Efficacy and Pharmacokinetics of Insulin Delivered to Pigs by Phase-transition Microneedle Transdermal Patch

Sixing Yang, Yan Feng, Xiaoyun Hong, Weien Yuan, and Tuo Jin
School of Pharmacy, Shanghai Jiao Tong University, Shanghai, 200240, China			
tomtjin@gmail.com

ABSTRACT SUMMARY

The ability of phase-transition microneedle patch, a newly invented system of us that releases drugs by water swelling, to achieve efficient and accurate transdermal delivery of insulin was confirmed by an efficacy and pharmacokinetic study using pig models. In a comparison with the commercial injection pen of lispro insulin, phase-transition microneedle patch reached a bioavailability 20% of the injection pen and a slightly delayed (20 min) initial response. The standard deviations in measuring the blood concentration were about the same for both of the two dosage forms, indicating their comparable dosing accuracy. Unlike the well-reported soluble microneedles, phase-transition microneedles, although formed of hydrophilic polymers too, do not rely on dissolving but swell to release cargos to the body fluid in the dermis layer. The swollen microneedles can be removed completely from the skin without leaving deposition of the needle tip materials, a favorable nature for frequent transdermal administration of drugs. In addition, the hydrogel network of the swollen microneedles is maintained by formation of nanocrystalline domains as the cross-linking junctions, for which exposure of delicate medicines to hazardous chemical or ionic cross-linking conditions can be avoided.

INTRODUCTION

Non-parenteral delivery of proteins and peptides has been a long and still standing objective of drug delivery studies. While the birth of microneedle technology has provided a great feasibility to achieve transdermal delivery of biomolecules without pain feeling and skin damage, existing microneedle systems all have some limitations for being a practical dosage form for frequent and chronic drug administration. The technical challenges to achieve frequent and chronic transdermal delivery of bio-medicines using microneedles comprise 1) insufficient bioavailability and dosing accuracy; 2) deposition of needle tip materials to the skin; 3) insufficient mechanical strength to ensure all-needle insertion and removal; and 4) exposing delicate medicines to hazardous fabrication conditions.

To address the above-mentioned issues, we developed an unique phase-transition microneedle patch, which showed a number of superior natures over previously reported microneedle systems. The microneedles are formed of polyvinyl alcohol (PVA) as the dominating matrix blended with some polysaccharide additives. Unlike soluble microneedles, phase-transition microneedles do not release water-soluble drugs by dissolving themselves in the body fluid of the dermic layer, but by swelling their network. Moreover, the post-swelling hydrogel network of our phase-transition microneedles are maintained by nano-crystalline cross-link junctions which are formed via a freeze-thaw treatment, a fabrication process free of stability hazards to delicate bio-medicines. In addition, the microneedles of cross-linked PVA are sufficiently hard and stiff to penetrate the epidermis at dry state and be removed completely at hydrated state without breaking.

An efficacy and pharmacokinetic trial involving normal and diabetic Pamma pigs was carried out in the present study to examine the expected characteristics of the phase-transition microneedle patch as a practical dosage form. Positive results in terms of bioavailability, dosing accuracy, and glycosylated hemoglobin level were obtained.

EXPERIMENTAL METHODS

The design of the microneedle patch includes an array of PVA needle tips loaded with insulin and an insulin-free supporting sheet made of the same materials.

Diabetic Pamma pigs (6 in each group) were fasted for 12 hours prior to experiment. To confirm the baseline, each pig was injected with 0.6g/Kg glucose right after insulin administration, followed by blood sugar measurement 30, 60, 120, and 180 min after the insulin dose. Then, the pigs were given various insulin formulations including microneedle patch, followed by measurement of blood sugar. Glycosylated hemoglobin and blood fat were assayed after two month insulin dose.

For pharmacokinetic study, four groups of normal pigs were given insulin injection pen (0.4IU/Kg), and three doses of insulin microneedle patches (Low, Medium and High dose equivalent to 1, 2 and 3IU/Kg). Blood samples were collected and measured as a function of time over 480 min.

RESULTS AND DISCUSSION

The morphology of a phase-transition microneedle patch is imaged in Figure 1. The ability of phase-transition microneedles to penetrate skins was examined by pressing a patch against a dissected pig skin, followed by imaging the cross-section of the skin dyed with Typan blue. As shown in Figure 1A, the microneedles penetrated into the pig skin by approximately 425µm in depth, half of the height of the needle tips (850µm). The result that a portion of the needle tips was unable to penetrate into the skin tissue might be due to deformation of the skin surface caused by static pressure.

