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RESEARCH PAPER

Erythropoietin-Coated ZP-Microneedle Transdermal System: Preclinical Formulation, Stability, and Delivery

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ABSTRACT

Purpose To evaluate the feasibility of coating formulated recombinant human erythropoietin alfa (EPO) on a titanium microneedle transdermal delivery system, ZP-EPO, and assess preclinical patch delivery performance.

Methods Formulation rheology and surface activity were assessed by viscometry and contact angle measurement. EPO liquid formulation was coated onto titanium microneedles by dip-coating and drying. Stability of coated EPO was assessed by SEC-HPLC, CZE and potency assay. Preclinical *in vivo* delivery and pharmacokinetic studies were conducted in rats with EPO-coated microneedle patches and compared to subcutaneous EPO injection.

Results Studies demonstrated successful EPO formulation development and coating on microneedle arrays. ZP-EPO patch was stable at 25°C for at least 3 months with no significant change in % aggregates, isoforms, or potency. Preclinical studies in rats showed the ZP-EPO microneedle patches, coated with 750 IU to 22,000 IU, delivered with high efficiency (75–90%) with a linear dose response. PK profile was similar to subcutaneous injection of commercial EPO.

Conclusions Results suggest transdermal microneedle patch delivery of EPO is feasible and may offer an efficient, dose-adjustable, patient-friendly alternative to current intravenous or subcutaneous routes of administration.

KEY WORDS EPO · erythropoietin alfa · pharmacokinetics · stability · transdermal microneedle patch

INTRODUCTION

Chronic renal failure (CRF) is an increasingly common condition that currently affects approximately 1 in 500 people in the United States (1). Many patients with CRF develop anemia primarily from decreased renal synthesis of erythropoietin, the hormone responsible for production of red blood cells (RBC) by the bone marrow. These patients have reduced or absent reticulocyte responses, decreased RBC survival and uremia-induced platelet dysfunction.

The current treatment for anemia in pre-dialysis CRF patients is with subcutaneous injection of EPOGEN®, erythropoietin alfa (EPO), or the long acting form ARANESP®, darbepoeitin. Currently, recommendations indicate the need for careful dosing to control the increase in patient hematocrit to avoid cardiovascular complications (2). This may require more individualized patient dose titration and shorter acting EPO administration. Many patients are reluctant to self-administer an injection. An alternative dose-adjustable, non-invasive microneedle patch delivery system could be a significant advancement.

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Administration of therapeutic proteins by methods other than intravenous or subcutaneous injection has been challenging for numerous reasons including transport across biological barriers (mucosal and skin), their large molecular size and instability (3,4). However, several transdermal and intradermal technologies are now being evaluated and show promise. The approaches involve skin ablation to breakdown the skin transport barrier layer, i.e. stratum corneum, to create aqueous pathways for peptide and protein transport. These technologies include laser (5), radio frequency (6) or heat ablation (7) treatment followed by transdermal patch application over the damaged skin site. Alternatively, transdermal microneedles are being developed for use as a skin pretreatment followed by a patch application (8), as hollow microneedles for aqueous formulation infusion (9,10) and as biodegradable drug microneedles (11–14).

The development of drug-coated microneedles (plastic and metal) is also advancing (15–21). When drug-coated microneedles are applied to the skin, they penetrate to a superficial depth into the epidermal/dermal layers thus bypassing the stratum corneum barrier. The drug can then dissolve in the skin interstitial fluid and diffuse to the adjacent micro capillary beds for efficient absorption into the systemic circulation. A representative drug-coated microneedle patch with applicator is shown in Fig. 1a, b. A drug-coated microneedle patch delivers drug directly into the skin and thereby offers several delivery advantages including efficient delivery (drug utilization from the patch) and the potential for very short patch wear time (minutes). Although the specific duration of application can depend on the therapeutic agent being administered and the desired delivery profile. This type of patch and applicator system has recently been validated in a Phase 2 clinical study for the delivery of parathyroid hormone (1–34) peptide for the treatment severe osteoporosis (19).

While significant progress is being made in the delivery of some peptides and proteins, transdermal delivery of EPO has remained a significant challenge because of its large molecular size (35 kDa), instability and tendency to form undesirable aggregates. In early attempts, EPO transdermal delivery using biodegradable microneedles (22–24) showed limited delivery and no evidence of a dose response.

