BioMetals 1997, **10**, 247–256

Liver iron depletion and toxicity of the iron chelator Deferiprone (L_1, CP20) in the guinea pig

A. Wong*, V. Alder†, D. Robertson*, J. Papadimitriou‡, J. Maserei§, V. Berdoukas¶, G. Kontoghiorghes‖, E. Taylor* & E. Baker*

**Department of Physiology, University of Western Australia, Perth, Western Australia, ¶Prince of Wales Children's Hospital, Sydney, Australia, †Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Western Australia, ‡Department of Pathology, QEII Medical Centre, Perth, Western Australia, §Department of Clinical Biochemistry, Royal Perth Hospital, Perth, Western Australia, and ‖Royal Free Hospital, London, UK*

Received 15 May 1997; accepted for publication 28 June 1997

The use of the iron chelator deferiprone (L_1, CP20, 1,2-dimethyl-3-hydroxypyrid-4-one) for the treatment of diseases of iron overload and other disorders is problematic and requires further evaluation. In this study the efficacy, toxicity and mechanism of action of orally administered L_1 were investigated in the guinea pig using the carbonyl iron model of iron overload. In an acute trial, depletion of liver non-heme iron in drug-treated guinea pigs (normal iron status) was maximal (approximately 50% of control) after a single oral dose of L_1 of 200 mg kg^{-1}, suggesting a limited chelatable pool in normal tissue. There was no apparent toxicity up to 600 mg kg^{-1}. In each of two sub-acute trials, normal and iron-loaded animals were fed L_1 (300 mg kg^{-1} day^{-1}) or placebo for six days. Final mortalities were 12/20 (L_1) and 0/20 (placebo). Symptoms included weakness, weight loss and eye discharge. Iron-loaded as well as normal guinea pigs were affected, indicating that at this drug level iron loading was not protective. In a chronic trial guinea pigs received L_1 (50 mg kg^{-1} day^{-1}) or placebo for six days per week over eight months. Liver non-heme iron was reduced in animals iron-loaded prior to the trial. The increase in *a* wave latency (electroretinogram), the foci of hepatic, myocardial and musculo-skeletal necrosis, and the decrease in white blood cells in the drug-treated/normal diet group even at the low dose of 50 mg kg^{-1} day^{-1} suggests that L_1 may be unsuitable for the treatment of diseases which do not involve Fe overload. However, the low level of pathology in animals treated with iron prior to the trial suggests that even a small degree of iron overload (two-fold after eight months) is protective at this drug level. We conclude that the relationship between drug dose and iron status is critical in avoiding toxicity and must be monitored rigorously as cellular iron is depleted.

Keywords: carbonyl iron, deferiprone (L_1), guinea pig, iron, iron chelators, iron-overload

impairment (de Virgillis *et al.* 1988), auditory sensori-neural damage (Albera *et al.* 1988) and visual neuropathy (Balkie *et al.* 1985) have also been described for DFO (Porter & Huehns 1989, Hershko 1994). Hence, intensive efforts are being made to find new alternative chelators (Baker *et al.* 1987, Baker 1988, Hider & Hall 1991, Kontoghiorghes *et al.* 1993a, Hershko 1994), preferably inexpensive and orally effective.

The α-ketohydroxypyridones are a promising group of Fe chelators (Hider & Hall 1991, Kontoghiorghes *et al.* 1993a). They have been shown to remove Fe from experimental animals such as mice (Kontoghiorghes 1986, Porter *et al.* 1990, 1991) and rabbits (Kontoghiorghes & Hoffbrand 1986) *in vivo* as well as from human patients (Bartlett *et al.* 1990, Kontoghiorghes *et al.* 1990, Agarwal *et al.* 1992). Here we report on 1,2-dimethyl-3-hydroxypyrid-4-one (L_1, CP20, Deferiprone; henceforth called L_1) which has been in extensive clinical trials (Bartlett *et al.* 1990, Kontoghiorghes *et al.* 1990, Agarwal *et al*, 1992, Kontoghiorghes 1995) despite some controversy (Hershko 1993, Kontoghiorghes *et al.* 1993b) and has shown evidence of toxicity (Al-Refaie *et al.* 1995).

