Minimally Invasive Transdermal Delivery of Iron–Dextran

ABHISHEK JULURI,[1] NARESH MODEPALLI,[1] SEONGBONG JO,[1] MICHAEL A. REPKA,[1] H. NANJAPPA SHIVAKUMAR,[2,3] S. NARASIMHA MURTHY[1]

[1]Department of Pharmaceutics, University of Mississippi, University, Mississippi 38677

[2]KLE University's College of Pharmacy, Rajajinagar, Bangalore, Karnataka, India

[3]Dermaperm Research Inc., Bangalore, Karnataka, India

Received 24 October 2012; revised 16 November 2012; accepted 30 November 2012

Published online 20 December 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23429

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

Keywords: Transdermal drug delivery; anemia; iron-dextran; macromolecular drug delivery; microarrays; microscopy; skin

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,

Correspondence to: S. Narasimha Murthy: (Telephone: +662-915-5164; Fax: +662-915-1177; E-mail: murthy@olemiss.edu)

Abhishek Juluri and Naresh Modepalli contributed equally to the work.

Journal of Pharmaceutical Sciences, Vol. 102, 987–993 (2013)

© 2012 Wiley Periodicals, Inc. and the American Pharmacists Association

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

with 200 μL of 50 mg/mL ID solution, and the receiver compartment was filled with 5 mL of freshly prepared PBS (pH 7.4). During all *in vitro* permeation studies, the temperature of receiver compartment was maintained at 37 ± 1 C by water circulation. Permeation studies were carried out for 6 h, and 1 mL samples were collected from the receiver compartment at predetermined time points (0, 1, 2, 3, 4, 5, and 6 h). Amount of iron permeated into the receiver compartment across skin was determined with the help of EZ201 UV spectrophotometer (PerkinElmer, Waltham, Massachusetts) using FerroVer® iron reagent (Hach Company) at 510 nm.[14]

To study the effect of microneedle pretreatment on the permeation of ID, freshly excised hairless rat skin was pretreated with microneedles for 2 min before mounting on the FDC apparatus.

ID Retained in the Skin. After *in vitro* permeation study, the active diffusion area (0.64 cm^2) was excised with biopsy punch, and the surface was washed thoroughly with normal saline to ensure complete removal of any ID adhering to the skin surface. The biopsied skin was then cut into small pieces and homogenized in a vial containing 5 mL of 1 N sodium hydroxide and incubated at 37°C for 24 h with intermittent shaking. The solutions were centrifuged to remove any interfering substance, and supernatant was collected and analyzed for iron content.

Iron Repletion Studies in Anemic Rats

Induction of Iron-Deficiency Anemia in Rats. In vivo studies were performed in hairless rats. The animals were housed in conventional cages with 12:12 h day–light cycles maintained in the facility during the entire study period. Rats ($n = 18$) were on normal diet and were allowed to adapt to the study environment for a week's time. Initially, 200 μL of blood samples were collected in ethylenediaminetetraacetic acid-coated Microvette® tubes (Sarstedt, Newton, North Carolina), and 0.5 mL of blood was collected into 1.5 mL centrifuge tubes (Eppendorf, Hauppauge, New York) from all animals by retro-orbital bleeding method. Blood samples collected were analyzed for hematological parameters such as hemoglobin (Hb), hematocrit (HCT), RBC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using VetScan HM2 hematology system (Abaxis, Union city, California). Serum was separated from blood sample collected in centrifuge tubes and analyzed for biochemical parameters such as SI, TIBC, and percent transferring saturation (%TS) (basal values in healthy normal rats). Following initial screening, rats were kept on diet with low iron content (∼2–6 ppm; Harlan Laboratories, Madison, Wisconsin) till the end of the study. Iron deficiency was induced in rats in 5 weeks since the inception of the custom-made low-iron diet, which was further confirmed by measuring the blood Hb and HCT. Later, rats were divided into three groups ($n = 6$), namely control group, microneedle-pretreated group, and intraperitoneal (i.p.) treatment group.

