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Biochimica et Biophysica Acta 1663 (2004) 61–73
BIOCHIMICA ET BIOPHYSICA ACTA
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Hydrocortisone and dexamethasone in very deformable drug carriers have increased biological potency, prolonged effect, and reduced therapeutic dosage

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Received 16 September 2003; received in revised form 20 January 2004; accepted 21 January 2004

Available online 11 February 2004

Abstract

We characterised biological properties of novel formulations of two low-potency glucocorticosteroids, dexamethasone and hydrocortisone, which have an equivalent dose ratio of 1:50 in vasoconstriction tests. The rate of such carrier-mediated, mainly non-diffusive glucocorticosteroids transport with very deformable lipid vesicles (Transfersomes®) through the skin, and the corresponding cutaneous drug biodistribution data, were complemented with the drug bio-efficacy studies. The minimum effective drug dose that reduces arachidonic acid-induced murine ear oedema by 50% was used as one bioactivity indicator. The minimum drug amount ensuring such an effect in mouse skin decreases appreciably when a corticosteroid is applied epicutaneously with very deformable vesicles rather than a lotion or a crème. Specifically, the minimum effective dose for hydrocortisone in very deformable carriers is 2–3 μg cm^{-2} whereas for the crème- or lotion-like preparations at least 10 μg cm^{-2} is required. Such three- to fivefold relative increase of hydrocortisone potency is accompanied by at least 13%, and more often >20%, absolute drug potency enhancement. The delivery of hydrocortisone with very deformable carriers moreover prolongs the suppression of the drug-induced oedema nearly 2-fold (to ~24 h per application). The effective dose of dexamethasone delivered with very deformable vesicles into murine skin is reduced >10 times compared with the crème- or lotion-based products. Specifically, less than 0.1 μg cm^{-2} dexamethasone in very deformable vesicles suppresses the arachidonic acid-induced murine ear oedema >50%, on the average. Dexamethasone use on the skin in such vesicles extends the duration of drug action fourfold, compared with a commercial crème, i.e. to >48 h per application. Epicutaneous use of glucocorticosteroids in very deformable vesicles also diminishes such drug's abrasion sensitivity and may increase the general robustness of drug effect. Lower frequency of skin treatment, which ensures adequate biological response, is a result of this. Topical corticosteroid delivery with very deformable vesicles, Transfersomes®, thus improves the therapeutic risk–benefit ratio, arguably due to better targeting into and longer drug presence in the skin.

Keywords: Hydrocortisone; Dexamethasone; Drug carrier

1. Introduction

Glucocorticoids were introduced in 1949 to treat rheumatoid arthritis. Since then, such drugs therapeutic applications grew in scope and now encompass numerous non-endocrine and endocrine diseases [1]. Decades of intense research effort also maximised the beneficial and minimised the adverse side effects of glucocorticoids.

The pharmacologic differences among various glucocorticosteroid derivatives result from structural alterations of the basic steroid nucleus and its side groups. Drug bioavailability, plasma half-life, and metabolic fate, but also the modified glucocorticosteroid ability to bind to the glucocorticoid receptor [2], and to modulate the transcription of glucocorticoid-responsive genes [3], consequently have been changed chemically. Furthermore, structural modifications diminished the natural cross-reactivity of glucocorticoids with the mineralocorticoid receptor and/or modified the drug solubility in water [1].

Glucocorticoid activity has been defined mostly in rat bioassays and does not always mimic the human response

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doi:10.1016/j.bbamem.2004.01.006

to such drugs. Most notably, preclinical data underestimate the growth-suppression by synthetic glucocorticoids. Results of animal and in vitro receptor-binding, or cell-stimulation studies, do not correlate with the tabulated topical potency of common glucocorticoids either. Glucocorticoid potency is therefore defined in terms of drug's maximum dermal vasoconstriction effect [1]. It encompasses ultra-high potency (Class I) drugs, including clobetasol- and halobetasol-propionate, and ends with low potency (Class VII) drugs, such as dexamethasone, prednisolone, and especially hydrocortisone.

Based on the duration of corticotropine suppression after single dose application [2], which correlates reasonably well with the biologic effect half-life, distinction is commonly made between the short-, intermediate-, or long-acting glucocorticosteroids. Hydrocortisone is a short-acting drug, for example, with plasma and biologic half-life of approximately 100 min and 8–12 h, respectively. Prednisolone has an intermediate duration of action, with corresponding half-lives of 115–200 min and 18–36 h. The long-acting dexamethasone has half-lives of approximately 200 min and 36–54 h, respectively.

Systemic absorption of epicutaneously administered glucocorticoids depends on the state of the treated skin. Drug absorption is highest in the damaged, inflamed, and occluded skin areas, or where the stratum corneum is thin (e.g. on the eyelids, genitalia, and face).

