Minimally Invasive Transdermal Delivery of Iron–Dextran

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ABSTRACT: Iron deficiency is one of the most prevalent and serious health issues among people all over the world. Iron–dextran (ID) colloidal solution is one among the very few US Food and Drug Administration (FDA)-approved iron sources for parenteral administration of iron. Parenteral route does not allow frequent administration because of its invasiveness and other associated complications. The main aim of this project was to investigate the plausibility of transdermal delivery of ID facilitated by microneedles, as an alternative to parenteral iron therapy. *In vitro* permeation studies were carried out using freshly excised hairless rat abdominal skin in a Franz diffusion apparatus. Iron repletion studies were carried out in hairless anemic rat model. The anemic rats were divided into intact skin (control), microneedle pretreated, and intraperitoneal (i.p.) groups depending on the mode of delivery of iron. The hematological parameters were measured intermittently during treatment. There was no improvement in the hematological parameters in case of control group, whereas, in case of microneedle pretreated and i.p. group, there was significant improvement within 2–3 weeks. The results suggest that microneedle-mediated delivery of ID could be developed as a potential treatment method for iron-deficiency anemia. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:987–993, 2013

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INTRODUCTION

Iron-deficiency anemia is one of the most prevalent and serious health issues among people all over the world. Iron is an essential element involved in the production of red blood cells (RBCs) and also plays a critical role in cellular metabolism, catalyzing many enzymatic reactions, mediating immune response, and helps in the production of connective tissues and neurotransmitters in brain.[1,2] Iron deficiency is known to be more prevalent in infants, children, and women of child-bearing age. Inadequate iron stores in the body could lead to several serious health consequences and even death.[3–6]

Treating iron deficiency and its anemia is still a challenge because of the limitations associated with existing oral and parenteral formulations. Despite the convenience and compliance associated with oral therapy, it is often associated with severe side effects such as gastric intolerance nausea and vomiting.[7,8] Also, oral iron supplements have limited potential in treating anemia associated with conditions such as accidental blood loss, hemodialysis, malabsorption syndrome, Crohn's disease, inflammatory bowel disease, and chronic bowel obstruction; hence parenteral iron therapy becomes inevitable. Iron–dextran (ID) complex was one among the very few products approved and extensively studied clinically for parenteral therapy to treat iron-deficiency anemia. ID is commercially available as stable, clear, viscous, and reddish-brown colloidal suspension containing 5% iron and 20% dextran.[9]

Parenteral ID administration is associated with immediate adverse events such as dyspnea,

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abdominal or back pain, nausea and vomiting, fever, and utricaria.[10] Fatal anaphylactic reactions were also reported with ID therapy.[11–13] Generally, parenteral iron therapy is considered to be safe and efficacious, but repeated administration could potentially result in toxic amounts of free iron in the blood; sometimes even could prove fatal. Slow and prolonged delivery of iron has been suggested as the best suited way to avoid supersaturation of iron-carrier protein, transferrin, and to control iron stores in the systemic circulation.[14]

Transdermal administration is generally intended for delivery of drugs across the skin over long duration simulating slow intravenous infusion.[15] However, transdermal delivery of therapeutic agents is limited because of the high molecular weight (>600 Da) and high hydrophilicity.[16] Chemical enhancers are known to possess limited ability to enhance the permeation of larger molecular weight therapeutic molecules. Use of microneedles is of great interest in recent days because of their unique ability to facilitate the delivery of macromolecules and colloidal drugs across the skin.[17,18] Microneedles can create micro conduits for transport of drug molecules across the stratum corneum.[19] In the current study, the feasibility of transdermal delivery of ID using microneedles was investigated. Successful delivery of ID via transdermal route could be a potential option for treating iron-deficiency anemia.

MATERIALS AND METHODS

Materials

Iron–dextran (50 mg/mL) with molecular weight in between 80 and 100 kDa was purchased from Sigma–Aldrich (St. Louis, Missouri). AdminPen 600 device was purchased from nanoBio Sciences LLC, Alameda, California. Phosphate buffered saline (PBS, pH 7.4) premixed powder was obtained from EMD Chemicals (Gibbstown, New Jersey). Ferrover® iron reagent was obtained from Hach Company (Loveland, Ohio). Serum iron (SI) and total iron binding capacity (TIBC) kit were obtained from Cliniqa Corporation (San Marcos, California), and all other chemicals were obtained from Fischer Scientific (Fairway, New Jersey).

Methods

Preparation of Rat Skin

Male hairless rats were used in both *in vitro* and *in vivo* studies, obtained from Charles River, Wilmington, Massachusetts. The use of hairless rat skin has been reported to be a good model for infants and children's skin. All the animals were 8 weeks old and weighing between 250 and 300 g. For the preparation of rat skin, the animals were asphyxiated with CO_2, and the abdominal skin was excised, subcutaneous fat was removed, and the skin pieces were cleaned carefully with normal saline. The rat skin was used on the same day for all *in vitro* experiments. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi (protocol #10-013).

Measurement of Hydrodynamic Radius of ID

Particle size (hydrodynamic radius) of the ID colloid was measured by dynamic light scattering (DLS) or photon correlation spectroscopy technique using Zetasizer 3000HSA (Malvern Instruments Ltd., Westborough, Massachusetts).

Skin Pretreatment with Microneedles

Freshly obtained rat skin was treated with AdminPen 600 stainless steel microneedles, having an area of 1 cm^2 containing 187 microneedles with height of 500 μm, for 2 min and embodied in optimum cutting temperature (OCT) medium (Tissue-Tek®, Sakura Finetek Inc, Torrance, CA, USA). Microneedles are designed as central hollow bore that are similar in shape to conventional hypodermic needles but much smaller.[20] The OCT medium with skin was subjected to freeze in dry ice bath and 30 μm thickness sections were prepared using a Leica 1800 cyrostat (Leica Biosystems, Buffalo Grove, IL). Skin specimens were allowed to dry and stained with hematoxylin and eosin. The developed stained specimens were observed under a high-resolution microscope (Axiolab A1; Carl Zeiss, Thornwood, NY, USA) with 10× magnification to evaluate the depth of penetration of microneedles. Images were captured with camera (Axio ICc 1; Carl Zeiss) attached to the microscope.

General In vitro Experimental Setup

In vitro studies were carried out in vertical Franz diffusion cell (FDC) apparatus (Logan Instruments, Boston, Massachusetts). The rat skin was sandwiched between the donor and receiver compartments of FDC, with stratum corneum facing the donor compartment of the cell. The active diffusion area was 0.64 cm^2. The AC electrical resistance of skin was measured with the help of an electric circuit consisting of a digital multimeter and waveform generator (Agilent Technologies, Santa Clara, California) having a load resistor R_L (100 kΩ) in series with the skin. The voltage drop across the whole circuit (V_0) and across the skin (Vs) was measured, and skin resistance was determined by applying a voltage of 100 mV at 10 Hz in the circuit. Skin pieces with a resistance of at least 20 kΩcm^2 were considered for permeation studies.[21]

In vitro Transdermal Permeation Studies

Permeation of ID. After measuring electrical resistance of the skin, the donor compartment was filled