Delivery of ID, In vivo. Animals in the control and microneedle pretreatment group were anesthetized using ketamine + xylazine (80 + 10 mg/kg) via i.p. route. In case of intact skin (control) group animals, a transdermal patch loaded with 200 μL of 50 mg/mL ID was placed on the dorsal side of the rat for 6 h. For microneedle-pretreated group, rats were pretreated with microneedles (10 cm^2) on dorsal surface for 2 min followed by the application of a transdermal patch, loaded with 200 μL of 50 mg/mL ID solution for a period of 6 h. The treatment was continued for a period of 3 weeks on alternate days. The i.p. group received 100 μL of ID solution (10 mg/mL) in saline on alternate days for 2 weeks.

RBC Morphology

Blood samples were withdrawn from rats in healthy, anemic, and posttreatment conditions from all the three groups, and the RBCs were visualized under high-resolution optical microscopy to study their cell morphology. In brief, a drop of venous blood was collected and smears were prepared by wedge slide method and stained using Wright–Giemsa stain. The RBCs were visualized using oil immersion microscopy and high-resolution light microscope (Axiolab A1; Carl Zeiss) at 100× magnification. Images of RBCs were captured using Carl Zeiss camera attached to the microscope.

Statistical Analysis

GraphPad InStat 3 software was used for statistical analysis. One-way analysis of variance was used to determine the level of significance for correlation between parameters, and a P value of less than 0.05 was considered as the significant difference.

RESULTS AND DISCUSSION

Skin is known to be a formidable barrier to the penetration of large molecular size therapeutic agents.[13] However, certain studies have demonstrated permeation of macromolecular substrates across the skin.[22] Some reports even demonstrated the penetration of particulate drug delivery systems, ranging from nanometer to micrometer size[23,24] into the skin. Recently, Sonavane et al.[25] have demonstrated the *in vitro* delivery of gold nanoparticles (NPs) of various sizes across rat abdominal skin. The authors reported that NPs with average size of 15 nm permeated across rat abdominal skin in significantly

higher amounts than those of 102 and 198 nm at the end of 12 h. The X-ray spectroscopy studies revealed deeper localization of 15 nm size particles than the larger particles.[25]

The hydrodynamic diameter of ID was measured using DLS technique and was found to be approximately 14 nm. In the present study, even at the end of 12 h (data shown up to 6 h), there was no ID detected in the receiver compartment, indicating practically no permeation across the rat skin. Moreover, there was no ID detected in the skin either. Despite all the *in vitro* experimental conditions being similar, at this stage, the reasons for conflicting observation between our studies and the studies by Sonavane et al.[25] are still unknown.

So far, many research groups have studied the effect of microneedles on transdermal delivery of wide range of molecules with different physicochemical properties.[26–29] Microneedles are micron-sized needles and have the potential to deliver molecules and macromolecules across the skin without causing significant pain.[30] Several research groups have studied the effect of microneedles on delivering both small and large molecules including proteins, peptides, and genes into the skin.[31] Several reports exist on the delivery of particulate systems utilizing microneedles via transdermal route. Kohli and Alpar[32] demonstrated that NPs of 50 nm or less in size can penetrate into the skin layers. Therefore, the effect of microneedle pretreatment of skin on the permeation of ID was investigated in this study.

In vitro Permeation of ID

In vitro permeation studies of ID were carried out for 6 h after pretreating the rat skin with microneedles. The cumulative amount of ID permeated at the end of 6 h was found to be 10.28 ± 0.45 μg/cm^2 (Fig. 1). After 6 h of *in vitro* permeation studies, 2.48 μg/mg of ID was found to have retained in the skin. *In vitro* permeation studies concluded that microneedle pretreatment could lead to the delivery of substantial amount of ID across the skin. The studies also clearly demonstrated that the colloidal ID neither penetrates nor permeates across the intact skin in detectable amounts.

