Qualitative and Quantitative Analysis of Lateral Diffusion of Drugs in Human Skin

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Purpose

The purpose of this study was to qualitatively as well as quantitatively analyze the lateral diffusion of drugs in dermatomed human skin.

Methods

Lateral diffusion of calcein and methylene blue dye in skin was investigated using confocal laser microscopy, calcein imaging, and histology studies. In vitro microdialysis studies were performed using vertical Franz diffusion cells. Two linear probes were inserted into the dermis of untreated, poly(D,L-lactide-co-glycolide) microneedle-treated and ablative laser-treated human skin such that one was in the center of the diffusion area (central probe), and the other probe was inserted parallel, at a distance of 8 mm from the central probe (lateral probe) (n=3). The depth of the probes in the skin was determined using dermascan (n=3). Skin samples with the probes were then mounted on Franz cells, sandwiched between donor chamber containing diclofenac sodium solution (4 mg/mL, 2mL) and receptor compartment containing 10 mM phosphate buffer saline (PBS) (pH 7.4). Probes were perfused with 10 mM PBS, pH 7.4 at 2 μL/min and the dialysate as well as receptor samples were collected at pre-determined intervals. After 24 h, drug levels in the skin were analyzed. Probe recovery factor was applied while calculating the drug amount in the dialysates. Statistical analysis was performed using Student’s t test at p values of 0.01 and 0.05 to conclude any significant difference.

Results

Microscopic images of confocal laser microscopy (Image 1), calcein imaging, and histology studies depicted lateral diffusion of the dyes in the microneedle and laser-treated skin. Dermascan images indicated insertion of probes into the dermis at an average depth of 346.67 ± 134.28 μm (Image2). The cumulative amount of diclofenac sodium permeated through laser-treated skin (659.71±107.26 μg/sq.cm) was significantly higher than untreated (125.95±23.75μg/sq.cm, p<0.01) and microneedle-treated skin (153.90 ±30.87μg/sq.cm, p<0.01). Rate of drug diffusion in the central probe observed after 24 h in the microneedle-treated skin (11.79 ± 2.54 μg/h) was significantly higher than untreated (p<0.01) as well as laser-treated skin (p<0.05). Rate of lateral diffusion in lateral probe after 24h in untreated group (0.69 ± 0.05 μg/h) was determined to be significantly lower than microneedle-treated (1.67 ± 0.32 μg/h, p<0.05) and laser-treated skin (1.32 ± 0.07 μg/h, p<0.01). Also, significantly higher amount of drug was observed in the epidermis and dermis of the central as well as lateral diffusion area in the laser treated skin as compared to passive and microneedle treated skin (p<0.05). Overall, observation of the drug levels in the lateral region of skin confirmed the phenomenon of lateral diffusion of drug in vitro.

Conclusion

In vitro microdialysis is a promising technique to quantify lateral diffusion of drugs in skin. The rate of lateral diffusion of diclofenac sodium was enhanced by microneedle and ablative laser treatment.