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Transdermal Delivery of Iron Using Soluble Microneedles: Dermal Kinetics and Safety

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ABSTRACT

Currently, the iron compounds are administered via oral and parenteral routes in patients of all ages, to treat iron deficiency. Despite continued efforts to supplement iron via these conventional routes, iron deficiency still remains the most prevalent nutritional disorder all over the world. Transdermal replenishment of iron is a novel, potential approach of iron replenishment. Ferric pyrophosphate (FPP) was found to be a suitable source of iron for transdermal replenishment. The safety of FPP was assessed in this project by challenging the dermal fibroblast cells with high concentration of FPP. The cell viability assay and reactive oxygen species assay were performed. The soluble microneedle array was developed, incorporated with FPP and the kinetics of free iron in the skin; extracellular fluid following dermal administration of microneedle array was investigated in hairless rats. From the cell based assays, FPP was selected as one of the potential iron sources for transdermal delivery. The microneedles were found to dissolve in the skin fluid within 3 hours of administration. The FPP concentration in the dermal extracellular fluid declined after complete dissolution of the microneedle array. Overall, the studies demonstrated the safety of FPP for dermal delivery and the feasibility of soluble microneedle approach for transdermal iron replenishment therapy.

Introduction

Transdermal delivery of iron with the help of various biophysical enhancement techniques was reported earlier.[1,2] Electrically mediated techniques such as iontophoresis and electroporation were able to enhance the transdermal delivery of ferric pyrophosphate (FPP) (a valuable transdermal iron source) significantly over the passive delivery. Microporation of the skin, that is, pretreatment with microneedles, was found to enhance the efficiency of iontophoresis even more in case of transdermal iron delivery.[3] Soluble microneedle system is an upcoming promising area of dermal/transdermal delivery of drugs. Soluble microneedle system consists of an array of microneedles prepared using water-soluble polymers loaded with the therapeutic agent. The therapeutic agent would be released into the skin tissue fluids on penetration of the microneedles. Development of a self-administrable dissolvable microneedle array system for delivery of iron can offer great advantages over oral and parenteral modes of administration. In the present study, soluble microneedles incorporated with FPP were developed to deliver iron to meet higher iron demand in case of moderate to severe anemic conditions. Higher doses requirements could be met by increasing the area of the patch, increasing the drug load in the microneedles, and by increasing the frequency of administration. FPP was incorporated into soluble microneedle array and the microneedle system was used to investigate the dermal kinetics of FPP in the hairless rat model. The safety of administered iron compound was evaluated using human dermal fibroblast (HDF) cell lines. Cell viability studies and reactive oxygen species (ROS) assay were performed to assess the safety of FPP at different concentrations.

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Materials and Methods

Materials

Soluble FPP was obtained from Sigma-Aldrich Inc. (St. Louis, MO). Phosphate-buffered saline (PBS, pH 7.4) premixed powder was obtained from EMD Chemicals (Gibbstown, NJ). Ferrover® iron was purchased from Hach Company (Loveland, OH). Serum iron and total iron binding capacity kit was procured from Cliniqa Corporation (San Marcos, CA). All other chemicals and solvents used were of pure grade obtained from Fisher Scientific (Fairway, NJ).

Methods

Preparation of Soluble Microneedles

Soluble microneedles containing FPP were prepared using water soluble polymers. Aqueous blends containing 15% wt/wt poly (methylvinylether/maleic acid) (Gantrez®AN-139, Ashland, Kidderminster, UK) and 143 mg of FPP/g were prepared and used to fabricate microneedle array using laser-engineered silicone micromold templates. The array was composed of 121 (11 × 11) needles per 0.5 cm^2 perpendicular to the base. Three hundred milligrams of the drug and polymer mixture was added into the laser-engineered silicon microneedle molds and subjected to centrifugation at 550 × *g* for 15 min followed by drying at room temperature for 48 h. On removal from molds, the sidewalls of the microneedle arrays were removed using a heated scalpel blade. An accurate measurement of the final percentage content of active compound in the microneedles was determined based on mass loss calculations after water evaporation from the array.

Fate of Microneedles in the Skin

The fate of microneedles was assessed after insertion into the skin tissue by scanning electron microscopy (SEM). Microneedle array was inserted into rat skin, *in vivo*, with mild force using the thumb. The array was removed carefully after 1 h and 3 h and SEM pictures were obtained. For SEM, the microneedle array was fixed on aluminum stubs using glued carbon tapes and coated with gold using Hummer 6.2 sputter coater (Anatech USA, Union City, CA). The sputter coating chamber was supplied with argon gas throughout the coating process. Photomicrographs of the microneedle array were acquired using a model JSM-5600 SEM (JEOL Ltd., Tokyo, Japan).

