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Sucrose Laurate Gels as a Percutaneous Delivery System for Oestradiol in Rabbits

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Abstract

In this study sucrose laurate was formulated in hydrogels and investigated as a suitable transdermal penetration enhancer for oestradiol. Using rabbits as an animal model, the absolute bioavailability and the skin irritation were evaluated after single and multiple application. Three hydrogels containing 60 mg% oestradiol were evaluated: Oestrogel, and two hypromellose gels containing 5 and 15% sucrose laurate (w/w), respectively.

No stability problem of the sucrose laurate was detected during a storage period of four months at $7 \pm 2°C$. After single application no significant difference ($P < 0.05$) was observed between the bioavailability parameters of Oestrogel and the 5% sucrose laurate gel. The values obtained for the 15% sucrose laurate gel were significantly higher than for the other gels. When applied on day 7 after a 6-day treatment, twice daily with the respective placebo gel, no significant difference was seen amongst the three formulations for any of the parameters evaluated. When the results after multiple application were compared with those after single application, a significant increase in oestradiol bioavailability was seen for the gel containing 30% ethanol and a significant decrease in oestradiol bioavailability was seen for the 5 and 15% sucrose laurate gels. Histological evaluation of the untreated and treated skin biopsies, showed a significantly higher incidence of infiltrate for all treated skin biopsies in comparison with the untreated ones. A significant increase in skinfold thickness was seen for the skin biopsies treated with gel containing 15% sucrose laurate.

It can be concluded that sucrose laurate shows a potential as an absorption enhancer for percutaneous drug delivery.

Biosurfactants such as sucrose esters are commonly used in the food industry, mainly as solubilizers, emulsifiers, crystal-growth and microbial-growth inhibitors (Breyer & Walker 1983; Marshall & Bullerman 1986; Matsunaga & Kainuma 1986; Vianen et al 1993; Akoh & Swanson 1994). Biosurfactants are especially of interest for their use as solubilizers and penetration enhancers in transdermal and topical drug delivery systems (Cheng et al 1990; Amkraut 1991; Cormier & Taskovich 1991). Several studies on the use of fatty acid esters as penetration enhancers showed that sucrose esters, and especially sucrose laurate, are a promising group of substances, being non-irritating and rapidly degraded (Klekner & Kosaric 1993; Lerk 1991). Recently sucrose esters have been investigated as adjuvants for cosmetic and pharmaceutical preparations (Desai & Lowicki 1985; Hahn & Sucker 1989; Lerk 1991). In this study oestradiol was chosen as the model drug. The rationale for the development of a transdermal oestradiol system for oestrogen replacement in post-menopausal women is associated with the metabolic and pharmacological effects of orally-administered oestrogen. Transdermal delivery of oestradiol successfully bypasses the first-pass effect and results in a more normal oestrogen blood profile (Good et al 1985). Oestrogel, an alcohol-based gel, was selected as a reference preparation (Simon et al 1990; Scott et al 1991). In this study the penetration-enhancing capacity of sucrose laurate for oestradiol in a topical formulation was investigated after single and multiple application.

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Materials and Methods

Oestradiol solubilization

To determine the solubilizing capacity of sucrose laurate for oestradiol (Sigma Chemical Company, St Louis, MO, USA) a calibration curve was prepared using the following oestradiol concentrations: 0·006, 0·03, 0·06, 0·12 and 0·24% (w/v) in ethanol. All solutions were prepared in triplicate. The concentration of oestradiol was measured by UV spectrophotometry (DU 65, Beckman Instruments, Fullerton, USA) at a wavelength of 282 nm. The calibration curve of oestradiol in ethanol was linear over the range 6–240 $\mu g\ mL^{-1}$. Sucrose laurate (Ryoto Sugar Ester, type L-1695, Mitshubishi Kasei Food Corporation, Tokyo, Japan) dispersions containing 1, 5, 10 and 15% (w/w) were prepared by stirring for 24 h with a magnetic stirrer at room temperature. Oestradiol (600 $\mu g\ mL^{-1}$) was added to each dispersion and additionally stirred for 24 h at room temperature. Next, the dispersions were filtered through a membrane filter (FP 030/3, Schleicher & Schuell, Germany) and the filtrate was collected in borosilicate tubes. The filtrate was diluted in ethanol (1:5) and the oestradiol concentration was measured. From these results, the minimal concentration of sucrose laurate necessary to solubilize 600 $\mu g\ mL^{-1}$ oestradiol was determined.