In this study the efficacy, toxicity and mechanism of action of orally administered L_1 were investigated *in vivo* in acute and chronic trials using normal guinea pigs or guinea pigs which were Fe-loaded using the carbonyl Fe model previously established in the rat (Park *et al.* 1987). The chronic trial was of particular interest as patients with transfusion-dependent disorders (e.g. thalassemia) require long-term chelation therapy. Hence, comprehensive analyses of body tissues, blood, ocular and auditory function were made at the end of the chronic trial to evaluate toxicity as well as effectiveness in Fe depletion.

The guinea pig was chosen as an animal model of Fe metabolism as there are similarities in Fe metabolism between the guinea pig and human (Bothwell *et al.* 1979, Chan *et al.* 1989), and considerable differences between the rat and man (Hershko *et al.* 1976, This study shows that the carbonyl Fe-loaded guinea pig may be a useful experimental model for investigating the interaction between chelators and tissue Fe pools (and vitamin C). It also shows that, as with DFO, the balance between level of Fe overload and dose of L_1 is critical in avoiding toxicity due to depletion of cellular Fe pools essential for normal metabolism.

Materials and methods

Deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one, L_1; CP20) was synthesised using a previously described method (Kontoghiorghes & Sheppard 1987). Carbonyl Fe was obtained from Sigma Chemicals, St. Louis, MO. Young adult, male, short-hair guinea pigs (English outbred strain) weighing approximately 300 g were used. This study was approved by the Animal Experimentation Ethics Committee of The University of Western Australia.

Iron overload with carbonyl iron

Guinea pigs were divided randomly into two groups. One group was placed on a normal chow diet. The second group was placed on a diet of 2% carbonyl Fe (w/w) mixed with normal guinea pig chow for two weeks, followed by another two weeks on a 3% carbonyl Fe diet. Animals were then allowed to equilibrate for one week. Five animals from each group were then exsanguinated for evaluation of diet-induced changes in liver non-heme Fe levels, hematology, serum biochemistry and histology of various tissues. Remaining guinea pigs were used in the sub-acute and chronic trials.

Acute toxicity trial

Six pairs of guinea pigs of normal Fe status each received a single oral dose of 100, 200, 400, 600, 800 or 1000 mg kg^{-1} and were compared with controls (given placebo gelatin capsules). Guinea pigs were observed for signs of toxicity over 48 h then sacrificed. Blood was removed for hematological analysis and serum biochemistry, and the liver and other organs were examined for drug-related toxicity. Liver non-heme Fe levels were also measured.

using the same protocol. Liver non-heme Fe levels were monitored in two or three guinea pigs from each of the four groups, sacrificed at the end of the trial or when showing severe signs of toxicity. The latter (all in the drug-treated groups) were recorded as dying during the trial.

Chronic trial

Thirty-six guinea pigs were started on an iron-deficient diet to prevent saturation of L_1 with Fe within the gut prior to absorption, and divided into two groups (normal or Fe-loaded). Guinea pigs within each group were given either L_1 ($50\ mg\ kg^{-1}\ day^{-1}$) or placebo (empty gelatin capsules) for six days per week. They remained on this drug regime and low-iron diet for eight months. The following parameters were assessed after this time (listed in the order in which they were studied):

1. General. The general behaviour and condition of the animals in each group, (i.e. fur quality, body weight, etc.) were monitored for any signs of drug-related changes.

2. Ocular assessment. The standard dark-adapted (scotopic) flash electroretinogram provided information about the gross functioning of the outer and inner retina of both eyes. The *a* wave is generated by the photoreceptor and the *b* wave by the inner nuclear layer of the retina. The latency of the *a* and *b* waves (msec) and their amplitude (μV) were determined for each animal using established techniques (Ben Nun *et al*. 1988). Animals were allowed to recover from anaesthesia for a period of five days before auditory assessment.

3. Auditory assessment. The electrical response of the cochlear branch of the VIIIth cranial nerve to acoustic stimuli was measured by a standard electrophysiological technique (Robertson & Wilson 1991). Briefly, this involves measuring the sound pressure level in decibels (dB SPL) required to elicit a just detectable response (compound action potential (CAP) threshold) from the auditory nerve when the animal's ear is presented with brief tone bursts over a range of frequencies from 2 to 30 kHz.