In this study we evaluated EPO coating formulations, coating feasibility and solid-state stability of EPO on the ZP-microneedle patch system. Preclinical pharmacokinetic performance of the ZP-EPO transdermal system was evaluated relative to commercial subcutaneous injection product.

MATERIALS AND METHODS

Materials

Recombinant human erythropoietin alfa (EPO) at a concentration of 1.9 mg/mL with 10 mM sodium phosphate buffer and 26 mM sodium chloride was purchased from AMEGA (Buenos Aires, Argentina). EPO activity was 110 IU/μg. The API solution was further processed prior to use as described in the METHODS section. Sucrose NF (High Purity Low Endotoxin Grade) was obtained from Pfanstiehl Laboratories (Waukegan, IL). Polysorbate 20 (Crillet 1 HP, high purity, low peroxide) was sourced from Croda (Edison, NJ). Titanium metal sheet (Commercially pure Grade 2, 25 μm in thickness) was obtained from Hamilton Precision Metals (Lancaster, PA).

The ZP-patch system consists of a titanium array of 650 microneedles per cm^2. Microneedles have an arrow head shape with a length of 215 μm, tip angle of 60°, coating surface with a height of 100 μm, width of 115 μm and thickness of 25 μm. The microneedle shaft is 115 μm long and 65 μm wide with thickness of 25 μm (Kemac, Azusa, CA) (Fig. 1c, d). Other patch components include a polycarbonate ring (Jatco, Union City, CA), adhesive patch (Adhesive Research Inc, Glen Rock, PA), 3 Å molecular sieve desiccant sachet (3.5 g Minipax, Multisorb, Buffalo, NY), and an aluminum foil pouch (Mangar, New Britain, PA).

EPOGEN® (epoetin alfa; recombinant human erythropoietin; 10,000 IU/mL; est.120 IU/μg) was produced by AMGEN, purchased from a commercial source and stored per the manufacturer's instructions.

Methods

Diafiltrated and concentrated EPO solution was prepared utilizing an Amicon Ultra-15 (Millipore, Billerica, MA) centrifugal filter unit with a 10 kDa MWCO regenerated cellulose membrane. Approximately 15 mL of the EPO solution was placed in the filter unit and centrifuged with a Beckman centrifuge model GS-15R (Beckman, Palo Alto, CA) at 4500 rpm for 30 min. The solution was maintained at 4°C during the centrifugation process. Following diafiltration the volume of the protein solution was reduced to a third of the original volume. Recovery of the EPO concentrate from the Amicon Ultra-15 Centrifugal filter units was high, typically >90% as determined UV spectroscopy. Sucrose was subsequently added to the concentrated EPO solution at 1:1 ratio and then lyophilized.

