ORIGINAL ARTICLE

Port J Nephrol Hypert 2011; 25(3): 219-224
Advance Access publication 13 September 2011

Assessment of dextran antigenicity of intravenous iron products by an immunodiffusion assay

Susann Neiser, Maria Wilhelm, Katrin Schwarz, Felix Funk, Peter Geisser, Susanna Burckhardt

Vifor (International) Inc. St. Gallen, Switzerland.

Received for publication: 26/07/2011
Accepted: 30/08/2011

ABSTRACT

The antigenicity of a number of different intravenous iron preparations was tested by reverse single radial immunodiffusion with antidextran antibodies. The tested products are low molecular weight iron dextran, ferumoxytol, iron isomaltoside 1000, sodium ferric gluconate, iron sucrose, and ferric carboxymaltose. Dextran-induced anaphylactic reactions have been clinically observed with low molecular weight iron dextran and with ferumoxytol, which contains a dextran derivative as ligand, whereas no dextran-induced anaphylactic reactions have been reported since its introduction in 2010 with iron isomaltoside 1000, which contains a ligand based on dextran 1. The results of the immunoassay confirmed that the dextran-free preparations sodium ferric gluconate, iron sucrose, and ferric carboxymaltose do not cross-react with antidextran antibodies. In contrast, low molecular weight iron dextran and ferumoxytol reacted with the antibodies, as demonstrated by the formation of a precipitation ring. As expected, dextran 1 did not cross-react with antidextran antibodies. Iron isomaltoside 1000 formed a precipitation ring, which suggests that if dextran 1 surrounds the polynuclear iron core, it can act as a polyvalent, higher molecular weight dextran and thus cross-react with the antibodies. Because of the limited number of patients and the exclusion criteria selected for clinical registration studies, e.g. previous hypersensitivity to iron dextran or other iron complexes, rare adverse events such as dextran-induced anaphylactic reactions often do not occur in controlled studies, as was the case with ferumoxytol. However, the presented immunodiffusion assay yielded results in agreement with post-marketing experience for five preparations, including ferumoxytol. Thus, this method could be a valuable tool for the determination of antidextran reactivity of new intravenous iron preparations.

Key-Words:
Iron dextran; iron isomaltoside 1000; iron sucrose; ferric carboxymaltose; ferumoxytol; sodium ferric gluconate.

INTRODUCTION

Intravenous (IV) iron preparations are effectively used for the treatment of iron deficiency especially in patients with chronic diseases with an inflammatory component such as chronic kidney disease, chronic heart failure, cancer, or inflammatory bowel disease[1]. Long-known IV iron preparations include iron dextran and iron sucrose. Iron dextran complexes are kinetically robust and have a low toxicity[2], but they carry the risk of inducing life-threatening dextran-induced anaphylactic reactions (DIAR)[3,4]. Therefore, before administration of the first dose of a dextran-containing iron preparation to a new patient, a test dose must be administered. The rate of life-threatening adverse drug events is reported to be higher for high molecular weight than for low molecular weight iron dextran complexes[5]. The dextran-free

alternatives iron sucrose and sodium ferric gluconate obviously do not carry the risk of DIAR, and they showed the lowest risk for life-threatening and total adverse drug events[4–6]. On the other hand, because of the lower complex stability[2,7] the maximum single infusion dose of iron sucrose (100-500 mg iron) and especially of sodium ferric gluconate (62.5-125 mg iron) is lower than that of iron dextran (up to 20 mg iron/kg body weight)[1]. The newest generation of IV iron complexes, ferric carboxymaltose, ferumoxytol, and iron isomaltoside 1000 can be administered relatively quickly with high maximal doses (1000 mg iron such as ferric carboxymaltose, 510 mg iron such as ferumoxytol[1], and 20 mg iron/kg body weight as iron isomaltoside 1000[8]). Being dextran-free, ferric carboxymaltose cannot induce DIAR, whereas the other two preparations were developed to minimise the risk of DIAR[9,10]. Nevertheless, iron isomaltoside 1000 is based on a very low molecular weight dextran[11], and ferumoxytol on a carboxymethylated dextran[10].

