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METABOLISM OF IRON FROM (3,5,5-TRIMETHYLHEXANOYL)FERROCENE IN RATS

A DIETARY MODEL FOR SEVERE IRON OVERLOAD

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Abstract—The feeding of diets enriched with (3,5,5-trimethylhexanoyl)ferrocene (TMH-ferrocene) has been shown recently to produce a severe experimental iron overload in rats and has been considered as an adequate animal model for hereditary haemochromatosis in humans. We synthesized three ^{59}Fe-labelled ferrocene compounds with different lipophilic characters {ferrocene, TMH-ferrocene, and 1,1'-bis(3,5,5-trimethylhexanoyl)ferrocene [$(TMH)_2$-ferrocene]} and studied the metabolism of iron from these compounds in comparison with the hydrophilic ferrous sulphate in rats with iron deficiency, and normal and increased iron stores. The bioavailability of iron from TMH-ferrocene (whole body retention, 48% from a 5 mg Fe dose) was twice as high as from ferrocene and six times higher than from $(TMH)_2$-ferrocene and ferrous sulphate. In contrast to the well-known iron salts (ferrous sulphate), the intestinal absorption of TMH-ferrocene iron was independent from the dose (1 or 5 mg Fe) and similar in iron-deficient and iron-loaded rats, indicating that the intestinal absorption of the TMH-ferrocene is not regulated by the body iron stores. After intestinal absorption, TMH-ferrocene iron in the portal blood is transported to the liver independently from transferrin. In contrast to absorbed ferrocene, iron from TMH-ferrocene is almost completely released from the hydrocarbon moiety within the liver. Depending on the body iron stores, TMH-ferrocene iron is then incorporated preferentially into haemoglobin (iron-deficient rats) or added to the iron stores in the liver (iron-loaded rats). A transient storage of the ^{59}Fe-label in fat tissue was observed only from oral ferrocene but not from TMH-ferrocene. Due to the outstandingly high bioavailability of TMH-ferrocene, the chronic feeding of this compound resulted in a fast and progressive iron overload in rats (liver iron: 16.9 mg Fe/g wet weight after 10 weeks of feeding a diet containing 0.5% TMH-ferrocene), and can be regarded as the best characterized and most useful animal model for severe hepatocellular iron overload in humans.

Ferrocenes (dicyclopentadienyliron) are a group of unusual iron-based organometallic compounds [1, 2]. Due to the limited intestinal absorption of ionic iron in humans, these lipophilic iron compounds became of theoretical interest as potential new haematinics in the therapy of iron deficiency anaemia. Following oral administration, some ferrocene derivatives were found to be well absorbed and then metabolized, leaving their iron available to be incorporated into haemoglobin [3–5].

It was shown in some of these former studies, that the feeding of some ferrocene compounds to rats, mice and dogs resulted in a distinct increase in the liver iron concentration [6, 7]. Therefore, the chronic administration of ferrocene compounds offered a new way to induce an experimental iron overload in animals. The feeding of a diet enriched with (3,5,5-trimethylhexanoyl)ferrocene (TMH-ferrocene†) for up to 6 weeks in rats has recently been suggested as a new animal model for hereditary haemochromatosis [8, 9]. This inborn error of iron metabolism in humans leads to a severe and progressive iron overload mainly of the liver [10]. In the past, the research on this disease has been hampered by the fact that no suitable animal model is known to date which mimics the typical organ damages found in human patients (liver cirrhosis, diabetes, cardiomyopathy). We have shown recently in rats that the chronic feeding of TMH-ferrocene for up to 8 months resulted in a very severe liver siderosis combined with a perisinusoidal as well as portal liver fibrosis [11]. At the commencement of iron storage, similar to hereditary haemochromatosis in humans, stainable iron was almost exclusively stored in the hepatocytes of iron-loaded rats. Although liver cirrhosis, the final stage of impairment in human liver iron overload, can so far not be induced in rats, the TMH-ferrocene model seems to be the most encouraging animal model to study various aspects of chronic iron overload diseases (e.g. mechanism of iron-induced lipid peroxidation and fibrogenesis, mode of action and efficacy of new oral iron chelators). The aim of the present investigation, using three ^{59}Fe-labelled ferrocene compounds with different lipophilicities, was to study in detail the metabolism of iron from TMH-ferrocene in rats with normal, decreased and increased iron stores.