Iron Repletion Studies in Anemic Rats

Initially, healthy animals were checked for all hematological and biochemical parameters and recorded as basal values. Iron-deficiency anemia was induced in all animals ($n = 18$) by feeding with low-iron diet for 5 weeks. There was a significant change in the hematological parameters, indicating successful induction of anemia. The first step in the typical diagnosis of iron-deficiency anemia includes measuring the Hb levels in the blood. Along with Hb levels, measurement of MCV, HCT, and other RBC indices are

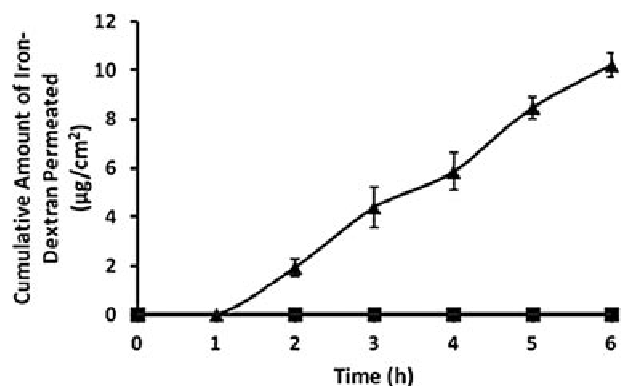

Figure 1. *In vitro* permeation studies of iron–dextran across the (■) intact hairless rat skin (Control) and (▲) skin pretreated with microneedles.

critical in assessing the status of iron-deficiency anemia. HCT measures the percent volume of RBCs in unit volume of whole blood. MCV gives the average volume and size of RBC, and in case of iron-deficiency condition, microcytic and hypochromic erythrocytes are usually observed. The RBC morphology in iron-deficient rat clearly shows the presence of microcytic RBC (Fig. 4b). MCH is the average mass of Hb in RBC, and MCHC is the measure of concentration of Hb in a given volume of packed RBCs. The RBC distribution width is a measure of the variation of RBC width and is used in combination with the MCV in differential diagnosis of various other anemic conditions. In this study, the mean Hb and RBC values at healthy condition were 14.43 ± 0.81 g/dL and $8.59 \pm 0.44 \times 10^{12}$/L, respectively, and were found to decrease to 10.06 ± 1.05 g/dL and $6.32 \pm 0.59 \times 10^{12}$/L when the rats were induced to anemic condition.

Serum samples were analyzed for biochemical parameters. The average values of SI before and after inducing anemia were found to be 187.96 ± 3.04 and 91.20 ± 10.58 μg/dL, respectively. The TIBC values increased from 351.60 ± 16.64 to 556.90 ± 58.39 μg/dL. There was a decrease in mean %TS from 42.43 ± 3.12 to 16.01 ± 0.76 after inducing anemia compared with healthy state. SI and %TS decreased significantly, whereas the TIBC values increased after inducing anemia. SI measures the transferrin bound iron in blood, and TIBC measures the amount of transferrin that is still available to bind and transport iron. %TS gives the ratio of SI to TIBC, which is an accurate measurement compared with individual measurements of SI and TIBC. Mean values of other hematological parameter of healthy and anemic condition of all the rats ($n = 18$) are shown in Table 1.

Transdermal Delivery of ID, In vivo

The anemic rats were divided into three groups and were treated with ID using different modes of delivery. Polyolefin foam patch of area 10 cm^2 was

Table 1. The Mean Values of Hematological Parameters, Prior to Inducing Anemia (Healthy), After Inducing Anemia, After Treating with ID in Case of Microneedle Pretreatment, and After Treating with ID via Intraperitoneal Route

