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SHORT COMMUNICATION

Effects of Desferrithiocin and Its Derivatives on Peripheral Iron and Striatal Dopamine and 5-Hydroxytryptamine Metabolism in the Ferrocene-Loaded Rat

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ABSTRACT. Iron overload disorders, such as β-thalassaemia, are currently treated with the iron chelator desferrioxamine (DFO) or 1,2-dimethyl-3-hydroxypyridin-4-one (L1), which is currently under clinical evaluation. However, DFO is inactive orally and needs to be administered by intramuscular infusion, whilst there are concerns over the long-term effectiveness and toxicity of L1. In addition, both DFO and L1 affect brain dopamine (DA) and 5-hydroxytryptamine (5-HT) metabolism. In this study, the 3,5,5-trimethylhexanoyl ferrocene rat model of iron overload was used to compare the iron-chelating capabilities of a novel orally active siderophore, desferrithiocin (DFT) and its desmethyl derivatives DFT-D and DFT-L, to that of DFO, along with their ability to affect brain DA and 5-HT metabolism. Chronic administration of ferrocene produced a 12-fold increase in liver iron levels, as assessed by electrothermal atomic absorption. Subsequent treatment with DFT over a two-week period produced a 37% reduction in liver iron levels, whereas similar treatment with DFT-D and DFT-L produced a more marked reduction in these levels (65% and 59%, respectively) in the ferrocene-treated animals. In contrast, using the same dosing regimen, DFO and L1 only produced a 16% and 18% reduction, respectively, in liver iron levels. Both DFT and its derivatives failed to affect either striatal DA or 5-HT metabolism when assessed by HPLC. In view of the previously described oral bioavailability of DFT, the marked ability of DFT and its derivatives to chelate hepatic iron, and their inability to affect brain DA or 5-HT metabolism, such siderophores appear potentially useful clinical iron chelators. BIOCHEM PHARMACOL **58**;1: 151–155, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. iron chelation; desferrithiocin; desferrioxamine; hydroxy pyridones; dopamine; 5-hydroxytryptamine; iron; brain

In haemochromatosis, the accumulation of iron occurs over a relatively long time period such that any toxicity, such as iron-induced diabetes, does not occur until middle age. In contrast, in thalassaemic patients large and repeated influxes of iron via blood transfusion over short periods of time to combat the anaemia can rapidly induce potentially lethal alterations in liver function [1, 2]. Removal of excessive amounts of iron is achieved by the administration of the iron-chelating drug DFO.‖ DFO selectively chelates Fe(III) with a binding affinity for iron higher than that of transferrin [3]. However, DFO lacks effectiveness when administered orally and needs to be administered by intramuscular infusion.

Consequently, there is a need for an orally active iron chelator which is similar or superior to DFO in its iron-chelating efficiency. To date, bidentate hydroxy pyridones such as L1 [4, 5] have shown the greatest promise due to their good oral availability and iron chelation activity and are in limited clinical use for the treatment of β-thalassaemia. However, there are still major concerns over whether such compounds will elicit long-term toxicity when administered for long periods of time. In addition, both DFO and hydroxy pyridones cross the blood–brain barrier and affect both brain dopamine and 5-HT metabolism [6, 7]. Such effects may be brought about by the removal of iron from key synthetic enzymes such as tyrosine hydroxylase and tryptophan hydroxylase, where iron acts as an essential cofactor. Alternatively, in the case of the hydroxy pyridones their catechol structure may coordinate in the iron centre of the enzyme, preventing the reduction

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‖ *Abbreviations:* HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; DFO, desferrioxamine B; DFT, desferriothiocin; 5-HT, 5-hydroxytryptamine; L1, 1,2-dimethyl-3-hydroxypyridin-4-one.

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of the iron to its active form (Fe^{2+}) by the tetrahydropterine cofactor. Indeed, the doses of used clinically (50–100 mg/kg/day) [8] produce inhibition of tyrosine and tryptophan hydroxylase in the rat [6]. The effects of such alterations in dopamine and 5-HT metabolism on brain function and behaviour remain to be determined. However, 70–80% inhibition of tyrosine hydroxylase by α-methyl-para-tyrosine causes dystonic reactions [9], anxiety [10], and panic attacks [10] in humans.

