IRON REMOVAL BY DESFERRIOXAMINE DURING HAEMODIALYSIS: IN VITRO STUDIES

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Summary

To find the best way of using desferrioxamine (DFO) in iron (Fe) overloaded patients on regular haemodialysis (HD) treatment, we investigated Fe removal in a standard in vitro haemodialysis model by measuring $^{59}$Fe activity in plasma and dialysate after the addition of DFO as a constant infusion during or as a bolus at different time intervals before haemodialysis. Data were compared with those obtained without DFO at normal or high plasma Fe concentrations and by haemofiltration (HF). In summary HF was superior to haemodialysis for Fe removal. No DFO was needed to dialyse high plasma Fe concentrations whereas the efficiency of removal of normal plasma Fe content increased with equilibration time of DFO before haemodialysis. For treatment of Fe overload DFO is therefore recommended to be given after each haemodialysis treatment.

Introduction

Iron overload is a common clinical problem in children on regular haemodialysis treatment – in contrast to aluminium overload. It is the consequence of a more pronounced anaemia compared with adults, making blood transfusion necessary at regular intervals [1]. Serum ferritin (SF) as the most reliable biochemical indicator of Fe stores was found to reflect Fe overload in three-quarters of haemodialysed children [2] and to be dependent not only on the number of blood transfusions but also on genetic conditions. In order to prevent its harmful consequences on heart, liver, endocrine system, muscle, blood and probably bone, severe hemosiderosis (SF $>$ 2,000µg/L) which occurs in about 25 per cent of children after 12 months on haemodialysis, has to be treated with the Fe chelating agent DFO. An increasing number of reports in adult patients on haemodialysis deals with the effect of DFO treatment [3–8], although no uniform mode of therapy is practised. To find out the most adequate schedule of DFO therapy in haemodialysis the following in vitro studies were performed. From ethical reasons we did not perform in vivo studies with radioactivity in children.
Methods

Imitating in vivo paediatric conditions pooled human plasma (11) was haemodialysed for four hours by a cuprophan hollow fibre dialyser (surface area 1.0m², Hemoflow MTS C, Fresenius) against a routine acetate containing dialysate (flow 500ml/min) without transmembrane pressure and a flow of 100ml/min by means of a Drake Willock monitor. The circulating volume was kept constant by a saline infusion. The concentration of plasma Fe was either unphysiologically high (2,500mmol/L) or normal (18mmol/L) with a constant transferrin of 250mg/dl. DFO of 1.0g was given either by an infusion during haemodialysis (3 hours) or as a bolus at the start of, or 30 minutes, two hours, or 24 hours before haemodialysis. At the time of DFO administration ⁵⁹Fe (0.1mCi) was additionally added to the plasma. Fe elimination was measured by the loss of radioactivity in plasma and its increase in the dialysate. Three plasma samples were taken at 0, 3, 15, 30, 60, 120, 180 and 240 minutes and three dialysate samples at 1, 2, 3, 8, 15, 30, 60, 120, 180 and 240 minutes respectively after start of dialysis. Radioactivity was measured three times per sample in a β-counter and plasma Fe loss expressed as per cent of initial (100%) activity = Δ. All data are given as mean values of simultaneous measurements. Additionally Fe concentrations were measured under the same conditions by atomic absorption spectrophotometry in two studies. Moreover modification of Fe removal was attempted by increasing the plasma transferrin content by 1,000mg/L addition of ascorbic acid (0.5g/L) or by using post dilution haemofiltration (cellulose triacetate filter, SM40041, Sartorius).

Results

At toxic plasma Fe concentrations (2,500µmol/L) Fe was eliminated almost completely within the first hour of haemodialysis both without (Figure 1A) and with DFO (Δ 97.1 and 99.1%). In contrast, at normal in vivo plasma Fe concentrations (18µmol/L) nearly no Fe could be removed without DFO by haemodialysis (Figure 1B), although about half the plasma content could be removed by HF (Δ 52%) (Figure 1C). By DFO infusion Fe removal by haemodialysis could only be increased to a minor extent (Δ 4%) (Figure 1D). Giving DFO as a bolus at the start of haemodialysis Fe removal, however, could be further augmented to 15.8 per cent (Figure 1E) and at 30 minutes before start to 48 per cent (Figure 1F). With this timing of DFO administration Fe removal could be further increased by HF (Δ 72%, Figure 1G), but not significantly by the addition of either transferrin (Δ51.1%, Figure 1H) or ascorbic acid (Δ 51.0%, Figure 1I). By increasing the interval between the application of DFO and start of haemodialysis a progressive increase of Fe removal could be detected which amounted to 82.4 per cent at an interval of two hours and to 99.7 per cent at 24 hours (Figures 1K, 1L).

The atomic absorption method revealed comparable results of Fe elimination by haemodialysis without DFO (Δ + 2.0%) as well as with DFO 24 hours before haemodialysis (Δ99.5%) (Figure 1M).