Hematological Parameter	Healthy Rats	Anemic Condition	After Microneedle Pretreatment	Intraperitoneal Group
Hemoglobin (g/dL)	14.43 ± 0.81	10.06 ± 1.05	13.96 ± 0.51	14.5 ± 0.22
RBC ($\times 10^{12}$/L)	8.59 ± 0.44	6.32 ± 0.59	10.25 ± 0.95	10.32 ± 0.25
Hematocrit (%)	42.65 ± 1.28	33.24 ± 3.37	41.87 ± 4.98	41.48 ± 0.91
Mean corpuscular volume (fL)	55.33 ± 4.12	45.31 ± 3.07	41.33 ± 2.25	46.03 ± 2.02
Mean corpuscular hemoglobin (pg)	19.30 ± 1.58	14.93 ± 0.68	14.86 ± 0.68	18.30 ± 0.75
Mean corpuscular hemoglobin concentration (g/dL)	35.39 ± 0.87	30.00 ± 1.83	32.03 ± 2.21	34.01 ± 0.73
Red blood cell distribution width (%)	16.47 ± 0.81	15.47 ± 3.87	16.73 ± 1.50	17.73 ± 0.73

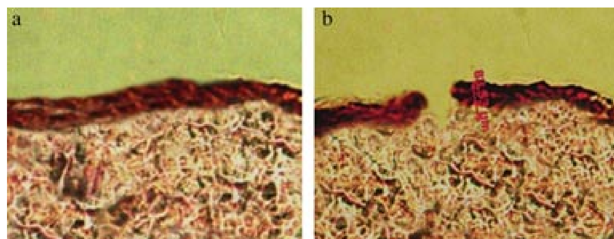

Figure 2. Histological sections of skin stained with hematoxylin and eosin stain (10× magnification) intact skin (a) and microneedle-pretreated skin (b).

loaded with 200 μL of ID solution and placed on the dorsal surface of the rats in case of intact skin (control) group, whereas in case of microneedle-pretreated group, the dorsal surface of the rat was pretreated with microneedles and then the patch was placed on the treated surface for 6 h. After removal of the patch, the skin surface was washed with soap and water to reduce the intensity of stain because of the adhering ID. The remaining mild stain completely disappeared eventually within a couple of days. This cosmetic factor could be one of the limiting factors for use of transdermal systems of ID. However, in case of patients who are critically anemic, this would be an insignificant issue. The microscopic pictures in Figure 2 represents the microneedle-treated and untreated sections of skin. The objective of the microneedle technology is to render the skin permeable by penetrating the needles through the epidermis but not far enough down to reach the deeper layers to minimize the discomfort to the subject. In the present study, the microneedles penetrated through a depth of 84.45 ± 10.32 μm. Figure 2b is a representative picture of the micropore created by microneedles.

The treatment with ID was continued for 3 weeks on alternate days. Blood samples were collected by retro-orbital bleeding method and subjected to all hematological and biochemical tests intermittently and at the end of weeks 2 and 3. In the case of control group, the hematological or biochemical parameters turned out to be severely poor at the end of week 2. This indicates that there is no feasibility of delivery of ID via passive transdermal delivery without microneedle pretreatment. Therefore, the passive transdermal delivery (control group) treatment was not continued to survive the rats. However, in the case of microneedle-pretreated group, there was a significant improvement in all the parameters at the end of week 3. The mean biochemical parameter values, prior to inducing anemia, after inducing anemia, and after treatment with ID, in case of microneedle-pretreated group and i.p. injection group, are shown in Figure 3.

The mean Hb value of 10.36 ± 0.57 g/dL at anemic condition increased to 13.96 ± 0.51 g/dL in microneedle-pretreated group. An increase in the Hb level of 1 g/dL for every 2–3 weeks is considered as an effective iron replacement therapy.[33,34] The mean SI, TIBC, and %TS at the end of the study in case of microneedle-treated group were found to be 137.63 ± 8.07 μg/dL, 334.23 ± 6.14 μg/dL, and 39.03 ± 4.75, respectively (Fig. 3). Mean values of all hematological parameters are shown in Table 1. There was a significant improvement in the morphology of RBCs in microneedle treatment group of rats compared with anemic condition (Fig. 4c).

Intraperitoneal Delivery of ID

Iron–dextran was administered via i.p. route on alternate days to one of the groups to serve as positive control. There was statistically significant improvement in all the biochemical and hematological parameters in the positive control group. The mean SI, TIBC, and %TS values at the end of the study was found to be 147.30 ± 15.56 μg/dL, 366.90 ± 14.07 μg/dL, and 40.29 ± 5.78 followed by i.p. injection of ID (Fig. 3). All other hematological parameters are shown in the Table 1.