Tridentate iron chelators have also shown excellent permeation of the gut mucosa as well as iron chelation capabilities [11]. However, early studies of the tridentate compound DFT showed renal toxicity at high doses, such that other derivatives have been synthesised and form the basis of the present study. Here, we have examined the properties of DFT and its desmethyl derivatives DFT-D and DFT-L. *In vitro* and *in vivo*, DFT is more effective at mobilising hepatic iron than DFO [12, 13], but only 50% of the oral dose is absorbed [12]. In contrast, DFT-D and DFT-L exhibit increased bioavailability and iron mobilisation but decreased toxicity. In our earlier studies, we showed the excellent iron chelator efficacy of DFT after oral administration [13]. We now compare the iron chelation capabilities of DFT, DFT-D, and DFT-L with those of DFO in the ferrocene-loaded rat model [14, 15]. Each of the compounds has been administered intraperitoneally such that it is possible to directly compare similar concentrations. In addition, the effects of DFT and its derivatives on striatal dopamine and 5-HT metabolism have been examined.

MATERIALS AND METHODS

Male Wistar rats (initial weight 75–100 g) were placed on either a control iron-free diet or a diet supplemented with 3,5,5-trimethylhexanoyl ferrocene (0.1 g/kg prepared in an iron-free diet) for 4 weeks, after which all animals were transferred onto an iron-free diet. For the iron chelation studies, DFO, DFT, or its derivatives DFT-D and DFT-L were administered at 30 mg/kg intraperitoneally three times per week for 2 weeks to the ferrocene-loaded rats. Control animals received injections of the drug vehicle, ethanol: water (1:9 v/v) at a pH of 7.

Prior to killing by cervical dislocation, the rats were anaesthetised with pentobarbital (50 mg/kg, i.p.), and blood was removed by cardiac puncture for the estimation of haematological parameters. The brain was rapidly removed from the cranium and placed on a chilled (0°) platform, and the striatum dissected and snap frozen in liquid nitrogen. Dopamine and its metabolites, HVA and DOPAC, and 5-HT and its metabolite 5-hydroxyindole-3-acetic acid were assayed by HPLC with electrochemical detection by a modification of the method of Rose *et al.* [16]. The striata were weighed and homogenised in 10 vol. (w/v) 0.4 M perchloric acid containing 1 mM EDTA and 0.5% sodium metabisulphite by a Microson tissue disrupter. The resulting homogenate was added to a solution of the internal standard dihydroxybenzylamine (DHBA) in a 9:1 (v/v) to give a final concentration of DHBA of 100 ng/mL. The homogenate was centrifuged at 10,000 *g* for 10 min at 4°. An aliquot of the supernatant was injected onto a Spherisorb ODS-2 reverse-phase column and the chromatographic peaks detected by a BAS LC-4B amperometric detector with a thin-layer electrochemical cell fitted with a glassy carbon working electrode and Ag/AgCl reference electrode. Iron determinations were carried out on the same samples.

The liver, spleen, and heart were removed and snap frozen in liquid nitrogen for the subsequent estimation of iron content by electrothermal atomic absorption spectroscopy (EASS). For iron determination, tissues were homogenised in water, 10% (w/v), using a glass/glass homogeniser, and further diluted with water to the standard reference range for iron of 0.1–0.4 μg Fe/mL prior to analysis by EASS.

The results are presented as means and standard deviations for N = 6 per group. Significance was assessed by the Student's *t*-test.

