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Reactive polymers

Iron(III)-chelating resins X. Iron detoxification of human plasma with iron(III)-chelating resins [1]

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Abstract

Iron detoxification of human blood plasma was studied with resins containing desferrioxamine B (DFO) or 3-hydroxy-2-methyl-4(1H)-pyridinone (HMP) as iron(III)-chelating groups. The behaviour of four resins was investigated: DFO–Sepharose, HMP–Sepharose and crosslinked copolymers of 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate (HEMA) and of AHMP with N,N-dimethylacrylamide (DMAA). The efficiency of iron detoxification of plasma of the resins was mainly dependent on the affinity of the ligands and the hydrophilicity of the resins. The results of a stability study in phosphate-buffered saline at a physiological pH indicated that AHMP–DMAA was the most stable resin, whereas the Sepharose gels had a relatively lower stability. Experiments with the AHMP–DMAA resin showed that the resin was able to remove iron from plasma with different iron contents, and from plasma poisoned with $FeCl_3$, iron(III) citrate or transferrin. A rapid removal from free serum iron was observed, whereas iron from transferrin was removed slowly afterwards. Only the overload iron was removed since in all cases the normal serum iron level of *ca.* 1 ppm was obtained.

Key words: Chelating resins; Iron overload; Iron detoxification of plasma

1. Introduction

Iron overload leads to toxic effects because the human body has no physiological route for the elimination of excess iron [1,2]. In the therapy of iron overload, excess iron must be removed by chelators. The current clinical drug for iron overload is desferrioxamine B (DFO), a naturally occurring iron chelator which is administered intravenously. DFO reduces iron levels by forming a stable, soluble complex with iron(III), which is eliminated in urine and stool [3,4].

Although DFO therapy is effective in removing large quantities of iron rapidly, there are several drawbacks in its use. DFO is not an orally active drug, and has a short plasma residence time [5,6]. Furthermore, the drug and its iron complex are toxic when present in large amounts [7], and the dose of DFO which can be administered intravenously is also limited by its adverse effects on blood pressure [8].

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In order to minimize DFO toxicity while maintaining its effectiveness, DFO was bound to polymers. These DFO-bound polymers had a lower toxicity than free DFO, whereas a similar effectiveness as DFO was observed [9,10]. In addition, DFO-containing resins and a device with immobilized DFO were developed for the extracorporeal removal of iron. However, the effectiveness of the immobilized DFO was low, and it seemed impossible to reduce the serum iron concentration to the normal level [11–13].

Iron(III) chelation by chelators other than DFO has also been studied [4,14–16]. It was found that 3-hydroxy-2-methyl-4(1H)-pyridinone (HMP) derivatives are strong iron(III) chelators and have a higher affinity towards iron(III) ($\log \beta_3 = 36$) [17] than DFO ($\log \beta = 31$) [18]. Water-soluble HMP derivatives are orally active and uncharged under physiological conditions both as the free ligands and as the iron complexes, and are relatively selective for iron(III) under *in vivo* conditions [19,20]. 1,2-Dimethyl-3-hydroxy-4(1H)-pyridinone was extensively studied in iron-overloaded animal models and in thalassaemia patients, and removed iron relatively efficiently at high dose [21,22]. However, the dose required to keep a previously well chelated patient in negative iron balance appeared to be unacceptably high. At a dose of 100 mg/kg, peak levels of iron-free drug exceeded 100 mM, significantly within the concentration range known to inhibit ribonucleotide reductase [14].

We recently reported on the synthesis and properties of new iron(III) chelators with immobilized DFO groups or HMP groups [23–27]. Because it was reported that with soluble HMP chelators high doses had to be used for iron removal [21,22], it seemed worthwile to study the applicability of iron(III)-chelating systems with immobilized HMP chelators in more detail.

In this paper, we report on the application of iron(III)-chelating resins with immobilized DFO or HMP groups for iron detoxification of human blood plasma. Their stability in phosphate-buffered saline (PBS, pH 7.4) and iron detoxification ability were compared. Results are also given on the iron removal from plasma with various iron levels and from plasma poisoned with $FeCl_3$, iron(III) citrate or transferrin, as well as the iron detoxification rate.