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† Abbreviations: TMH-ferrocene, (3,5,5-trimethylhexanoyl)ferrocene; $(TMH)_2$-ferrocene, 1,1'-bis(3,5,5-trimethylhexanoyl)ferrocene.

MATERIALS AND METHODS

3,5,5-Trimethyl-1-hexanal was obtained from the

Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). All other chemicals of analytical grade were from E. Merck (Darmstadt, Germany). 3,5,5-Trimethylhexanoyl chloride was prepared from the respective aldehyde as described [12]. $^{59}FeCl_3$, sp. act. 12 mCi/mg, was from Amersham Buchler (Braunschweig, Germany). TLC was performed using HPTLC aluminium sheets silicagel 60 (E. Merck) and toluene/ethyl acetate (45:5 v/v) as mobile phase. Reverse-phase HPLC was performed using a Lichrosorb-7-phenyl-column (E. Merck) with methanol/water (80:20, v/v) as eluant.

The mass spectra of ferrocene compounds were performed using a double focussing instrument Type VG ZAB1-F (Vacuum Generators, Wythenshawe, U.K.) equipped with a saddle field atom gun (Iontech, Middlesex, U.K.) operated with xenon as reagent gas (Linde, Unterschleissheim, Germany). ^{1}H-NMR spectroscopy was performed using a WP 80 NMR-Spectrometer from Bruker with $CDCl_3$ and tetramethylsilan as internal standard. Elemental analyses were performed by the "Mikroanalytisches laboratorium Beller" (Göttingen, Germany).

TMH-ferrocene. (Modified procedure according to a general description [13].) To a reaction mixture of 250 g (1.34 mol) ferrocene and 141 g (1.06 mol) aluminium chloride in 1.4 L methylenechloride were added drop-wise 153 g (0.87 mol) 3,5,5-trimethylhexanoyl chloride under stirring and ice-water cooling. The reaction mixture was allowed to warm up and was poured onto 300 g ice. The organic phase was separated and concentrated by evaporation under reduced pressure. The oily residue was dissolved in 50 mL of toluene and put onto a column of silicagel (9 × 73 cm). The column was developed with toluene and the eluate was analysed by TLC. Fractions containing TMH-ferrocene ($R_f = 0.53$) were collected and toluene was removed by evaporation under reduced pressure. The oily residue was dissolved in ethanol, filtered and lyophilized, resulting in a red-brownish solid.

Yield: 211 g (74.7%); m.p. 43–45° Anal. ($C_{19}H_{26}OFe$, M_r 326.26) calc.: Fe, 17.12; C, 69.95; H, 8.03. Found: Fe, 17.03; C, 69.29; H, 8.04. Mass spectrum m/e 326 (M^+). NMR ($CDCl_3$) δ 0.97–1.28, 2.16–2.69 (m, 16 H, sidechain H), 4.45–4.79 (s, 5H, aromat. H), 4.19 (m, 4H, aromat. H).

[^{59}Fe]*TMH-ferrocene.* [^{59}Fe]TMH-ferrocene was prepared from 0.94 g (5.0 mmol) [^{59}Fe]ferrocene, 0.7 g (5.2 mmol) aluminium chloride and 1.2 mL 3,5,5-trimethylhexanoyl chloride in 65 mL methylene chloride as described above. After chromatography on silicagel (column, 3.3 × 80 cm; eluant, toluene) 1.02 g (50.8%) of a red oil was obtained. Specific activity 5.83 μCi/mg Fe, radiochemical purity >99% as judged by TLC or >98% as judged by HPLC.

1,1'-bis(3,5,5-trimethylhexanoyl)ferrocene [*$(TMH)_2$-ferrocene*] *and* [^{59}Fe]*$(TMH)_2$-ferrocene.* These compounds were prepared as described for TMH-ferrocene but using a ratio ferrocene/$AlCl_3$/3,5,5-trimethylhexanoyl chloride of 1:2:2. Toluene/ethyl acetate (45:5, v/v) was used as eluant for the chromatography.