Formulation development

The solution for intravenous administration was prepared by dissolving 60 mg oestradiol in 100 mL absolute ethanol (analytical grade, Merck, Darmstadt, Germany). Before administra-

Table 1. Composition of the topical formulations.

	Oestrogel	Gel 5%	Gel 15%
Oestradiol	300 mg	300 mg	300 mg
Sucrose laurate	–	25 g	75 g
Ethanol	150 g	–	–
Methocel	–	11·5 g	11·5 g
Carbopol	9 g	–	–
Triethanolamine	~3 mL to pH 6	–	–
Germall 115	2·5 g	2·5 g	2·5 g
Water	To a final weight of 500 g		

The placebo formulation was the same as the active without oestradiol.

tion, the solution was sterilized by filtration (FP 030/3, Schleicher & Schuell, Germany).

The composition of the topical formulations used is reviewed in Table 1. Oestrogel (Piette International Laboratoria NV, Drogenbos, Belgium) was used as a reference preparation. Oestrogel is an alcohol-based gel containing 30% ethanol (w/v). For the preparation of the placebo Oestrogel, a mixture of water/ethanol (70:30; v/v) was prepared and 0·5% (w/v) Germall 115 (Sutton Lab., Inc., Catham, NJ, USA) was added as a preservative. Next, Carbopol 940 (The BF Goodrich Company, Cleveland, OH, USA) was added and mixed with a Silverson mixer until a homogeneous gel was obtained. The pH was adjusted to 6·5 using triethanolamine (UCB, Leuven, Belgium). For the preparation of the sucrose laurate gels, oestradiol was micronized and a particle size analysis by laser diffractometry (1·2–118·4 μm, small volume cell, 1% Tween 80 dispersion in distilled water) using a Malvern Particle Sizer (Malvern Instruments, Malvern, UK) showed that less than 10% of the particles had a diameter exceeding 10 μm. A dispersion of sucrose laurate (40%; w/w) in water was prepared by stirring with a magnetic stirrer for 24 h at room temperature. Next the oestradiol was added to the sucrose laurate concentrate and the dispersion was stirred with a magnetic stirrer for an additional 24 h at room temperature. As a preservative agent 0·5% (w/v) Germall 115 (Sutton Lab., Inc., Catham, NJ) was dissolved in the remaining part of the water. The hypromellose (hydroxypropyl methylcellulose, Methocel K 100M, USP Premium, Colorcon Ltd, Kent, UK) was added to this solution and mixed for 15 min at 3000 rev min^{-1}. Next, the sucrose laurate solution was added to the gel and mixed. For the preparation of the respective placebo gels the same procedure was followed.

The pH of the sucrose laurate gels was measured using a Consort pH meter (D 814, Consort, Belgium). The pH values of the gels were initially $6{\cdot}0 \pm 0{\cdot}1$, and did not alter after four months of storage at $7 \pm 2°C$. The freshly prepared sucrose laurate solutions 5 and 15% (w/w) contained 1·16 and 0·92% (w/w) free fatty acid, respectively. The stability of sucrose laurate in hydrophilic gels after four months of storage at $7 \pm 2°C$ was evaluated by a gas chromatographic analysis of the gel containing 5 and 15% sucrose laurate (w/w), and revealed free fatty acid levels of 1·45 and 1·74% (w/w), respectively.

Serum assay

After storage for 24 h at room temperature, blood samples were centrifuged for 10 min at 3000 rev min^{-1}. The supernatant was transferred to borosilicate tubes and stored at 20°C until analysis. Oestradiol was isolated from the serum (1 mL) by extraction with dichloromethane (5 mL). The average recovery ($\pm$ s.d.) of the extraction procedure was $94{\cdot}6 \pm 2{\cdot}8\%$. The extract was evaporated to dryness at 50°C under nitrogen. The residue was dissolved in 0·5 mL water/ethanol (5:95, v/v). Oestradiol was determined by radioimmunoassay (RIA), using an antiserum of high specificity raised in the goat with oestradiol 6-(*O*-carboxymethyl)-oxime:BSA (oestradiol-6-CMO-BSA, Sigma Chemical Company, St Louis, MO, USA).

