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Rapid Communication

The influence of Azone® on the percutaneous absorption of methotrexate

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Summary

The percutaneous absorption of the polar drug methotrexate has been examined in vitro. Two alcoholic gel formulations containing 1% methotrexate with or without Azone® were applied to full-thickness abdominal human skin mounted in all-glass Franz-type diffusion cells. In the absence of Azone® no percutaneous penetration of methotrexate was observed. In the presence of Azone® 190 ng/cm^2 permeated after 48 h. Azone® appears to be acting as an efficient penetration enhancer for this drug.

Methotrexate was examined since it has been shown to be an effective systemic therapy for many proliferative disorders. It acts by inhibiting dihydrofolate reductase, thereby disrupting DNA synthesis (Hitchings and Burchall, 1965). It has proved useful in the treatment of neoplastic disorders (Calabresi and Parks, 1985) and has been successfully used in the treatment of cutaneous proliferative disorders, e.g. psoriasis (McCullough and Weinstein, 1983), and cutaneous T cell lymphoma. Its primary limitation is the risk of acute toxicity in rapidly proliferating tissues such as the gastrointestinal tract and the bone marrow. At the present time, methotrexate is available in oral and parenteral dosage forms. The availability of a topical preparation would theoretically limit systemic toxicity while providing effective local drug concentrations to treat a variety of proliferative skin disorders.

In order to increase the effectiveness of drugs that are delivered topically or transdermally it is beneficial to identify safe and pharmaceutically acceptable penetration enhancers. The mode of action of penetration enhancers has been the matter of debate over the past few years. It is generally thought that materials such as Azone® interact with the structured lipids in the intercellular channels of the stratum corneum (Beastall et al. 1988; Bouwstra et al., 1989). Disorder is increased in the ceramide bilayers with an increase in the diffusion coefficient of the drug through the stratum corneum. It has also been proposed that there are two distinct routes of penetration through the skin: the polar route and the lipid route (Berner and Cooper, 1987). Hydrophilic drugs would be expected to have a slow flux through the skin (at

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constant concentration) that is independent of partition coefficient. It may be anticipated that their flux would also be independent of enhancer effect if the enhancer acts on the lipid regions of the skin.

Azone® was developed by Whitby Research, Inc. as a penetration enhancer. The results of numerous pharmacological and toxicological studies demonstrate that Azone® possesses a safe toxicological profile. A methotrexate gel formulation has been formulated which contains 1% of the active drug and 3% Azone® and this paper provides evidence that the presence of the Azone® in the gel enhances the skin penetration of antimetabolite.

Simple alcoholic carbomer gel formulations containing 1% methotrexate and 0 or 3% Azone® were supplied by Whitby Research, Inc. Assay validation was conducted using methotrexate USP (Mack). Separation by HPLC was performed on a μ Bondapack C18 column (25 cm × 4 mm) using a mobile phase of: acetonitrile 15/(0.05 M sodium phosphate buffer, pH 7.0 + 0.005 M TBAS) 85 at ambient temperature and UV detection at 302 nm. The retention time of methotrexate was 6.1 min. The detection limit was found to be 15 ng/ml with no interference from leached skin or formulation components.

Human female abdominal skin (age range 27–35) was obtained and the subcutaneous fat removed by blunt dissection. Permeation assessment was carried out on the two formulations using paired samples from the same donor. The full-thickness epidermal membranes were clamped in all-glass Franz-type diffusion cells with an available diffusion area of approx. 2 cm^2 and receptor volume of approx. 6 ml. The receptor phase (pH 7.4 phosphate buffer) was stirred continuously and thermostated at 37 °C. The skin surface temperature was 32 ± 1 °C. The gel formulations were applied to each donor chamber at a dosing of approx. 100 mg/cm^2. The experiments were conducted six times. Samples from the receptor phase were removed at periodic intervals and analysed for methotrexate content.

After a 48 h period, no methotrexate was detected in the receptor compartments of the diffusion cells in which the formulation with no Azone® was used. This can be compared with the results for the formulation with Azone® in which 189 ± 98 ng/cm^2 (mean ± SE) was found. Statistical analysis of the data using Super-Anova and Fisher's Protected LSD post hoc indicated that the results for the two formulations were significantly different at the $p = 0.088$ level. Considering the detection limit of 15 ng/ml, the enhancement factor for Azone® can be calculated to be not less than a factor of 5. The permeation profile for the most permeable skin sample with the formulation containing Azone® is shown in Fig. 1. This graph can be used to estimate the pseudo-steady state permeation rate of 19 ng/cm^2 per h.

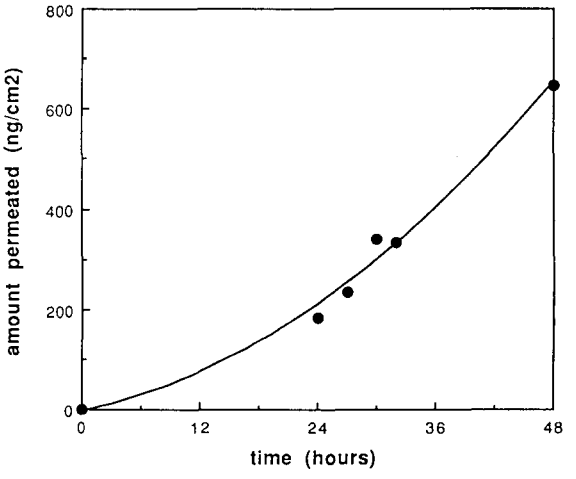

Fig. 1. The amount of methotrexate permeating human skin (in vitro) as a function of time.

It is possible to predict the steady-state diffusion flux of methotrexate using the kinetic model of Guy and Hadgraft (1985). A solubility constraint in the stratum corneum (Hadgraft et al., 1990) will be significant for this hydrophilic compound. In the model the important factors are MW (454), log P at pH 7.4 = −2.52 * (measured) and the octanol solubility [oct]. The solubility in octanol can be estimated from the aqueous solubilities and the measured partition coefficients given by Wallace and Barnett (1978). The esti-

* Medchem Software, Daylight Chemical Information SystemsInc., Claremont, CA 91711, U.S.A.

mated octanol solubility is 0.01 g/l which can be related to the amount of drug in the stratum corneum [sc] lipids (Hadgraft et al., 1990) by

$$\log[sc] = 1.31 \log[oct] - 0.13$$

giving a value of 2.18×10^{-3} $\mu g/cm^2$. This then gives an estimated maximum flux of methotrexate through the skin of 0.26 ng/cm^2 per h. This very slow flux is due to the very hydrophilic nature of methotrexate which does not partition favourably into the stratum corneum lipids.

The calculated value can be compared with experimental values given by Siddiqui et al. (1985) where, at pH 8, the steady-state flux was found to be 2.2 ng/cm^2 per h. Both the predicted rate and the experimentally determined rate are below the levels of detection used in this publication.

Significant enhancement by Azone® has been produced and this lipophilic enhancer, log $P =$ 6.2 * (estimated), would be expected to locate in the skin lipids. The enhanced flux is most likely to be caused by increased diffusion through the stratum corneum and it seems implausible that a molecule like Azone® would influence a polar route.

Guy and Hadgraft (1988) have already questioned the existence of a polar route through the stratum corneum. It seems that the enhancer effects of Azone® on methotrexate support the view that polar molecules do not have a unique diffusional pathway through the intercellular channels.

The in vitro enhancement shown by Azone® in this publication and preliminary clinical data indicate that the combination of Azone® and methotrexate may possess sufficient clinical efficacy to warrant further development (Weinstein et al., 1989).

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