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Application of sucrose laurate in topical preparations of cyclosporin A

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Summary

The skin irritating potential of sucrose laurate was studied in the normal guinea pig model. The results showed that sucrose laurate exhibited no skin irritating effect at all. The application of sucrose laurate as an excipient in hydrophilic dermal preparations was investigated, by measuring the inhibition of contact sensitivity skin reactions to dinitrofluorobenzene (DNFB), after topical application of a cyclosporin A hydrogel. The findings showed that the sucrose laurate hydrogel formulation was somewhat less effective as compared to a sucrose laurate or ethanolic solution, but still superior to a reference paraffin suspension. The sucrose laurate/cyclosporin A hydrogel significantly reduced the skinfold thickness after 24, 32, 48 and 56 h of sensitization ($p < 0.01$–0.001). Skin permeability experiments indicated that the sucrose laurate has some intermediate skin permeability enhancing properties. Conclusively, these findings showed that sucrose laurate is a suitable, non irritating excipient for the dermal formulation of the poorly water soluble cyclosporin A. In order to obtain an optimal sucrose laurate (trans)dermal hydrogel formulation, however, the effect of surfactant or cyclosporin A concentration and the presence or absence of occlusion must be subjected to further investigation.

Introduction

Surfactants are frequently used in dermal preparations for dermatological and cosmetic use, to improve physico-chemical characteristics such as stability and appearance. In hydrophilic dermal preparations, surface active agents are also employed to raise aqueous solubility of poorly soluble drugs. The surface active agent sucrose laurate, a new promising excipient, was found to have high solubilizing capacity (Hahn, 1988; Lerk, 1991). Because sucrose esters are only applied in the food industry, there is no information regarding the topical application of sucrose laurate. Therefore, this investigation presents the results of a study on the application of sucrose laurate as an excipient in hydrophilic dermal preparations. Accordingly, the inhibition of contact sensitivity skin reactions to dinitrofluorobenzene (DNFB)

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by topical application of cyclosporin A, a poorly water soluble drug substance, is investigated.

In addition to their effect of raising the solubility of drug substances, surface active agents can also have a profound influence on cutaneous membrane permeability. The property of nonionic surfactants of changing skin permeability can be explained, firstly, by their penetration into the intercellular regions of the stratum corneum, increasing membrane fluidity and eventually extracting lipid components (Golden et al., 1986). Secondly, the surfactants may penetrate into the intracellular matrix and interact with keratin filaments which results in the disruption of order within the corneocyte (Walters et al., 1988). Since there is no information regarding the skin permeability enhancing properties of sucrose laurate, an additional objective of this study is to investigate the use of sucrose laurate as a permeation enhancer.

All surface active agents are potential irritants. Although the group of nonionic surfactants have been found to be much less irritating (Ashton et al., 1986; Tupker et al., 1989), as compared to ionic surfactants, this study will start with an evaluation of the skin irritating potential of sucrose laurate.

Materials and Methods

Materials

Carbopol 934P (Dr Buser Co., Zürich, Switzerland), propylene glycol (Prochem AG, Zürich, Switzerland), dextran sulfate (Mol. Wt 500 000; Fluka AG, Buchs, Switzerland), sorbic acid (Pluess-Staufer AG, Oftringen, Switzerland), 2,4-dinitro-1-fluorobenzene (Fluka AG, Buchs, Switzerland), and sodium hydroxide (Merck, Darmstadt, Germany) were all of commercial grade. Ethyl acetate, methanol and hexane were obtained from Merck, Uvasol (Darmstadt, Germany). Acetonitrile was also purchased from Merck, trademark LiChrosolv. Cyclosporin A was derived from Sandoz Pharma AG (Basel, Switzerland). Sucrose laurate (L-1695) was a gift from Ryoto Co. (Chyodo-Ku, Tokyo, Japan) and conformed to the Japanese Food standards. The remaining materials were purchased from either Fluka (Fluka AG, Buchs, Switzerland) or Merck (Darmstadt, Germany) and were chemically pure.