Dextran is composed of $\alpha(1\rightarrow6)$-linked polyglucose and is produced by bacteria, whereas animal- or plant-derived polysaccharides such as glycogen and starch consist of mainly $\alpha(1\rightarrow4)$-linked polyglucose. Since dextran is produced not only by the industrially relevant *leuconostoc mesenteroides* strains but also by caries-inducing *streptococcus* species and intestinal bacteria, a sensibilisation often results from naturally occurring dextran, and most people have dextran-reactive antibodies[12,13]. Immune reactions to dextran may therefore occur at the first clinical administration of dextran as well as after subsequent doses. It has been shown that the titer of dextran-reactive antibodies correlates positively with the severity of DIAR, but does not allow to predict whether a reaction will occur in an individual patient[12,13].

Dextran has also been used in plasma replacement therapy and thromboprophylaxis[14]. In this context, it has been shown that severe DIAR are caused most importantly by IgG antibodies which suggests an immune complex anaphylaxis as underlying mechanism for severe DIAR[13,15,16]. It is known that most antidextran antibodies bind to the linear $\alpha(1\rightarrow6)$ glucose-sequence[12,17], which explains the cross-reactivity of pre-existing antidextran antibodies with synthetic, linear dextran fractions[15]. The immunogenicity of dextran depends on its size, with low molecular weight dextrans being less immunogenic than high molecular weight dextrans[18]. Very low molecular weight dextran of molecular weight of about 1000 Da (dextran 1, Promit®) was successfully used as hapten: dextran 1 blocks the binding sites of antidextran antibodies and can thus minimise[13–15], though not entirely prevent[19], DIAR if injected prior to higher molecular weight dextrans.

An in vitro antibody test, the reverse single radial immunodiffusion assay, has been shown to indicate the existence of an antigenic dextran structure with an immunogenic potential in dextran-containing preparations[20,21]. In the present study, the same assay was used to investigate the antidextran reactivity of currently available IV iron preparations. The tested preparations were low molecular weight iron dextran (CosmoFer®), dextran 1-based iron isomaltoside 1000 (MonoFer®), the iron dextran derivative ferumoxytol (Feraheme®), and three preparations free of dextran and dextran derivatives, i.e. iron sucrose (Venofer®), sodium iron gluconate (Ferrlecit®), and ferric carboxymaltose (Ferinject®).

MATERIALS AND METHODS

Dextran detection by reverse single radial immunodiffusion

The method is based on that described by Richter[20,21] and employs monoclonal dextran antibodies deposited in a well punched into an agar plate that contains test substance. If immunologically active dextran or a cross-reacting dextran derivative is present in the test substance, it forms a precipitate with the antibody upon its diffusion into the agar. *Preparation of agar plates:* The supporting gel was prepared from 1% agarose (m/v) (Fluka, art. no. 05065, lot 1098455) and 1% polyethylene glycol 6000 (m/v) (Fluka, art. no. 03394, lot 146875) in phosphate buffer (pH 8.0 aqueous solution of 1.725% (w/v) $Na_2HPO_4 \cdot 2H_2O$ and 0.055% (w/v) $NaH_2PO_4 \cdot 2H_2O$). For the preparation of the agar sample plates, the supporting gel was completely melted on a steam bath, and 8 mL supporting agar were mixed with 2 mL sample solution and poured into petri dishes. Circular wells of 3 mm diameter were punched with a steel tube into the hardened agar, and the agar cylinders were removed by suction with a Pasteur pipette.