Unlabelled $(TMH)_2$-ferrocene; yield, 40.8 g (72.9%) red-brownish oil. Anal. calc. for $C_{28}H_{42}O_2Fe$, M_r 466.49: Fe, 11.97; C, 72.09; H, 9.07. Found: Fe, 10.60; C, 70.56; H, 9.35. Mass spectrum m/e 466 (M^+). NMR ($CDCl_3$) δ 0.94–1.29, 2.16–2.62 (m, 32H, sidechain H), 4.45–4.78 (s, 8H, aromat. H).

[^{59}Fe]$(TMH)_2$-ferrocene: sp. act., 4.3 μCi/mg Fe; radiochemical purity >96%.

[^{59}Fe]*ferrocene.* To a suspension of 343 mg iron powder (6.1 mmol; SCM Glidden A-131) in 6 mL of 0.1 M hydrochloric acid was added a solution of $^{59}FeCl_3$ (74.0 MBq) in 0.1 M HCl under stirring and N_2 atmosphere. The blue-green solution was lyophilized and the residue was dissolved in 14 mL of water-free dimethyl sulphoxide. The iron(II) solution was added drop-wise to a suspension of 2.5 g potassium hydroxide in 25 mL of dry dimethoxyethane and 4 mL of freshly distilled cyclopentadiene (48 μmol) under nitrogen. After stirring at room temperature for 60 min, the reaction mixture was poured onto a mixture of 40 g ice, 20 mL water and 3 mL concentrated hydrochloric acid. The yellow substance formed was filtered on a glass disc by suction and dried over P_2O_5. Yield: 0.95 g (82.9%) [^{59}Fe]ferrocene.

Animals, diets and experimental groups. Female Wistar rats (260–280 g) were obtained from Wiga (Hannover, Germany). Rats were housed in polyethylene cages with stainless steel wire tops or in metabolic cages (polyethylene cage with stainless steel grid floors) to collect urine and faeces separately. Iron deficiency was induced by feeding an iron-restricted diet (Altromin C1038, 6.3 μg Fe/g, Altromin, Lage, Germany) to growing rats for 5–6 weeks. Iron deficiency was evaluated by a low haemoglobin concentration (<11 g/dL) and the absence of stainable bone marrow iron [14]. Groups of rats received the iron-restricted diet in pellet form or the same diet supplemented with different ferrocene compounds and dosages (see Tables 2 and 3). Tap water was given *ad lib.* At the end of the feeding period, after an overnight fast, rats were killed by exsanguination from the abdominal artery while under light ether anaesthesia. The organs were quickly excised and weighed. Samples of the liver, spleen and heart were used for the evaluation of tissue iron concentration using a wet ashing technique as described [11].

Written consent was obtained from the local animal research council for the care and use of laboratory animals in all animal experiments.

Iron absorption test. Iron absorption tests were performed in fasted rats with normal, decreased or increased iron stores. Amounts of ^{59}Fe-labelled compounds, equivalent to 1 or 5 mg Fe (0.5–5 μCi ^{59}Fe), were dissolved in 0.5 mL of water (ferrous sulphate) and in 0.5 mL of miglyol 812 (ferrocenes), respectively. All test compounds were administered through a gastric tube. Thereafter, the intubation tools were rinsed with water or miglyol up to a total volume of 1.0 mL ingested. Rats were fasted for 24 hr prior to and 6 hr after oral application and afterwards housed in metabolic cages, to collect urine and faeces separately, for 7 days. The activity, measured immediately after administration of ^{59}Fe, was taken as the 100% reference value. The ^{59}Fe whole body retention was measured 7–10 days after oral administration of the iron compound in the

Table 1. Whole body retention and faecal and urinary excretion of ^{59}Fe, and intestinal ^{59}Fe absorption 7–10 days after oral administration of [^{59}Fe]ferrocene, [^{59}Fe]TMH-ferrocene, [^{59}Fe](TMH)$_2$-ferrocene and [^{59}Fe]iron(II) sulphate (5 mg Fe) in fasted rats with normal iron stores