4. Hematology. After visual and auditory tests, blood samples (0.5 ml) were taken into 0.5 M EDTA (20 μl) by heart puncture of the anaesthetised animals and used for

6. Histology. Animals were sacrificed and the liver removed, washed, blotted dry and weighed. Small samples of the liver were fixed in 2.5% (w/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.45) and processed for electron microscopy. The spleen, kidney, heart, lungs, brain, eyes, adrenal glands and pancreas were also removed and weighed, and samples of skeletal muscle, bone marrow, skin, colon and ileum were taken. The major organs were formalin fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin or Prussian blue (for Fe). A blinded review of histology was then made. Remaining tissue samples were assayed for non-heme Fe content.

7. Non-heme iron. Tissue non-heme Fe was measured using the method of Torrance & Bothwell (1968).

Statistical analysis

Student's *t*-test was used to compare the results obtained in the drug-treated versus control normal diet groups and similarly in the drug-treated versus control Fe-loaded groups. Differences were considered statistically significant when $P < 0.05$. Results are expressed as mean ± SEM unless stated otherwise.

Results

A. Effects of dietary carbonyl iron

No difference between controls and Fe-loaded animals was observed in general behaviour and appearance of the animals nor in organ weights at five and 45 weeks after starting the diet.

Five weeks (i.e. Start of sub-acute trial). There was a 13-fold increase in liver non-heme Fe concentration from 53.4 ± 12.7 to 682.1 ± 29.8 μg Fe g^{-1} wet weight ($P < 0.01$) and a 14-fold increase in total liver Fe from 864 ± 195 to 12358 ± 382 μg Fe g^{-1} wet weight ($P < 0.01$). The histology of the animals receiving a normal diet or an Fe-enriched diet was similar except that the animals fed an Fe-enriched diet contained higher tissue Fe levels (Prussian blue stain) in the liver and spleen and the mucosa of

levels. There was still a marked difference between normal and Fe-loaded animals with respect to liver non-heme Fe concentration (51.4 (48.6, 54.2) $\mu g\ g^{-1}$ wet weight and 191.8 (209.0, 173.6) $\mu g\ g^{-1}$ wet weight) and total liver non-heme Fe (1138 (1164, 1111) μg and 6147 (6045, 6250) μg), respectively. However, relative Fe loading had decreased from approximately 13-fold at five weeks to fivefold at nine weeks.

Forty-five weeks (i.e. end of chronic trial after eight months on a low Fe diet). Compared with controls ($82.7 \pm 8.9\ \mu g\ g^{-1}$ wet weight), the originally Fe-loaded group still contained a significantly higher concentration of liver non-heme Fe ($157.3 \pm 23.0\ \mu g\ g^{-1}$ wet weight) ($P < 0.01$). A similar trend was seen with respect to liver total non-heme Fe ($2273 \pm 390\ \mu g$ versus $3875 \pm 665\ \mu g$, $P = 0.057$). Animals which originally received the Fe-enriched diet still displayed more stainable Fe in the liver and mucosa of the small bowel after 45 weeks than those originally on a normal diet. However, differences in the spleen were marginal. No significant differences were observed between Fe-loaded and control animals with regard to any hematological or biochemical parameters.

B. Acute toxicity trial

General. The guinea pigs appeared healthy and active after receiving a single oral dose of up to 800 $mg\ kg^{-1}$of L_1. However, animals were very ill after 1000 $mg\ kg^{-1}$ (weak, eye discharge, lethargy and anorexia), with one death. There was evidence of internal bleeding, particularly in the gut. No marked changes in organ weights were observed.

Hematology. No drug-related changes were detected in serum biochemical or hematological parameters. There was no apparent change in serum Fe.

Histology. Tissues and organs that were sampled in the heart and a few vacuolated cardiac myocytes were seen. Signs of interstitial pneumonia in these animals, probably due to *Bordetella* infection in the original guinea pig colony, were more severe than in controls, while bronchial associated lymphoid tissue was also prominent. No other structural lesions were detected.