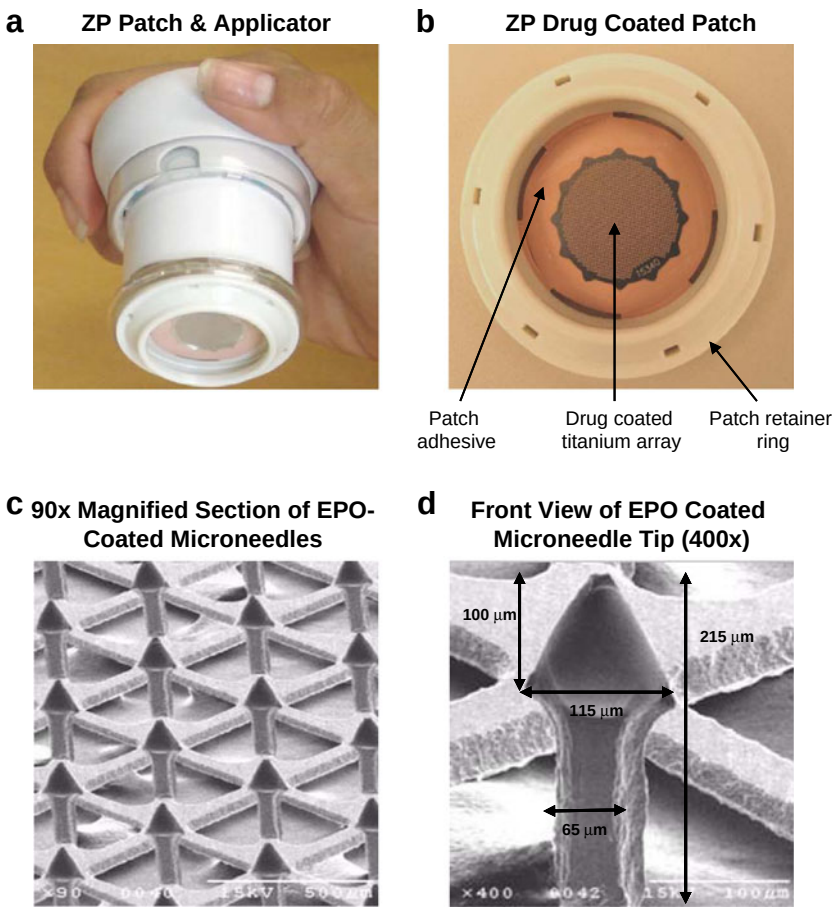

Fig. 1 Transdermal microneedle patch delivery system: (**a**) ZP-patch and applicator; (**b**) 5 cm^2 adhesive patch with microneedle array (2 cm^2) in applicator ring; (**c**) 90x magnification of EPO coated microneedles, EPO coated at 3,000 IU (30 μg)/3 cm^2 array; (**d**) Front view of an individual EPO coated microneedle (400x magnification). The length of each microneedle is 215 μm, the thickness is 25 μm, the length and the width of the microneedle arrowhead is 100 μm and 115 μm, respectively, with a tip angle of 60°. The microneedle shaft is 115 μm long and 65 μm wide.

Lyophilization

Freeze drying was performed using a LyoStar II lyophilizer (FTS Systems, Stone Ridge, NY) using the following cycle: freezing at -40°C for 2 h; primary drying for 2 h under the vacuum of 350 mTorr at each temperature of -40, -30, -20, -10, and 0°C; secondary drying at 10°C/350 mTorr for 2 h, 20°C/350 mTorr for 2 h, 30°C/350 mTorr for 1 h, 30°C/50 mTorr for 0.5 h, and 30°C/0 mTorr for 0.5 h. Temperature was ramped up at 5°C/min consistently.

Size Exclusion Chromatography

Soluble aggregates of EPO were determined by the SEC-HPLC method specified in European Pharmacopoeia (7.0) monograph for erythropoietin (25).Chromatography for the assay was performed using a TSK-gel G3000 SW column (7.8 mm ID x 60 cm) (TOSOH Bioscience,) with an isocratic mobile phase (1.5 mM potassium dihydrogen phosphate, 8.1 mM disodium hydrogen phosphate and 0.4 M sodium chloride at pH 7.4) at a flow rate of 0.5 mL/min, on a HPLC system (1100 series, Agilent Technologies, Inc., CA, USA) equipped with a binary pump, a thermostatted autosampler and column compartment, and a multiple wavelength DAD/UV detector. Data were collected and analyzed using Empower Pro (Empower 2 software, Waters Corporation, USA).

Capillary Zone Electrophoresis

EPO isoform distribution was determined by the capillary zone electrophoresis (CZE) method specified in European Pharmacopoeia (7.0) monograph for Erythropoietin (25).

UV-Visible EPO Quantification

EPO content was measured by absorbance at 275 nm using an Agilent 8354 UV/Visible Spectrophotometer (Agilent, Wilmington, DE). The samples were measured in quartz micro-cuvettes without dilution after the extraction of the coated microneedle arrays with ultra-pure water (Milli-Q, Millipore Corporation, Billerica, MA) and blanked against the water. Quantitation was done based on a three-point standard curve (typically 80, 120 and 200 μg/mL) prepared from EPO reference standard (in-house qualification) in sodium phosphate buffer.

Rheology

Rheological characterization of the liquid formulations was conducted utilizing a rheometer (model CVOR150, Bohlin Instrument, Cranbury, NJ) configured with a cone and plate geometry (a cone angle of 1° and radius 10 mm). Seventy μL of the liquid formulation was utilized for each experiment.

Contact Angle Measurement

Static contact angle of drug solution formulations on titanium surface was determined using a FDS contact angle meter (Model OCA15) employing the "Sessile drop" optical contact angle method. For static contact angle measurement, a photo snapshot was taken once a drop of the solution (5 μL) is dispensed from the syringe and laid on a clean titanium foil surface. The angle between the baseline of the drop and the tangent at the drop boundary is measured on both sides. Complete measurement was obtained by averaging the two numbers. A minimum of five readings were recorded for each sample.