Laboratory animals

For evaluation of the skin irritation potential and anti-inflammatory efficiency, female Dunkin Hartley guinea pigs (Charles River Wega, Sulzfeld, Germany) were used. The animals were housed in groups in Macrolon type IV cages under standard conditions for a period of 7 days for acclimatization (12 h light/12 h dark cycle, 23 ± 1°C room temperature, 50 ± 20% relative humidity). The guinea pigs had an average weight of approx. 450 g. After sensitization the animals were kept individually in Macrolon type III cages. Groups of three or six pigs were used.

In the case of penetration experiments, hairless female rats (strain: ICO: OFA hr-hr) in the age range 6–8 weeks were used. The rats were supplied by Iffa-Credo (Lyon, France). After an acclimatization period of at least 1 week, the rats were kept individually in Macrolon type III cages. All animals were allowed free access to food and water.

Skin irritant potential

The skin irritant potential of the placebo formulation was tested by measurement of skinfold thickness in normal guinea pigs (450–700 g body weight) after 20 min, 8, 24, 32 and 48 h following challenge. Treatment was performed on three animals per group by repeated topical applications of 250 ± 10 mg of the formulations to the left and right flanks.

Anti-inflammatory efficiency

To initiate sensitization, 50 μl of a 2% solution of 2,4-dinitro-1-fluorobenzene (DNFB), prepared in acetone/olive oil (1:1), was applied epicutaneously to the dorsum of the right pinna. Skin testing was started 7 days after initiation, by application of 20 μl of 0.5% DNFB, dissolved in acetone/olive oil (4:1), to circular areas of 1.5 cm diameter of both shaved flanks. In contrast to the evaluation of skin irritant potential, six animals per group were used. The cyclosporin A formulations were applied to the right flanks, the

left flanks being treated likewise with the corresponding placebo formulation. The anti-inflammatory efficiency of the formulations was determined after 20 min, 8, 24, 32 and 48 h following challenge.

Skin reactions were determined by measurements of the skinfold thickness of the exposed skin with calipers (Schnelltaster, Kroeplin, range: 0–10 mm, readability 0.05 mm) before and 8, 24, 32, 48 and 56 h after challenge. Cutaneous redness was quantified before challenge and after 24 and 32 h, using a reflected-light colorimeter (Chroma-meter II Reflectance, Minolta). Measurements 8 h after skin testing were not performed, since these data would have been rendered inaccurate by the DNFB-related discoloration of the skin. Regrowth of hair impeded follow-up measurements after more than 32 h. Data on skinfold thickness and erythema were analyzed by three-way analysis of variance. The efficacy of the cyclosporin A formulations was calculated as follows:

$$\text{Efficacy}(\%) = \left(1 - \frac{\delta(\text{verum})}{\delta(\text{placebo})}\right) \qquad (1)$$

where δ(verum) is the difference in skinfold thickness of cyclosporin A treated skin and δ(placebo) denotes the change in skinfold thickness of the corresponding placebo treated skin.

The relative efficacy was defined as the enumeration of the efficacy after 24, 32 and 48 h.

Skin penetration assay

For the skin penetration experiments, abdominal and dorsal skin was used. Subcutaneous fat was removed from the skin patches as far as possible. The remaining skin thickness (dermis and epidermis) was 0.30–0.35 mm. After preparation the skin was transferred, upside down, onto an aluminum foil. Up to four skin samples with a diameter of 35 mm were punched from the samples obtained.

Experiments were set up according to Franz (1975, 1978) and Cooper (1984). The modified Franz diffusion cell used, is shown schematically in Fig. 1. The acceptor compartment contained 5.8 ml of a 0.86% saline/methanol (75 : 25) solution. The skin samples were placed upside down upon the acceptor chamber. The effective diffusion area was 2.54 cm^2. The donor compartment was filled with about 300 μl donor solution or, in the case of the semisolid oleogel, with approx. 300 mg. The temperature was maintained at 32°C by circulating water through a jacket which surrounded both chambers. The acceptor compartment was stirred magnetically.