In Vivo *Cutaneous Microdialysis Studies*

Implantation of Microdialysis Probe. All animal studies were approved by the institutional animal care and use committee at the University of Mississippi (Protocol # 11-016). Linear microdialysis probe (BASi, West Lafayette, IN) with 5-mm length and 30-kDa cutoff molecular weight was used to perform dermal microdialysis studies. A 30G needle was inserted intradermally parallel to the stratum corneum surface, through a distance of 1 cm in hairless rats. The microdialysis probe was inserted through this needle, and the needle was withdrawn leaving the probe implanted in the dermal tissue. The inlet tube was connected to an injection pump (BASi), and the outlet was placed in a sample collection vial.

Recovery of Microdialysis Probe. A microdialysis probe recovery study was performed *in vivo* using the retrodialysis method in hairless rats. A flow rate of 2 μL/min was chosen for the entire study. The microdialysis probe was equilibrated with PBS (pH-5) for 30 min after implantation of the probe, and later, a known concentration of drug was perfused and dialysate was collected at different time points.[4] The recovery was calculated using the following formula:

$$\text{Recovery}(\%) = 100 - \left(\frac{\text{concentration of dialysate}}{\text{concentration of perfusate}} \times 100\right)$$

Dermal Kinetics of FPP. After implantation of the probe, the buffer was perfused for 30 min to equilibrate the probe with skin tissue fluid. The microneedle array was applied on the rat abdominal skin exactly at the site the probe was implanted, by firmly pressing it against the rat skin using the thumb, and the array was secured with the help of a surgical tape. Dialysate fluid was collected every hour and the array was removed after 3 h, and microdialysis sampling was continued up until 10 h. The microdialysis samples were analyzed using inductively coupled plasma mass spectrometry.

Evaluation of Safety and Toxicity of FPP in Human Skin Cell Lines

Cell Culture. The safety studies were carried out using human skin fibroblast (HDF) (CCD1093Sk [ATCC® CRL2115™]) cell lines (ATCC, Manassas, VA). HDF cells were grown in Eagle's Minimum Essential Medium (ATCC-302003) with 10% fetal bovine serum in cell culture flasks (75 cm^2) to approximately 80% confluence in a 37°C, humidified 5% CO_2 incubator. The cell media was supplemented with penicillin (10,000 units) and streptomycin (10 mg/mL) solution. The cells were seeded into clear/black wall 96-well microplates at a density of 200,000 cells/mL (200 μL) and cell proliferation/viability assay and ROS measurement assay were performed. Cell count was obtained with Bio-Rad automatic cell counter (Bio-Rad, Hercules, CA) after staining a 10-μL aliquot of cell suspension with 10 μL of Trypan blue stain (0.4%) (Life Technologies, Grand Island, NY). The passage number 6 was used for HDF cell lines in all the experiments.

Cell Viability Assay. Cell proliferation assay was carried out using the CellTiter 96® AQueous one solution reagent (Promega, Madison, WI), which can be used to measure the number of viable cells by colorimetric method. After reaching approximately 80% confluence of the cells in microplates, the media in all the wells were replaced with 100 μL of fresh medium (without serum and penicillin streptomycin solution) cell lines that were exposed to 100 μL of either basal medium (control) or serial dilutions of FPP prepared at different concentrations (31.25, 15.62, and 7.81 mg/mL) or digitonin (positive control) (Promega, Madison, WI) prepared at different concentrations (60 μg/mL-1.87 μg/mL) with serial dilutions for 24 h. FPP solutions were prepared using basal medium, and digitonin solutions were prepared from a stock of 20 mg/mL using cell culture grade dimethylsulfoxide (ATCC). Untreated cells served as a control in this study. The CellTiter 96® AQueous one solution reagent was completely thawed in water bath at 37°C for 10 min before use, and 40 μL of this reagent was added to each well in the 96-well plate containing the cells and the plate was incubated at 37°C in a humidified, 5% CO_2 atmosphere for 4 h. After incubation, the absorbance at 490 nm was recorded using microplate Reader (SpectraMax® M5; Molecular Devices, LLC., Sunnyvale, CA). Testing at each concentration of the FPP and positive standard was performed in triplicates.

Measurement of ROS Activity/Oxidative Stress Markers. Possible generation of ROS and the intracellular ROS activity on treating HDF cell lines with FPP at different concentrations was measured using Oxiselect™ intracellular ROS assay kit (Cell Biolabs, San Diego, CA). After reaching approximately 80% confluence in 96-well microplates, both the cell lines were treated with cell permeable fluorogenic probe 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA). Cells loaded with DCFH-DA were washed gently with D-PBS