Microneedle Array Coating and Packaging

Titanium microneedle arrays were fabricated by a photo/chemical etching and formed using a controlled manufacturing process (26). Drug formulation (15% w/w EPO, 15% w/w sucrose and 0.2% w/w polysorbate 20) coating on the microneedle array was conducted at ambient temperature utilizing a roller drum, rotating at 50 rpm, in a drug formulation reservoir (2 mL in volume) to produce a thin drug formulation film of controlled thickness of ~100 μm in thickness (27). The general schematic for this process is described in reference 27.

Microneedle tips on the array are dipped into the thin film and the coating per area controlled by the number of dips (passes) through the drug film. The time between each dip coating was less than 5 s which was sufficient to dry the formulation coating. The dose per patch was also adjusted by the microneedle array area (from 0.3 to 3 cm^2).

The EPO-coated microneedle array was assembled with adhesive and retainer ring (Fig. 1b). The patch in retainer ring was packaged in an aluminium pouch (Mangar, New Britain, PA) with a 3 Å molecular sieve desiccant sachet, purged with dry nitrogen and heat sealed with a Van der Stahl heat sealer (model MFG-18).

Stability Experiments on Packaged Drug-Coated Microneedle Delivery Systems

The ZP-EPO patch in the sealed pouch was stored in a stability chamber (Model 6010, Caron, Marietta, OH) controlled at 25°C/60% RH. At each time point, three pouch samples were pulled from the stability chamber for HPLC analysis. To extract EPO from the coated array, the array was first separated from the adhesive by exposing to liquid nitrogen vapor and then peeled from the adhesive. The coated array was then placed in a vial containing 0.5 mL of sodium phosphate buffer (pH 7.4) and mixed for a period of 5 min, of which 250 μL of the resulting solution was transferred into a secondary vial for SEC analysis. Extraction efficiency was determined to be 98%–100% based on EPO spike and recovery experiments.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) using a Hitachi scanning electron microscope (Model S-2460 N) was used to determine the morphology and distribution of the coating on the microneedles.

EPO Bioactivity Assay

EPO bioactivity was determined using the *in vivo* reticulocyte stimulation assay specified in European Pharmacopoeia (7.0) monograph for Erythropoietin (25). Briefly, normocythemic mice received a single SC injection of EPO followed by blood sampling 96 h later. Reticulocyte counts were determined by flow cytometry and compared to the counts obtained using the European Pharmacopoeia Biological Reference Preparation of EPO.

Preclinical Pharmacokinetic Studies

Animal Model and Skin Preparation

Adult Male Sprague Dawley Rats (body weights 0.4–0.5 kg) were obtained from Harlan Laboratories (Livermore, CA). All animal studies adhered to the NIH Principles of Laboratory Animal Care (28) and were in compliance with the animal welfare regulations in 9 CFR 1-3, the National Research Council Guide for the Care and Use of Laboratory Animals 1996 (29) and an approved institutional animal care and use committee protocol.

Animals were anesthetized using ketamine/xylazine (intramuscular, 40 mg/kg ketamine, 6 mg/kg xylazine, respectively). To minimize the stress of anesthesia, the animals were kept warm on a circulating water pad at 37°C and carefully monitored. The lateral thorax of each animal was shaved and cleaned with 70% isopropyl alcohol to facilitate both ZP-EPO patch applications and subcutaneous (SC) dosing. The lateral thorax dosing site was chosen because it provided a large and relatively flat surface for patch application.

ZP-EPO Patch Application and SC Dosing

ZP-EPO patches were applied to the shaved area of the lateral thorax of adult male Sprague Dawley Rats using a hand held re-usable applicator. The patch is attached to the applicator and the applicator is pressed on the skin, releasing the patch and applying it with a predetermined force using a previously described method (20). ZP-EPO patches were applied and worn for 15 min to 2 h then removed. The ZP-EPO patch doses tested were; 750 IU, 3,000 IU, 11,000 IU and 22,000 IU corresponding to approximately 7 μg, 27 μg, 100 μg and 200 μg, respectively. Patches were prepared with the same EPO coating formulation and the same coated amount per cm^2. The microneedle array area was used to vary dose. Array areas of 0.3 cm^2 to 3 cm^2 were used for the EPO doses tested with two patches of 3 cm^2 used for the highest dose of 22,000 IU.

