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Differences in tissue distribution of iron from various clinically used intravenous iron complexes in fetal avian heart and liver.

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ABSTRACT

Nanomedicines are more complex than most pharmacologically active substances or medicines and have been considered as non-biological complex drugs. For nanomedicines pivotal pharmacokinetic properties cannot be assessed by plasma concentration data from standard bioequivalence studies. Using intravenous iron complexes (IICs) as model we show that fetal avian tissues can be used to study time dependent tissue concentrations in heart and liver. Clear differences were found between equimolar doses of sucrose, gluconate or carboxymaltose coated iron particles. The range in tissue iron concentrations observed with these clinically widely used IICs provides an orientation as to what should be acceptable for any new IICs. Moreover, sensitivity of the experimental model was high enough to detect a 20% difference in tissue iron concentration. For the authorization of generic products under Article 10 (1) of Directive 2001/83/EC a plasma concentration of an active substance in the range of 80%–125% versus the reference product is usually considered acceptable. Based on its high discriminatory sensitivity this method was used to support a positive marketing authorization decision for a generic nanomedicine product.

1. Introduction

Nanomedicines (NMs[1]) are presumed to achieve an improved benefit-risk balance by an increased efficacy in pharmacological target organs and a reduced uptake in toxicological target organs resulting in decreased adverse effects.

Several decades before the scientific community and regulatory authorities recognized the particularities of NMs, iron nanoparticles have been introduced in clinical practice as intravenous iron complexes (IICs) for iron supplementation (Macdougall, 2009). Exceeding their use for the treatment of anemia, iron oxide nanoparticles have been or are currently developed for a broad range of medical uses (Liu et al., 2013, Ling and Hyeon, 2013). As IICs are solid nanoparticles of a rather toxic substance (iron oxide) they can be used only with a biocompatible complex carbohydrate coating (Ai et al., 2011) differences of which may exert pronounced effects on the cellular uptake of iron nanoparticles (Jahn et al., 2012). Although the active substance is iron in all products, clear differences in biodistribution and toxicity between the different complexes have been shown in clinical (Anirban et al., 2008; Hayat, 2008; Stefánssin et al., 2011; Okam et al., 2012) and non-clinial studies (Gupta et al 2010; Roth et al., 2008; Sturm et al., 2010; Toblli et al., 2010; Fütterer et al., 2013).

When NMs contain the same active substance (e.g. iron) as a previously approved NM in presumably the same pharmaceutical form and with equivalent pharmaceutical quality they might be considered as generic products to the reference NM. For their efficacy and safety no new data would be necessary but the data of the reference product could be used assuming essential similarity. As the administration route for most NMs is intravenous, it was even suggested that no clinical studies at all, not even the demonstration of bioequivalence would be required. The potentially pronounced effects that differences of the carbohydrate shell exert on the cellular uptake of iron nanoparticles (Jahn et al., 2012)

Abbreviations: IIC, intravenous iron complex; IICs, intravenous iron complexes; NM, nanomedicine; NMs, nanomedicines; NBCDs, non-biological complex drugs; CAM, chorioallantoic membrane; FCM, ferric carboxymaltose; IS, iron(III)-hydroxide sucrose complex; SFG, sodium ferric gluconate in complex.

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exclude this approach. But even for presumably identical or similar complexes from different manufacturers differences in the toxicological properties have been reported in clinical (Stein et al., 2012; Lee et al., 2013) and non-clinical studies (Toblli et al., 2012). Subtle differences in the pharmaceutical quality of NMs may conceivably result in relevant changes in the stability, cellular uptake and redistribution of the complex and alter efficacy and safety of the product. The immunological properties of IICs may cause anaphylactic and anaphylactoid reactions. For this dreaded effect, low-molecular-weight iron dextran has come in the focus of interest, but only the pharmacovigilance measures currently under way are expected to provide a meaningful answer to this issue. Differences in the organotropy may be relevant for various organs (e.g. heart, kidney). Iron-containing particles as used for the parenteral treatment of iron deficiency will predominately be taken up by the reticulo-endothelial system and may remain there for prolonged periods of time (Almeida et al., 2011), or may accumulate in other organs (Ghoti et al., 2012; Kobayashi et al., 2013). Any damage may become manifest only after prolonged treatment of the patients (Auerbach and Ballard, 2010) and is difficult to perceive in clinical studies or in clinical practice. Any generic NM to a reference NM will therefore require appropriately designed bridging studies for the demonstration of essential similarity to the reference product. Alleged essential similarity and bioequivalence concluded from the measurement of plasma concentration in humans will not be sufficient to detect potential differences in the distribution of iron in different tissues. In 2011 the European Medicines Agency published a reflection paper (EMA, 2011) detailing why the authorization of IIC products as generic products is not appropriate and what kind of non-clinical studies would be expected in support of generic nanoparticle iron medicinal product applications, followed four years later by an expanded reflection paper on both clinical and non-clinical data requirements.