Compound	N	Whole body retention	Urine excretion	Absorption	Faecal excretion	Recovery
Ferrocene	6	27.4 ± 3.0	61.3 ± 2.7	88.7 ± 10.2	10.2 ± 2.3	98.7
(TMH)$_2$-ferrocene	10	6.33 ± 1.51	0.31 ± 0.20	6.58 ± 1.61	90.3 ± 3.1	96.9
TMH-ferrocene	10	47.8 ± 9.7	2.51 ± 0.92	50.9 ± 10.4	46.9 ± 8.7	97.2
Iron(II) sulphate	5	7.48 ± 1.9	0.07 ± 0.05	7.55 ± 1.81	90.7 ± 2.0	98.3

Values are % of dose; means ± SD.

centre of a 4 π-geometry whole body counter for humans with liquid organic scintillator in the energy range of 980–3000 keV [15]. ^{59}Fe Activity in the excrement and tissues of rats was measured in a whole body counter or for larger sensitivity in an 3″ × 3″-NaJ detector (autogamma 5260, Canberra-Packard, Frankfurt, Germany). The incorporation of ^{59}Fe into erythrocytes was calculated by ^{59}Fe in the total blood volume (5.6% of body weight) as a percentage of the whole body retention of ^{59}Fe.

The biological half-life of ^{59}Fe was calculated from a double term exponential fit algorithm to the measured whole body retention of ^{59}Fe values in a period of 14–120 days after oral administration of ^{59}Fe-labelled TMH-ferrocene.

HPLC. Anion-exchange HPLC of serum samples, collected from the portal vein and the abdominal artery, was performed using an iron-free system type Ultrochrom GTI (Pharmacia LKB, Freiburg, Germany), equipped with a Mono Q column (Pharmacia LKB). LC effluent was monitored at 280 nm. Tris–hydrochloric acid (10 mM) pH 7.5 with a linear gradient of 0–0.5 M NaCl was used as eluant (flow rate 1.0 mL/min). Fractions of 1.0 mL were collected and measured for ^{59}Fe activity in an automated γ-scintillation spectrometer (Packard Auto γ 5360) and for ^{55}Fe activity in a β-scintillation counter (Packard).

Under these HPLC conditions, a part of serum transferrin did not bind to the column and was eluted in the first two fractions (peak I, Fig. 2). Rechromatography of this fraction as well as polyacrylamide gel electrophoresis established the presence of transferrin in this fraction.

RESULTS

Three ^{59}Fe-labelled compounds (ferrocene, TMH-ferrocene, (TMH)$_2$-ferrocene) with different lipophilicities were synthesized and their iron bioavailability was studied in rats.

Intestinal absorption of iron from ferrocene compounds

The intestinal absorption, urinary excretion and whole body retention of ^{59}Fe from oral ferrocene, TMH-ferrocene, (TMH)$_2$-ferrocene and iron(II) sulphate (5 mg Fe dosage) were studied in starved female rats with normal body iron stores by intra-individual comparison (Table 1). The whole body

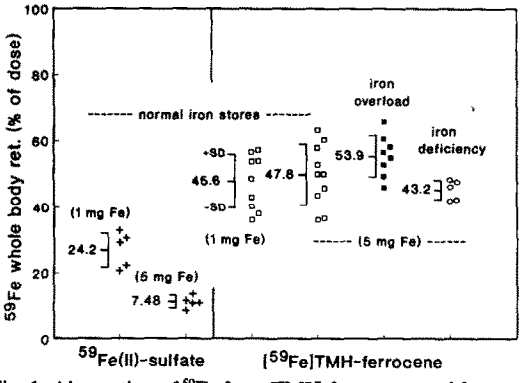

Fig. 1. Absorption of ^{59}Fe from TMH-ferrocene and ferrous sulphate (1 or 5 mg Fe dosage) in rats with depleted (liver Fe < 0.05 mg Fe/g wet weight), normal (liver Fe, 0.4 mg/g wet wt) and increased iron stores (liver Fe, 8–16 mg/g wet wt) as judged by the whole body retention of ^{59}Fe after 7–10 days.

retention of ^{59}Fe was significantly higher ($P < 0.001$) after oral TMH-ferrocene compared to ferrocene, iron(II) sulphate and (TMH)$_2$-ferrocene. The urine excretion of ^{59}Fe was low after administration of TMH-ferrocene, (TMH)$_2$-ferrocene or ferrous iron, whereas more than 60% of the ^{59}Fe activity was found in the urine within 7 days of oral [^{59}Fe]-ferrocene. Reverse-phase HPLC separation of urine samples from rats receiving oral [^{59}Fe]TMH-ferrocene demonstrated several small, iron-containing fractions (data not shown). Unmetabolized TMH-ferrocene was not detectable in the urine.