Sample solutions

The tested iron preparations were diluted to 40 µg Fe/mL in 0.9% (m/V) sodium chloride solution: Iron sucrose (Venofer®, lot 10674663, Vifor(International) Inc., St. Gallen, Switzerland), ferric carboxymaltose (Ferinject®, lot 10667273, Vifor(International) Inc., St. Gallen, Switzerland), low molecular weight iron dextran (CosmoFer®, lot 1009019, TEVA GmbH, Radebeul, Germany), ferumoxytol (Feraheme®, lot 09060402, AMAG Pharmaceuticals, Cambridge, MA, USA), iron isomaltoside 1000 (MonoFer®, lot 949171-1, Pharmacosmos, Holbæk, Denmark), and sodium ferric gluconate (Ferrlecit®, lot D7A743A, Sanofi Aventis Deutschland GmbH, Frankfurt, Germany). Dextran 5 (Dextran 5, lot 00309, Serumwerke Bernburg AG, Bernburg, Germany, weight average molecular weight 4'500 Da) and dextran 1 (dextran standard 1000 from *leuconostoc mesenteroides* for GPC, product no. 31416 from Fluka, Sigma-Aldrich, Buchs, Switzerland, weight average molecular weight of 1'270 Da) were tested in a concentration of 3 µg dextran/mL in 0.9% (m/V) aqueous sodium chloride solution. These solutions were mixed with the supporting gel as described above. *Antigen test:* 5 µL dextran antibody solution was added to each well (100 mg/mL, Midland MCA Antibody, M9010, Kansas, USA). The plates were incubated at 4°C for 48 hours. The presence of dextran was visually detected as circular precipitate ring of antigen/antibody complex around the wells. All reported experiments were conducted at least in duplicate.

Molecular weight determination by gel permeation chromatography (GPC)

The method is based on permeation chromatography on a poly(methylmethacrylate) gel, calibrated with pullulan standards. The method has been described in detail elsewhere[2] and corresponds to that described in the United States Pharmacopeial Convention for iron sucrose[22].

RESULTS

Reverse single radial immunodiffusion was used to identify products with antidextran antibody reactivity (Figure 1, Table I). As expected, the dextran 5 positive control solution reacted with the antibody and formed a distinct precipitation ring, whereas the dextran 1 negative control did not form a precipitate with the antibody. Venofer® (iron sucrose), Ferinject® (ferric carboxymaltose), and Ferrlecit® (sodium iron gluconate) did not form precipitates with antidextran antibodies.

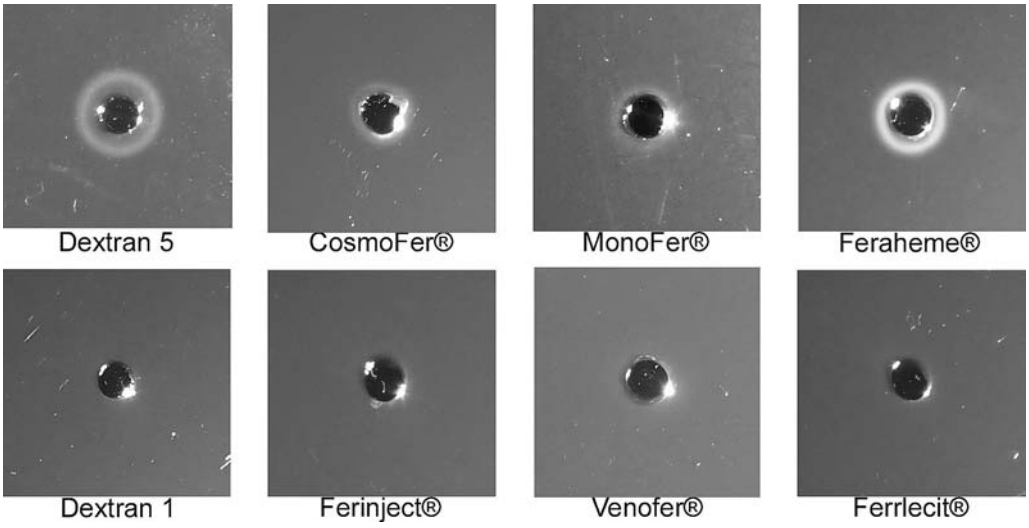

Figure 1

Assessment of dextran antigenicity of different IV iron preparations by reverse single radial immunodiffusion. A positive antigen/antibody reaction is indicated by circular turbidity around the well. Dextran 5 served as a positive control.