Prior to the publication of EMA's first reflection paper on this issue (EMA, 2011), national authorities authorized generic IIC products. The data submitted in these MAA may seem insufficient from today's perspective and with today's knowledge. BfArM has been responsible as Reference Member State (RMS) for the marketing authorization for FerMed®. This product was licensed in Germany in 2009 as essential similar i.e. a generic to the reference product Venofer® without comparative data on the time dependent tissue concentrations of iron. It would seem prudent and in the interest of patients' safety to reconsider this decision or request additional data that would provide reassurance that the generic IIC product and the reference IIC product are essentially similar. Legally, however, the authorization is valid and no additional studies e.g. on tissue distribution of iron can be required from the marketing authorization holder unless new data relevant for the product become available that were not assessed previously during the approval procedure in 2009. In their responsibility as RMS for patients' safety both in Germany and in the other Members States where FerMed® is marketed BfArM initiated experimental studies on comparative iron tissue concentrations. These studies were expected to either support the presumed essential similarity of the approved generic product or else provide grounds to reconsider the marketing authorization and demand further studies from the marketing authorization holder.

2. Materials and methods

2.1. Hatching eggs

Chicken eggs (Clean Eggs®) were purchased from ValoBiomedia; Osterholz-Scharmbeck, Germany and incubated at 37.5 °C, 65% relative humidity and turned four times a day unless the egg shell had been opened and the CAM exposed.

All IIC products were purchased from a pharmacy: iron(III)-hydroxide sucrose complex (IS) as Venofer®, sodium ferric gluconate (SFG) as Ferrlecit®, ferric carboxymaltose (FCM) as Ferinject® and iron(III)-hydroxide sucrose complex as the generic product (ISg) as FerMed® (reference product Venofer®). For more details see Supplementary Table S1 in supplementary material.

2.2. Intravascular administration in blood vessels of the CAM

On the 7th day of incubation the apical pole of the egg was swabbed with alcohol. A small hole of less than a millimeter in diameter was drilled in the egg shell at the apical pole of the egg. The hole was sealed with adhesive tape (Leukosilk®-Klebeband from BSN medical GmbH, Hamburg, Germany). Eggs were returned to the incubator and incubation continued with the eggs in upright position (apical pole up) without rotation. On the 14th day of incubation, the hole was extended to approximately 2.5 cm in diameter. The suitability of CAM vessels for injection was assessed with a binocular. Eggs without or with insufficient vascularization were discarded. Injection needles Kanuele 30G 0.30 × 12 mm PZN 1410036, Sterican, Braun, Melsungen, Germany were used in all experiments. For all test products, equimolar doses of iron (400 μg, 200 μg or 100 μg Fe^{3+}) were administered in equal injection volume (volume adjustment with 0.9% saline) of 40 μl per egg into CAM vessels. Eggs with significant bleeding at the injection site or extravasation of the very dark injection solutions were discarded. The hole in the shell was covered with a watch glass fixed with adhesive tape and incubation was continued with the eggs in upright position (apical pole up) without rotation.

2.3. Tissue sampling

At 1, 4, 24, 48, 72, and 120 h after injection the fetuses were removed and decapitated. The abdominal cavity was opened and the whole liver and heart were removed, weighed and fixed in 10% phosphate buffered formalin (containing 4% formaldehyde) for histology or used for the measurement of tissue iron content.

2.4. Iron content

Iron in liver and heart tissue was measured as described by Rebouche et al., 2004. Histological sections were used for histochemical demonstration of tissue iron according to Mulisch and Welsch 2010.

3. Results

3.1. Time and dose dependent uptake of iron sucrose in liver tissue and distribution in different cell types

After injection of IS into CAM vessels the IIC nanoparticles are rapidly taken up by phagocytosis. High liver iron concentrations were seen as early as 1 h after injection. The histochemical demonstration of intracellular iron by the Prussian Blue reaction shows very dense iron deposits in the cells of the RES during the first few hours after administration (supplementary material, Fig. S1). Later, when iron has been redistributed to the parenchymal cells the iron deposits appear much less dense and are more diffusely distributed in the cytoplasm of the hepatocytes, presumably indicating an increasing degradation of the nanoparticles (supplementary material, Fig. S2).

Liver iron concentrations were highest 4 h and 24 h after injection and clearly decreased at 72 h and 120 h (Fig. 1). Liver iron concentration values in controls increased with increasing age of