EPOGEN® SC injections of 2,000 IU and 5,000 IU were used as comparators. Injections were made on a shaved area of the lateral thorax consistent with the patch application sites.

ZP-EPO patch delivery efficiency was assessed by residual drug analysis. The amount of EPO left on the microneedle array and skin surface after each application were compared against the original coated amount on the microneedle array.

Local skin tolerability of the ZP-EPO patch was evaluated using the Draize scoring system (30). Skin site evaluations were made immediately following patch removal and at 12, 24, 48 and 72 h.

Pharmacokinetic Studies

Pharmacokinetic samples were collected at 0, 1, 2, 4, 6, 12, 24, 48 and 72 h to evaluate systemic absorption of EPO. At each time point, a 0.2 mL blood sample was collected from either the lateral tail vein or retro-orbital plexus. Whole blood samples were centrifuged (4,000 rpm, 8 min, 4°C), plasma collected and frozen (–80°C) until assayed. Plasma levels of EPO were measured using an ELISA (R&D Systems, Minneapolis MN) modified for use with rat plasma. The lower limit of quantitation of the assay was 2.5 mIU/mL. Values below this limit were treated as zero for pharmacokinetic analysis.

Plasma samples were obtained from alternating groups of animals to minimize the effect of sampling with a minimum of 3 animals per time point. Pharmacokinetic parameters were determined by noncompartmental analysis.

Statistical Analyses

Results are presented as the mean ± SE.

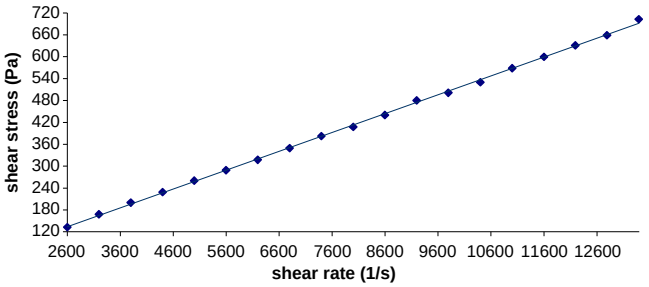

Fig. 2 Shear stress *vs.* shear rate for 15% w/w EPO, 15% w/w sucrose, 0.2% w/w polysorbate 20 liquid formulation. Shear stress is proportional to shear rate suggesting Newtonian behavior for the EPO liquid formulation.

RESULTS AND DISCUSSION

Formulation Characterization, Microneedle Coating, and Stability

There are two processes, as defined by the coating operation, to produce the drug-coated patch dip coating of microneedles into a high concentration liquid drug formulation as well as drying and packaging of the solid-state drug formulation, which have been discussed previously (31). Briefly, a liquid formulation is prepared to primarily satisfy three key coating formulation parameters: drug concentration, viscosity, and surface activity.

On this basis a liquid 15% w/w EPO, 15% w/w sucrose and 0.2% w/w polysorbate 20 was prepared. Arriving at this formulation composition was partially driven by chemical stability considerations. The EPO liquid formulation was formulated to a high concentration (200 mg/mL; 15%w/w) to ensure that each dip of microneedles into the liquid formulation can pick up sufficient volume of liquid for drying, which can achieve the desired drug dose with a minimum number of dips. In addition the viscosity of the coating solution, 70 cP,

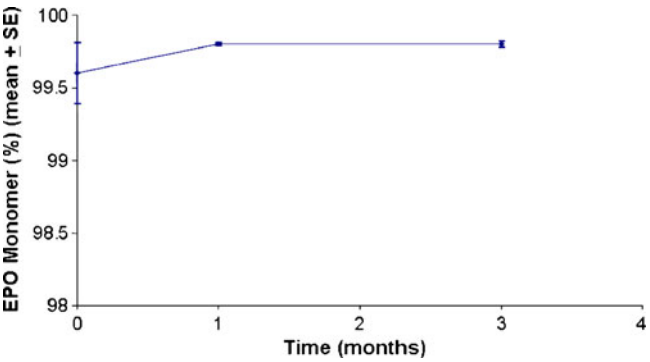

Fig. 3 Stability of EPO-coated microneedle system where the titanium microneedle array was coated with 11,000 IU EPO dose, assembled on a 5 cm^2 adhesive patch within a polycarbonate retainer ring, and heat sealed in a nitrogen-purged foil pouch containing a 3.5 g molecular sieve desiccant sachet. The final EPO-coated microneedle packaged systems were stored at 25°C/60% RH.

