model.

2. To evaluate the pharmacokinetics of deferiprone in the treatment of acute iron intoxication.
3. To study the effects of dosing regimens (sustained compared to bolus) on urinary iron excretion in acutely iron-intoxicated rats.

6.3 Hypotheses

1. Mortality will be reduced in acutely iron-intoxicated rats receiving a toxic dose of iron and treated with deferiprone.
2. Systemically administered deferiprone will chelate orally administered iron in toxic doses and the chelate will be excreted in the urine.
3. In acutely iron-intoxicated rats, single-dose bolus injections with deferiprone will induce greater urinary iron excretion than repeated injections (sustained exposure) of a similar total dose.

6.4 Animals and Methods

6.4.1 Rats

Male Wistar rats, weighing between 150 g and 250 g, were purchased from Charles River Canada, Inc. (Quebec, Canada). Upon arrival at the HSC Lab Animal Services (L.A.S.) facility, animals were double-housed in plastic shoe box-type cages. At approximately 1800 hours of the evening prior to the experiment, standard lab rat chow was removed from the cages and the rats were numbered by numerical bands with permanent magic marker on the tails. At approximately 0800 hours the following morning, (after approximately 14 hours of food deprivation), the rats were weighed.

6.4.2 Iron preparation

One hour prior to the experiment, hydrous ferrous sulphate (Fisher Scientific, New Jersey) was dissolved in double-distilled Milli-Q water (Millipore, Massachusetts) for a final concentration of elemental iron of 0.082 g/ml. The solution was transported to the L.A.S. facility in 50 ml plastic, conical tubes (Beckton Dickinson, New Jersey).

6.4.3 Deferiprone synthesis and solution preparation

Deferiprone was synthesized at The University of Toronto, Department of Chemistry, by Dr. R. McClelland according to previously published methods (Kontoghiorghes and Sheppard, 1989). In the afternoon of the day before the experiment, crystalline deferiprone was dissolved in distilled water to a final concentration of 10 mg/ml and sterilized by suction-filtration through a GS 0.22 micron filter (Millipore, Massachusetts) in a laminar flow hood. The filtrate was stored at room temperature in sterile 100 ml injection vials (Bencard Labs, Ontario). The deferiprone solution was always prepared fresh the day before each experiment and the excess volumes of the deferiprone solution discarded.

6.4.4 Experimental procedure

6.4.4.1 Efficacy study

Animals were assigned to one of three groups. Group I rats (n=19) received 612 mg/kg of elemental iron by gavage alone, while those in group II (n=20) received the same dose of iron by gavage followed by four intraperitoneal injections of deferiprone solution, for a cumulative dose of 800 mg/kg deferiprone. A third group of rats were gavaged with distilled water, and were used as controls for a number of biochemical parameters or for the examination of pathological slides.

At the commencement of the experiment rats in groups I and II were gavaged, by a

L.A.S. animal technician, with a volume of ferrous sulphate solution corresponding to 612 mg/kg of elemental iron. Following the administration of the iron solution rats were closely observed. Immediately after the iron gavage, standard laboratory rat chow was returned to each cage.

Fifteen minutes following gavage, rats in group II were injected intraperitoneally with an initial loading dose of 400 mg/kg of deferiprone solution. Thereafter three intraperitoneal injections of deferiprone were administered at one-hour intervals in doses of 200, 100, and 100 mg/kg, respectively. Eight hours following the initial injection of deferiprone, the rats were placed in clean plastic cages without bedding. Single timed urine specimens were collected from the plastic cage floor using plastic transfer pipettes (Samco Scientific Inc., California), and transferred to labelled Eppendorf tubes (Rose Scientific Ltd., Alberta). The rats were then returned to their original cages.

Rats that required euthanasia during the 14-hour observation period were exsanguinated under deep ethrane-induced anesthesia and dissected immediately. Euthanasia before the end of the 14-hour observation period was performed if a rat exhibited any one [or more] of the three following symptoms: 1) gasping for air, 2) seizure, or 3) limp body or unresponsiveness to a loud slap on the side of the cage.

Rats that remained alive until the end of the observation period were euthanised with Ethrane gas (Anaquest, Ontario) in a gas delivery apparatus (Ohio Medical Products, Wisconsin). The rats were exsanguinated by needle aspiration. Blood samples were centrifuged at 2500 rpm for ten minutes. Serum was collected using plastic transfer pipettes, placed in prelabelled Eppendorf tubes, frozen at -20°C, and later transferred to a -80°C freezer. Residual bladder urine was also collected by needle aspiration, placed into separate prelabelled Eppendorf tubes, frozen at -20°C and stored at -80°C.

6.4.4.2 Pharmacokinetic and urinary iron excretion studies

Rats were assigned to one of four groups:

Group I rats (n=8) received 100 mg/kg of elemental iron by gavage followed by a subcutaneous injection of 2.5 mg/kg of diazepam (Sabex Inc., Quebec), [to sedate the animals and to minimize suffering], and 400 mg/kg of deferiprone in four equally divided intra-peritoneal injections. Diazepam was not administered in the efficacy study because it reduced the incidence of mortality of rats that received the toxic iron meal (unpublished data).

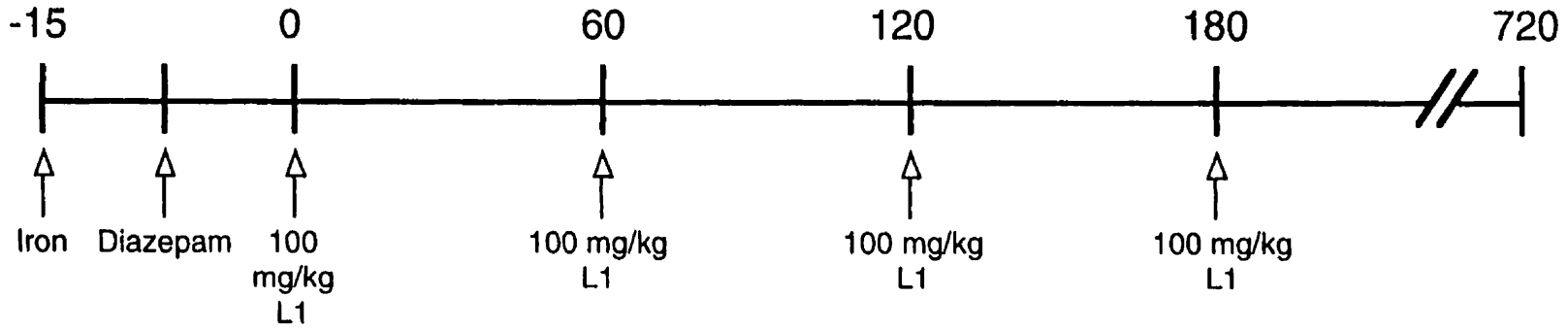
In this experiment deferiprone was prepared to a final concentration of 10 mg/ml in saline, not distilled water. The first injection was administered within 15 minutes of iron gavage (Fig. 18). Each subsequent intraperitoneal injection was administered at one-hour intervals. Animals received standard rat chow and were single-housed in Nalgene metabolic cages (Rochester, New York) for twelve hours of observation. Heat lamps were placed six feet from the metabolic cages to keep the sedated animals warm. All urine was collected at the end of the observation period in conical Falcon tubes (Becton Dickinson, New Jersey). Rats were subsequently euthanised with Ethrane (Anaquest, Ontario) overdose in a gas delivery apparatus (Ohio Medical Products, Wisconsin).

Group II rats (n=8) underwent exactly the same procedure as Group I, except that these rats received a single intraperitoneal injection of 400 mg/kg deferiprone within 15 minutes of being gavaged with iron (Fig. 18). Deferiprone was prepared and filtered in a manner similar to that mentioned in Section 6.3.3, except that deferiprone was dissolved in saline to a final concentration of 16 mg/ml.

Group III rats (n=18) underwent a procedure identical to those of rats in Group I, except that these rats were double-housed in plastic shoebox-type cages with bedding. Rats were exsanguinated at specified times (t=15, 75, 115, 135, 175, 195, 235, 360, 720 minutes). Serum samples were analyzed for deferiprone concentrations by HPLC. Two

Iron Intoxication Study (Part IIa)

Group I



Group II

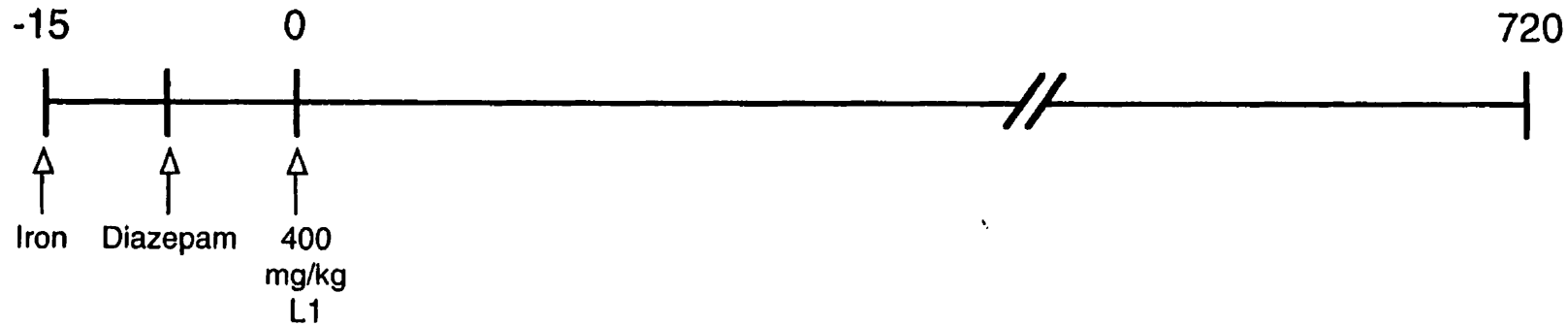


Fig 18: Diagrammatic representation of experimental procedure of rats receiving 100 mg/kg elemental iron with 400 mg/kg deferiprone intra-peritoneally either in divided doses (top) or bolus injection (bottom)

rats were euthanised for every time point on the pharmacokinetic profile (Fig. 19).