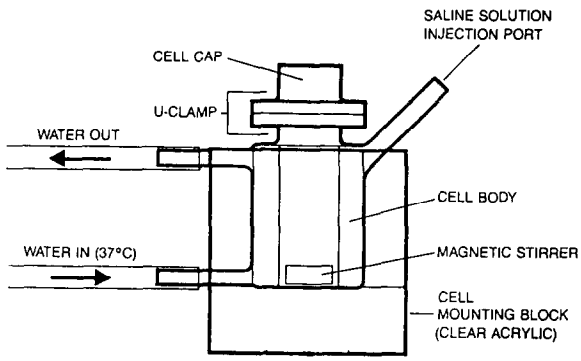

Fig. 1. Schematic representation of the modified Franz diffusion cell.

Samples of 100 μl were taken after 4, 8, 24, 28, 32 and 48 h, and were replaced by an equivalent amount saline/methanol solution. The two half cells were separated after 48 h and the excess donor formulation removed with soft tissues. The skin sample was peeled off from the cell and the stratum corneum stripped 15 times with adhesive Tesa-tape.

The remaining skin was punched with a 7 mm punch. The sample was weighed and homogenized in a glass homogeniser in 0.2 M phosphate buffer (pH 7.0). An internal standard (100 μl) comprising 100 μg/ml cyclosporin A was added. The homogenized sample was quantitatively transferred into a 50 ml centrifuge tube and the residue removed by washing four times with 5 ml phosphate buffer (pH 7.0). The resulting aqueous solution was stirred for 30 min with 10 ml of an ethyl acetate/methanol (9 : 1) solution. The aqueous layer was discarded after centrifugation for 10 min at 5000 rpm (Christ UJ2 or Heraus Minifuge RF). A 10 ml aliquot of the organic phase was transferred into a 25 ml conical flask and evaporated to dryness at 35°C (Buechi rotary evaporator). The residue was dissolved in 1 ml

acetonitrile and 5 ml aqueous dextran sulfate solution (2%). The sample was quantitatively transferred into an extraction tube (Supelclean LC-CN (3 ml) Supelco Inc.) and consecutively extracted with 4×3 ml distilled water, 4×3 ml of a 40% methanol solution and 3×2 ml of hexane. The tube was extracted under reduced pressure (Vacelut Analytichem International) for 10 min. The purified sample was eluted with 4×0.5 ml acetonitrile into a conical flask and evaporated to dryness on a rotary evaporator at 32°C. The sample was redissolved in 500 μl eluent (HPLC, see below).

The skin extracts and receptor solutions were analyzed using a liquid chromatographic system (Waters Associates, Milford, MA, U.S.A.) consisting of an M-6000A pump, a WISP 710B automatic sample injector, an RP8 7 μm pre-packed column (RT 250-4; Merck, Darmstadt, Germany), and a Kratos Spectroflow 757 absorbance detector. The injection volume was 20 μl. The mobile phase consisted of 60% acetonitrile and 40% 0.01 M aqueous ammonium sulfate solution. Data processing was performed by an HP3393-A integrator (Hewlett Packard). Penetration rates were calculated by linear regression analysis of the concentration data.

Preparation of the formulations

The cyclosporin A reference formulations were prepared by dissolving or suspending the drug substance in ethanol or paraffin, respectively. The cyclosporin A solubilizate examined in the skin penetration assay was prepared by dissolving 5 g of cyclosporin A in 100 ml of an aqueous sucrose laurate solution (30%). The reference oleogel comprised 5% cyclosporin A, 20% oleyl alcohol, 68% Miglyol and 7% Aerosil. The alcoholic solution was prepared by dissolving 5 g cyclosporin A in 100 ml isopropyl alcohol. All formulations were freshly prepared and stored at 5°C.

