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CONTRASTING UPTAKES OF ^{59}Fe INTO SPLEEN, LIVER, KIDNEY AND SOME OTHER SOFT TISSUES IN NORMAL AND HYPOTRANSFERRINAEMIC MICE

INFLUENCE OF AN ANTIBODY AGAINST THE TRANSFERRIN RECEPTOR

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Abstract—Uptake of iron-59 from blood into various soft tissues of anaesthetized mice was investigated by continuous intravenous infusion of the radiotracer during 2 hr. The ^{59}Fe was given either as ferrous chloride with ascorbate or as ^{59}Fe-transferrin. Infusions were made into adult mice with and without pretreatment with a monoclonal antibody against transferrin receptors, and into hypotransferrinaemic mice and appropriate controls. In normal mice, ^{59}Fe uptake into spleen was much higher than into other tissues and was 94–96% inhibited by the antibody. Inhibitions due to the antibody were less complete in liver and renal cortex, and there was evidence of some non-transferrin-mediated transport during infusion of ^{59}Fe/ascorbate. In the hypotransferrinaemic mice, tissue uptakes of ^{59}Fe during infusion of ^{59}Fe/ascorbate were enormous, being two to three orders of magnitude greater than in the normal controls. The rank order for size of uptake was liver > renal cortex > pancreas > spleen > other tissues. All tissues examined have a considerable potential capacity for uptake of non-transferrin-bound iron, this being greatest in liver and renal cortex.

Key words: iron transport; ^{59}Fe tissue uptake; hypotransferrinaemic mice; transferrin receptor; antibody agonist

It is accepted that the major route of iron uptake into cells of different tissues is via specific binding and endocytosis of iron transferrin [1, 2]. However, the effect of antibodies against the transferrin receptor on radioactive iron uptake into different tissues *in vivo* has not to our knowledge been measured quantitatively. There is also only limited information available on radioactive iron uptake into different tissues in the absence or near absence of transferrin. In one study this has been examined in hypotransferrinaemic mice after intragastric administration of ^{59}Fe [3].

In a recent study [4], we have examined transport of ^{59}Fe into brain in the mouse and rat. This involved infusing ^{59}Fe either as ferrous iron in the presence of ascorbate or as Fe-transferrin. Both forms of iron were infused intravenously into control mice and into mice given a prior saturating dose of an immunoglobulin M (IgM) antibody, prepared in the rat against the mouse transferrin receptor. Infusions of ^{59}Fe with ascorbate were also made into hypotransferrinaemic (hpx/hpx) mice, referred to below as hpx mice.

The opportunity was taken in the above experiments to sample various soft tissues from the mice, as well as different regions of brain. The results yield interesting clues as to the behaviour of an antibody against the transferrin receptor after its administration into the circulating blood and as to the magnitude of potential non-transferrin-bound iron transport into different tissues.

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MATERIALS AND METHODS

Preparation of animals. Male and female mice (To) of 30–45 g body wt were anaesthetized with 0.01 mL/30 g of fentanyl/fluanisone (Hypnorm, Janssen) plus diazepam 5 mg/kg (i.p.). In several experiments hypotransferrinaemic mice (16–26 g), homozygous for the recessive gene hpx, were used. In relation to the hypotransferrinaemic mice, similar aged mice which either lacked the hpx gene or were heterozygous for the gene were also used as controls. The left external jugular vein and external ileac artery were cannulated by using polyethylene tubing. ^{59}Fe (NEN Research Products, Dupont) was infused via the jugular vein and blood samples were collected from the external ileac artery.