Non-heme iron. Hepatic non-heme Fe was measured at the end of the 48 h observation period. In animals treated with 200 $mg\ kg^{-1}$ of L_1 there was a marked decrease in liver Fe levels to approximately 50% of those seen in controls (Figure 1). No further decrease in non-heme Fe concentration or total liver non-heme Fe levels was observed at doses up to 1000 $mg\ kg^{-1}$.

C. Sub-acute trial (five weeks after starting Fe-enriched diet).

Guinea pigs were given 300 mg $L_1\ kg^{-1}\ day^{-1}$ or placebo. Drug administration was stopped after six days due to illness (weakness, eye discharge and anorexia) in some of the drug-treated animals (both normal and Fe-loaded). Gross disturbances of central nervous system function (e.g. convulsions) were not seen. By Day 14, three of the drug-treated/normal diet animals had died (Table 1). There were no mortalities in the other three groups. After a recovery period of one week the trial was recommenced (Day 21) using the same protocol with L_1 given for six days. As noticed in the first trial,

Table 1. Mortalities in sub-acute toxocity trial

	Number of surviving animals			
	Normal		Fe-loaded	
	Placebo	Drug	Placebo	Drug
Day 0	10	10	10	10
Day 7	10	9	10	10
Day 14	10	7	10	10
	One week recovery			
Day 21	10	7	10	10
Day 28	10	4	10	4

Five weeks after the start of iron loading, animals were divided into four groups of ten, of which two groups were previously iron-treated. Drug-treated animals were given oral doses of L_1 in gelatin capsules at 300 mg kg^{-1} for Days 1–6 and observed over Days 1–14. Control animals received placebo capsules. After a one week recovery period, the trial was repeated on the remaining guinea pigs using the same protocol (i.e. L_1 given on Days 1–6 and 22–27). Day 0 refers to the day before drug treatment was started.

drug-treated animals showed toxic side-effects. This time both normal and Fe-loaded animals were seriously affected (Table 1). By Day 28, three more of the drug-treated/normal diet animals and six of the drug-treated/Fe-loaded diet animals had died. No further deaths were observed. Two animals from each group were sacrificed at this stage (including several drug-treated animals showing signs of severe toxicity, counted as dead from drug) to monitor changes in body and tissue weights. Guinea pigs in the placebo/Fe-loaded group had a higher mean liver weight at the end of the study compared with the other groups or the same group at Day zero. No other change was observed.

Liver non-heme iron was also monitored. Total non-heme iron levels in the drug-treated normal group (481 ± 120 μg) had decreased to about 50% of the value observed in the placebo–treated normal diet group (1138: 1164, 1111 μg), in agreement with changes seen in the acute trial. However, differences in total iron levels between the drug-treated (4534: 3911, 5156 μg) and control iron-loaded groups (6147: 6065, 6250 μg) were less marked.

D. Chronic toxicity trial

Beginning 13 weeks after the start of Fe-loading, test animals (18) were given oral doses of L_1 (50 mg kg^{-1} day^{-1}; six days per week) for eight months. Controls (18) received placebo for the duration of the trial.

During this lengthy trial animals died in both placebo (6/18) and drug-treated (6/18) groups (mainly due to fighting), but several statistically significant changes are still apparent in the drug-treated groups. Furthermore, the differences between placebo-treated and drug-treated groups are unlikely to reflect nutritional deficiencies in the latter since there were no apparent differences in body or organ weights between any of the groups nor in general condition and behaviour.

Ocular assessment Four quantitative parameters were derived from the retinogram (Table 2). There was a significant increase in the *a* wave latency ($P < 0.05$) for the drug-treated/normal diet group compared with the placebo/normal diet group (Table 2) and a similar trend in the Fe-loaded group. The mean *b* wave amplitude was lower in both drug-treated groups compared with the corresponding placebo groups (Table 2). This difference approached statistical significance in the previously Fe-loaded animals ($P = 0.05$).