Results and Discussion

Skin irritation potential

The results of the skin irritant potential measurements are shown in Fig. 2. The data demonstrate that a 2% sucrose laurate placebo formulation caused no change in skinfold thickness after repeated applications on the left and right flanks. In similar experiments, Miglyol 812, Albil K4 and/or oleyl alcohol containing formulations proved to induce irritation of various degrees (Schmook and Pecanka, 1988). Also, Brij 96 was found to cause erythema and thickening of rabbit or human skin (Mezei et al., 1966). In principle, all nonionic surfactants are regarded as potential irritants. The finding that sucrose laurate was surprisingly well tolerated on guinea pig skin could be explained either by the fact that sucrose laurate is indeed a non-irritant, or by the method of application; the irritating effect of surfactants depends strongly on the conditions of the investigation and the mode of application (e.g., degree of skin hydration, occlusion). The resolution of

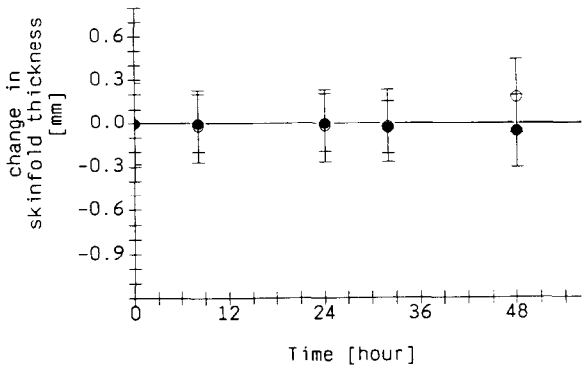

Fig. 2. Change in skinfold thickness after repeated application of a 2% sucrose laurate (L-1695) solution to the left flank (○) and right flank (●) of normal guinea pigs. The results are expressed as the means ± S.D. of three experiments.

TABLE 1

Formulations of the different hydrogels used

Hydrogel (number)	I	II	III	IV	V
SIM stock solution	40 ml	40 ml	40 ml	40 ml	40 ml
Methocel E4M	2.5 g	–	–	–	–
Na-carboxymethyl-cellulose	–	1.6 g	–	–	–
Carbopol 934	–	–	1.0 g	–	–
Na-alginate	–	–	–	1.0 g	–
Gelatin A	–	–	–		1.3 g
Propylene glycol	5.0 g	5.0 g	5.0 g	5.0 g	5.0 g
Aqua dest.	ad 50.0 g	50.0 g	50.0 g	50.0 g	50.0 g

TABLE 2

Composition of the different test formulations, used in the evaluation of the anti-inflammatory efficacy

Excipient	Test formulations		
	Solution	Hydrogel I	Hydrogel II
Cyclosporin A	50.0 mg	50.0 mg	50.0 mg
Sucrose laurate (L-1695)	1.00 g	1.00 g	5.00 g
Carbopol 934	–	1.00 g	1.00 g
NaOH (5%)	–	qs.	qs.
Propylene glycol	–	4.00 g	4.00 g
Aqua demi.	ad 50.0 g	50.0 g	50.0

TABLE 3

Composition of both reference formulations, used in the evaluation of the anti-inflammatory efficacy

Reference formulation			
Ethanolic solution		Paraffin ointment	
Cyclosporin A	50.0 mg	cyclosporin A	50.0 mg
Ethanol	ad 50.0 g	paraffin	ad 50.0

this problem requires an investigation which would exceed the objectives of this study. However, it can be concluded that, under the conditions employed in this investigation, sucrose laurate shows no skin irritation potential.