In-vivo bioavailability study

Intravenous administration. Twenty-two male rabbits were used (White New Zealand, 3 months $\pm$ 1 week, $3 \pm 0{\cdot}5$ kg). Food was withheld during 24 h before drug administration and during the sampling period. Water was freely available. A bolus injection of 100 μL of an ethanolic solution (60 μg oestradiol) was given in the marginal ear vein. Blood samples were taken from the other ear immediately and 5, 10, 20, 30, 60, 120 and 240 min after the injection.

Topical administration. Nine male rabbits were used for each experiment (White New Zealand, 3 months $\pm$ 1 week, $3 \pm 0{\cdot}5$ kg). Food was withheld during 24 h before the application of the gel and during the sampling period. Water was freely available. Each morning at both sides of the back a surface of 5 × 5 cm was electrically shaved. On day 1 and day 7, 100 mg of a gel, equivalent to 60 μg oestradiol, was applied on a surface of 3 × 3 cm. From the second until the sixth day, 100 mg of the respective placebo gel was applied twice daily on the same surface. The following formulations were used: Oestrogel containing 30% ethanol (w/v), the hypromellose gel containing 5% sucrose laurate (w/w) and the hypromellose gel containing 15% sucrose laurate (w/w). Blood samples were taken from the marginal ear vein 0.5, 1, 1.5, 2, 3, 6, 9 and 12 h after the topical application. After the final blood sampling the rabbits were killed by exsanguination. Skin biopsies were taken from the site of application and from the untreated skin at the other side of the back. The biopsies were fixed in a mixture of formaldehyde (4% in distilled water) buffered with NaH_2PO_4/Na_2HPO_4 (0·4:0·65, w/w), embedded in paraffin, sectioned at 5 μm and stained with haematoxylin eosin. The biopsies were evaluated blind by light microscopy (4 × 4, Photomicroscope Optovar, Carl Zeiss, Oberkochen, Germany). The skin biopsies were evaluated for epidermal thickness (0 = thin, 1 = normal, 2 = thick, 3 = very thick) and infiltrate (0 = normal numbers, 1 = focal, 2 = diffuse, 3 = heavily diffuse).

Pharmacokinetic analysis

Pharmacokinetic evaluation of the average serum levels of oestradiol after intravenous and transdermal administration was performed using the MW-Pharm program (1991). The AUC_{0-12} was calculated using the trapezoidal rule. The absorption rate constant, k_1, was calculated after optimization of the calculated input using the weighed least squares reconvolution method. The C_{max} was determined from the data points.

Statistical analysis

The results in-vivo were evaluated using non-parametric statistics (Siegel & Castellan 1988). The two-tailed Wilcoxon–Mann–Whitney test was used for comparison of the parameters amongst the three treatment groups. The two-tailed Wilcoxon

signed ranks test was employed for the comparison of the parameters after single application with the parameters after multiple application. Differences between two parameters were considered statistically significant for P values less 0·05.

Results and Discussion

A comparative study of the penetration-enhancing capacities of sucrose laurate and ethanol for oestradiol was performed. In Oestrogel, ethanol 30% (w/v) was used as a solubilizer and penetration enhancer for oestradiol. This high ethanol concentration is necessary to obtain a maximal oestradiol flux across the skin (Knutson et al 1993). As the amount of oestradiol is completely solubilized in the reference formulation Oestrogel, the minimal concentration of sucrose laurate necessary to solubilize 600 μg mL^{-1} oestradiol was determined. The amount of oestradiol that could be solubilized was directly proportional to the concentration of sucrose laurate. From these data the minimal concentration of sucrose laurate necessary to solubilize 600 μg mL^{-1} oestradiol in distilled water was determined as being 15% (w/w). To evaluate the effect of the concentration of sucrose laurate on the bioavailability, two different formulations were prepared containing 5 and 15% sucrose laurate (w/w), respectively.