Table I

Overview of the names of the tested IV iron preparations, their active ingredients, chemical classification of the ligands, weight average molecular weight of the complex, and their reactivity with antidextran antibodies.

Active ingredient	Name	Ligand	Molecular weight (Da)[1]	Dextran antibody reaction
Low molecular weight iron dextran	CosmoFer®	Dextran	80'000[2]	Yes
Iron isomaltoside 1000	MonoFer®	Very low molecular weight dextran (3-5 glucose units)	69'000[2]	Yes
Ferumoxytol	Feraheme®	Carboxymethylated, reduced dextran	185'000[3]	Yes
Ferric carboxymaltose	Ferinject®	Carboxymaltose	150'000[3]	No
Iron sucrose	Venofer®	Sucrose	43'000[3]	No
Sodium ferric gluconate	Ferrlecit®	Gluconate / sucrose	38'000[3]	No

[1] Weight average molecular weight of the complex according to the method described in the USP for Iron sucrose injection[22], i.e. relative to a pullulan standard.
[2] This work.
[3] Ref. 28.

This result is in agreement with their chemical specifications and was anticipated, because these complexes are free of dextran or dextran derivatives. CosmoFer®, a low molecular weight iron dextran, expectedly formed precipitates with the antibody. Also Feraheme® and MonoFer® clearly formed precipitates.

The weight average molecular weights determined according to the method described in the USP for Iron sucrose injection (i.e. relative to a pullulan standard) were 69'000 Da for MonoFer® and 80'000 Da for CosmoFer® (Table I).

DISCUSSION

All the tested IV iron products were developed either to minimise the risk of DIAR, by lowering the molecular weight of the iron dextran complex (CosmoFer®), by using very low molecular weight dextran as a ligand (MonoFer®), by derivatization of dextran (Feraheme®), or to exclude the risk of DIAR by complete absence of dextran or dextran derivatives (Venofer®, Ferinject®, Ferrlecit®). However, all the dextran-based preparations tested formed precipitates with the antidextran antibody, whereas the three dextran-free preparations did not. CosmoFer® is classified by the manufacturer as low molecular weight iron dextran, and DIAR are known to occur[4]. The positive reaction of the immunodiffusion assay is therefore not surprising and is in agreement with clinical experience.

Feraheme® contains polyglucose sorbitol carboxymethylether iron oxide, i.e. has a dextran derivative as a ligand[9,10]. The degree of carboxymethylation lies above the threshold that has been determined as non-immunogenic by measuring the extent of rat paw edema response, as an indicator of the potential to induce human adverse reactions upon intravenous injection[10]. No DIAR were observed in the 568 patients who received Feraheme® during clinical registration studies – all of which excluded patients with known hypersensitivity to other iron preparations, and thus to iron dextran, or with multiple drug allergies[23]. However, shortly after the approval in the US in June 2009, the FDA raised safety concerns[23], and, subsequently, a case of DIAR was reported in a patient with prior adverse reaction to iron dextran[9], which led to a hypersensitivity warning for ferumoxytol. The positive antigen-antibody reaction in the immunoassay reported in this work clearly suggests the possibility of DIAR and thus confirms the clinical experience with ferumoxytol.

The rationale for the development of MonoFer® was the creation of a stable iron dextran complex that, due to the non-immunologic properties of its ligand isomaltoside 1000, would not induce DIAR [8,24]. This carbohydrate is prepared from dextran 1, a linear dextran oligomer of very low molecular weight (with 3-5 glucose units), which has been shown to be nonanaphylactogenic[25]. Moreover, dextran 1 acts as a monovalent hapten and has been shown to minimise[14,15], though not entirely prevent[19], DIAR if injected prior to higher molecular weight dextrans. As expected, and in agreement to previous reports[21], Dextran 1 did not show a precipitation ring in the immunodiffusion assay.

