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Note

In vitro skin penetration of dazmegrel delivered with a bioelastic matrix

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Abstract

The penetration of dazmegrel, a selective thromboxane synthetase inhibitor, through excised human and greyhound skin was measured. A bioelastic matrix was used for topical delivery. Results demonstrated that dazmegrel readily penetrated the skin. Penetration through greyhound skin was significantly greater than penetration through human skin. Penetration through greyhound skin was not significantly different between 4, 24, and 48 h of exposure for the low and intermediate doses studied.

Keywords: Dazmegrel; Thromboxane synthetase inhibitor; Greyhound dog skin; Bioelastic matrix; Dermal pressure ulcer; Skin penetration

Elevated thromboxane levels are associated with a number of disease states including dermal pressure ulcers (Robson et al., 1992). Prevention of tissue thromboxanes could help prevent development of these ulcers. Dazmegrel is a selective thromboxane synthetase inhibitor (Cross et al., 1986), with the potential for negating thromboxane development. Dazmegrel is rapidly excreted after oral administration to humans, and has a half-life of 0.88 ± 0.17 h (Lorenz et al., 1986). The four times daily dosing regimen to maintain effective tissue levels could be simplified by topical application with controlled release of the drug. When dazmegrel was orally administered to greyhound dogs wearing leg casts, it resulted in a sparring effect on the skin in areas of potential pressure ulcer development (Swaim et al., 1994). The present study was undertaken to determine if dazmegrel penetrates greyhound dog skin. Greyhound dog skin was used in this study because the thin skin, angular body conformation, and limited body fat of the dogs is similar to geriatric humans and predisposes them to pressure ulcers. Penetration of dazmegrel through human skin was studied to determine the relationship of skin penetration in greyhound dogs to skin penetration in humans. The study was performed to investigate dazmegrel's potential in preventing dermal pressure ulcers.

Dazmegrel was supplied by Pfizer (Sandwich, Kent, England). The bioelastic matrices were comprised of protein-based polymers, i.e., $[\text{Glycine-Valine-Glycine-Valine-Proline}]_{251}$ (Urry et al., 1993). The protein polymers were prepared by means of recombinant

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DNA technology, synthesized by transformed *Escherichia coli*, and gamma-irradiated cross-linked to form elastic matrices containing 63% water by weight. The pH-dependent solubility of dazmegrel in water was used to control loading of the bioelastic matrix over a 100-fold concentration range from approximately 0.1 mg/cm^2 to 10 mg/cm^2. Full-thickness human breast skin was obtained from the National Disease Research Interchange, Philadelphia, PA, USA. Full-thickness greyhound skin was obtained from dogs that had recently (<1 h) been euthanized as part of other ongoing research projects. Hair on the dorsolateral aspect of the trunk of the greyhound dogs was clipped; the skin was washed, and depilatory cream was used to remove the remaining stubble. The fat was removed from the dermal side and the skin was placed horizontally between a donar (epidermal) and receptor (dermal) chamber of the percutaneous penetration cells (Kemppainen, 1993). The receptor fluid was continually stirred by a motor driven, Teflon-coated magnetic stir bar. The epidermal surface was exposed to room air, had a surface area of 1.9 cm^2, and the volume of the receptor chamber was 2 ml. The receptor fluid was tissue culture media, (RPMI media, GIBCO BRL, Grand Island, NY, USA) supplemented with sodium bicarbonate, HEPES buffer, and ciprofloxacin antibiotic (Miles, West Haven, CT, USA), and had a pH of 7.4. The solubility of dazmegrel in the receptor fluid was 0.143 mg/ml. It has been shown that there is good correlation between in vitro and in vivo skin absorption of lipophilic compounds when skin penetration is calculated by summing the penetrant in the dermis and receptor fluid (Hawkins and Reifenrath, 1986). In Experiment 1, the epidermal surfaces of the human skin ($n = 3$) and greyhound skin ($n = 3$) were dosed with 40 μg [^{14}C]dazmegrel dissolved in 60 μl water pH 8.4, and the time of exposure was 24 h. In Experiment 2, the epidermal surfaces of the greyhound skin were exposed to 0.19 ± 0.32 mg dazmegrel/bioelastic membrane ($n = 3$), 1.9 ± 0.63 mg dazmegrel/bioelastic membrane ($n = 3$), 19 ± 5.7 mg dazmegrel/bioelastic membrane ($n = 3$) for 4, 24, and 48 h. [^{14}C]Dazmegrel was measured with standard liquid scintillation techniques. Dazmegrel was measured with high-pressure liquid chromatography using a Zorbax Phenyl 5 μm analytical (4.6 mm i.d. × 15 cm, MAC-MOD Analytical Inc., Chadds Ford, PA, USA) column, mobile phase of 0.1 M pH 7.5 tetramethyl-ethylenediamine citrate buffer:methanol (50:50) and UV detection at wavelength of 282. Skin penetration was calculated by summing the amount of dazmegrel recovered from the dermis and receptor fluid (Hawkins and Reifenrath, 1986). Statistical analysis of the data consisted of two-way analysis of variance. If the ANOVA indicated a significant effect of variable, a post hoc test (Tukey test) by SAS^R version 6.12 (SAS Institute Inc., Cary, NC, USA, 1996) was used to identify groups with the significant differences.