2. Experimental

2.1. Materials

Human blood plasma containing 21.7 mM citrate for anticoagulation (ACD plasma) was provided by the Blood Bank (Enschede, Netherlands). $FeCl_3 \cdot 6H_2O$, iron(III) citrate and human apotransferrin (containing only 4.9 ppm of iron) were obtained from Merck, Janssen and Sigma, respectively, and used as received.

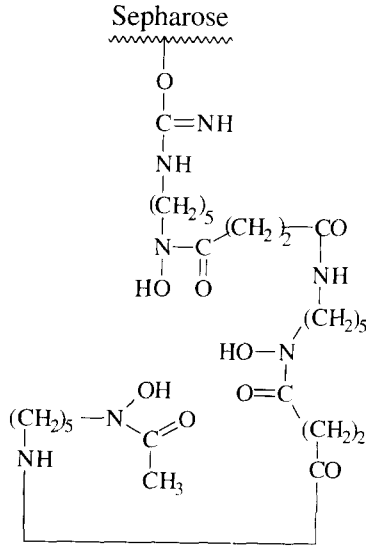
2.2. Preparation of iron(III)-chelating resins

DFO–Sepharose gel and HMP–Sepharose gel were synthesized as described previously [23,24], with ligand densities of 20.8 and 18.3 μmol/ml, respectively. AHMP–HEMA and AHMP–DMAA resins were derived from copolymerization of 1-(β-acrylamidoethyl)-3-hydroxy-2-methyl-4(1H)-pyridinone (AHMP) with 2-hydroxyethyl methacrylate (HEMA) or N,N-dimethylacrylamide (DMAA) in the presence of a crosslinking agent [25,26], and their ligand densities were 214 and 460 μmol/g, respectively.

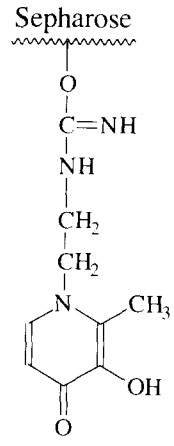
2.3. Stability study of the resins

As an indication for the stability of the resins at physiological pH, the release of the ligands from the resins was determined in PBS (pH 7.4). The DFO–Sepharose and HMP–Sepharose gels were first washed with

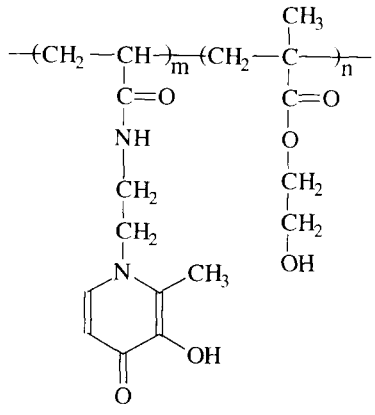
distilled water. About 0.3 g of resin or 0.5 ml of gel was placed into 20 ml of PBS, and the mixture was rotated at 20°C. After a determined time interval rotating was stopped for 5–10 min, allowing the resin or gel to precipitate. A part of the supernatant was removed for the determination of the concentration of released ligands. The amount of DFO in the aqueous solution was determined by its maximal absorbance in the range 195–210 nm, whereas the amount of HMP was determined by its maximal absorbance at 280 nm with an ultraviolet–visible spectrophotometer.

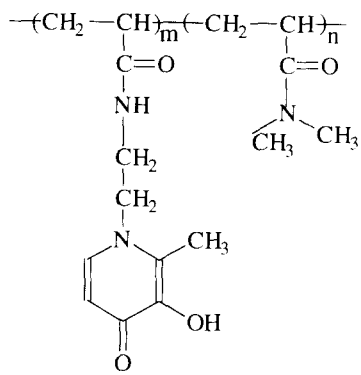


DFO-Sepharose gel



HMP-Sepharose gel





Crosslinked copolymers of

AHMP and HEMA

AHMP and DMAA

Fig. 1. Structures of various iron(III)-chelating resins.

2.4. Iron detoxification

Ferric trichloride and iron(III) citrate solutions were freshly prepared by adding appropriate amounts of $FeCl_3 \cdot 6H_2O$ and iron(III) citrate in distilled water, respectively. Apotransferrin was saturated with iron(III) by dissolving apotransferrin in the $FeCl_3$ solution, and incubated at 20°C for 4 h.