Table I CZE Electropherogram Data of ZP-EPO Patches Stored at 40°C/75% RH for 2 months and rhEPO API Stored at -80°C

Isoform	Ph. Eur. Range (%)	ZP-EPO (%)	rhEPO API (%)
1	0–15	0.5	0.4
2	0–15	4	4.5
3	1–20	18.9	18.8
4	10–35	30.4	30.3
5	15–40	24.1	24
6	10–35	14.6	14.7
7	5–25	5.8	5.4
8	0–15	2.1	1.7

was high enough so that the coated liquid will not quickly drip back after dipping but before drying. Sucrose was the primary protein stabilizer and its content was limited to a 1:1 sucrose: EPO w/w ratio because increasing sucrose content would add more solid to the coating of the same EPO dose on the microneedle tips which would eventually blunt the sharpness and hinder skin penetration. A surfactant, polysorbate 20, (0.2%) was added to the liquid EPO formulation to decrease the contact angle to 45 ° and improve the wetting and coating on the titanium microneedle surface. The EPO liquid formulation exhibited Newtonian behavior (Fig. 2), with the shear stress proportional to the shear rate. This formulation property facilitates a uniform coating of the microneedles as described previously (27).

The EPO liquid formulation was then coated on titanium microneedle array. Figure 1c shows the uniform formulation coating on the arrowhead tip of each microneedle within the array as demonstrated by scanning electron microscopy (SEM). No EPO coating is observed on any other part of the microneedle or the array structure. The localization of the coating to the arrowhead (Fig. 1d) is consistent with the thickness of the thin drug film established by the roller drum. The coating surface morphology is smooth. The EPO coated amount per 1 cm^2 of microneedle array was 3.0 μg±0.3 μg and was consistent from batch to batch.

The assembled ZP-EPO microneedle patches (Fig. 1b) were stored at 25°C/60% RH. Figure 3 summarizes EPO% monomer data for 25°C/60% RH up to 3 months. Within the time course of the study ZP-EPO systems held excellent stability; EPO% monomer at the 3 month time point is similar to that at T=0 (~99%) and showed no obvious trend of decreasing. The ZP-EPO patches after 2 months storage at 40°C/75% RH were subjected to CZE analysis, which exhibited the expected eight peak distribution, which were within the given specification of the European Pharmacopoeia 7.0 (Table I) and similar to the control, EPO API which was stored at -80°C. This result indicates that storage at 40°C did not lead to any degradation of the carbohydrate structures, thus within the study period the ZP-EPO patches are stable.

The tertiary structure of therapeutic proteins, such as EPO, may change during the manufacturing process and