Standard infusion and sampling procedure. Mice were infused with 2 μCi ^{59}Fe (1 nmol total Fe) in physiological saline containing ~4 mg ascorbic acid for a period of 2 hr. In some experiments ^{59}Fe-labelled mouse serum transferrin was used in the infusing solution without ascorbate. The preparation of ^{59}Fe-labelled mouse serum transferrin has been described in detail in the previous report [4]. The infusions were performed at a diminishing rate with a Harvard syringe pump so as to maintain a near constant ^{59}Fe level in serum during the experiment. In the case of administration of a monoclonal

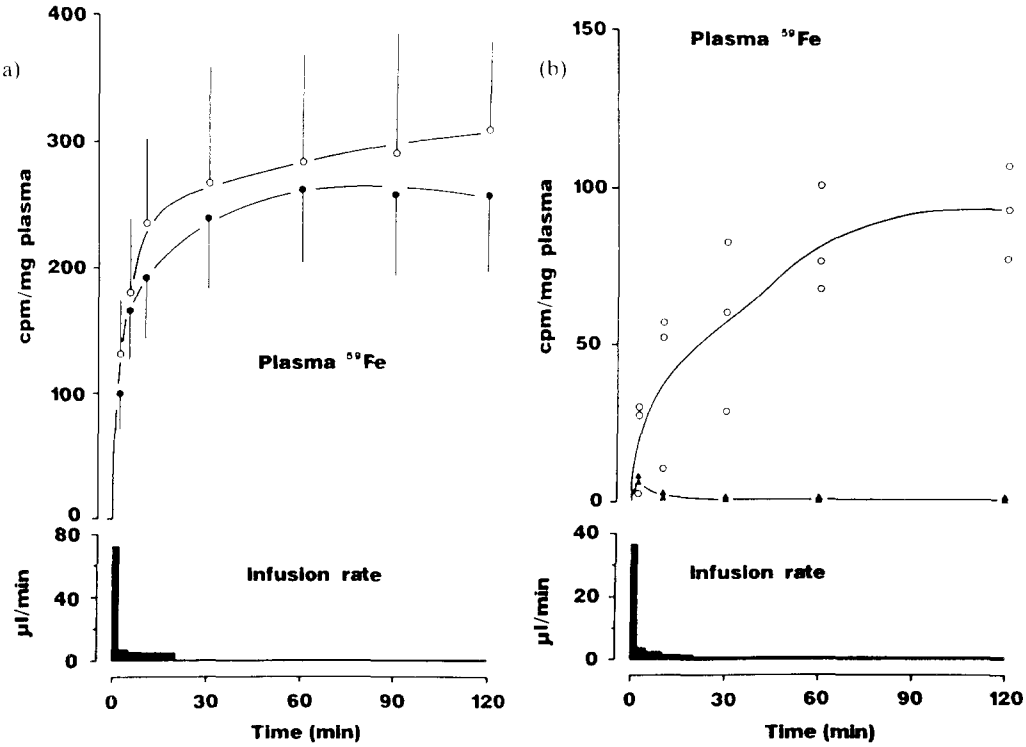

Fig. 1. (a) Plasma ^{59}Fe in adult mice during a 2 hr intravenous infusion of ^{59}Fe ferrous chloride with ascorbate: three controls (●); three mice after monoclonal antibody IgM RI7 208 pre-treatment (○). The infusion rate is indicated by lower black blocks. Each point and vertical bar represents mean + SE. (b) Plasma ^{59}Fe in three small mice (16–26 g) and in two hypotransferrinaemic mice during a 2 hr intravenous infusion of ^{59}Fe ferrous chloride with ascorbate: controls (○); hypotransferrinaemic mice (▲). The infusion rate is indicated by lower black blocks.

antibody, a single intravenous injection of antibody of 1 mg was made 10 min before the infusion. Five to seven arterial blood samples of approximately 0.04 mL were collected into capillary tubes during each experiment and were spun immediately. Samples of serum and packed red cells were placed in tared vials for weighing and counting.