Auditory assessment Due to bony overgrowth related to the extended length of this study, the small number of usable ears prevented statistical analysis. In the normal diet groups, however, there were some aspects of the data that encourage the view that L_1 did not cause major hearing losses. Among these were: (i) the occurrence of a number of good

audiograms in the test group; and (ii) the occurrence of unilateral losses in the test group, making it unlikely that systemic factors were responsible. However, it should be noted that only peripheral hearing function is tested by the CAP threshold method.

Hematology. Significant differences between placebo and drug-treated normal diet groups were observed in white blood cells and platelets. The number of white blood cells was reduced by approximately 40% ($P < 0.05$) from $6.3 \pm 0.8 \times 10^9$ l^{-1} to $3.9 \pm 0.6 \times 10^9$ l^{-1} in the drug-treated/normal diet animals compared with the placebo/normal diet group (Table 3). The platelet count was increased by approximately 80% ($P < 0.05$) from $561.5 \pm 165.5 \times 10^9$ l^{-1} to $1012.2 \pm 87.9 \times 10^9$ l^{-1} in the drug-treated/normal diet group (Table 3) compared with the placebo/normal diet group. By contrast, the only significant difference between the drug and placebo groups of Fe-loaded animals was in the mean cellular volume (MCV), which increased from $(76.6 \pm 1.1) \times 10^{5}$ L in the placebo to $(83.3 \pm 1.8) \times 10^{-15}$L in the drug group ($P < 0.05$).

Biochemistry. Within the Fe-loaded groups, there was a significant increase in albumin and bicarbonate when given the drug ($P < 0.05$; Table 3). However, no other statistically significant differences were observed (Table 3).

Histology. The light microscopic appearance of tissues from all groups of control animals were similar whether they were taken from the acute, sub-acute or chronic toxicity trial. Some of the control animals showed scattered small foci of necrosis in striated skeletal muscles (myositis; Table 4). Animals which received an Fe-enriched diet prior to the trial still displayed more stainable Fe in their liver and mucosa of small intestine than those on a normal diet; differences in the spleen were marginal.

Animals receiving a normal diet and the drug exhibited severe lesions which were either not seen or were much less severe in placebo/normal diet animals. The most obvious were foci of striated muscle necrosis, foci of necrosis with inflammatory infiltrates in their livers and myocardial necrosis (Table 4). Normal histology was seen in animals fed an Fe-enriched diet and the drug. Striated muscle necrosis was detected in some animals that were on the drug as well as some that were not. However, the necrosis was much more severe in those receiving the drug. Such effects may perhaps be

Table 4. Incidence of histological changes observed in guinea pigs after eight months oral administration of placebo or L_1 (50 mg kg^{-1} day^{-1}; six days per week)

	Normal		Fe-loaded	
	Placebo	Drug	Placebo	Drug
Myositis	4 (slight)	5 (severe)	2 (slight)	0
Hepatic necrosis	6 (slight)	3 (severe)	1 (slight)	0
Myocardial necrosis	0	1	0	0
Number examined (*n*)	7	9	5	3

Table 3. Chronic toxicity trial: Serum biochemistry and hematology data from guinea pigs after eight months oral administration of L_1 (50 mg kg^{-1} day^{-1}; six days per week)

	Normal diet		Fe-loaded	
	Placebo	Drug	Placebo	Drug
Sodium (mmol l^{-1})	138.4 ± 2.4	137.8 ± 3.2 (6)	133.0 ± 3.8	141.33 ± 1.2
Potassium (mmol l^{-1})	5.68 ± 0.51	6.00 ± 0.51 (6)	4.98 ± 0.64	7.90 ± 3.06
Creatinine (μmol l^{-1})	108.2 ± 7.8	105.3 ± 11.0 (6)	111.5 ± 12.4	213.67 ± 70.94
Gl (11 ^{1})	13 73 0 75 (3)	11 18 0 84 (6)	12 32 0 86	9 53 5 06

dietary if the drug-treated group became less interested in food (because of drug effects) than the untreated animals. However, there were no apparent changes in body weight (not shown).

Non-heme iron. Table 5 shows the non-heme Fe levels in various tissues. None of the differences in Fe levels between the drug-treated groups and the corresponding placebo-treated groups were statistically significant. However, the mean total liver non-heme Fe level in the drug-treated/Fe-loaded group was nearly 50% lower than the placebo-treated/Fe-loaded group. Splenic total Fe levels were lower in both test groups compared with the corresponding control groups. In all other tissues, the drug-treated and placebo-treated groups contained similar levels of Fe. There was no evidence of redistribution of Fe from storage sites to other tissues (e.g. brain).

