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In vitro permeation of azelaic acid from viscosized microemulsions

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Summary

The transport of azelaic acid, a bioactive molecule used in treating acne and many skin disorders, from a viscosized microemulsion and from a gel through full thickness abdominal skin was examined. A lag time was evident in both systems. The percentage of azelaic acid transported from the microemulsion was several times higher than that from the gel. The effect of dimethyl sulfoxide, chosen as a model enhancer, on transport was also investigated. After 8 h from the viscosized microemulsions, 43 and 64% of the initial amount passed through hairless skin in the presence of 1 and 2% of dimethyl sulfoxide, respectively.

Introduction

Azelaic acid (nonanedioic acid) has been shown to have positive therapeutic effects after topical application. It has beneficial effects in some pigmentary disorders such as lentigo maligna, on acne vulgaris and in some hyperpigmentary disorders (Nazzaro-Porro et al., 1979; Breathnach et al., 1984, 1989). The mechanism of action of the acid is still under investigation, but in all the conditions considerable amounts of azelaic acid are required for a number of months in order to produce therapeutic effects. The necessity of achieving and maintaining high intralesional concentrations of the diacid for a long period is a limiting factor, also because, at the moment, only the topical route of administration has given good results.

In a previous work (Gallarate et al., 1990) fluid o/w microemulsions carrying azelaic acid were studied in order to achieve two different objectives: (i) a sufficient reservoir of the acid in the internal phase and (ii) a fast release of the acid. The environment of the external hydrophilic phase was studied in order to achieve a lower extent of dissociation of azelaic acid and favour its transport through a lipophilic membrane. High rates of transport through lipophilic membrane were obtained but the microemulsions could not be applied on the skin because of their fluidity.

In the present work we refer to the viscosization of a microemulsion and permeation of azelaic acid through hairless mouse skin. Moreover, the influence of dimethyl sulfoxide as a model enhancer was also examined.

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Experimental

Materials

Azelaic acid (nonanedioic acid), 1-decanol, 1-dodecanol, sorbitan(20)OE-monolaurate (Tween 20), and 2-bromoacetophenone (phenacyl bromide) were from Fluka; 1-butanol, 1,2-propylene glycol, dimethyl sulfoxide (DMSO), dichloromethane, *n*-hexane, *n*-heptane and *n*-tributyl phosphate (TBP) were from Merck; tetrahexylammonium chloride (THA) was from Aldrich; and Carbopol 934 was from Biochim.

Phenacyl bromide was recrystallized in *n*-heptane and stored in a desiccator before use. Dichloromethane was bidistilled and washed with water before use, except when used as mobile phase. All chemicals were of analytical grade.

Microemulsion preparation

The microemulsion, consisting of azelaic acid, water-propylene glycol (1.1 : 1 w/w) at pH 3.0, decanol-dodecanol (2 : 1 w/w), Tween 20, 1-butanol and Carbopol 934, was prepared as previously described (Gallarate et al., 1990).

A microemulsion containing Sudan Red (0.05%) instead of azelaic acid was also prepared.

Microemulsions containing the enhancer were prepared by substituting 1 or 2% of the aqueous phase with DMSO. The final concentration of azelaic acid was always 6.4%.

A gel described in the literature (Marn et al., 1982), consisting of water, propylene glycol, ethanol, Carbopol 934 and 15% (w/w) azelaic acid was also studied. The pH was corrected at 3.0.

Partition studies

The apparent partition coefficient in the presence of cosurfactant (P_{cos}) of Sudan Red at pH 3.0 was determined as previously described (Trotta et al., 1989). The concentration of Sudan Red in the aqueous phase was determined spectrophotometrically at 472 nm.

In vitro diffusion studies

Skin specimen The full-thickness abdominal skin of eight male hairless mice (Hsd:athymic nude-nu Br-NOSSAN) aged 4–5 weeks was used. The skin was excised, washed, examined for integrity and used for permeation experiments.