At the end of the experiment, the vascular system was washed out, the mouse decapitated and tissue samples were removed. The wash was made with saline containing 1 mM EDTA infused into the arterial cannula under high pressure, as described in detail by Bradbury and Deane [5]. Fluid and tissue samples were counted in a Nuclear Enterprises N 8311 or LKB Compu Gamma automatic counter, where possible, to a total of at least 20,000 counts. Counts were corrected for background and decay. Uptake into tissues was expressed as cpm/mg tissue or as a space (mL/g), the tissue cpm/g (C_{tissue}), at the end of experimental time (T), being related to the plasma cpm/mL (C_{pl}) integrated over time, as:

$$\text{Uptake space} = C_{tissue} \cdot T/\int_0^T C_{pl}\, dt.$$

The ^{59}Fe uptake into tissues was compared with that in the presence of a monoclonal antibody against mouse transferrin receptors, RI7 208 anti-mouse (provided by Dr Ian Trowbridge). Uptake into tissues of hypotransferrinaemic mice was compared with that into tissues of the litter-mate controls.

Results between mice receiving the two types of infusion with or without antibody pretreatment were compared by analysis of variance, the data being treated as if the cell sizes were all equal to the harmonic mean of the cell sizes. The significance of differences between individual means of a pair were estimated by the Bonferroni test. Results are represented as the mean value + SEM for (N) experiments.

RESULTS

Level of ^{59}Fe *in mouse plasma*

The infusion rate in the larger mice was 72.5 µL/min over the first 2 min and was then gradually reduced to 1.8 µL/min during 20–120 min. The serum ^{59}Fe level increased rapidly for the first 15 min and then reached 250 cpm/mg serum at about 30 min (Fig. 1a). Thereafter, it remained tolerably constant. Prior injection of the antibody did not appreciably influence the ^{59}Fe level in serum. In the younger and smaller mice (15–25 g), the infusion rate was reduced so that they received a similar amount of ^{59}Fe and volume of fluid per unit body weight to those given to the older and larger mice. The increases in serum ^{59}Fe were, however, more gradual than for the larger animals. In the hypotransferrinaemic mice, the initial ^{59}Fe level was very low and remained so, in spite of the same infusion rate over the 2 hr (Fig. 1b).

Table 1. Residual plasma in various tissues of large mice, determined by infusion of ^{125}I-albumin over 10 min (N = 3)

Tissue	Space (mL/100 g)
Spleen	2.20 ± 0.47
Liver	0.59 ± 0.16
Kidney	2.29 ± 0.65
Muscle	0.13 ± 0.08

Estimates of the plasma albumin space in various tissues after intravenous infusion of ^{125}I-albumin (Table 1) indicate that the presence of residual blood containing ^{59}Fe in tissues after the washout and sampling procedure is not likely to interfere significantly with the measured tissue uptakes of the later radiotracer.

^{59}Fe uptake into various tissues and effects of antibody

After infusion of ^{59}Fe with ascorbate or as iron-transferrin, uptake into spleen was an order of magnitude greater than into other tissues and was of similar size in ^{59}Fe/ascorbate-infused and in ^{59}Fe-transferrin-infused mice (Fig. 2). The uptake into liver and kidney in the controls was also not significantly different between the two types of ^{59}Fe infusion. The presence of antibody inhibited ^{59}Fe uptake into the spleen by 93–95% after infusions with either ascorbate or as iron transferrin. The antibody was without significant effect on uptake into liver or kidney during either of the ^{59}Fe/ascorbate infusions, though in the case of the ^{59}Fe-transferrin infusions the uptakes were 30% and 48% less in the presence of antibody in liver and kidney, respectively. When the two groups of mice receiving antibody were compared, liver and kidney uptakes of ^{59}Fe in the ascorbate-infused group were substantially higher than in the Fe-transferrin group ($P < 0.01$ and ~0.05, respectively).