Discussion

There is considerable pressure on clinicians to provide an inexpensive and orally effective chelator to replace DFO. However, the current and potential use of L_1 for chelation therapy in patients with iron overload disease is controversial. Short- and long-term efficacy and safety of L_1 have been described previously in several groups of thalassemic patients and patients with other Fe loading states (Bartlett *et al*, 1990, Kontoghiorghes *et al.* 1990, Olivieri *et al.* 1990a, Agarwal *et al.* 1992, Hershko 1994). Limited clinical trials in some centres have shown a significant increase in urinary Fe excretion (Kontoghiorghes *et al.* 1990, Olivieri *et al.* 1990a, b, Singh *et al.* 1992) or decrease in serum ferritin (Agarwal *et al.* 1992, Olivieri *et al.* 1992, Al-Refaie *et al.* 1995), a commonly used indicator of Fe status. However, there is evidence of toxicity after L_1 administration in humans (Bartlett *et al.* 1990, Agarwal *et al.* 1992, Al-Refaie *et al.* 1992, Porter *et al.* 1993, Al-Refaie *et al.* 1995, Kontoghiorghes 1995) and in mice, rats and rabbits (Kontoghiorghes 1986, Kontoghiorghes & Hoffbrand 1986, Porter *et al.* 1990, 1991, Kontoghiorghes *et al.* 1993a, b). Clearly, there is a need for further evaluation in animal species and a greater understanding of its mechanism of action before L_1 is applied more widely in clinical practice. As mentioned earlier, the guinea pig offers advantages over other animal species for this purpose, due to similarities to humans in its Fe metabolism and inability to synthesise vitamin C. Guinea pigs have been used previously in only one investigation on the pyridinones (Porter *et al.* 1993) comparing the efficacy of CP94 in the rat and guinea pig, which were markedly different in their responses.

The results of the acute trial seemed quite encouraging for several reasons. Firstly, there was the marked reduction in liver non-heme Fe in normal guinea pigs after oral administration of a single dose of L_1 of 200 mg kg^{-1} or more (Figure 1) to approximately 50% of the placebo-treated control. Secondly, the efficacy of L_1 was maximal at *c.* 200 mg kg^{-1} while there was no apparent change in appearance or behaviour of the animals up to 800 mg kg^{-1} and only slight changes in striated skeletal muscle histology at 600 mg kg^{-1}, with no detectable effect on other tissues. Obvious pathological change was not observed until a dose of 800 mg kg^{-1}. This suggested that there was a safety margin between the drug concentration producing maximal chelation efficiency, and that producing detectable toxicity, even in animals with normal Fe levels. It also suggested that repeated lower doses of the drug may be a more efficient and safer strategy than a single large dose per day.

Table 5. Total non-heme iron in tissues from guinea pigs after eight months oral administration of L_1 (50 mg kg^{-1} day^{-1}; six days per week) or placebo

A daily oral dose of 300 mg kg^{-1} was chosen for the sub-acute trial for the guinea pig. This dose is slightly higher than the maximum dose which has been given to patients in any one day (Kontoghiorghes *et al.* 1987, Olivieri *et al.* 1990a, b) but well below that which caused toxicity in the acute trial in the normal guinea pig. The drug was administered to normal animals and to guinea pigs in which Fe overload was induced with carbonyl Fe. Evaluation of a chelator in both normal and Fe-loaded animals is important in view of clinical evidence that the toxic side effects of DFO (Hershko 1994) are most apparent in patients with relatively little Fe overload or when given high doses of DFO relative to their Fe status. However, while deaths only occurred in the present study in the drug-treated/normal Fe guinea pigs in the first sub-acute trial, several of the drug-treated/Fe-loaded animals were ill, but recovered and there were deaths in each drug-treated group when the trial was repeated. In addition, data from the few animals sacrificed at this stage to monitor changes in liver non-heme Fe levels suggested that Fe removal from the normal diet guinea pigs treated with 300 mg kg^{-1} day^{-1} for a total of 12 days, was no greater (*c.* 50% control) than observed in the acute trial with a single dose of 200 mg kg^{-1}.