In contrast to ferrous iron, the intestinal absorption of iron from TMH-ferrocene was found to be independent of the iron dosage (1 or 5 mg Fe) and the body iron stores (Fig. 1).

In a period between 30 min and 4 hr after oral loading with [^{59}Fe]TMH-ferrocene, the ^{59}Fe activity measured in the blood and serum from the abdominal artery was significantly lower (40–70%) than in blood (serum) samples removed from the portal vein, indicating a first pass uptake of TMH-ferrocene iron into the liver. In anion-exchange chromatography of serum samples (Mono Q column) from portal blood, most of the ^{59}Fe activity was strongly retained on the column and could only be eluted using ethanol

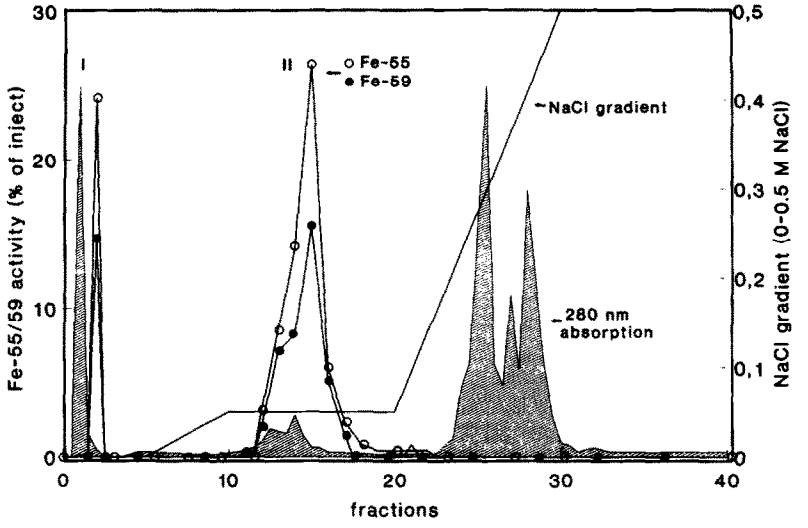

Fig. 2. Anion-exchange HPLC of serum (from portal vein) from a rat 120 min after oral preloading with ferrous sulphate (5 mg Fe) and 60 min after oral [^{59}Fe]TMH-ferrocene (5 mg Fe). The ferrous sulphate was labelled with ^{55}Fe to identify transferrin-bound Fe and to calculate the recovery of the label during chromatography. Column, Mono Q, Pharmacia; eluant, 10 mM Tris–HCl, pH 7.5; gradient 0–0.5 M NaCl. Peak I and II, serum transferrin (peak I represents transferrin that did not bind to the column under these conditions).

as eluant. This ^{59}Fe fraction comigrated with unlabelled TMH-ferrocene in reverse phase HPLC and in TLC, thus representing unmetabolized TMH-ferrocene. Unexpectedly, a substantial part of the ^{59}Fe activity in serum was tightly bound to transferrin (Fig. 2) but without forming a gradient before and after the liver. Transferrin-bound ^{59}Fe in serum was also found in a rat which was preloaded with oral ferrous iron before administration of [^{59}Fe]TMH-ferrocene (Fig. 2). Therefore, it seems unlikely that this ^{59}Fe fraction is derived from intestinal absorption of an ionic iron impurity from the [^{59}Fe]TMH-ferrocene preparation. As it could also be isolated from serum after incubation with [^{59}Fe]TMH-ferrocene *in vitro*, the transferrin-bound ^{59}Fe probably represents a small amount of ^{59}Fe-label that has been broken down (enzymatically?) from TMH-ferrocene in serum or during the anion-exchange chromatography.