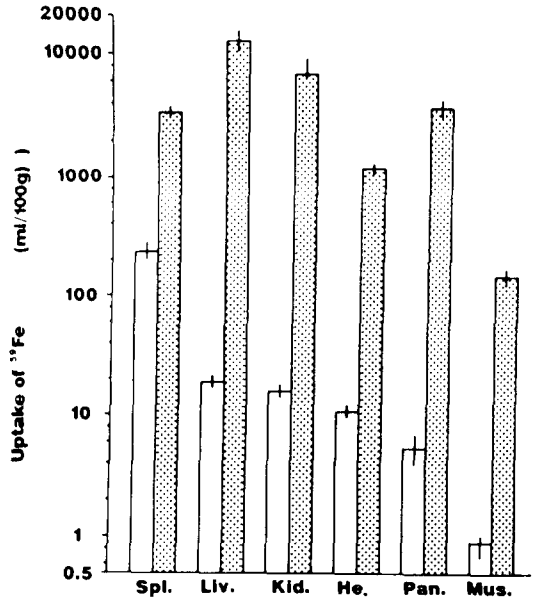

Fig. 3. Uptake of ^{59}Fe as a space (mL/100 g) in spleen (Spl), liver (Liv), renal cortex (Kid), heart (He), pancreas (Pan) and triceps (Mus) of three small control mice (open blocks) and three hypotransferrinaemic mice (shaded blocks). The uptakes are means ± SE after 2 hr of ^{59}Fe/ascorbate infusion. Note log scale of ordinate.

Tissue uptake of ^{59}Fe in hypotransferrinaemic mice

Uptake of ^{59}Fe into tissues of the genetically hypotransferrinaemic mice was compared with that in litter-mate controls (Fig. 3). The ^{59}Fe uptake, expressed as a space, was 14 times higher in the

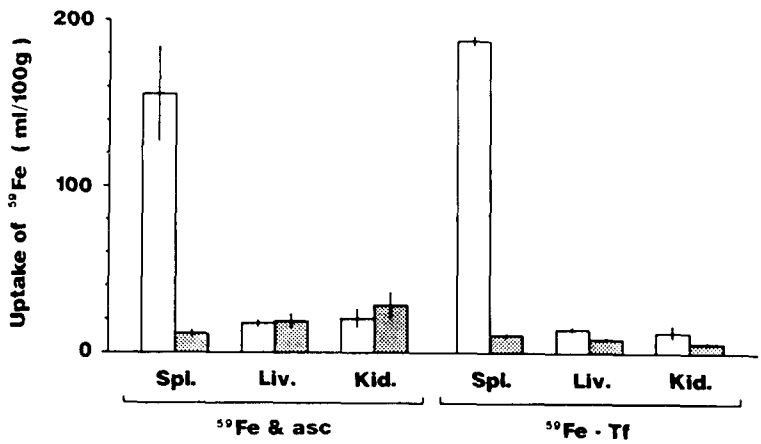

Fig. 2. Uptake of ^{59}Fe as a space (mL/100 g) in spleen (Spl), liver (Liv) and renal cortex (Kid) of large control mice (open blocks) and mice pretreated with monoclonal antibody IgM RI7 208 (shaded blocks). The uptakes are means ± SE after 2 hr of ^{59}Fe infusion. ^{59}Fe/ascorbate infused: seven controls and five with antibody; ^{59}Fe-transferrin infused: three in each group.

Table 2. Uptake of ^{59}Fe into various tissues of hypotransferrinaemic mice (hpx) and small controls after 2 hr infusion