The presence of myositis could explain the musculo-skeletal pain experienced by up to 30% of patients taking L_1 in some trials (Kontoghiorghes 1995). It is of some concern that similar pathology (e.g. myositis) was seen after prolonged exposure to a low dose (chronic trial) as after a single large dose (acute trial), without a significant drop in liver Fe. However, several aspects of this toxicity assessment were encouraging in that severe pathology and significant changes in the numbers of platelets and white blood cells were only seen in the drug-treated animals with normal Fe stores. There were few changes apparent in serum biochemistry and other hematological parameters in any treatment group which included additional Fe in the diet. This suggests that even a small excess of Fe is protective

auditory function are not so clear cut. The data comparing retinal function revealed two significant differences. The longer *a* wave latency observed in the drug group with normal Fe status implies that photoreceptor processing is slowed. This difference was not significant in the drug-treated/Fe-loaded group which might again be taken as evidence that Fe loading had a protective effect against the drug. However, there was a significant reduction in the *b* wave amplitude in the drug-treated/Fe-loaded group. The electroretinogram potentials are notoriously labile, which is why more importance is always placed on latencies rather than on amplitudes. Obviously, more experiments are required to further characterise these differences and also changes in auditory function.

The deaths observed in the sub-acute trial at a relatively high dose of L_1 in both normal and Fe-loaded guinea pigs, as well as the toxic side effects observed after administration of repeated low doses of L_1 to guinea pigs with normal Fe loading (in the chronic trial), suggest that, as with DFO, each patient must be monitored individually and regularly with regard to the relationship between dose of L_1, frequency of dose and the patient's liver Fe status. In addition, the apparent drug-induced changes in the retinograms of both normal and Fe-treated animals indicate that frequent opthalmological assessment should be made.

Acknowledgements

These studies were supported by Prince of Wales Children's Hospital Thalassemia Research Fund and National Health & Medical Research Council of Australia research project grants 920282 to E. Baker. Professor E.H. Morgan and Dr. D. Richardson are thanked for their constructive comments on the manuscript.

References

Al-Refaie FN, Wonke B, Hoffbrand AV, *et al*. 1992 Efficacy and possible adverse effects of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1) in thalassemia major. *Blood* **80**, 593–599.

Al-Refaie FN, Hershko C, Hoffbrand AV, *et al*. 1995 Results of long-term deferiprone (L_1) therapy: a report by the International Study Group on oral iron chelators. *Br J Haematol* **91**, 224–229.

Baker E. 1988 Biological screens for iron chelators. In: *Thalassemia: Pathophysiology and Management*. New York Alan R. Liss Inc. March of Dimes Birth Defects Foundation, Original Article Series; **23(5b)**: 49–62.

Baker E, Richardson DR, Gross S, *et al*. 1987 Evaluation of new iron chelators in the hepatocyte in culture. *Eighth International Conference on Proteins of Iron Transport and Storage*, 143 Montreal, P. Ponkan, McGill University.

Bartlett AN, Hoffbrand AV, Kontoghiorghes GJ. 1990 Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1) II: Clinical observations. *Brit J Hematol* **76**, 301–304.

Ben Nun J, Alder VA, Cringle SJ, Constable, IJC. 1988 A new method for oxygen supply to acute ischemic retina. *Invest Ophthalmol & Vis Sci* **29**, 298–304.

Blake DR, Winyard P, Lunec J, *et al*. 1985 Cerebral and ocular toxicity induced by desferrioxamine. *Quart J Med* **56**, 345–355.

Bothwell TH, Charlton RW, Cook JD, Finch CA. 1979. *Iron Metabolism in Man.* Oxford: Blackwell Scientific Publications.

Chan BE, Roeser HP, Nikles AC. 1989 Cytosolic ferritin and lipid-associated ferritin are metabolically different in guinea-pig livers. *Biochem J* **263**, 989–992.