Group IV rats (n=20) underwent a procedure identical to those rats in Group II, except that these rats were double-housed in plastic shoebox-type cages with bedding. Rats were exsanguinated at specified times (t=15, 30, 45, 60, 90, 120, 180, 240, 360, 720 minutes). Serum samples were analyzed for deferiprone by HPLC and a pharmacokinetic profile was generated. Two rats were euthanised for every time point (Fig. 19).

6.4.5 Pathological studies

Cross-sectional slices were obtained for pathological examination: of heart, liver, lung, stomach, duodenum, and kidney. Tissue samples were stored in pre-labelled vials containing 10% buffered formalin solution (BDH Chemicals Inc., Mississauga). The tissue samples were stained with hematoxylin/eosin or with Prussian Blue for iron. Photographs of the organ slices were taken with a 35 mm camera mounted atop a microscope (Leitz, Germany), using 64 ASA film. Slides of tissues stained for iron were scored from 0 (no iron) to 4 (maximal iron score) by a pathologist, blinded to the groups.

6.4.6 Assays and Statistical Analysis

Serum iron samples were analyzed on a Kodak Ektachem 700 Analyzer (Rochester, New York) and urine iron samples on a Spectr AA-10 atomic absorption spectrometer (Varian Techtron Pty Ltd., Australia) by biochemistry technicians at The Hospital for Sick Children (Toronto, Canada). Plasma samples were analyzed for deferiprone concentration according to the HPLC method of Klein et al. (1991) in the Division of Clinical Pharmacology and Toxicology. Survival between groups was compared by means of a two-tailed Fisher's exact test. Results of laboratory parameters between animal groups were compared by the Mann-Witney U test.

Iron Intoxication Study (Part IIb)

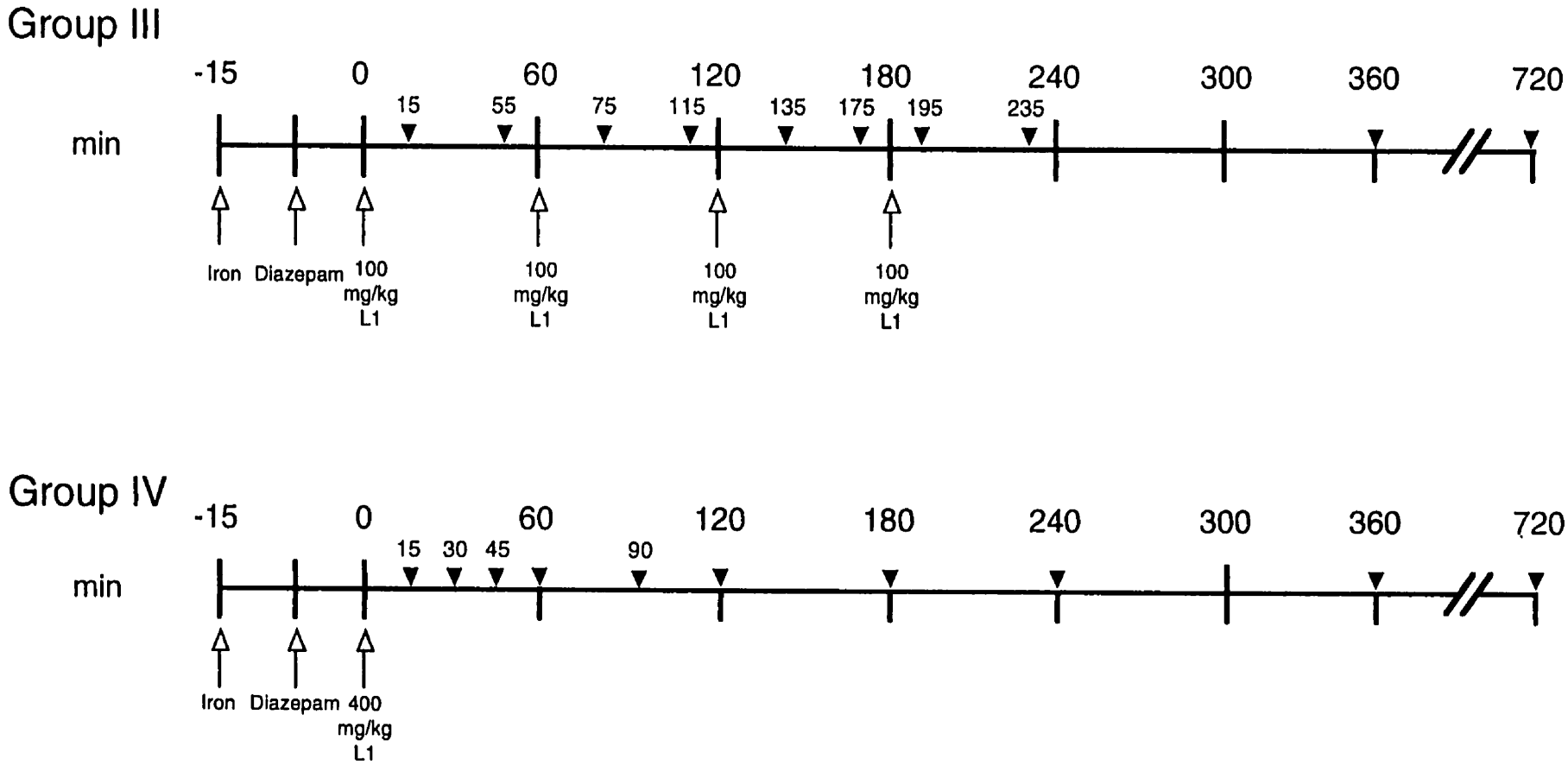


Fig 19: Diagrammatic representation of pharmacokinetic procedure (solid arrows) of rats receiving 100 mg/kg elemental iron with 400 mg/kg deferiprone (intra-peritoneally) either in divided doses (top) or bolus injection (bottom)

6.5 Results

6.5.1 Efficacy study

At the conclusion of the fourteen hour observation period, only 3 out of 20 (15%) of the rats that were given iron and deferiprone had died, compared to 11 out of 19 (58%) of the rats that received only iron (p=0.013). A survival curve depicts this difference (Fig. 20). None of the rats that received deferiprone died within the first seven hours after the iron meal, whereas six of the untreated rats died during this period (p=0.0064). All animals in Group II had a strong red discoloration of urine. No such colour was evident in either the urines collected from the rats in Group I or from the rats in the control group.

Serum iron levels in animals receiving elemental iron alone (mean ± SD) (331 ± 626 μmol/L) did not differ significantly from three of the controls (52.7 ± 25.9 μmol/L) (p = 0.32). The group receiving iron followed by injections of deferiprone, had levels (806 ± 1143 μmol/L), which were not significantly different from the levels in animals receiving iron alone (p = 0.59).

In control animals (those not injected with either deferiprone or gavaged with iron), urine iron levels were 10.2 ± 0.8 μmol/L. Animals receiving iron alone had a mean ± SD urine iron concentration of 21.9 ± 27.4 μmol/L. Conversely, animals receiving iron with IP injections of deferiprone had a mean ± SD urine iron concentration of 3022 ± 969 μmol/L, eight hours following injection (p=0.0001, compared to rats receiving iron). These data are summarized in Table 6.

Histological examination of the tissues collected from the animals showed the apparent absence of iron from the liver, kidney and heart tissues when deferiprone was administered. Tissue iron scores are presented in Table 7.

Pancreatic iron staining scores also dropped dramatically with deferiprone treatment. In the stomach and duodenum, iron was still present in the superficial lining, yet there was less iron in the capillary endothelium, muscles, serosa and it was absent in nuclei

Figure 20: Survival curves of rats receiving 612 mg/kg elemental iron with and without deferiprone.

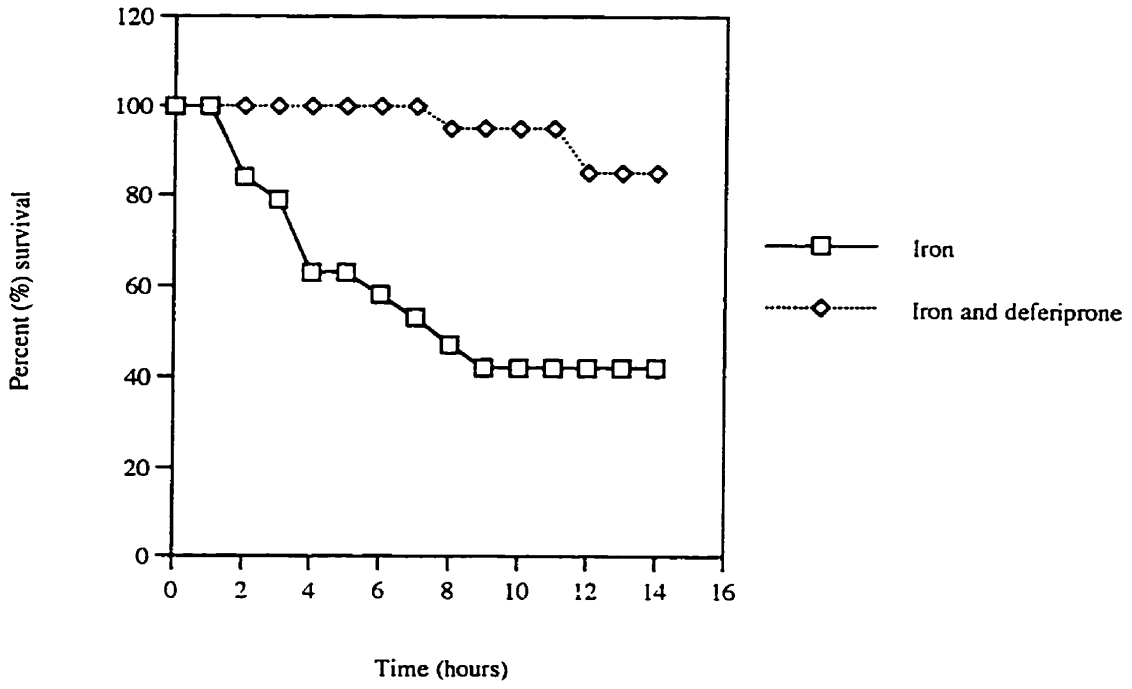


Table 6: Urine iron concentration data of control rats and of rats receiving 612 mg/kg iron or 612 mg/kg iron with 800 mg/kg deferiprone [in a four-hour period] solution.