Tissue	^{59}Fe radioactivity (cpm/mg wet wt)		^{59}Fe space (mL/100 g)	
	Control (N = 3)	hpx (N = 3)	Control	hpx
Spleen	213.5 ± 48.5	43.5 ± 9.4	244.3 ± 39.0	3431 ± 381
Liver	17.0 ± 3.5	160.6 ± 35.7	19.6 ± 2.6	13063 ± 2534
Kidney	13.8 ± 2.3	92.8 ± 20.8	16.2 ± 2.1	7258 ± 710
Heart	9.5 ± 1.5	16.0 ± 4.2	11.1 ± 1.3	1242 ± 144
Pancreas	4.6 ± 1.5	48.9 ± 12.3	5.5 ± 1.6	3882 ± 635
Muscle	0.78 ± 0.16	1.9 ± 0.49	0.9 ± 0.1	153 ± 24

spleens of the hpx mice than in those of the controls. However, when expressed as cpm/mg, spleen activity was one fifth of the control value (Fig. 3 and Table 2). The difference between the space and cpm/mg is related to the very low plasma cpm in the hpx mice. In liver and kidney, the cpm/mg were nine and seven times higher in the hpx mice than in the controls, whilst the spaces were nearly three orders of magnitude greater. The rank order for ^{59}Fe uptake in hpx mice was liver > kidney > pancreas > spleen > other tissues.

DISCUSSION

The measured uptakes of ^{59}Fe into various tissues of normal and hypotransferrinaemic mice under the different conditions give indications about differences in the mechanisms available for iron transport into these tissues. In all three control groups, i.e. ^{59}Fe/ascorbate into large and small normal mice and ^{59}Fe-transferrin infusions into large mice, accumulation of ^{59}Fe in the spleen was greater than in any other tissue investigated, usually by at least an order of magnitude. Inhibition of this uptake by the monoclonal antibody against transferrin receptors was effectively complete whether the infusion contained ^{59}Fe/ascorbate or ^{59}Fe-transferrin. The spleen results, taken on their own, indicate that iron transport into this organ is virtually all via transferrin receptors in normal mice. It also follows from the uniformly high uptakes and similar inhibitions by antibody, whatever the composition of the infusion fluid, that a high proportion of ^{59}Fe in blood serum must be bound to transferrin in the mice infused with ^{59}Fe/ascorbate.

In the hpx mice, although spleen uptake of ^{59}Fe expressed as a space was 14 times greater than in the controls, this relative increase was much smaller than in any other tissue investigated. Hence, if tissue uptake of iron from ^{59}Fe/ascorbate is via a non-transferrin-mediated mechanism in hpx mice, the potential for such transport must be less in the spleen than in other tissues. Since the capillaries in the spleen are discontinuous [6], the cells within it are likely to be fully exposed to the monoclonal antibody. The cells within the spleen likely to have a need for iron are the dividing lymphocyte and erythrocyte precursors [7].

Radioactive iron uptake into liver and kidney cortex contrasted markedly with that into spleen. Not only was it much less, expressed as space, uptake being from 10% to 40% in the intact mice without antibody, but inhibitions due to the antibody, if present, were much less complete. The apparent inhibitions due to antibody on liver and kidney uptake were in the mice infused with ^{59}Fe-transferrin as low as 30% and 48% for liver and kidney, respectively. If ^{59}Fe in residual plasma in the tissues is corrected for by subtraction of the ^{125}I-albumin spaces (Table 1), these inhibitions due to antibody on liver and kidney become higher, 32% and 59%, respectively. It is unlikely that the non-inhibitable transport represents non-transferrin-mediated transport in the mice infused with ^{59}Fe-transferrin. Although non-specific fluid phase endocytosis has been observed in cultured hepatocytes [8], iron uptake into liver from plasma transferrin *in vivo* has been shown to be largely or wholly dependent on specific transferrin receptor interaction [9]. Hence in our experiments, the transferrin receptors on the parenchymal cells in each organ must be incompletely blocked with antibody. Antibody might be anticipated to have access to interstitial fluid and thus to the hepatocytes via the discontinuous capillaries of the liver. However, much of the blood flow to the liver comes from the hepatic portal vein and will have already passed through the circulation of the gut. Hence, much of the available antibody may have already bound to receptors in the latter tissue before the blood originally containing it reaches the liver. In the renal cortex, the capillaries supplying the tubules are known to be continuous or fenestrated [6]. Such capillaries are known to have a very high reflexion coefficient to albumin and [10, 11] hence would allow little access of an immunoglobulin to the transferrin receptors on the renal tubular cells.