Anti-inflammatory efficiency

The formulations of the different hydrogels are shown in Table 1. The sucrose laurate/cyclosporin A stock solution was prepared by successively dissolving 150 g of sucrose laurate and 22.5 g of cyclosporin A in 1000 ml of freshly distilled water. All hydrogels were prepared lege artis. Methylcellulose (I), sodium carboxymethylcellulose (II) and gelatin (V), respectively, required prewetting with a hot cyclosporin/sucrose laurate stock solution. Due to the inverse temperature-solubility relationship of cyclosporin A, part of the drug precipitated during the heating of the stock solution. However, the precipitate resolved after mixing and cooling of the formulations. Carbopol (III) and sodium alginate (IV) could be processed with a cold cyclosporin A stock solution. Propylene glycol was admixed to the formulation in order to prevent desiccation of the gel. Common preservatives could not be used, due to the possible competitive solubilization of the preservative by the surface active agent (Barr and Tice, 1957). The final pH of the sodium methylcellulose and carboxymethylcellulose hydrogel precluded the use of the hydrophilic sorbic acid. At a concentration of 10 g/100 ml, however, propylene glycol also acts as an antibacterial agent.

Based on the simple manufacture and consistent quality of the hydrogel, the Carbopol hydro-

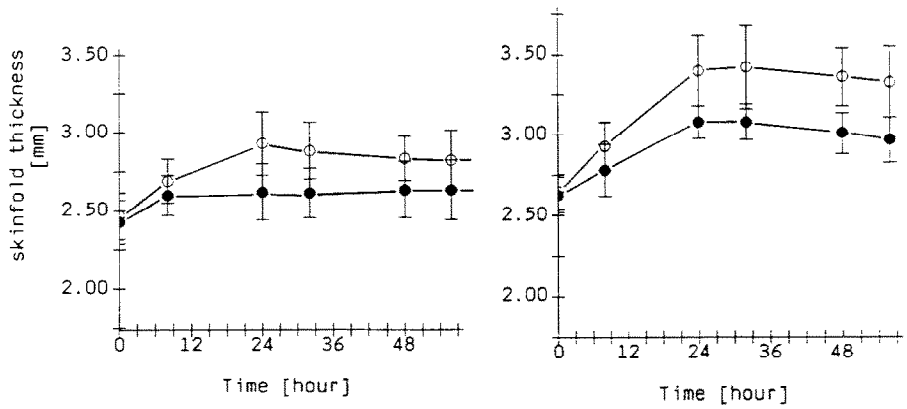

Fig. 3. Effect of two cyclosporin A reference formulations on skin test reactivity to DNFB. Change in skinfold thickness after repeated application of (a) a solutions of 0.1% cyclosporin A in ethanol and (b) a suspension of 0.1% cyclosporin A in paraffin. The open symbols (○) refer to the placebo formulations, the closed symbols (●) represent the formulations comprising cyclosporin A. The results are expressed as the means ± S.D. of six experiments.

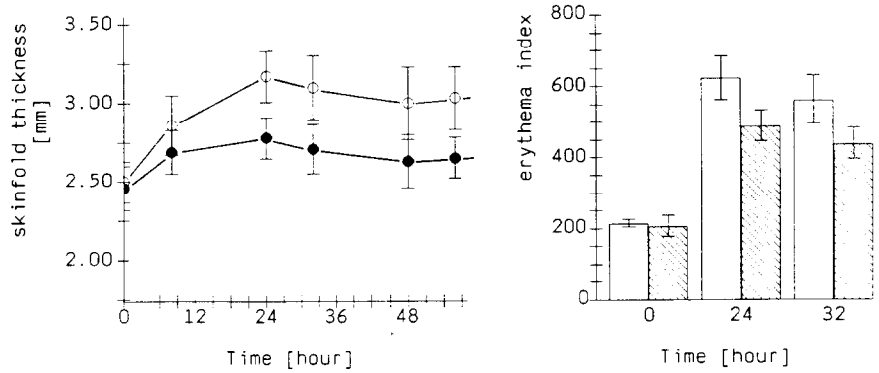

Fig. 4. Effect of the micellar solution comprising 0.1% cyclosporin A and 2.0% sucrose laurate, on the skin test reactivity to DNFB. Change in (a) skinfold thickness and (b) erythema index after repeated application of a verum (○, □) and placebo (●, ▨) formulation. The results are expressed as the means ± S.D. of six experiments.

gel was chosen as the sucrose laurate/cyclosporin A test formulation. The methylcellulose, sodium carboxymethylcellulose and gelatin formulations were rejected owing to the (temporary) precipitation of cyclosporin A. The sodium alginate formulation was also rejected due to the varying quality of the excipient. The formulations of the different test and reference preparations are listed in Tables 2 and 3, respectively.