Rat exposure	**Urinary iron concentration ± SD (range) (µmol/L)**
Control[a]	10 ± 1 (10 - 13)
Iron[a,b]	22 ± 27 (10 - 89)
Iron + deferiprone (t=8h)[b]	3022 ± 969 (1095 - 4260)

a p=0.3
b p=0.0001

Table 7: Tissue iron scores (0=no iron, 4=maximal iron score) of rats receiving 612 mg/kg elemental iron with and without deferiprone treatment.

Organ	Location	Iron Score	Iron+ deferiprone Score
LIVER	Kupffer cells	<1	0
	vascular endothelium (periportal)	2	0
KIDNEY	vascular endothelium	2	0
	crystals in tubules	4	0
HEART	vascular endothelium	1	0
	myocardium	0	0
LUNG	pleural lining	2-3	2
	alveolar lining	0	3
STOMACH	superficial mucosa/lumen	4	4
	vascular endothelium	4	1
	between muscle cells	4	0
	between myocytes	4	0
	serosa	4	1
PANCREAS	vascular endothelium	4	0
	serosa	4	1
	Islets	0	0
DUODENUM	serosa	4	2
	superficial mucosal lumen	4	4
	absorptive cells	4	4
	crypts	1	3
	vascular endothelium	4	1
	between muscle cells	4	2

when deferiprone was administered.

Iron stains of kidney sections showed complete removal of iron crystals in renal tubules when deferiprone was administered (Fig. 21c). With iron treatment alone, large iron crystals were deposited in renal tubules (Fig. 21b). Whether deferiprone was administered or not, there did not appear to be any signs of tubular or glomerular necrosis (figures not shown).

Lung tissue of animals treated only with iron show iron deposition in the serosa but nowhere else (figures not shown). In the presence of deferiprone there appears to be greater amounts of iron in the lungs. Structurally there was no evidence of alveolar damage whether or not deferiprone was administered.

Iron stains of the pancreas show iron deposition in the endothelium and serosa (Fig. 22b) in the absence of deferiprone. Iron virtually disappears from blood vessels and the serosa in the deferiprone-treated rats. Neither the pancreatic tissue nor the islets appear to be damaged by the iron, whether deferiprone is administered or not (not shown).

There was no iron deposition anywhere in the myocardium in the presence or absence of deferiprone (figures not shown). Despite the absence of iron deposited in the myocardium there was apoptosis or early stages of cell death. Even when deferiprone was adminsitered, there was evidence of apoptosis (not shown).

Iron staining of the liver showed iron localized in the vascular endothelium and in Kupffer cells (figures not shown). There were traces of iron deposition in hepatocytes. The administration of deferiprone removed all stainable iron from the liver endothelium. Despite the lack of iron deposition in hepatocytes and the absence of iron in the vascular endothelium following deferiprone administration, some hepatocytes exhibited early signs of death, in the form of darker stained cells (not shown).

In the stomach of rats that were given only iron, there was iron deposition in the gastric serosa and also within the stomach cells (specifically in the cytoplasm and within

Figure 21: Prussian blue iron stains of rat kidney tissue (400X magnification):

a) control rats

b) rats treated with 612 mg/kg elemental iron (by gavage)

c) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.

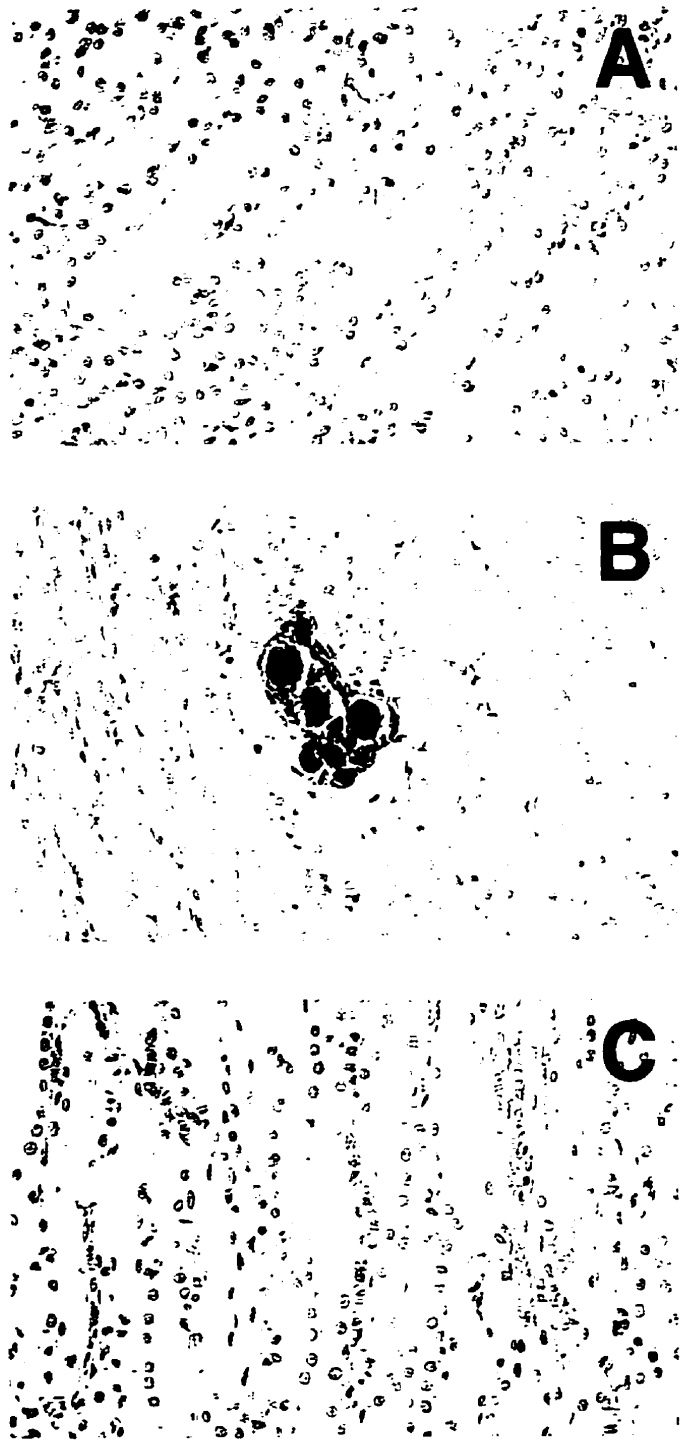
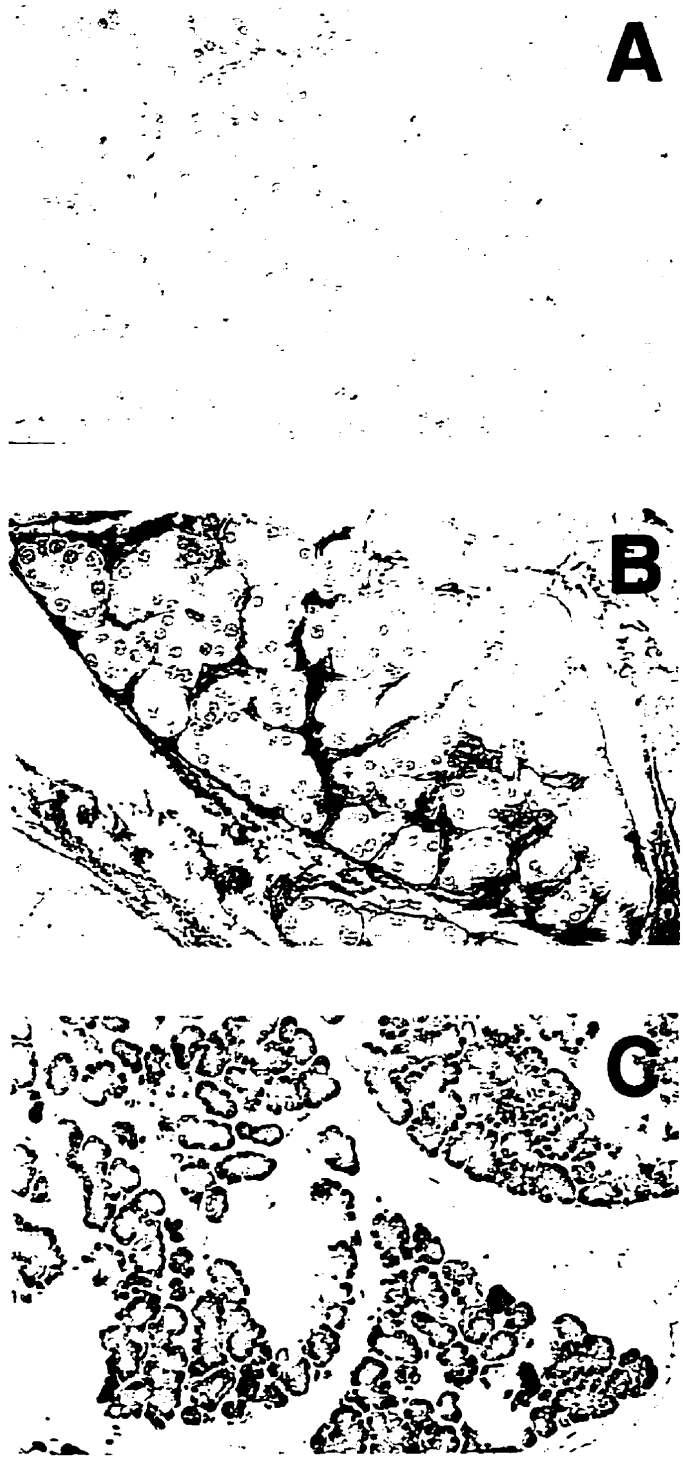

A
B
C

Figure 22: Prussian blue iron stains of rat pancreatic tissue (400X magnification):

a) control rats

b) rats treated with 612 mg/kg elemental iron (by gavage)

c) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.


