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The influence of receptor fluid on in vitro percutaneous penetration

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Summary

The in vitro percutaneous penetration of hydrocortisone through full-thickness human mammary skin into 3 receptors has been assessed. The receptor fluid was found to influence the extent of penetration These results suggest that the osmotic strength of the receptor used for in vitro experiments, and its ability to exert any delipidizing/back-flushing effects, will influence the amount of penetrant able to traverse the skin barrier.

Introduction

The FDA has recently published guidelines appropriate to measuring in vitro percutaneous penetration, following a Transdermal Workshop (Skelly et al., 1987). The guidelines are required since experimental conditions used in order to measure percutaneous absorption vary between laboratories. Skin type and thickness will certainly influence the quantity of material appearing in the receptor fluid. The type of receptor fluid employed might not appear to have the ability to influence the results to the same extent. However, prior to the Transdermal Workshop, we have investigated the influence of receptor fluid on hydrocortisone.

The prime objective of a receptor fluid is to provide sink conditions for the penetrating molecule. In order that transfer of the penetrant from the skin into the receptor does not influence the rate of release, it is generally accepted that sink conditions exist provided that the concentration of penetrant in the receptor phase does not exceed 10% of its saturated solubility.

Isotonic saline is probably the most popular receptor fluid, and has been used by Bronaugh et al. (1986), Okamoto et al. (1986) and Scott et al (1986a). Other reported receptor fluids used for in vitro percutaneous penetration experiments include pH 7.4 buffer (Akhter and Barry, 1985; Swarbrick et al., 1982); isotonic pH 7.4 buffer (Keister and Kasting, 1986; Guy et al., 1986; Southwell and Barry, 1984); 50% aqueous ethanol (Akhter et al., 1984; Scott et al., 1986b); polyethylene glycol 400 (Sarpotdar et al., 1986); Volpo

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N20 (Bronaugh and Stewart, 1986); and water (Akhter et al., 1984; Cooper, 1984).

This gives an indication of the range of receptor fluids that have been employed.

Of all the receptor fluids, isotonic saline, pH 7.4 buffer and 50% aqueous ethanol appear to be the most widespread. Volpo N20 is also increasing in popularity. These receptor fluids are all potentially capable of providing sink conditions for hydrocortisone and were therefore chosen to examine the effect of receptor fluid on the in vitro penetration of hydrocortisone from a dry film deposited from ethanol through full-thickness human mammary skin.

Materials and Methods

Materials

Isotonic saline (0.9%) was prepared using sodium chloride (Fisons A.R.). Ringer–Locke pH 7.4 physiological buffer was prepared to contain 0.9% sodium chloride, 0.042% potassium chloride, 0.1% glucose, 0.05% sodium bicarbonate (all Fisons A.R.) and 0.016% calcium chloride (BDH Analar). Both these receptors contained 0.01% thiomersal (Sigma Chemicals) as preservative. Ethanol (James Burrough) and Volpo N20 (PEG-20 oleyl ether, gift from Croda Chemicals Ltd.) were prepared in water at concentrations of 50% v/v and 6% respectively. All percentages are w/v unless stated otherwise.

Permeation studies using excised human skin

Human skin was obtained from a breast reduction operation (patient age 26 years) and stored at −20 °C prior to use. Subcutaneous fat was removed using blunt dissection, and the skin was mounted into Franz-type diffusion cells of the classical design (Franz 1975), diffusional area 0.79 cm^2. After equilibration for 1 h with the respective receptor fluid at 37 °C, the skin was dosed with approximately 1 μmol hydrocortisone (Beecham Pharmaceuticals Laboratory Reference Standard) dissolved in 50 μl ethanol. The ethanol evaporated within a few minutes, leaving a dry film of drug deposited on the skin surface open to ambient conditions. 1 ml samples of receptor fluid were taken via the side-arm at appropriate intervals, filtered through a 0.45 μm disposable filter (Gelman Sciences Ltd.) and analyzed by HPLC. Receptor fluid was replaced, and dilutions were taken into account at the calculation stages. The studies were carried out in quadruplicate.