Test formulation III was selected for investigating the effect of additional sucrose laurate. Excess sucrose laurate is thought to reduce the amount of free, non-solubilized cyclosporin A. Since it is generally assumed that only the non-solubilized drug can exert its activity (Dalvi and Zatz, 1981; Komatsu, 1984; Ashton et al., 1986; Sarpotdar and Zatz, 1986), additional sucrose laurate is expected to reduce the anti-inflammatory efficiency. The commercial cyclosporin A drinking solution was not included in the reference solutions, as the vehicle is known to cause severe irritation.

The effect of two cyclosporin A reference formulations on the skinfold thickness of guinea pigs, previously challenged with DNFB, is shown in Fig. 3. Both formulations significantly reduced the skinfold thickness after 24, 32, 48 and 56 h of sensitization. Adding the efficacy after 24, 32 and

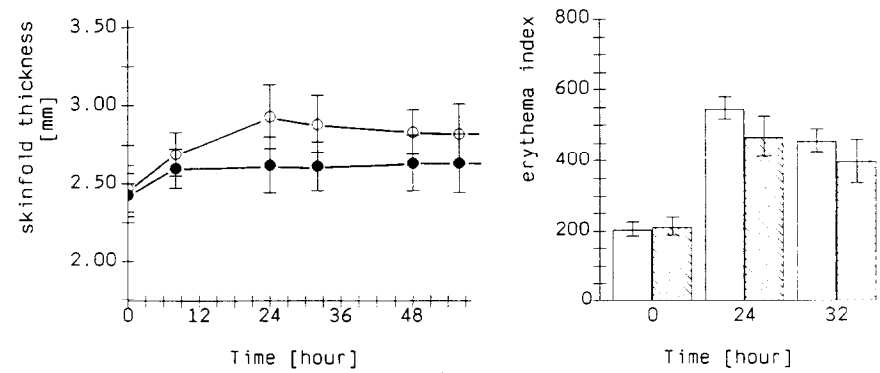

Fig. 5. Effect of the Carbopol hydrogel comprising 0.1% cyclosporin A and 2.0% sucrose laurate, on the skin test reactivity to DNFB. Change in (a) skinfold thickness and (b) erythema index after repeated application of a verum (○, □) and placebo (●, ▨) formulation. The results are expressed as the means ± S.D. of six experiments.

48 h, a relative efficiency of 180 and 128 was calculated for the ethanolic solution and paraffin suspension, respectively. Although the ethanolic cyclosporin A solution has a high relative efficacy, this formulation can only serve as a reference formulation. An ethanolic solution will never be acceptable as a dermal preparation for daily application. The difference in efficiency between the ethanolic solution and the paraffin suspension is in accordance with Fick's first law of diffusion (Eqn 2):

$$\phi = -D \cdot A \cdot \frac{\delta c}{\delta x} \qquad (2)$$

Changing a solution into a suspension results in a reduced concentration gradient between the vehicle and skin. Furthermore, the flux from the paraffin vehicle is also diminished by its relatively high viscosity.

The results of the cyclosporin A test formulations are depicted in Figs 4–6.

Fig. 4 shows that the skinfold thickness after more than 24 h was significantly reduced by the micellar solution. According to the relative efficiency of 176, the micellar solution was almost as effective as the alcoholic solution. Like the decrease in skinfold thickness, a profound reduction of the erythema index was found ($p < 0.001$).