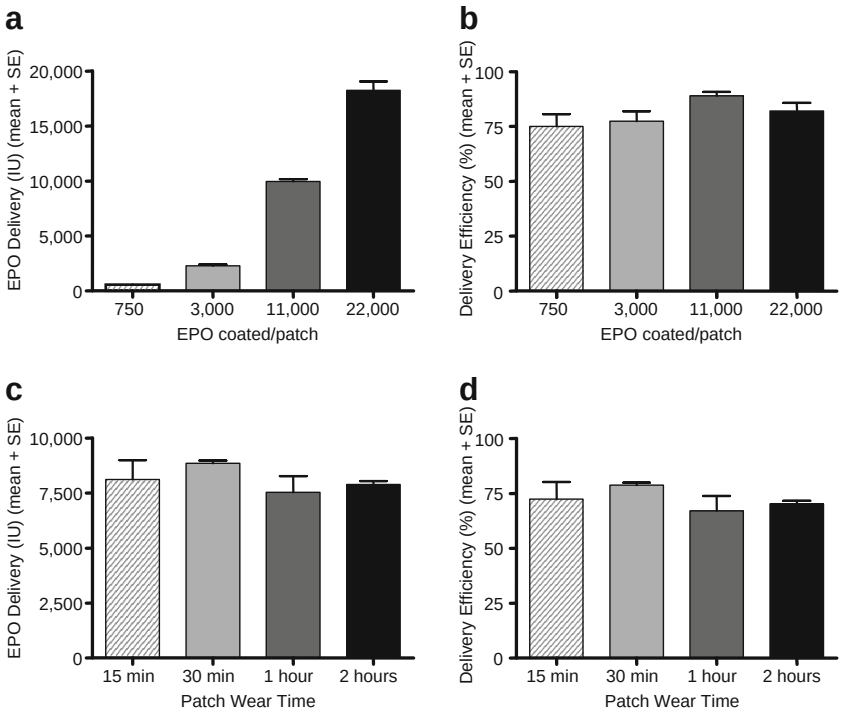

Fig. 4 ZP-EPO delivery performance: (**a**) delivery into rat skin, (**b**) delivery efficiency (%) with ZP-EPO patch wear time of 2 h: Patches were tested at doses of 750–22,000 IU; and, (**c**) delivery into rat skin, (**d**) delivery efficiency (%) with 11,000 IU EPO-coated patches worn for 15 min to 2 h.

Table II Comparison of PK Parameters for ZP-EPO and EPOGEN SC Injection

	ZP-EPO				SC Epogen	
	750 IU	3,000 IU	11,000 IU	22,000 IU	2,000 IU	5,000 IU
C_{max} (IU/mL)	2.2	7.9	27.5	76.3	9.0	11.0
T_{max} (h)	12	12	6	12	12	6
$T_{1/2}$ (h)	9.8	10.0	11.8	9.3	12.2	18.2
AUC_t (IU/mL*h)	65.8	213.7	784.9	1,505.9	191.0	395.3
Relative Exposure (%)*	109	92	89	86	120	Reference

AUCt (t=0-72 h)

* Presented using the calculation with dose normalization

subsequent storage. Conformational changes may affect bioactivity. The bioactivity of the coated ZP-EPO patches was compared to that of the EPO API. Bioactivity was 93.8% and 103.6% for ZP-EPO and EPO API, respectively and thus both samples fall within the acceptable range set for European Pharmacopoeia Biological Reference Preparation of EPO) This confirms that bioactivity is maintained during the microneedle coating and packaging process.

EPO-Coated Microneedle Delivery Performance and Release Kinetics

EPO delivery into skin with a patch wear time of 2 h was: 573±43; 2,272±132; 9,961±193 and 18,228±791 IU (mean ± SE) for EPO-coated doses of 750, 3,000, 11,000 and 22,000 IU, respectively. Delivery efficiency was excellent with 75%–80% of the EPO-coated dose delivered into the skin (Fig. 4a, b).

Further examination of the release kinetics of ZP-EPO indicated maximal delivery performance could be obtained with a patch wear time of as little as 15 min (Fig. 4c, d). There was no additional benefit from wearing the patch longer. Shorter patch wear times were not evaluated. Using the 11,000 IU patch dose, mean EPO delivery into skin was: 8,106±882; 8,850±120; 7,518±755 and 7,881±150 IU (mean±SE) for patch wear times of 15, 30, 60 and 120 min, respectively.

Local skin tolerability was rated as very good. The erythema scores (maximum possible score: 4) (30) never exceeded 2 and were predominately 0 -1 representing no or barely perceptible erythema. By 24–48 h skin sites appeared normal. There was no evidence of topical bleeding with any of the treatment conditions. Primary Irritation Indices (PII) (maximum possible score: 8) were 0 to 0.6 and thus categorized as negligible to slight.

Pharmacokinetic and Pharmacodynamic Data

Preclinical *in vivo* evaluation showed rapid systemic absorption of EPO with patch administration. There was a linear dose response in terms of both area under the curve (AUC_t) and