The distribution of ^{59}Fe in the tissues of rats, 1 hr–7 days after oral dosing of ^{59}Fe-labelled ferrocene compounds, was remarkably different from the respective distribution of ^{59}Fe after ferrous iron absorption (Fig. 3). Significantly higher amounts of the label from oral [^{59}Fe]TMH-ferrocene and [^{59}Fe]-ferrocene were found in the liver as compared to the experiment with ferrous iron. On the other hand, ^{59}Fe incorporation into erythrocytes was lower after oral administration of all the ferrocene compounds under study. The particular lipophilic character of ferrocene became obvious by a noteworthy transient storage of the ^{59}Fe-label in fat tissue within 7 days of oral administration of [^{59}Fe]ferrocene, whereas the slightly more polar [^{59}Fe]TMH-ferrocene (19 mg Fe/kg dosage) was not enriched in fat tissue.

After parenteral (i.p.) administration of [^{59}Fe]-TMH-ferrocene (2.5 mg Fe), the tissue distribution and erythrocyte incorporation of the ^{59}Fe-label were similar to in the oral experiment (Fig. 4).

In iron-deficient rats, the absorbed TMH-ferrocene iron was incorporated into the haemoglobin of circulating erythrocytes (erythrocyte incorporation) very efficiently (Table 2), indicating that this iron is highly bioavailable in case of need. In rats with normal iron stores and, to a greater extent in already iron-overloaded rats, the absorbed TMH-ferrocene iron was mainly stored in the liver. Thus, in contrast to iron-deficient rats, the erythrocyte incorporation was low in iron-loaded animals.

Experimental iron overload

A diet supplemented with different doses of TMH- and $(TMH)_2$-ferrocene was fed to groups of rats for 2–10 weeks (Table 3). The daily provided amount of 15 g/rat was tolerated quite well in all except one group. In this group, the diet consisting of 2% (w/w) TMH-ferrocene was poorly accepted and only minor amounts (<5 g/day) were consumed. These animals lost weight and as one rat died after 2 weeks this feeding experiment was terminated.

As seen from the liver weight and liver to body weight ratio (Table 3), the iron-overloaded rats showed a true hepatomegaly. Feeding a diet enriched with TMH-ferrocene resulted in a dose-dependent progressive liver iron overload. After 10 weeks of feeding, splenic iron was also increased to a certain degree, whereas the amount of iron in the kidneys, heart and lungs was not different compared to the control.

DISCUSSION

Compared and in contrast to ferrous iron, an iron

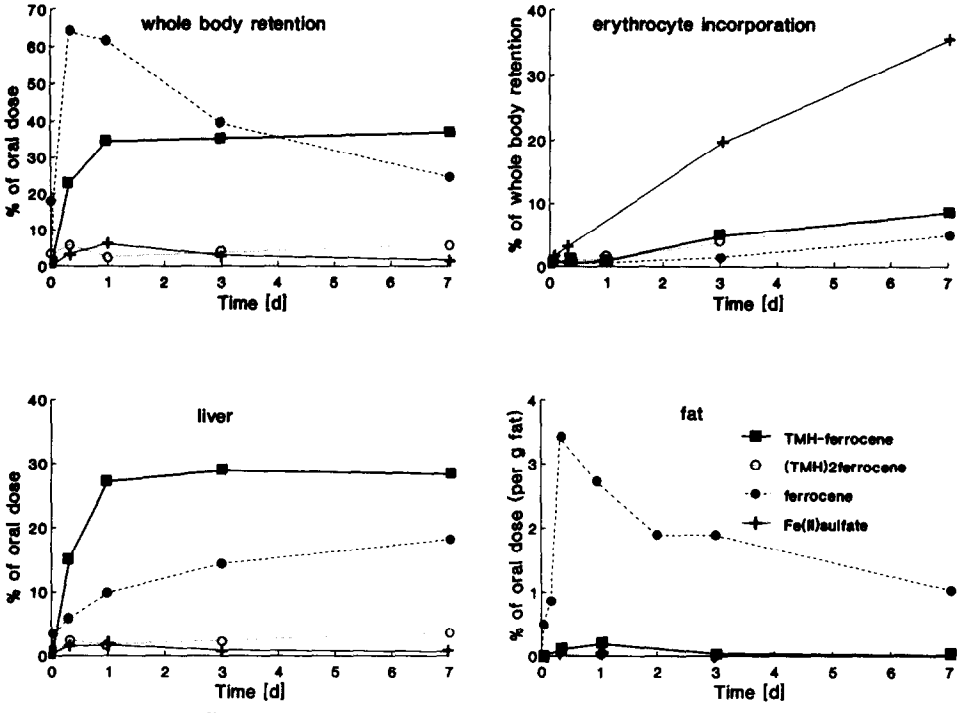

Fig. 3. Distribution of ^{59}Fe 1 hr–7 days after oral ^{59}Fe-labelled ferrocene compounds or ferrous sulphate (5 mg Fe dosage) in the whole body, erythrocytes, liver or fat tissue of rats with normal iron stores (mean values from 2–3 rats, respectively).