A
B
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the nuclei - Fig. 23b). The concomitant administration of deferiprone, dramatically reduced the intensity of the iron staining within the serosal lining and iron was virtually absent in the nuclei. Although the iron continued to adhere to the surface lining of the stomach, there was little or no iron within the gastric mucosal cells. Hematoxylin/eosin staining depict the stomach lacking any visible signs of cell damage in the presence of deferiprone.

Iron staining of the duodenum of rats treated only with iron (Fig. 24b) shows a similar pattern to that of the stomach. Iron adheres to the villous surfaces and is also present in the nuclei, cytoplasm, serosa, and smooth muscle layers. Yet in animals that received deferiprone, there was virtually no iron within the cells (Fig. 24c) or in the blood vessels and iron appeared to be completely absent from the nuclei.

6.5.2 Pharmacokinetic study

Rats that received 400 mg/kg deferiprone (intraperitoneally) as a single injection following a 100 mg/kg elemental iron gavage meal, had a mean peak deferiprone concentration of 293.4 ± 63.7 μg/ml. The mean peak deferiprone concentration after four (intraperitoneal) injections of 100 mg/kg deferiprone, following the iron meal was 104.9 ± 2.4 μg/ml. The deferiprone pharmacokinetic profiles from the two regimens are presented in Figure 25.

Rats receiving the bolus injection of 400 mg/kg deferiprone had a mean urinary iron excretion of 3.65 ± 1.69 mg/kg, which was significantly greater than the urinary iron excretion of those rats that received four injections of 100 mg/kg deferiprone (1.9 ± 0.6 mg/kg [p=0.0164]) (Fig. 26).

6.6 Discussion

In this study, 58% of the rats that were gavaged with a toxic iron meal died following the intoxication. Yet only 15% of the rats treated with intraperitoneal deferiprone

Figure 23: Prussian blue iron stains (a-c) and hematoxylin/eosin stains (d-f) of rat stomach tissue (400X magnification):

a) control rats

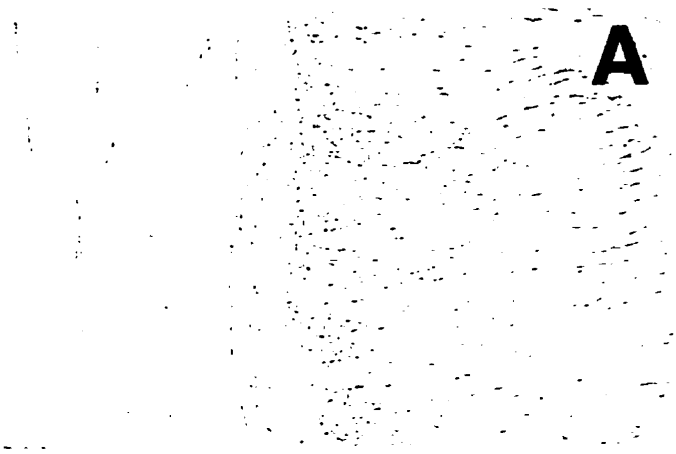
b) rats treated with 612 mg/kg elemental iron (by gavage)

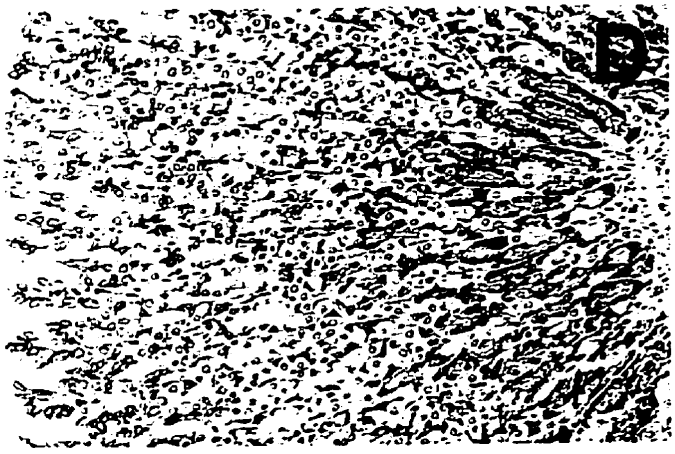
c) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.

d) control rats

e) rats treated with 612 mg/kg elemental iron (by gavage)

f) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.


A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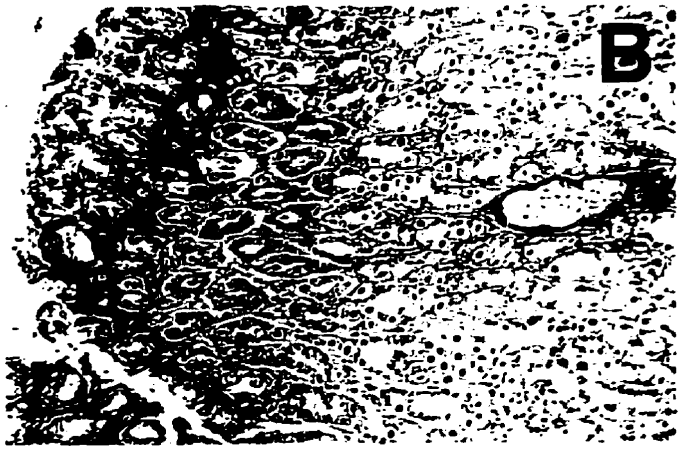
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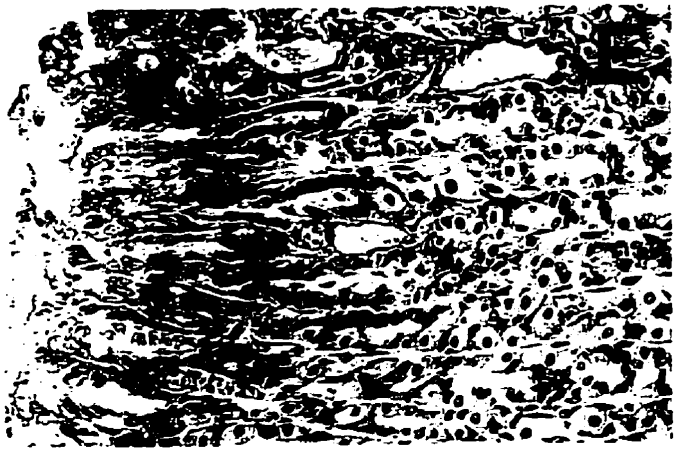
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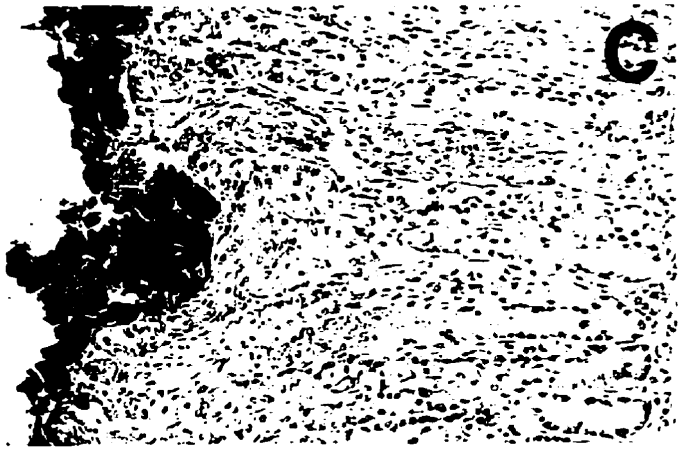

C


F

Figure 24: Prussian blue iron stains (a-c) and hematoxylin/eosin stains (d-f) of rat duodenal tissue (400X magnification):