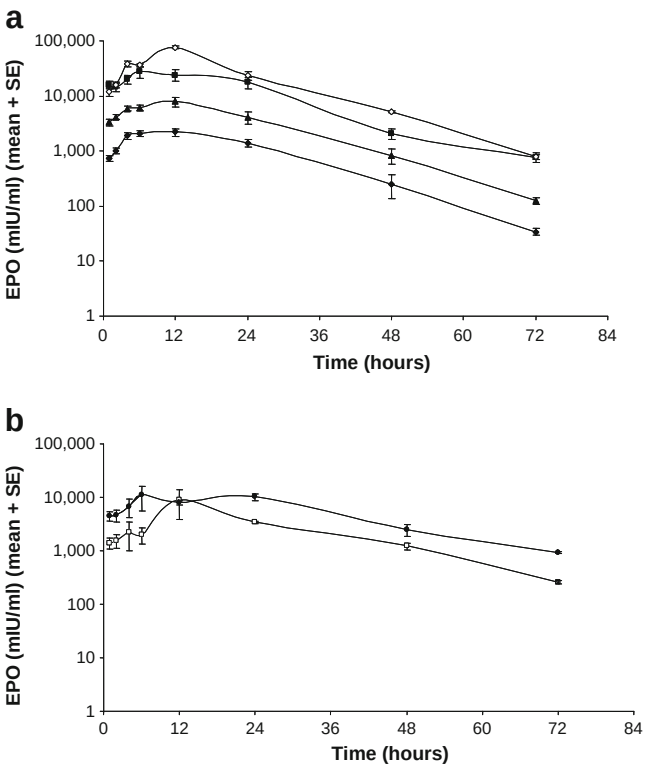

Fig. 5 PK profile of EPO delivered by microneedle patch or SC injection: (**a**) ZP-EPO patch (♦ 750 IU, ▲ 3,000 IU, ■ 11,000 IU, ◊ 22,000 IU); (**b**) EPOGEN® SC injection (□ 2,000 IU, ● 5,000 IU).

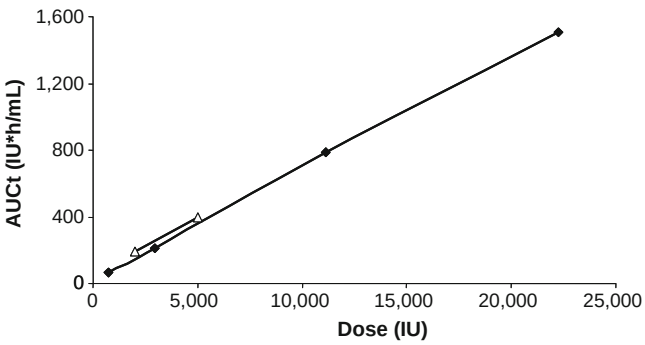

Fig. 6 EPO plasma concentration verses administered dose. ZP-EPO patch over the range of 750–22,000 IU (♦) *vs.* EPOGEN® SC injection over the range of 2000–5000 IU (Δ). For patch dosing r^2=0.997.

maximum observed plasma concentration (C_{max}). Plasma levels were maintained over 72 h with T_{max} at 6–12 h and a plasma half-life of 9–12 h (Table II, Fig. 5). These values were comparable to subcutaneous EPOGEN® injection (Fig. 6). These data are also comparable to published studies evaluating the PK/PD of injected EPOGEN® in a rat model (32,33). EPO patches had relative exposure values of 86–109% to the 5,000 IU SC EPOGEN® comparator.

Delivery into skin correlated well with systemic absorption. Plasma levels of EPO continued to rise following the removal of the ZP-EPO patches indicating that the rate of systemic absorption from the delivery site determines the overall PK profile of EPO. Published data with other peptides suggest microneedle delivery produces a rapid-on, rapid off PK profile (12,17,20,34). The present data are the first demonstration of a microneedle patch matching the PK profile of a subcutaneous injection. The current observation of more prolonged absorption and elimination phases of the pharmacokinetic profile of ZP-EPO patch compared to the previously reported rapid absorption and elimination with ZP-PTH (20) demonstrates that absorption from the ZP-microneedle system is drug dependent.

CONCLUSION

These studies demonstrate for the first time that the EPO can be formulated at high concentration and coated onto a microneedle patch without loss of potency or generation of soluble aggregates. The diafiltration and concentration pre-formulation process developed was highly efficient resulting in a 90% process yield and no increase in aggregate formation. The EPO coating formulation at 200 mg/mL allowed uniform and localized microneedle coating. ZP-EPO patches stored in sealed nitrogen purged foil pouches with desiccant were stable at 25°C storage for at least 3 months with no significant changes in aggregation or EPO isoform pattern.

Preclinical delivery studies in a rat model showed high drug delivery efficiency and a linear dose response at therapeutically relevant doses. The PK profile was similar to the commercially available subcutaneous injectable product, EPOGEN®. These results suggest that EPO-coated microneedle patch development may be feasible and warrants further study.

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