RESULTS

Administration of ferrocene for 4 weeks or subsequent treatment with the iron chelators DFO, DFT, or its derivatives for 2 weeks had no effect on the body weight of the animals or on the weight of the liver and heart used for iron analysis (Table 1). Transferrin saturation, total iron-bind-

TABLE 1. Animal and tissue weights + haematological parameters in rats supplemented with the ferrocene derivative followed by chelation

	Animal and tissue weights (g)			Haematological parameters			
Treatment	Animal	Liver	Heart	Transferrin saturation (%)	Total iron-bindingcapacity (ug/dL)	Serum iron (ug/dL)	Haemoglobin (g/dL)
Control	324 ± 13	15.9 ± 2.2	0.7 ± 0.2	27.5 ± 3	731 ± 44	215 ± 21	15.1 ± 0.3
Ferrocene	329 ± 16	17.4 ± 1.1	0.7 ± 0.1	28.5 ± 0.5	685 ± 56	198 ± 22	14.3 ± 0.4
Ferrocene + DFT	315 ± 12	15.0 ± 1.4	0.6 ± 0.1	30.0 ± 2.4	718 ± 38	214 ± 11	13.5 ± 0.4
Ferrocene + DFT-D	306 ± 23	16.0 ± 1.5	0.8 ± 0.2	24.0 ± 1.1	700 ± 61	168 ± 25	13.8 ± 0.4
Ferrocene + DFT-L	315 ± 23	14.3 ± 2.3	0.8 ± 0.1	26.5 ± 3.0	701 ± 48	174 ± 26	13.9 ± 0.4
Ferrocene + DFO	333 ± 13	14.1 ± 1.4	0.8 ± 0.1	30.5 ± 1.8	758 ± 14	226 ± 32	13.7 ± 0.7

The rats were supplemented with the ferrocene derivative 3,5,5-trimethyl hexanoyl for 4 weeks followed by chelation with DFT, DFT-D, DFT-L, and DFO, 30 mg/kg i.p. for 2 weeks. The results are expressed as means ± standard deviation, N = 6.

TABLE 2. Iron content of rat tissues after ferrocene supplementation followed by chelation

Treatment	Total iron ug/g tissue		
	Liver	Spleen	Heart
Control	213 ± 18	180 ± 16	48 ± 4
Ferrocene	2557 ± 32	368 ± 26	61 ± 4
Ferrocene + DFT	1620 ± 19*	396 ± 50	66 ± 3
Ferrocene + DFT-D	883 ± 27*	387 ± 36	62 ± 2
Ferrocene + DFT-L	1057 ± 34*	408 ± 78	54 ± 3*

The rats were supplemented with the ferrocene derivative 3,5,5-trimethyl hexanoyl for 4 weeks followed by chelation with DFT, DFT-D, DFT-L, and DFO, 30 mg/kg i.p. for 2 weeks. The results are expressed as means ± standard deviation, N = 6.

*$P < 0.05$ compared to rats treated with the ferrocene derivative alone.

ing capacity, serum iron levels, and haemoglobin levels remained unchanged with iron loading using ferrocene or subsequent iron chelation therapy using DFO, DFT, or its derivatives (Table 1).

Dietary treatment with ferrocene resulted in marked increases (12-fold) in liver iron levels when compared to animals fed an iron-free diet (2557 ± 32 μg/g tissue, compared to the control values of 213 ± 18 μg/g tissue); smaller increases in iron levels occurred in the spleen and heart (2-fold and 1.3-fold, respectively) of ferrocene-treated animals compared to animals fed the iron-free diet (Table 2). The subsequent administration of DFT or its derivatives DFT-D and DFT-L to the ferrocene-treated animals for 2 weeks resulted in a significant reduction in liver iron content when compared to the ferrocene animals treated with the drug vehicle alone (Table 2). Hepatic iron removal in ferrocene-treated animals was more marked with DFT-D and DFT-L (65% and 59%, respectively) compared to DFT itself (37%). Administration of DFT, DFT-D, or DFT-L failed to reduce the increase in spleen iron levels induced by ferrocene treatment, and only DFT-L produced a small but significant reduction (11%) in heart iron levels in ferrocene-loaded rats (Table 2). In contrast, administration of DFO produced only a 16% reduction in liver iron levels in the ferrocene-treated rat (Table 3).