The active ingredient of MonoFer®, however, consists of a polynuclear iron(III)-oxyhydroxide core

surrounded by a number of dextran 1 molecules[8] with an overall molecular weight close to that of the low molecular weight iron dextran complex in CosmoFer® (Table I). Thus, iron isomaltoside 1000 could immunologically resemble a polyvalent, higher molecular weight dextran and thus cross-react with the antidextran antibodies. This effect has been proposed previously since positive in vitro antigen/antibody reactions were observed for similar iron dextran complexes composed exclusively of dextran 1[26]. The positive reaction for Monofer® in the immunodiffusion assay shown in this work supports this theory. Taken together, the results imply that, despite the nonanaphylactogenic properties of isomaltoside 1000, a DIAR with the iron complex MonoFer® is theoretically possible, although no clinical cases have been reported up to now.

Ferric carboxymaltose (Ferinject®), iron sucrose (Venofer®), and sodium ferric gluconate (Ferrlecit®) do not contain dextran or dextran derivatives and as expected did not react in the dextran immunoassay. This is in agreement with the clinical experience; neither preparation has been reported to evoke DIAR. In spite of the good safety profile of Venofer®, it can, similar to Ferrlecit®, only be administered at relatively low doses. Thus, the only IV iron preparation on the market that does not react with dextran antibodies and that can be administered at high doses is Ferinject®.

The results from reverse single radial immunodiffusion in combination with the chemical characterisation and clinical experience suggest that this method could be used to estimate whether there is a risk of DIAR for a given preparation. Clearly, the assay may not identify all immunologically active dextrans and dextran derivatives. A main limitation is the choice of antibody for the immunoassay since a number of different dextran antibodies have been found in humans[27].

CONCLUSION

The reported immunoassay data agree well with clinical observations and thus represent a possible approach for the evaluation of the risk of DIAR. The two tested products that are proven to induce DIAR showed a positive reaction (CosmoFer®, Feraheme®), whereas the three preparations that are free of dextran or dextran derivatives tested negative, in agreement with clinical experience (Venofer®, Ferinject®, Ferrlecit®). For MonoFer®, despite the nonanaphylactogenic property of the isomaltoside 1000 ligand, the test result was positive, which suggests the possibility of a reaction with pre-existing antidextran antibodies.

Conflict of interest statement. The authors are employed by Vifor (International) Inc. St. Gallen, Switzerland.

Acknowledgments. We thank Ms. Maja Thum for technical assistance.

References

1. Qunibi WY. The efficacy and safety of current intravenous iron preparations for the management of iron-deficiency anaemia: a review. Drug Res 2010;60(6a):399-412
2. Geisser P, Baer M, Schaub E. Structure/histotoxicity relationship of parenteral iron preparations. Drug Res 1992;42(II),12:1439-1452
3. Fletes R, Lazarus JM, Gage J, Chertow GM. Suspected iron dextran-related adverse drug events in hemodialysis patients. Am J Kidney Dis 2001;37:743-749
4. Bailie GR, Clark JA, Lane CE, Lane PL. Hypersensitivity reactions and deaths associated with intravenous iron preparations. Nephrol Dial Transplant 2005;20:1443-1449
5. Chertow GM, Mason PD, Vaage-Nilsen O, Ahlmén J. Update on adverse drug events associated with parenteral iron. Nephrol Dial Transplant 2006;21:378-382
6. Bailie GR, Hörl WH, Verhoef JJ. Differences in spontaneously reported hypersensitivity and serious adverse events for intravenous iron preparations: comparison of Europe and North America. Drug Res 2011;61:267-275
7. Van Wyck D, Anderson J, Johnson K. Labile iron in parenteral iron formulations: a quantitative and comparative study. Nephrol Dial Transplant 2004;19:561-565
8. Jahn MR, Andreasen HB, Fütterer S, *et al*. A comparative study of the physicochemical properties of iron isomaltoside 1000 (MonoFer®), a new intravenous iron preparation and its clinical implications. Eur J Pharm Biopharm 2011;DOI:10.1016/j.ejpb.2011.03.016
9. Santosh S, Podaralla P, Miller B. Anaphylaxis with elevated serum tryptase after administration of intravenous ferumoxytol. Nephrol Dial Transplant Plus 2010;3:341-342
10. Groman EV, Paul KG, Frigo TB, Bengele H, Lewis JM. Heat stable colloidal iron oxides coated with reduced carbohydrates and carbohydrate derivatives. US Patent 2003;No.6,599,498
11. Andreasen HB, Christensen L. Iron-dextran compound for the use as component in a therapeutical composition for prophylaxis or treatment of iron-deficiency. US patent 2001;No. 6291,440
12. Richter AW, Hedin HI. Dextran hypersensitivity. Immunol Today 1982;3:132-138
13. Hedin H, Richter W. Pathomechanisms of dextran-induced anaphylactoid/anaphylactic reactions in man. Int Archs Allergy appl Immunol 1982;68:122-126
14. Messmer K. Risiken der Infusion kolloidaler Lösungen. Infusionsther Transfusionsmed 1993;20:284-285
15. Richter W, Hedin H, Ring J, Kraft D, Messmer K. Anaphylaktoide Reaktionen nach Dextran. Allergologie 1980;3:51-58