Analysis

HPLC analysis was conducted using a Severn Analytical 6410 Solvent Delivery System equipped with a Kratos Spectroflow 773 high sensitivity variable wavelength detector at 240 nm and a Spark-Holland PROMIS Autosampler with a rheodyne injection valve and 100 μl injection loop. The column used, 160 × 4.6 mm, was packed with Spherisorb 5 ODS (Phase Sep) reverse-phase column packing. HPLC eluent was 70/30 acetonitrile/pH 6.3 phosphate buffer, so that the hydrocortisone retention time was 4 min. Solvent flow was 2.0 ml/min. All chemicals were of chromatographic grade.

Results and Discussion

HPLC analysis of 6% Volpo N20 receptor fluids for hydrocortisone was not possible. Receptor fluid that had been in contact with the skin contained a gross contaminant that completely obscured the hydrocortisone peak. Since analysis of the drug was possible for receptor fluid that had not been in contact with skin, it must be assumed that the Volpo N20 is extracting the contaminant from the skin barrier. This raises questions as to the integrity of the skin that had been in contact with Volpo.

Fig. 1 shows the penetration profiles of hydrocortisone into each of the other 3 receptor fluids. For isotonic saline, approximately 2.5% of the applied dose appeared in the receptor fluid after 118 h, and correspondingly less for 50% aqueous ethanol and pH 7.4 buffer. Penetration was most rapid through skin bathed by isotonic saline, then 50% aqueous ethanol, and least rapid into pH 7.4 buffer.

The difference in penetration of hydrocortisone through full-thickness skin according to receptor fluid may be explained by considering the relative

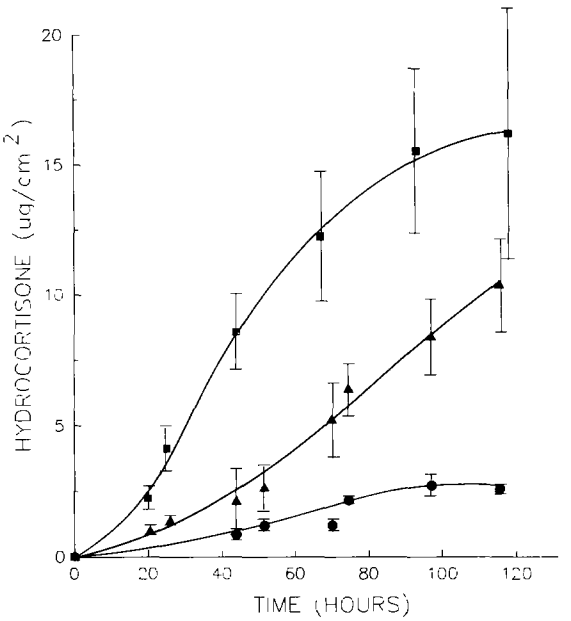

Fig 1 The effect of receptor fluid on the in vitro percutaneous penetration of hydrocortisone (measured as $\mu g/cm^2$ hydrocortisone in the receptor fluid) through full thickness human mammary skin, as a function of time Receptor fluids (■———■), isotonic saline, (▲———▲), 50% aqueous ethanol, (●———●), pH 7 4 buffer Each point is the mean ± S E M , $n > 3$

hydration of the skin in contact with the receptor fluid. Skin hydration (normally achieved by occlusion) is well documented in increasing the permeability of the skin to drug molecules (see Barry (1983) and references therein). Dehydration of the skin may therefore be predicted to have the reverse effect. Any hypertonic solution in contact with an aqueous layer of a skin membrane (i.e. dermis) will, in comparison to an isotonic solution, have a dehydrating action.

Aqueous ethanol (50%) and pH 7.4 buffer are both hypertonic relative to isotonic saline. The resulting relative dehydration of the skin therefore retards penetration of hydrocortisone for these two receptor fluids, compared with isotonic saline. Additionally, ethanol is capable of exerting a delipidizing and back-flushing effect on the skin (Barry, 1983). This further impairs the barrier function of the skin, enhancing the permeability of the skin to hydrocortisone, and is additional to the dehydration properties exerted by an aqueous ethanol receptor fluid.

The receptor fluid has therefore been shown to affect the extent of penetration, and should be chosen with care.

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