The pharmacokinetic parameters for oestradiol after intravenous and topical administration are shown in Table 2. After intravenous administration the serum concentration vs time profile can be described by a two-compartmental model:

$$C = a \cdot e^{-\lambda_1 t} + b \cdot e^{-\lambda_2 t}$$

with a = 139·2, λ_1 = 19·6, b = 9·9 and λ_2 = 0·8 (r^2 = 0·99032).

The serum concentrations of oestradiol after a single topical administration of oestradiol in Oestrogel and in the gels containing 5 and 15% sucrose laurate (w/w), respectively, are shown in Fig. 1.

After topical administration of oestradiol on day 1 the AUC_{0-12}, C_{max} and k_1 values were significantly higher when administered in the gel containing 15% sucrose laurate (w/w) compared with the Oestrogel and the gel containing 5% sucrose laurate (w/w), respectively. There was no significant difference for any of these parameters after single administration of oestradiol in Oestrogel and the gel containing 5% sucrose laurate (w/w). Therefore we could assume that sucrose laurate enhanced the skin penetration of non-solubilized oestradiol after single application. To establish whether the percutaneous absorption of oestradiol is directly proportional to the concentration of oestradiol solubilized, further experiments are required. The AUC_{0-12}, C_{max} and k_1 values after multiple application of the placebo gel containing 5% sucrose laurate (w/w) were significantly lower in comparison with either of the other formulations. Although the oestradiol levels reached after multiple application of the placebo gel containing 15% sucrose laurate (w/w) were higher in comparison with the drug levels after multiple application of the placebo gel containing 30% ethanol (w/v), no significant difference was observed between the pharmacokinetic parameters. After multiple application of the placebo gel containing 30% ethanol (w/v) and of the placebo gel containing 15% sucrose laurate (w/w) a significant increase and a significant decrease was observed, respectively, as compared with single application. The decrease of the bioavailability after multiple application of the placebo gel containing 15% sucrose laurate (w/w) could be due to the formation of incrustations. This phenomenon was also described by Lerk (1991), where skinfold thickness could not be measured after application of a gel with 10% sucrose laurate

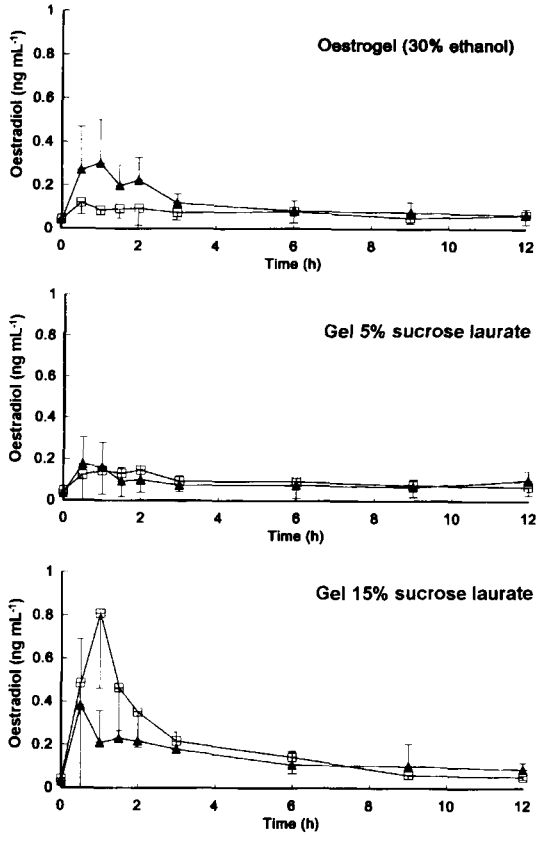

FIG. 1. Serum concentration–time profiles (mean ± s.d., n = 9), after application of (a) Oestrogel, (b) gel containing 5% sucrose laurate (w/w), and (c) gel containing 15% sucrose laurate (w/w) on day 1 (□) and on day 7 (▲).