a) control rats

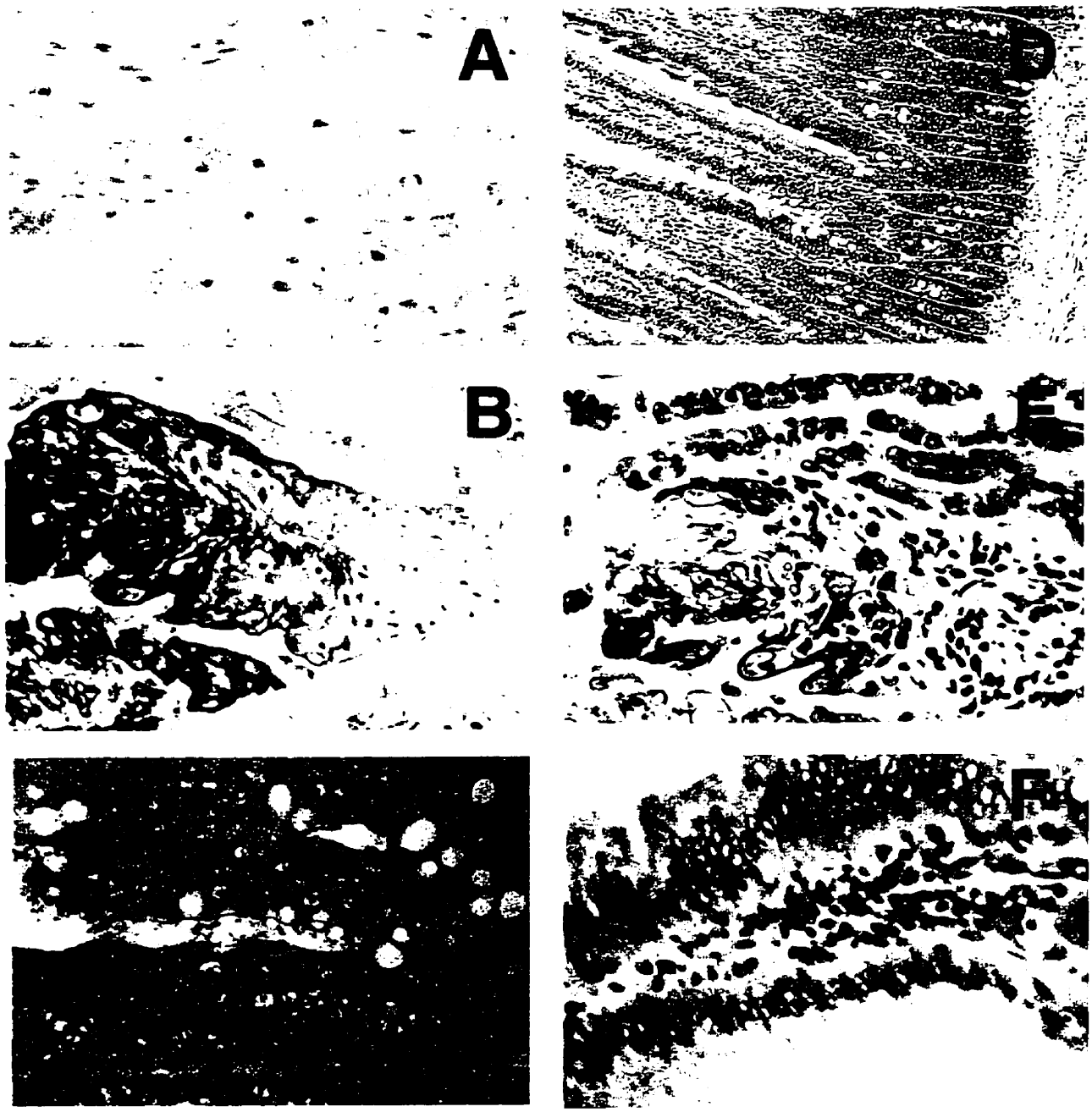
b) rats treated with 612 mg/kg elemental iron (by gavage)

c) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.

d) control rats

e) rats treated with 612 mg/kg elemental iron (by gavage)

f) rats treated with 612 mg/kg elemental iron (by gavage), followed by 800 mg/kg intraperitoneal injections of deferiprone solution.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F

Figure 25: Deferiprone concentrations in the sera of rats after receiving 400 mg/kg deferiprone either as a single injection or four 100 mg/kg injections.

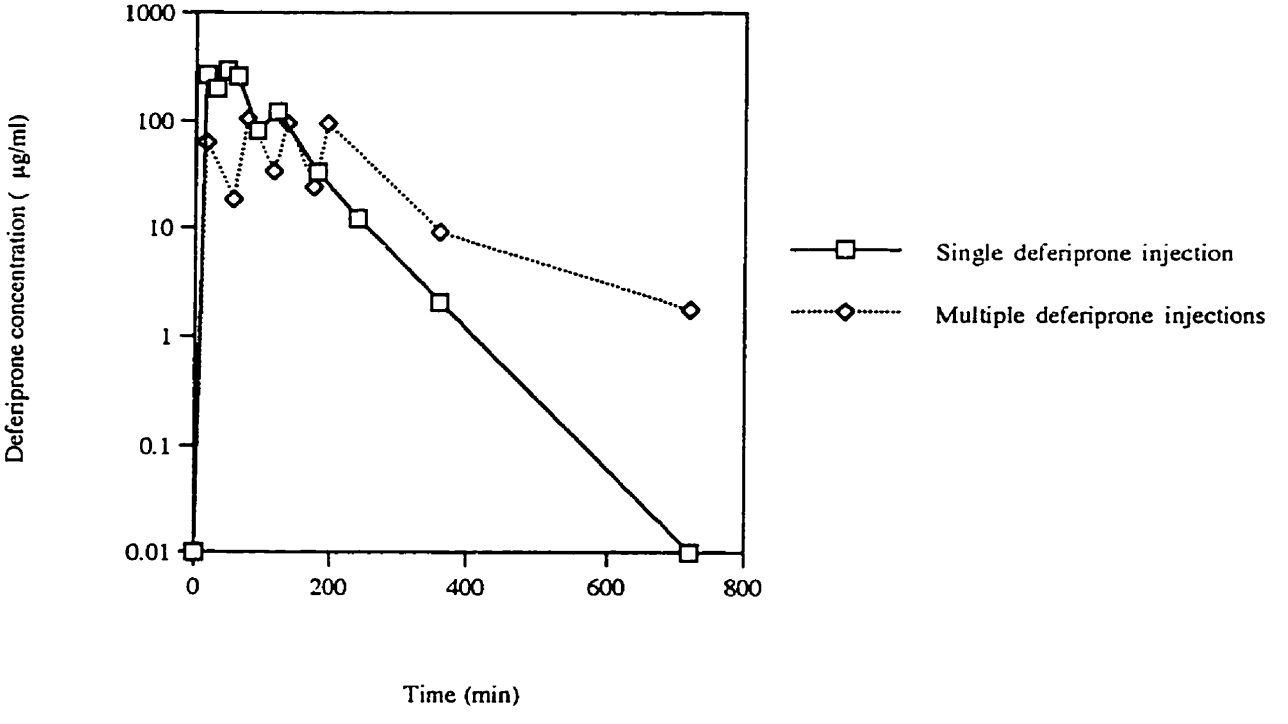
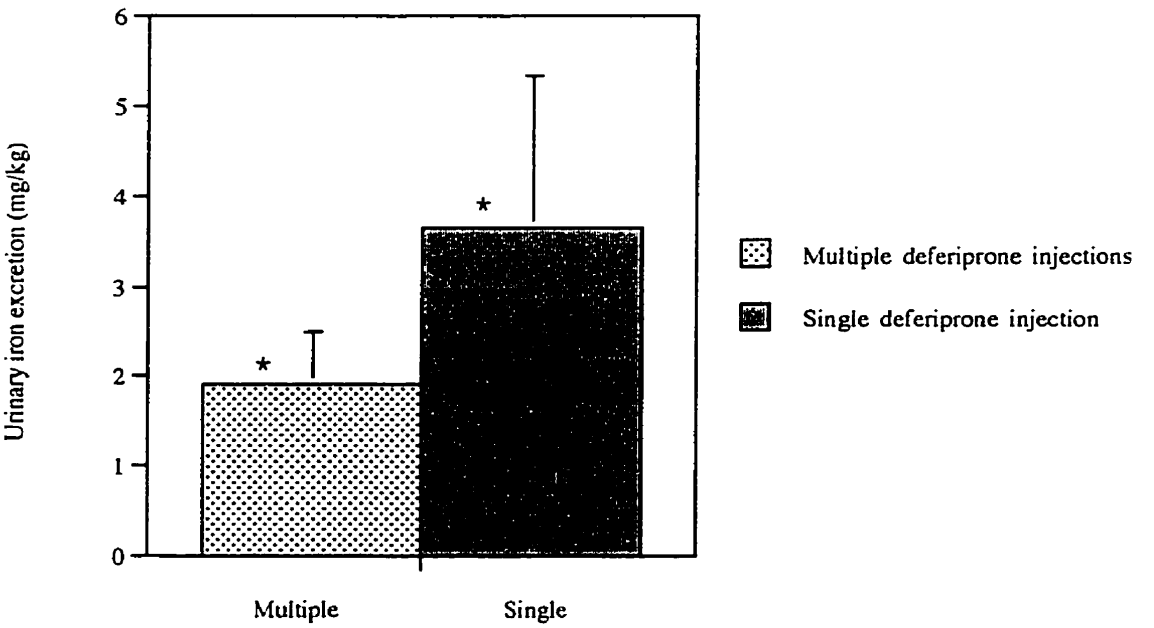


Figure 26: Urinary iron excretion in rats receiving 400 mg/kg deferiprone either as a single injection or as four 100 mg/kg injections following treatment with 100 mg/kg oral elemental iron.



* p=0.0164

died after a same-dose iron meal. The presence of the distinct red colour of the urine samples of deferiprone-treated rats signalled the presence of the deferiprone-iron complex. Thus deferiprone chelated the absorbed iron and was subsequently cleared from the circulation. Those rats that were not treated with deferiprone had no urinary iron excretion compared to those rats that received deferiprone. This decrease in body iron burden was reflected in the iron stains of stomach and intestine sections of deferiprone-treated rats. Chelation treatment with deferiprone resulted in a greatly reduced intracellular iron staining. Thus, deferiprone treatment may have protected intracellular organelles from the toxic effects of free cytosolic iron and contributed to the decrease in mortality of iron-intoxicated rats. Future studies will have to include transmission electron microscopy slides to determine whether or not the mitochondria are swollen -- an indirect method of looking for mitochondrial dysfunction.

Although the cytosol may have been protected by the deferiprone treatment, hematoxylin/eosin stains of rat myocardial and hepatic tissues showed evidence of early apoptosis. Some of the myocardial cells had lost their nuclei and were darker. Periportal hepatocytes also exhibited darker staining than other hepatic regions. The finding of periportal hepatocellular damage are consistent with the findings of other authors (Ganote and Nahara, 1973). The efficacy study was however acute. In future studies the chronic survival of deferiprone-treated rats and the sequellae that are a result of the iron intoxication should be examined.

Not only did the deferiprone treatment reduce the mortality of the rats following iron intoxication, but the drug treatment delayed death for up to seven hours compared to the untreated group. The administration of large doses of deferiprone may provide protection from the free radicals induced by the circulating free iron, as seen by Artman et al. (1984b). Deferiprone can be administered rapidly and in large doses in rats. Conversely, DFO can only be administered as a slow infusion of 15 mg/kg/hour following

acute iron intoxication to prevent hypotension (Peck et al., 1982). Thus deferiprone offers a potentially valuable advantage to iron chelation therapy following acute iron intoxication.