Plasma was poisoned by adding the above-mentioned iron(III)-containing solutions to various iron levels, and the plasma was rotated at 20°C for 1 h. DFO–Sepharose and HMP–Sepharose gels were washed with distilled water before use.

Iron detoxification of plasma was carried out at 20°C by rotating the mixture of an iron-chelating resin and plasma. The rotating was stopped and the resin or gel was allowed to precipitate. The supernatant of the plasma was removed for the determination of the iron content with a Perkin-Elmer Zeeman 5000 atomic absorption spectrophotometer (AAS). For comparison, a blank control was carried out and determined at the same conditions.

3. Results and discussion

Various iron(III)-chelating systems were investigated, and their structures are shown in Fig. 1. The stability of the iron(III)-chelating resins at a physiological pH was studied (Fig. 2), and the results show that the Sepharose gels with immobilised ligands had a relatively lower stability probably due to hydrolysis of bonds between the ligands and the matrix, which is consistent with previous observations in distilled water [23,24]. It is noted that only small amounts of ligands were released from the AHMP–HEMA and AHMP–DMAA resins, which probably resulted from some unreacted monomers encapsulated in the polymer networks. Because of the high stability of the resins in PBS and in water [25,26], a similar behaviour in plasma was expected.

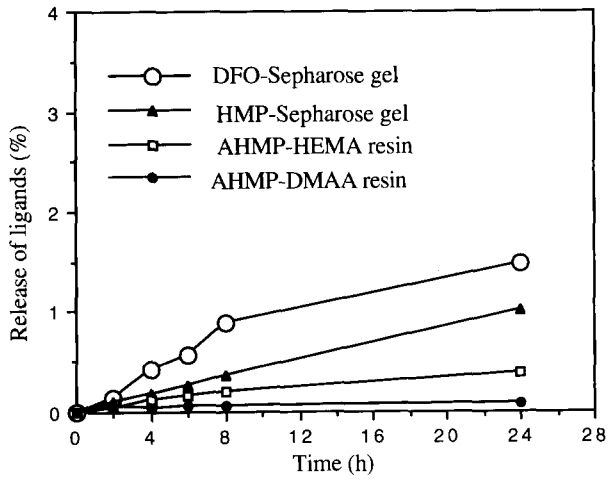


Fig. 2. Stability of iron(III)-chelating resins in PBS (pH 7.4).

Table 1
Iron detoxification of plasma [a] by various iron chelating resins [b]

Iron chelating resin			Iron content in plasma [c] (ppm)		Iron removal (%)
Code	Amount	Capacity (μmol)	Initial [d]	Remaining	
DFO–Sepharose	0.5 ml	14.4 [e]	4.26	1.57	63
HMP–Sepharose	1.0 ml	6.1 [f]	4.26	0.41	90
AHMP–HEMA	75 mg	5.4 [f]	4.26	3.86	9
AHMP–DMAA	40 mg	6.1 [f]	4.26	0.98	77

[a] Poisoned by adding $FeCl_3$ solution to 5 ml of plasma.
[b] Performed at 20°C for 24 h.
[c] Mean value of three determinations.
[d] After addition of $FeCl_3$.
[e] (Amount of resin) × (ligand density).
[f] (Amount of resin) × (ligand density) × 1/3.

Iron detoxification by various resins was studied with plasma poisoned with ferric chloride (Table 1). The resins were effective in iron removal from poisoned plasma, but differed in the efficiency. The HMP–Sepharose gel had a higher efficiency than the DFO–Sepharose gel, which may be due to a higher affinity of the HMP ligands than DFO. Comparing the results between AHMP–HEMA, AHMP–DMAA and HMP–Sepharose, it can be seen that the efficiency of AHMP–HEMA was much lower than that of AHMP–DMAA or HMP–Sepharose, which may be explained by their difference in iron(III)-chelating ability as observed previously [25–27]. This difference in the ability between AHMP–HEMA and AHMP–DMAA or HMP–Sepharose is probably due to differences in the type of the polymeric matrices which may influence the hydrophilicity or swelling extent, the diffusion rate of iron(III) into the resin and the steric hindrance of the ligands. In addition, AHMP and HEMA would not copolymerize well, which may have some bad effect on site distribution of AHMP and hence on the accessibility of iron.