In Experiment 1, penetration of dazmegrel was significantly greater through greyhound dog skin than through human skin (results not shown). The permeation of dazmegrel through greyhound dog skin (20.5 ± 1.9 percent of the dose) was five times greater than the permeation of dazmegrel through human skin (4.1 ± 1.2 percent of the dose). This is consistent with other reports that dog skin is more permeable than human skin (Wester and Maibach, 1985). The penetration of dazmegrel through greyhound dog skin (Experiment 2) is shown in Fig. 1. The low and intermediate dose patches provided constant permeation concentrations of dazmegrel at 4, 24, and 48 h. The low dose had a tendency toward greater penetration at 24 h, but this was not significant because of the amount of variation. Variation in skin penetration studies can be caused by stratum corneum thickness, lipid content, and the keratinocyte dimensions, all known to vary

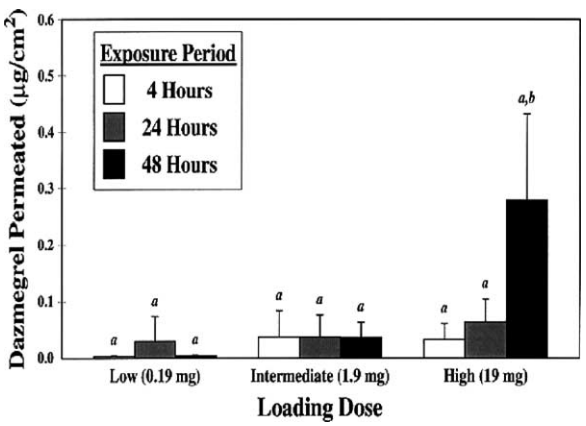

Fig. 1. In vitro greyhound skin permeation of dazmegrel. Compare the amount of loading dose and length of exposure period on permeation of dazmegrel (expressed as mean μg/cm^2 ± S.E.). Different superscripts (a and b) indicate when there is a significant effect of exposure period (4, 24, 48 h) on penetration.

between individuals and different locations on an individual (Johnson et al., 1995). The high-dose patches provided constant permeation concentrations at 4 and 24 h, but there was a significant increase at 48-h topical exposure. The increased penetration with increased time is a phenomena that has been seen before. When percutaeous absorption of glucose was studied in the rat there was a increase in flux with time (Ferreira et al., 1995). The amount of dazmegrel that permeated the greyhound dog skin ranged from $7.5 \times 10^{-4} \pm 2.5 \times 10^{-4}$ μg/cm^2/h for the low dose after 4 h of topical exposure to $5.8 \times 10^{-3} \pm 3.1 \times 10^{-3}$ μg/cm^2/h for the high dose after 48 h of topical exposure. The 50% inhibitory concentration (IC_{50}) for dazmegral for thromboxane synthetase is 7.4×10^{-3} μg/ml (Cross et al., 1986). The total recovery of the applied dose did not differ between groups, and averaged 88 ± 13%. The largest amount of the applied dose was recovered in the bioelastic matrices (averaging 67 ± 9.0).

This research demonstrated that dazmegrel penetration is greater through greyhound dog skin than through human skin. In addition, constant levels of dazmegrel were achieved in the skin at 4, 24, and 48 h by using the bioelastic matrices for topical delivery of the low and intermediate doses. Current in vivo work in our laboratory shows promise for the use of bioelastic matrices loaded with dazmegrel for preventing skin pressure lesions in greyhound dogs.

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References

Cross, P.E., Dickenson, R.P., Parry, M.J., Randall, M.J., 1986. Selective thromboxane synthetase inhibitors. 2. 3-(1*H*-Imidazol-1-ylmetyl)-2-methyl-1*H*-indole-1-propanoic acid and analogues. J. Med. Chem. 29, 1643–1650.

Ferreira, L.A.M., Doucet, J., Seiller, M., Grossiod, J.L., Marty, J.P., Wepierre, J., 1995. In vitro percutaneous absorption of metronidazole and glucose: comparison of O/W, W/O/W and W/O systems. Int. J. Pharm. 121, 169–179.

Hawkins Jr., G.S., Reifenrath, W.G., 1986. Influence of skin source penetration cell fluid and partition coefficient on in vitro skin penetration. J. Pharm. Sci. 75, 378–381.

Johnson, M.E., Blankschtein, D., Langer, R., 1995. Permeation of steroids through human skin. J. Pharm. Sci. 84, 1144–1146.

Kemppainen, B.W., 1993. Penetration studies with excised human and animal skin. In: Tyson, C.A., Frazier, J.M. (Eds.), In Vitro Biological Systems, vol. 1. Academic Press, San Diego, pp. 504–514.

Lorenz, R.L., Fischer, S., Wober, W., Wagner, H.A., Weber, P.C., 1986. Effects on prostanoid formation and pharmacokinetics of dazmegrel (UK-38,485), a novel thromboxane synthetase inhibitor, in man. Biochem. Pharmacol. 35, 761–766.

Robson, M.C., Heggers, J.P., 1992. Eicosanoids, cytokines, and free radicals. In: Cohen, I.K., Diegelmann, R.F., Linblad, W.J. (Eds.), Wound Healing: Biochemical and Clinical Aspects. W.B. Saunders, Philadelphia, pp. 292–304.

Swaim, S.F., Bradley, D.M., Vaughn, D.M., Powers, R.D., Hoffman, C.E., Beard, M.L., 1994. Evaluation of thromboxane synthetase inhibitor in the prevention of dermal pressure lesions. Wounds 6, 74–82.

Urry, D.W., Nicol, A., Gowda, D.C., Hogan, L.D., McGee, A., Williams, T., Olson, D.B., Cox, B.A., 1993. Medical applications of bioelastic materials. In: Gebelein, C.G. (Ed.), Biotechnological Polymers: Medical, Pharmaceutical and Industrial Applications. Technomic Publishing, Lancaster, PA, pp. 82–103.

Wester, R.C., Maibach, H.I., 1985. In vivo animal models for percutaneous absorption. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption. Mechanisms-Methodology-Drug Delivery, vol. 6. Marcel Dekker, New York, pp. 251–266.