Having proven the efficacy of deferiprone in preventing and delaying mortality in acutely iron-intoxicated rats, I set out to determine if greater urinary iron excretion can be induced by either a sustained deferiprone exposure or a bolus exposure of a similar total dose. In this latter study, rats received a sub-lethal dose of 100 mg/kg iron and only 400 mg/kg deferiprone.

Following the administration of 100 mg/kg iron and deferiprone, greater urinary iron excretion resulted when deferiprone was administered as a single, large dose compared to four, smaller injections. These data indicate that the administration of deferiprone as a bolus to acutely iron intoxicated rats more effectively chelated the bolus of iron as it was being absorbed into the systemic circulation and prevented the iron from entering and damaging the intracellular organelles. This finding supports the suggestion that deferiprone may be optimally used at larger doses in acute iron intoxication to chelate the sudden influx of free iron, thereby minimizing the toxic effects of the iron.

6.7 Conclusions

1. Mortality is reduced in acutely iron-intoxicated rats treated with deferiprone.
2. Systemically administered deferiprone results in the chelation of toxic doses of orally administered iron, iron removal from target organs, and iron excretion into the urine.
3. In acutely iron-intoxicated rats, single-dose bolus injections of deferiprone induce greater urinary iron excretion than smaller, repeated injections (sustained exposure) of a similar dose.

7.0 Overall Discussion

The experiments in this thesis were designed to examine three different pharmacodynamic endpoints in an attempt to increase our understanding of the ways by which the new oral iron chelator, deferiprone, exerts its clinical effects. All three pharmacodynamic endpoints chosen stem from the ability of deferiprone to chelate circulating iron that is not bound to transferrin. These pharmacodynamic endpoints involve patients with chronic iron overload (thalassemia and sickle cell disease), patients with malaria and animals with acute iron intoxication.

In the cases of thalassemia and sickle cell disease, chronic iron overload is the result of repeated blood transfusions. In conditions of chronic iron-overload, an understanding of the mechanisms that lead to sustained and effective iron chelation is crucial in removing sufficient amounts of iron and preventing irreversible damage to vital organs such as the heart and liver. In acute iron intoxication, the damage caused by free iron is rapid -- after the total iron binding capacity is exceeded -- and any attempt to prevent severe morbidity and mortality must be based on rapid and efficient iron chelation.

The third endpoint chosen is very different from the first two. The survival of plasmodium parasites that are responsible for causing malaria depends on the availability of iron. I hypothesized that iron chelation would be a rate-limiting factor in the survival of this microorganism. In the case of plasmodia, the pharmacodynamic effect of deferiprone is targeted towards a non-mammalian cell. Moreover, patients with malaria do not suffer from iron-overload, making it important to verify that chelation therapy with deferiprone does not deplete them of iron. This issue was addressed in our laboratory several years ago when Stobie et al. (1993) showed that in volunteers with normal blood iron levels, the administration of deferiprone does not result in any measurable iron excretion in the urine. This is not surprising, because transferrin has a greater affinity for iron than does deferiprone. Consequently, the targeted use of deferiprone to chelate plasmodial iron

should not affect the pool of iron available for hematopoiesis and various vital cellular functions.

The central hypothesis examined in this thesis was that iron chelation by deferiprone can be optimized, by matching the administration regimen of the drug to the kinetic profile of the iron (either by administering larger, less frequent doses or smaller, more frequent doses).

For the first part of the experiments, I examined whether high peak concentrations of deferiprone are more effective at inducing greater urinary iron excretion than lower, but more sustained levels of the drug in chronic iron overload. The answer to this question is of immediate clinical importance as it should direct clinicians how to employ this chelator. This part of the study should assist in the understanding of the interactions between deferiprone and the iron pool available for chelation. This protocol was preceded by unpublished claims by Kontoghiorghes that large single daily doses induced greater urinary iron excretion in his patients, than did smaller divided doses. Should larger doses prove to be more effective, than those findings would support the hypothesis that the iron pool in chronic iron overload is not readily accessible and that a concentration gradient is needed to reach it.

My study showed the superiority of smaller, but more frequent doses of deferiprone, suggesting that this may be due to its lipophilicity and its ability to enter cells readily. A large concentration gradient of deferiprone is thus not needed. Instead, more sustained levels are needed in chronic iron overload. These findings also suggested that the chelatable iron pool is constantly being replenished by iron from intracellular iron deposits, necessitating sustained levels of deferiprone to allow effective chelation.

Urinary iron excretion has been previously shown to decrease while fecal iron excretion increased, after desferrioxamine was administered to chronically iron-overloaded patients who had high hemoglobin levels. Conversely, lower levels of hemoglobin produced an increase in UIE and a decrease in fecal iron excretion with DFO use (Pippard

et al., 1982). This response has been attributed to more iron being available, or being "accessible", for chelation when hemoglobin levels are low and erythropoietic activity is high. To control for this potential confounder, all of the patients were transfused prior to entering this part of the study so that all patients had high and similar levels of hemoglobin during the two arms of the study (ie. q6h and q12h regimens).

In this study the mean UIE achieved by taking deferiprone q6h was greater than that after the same total dose of deferiprone was given q12h. Since the AUC_{0-24} of deferiprone did not differ between the two regimens, the changes in UIE are important in understanding the mechanism of iron chelation by deferiprone. Our data indicate that, given equal systemic exposure (in terms of similar AUC_{0-24}), higher peak levels achieved with the q12h regimen are not necessary to penetrate iron-overloaded cells; rather, sustained intermediate levels throughout the day result in greater iron chelation. These results may also suggest that in iron-overloaded patients with thalassemia major, the pool of iron chelated by deferiprone is immediately available (ie. in the blood or in easily accessible tissue compartments), and that the high gradient between plasma and tissue concentration (such as the one created after q12h administration) may not be needed for effective chelation. There were no statistical differences in the amount of deferiprone glucuronidation occurring between the q6h and the q12h regimen, which further support the similar AUC_{0-24} of the parent drug itself.

These data further indicate that the q6h regimen provides more effective iron chelation, despite the danger of compromised compliance with more frequent drug administration.

Since this first part of my protocol was performed on patients who had immediately been transfused, when their hemoglobin levels were high, it was important to verify that the above conclusions held true during lower hemoglobin levels. Without performing a study at lower hemoglobin levels, our findings may not be valid four weeks after the

transfusion when hemoglobin levels are lower. Furthermore, any conlusion of the pharmacokinetic-pharmacodynamic relationship of deferiprone would be tenuous at best.

To date, information on the effect of hemoglobin levels on iron excretion is derived from studies with DFO. It has been previously shown (Pippard et al., 1982) that urinary iron excretion with DFO correlates directly, while stool iron excretion correlates inversely, with erythropoietic activity in HBT patients (Pippard et al., 1982). They attributed these findings to the suppression of erythropoietic activity and reduced iron utilization during high hemoglobin levels.

In contrast to the first study in our protocol, urinary iron excretion achieved when deferiprone was administered q6h *did not* differ from that observed when the same dose of deferiprone given q12h, when hemoglobin levels *were low*. Unexpectedly, the $AUC_{0\text{-}24}$ of deferiprone differed significantly between the two regimens, with the q6h $AUC_{0\text{-}24}$ being significantly lower, than that with the q12h regimen. Thus urinary iron excretion due to deferiprone may depend not only on the intervals of administration of the drug, it may also be dependent on the total deferiprone $AUC_{0\text{-}24}$ in the systemic circulation. That a lower $AUC_{0\text{-}24}$ with the q6h regimen resulted in similar iron excretion to a significantly greater $AUC_{0\text{-}24}$ with the q12h regimen, agrees with the results of our previous studies at high hemoglobin levels. In the first [post-transfusion] study, similar $AUC_{0\text{-}24}$ in the two regimens resulted in a higher UIE with the q6h regimen. Taken together, these findings suggest that when deferiprone is administered q6h, the amount of iron excreted per standard unit of deferiprone $AUC_{0\text{-}24}$ is greater than if the drug is administered q12h.

I propose the following model in an attempt to synthesize the observations made in these two studies into a unified theory for a mechanism of iron chelation with deferiprone.

1) Prior to transfusion when hemoglobin levels are low, more iron may be accessible for chelation because of increased erythropoietic activity. The sustained levels of deferiprone (q6h) may result in greater iron excretion during a first-pass of the drug

through the liver. The chelated iron may then enter the biliary system, and may result in lower systemic AUC_{0-24} of deferiprone, but in higher fecal iron excretion. Alternatively, high deferiprone (q12h) concentrations before transfusion, may result in greater formation of the complete 3:1 deferiprone:iron complex, which may result in diffusion into the systemic circulation and may explain the higher systemic AUC_{0-24} of deferiprone.

2) When hemoglobin concentrations are high after a transfusion, the chelatable iron pool may be substantially smaller than it is when hemoglobin levels are low. If this is true, deferiprone may not chelate iron during its first pass (presystemically) and not enter the biliary system. Thus, deferiprone may not leave the systemic circulation, resulting in similar AUC_{0-24} between the q6h and q12h regimens.

The two studies discussed above were not designed to prove the hypothesis presented in points 1 and 2. However they led me to design the next phase of the experimental protocol, the aim of which was to examine whether or not deferiprone can induce biliary excretion of iron -- a key element in the hypothesis.

The balance study examined whether deferiprone leads to fecal iron excretion in iron-overloaded sickle cell disease patients. Desferrioxamine has been shown to result in clinically important fecal iron excretion. However the comparison of DFO-induced fecal iron excretion to deferiprone-induced fecal iron excretion, if any, has not been examined in sickle cell disease. Should the fecal iron excretion vary among chronically transfused, iron-overloaded sickle cell disease patients receiving deferiprone, then it would support the hypothesis that fecal iron excretion with deferiprone may occur at low hemoglobin levels in the thalassemia patients who were studied. In fact the results indicate clearly that deferiprone not only induces a negative net iron balance in sickle cell disease patients, but also results in fecal iron excretion ranging from 3-33%. These findings are consistent with results by others who examined fecal iron excretion induced by deferiprone (Olivieri et al., 1990; al-Refaie et al., 1992). Perhaps these values of fecal iron excretion were not nearly

as high as those induced by desferrioxamine in the previous studies because of differences in total body iron load or because chelation occurred from a different iron compartment. The high compliance of thalassemia patients with deferiprone (Olivieri et al., 1992) and the ability of the drug to reduce iron in thalassemia patients (Olivieri et al., 1995) suggests that sickle cell patients could also benefit from deferiprone therapy.