Considering the stability as well as the efficiency, AHMP–DMAA had the most favourable properties, and for this reason more experiments were carried out with the AHMP–DMAA resin.

The AHMP–DMAA resin was used in iron detoxification of plasma with various iron contents and the results are shown in Fig. 3. For unpoisoned plasma (iron content in this case was 0.86 ppm), only a small amount of iron was removed, while iron was removed at higher iron contents. It is interesting to note that by using different initial iron contents (0.86, 2.02, 4.26 and 6.52 ppm) the iron contents of the treated plasma were always in the region 0.6–1.2 ppm, the region of normal iron contents.

It is known that the iron in plasma is tightly bound to its transport protein, transferrin. Normal serum iron levels fluctuate around 1 ppm, and typically only 30% of the iron binding sites of transferrin are occupied, making the total iron binding capacity (TIBC) *ca.* 3 ppm. From the results shown in Fig. 3 it can be seen that the iron contents of plasma after treatment with the AHMP–DMAA resin was lower than *ca.* 3 ppm, which indicates that the resin was able to remove iron from transferrin.

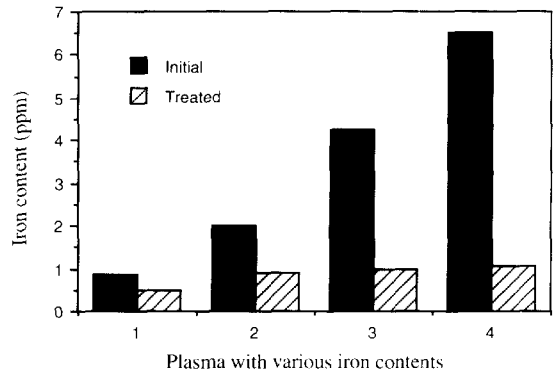


Fig. 3. Iron detoxification of plasma with various iron contents. Experiments were carried out at 20°C for 24 h by rotating 40 mg of AHMP–DMAA resin and 5 ml of plasma with iron contents of 0.86 ppm (1, unpoisoned plasma), 2.02 ppm (2), 4.26 ppm (3) and 6.52 ppm (4), respectively. The poisoned plasma was prepared by adding appropriate amounts of a $FeCl_3$ solution.

The two major causes of iron overload are iron poisoning and β-thalassemia [14]. Iron poisoning most often arises in small children through the inadvertent ingestion of iron preparations [14]. The only effective treatment of β-thalassemia is to administer blood transfusion throughout life, which introduces large quantities of iron and transferrin [14]. In order to investigate the effect of the type of iron(III)-containing substance which poisons the plasma, iron detoxification by the AHMP–DMAA resin was studied with plasma to which was added $FeCl_3$, iron(III) citrate or transferrin (100% saturated with iron, Fe-binding form), respectively.

In Fig. 4 the TIBC values and the iron contents of initial poisoned plasma as well as of treated plasma are given. For comparative

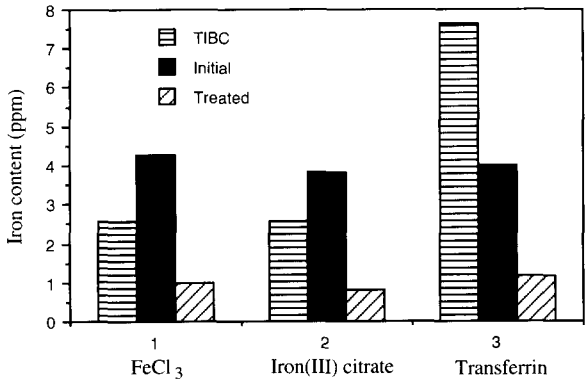


Fig. 4. Iron detoxification of plasma poisoned with $FeCl_3$, iron(III) citrate or transferrin (100% iron saturated transferrin). Detoxification was performed with 40 mg of AHMP–DMAA resin and 5 ml of plasma at 20°C for 24 h.

purposes the iron content of the initial poisoned plasma in all three cases was brought to *ca.* 4 ppm. In the plasma poisoned with $FeCl_3$ and iron(III) citrate, the calculated TIBC based on the iron content of unpoisoned plasma was 2.6 ppm, whereas the TIBC in the plasma poisoned with transferrin was 7.7 ppm, which was calculated by taking into account the TIBC of unpoisoned plasma and the amount of transferrin added. It is obvious that the resin was effective in iron removal from the poisoned plasma, and in all cases the iron content was reduced to *ca.* 1 ppm, even with different TIBCs of the plasma and iron sources used. These results indicate that iron detoxification of plasma with the AHMP–DMAA resin was independent of the form of poisoning iron. In addition, it is important to note that only the overload iron was removed by the resin since in all cases the normal iron content in plasma of *ca.* 1 ppm was obtained.