16. Ljungström KG, Renck H, Hedin H, Richter W, Wiholm BE. Hapten inhibition and dextran anaphylaxis. Anaesthesia 1988; 3:729-732

17. Newman BA, Kabat EA. An immunochemical study of the combining site specificities of C57BL/6J monoclonal antibodies to α(1→6)-linked dextran B512. J Immunol 1985;135:1220-1231

18. Kabat EA, Bezer AE. The effect of variation in molecular weight on the antigenicity of dextran in man. Arch Biochem Biophys 1958;78:306-318

19. Allhoff T, Lenhart FP. Schwere dextraninduzierte anaphaylaktische/anaphylaktoide Reaktion (DIAR) trotz Haptenprophylaxe. Infusionsther Transfusionsmed 1993;20:301-306

20. Richter W, Kågedal L. Preparation of dextran-protein conjugates and studies of their immunogenicity. Int Arch Allergy Immunol 1972;42:885-902

21. Richter W. Micromethod for immunochemical quantitation of dextran and studies on role of antigen size in single radial immunodiffusion. Int Arch Allergy Immunol 1972;43:700-715

22. United States Pharmacopeial Convention: Iron Sucrose Injection, official monograph in: The United States Pharmacopeia. United States Pharmacopeial Convention, Tockville, MD, US 31,2008:2449-2451

23. Lu M, Cohen MH, Rieves D, Pazdur R. FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease. Am J Hematol 2010;85:315-319

24. Public Assessment Report, Scientific discussion, MonoFer® 100 mg/ml solution for injection/infusion (iron(III)isomaltoside 1000). SE/H/734/01/DC, 2009. http://www.lakemedelsverket.se/SPC_PIL/Pdf/par/Monofer%20solution%20for%20infusion-injection.pdf

25. Richter W. Minimal molecular size of dextran required to elicit heterologous passive cuteaneous anaphylaxis in guinea pigs. Int Arch Allergy 1972;43:252-268

26. Crichton RR, Danielson BG, Geisser P. Iron therapy with special emphasis on intravenous administration. 3rd ed.Bremen: UNI-MED Verlag, Germany, 2005:83

27. Kabat EA, Mayer MM. Experimental Immunochemistry. 2nd ed. Springfield, Illinois USA: Charles C Thomas, 1961:241-267

28. Geisser P, Burckhardt S. The pharmacokinetics and pharmacodynamics of iron preparations. Pharmaceutics 2011;3:12-33

Correspondence to:
Dr Susanna Burckhardt
Vifor (International) Inc.
Rechenstrasse 37
9001 St. Gallen,
Switzerland
e-mail: susanna.burckhardt@viforpharma.com