The second pharmacodynamic model used in my thesis, involved an examination of the effects of deferiprone on plasmodial survival in asymptomatic malaria patients. I had a rare opportunity to examine the data derived from malaria patients and to contrast them with *in vitro* studies on the susceptibility of *Plasmodia* parasites to deferiprone.

Although continuous subcutaneous DFO has been shown to have an antimalarial effect (Gordeuk et al., 1992a; 1992b; 1993), in no study so far has the potential role of orally active iron chelating agents as anti-malarial agents been examined. In this study, the deferiprone was administered to asymptomatic male Zambian volunteers, screened for the presence of malarial parasitemia. These patients received either placebo or deferiprone (75 or 100 mg/kg/day) in a randomized, double-blind, crossover manner.

In this study, deferiprone did not have any clinically measurable anti-malarial efficacy at the dose schedules used, in contrast to *in vitro* studies with the drug. This discrepancy could be explained adequately by a close analysis of the results. Effective antimalarial deferiprone peak serum levels of 15.15 μg/ml (108 μmol/L) were reached with a regimen of 75 mg/kg/day of oral deferiprone (ie. 25 mg/kg, q8h). Such levels were shown to have an antimalarial effect *in vitro* following a 48-hour exposure (Heppner et al., 1988; Hershko et al., 1991; 1992). The mean serum deferiprone 24-hour AUC in our patients was 7747 ± 1510 mg·min/L, such that the 48-hour *in vivo* AUC would have been 15494 mg·min/L. In comparison, the 48-hour *in vitro* AUC is 40320 mg·min/L and the ratio of the 48-hour *in vivo* serum deferiprone AUC to the *in vitro* AUC yields a patient exposure of only 39%. Although the desirable peak deferiprone levels were reached with

this regimen *in vivo*, there was not enough sustained exposure to the drug to produce an antimalarial response in the patients.

To achieve an AUC *in vivo* corresponding to *in vitro* conditions associated with antimalarial efficacy, one can predict that 2.60 times more deferiprone must be administered in order to reach the AUC achieved *in vitro* (ie. 40320/15494=2.60). Hence it can be predicted that a final 'efficacious dose' of 75 mg/kg/day*2.60 = 195 mg/kg/day. Thus, a deferiprone exposure of approximately 200 mg/kg/day in divided doses and administered every 2 hours would predictably provide asymptomatic individuals with serum deferiprone levels similar to those attained *in vitro* and may produce an antimalarial effect. This level of exposure to deferiprone is not possible because of its narrow therapeutic window. Moreover, because malaria patients do not have chronic iron overload to buffer a dose of even 75 mg/kg/day of oral deferiprone, they would be exposed to the possibility of higher rates of toxicity than seen in thalassemia patients, if given a dose of 200 mg/kg/day.

Thus, the calculated dose of oral deferiprone needed to achieve plasmodicidal concentrations led me to predict that this drug cannot be given for this indication at a dose of 200 mg/kg/day. At this dose the drug is toxic and potentially fatal. This prediction will prevent patients from being exposed to a drug which cannot combat their illness, but which may also predispose them to serious adverse effects. Moreover, the results of this study is likely to save money because further studies should not be initiated with oral deferiprone in malaria patients.

The third pharmacodynamic endpoint of my thesis examined the ability of deferiprone to chelate iron in acute iron intoxication. Acute iron intoxication is entirely different from chronic iron-overload in which patients die from free-radical-induced cardiac dysfunction. In acute iron overload, the main causes of death are corrosive damage to the gastrointestinal tract, cardiovascular shock, and mitochondrial damage. At the present time desferrioxamine is the only drug used for acute iron intoxication; its efficacy is directly related to its ability to chelate iron in the systemic circulation.

To examine whether deferiprone acts similarly, the drug was administered to rats intraperitoneally. By doing this, any potential effect would be due to chelation in the systemic circulation and not due to the inhibition of iron absorption -- a scenario that might have presented itself if deferiprone had been given orally.

In the first part of this protocol, 58% of male Wistar rats that received 612 mg/kg elemental iron in the form of oral ferrous sulphate solution by gavage died before the end of the 14-hour observation period, compared to 15% of rats that received iron followed by deferiprone injections. These data show that the administration of deferiprone has a protective effect in rats receiving toxic doses of iron. In addition to the overall prevention of mortality, it was also noted that the time of death of the deferiprone-treated rats was delayed for several hours. This last point may 'buy' time for children and adults suffering from acute iron intoxication, during transport to a tertiary care facility.

The decreased mortality with deferiprone was associated with several biochemical and pathological observations which prove the direct role of deferiprone in chelating the excess iron and thus preventing morbidity and mortality.

The deferiprone-iron complex has a noticeable red colour. Hence, its appearance in the urine of animals receiving toxic doses of iron with deferiprone reflects the presence of the deferiprone-iron complex. Although only minute quantities of iron are excreted by the kidney, the iron chelated by deferiprone is excreted in the urine, thus decreasing the iron load.

The increase in survival of the group of animals receiving deferiprone with iron was associated with significantly less iron retention in the tissues. Of particular interest are organs most commonly being responsible for morbidity and mortality in acute iron intoxication -- stomach, intestine and liver. Thus my initial hypothesis that deferiprone increases survival in acutely iron-overloaded rats has been supported by the experimental findings. Furthermore, the second hypothesis, that orally administered iron will be

chelated and excreted in the urine by systemically administered deferiprone, was also supported by my experimental findings.

The next step of this protocol determined that a sustained exposure to the drug is less effective at inducing greater UIE than a bolus injection. Thus in acute iron intoxication, I predicted that better chelation would occur when a bolus dose of the drug encountered a bolus dose of iron. These findings suggest that in the future when deferiprone therapy is given for acute iron intoxication, deferiprone should be administered in bolus doses and not as smaller, divided doses.

These encouraging results from the acute iron intoxication study should lead to the development of a protocol for human studies. Deferiprone has been used safely by the Iron Chelation Group at The Hospital for Sick Children and The Toronto Hospital on a daily basis for over five years in chronically transfused iron-overloaded patients. Thus it is fair to assume that its use will be safe also in the short treatment of acute iron overload. Due to the high cost of desferrioxamine, and the need to mix the drug with sterile water and administer it parenterally, the drug is given only in large medical centres of First World nations. A recent survey reveals that many community hospitals do not stock desferrioxamine even in towns of 10 000 people (Koren, personal communication). Hence a typical patient at a distant location may spend several hours on the way to a centre, where desferrioxamine can be administered. The oral administration of deferiprone should make it an easily accessible antidote that may be used even in remote areas.

In summary, sustained exposure to deferiprone can achieve greater iron chelation in chronically iron-overloaded patients with homozygous beta-thalassemia and sickle cell disease than high exposure to the drug. These findings set the stage for the development of a sustained-release preparation. Conversely, in acute iron poisoning the experimental findings show that larger deferiprone doses result in greater iron chelation than smaller, sustained doses of the drug.

In conclusion, my studies indicate that the effective administration of deferiprone is dependent on the kinetic profile of iron in the patient [or animal]. Constant serum iron levels (chronically iron-overloaded homozygous beta-thalassemia patients) necessitate sustained exposure to deferiprone, while high peak serum iron levels (acute iron intoxication) require large immediate doses of deferiprone. These findings suggest the need for the development of a stable deferiprone solution that can be readily administered as a bolus injection.

7.1 Future studies.

In this section I wish to indicate directions for future research regarding the role of deferiprone in iron chelation.

1) The correlation between deferiprone pharmacokinetics and the clinical response in chronic iron-overload. In the present studies urine and fecal iron excretions served as short-term indicators of iron chelation and the efficacy of deferiprone. However, the effectiveness of deferiprone in removing iron from vital organs may take years to complete. We predict that at least some of the variability in the patient's clinical response to deferiprone will depend on the pharmacokinetics of the drug. Specifically, patients with greater systemic exposure (in terms of greater AUC_{0-24} with similar daily doses) will have a greater survival.

2) The efficacy of deferiprone in acute iron poisoning in humans: *In vitro* testing of higher levels of deferiprone after various time exposures of heparinized whole blood to concentrations of elemental iron would prove invaluable in deciding whether these higher levels would be toxic in cases of acute iron intoxication. These findings could be instrumental in determining the feasibility of administering deferiprone as an intravenous

preparation for infusion, or a bolus injection with either saline or plasma expanders, as required. These studies maybe the basis of clinical studies with deferiprone in acute iron intoxication. This is likely to result in a new, universally available antidote for severe intoxication.

3) A deferiprone challenge test: Since deferiprone can be used in remote areas, it could also be used to determine whether or not a patient ingested sufficient iron to surpass the saturation of binding capacity compared to those whose levels are clinically insignificant. The appearance of red urine of acutely iron-overloaded animals, and of chronically iron-overloaded patients treated with deferiprone may lead to an important clinical use of the drug. Clinicians rarely know the exact amount of iron taken accidentally by a child or consumed intentionally by an adult during a suicide attempt. This amount may range from a negligible dose that will be readily bound to transferrin to dangerously large quantities resulting in circulating free iron and subsequent toxic effects. A deferiprone challenge-test may allow a clinician to decide whether the ingestion is life-threatening with the presence of the red deferiprone-iron complex in the urine, or not dangerous, with the absence of free iron. In the former case a clinician may wish to mobilize costly means (ie. helicopter transportation), whereas in the latter case the patient may be treated locally, and thus avoid excessive costs.