Swelling of the needle tips by body fluid, essential for phase-transition microneedles to deliver biologics across epidermis, was examined by applying the drug-free patch on live human skins directly with a moisture preserving
backing membrane (3M 9860, Fig. 1E). As shown in Fig. 1, the hard and sharp microneedles (Fig. 1B) turned to be soft and thicker (Fig. 1C) after three hours patching on live human skin. Attaching a same patch except all the needle tips were cut off with the same moisture-preserving baking membrane did not result in the same phase transition (Fig. 1D), suggesting that swelling of the microneedle patch was not resulted from the moisture on skin surface (such as sweat). Clearly, the phase transition from the glassy state to the hydrogel state of the polymers was resulted from absorbing body fluid by the microneedle tips in the dermis layer. This result is also evident for that the microneedles are able to penetrate the epidermis of live human skin.

Fig. 1. Characteristics of phase-transition microneedle patch. A: a histological slice of dissected pig skin pressed by microneedles and dyed with trypan blue; B: a microneedle patch with an array of needles 0.85mm in length; C: a swollen microneedle patch applied on live human skin for 3 hours; D: a patch with all the microneedles cut off and applied on live human skin for 3 hours; E: a microneedle patch with an adhesive backing membrane being pressed on live human skin; F: a spot on human skin right after a microneedle patch removal; G: the spot of skin 15 min after patch removal; H: the spot 60 min after patch removal.

Skin compatibility of the phase-transition microneedles was examined by flowing the recovery process of the spot of live skin where a drug-free microneedle patch was applied. Removal of the microneedle patch from the skin left an impress of an array of micro-holes created by the needle tips (Fig. 1F). These micro-holes disappeared within 15 min (Fig. 1G), and the reddish spot became invisible three hours after (Fig. 1H), suggesting the feasibility of using the patch for frequent administration.

To examine hypoglycemic effect of the microneedle patch, the formulation was given to diabetic Bama pigs at three insulin doses (1.0, 2.0, and 3.0 IU/Kg), and compared with those given insulin injection (0.44 IU/Kg). The pigs, 6 in a group, were injected 0.6g/Kg glucose right before insulin administration. As shown in Fig. 3A, blood sugar dropped responsively to the insulin dose for all the formulations. The pigs received two patches (2 IU/Kg) showed similar profile as those received injection pen, except the initial response delayed for about 20 min, but hypoglycemic efficacy sustained after 3 h (Fig. 2A). Glycosylated hemoglobin was measured after two months treatment. The pigs treated with microneedle patch of equivalent insulin dose as injection pen showed lower glycosylated hemoglobin level (Fig. 2B).

Fig. 2 Hypoglycemic efficacy of insulin-loaded phase-transition microneedle patch in pigs. A: Comparison of various dosage forms in sugar tolerance test; B: Comparison in glycosylated hemoglobin level.

The profiles of blood concentration of insulin resulted from different dosage forms were measured by giving he formulations to each animal group after ruling out the effect of experimental procedures such as the blood drawing operation on the baseline of blood insulin. The three groups of normal pigs (6 in each group) given three different doses of the microneedle patches (1, 2 and 3 patches) showed corresponding AUC (718, 1054, and 1541 ng·min/mL), respectively (Fig. 3A). The pigs received two microneedle patches showed similar blood insulin profile as those received injection pen (Fig. 3B). This result is consistent with the dose dependent hypoglycemic efficacy shown in Fig. 2A. It should be noted that the standard deviation of the blood insulin measurement for the microneedle patch was not higher than the injection pen group (Fig. 3B), suggesting the feasibility of the patch to give accurate dose.

Fig. 3 Blood insulin profiles in pigs received different insulin dosages. A: dose dependency in normal pigs; B: comparison between microneedle patch and injection pen in diabetic pigs.

CONCLUSION

The phase-transition microneedle patch formed of PVA-based hydrophilic polymers through nano-crystalline cross-linking may offer efficient and accurate transdermal delivery of insulin without depositing needle tip materials.

ACKNOWLEDGMENTS

This study was financially supported by National Grand New Drug Program of China (2009ZX09310-007), National Science Foundation of China (80073001), and BioPharm Solutions, Inc.