Table 2. Pharmacokinetic parameters after intravenous administration of oestradiol (n = 22) and after topical application of 60 μg oestradiol on day 1, and on day 7, on skin treated for seven days twice daily with the respective placebo gel. Three different formulations were used: Oestrogel, a gel containing 5% sucrose laurate, and a gel containing 15% sucrose laurate (n = 9 for each formulation).

	AUC_{0-12} (μg h mL^{-1})	C_{max} (ng mL^{-1})	k_1 (h^{-1})
Intravenous	19·57 ± 7·67		
Topical			
Oestrogel			
day 1	0·90 ± 0·35	0·16 ± 0·06	0·02 ± 0·02
day 7	1·33* ± 0·40	0·38* ± 0·21	0·10* ± 0·10
Gel 5%			
day 1	1·10 ± 0·41	0·26 ± 0·16	0·05 ± 0·06
day 7	0·99† ± 0·43	0·19† ± 0·12	0·03† ± 0·03
Gel 15%			
day 1	2·24‡ ± 0·56	0·89‡ ± 0·27	0·30‡ ± 0·22
day 7	1·70* ± 0·38	0·52* ± 0·21	0·10* ± 0·04

Values are presented as means ± s.d. *P < 0·05 compared with day 1· †P < 0·05 compared with Oestrogel (30% ethanol) and gel (15% sucrose laurate). ‡P < 0·05 compared with Oestrogel (30% ethanol) and gel (5% sucrose laurate).

due to the formation of incrustations. Although sucrose laurate has been defined as a skin moisturizer and an increase of skin hydration enhances the skin permeability, the main mechanism of penetration enhancement is probably due to the immediate action of sucrose laurate on the stratum corneum lipids. The histological sections of treated and untreated skin examined are shown in Fig. 2 (1–4). The results of the evaluation of the epidermal thickness and infiltration are shown in Table 3. The untreated skin showed a multilayered, rather thin epidermis. There was only focal infiltration of neutrophilic polymorphonuclear cells and a low number of lymphocytes and eosinophils in the upper dermis. No significant change in epidermal thickness was seen in biopsies from the skin treated with the gel containing 30% ethanol (w/v). Sometimes the epidermis was interrupted and small crusts of serum necrotic material were seen. A significantly higher incidence of grouped lymphocytes, eosinophils and polymorphonuclear cells was seen in the superficial skin of all biopsies treated with the gel containing 30% ethanol (w/v) in comparison with the untreated skin biopsies. Although high concentrations of ethanol are known to extract stratum corneum lipids after a long-term exposure, no symptoms of dryness of the epidermal layer were seen after multiple application of the gel containing 30% ethanol (w/v).

Table 3. Epidermal thickness and infiltration in rabbit skin, untreated and after seven days treatment with a gel containing 30% ethanol (w/v), a gel containing 5% sucrose laurate (w/w), and a gel containing 15% sucrose laurate (w/w).

	Epidermal thickness	Infiltration
Untreated	0·61 ± 0·33	0·44 ± 0·53
Gel 30% ethanol	0·61 ± 0·33	1·33* ± 0·44
Untreated	0·50 ± 0·00	0·66 ± 0·71
Gel 5%	1·16* ± 0·50	2·10* ± 0·60
Untreated	0·61 ± 0·33	0·55 ± 0·73
Gel 15%	1·27* ± 0·67	1·77* ± 0·67

The results are given as means ± s.d. (n = 9). *$P < 0.05$ compared with the respective untreated skin biopsies.