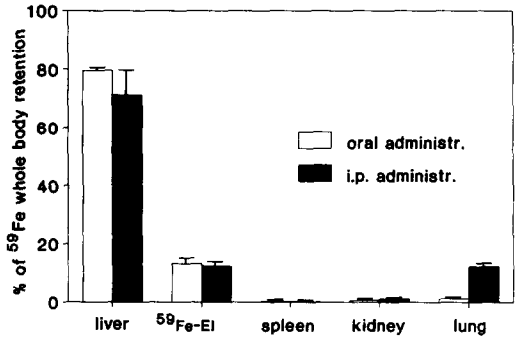

Fig. 4. Distribution of ^{59}Fe in the tissues of rats 14 days after oral (5 mg Fe) or i.p. (2.5 mg) administration of [^{59}Fe]TMH-ferrocene (N = 5, mean ± SD).

compound with known high bioavailability, the intestinal absorption of iron from TMH-ferrocene was much higher, independent of the dosage (1 or 5 mg Fe) and showed no significant differences between animals with depleted and increased iron stores. In the series ferrocene, TMH-ferrocene and $(TMH)_2$-ferrocene, the absorbability of iron runs parallel with the lipophilic character of the compounds (ferrocene being the most lipophilic compound). All these findings indicate that TMH-ferrocene is not subjected to the regulation of ionic iron absorption and is probably absorbed via the lipid absorption mechanism. Different lines of evidence were found that TMH-ferrocene is not already metabolized in the intestinal mucosa but in the liver (similar distribution of ^{59}Fe after oral and i.p. administration; isolation of TMH-ferrocene from the serum of portal blood).

Although a fraction of transferrin-bound ^{59}Fe was found in serum after oral administration of [^{59}Fe]-TMH-ferrocene, most of the absorbed TMH-ferrocene is transported to the liver via portal blood independent from transferrin.

Whereas most of the absorbed ferrocene is hydroxylated in the liver and excreted by the kidneys [16], the metabolism of the hydrocarbon moiety of TMH-ferrocene is obviously quite different to that of ferrocene (Table 1) and only small amounts of a variety of lipophilic, iron-containing metabolites were found in the urine.

The rate of ^{59}Fe incorporation from labelled TMH-ferrocene into the haemoglobin of circulating erythrocytes was correlated to the body iron stores, being high in iron-deficient and low in iron-overloaded animals. In the iron-loaded rats most of the body iron is non-erythron iron and stored in the liver. We have shown recently that the serum iron concentration and the transferrin saturation are strongly increased in these animals and that large amounts of an iron-rich ferritin form a substantial fraction of non-transferrin-bound serum iron [17].

In the TMH-ferrocene-fed rat, iron accumulation in the liver occurs initially in parenchymal cells [6, 8, 9, 11] and is preferentially located in the

Table 2. Tissue distribution, erythrocyte incorporation and biological half-life of ^{59}Fe after oral administration of [^{59}Fe]TMH-ferrocene (5 mg Fe) in rats

Time* (days)	N	Liver Fe (mg Fe/g wet wt)	^{59}Fe Distribution Liver (% of ^{59}Fe-WBR)	Spleen	Erythrocyte incorporation†	[^{59}Fe]TMH-ferrocene biological half-life (days)
Group A: iron-deficient, kept further on low iron diet						
14	3	0.06 ± 0.03	37.5 ± 2.72	0.78 ± 0.12	48.5 ± 14.6	
118	3	0.10 ± 0.03	4.53 ± 1.59	3.00 ± 1.65	92.1 ± 8.7	318 ± 48
Group B: normal iron stores, kept on low iron diet						
14	4	0.42 ± 0.11	79.6 ± 0.63	0.28 ± 0.05	13.3 ± 1.57	
118	4		10.2 ± 1.61	5.39 ± 1.25		302 ± 22
Group C: iron-overloaded, kept on low iron diet						
124	4	14.3 ± 3.0	87.3 ± 2.4	3.22 ± 1.32	4.03 ± 0.61	468 ± 167
Group D: iron-overloaded, kept further on TMH-ferrocene						
14	3	22.1 ± 2.6	94.9 ± 0.12	0.16 ± 0.01	3.17 ± 0.40	
209	3	29.9 ± 3.5	91.0 ± 0.57	2.39 ± 0.33	0.56 ± 0.13	919 ± 244