Skin permeation studies Vertical cells, proposed by Gummer et al. (1987), were used in the permeation experiments. The skin, previously rinsed with normal saline, was sandwiched between two areas of ground glass and the diffusion cell assembly was secured in place with a clamp. After equilibrating the receptor chamber with phosphate buffer (0.1 M, pH 7.0), 180 mg of the system to be studied (microemulsion or gel) was applied on the skin (area 1.97 cm^2). The contents of the receptor cell were stirred continuously at 600 rpm by a rod-shaped rotating magnet and thermostated at 37 °C. At fixed times, the entire contents of the receptor chamber (6 ml) were removed by gravity and substituted for fresh phosphate buffer.

Diffusion experiments The in vitro permeation studies were performed using as donor phases: (a) gel containing 15% azelaic acid (Marn et al., 1982); (b) microemulsion containing azelaic acid (6.4%); (c) microemulsion containing azelaic acid (6.4%) and DMSO (1 and 2%); and (d) microemulsion containing Sudan Red (0.05%).

HPLC analysis

Azelaic acid was analyzed as the phenacyl ester according to Furängen et al. (1986). The derivative was prepared by extractive alkylation: the reaction, carried out at 25 °C, required about 1 h. The derivative samples were dissolved in the mobile phase before HPLC analysis.

The analysis of azelaic acid after derivatization was performed on a Perkin Elmer HPLC apparatus consisting of a UV detector (LC 90), a pump control unit (LC 250) and a data station equipped with the OMEGA 2 software. The chromatographic conditions were as follows: column, 125 × 4.6 mm LiChrosorb CN 5; mobile phase, *n*-hexane/dichloromethane/TBP (90 : 10 : 1); flow rate, 1.3 ml/min; UV detector, 254 nm; sample volume, 6 μl.

Results

Partition coefficient

The apparent partition coefficient (P_{cos}) of Sudan Red between decanol-dodecanol and

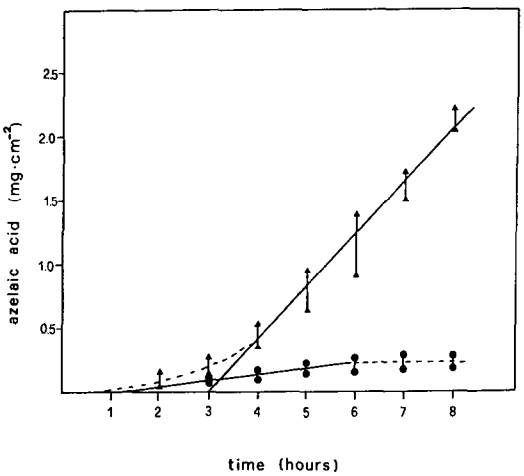

Fig. 1. Permeation profiles of azelaic acid from a viscosized microemulsion (▲) and from a gel (●). Vertical bars indicate S.D.

water-glycol solutions in the presence of butanol was 159.4.

In vitro diffusion studies

The azelaic acid release rate data using hairless mouse abdominal skin and pH 7.0 phosphate buffer as receptor phase are reported in Figs. 1 and 2. In Fig. 1, data on the diffusion of azelaic acid (6.4%) from the microemulsion without the enhancer are compared to those from the gel. The amount of drug penetrating through the skin per unit area was plotted vs time. Each data point is the mean of three experimental trials. The bars indicate the S.D. and the best-fit line, which represents the mean steady-state flux, was determined by linear regression. The steady-state flux of azelaic acid was 0.414 mg/cm^2 per h ($r^2 = 0.9985$) from the microemulsion and 0.0459 mg/cm^2 per h ($r^2 = 0.966$) from the gel.