4) The development of a sustained-release preparation of deferiprone to determine the optimal dose schedule in chronic iron overload.

5) The development of a stable aqueous solution of deferiprone to determine the optimal dose schedule in acute iron intoxication.

8.0 Conclusions

1. Smaller, more frequent doses of the same total dose of deferiprone produce greater UIE than larger, less frequent doses of the same total dose of deferiprone (when hemoglobin concentrations are high), in iron-overloaded thalassemia patients.
2. A difference in the systemic exposure of deferiprone occured when hemoglobin concentrations were low, while UIE remains similar regardless of the frequency of drug administration.
3. The deferiprone-iron complex is excreted into the bile. Variable fecal iron excretion occurs in chronically-transfused iron-overloaded sickle cell disease patients following treatment with deferiprone.
4. Deferiprone did not induce a plasmodicidal effect in asymptomatic malaria patients. The pharmacokinetics of deferiprone can predict a lack of plasmodicidal effect when used at the clinically recommended dose.
5. A greater systemic exposure of deferiprone in humans would likely be required to produce plasmodicidal activity.
6. Mortality is reduced in acutely iron-intoxicated rats treated with deferiprone.
7. Systemically administered deferiprone results in the chelation of toxic doses of orally administered iron, its removal from target organs and its excretion into the urine.
8. In acutely iron-intoxicated rats, single-dose bolus injections of deferiprone induced greater urinary iron excretion than smaller, repeated injections (sustained exposure) of a similar dose.

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APPENDICES

PREQ6H					PREQ12H				
Time (min)	Mean L1	SD	Mean Gluc	SD	Time (min)	Mean L1	SD	Mean Gluc	SD
0	2.1	1.0	0.5	0.9	0	1.5	1.0	0.5	0.3
15	9.1	3.3	2.1	1.9	15	30.5	14.8	1.6	1.4
30	7.7	3.8	2.9	3.2	30	26.1	13.3	0.0	0.0
45	7.4	3.1	2.6	3.2	45	26.0	5.4	1.3	3.2
60	7.1	2.9	1.1	1.3	60	22.0	6.1	2.1	2.6
90	7.7	3.2	1.7	1.4	90	19.1	1.4	1.7	2.4
120	6.8	1.8	0.5	1.0	120	14.0	2.9	5.8	7.0
180	4.8	2.2	1.1	1.1	180	9.7	2.3	3.0	3.3
240	3.3	1.8	0.5	0.4	240	6.8	2.0	2.2	3.2
300	2.2	1.1	0.9	1.3	300	4.3	1.7	1.6	1.4
360	1.8	1.1	0.7	0.5	360	3.7	2.0	0.5	0.4
POSTQ6H					POSTQ12H				
Time (min)	Mean L1	SD	Mean Gluc	SD	Time (min)	Mean L1	SD	Mean Gluc	SD
0	2.1	1.2	1.0	0.7	0	1.2	1.0	0.4	0.3
15	10.3	11.7	1.1	1.2	15	29.4	25.2	0.6	0.8
30	8.5	6.3	2.1	3.1	30	18.4	9.1	9.2	9.2
45	10.0	4.8	1.6	2.1	45	20.7	8.8	8.4	14.4
60	9.0	4.3	1.1	1.4	60	23.2	9.1	5.7	8.7
90	7.2	2.0	2.9	2.5	90	16.0	1.9	5.7	2.3
120	7.1	2.0	1.9	1.5	120	13.1	2.6	4.5	7.2
180	5.0	1.8	0.8	0.8	180	9.3	2.5	4.9	7.4
240	3.0	1.2	1.4	1.4	240	5.4	1.6	3.4	2.7
300	2.3	1.0	0.8	0.8	300	4.0	1.8	2.3	1.9
360	1.8	0.9	0.7	0.7	360	2.9	1.5	1.5	1.4

		L1 (ug/ml)		
		Time (min)	MEAN LEVEL	SD
		0	1.8	1.0
		30	9.7	7.1
		60	10.1	5.1
		90	12.4	5.0
		120	11.1	1.7
		180	7.5	0.8
		240	4.5	0.7
		300	3.0	0.4
		360	1.7	0.4
		420	1.2	0.3
		480	0.7	0.3
		L1 metab (ug/ml)		
		Time (min)	MEAN LEVEL	SD
		0	0.2	0.4
		30	1.2	2.7
		60	0.8	1.1
		90	0.4	0.9
		120	2.5	2.6
		180	0.4	0.6
		240	0.5	0.5
		300	0.2	0.2
		360	0.3	0.2
		420	0.3	0.2
		480	0.1	0.1

Appendix III

1) Part of the post-transfusion study (Chapter 3) was reprinted from the following published reference by courtesy of Mosby-Yearbook Inc.

Fassos FF, Klein J, Fernandes D, Matsui D, Olivieri NF and Koren G. Urinary iron excretion depends on the mode of administration of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in patients with homozygous β-thalassemia. Clin. Pharm. Ther. 1994;55:70-5.

2) Part of the pre-transfusion study (Chapter 3) was reprinted from the following reference by courtesy of Dustri-Verlag Dr. Karl Feistle.

Fassos FF, Klein J, Fernandes D, Matsui D, Olivieri NF and Koren G. The pharmacokinetics and pharmacodynamics of the oral iron chelator deferiprone (L1) in relation to hemoglobin levels. Int. J. Clin. Pharm. Ther. 1996;34:288-292.

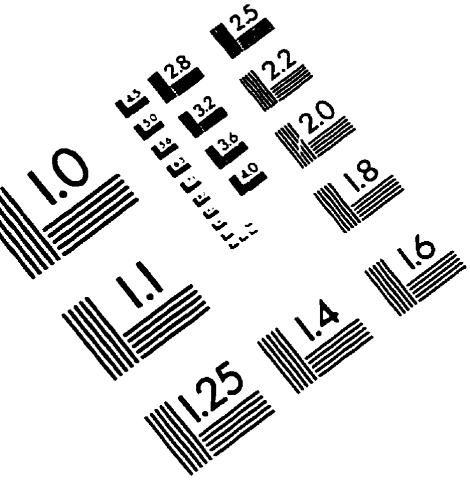
3) Part of the sickle cell disease balance study (Chapter 4) was reprinted from the following published reference by courtesy of WB Saunders Co. (and The American Society of Hematology).

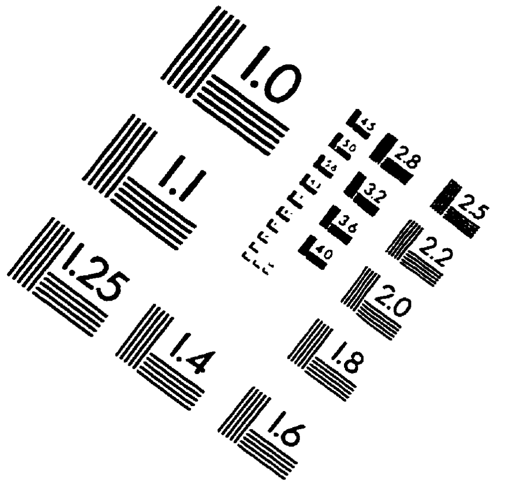
Collins AF, Fassos FF, Stobie S, Lewis N, Shaw D, Fernandes D, Fry M, Templeton DM, Koren G and Olivieri NF. Iron balance and dose-response studies of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in iron-loaded patients with sickle cell disease. Blood. 1994;83(8):2329-2333.

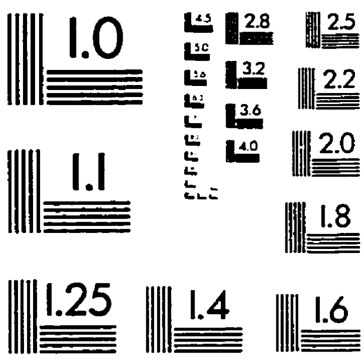
4) Part of the iron intoxication study (Chapter 6) was reprinted from the following published reference by courtesy of Marcel Dekker Inc.

Fassos FF, Berkovitch M, Daneman N, Koren L, Cameron R, Klein J, Falcitelli C, St. Louis P, Daneman R and Koren G. The efficacy of the oral iron chelator deferiprone in the treatment of acute iron intoxication in rats. J. Tox. Clin. Tox. 1996;34(3):279-287.

IMAGE EVALUATION TEST TARGET (QA-3)







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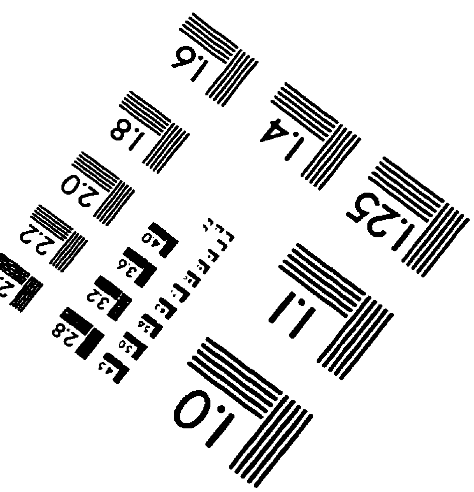

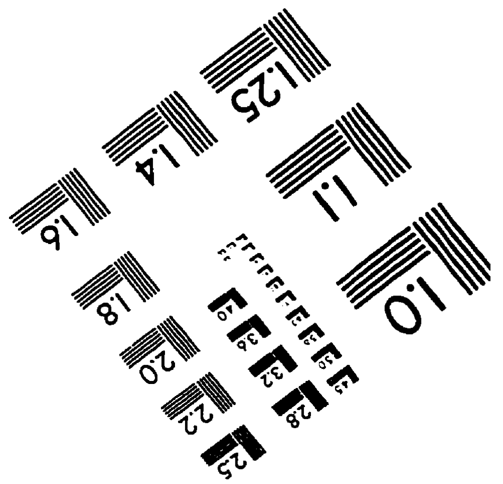

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