Iron-deficient rats (group A) or rats with normal iron stores (group B) were kept further on a low iron diet. After ^{59}Fe-labelling, iron-loaded rats (25 weeks on 0.5% TMH-ferrocene diet) were fed a low iron diet (group C) or TMH-ferrocene (0.5%) enriched diet (group D).

Values are means ± SD.

^{59}Fe-WBR, ^{59}Fe retained by whole body.

* Time after oral administration of [^{59}Fe]TMH-ferrocene and start of diet.

† (^{59}Fe activity in blood/^{59}Fe-WBR) × 100.

Table 3. Tissue iron concentrations in female rats on an iron-enriched diet for 10 weeks (2% TMH-ferrocene, 2 weeks)

Food additive	N	Body wt (g)	Liver wt (g)	Liver/body wt (%)	Liver Fe (mg Fe/g wet wt)	Spleen Fe (mg Fe/g wet wt)
None	5	335 ± 28	8.5 ± 1.5	2.4 ± 0.4	0.44 ± 0.05	1.47 ± 0.73
$(TMH)_2$-ferrocene (2%, w/w)	5	298 ± 15	16.1 ± 0.3	5.4 ± 0.2	5.23 ± 0.42	2.09 ± 0.06
TMH-ferrocene (0.15%, w/w)	5	304 ± 21	10.9 ± 1.4	3.6 ± 0.3	8.43 ± 1.34	2.53 ± 0.52
TMH-ferrocene (0.5%, w/w)	5	308 ± 18	14.8 ± 1.2	4.8 ± 0.1	16.88 ± 2.25	4.76 ± 0.26
TMH-ferrocene (2%, w/w)	4	227 ± 8	20.0 ± 2.0	8.7 ± 0.6	4.11 ± 0.21	2.80 ± 0.07

Values are means ± SD.

periportal area of the acinus of the liver [11]. This pattern of iron storage in the liver is also similar to that described in dietary iron overload with carbonyl iron in rats [18] as well as in the early stages of hereditary haemochromatosis [19]. With advancement of iron-loading, Kupffer cells proliferate and store increasing amounts of iron [11]. Unlike haemochromatosis in humans, the liver macrophages finally exceed by far the hepatocytes with respect to iron storage. It is not yet clear whether or not Kupffer cells can take up and metabolize TMH-ferrocene directly. We found however the distribution of ^{59}Fe-label from oral TMH-ferrocene to be similar to that from i.v. injected rat ferritin (unpublished results), indicating a predominantly hepatocellular uptake of TMH-ferrocene, as [^{59}Fe]ferritin is a selective radio-iron probe for iron stores in hepatocytes [20]. Due to the high bioavailability of TMH-ferrocene even in already iron-loaded rats, the range of iron-loading is much greater than in any other dietary iron overload model, including the carbonyl iron model. The latter has so far been widely used as an animal model for hereditary haemochromatosis [18, 21, 22]. It was shown recently that the absorption of ^{59}Fe-labelled ferrous iron is low in carbonyl iron-loaded rats [23]. Using a [^{59}Fe]-carbonyl iron-enriched diet in rats, we have demonstrated that the absorption of carbonyl iron is also markedly depressed in moderately iron-loaded animals (unpublished results). As a consequence, higher liver iron concentrations, which are probably essential for the severe hepatocellular damage and subsequent liver fibrosis and cirrhosis found in hereditary haemochromatosis, can hardly be achieved in the carbonyl iron model.

Due to the fast, progressive and severe iron overload that can be induced, and the similarity to the hepatocellular iron distribution in human

haemochromatosis, the TMH-ferrocene model seems suitable for studying various aspects of iron overload diseases in humans.

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