Radioactive counts of the dialysate/filtrate gave an initial peak three minutes
Figure 1. Kinetics of plasma iron (Fe) removal by haemodialysis (HD) or haemofiltration (HF) at different modes of desferrioxamine (DFO) application. Final removal is given by Δ of initial $^{59}$Fe radioactive counts or of total Fe as measured by atomic absorption (M). The pretreatment plasma Fe concentration is presented in brackets. For details see text. • represent mean values
after start of treatment and a consecutive hyperbolic decrease with a minimum usually achieved by two hours.

Discussion

From the data presented it becomes evident that the efficiency of Fe removal by extracorporeal blood purification depends on its plasma concentration, the purification method used and the mode and timing of DFO administration. In the case of high, toxic plasma values, generally occurring in accidental oral Fe intoxication, Fe elimination can efficiently be achieved by the performance of haemodialysis alone without any DFO. The addition of the chelator does not lead to further significant decrease in plasma Fe. The reason for this obvious effect of haemodialysis on toxic Fe concentration is probably due to the fact that at high Fe concentrations the majority of ions are unbound to transferrin or other proteins such as albumin and therefore easily dialysable because of their low molecular weight. This is of great importance for the clinical management of acute Fe intoxication.

In the case of chronic Fe overload on the other hand plasma Fe is normal or elevated to only a minor extent, so that it is totally bound to transferrin, whereas the majority of the metal is intracellularly stored in the cavity of an apo-ferritin shell as ferritin. That means if Fe removal is needed in the case of normal plasma concentrations it has to be mobilised from its binding sites in advance otherwise it is not dialysable because of its protein binding. This is the reason for the ineffective Fe removal by haemodialysis at normal plasma concentrations we found without DFO and also probably for the successful elimination by the more permeable membrane used in HF. However, the cellulose triacetate membrane is reported to have a molecular cut-off around 30,000 Daltons, which should not allow filtration of Fe bound to at least intact transferrin molecules. Nevertheless, in the case of normal plasma values, Fe elimination by HF seems to be superior to haemodialysis in the absence of additional DFO.

By the administration of DFO Fe removal occurs by haemodialysis and increases in HF at physiological plasma concentrations. This better Fe clearance can be explained by a mobilisation of Fe from its binding to undialysable proteins and its subsequent chelation in a 1:1 molar ratio ($K_D = 10^{30}$) forming the dialysable ferrioxamine complex with a molecular weight of 611 Daltons. The lack of an additional effect with ascorbic acid, which is known to increase Fe removal in vivo seems to be due to the in vitro conditions of this study being unable to imitate Fe release from its storage. The failure of inhibition of Fe removal by additional transferrin administration suggests that there is no competition between this transport protein and DFO.

Although DFO treatment has progressively been used as the treatment of choice in severely Fe overloaded haemodialysis patients, no uniform regimen has either been reported or accepted up to now [3–8]. From our studies we cannot recommend DFO infusion during haemodialysis because of its minor effect on Fe removal, probably due to the simultaneous dialysis of the chelator itself. The better effect of the bolus application and results with increasing time intervals between DFO application and start of haemodialysis is in favour
of an Fe mobilising and chelating reaction which is time dependent. Although in vitro studies of DFO binding are not totally representative of in vivo conditions because of their multiple compartment character it seems reasonable from the practical clinical point of view to apply DFO at the end of each haemodialysis session. By this procedure there is time enough for DFO to react with plasma and certainly storage Fe to lead to an efficient Fe removal via the dialysis membrane. This therapeutic regimen is in accordance with the subcutaneous DFO infusion used successfully in hemosiderotic children suffering from homozygous β-thalassaemia. As the elimination depends on membrane permeability an effect is achieved more quickly and better not only by HF as demonstrated by us and other investigators [9] but also by additional haemoperfusion [10]. As Fe removal could not be optimised after DFO application 24 hours before haemodialysis we did not investigate the effect of other haemodialysis membranes [5] with this regimen.

The plasma $^{59}$Fe removal accorded with the elimination kinetics found by dialysate counting. The data obtained are not artificially influenced by radioactive Fe because the results by atomic spectrophotometry were similar.

References

5 Rembold CM, Krumlovsky FA, Roxe DM et al. Trans ASAIO 1982; 28: 621
10 Chang TMS, Barre P. Lancet 1983; ii: 1051

Open Discussion

CAROZZI (Genoa) Did you observe in your study a clinical improvement in the anaemia or in the bone marrow iron incorporation?

MULLER-WIEFFEL. We only have the clinical impression that in some patients who are on long-term desferrioxamine there is some improvement in anaemia. We have not routinely looked at the bone marrow of these patients and so I can’t comment on marrow iron.

KERR (Chairman) How do you explain the removal of half of your serum iron by haemofiltration, even without desferrioxamine, when you get almost no removal by haemodialysis suggesting that it is all protein bound. Have you looked at the iron content of the filtrate?

MULLER-WIEFFEL. By our methods we were unable to differentiate between bound and unbound iron. It has been shown by other investigators that the removal of iron increases with the permeability of the membrane.