Ferrocene treatment had no effect on striatal concentrations of dopamine, its metabolites DOPAC and HVA, or dopamine turnover when compared to control animals. Similarly, concentrations of 5-HT and its metabolite 5-hydroxyindole-3-acetic acid and 5-HT turnover were unaf-

TABLE 3. Iron content of rat tissues after ferrocene supplementation followed by chelation

Treatment	Total iron ug/g tissue		
	Liver	Spleen	Heart
Control	189 ± 22	242 ± 21	27 ± 7
Ferrocene	2838 ± 6.25	550 ± 112	145 ± 9
Ferrocene + DFO	2405 ± 187*	487 ± 38	166 ± 8

The rats were supplemented with the ferrocene derivative 3,5,5-trimethyl hexanoyl for 4 weeks followed by chelation with DFT, DFT-D, DFT-L, and DFO for 2 weeks. The results are expressed as means ± standard deviation, N = 6.

*$P < 0.05$ compared to rats.

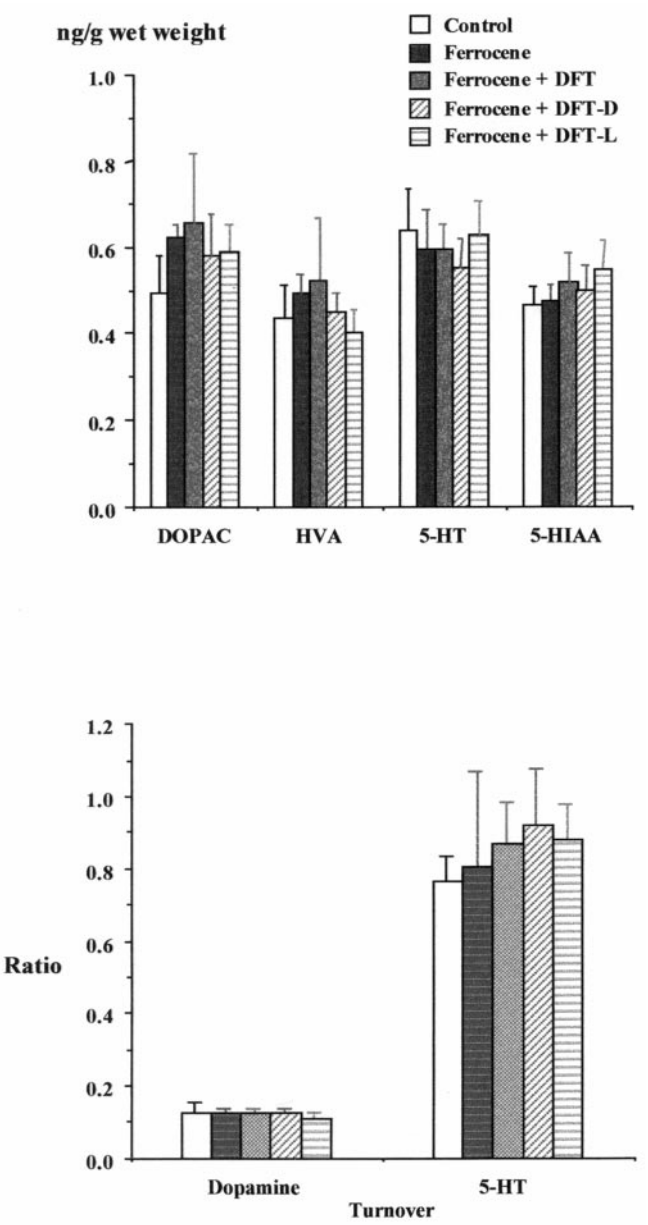

FIG. 1. Levels of dopamine, serotonin, and their metabolites. (A) The striatal levels of dopamine, serotonin, and their metabolites are expressed in terms of the ratio

$$\frac{\text{HVA + DOPAC}}{\text{DOPAMINE}} = \text{dopamine turnover}$$

$$\frac{\text{5-HIAA}}{\text{5HT}} = \text{serotonin turnover}$$

(B) The rats were supplemented with ferrocene derivative for 4 weeks and then received 30 mg/kg i.p. of either DFT or its derivative DFT-D or DFT-L, for 2 weeks. The results are expressed as means ± standard deviation, N = 6.

fected by ferrocene treatment (Fig. 1). Subsequent treatment of the ferrocene-treated animals with either DFT or its derivatives DFT-D or DFT-L did not affect striatal concentrations of dopamine, 5-HT and their metabolites,

or turnover (Fig. 1). Previous studies [7] demonstrated that DFO had an effect on dopamine metabolism.