Chan BE, Roeser HP, Nikles C. 1990 Ascorbin acid deficiency affects the metabolism of cytosolic ferritin but not lipid-associated ferritin in livers of guinea pigs. *Biochem J* **267**, 535–537.

de Virgillis S, Congia M, Frau F, *et al*. 1988 Deferoxamine-induced growth retardation in patients with thalassemia major. *J Pediatr* **113**, 661–669.

Hershko C. 1993 Development of oral iron chelator L_1. *Lancet* **341**, 1088–1089.

Hershko C. 1994 Iron chelators. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. *Iron Metabolism in Health and Disease*. 391–426 London, W.B. Saunders Co. Ltd.

ketohydroxypyridine iron chelators intended for clinical use: *in vivo* studies in rabbits. *Br J Hematol* **62**, 607–613.

Kontoghiorghes GJ, Sheppard L. 1987 Simple synthesis of the potent iron chelators 1-alkyl-3-hydroxy-2-methylpyrid-4-ones. *Inorganica Chimica Acta* **136**, L11–L12.

Kontoghiorghes GH, Aldouri MA, Hoffbrand AV, *et al*. 1987 Effective chelation of iron in beta-thalassaemia with the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1). *Br Med J Clin Res* **295**, 1509–1512.

Kontoghiorghes GJ, Bartlett AN, Hoffbrand AV, *et al*. 1990 Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1): Iron chelation and metabolic studies. *Brit J Hematol* **76**, 295–300.

Kontoghiorghes GJ, Barr J, Nortey P, Sheppard L. 1993a Selection of a new generation of orally active a-keto-hydroxypyridine iron chelators intended for use in the treatment of iron overload. *Am J Hematol* **42**, 340–349.

Kontoghiorghes GJ, Agarwal MB, Kersten MJ, *et al*. 1993b Future of oral iron chelator deferiprone (L_1). *Lancet* **341**, 1479–1480.

Lipschitz DA, Bothwell TH, Seftel HC, *et al*. 1991 The role of ascorbic acid in the metabolism of storage iron. *Br J Hematol* **20**, 155–163.

Modell B, Berdoukas V. 1984 *The Clinical Approach to Thalassemia.* New York: Grune & Stratton, Inc.

Olivieri NF, Koren G, Hermann C, *et al*. 1990a Comparison of oral iron chelator-L_1 and desferrioxamine in iron-loaded patients. *Lancet* **336**, 1275–1279.

Olivieri NF, Templeton DM, Koren G, *et al*. 1990b Evaluation of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1) in iron-loaded patients. *Ann NY Acad Sci USA* **612**, 369–377.

Olivieri NF, Koren G, Matsui D, *et al*. 1992 Reduction of tissue iron stores and normalization of serum ferritin during treatment with the oral iron chelator L_1 in thalassemia-intermedia. *Blood* **79**, 2741–2748.

Park CH, Bacon BR, Brittenham EM, Tavill AS. 1987 Pathology of dietary carbonyl iron overload in rats. *Lab Invest* **57**, 555–563.

Pippard MJ, Jackson MJ, Hoffman K, *et al*. 1986 Iron chelation using subcutaneous infusions of diethylene triamine penta-acetic acid (DTPA). *Scand J Hematol* **36**, 466–472.

differing metabolism of the iron chelating site. *Br J Hematol* **85**, 159–168.

Robertson D, Wilson SA. 1991 Changes in cochlear sensitivity do not alter relative thresholds of different spontaneous rate categories of primary auditory nerve fibres. *Hearing Res* **51**, 29–32.

Singh S, Epemolu RO, Dobbin PS, *et al*. 1992 Urinary metabolic profiles in human and rat of 1,2-dimethyl-substituted and 1,2-diethyl-substituted 3-hydroxypyridin-4-ones. *Drug Metabolism and Disposition* **20**, 256–261.

Torrance JD, Bothwell TH. 1968 A simple technique for measuring storage iron concentrations in formalised liver samples. *S Afr J Med Sci* **33**, 9–11.

Ward C, Saltman P, Ripley L, *et al*. 1977 Correlation of serum ferritin and liver ferritin iron in the anemic, normal and iron-loaded rat. *Am J Clin Nutr* **30**,