Fig. 5 gives data on the rate of iron detoxification of iron poisoned plasma by the AHMP–DMAA resin. It can be seen that removal of free iron (not bound by transferrin) was fast and complete within 1 h, resulting in a serum iron concentration on the level of the TIBC. The iron from transferrin was removed slowly afterwards, and the iron concentration ultimately reached the normal iron level (in unpoisoned plasma).

From the data shown in Table 1 it can be concluded that part of the iron from transferrin could be removed by the DFO–Sepharose gel. The remaining iron concentration (1.57 ppm) by using the gel was lower than that with DFO-immobilized crosslinked polystyrene or polyacrolein observed by other workers [11,13] notwithstanding the different operation conditions. In their iron detoxification of plasma with a column process, only small amounts of iron could be removed from transferrin in poisoned plasma. They proposed that it was not possible to remove iron from transferrin because of the relatively low affinity of free DFO and the steric hindrance induced in the coupling of DFO [11,13]. The difference in behaviour of the DFO–polystyrene or the polyacrolein system and our DFO–Sepharose gel might be due to the high hydrophilicity of Sepharose gel and less steric hindrance between DFO and the matrix. It is noteworthy that using the AHMP–DMAA resin, the iron content in plasma could be reduced to 1 ppm, which showed a significant improvement in the iron detoxification of plasma compared with the reported results in a column process (the final iron content was 2–4 ppm) [11,13]. Although a difference in the iron removal by

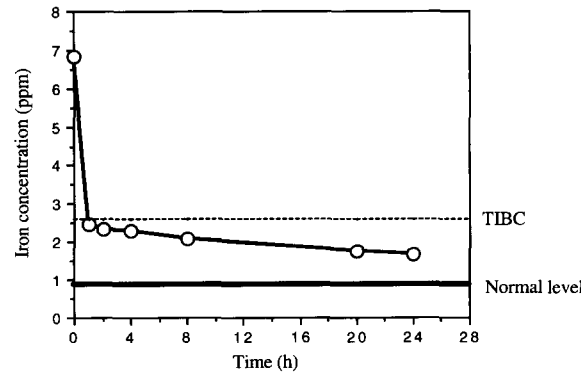


Fig. 5. Iron detoxification rate by the AHMP–DMAA resin. Experiments were performed at 20°C for 24 h by rotating 150 mg of AHMP–DMAA resin and 25 ml of plasma poisoned with $FeCl_3$ solution.

using batch or column process may be present, the ability of the AHMP–DMAA resin in the iron removal is not supposed to change much when using it in a column process because the iron(III)-chelating capacity and ability of the AHMP–DMAA resin in a continuous process with a column was found to be as high as in a batch process [26].

As shown, our iron-chelating resins, especially the AHMP–DMAA resin, removed free serum iron completely and also part of the iron from transferrin, resulting in an iron concentration in the treated plasma of *ca.* 1 ppm. The ability of the iron(III)-chelating resins to remove iron from plasma indicates the possibility of applying the resins in treatment of iron overload in blood, especially in extracorporeal detoxification of severe acute iron poisoning cases.

4. Conclusions

AHMP–HEMA and AHMP–DMAA resins, synthesized by copolymerization of AHMP with HEMA or DMAA in the presence of a crosslinking agent, showed a higher stability in PBS than DFO- or HMP-immobilized Sepharose gels.

All iron(III)-chelating resins were able to remove iron from poisoned plasma, and resins with a high hydrophilicity and with HMP ligands were more effective.

By using the AHMP–DMAA resin, it was possible to reduce the iron contents of poisoned plasma to *ca.* 1 ppm, the normal serum iron level in unpoisoned plasma.

The iron(III) chelators, especially the AHMP–DMAA resin, had much better properties in iron detoxification than the DFO-immobilized resins reported previously.

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