Iron–dextran is known to undergo ready uptake by the reticuloendothelial system from the intravascular fluid compartments and processed to make the iron available to the body for different biochemical and physiological needs.[35] Treatment of iron deficiency with ID solution via intravenous route is well established.[36] In the present case, the levels of Hb and other parameters were close to that of normal condition (baseline values) at the end of week 2 in

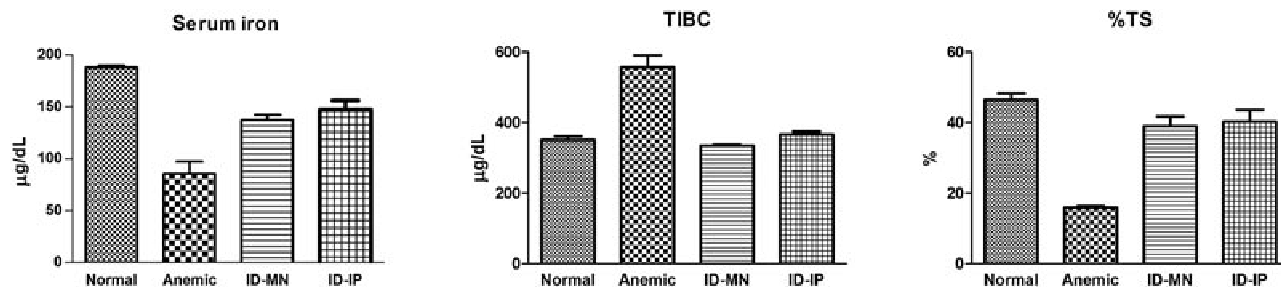

Figure 3. The mean values of all biochemical parameters, prior to inducing anemia (normal), after inducing anemia (anemic), after treating with ID [microneedle pretreatment (ID-MN)] and treatment with intraperitoneal injection of ID (ID-IP).

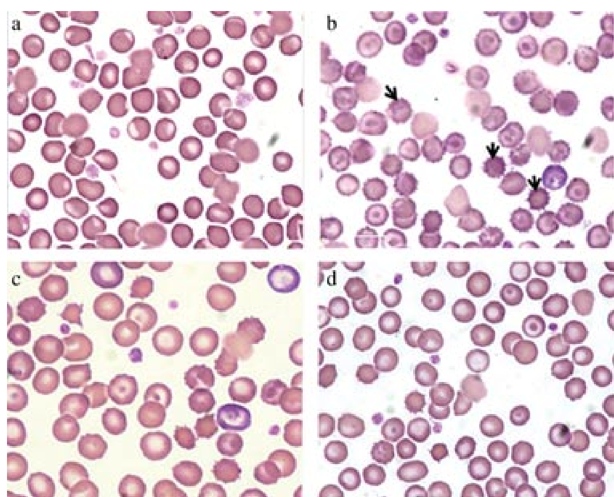

Figure 4. Representative pictures of morphology of RBCs. (a) RBCs in healthy rats, (b) RBCs of anemic rats (arrows point the microcytic and hypochromic RBC), (c) RBCs of rats treated with ID following microneedle pretreatment, and (d) RBC of rats received ID through intraperitoneal injection.

case of positive control group (i.p.). On the other hand, in case of microneedle-treated group, biochemical and hematological parameters became statistically insignificant from its basal levels at the end of third week.

CONCLUSIONS

Transdermal delivery of therapeutic agents allows slow and prolonged delivery, which is essential for delivering iron to avoid the saturation of transferrin and to control the free iron stores in the systemic circulation. This study demonstrates the feasibility of microneedle-based transdermal delivery as one of the potential routes for iron replenishment. *In vitro* permeation studies resulted in the delivery of substantial amount of ID across the hairless rat skin, when it was pretreated with microneedles. Iron repletion studies in anemic hairless rat model revealed that there was no improvement in the hematological parameters in case of control group, whereas, in case of i.p. and microneedle-pretreated group, there was a significant improvement in all the hematological parameter at the end of weeks 2 and 3, respectively. ID delivered transdermally in the form of poke and patch is likely to overcome the limitations of parenteral delivery of ID and is believed to be relatively more patient compliant because of the minimal invasive nature of the mode of delivery.