The much greater ^{59}Fe uptakes into inhibited liver and spleen during infusion with ^{59}Fe/ascorbate as opposed to during infusion with ^{59}Fe-transferrin indicate that non-transferrin-mediated transport into these organs occurs readily, as do the results in the hpx mice in which liver and spleen uptakes during

^{59}Fe/ascorbate infusions were enormous. Taken together, these results with liver and spleen suggest that there must be some non-transferrin-bound ^{59}Fe present in the blood plasma of normal mice infused with ^{59}Fe/ascorbate. Since the capacity for transport of non-transferrin-bound iron is so very large in liver and kidney, as judged by the results in hpx mice, the concentration of such iron in normal ^{59}Fe/ascorbate-infused mice is probably very small. It is pertinent that whilst 99% of plasma ^{59}Fe in these mice was shown to be transferrin bound at 2 hr, this percentage was much less at 1 min of infusion, namely 90% [4].

An additional possible mechanism whereby an IgM antibody may influence iron distribution *in vivo* is via the acute phase response. The IgM molecule is a pentamer and hence can very effectively activate complement [12]. The acute phase response is associated with a reduction in plasma iron [13, 14]. This is presumably due to a shift of iron-transferrin or iron from iron-transferrin in plasma into cells. We do not know whether such an inflammatory reaction in the presence of RI7 208 occurred in our experiments or whether, if present, it influenced uptake of ^{59}Fe. We consider it unlikely, since a similar distribution of the influence of an IgG against transferrin receptors, OX-26, on ^{59}Fe uptake into tissues has been observed in the young rat (M. W. B. Bradbury, unpublished observations).

In general, our results for tissue uptake of ^{59}Fe in the hpx mouse contrasted with those in the normal mouse are in the same direction as those of Craven *et al.* [3]. The quantitatively greater increases in ^{59}Fe uptake observed in the present study are no doubt due to the different routes of adminstration of the radiotracer in the two studies—intragastric by Craven *et al.* [3] and intravenous infusion by us. It is likely that non-transferrin-bound Fe uptake into tissues occurs by a similar process in the near absence of transferrin and when most transferrin is saturated with iron, i.e. in iron overload [3]. It is of interest, therefore, that we observed especially high ^{59}Fe uptakes in liver, pancreas and heart, since hepatic cirrhosis, diabetes and congestive heart failure are common complications of haemochromatosis [15]. The very high uptake of ^{59}Fe in renal cortex in hpx mice cannot be compared with the incidence of renal complications in haemochromatosis, since these do not to our knowledge occur.

The question arises as to the chemical form in which non-transferrin-bound iron is so readily transported into liver and kidney. In the hpx mice, there is certainly a large excess of iron in plasma over the binding capacity of its transferrin. Thus, in hpx mice the transferrin concentration in plasma is extremely low, i.e. 4–7 mg/dL or 1/40–1/80 of the normal level [16]. The total iron measured by atomic absorption spectrometry is surprisingly high at ~65 μM [17]. There is, of course, no certainty that the same complex or ion is being handled in the experiments with normal and hypotransferrinaemic animals. However, ferrous iron was rapidly taken up into liver perfused with Krebs solution. Ferric iron was also removed from such a perfusate, especially in the presence of the complexing agent citrate [18]. Morgan [19] has observed that non-transferrin-bound ferrous iron enters human reticulocytes by a saturable process which is inhibitable by divalent ions such as Co^2, Mn^{2+} and Ni^{2+}.

In conclusion these experiments confirm the potential for transport of non-transferrin-bound iron into a number of tissues. They provide no good evidence that this occurs in normal mammals in the absence of iron overload. Under normal conditions, there are likely to be insignificant concentrations of non-transferrin-bound iron in blood plasma.

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