For the skin treated with the gel containing 5 and 15% sucrose laurate (w/w) a significant increase in epidermal thickness was seen in comparison with the untreated skin. In a study on the influence of the application of sucrose laurate gels on guinea-pigs, no changes in epidermal thickness were described for the gels containing 2% sucrose laurate. Skinfold thickness could not be measured after application of the gels containing 10% sucrose laurate due to the formation of incrustations (Cormier & Taskovich 1991). In our study there was no significant

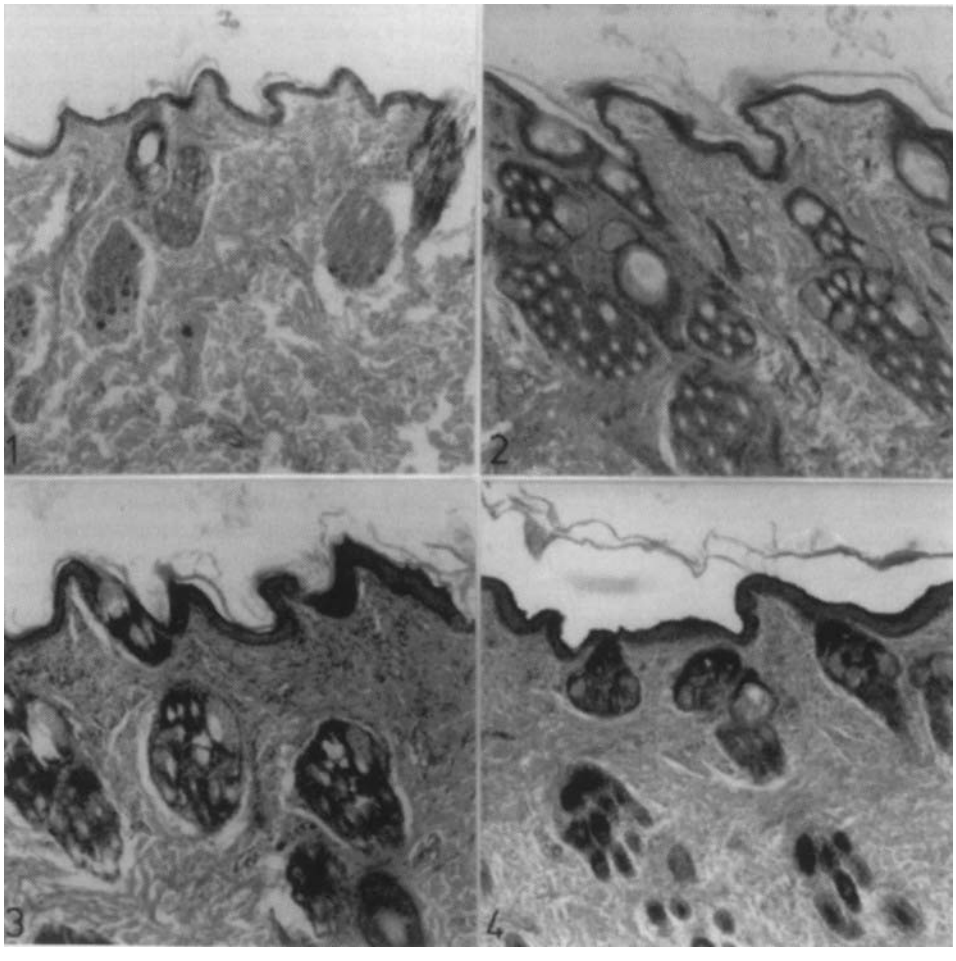

FIG. 2. Histological sections of rabbit skin: untreated (1) and after seven days treatment with a gel containing 30% ethanol (w/v) (2), a gel containing 5% sucrose laurate (w/w) (3), and a gel containing 15% sucrose laurate (w/w) (4).

difference in epidermal thickness between the skin treated with the gel containing 5 and 15% sucrose laurate (w/w), respectively. In all biopsies from a skin treated with the gel containing 5 and 15% sucrose laurate (w/w), a significantly higher incidence of single or grouped eosinophils, lymphocytes and polymorphonuclear cells was seen in comparison with the untreated skin. In relation to the infiltration there was no difference between the three formulations. All biopsies of treated skin showed a significantly higher incidence of infiltration in comparison with the biopsies of untreated skin. The findings that sucrose laurate was well-tolerated by the rabbit skin, confirm the results of other studies on the skin irritation potential of sucrose laurate (Lerk 1991). It should be emphasized that the method of application and the investigation conditions have an important influence on the skin irritation effect of surfactants. It can be concluded, within the experimental conditions of this study, that sucrose laurate showed absorption enhancing properties and has some skin irritation potential.

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