ACKNOWLEDGMENTS

This project was partially funded by a grant number HD061531A from Eunice Kennedy Shriver National Institute of Child Health and Human Development.

The authors would like to thank Dr. Mohammad Khalid Ashfaq (Senior Research Scientist, NCNPR, University of Mississippi) for the VetScan HM2 hematology instrument and Mr. Rajnish Sahu (NCNPR, University of Mississippi) for his help with RBC morphology studies. The authors would also like to acknowledge the valuable input from Dr. Phaniraj Cegu, Pharmacist, Wallgreens, Memphis, Tennessee.

Authors report no conflict of interest.

REFERENCES

1. Chitambar CR. 2005. Celluar iron metabolism: Mitochondria in the spotlight. Blood 105(5):1844–1845.
2. Cherayil BJ. 2011. The role of iron in immune response to bacterial infection. Immunol Res 50(1):1–9.
3. Brabin BJ, Premji Z, Verhoeff F. 2001. An analysis of anemia and child mortality. J Nutr 131(2):6365–6485.
4. Li FP, Alter BP, Nathan DG. 1972. The mortality of acquired aplastic anemia in children. Blood 40(2):153–162.
5. Lozoff B, Brrittenham GM, Wolf AW, McClish DK, Kunhert PM, Jimenez E, Jimenez R, Mora LA, Gomez I, Krauskoph D. 1987. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics 79(6):981–995.
6. Brabin BJ, Hakimi M, Pelletier D. 2001. An analysis of anemia and pregnancy related maternal mortality. J Nutr 131(2):6045–6155.
7. Barton JC, Barton EH, Bertoli LF. 2000. Intravenous iron dextran therapy in patients with iron deficiency and normal