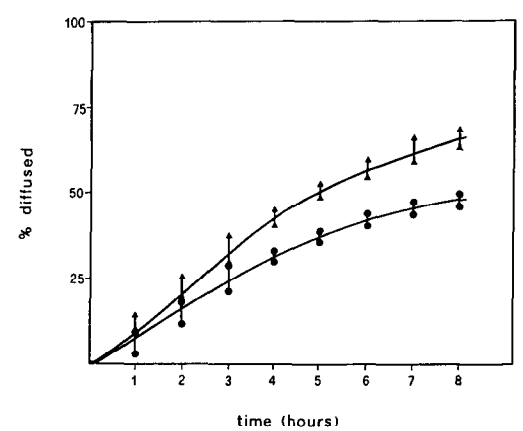

Fig. 2. Azelaic acid permeated from viscosized microemulsions [containing 1% (●) and 2% (▲) of dimethyl sulfoxide] against time. Vertical bars indicate S.D.

The amounts of azelaic acid transported through hairless mouse skin from microemulsions containing different percentage of DMSO are reported in Fig. 2. Each point represents the mean ± S.D. of three trials.

The diffusion experiments on microemulsion containing Sudan Red showed no appreciable transport of the dye through hairless mouse skin.

Discussion

In a previous work (Gallarate et al., 1990), fluid o/w microemulsions containing azelaic acid were studied and found to result in high rates of release of the drug through a double membrane. In the present study a viscosized microemulsion was examined; an amount of azelaic acid up to 6.4% was carried by the system. The concentration was lower than those commonly used in topical formulations of azelaic acid (Marn et al., 1982; Breathnach et al., 1989).

However, comment is necessary regarding two aspects: all the azelaic acid was dissolved, not suspended as occurs in the topical systems usually applied; moreover, the highest amount of drug was present in the reservoir constituted by the internal phase (including also the interphase in the internal phase) of the microemulsion. A previously reported gel (Marn et al., 1982) was chosen as a reference, also because some of its components (propylene glycol, Carbopol 934, water) were the same as those of the microemulsion.

In Fig. 1 the cumulative transport of azelaic acid from equal amounts of gel and microemulsion through hairless skin membrane is reported. Even if the microemulsion and the gel were 6.4 and 15%, respectively, in azelaic acid, the transport of the drug was very different; after 8 h about 35% of the azelaic acid present in the microemulsion had been transported, while transport

from the gel was only 1.8%. The release of azelaic acid from the viscosized microemulsion was higher than that from a fluid microemulsion (Gallarate et al., 1990), most likely as a consequence of the different permeability of the two barriers.

To exclude transport through hairless mouse skin of whole droplets of the microemulsion, a highly lipophilic dye (often used in the field of emulsions for their characterization) was vectorized in the microemulsion. The partition coefficient (P_{cos}) of the dye in the presence of the cosurfactant was determined as previously performed for azelaic acid (Gallarate et al., 1990). Also, if P_{cos} only roughly reproduced the real partition of the drug between the phases of the microemulsion, the result ($P_{cos} = 159.4$) suggested that practically all the dye was in the internal phase. Therefore, the dye should be in the receptor phase only if the droplets passed through the hairless skin; but no dye could be detected in the receptor phase. Therefore, the considerable and rapid transport of azelaic acid through the skin should be due to the continuous partition of the drug from the internal to the external phase.

A lag time was evident in both systems, being rather longer for the microemulsion than for the gel (Fig. 1). The addition of different amounts of DMSO to the viscosized microemulsion was performed in order to examine how an enhancer could affect the trend in the transport of azelaic acid.

In Fig. 2 the percentages of azelaic acid diffused from microemulsions vs time, in the presence of DMSO, are reported; no lag time is detectable. The trend in the amounts of azelaic acid transported through the skin vs time was quite different from that observed previously; a linear relationship lasting for some hours between the amounts transported vs time could be observed. The effect of the enhancer was evident; after 8 h the percentage of azelaic acid released from microemulsions containing 1 and 2%, respectively, of DMSO reached 43 and 64% of the initial value.

The high transport of azelaic acid from microemulsions should be ascribed to the considerable partition of the acid in the internal phase of the microemulsions. Owing to the infinite stability of microemulsions, the real amount of azelaic acid present in the internal phase cannot be determined. However, the trend in its transport, in the presence and absence of enhancers, appears to show that almost all the acid is dissolved in the disperse phase.

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