DISCUSSION

Dietary treatment with ferrocene resulted in a marked increase in iron loading in the liver compared to iron loading in the spleen and heart, as previously reported [13]. The periodic administration of DFT over a two-week span resulted in a marked reduction in hepatic iron loading, but had no affect on iron levels in the heart or spleen. The decreased iron load in the liver produced with DFT was greater than that produced by similar treatment with DFO. It was also greater than that previously reported for the hydroxy pyridone chelators L1 and CP94 [17]. However, the DFT derivatives DFT-D and DFT-L both had a greater effect in reducing the liver iron load than DFT. This may relate to an increased bioavailability of the two DFT derivatives after administration and/or a higher iron-binding potential. In addition, DFT-L caused a significant reduction in the iron loading of the heart, one of the major sites of toxicity in iron overload disorders.

Despite the high iron chelation capabilities of DFT and its derivatives, they had no effect on either dopamine or 5-HT levels or turnover in brain. Previous studies showed that DFO and the hydroxy pyridone chelators (L1, CP94, and CP 24) decreased striatal levels of dopamine, 5-HT, and their metabolites and turnover of dopamine and 5-HT [6, 7] as a result of inhibition of the rate-limiting enzymes tyrosine and tryptophan hydroxylase [6]. Such alterations in enzyme activity may relate to the removal of iron acting as an essential cofactor [18, 19]. Indeed, the activity of tyrosine hydroxylase *in vitro* and *in vivo* is markedly inhibited by the presence of iron chelators such as bipyridyl [20]. In addition, the ability of the hydroxy pyridone compounds to inhibit tyrosine and tryptophan hydroxylase activity may relate to their catechol structure [6]. Indeed, both enzymes are inhibited by catechol derivatives [21, 22], and this may be due to catechol coordination in the iron centre [23, 24]. There are two sites on the iron coordination sphere of such enzymes which are accessible to catechols. They appear to bind to the (inactive) Fe (III) form of the enzyme, preventing the reduction to the (active) Fe (II) form by the tetrahydropterine cofactor by stabilising the Fe (III) form or impeding the access of the cofactor to the iron centre.

DFT, like DFO, crosses cell membranes by diffusion and is more rapidly taken up than DFO in isolated hepatocytes [25]. Such differences may relate to DFT's smaller molecular size and higher lipophilicity when compared to DFO. Consequently, like DFO, DFT may penetrate the blood–brain barrier but if so, the lack of effect of DFT and its derivatives on dopamine and 5-HT metabolism may be explained by their ability to chelate iron from different cellular iron compartments. Indeed, in hepatocyte cultures the subcellular distribution of DFO and DFT are different. DFT is predominantly present in the cytosol and, to a lesser extent, in mitochondria, whereas DFO is evenly distributed between lysosomes and the cytosol [24]. However, such studies have not been conducted in the brain. Further studies are required to determine the cellular and subcellular distribution of DFT.

From these studies, we can confirm that DFT, previously shown to be orally active [13], is an effective iron chelator at a dose which is non-toxic [13]. In addition, the two desmethyl derivatives of DFT, DFT-D and DFT-L, were shown to be more effective at reducing iron overloading in the liver. DFT and its derivatives also had no effect on striatal dopamine or 5-HT metabolism, unlike those chelators in clinical use. In view of the high iron-chelating efficacy of DFT and its derivatives and their lack of effect on brain function, it would seem reasonable to pursue studies on DFT which, by virtue of its potential for oral administration compared to DFO, seems to represent a considerable advance in the development of iron chelators for the treatment of iron overload disorders such as β-thalassaemia. It is interesting to note that recent developments in the pharmaceutical industry have selected a tridentate ligand for further development, with the goal of marketing an orally active iron chelator.

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