renal function who failed to respond to or did not tolerate oral iron supplementation. Am J Med 109:27–32.
8. Henry DH, Dahl NV, Auerbach M. 2007. Intravenous ferric gluconate significantly improves response to epoetin alfa versus oral iron or no iron in anemic patients with cancer receiving chemotherapy. Oncologist 12:231–242.
9. Report on carcinogens. 2011. Iron dextran complex. 12th ed. pp 246–247.
10. Burns DL, Pomposelli JJ. 1999. Toxicity of parenteral iron dextran therapy. Kidney Int 55(69):S-119–S-124.
11. Auerbach M, Witt D, Toler W, Fierstein M, Lerner RG, Ballard H. 1988. Clinical use of the total dose intravenous infusion of iron dextran. J Lab Clin Med 111:566–570.
12. Hamstra RD, Block MH, Schocket AL. 1980. Intravenous iron dextran in clinical medicine. JAMA 243(17):1726–1731.
13. Blake DR, Lunec J, Ahern M, Ring EF, Bradfield J, Gutteridge JM. 1985. Effect of intravenous iron dextran on rheumatoid synovitis. Ann Rheum Dis 44:183–188.
14. Murthy SN, Vaka SR. 2009. Irontophoresis: Transdermal delivery of iron by iontophoresis. J Pharm Sci 98(8):2670–2676.
15. Lawrence AH. 2009. Transdermal pharmaceuticals: Unique aspects of clinical development. Transderm Mag 1(1):14–18.
16. Potts RO, Guy RH. 1992. Predicting skin permeability. Pharm Res 9(5):663–669.
17. Sachdeva V, Banga AK. 2011. Microneedles and their applications. Recent Pat Drug Deliv Formul 5(2):95–132.
18. Coulman SA, Barrow D, Anstey A, Gateley C, Morrissey A, Wilke N, Allender C, Brain K, Birchall JC. 2006. Minimally invasive cutaneous delivery of macromolecules and plasmid DNA via microneedles. Curr Drug Deliv 3(1):65–75.
19. Prausnitz MR. 2004. Microneedles for transdermal drug delivery. Adv Drug Deliv Rev 56:581–587.
20. Vadim VY. 2010. The AdminPen^{TM} microneedle device for painless and convenient drug delivery. Adv Deliv Dev 10(4):32–36.
21. Sammeta SM, Vaka SRK, Murthy SN. 2010. Transcutaneous electrporation mediated delivery of doxepin–HPCD compels: A sustained release approach for treatment of postherpetic neuralgia. J Cont Rel 142:361–367.
22. Praunitz MR. 1997. Transdermal delivery of macromolecules: Recent advances by modification of skin's barrier properties. In Therapeutic protein and peptide formulation delivery; Shahrokh Z, Sluzky V, Cleland JL, Shire SJ, Randolph TW, Eds. Vol. 675. Washington, DC: American Chemical Society, pp 124–153.
23. Cappel MJ, Kreuter J. 1991. Effect of nanoparticles on transdermal drug delivery. J Microencapsul 8(3):369–374.
24. Prow TW, Grice JE, Linn LL, Faye R, Butler M, Becker W, Wurm EM, Yoong C, Robertson TA, Soyer HP, Roberts MS. 2011. Nanoparticles and microparticle for skin drug delivery. Adv Drug Deliv Rev 63(6):470–491.
25. Sonavane G, Tomoda K, Sano A, Oshima H, Terada H, Makino K. 2008. *In vitro* permeation of gold nanoparticles through rat skin and rat intestine: Effect of particle size. Colloids Surf B Biointerfaces 65(1):1–10.
26. Martanto W, Davis SP, Holiday NR, Wang J, Gill HS, Prausnitz MR. 2004. Transdermal delivery of insulin using microneedles *in vivo*. Pharm Res 21:947–952.
27. Badran MM, Kuntsche J, Fahr A. 2009. Skin penetration enhancement by a microneedle device (Dermaroller$^{®}$) *in vitro*: Dependency on needle size and applied formulation. Eur J Pharm Sci 36:511–523.
28. Ding Z, Verbaan FH, Bivas BM, Bungener L, Huckriede A, Van den Berg DJ, Kersten G, Bouwstra JA. 2009. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. J Control Rel 136:71–78.
29. Donnelly RF, Morrow DI. McCarron PA, Woolfson AD, Morrissey A, Juzenas P, Juzeniene A, Iani V, McCarthy HO, Moan J. 2008. Microneedle mediated intradermal delivery of 5-aminolevulinic acid: Potential for enhanced topical photodynamic therapy. J Control Rel 129:154–162.
30. Kaushik S, Hord AH, Denson DD, Mcallister DV, Smitra S, Allen MG, Prausnitz, MR. 2001. Lack of pain associated with microfabricated microneedles. Anesth Analg 92:502–504.
31. Chang RI, Moon SK, Lee HB, Han KI, John MR, Gilson K. 2007. The effect of molecular weight of drugs on transdermal delivery system using microneedle device. Key Eng Mater 342(3):945–948.
32. Kohli AK, Alpar HO. 2004. Potential use of nanoparticles for transcutaneous vaccine delivery: Effect of particle size and charge. Int. J. Pharm 275:13–17.
33. Provan D. 1999. Mechanisms and management of iron deficiency anemia. Br J Haematol 105 (Suppl 1):19–26.
34. Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, Roger SD, Savoia HF, Tampi R, Thomson AR, Wood EM, Robinson KL. 2010. Diagnosis and management of iron deficiency anaemia: A clinical update. Med J Aust 193(9):525–532.
35. Auerbach M, Ballard H. 2010. Clinical use of intravenous iron: Administration, efficacy, and safety. Hematol Am Soc Hematol Educ Prog 18:338–347.
36. Reddy DK, Moore HL, Lee JH, Saran R, Nolph KD, Khanna R, Twardowski ZJ. 2001. Chronic peritoneal dialysis in iron-deficient rats with solutions containing iron dextran. Kidney Int 59:764–773.