The application of the hydrogel containing 0.1% cyclosporin A and 2.0% sucrose laurate also reduced both skinfold thickness and erythema index (Fig. 5). The relative efficacy of 159 indicated that this formulation was somewhat less effective as compared to the micellar solution or ethanolic solution, but still superior to the paraffin suspension. The reduction of the relative efficacy from 176 for the micellar solution to 159 for the sucrose laurate hydrogel can also be explained on the basis of Fick's first law (Eqn 2).

The sucrose laurate hydrogel, however, might be a suitable alternative dermal cyclosporin A preparation.

The formulation comprising 0.1% cyclosporin A and 10% sucrose laurate (Fig. 6) was inappropriate for testing in the model under investigation. Due to the formation of incrustations, an increase in skinfold thickness was apparent which interfered with skinfold measurements. Consequently, the effect of additional sucrose laurate on the anti-inflammatory efficiency of a sucrose laurate hydrogel could not be investigated.

TABLE 4

Effect of different formulations on the in vitro skin permeability of hairless rats to cyclosporin A

Formulation	v_p (μg ml^{-1} h^{-1})	t_{lag} (h)	C_{skin} (g cm^{-2})
Isopropyl alcohol solution	0.015	5.9	6.1 (1.3)
Oleyl alcohol/Miglyol gel	0.105	6.8	19.0 (6.6)
Sucrose laurate hydrogel	0.153	9.4	30.9 (18.4)

v_p, penetration rate; t_{lag}, lag time; C_{skin}, concentration (±S.D.) (%).

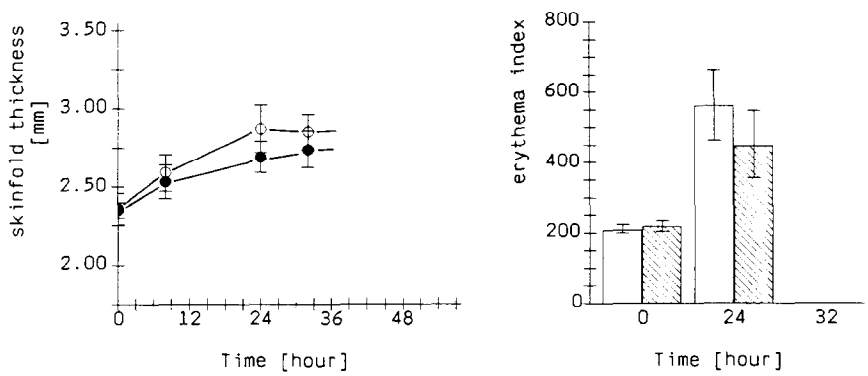

Fig. 6. Effect of the Carbopol hydrogel comprising 0.1% cyclosporin A and 10.0% sucrose laurate, on the skin test reactivity to DNFB. Change in (a) skinfold thickness and (b) erythema index after repeated application of a verum (○, □) and placebo (●, ▨) formulation. The results are expressed as the means ± S.D. of six experiments.

The results of the skin penetration essay are depicted in Table 4. It is conceivable that, as would be expected, the isopropyl alcohol containing reference solution has no penetration enhancing properties. In addition to an almost negligible penetration rate of 0.015 $\mu g\ ml^{-1} \cdot h^{-1}$, skin concentrations of only 6.1 $\mu g\ cm^{-2}$ were found after 48 h of incubation. The oleogel and sucrose laurate hydrogel demonstrated penetration rates and skin concentrations of 0.105 and 0.153 $\mu g\ ml^{-1} \cdot h^{-1}$ and 19.0 and 30.9 $\mu g\ cm^{-2}$, respectively. Lag times, which were calculated by extrapolation of the linear penetration phase to zero concentration, amounted to 6.8 and 9.4 h. Comparing these findings with similar previous experiments (Schmook and Pecanka, 1988), using alternative penetration enhancers, it can be concluded that the sucrose laurate hydrogel might be a suitable alternative formulation for the dermal application of cyclosporin A. However, in order to obtain an optimal sucrose laurate (trans)dermal hydrogel formulation, additional factors such as surfactant or cyclosporin A concentration and the presence or absence of occlusion must be subjected to further examination.

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