Most corticosteroid dermatics contain skin permeation enhancers [2] to promote drug absorption. Agent transfer across the skin is further modified by changing the thermodynamic activity of the drug on the skin, e.g. by choosing a less potent (e.g. crème/lotion) or a more potent (e.g. ointment) formulation, and occasionally by drug supersaturation [4].

Lipid vesicles (liposomes) were proposed by some [5–10] but not confirmed by other [11] researchers to increase glucocorticosteroid concentration in the skin. Therapeutic benefit of liposome-based corticosteroid dermatics in humans was also found to be inconsistent [12]. This may explain why no liposome-based corticosteroid formulation has reached the market to date.

We developed self-regulating, lipid-based drug carriers, so-called Transfersomes®[2] ('carrying bodies') for targeted and noninvasive delivery of agents into or through the skin [13–18,25]. We also elucidated how such specially designed vesicles overcome the skin permeability barrier in the stratum corneum. The prerequisites are carrier stability and self-deformation under stress [13–15] and virtual pathways opening through the organ [16,17]. The former two processes rely on local adjustment of the very deformable vesicle composition to the surrounding stress [13]. The latter originates from the transepidermal water concentration gradient [15] and pushes the vesicles through the natural hydrophilic passages through the skin [16], through which normally water evaporates. Very deformable vesicles consequently transport drugs spontaneously into and across the non-occluded skin barrier better than the previously tested vehicle systems [14,19–21]. This increases biological activity of the preparations based on very deformable vesicle over that of more conventional topical corticosteroid formulations, and also offers a means for controlling drug deposition into the skin [18].

We previously published results of bioactivity measurements with an intermediate potency glucocorticosteroid, triamcinolone acetonide, delivered through the skin of mice [19] and humans [20] with very deformable vesicles. To complement the data, we now describe the corresponding findings with two low potency corticosteroids, the short-acting hydrocortisone and the very long-acting dexamethasone. For completeness we include some relevant drug biodistribution data, which are described in greater detail in Ref. [18].

Formulating either hydrocortisone or dexamethasone in a suspension of very deformable vesicles significantly lowers the therapeutically relevant concentration range to around 0.1 wt.% and below 0.01 wt.%, respectively. This is less than the respective concentrations of 0.25–2.5 wt.% (mainly 1%) and 0.03–0.1 wt.% used in commercial hydrocortisone and dexamethasone products. The biological response time for the local corticosteroid action is prolonged and the sensitivity of dexamethasone in very deformable vesicles to abrasion is diminished. These findings confirm our expectation that very deformable carriers offer several advantages for topical delivery of glucocorticosteroids in the skin.

2. Materials and methods

2.1. Formulation ingredients

Lipoid KG (Ludwigshafen, Germany) or Nattermann Phospholipids-Rhone Poulenc Rorer (Cologne, Germany) were our sources of soybean phosphatidylcholine (SPC) with at least 95% purity. The membrane softening agent, polysorbate, with nominally 20 oxyethylene units on each molecule, was purchased from Henkel (Düsseldorf, Germany) in pharmaceutical grade quality, and was included in 1:1 molar ratio relatively to SPC, unless specified otherwise. Dexamethasone and hydrocortisone were obtained from SynoPharm (Hamburg, Germany). Injectable quality, bi-distilled water was acquired from a local pharmacy and used to prepare all suspension buffers. Commercial drug formulations were used for controls: Anemul creme (Pharmasal, Gräfeling, Germany), containing dexamethasone, and Hydrocortisone-Wolff crème or lotion (Wolff, Bielefeld, Germany). Tritiurated corticosteroids were obtained from Amersham or ICN, and were used as received.

[2] Transfersome is a trademark of IDEA AG.

2.2. Test formulations

Test formulations used in animal experiments were prepared with conventional film method. In short, a homogeneous solution of all water-insoluble components in methanol/chloroform (1:1 v/v) was dried under a stream of nitrogen. This yielded a thin, mixed, lipid plus agent film in a round-bottom glass flask. The film was further evacuated with a diffusion pump (10 Pa, 12 h) and subsequently taken up in a buffer with pH = 6.5. This yielded heterogeneous vesicles suspension, which was homogenised by gentle sonication with a titanium microtip (Heat Systems W 380, USA, < 30 min and 4 °C, under nitrogen). The resulting suspension contained vesicles with an average diameter between 100 and 200 nm, depending on preparation, as determined with photon correlation spectroscopy (ALV-5000, ALV-Laser Vertriebsgesellschaft, Langen, Germany) using 90° scattering. The typical vesicle size distribution width was around 30%. When required, the bulk lipid suspension was diluted with the buffer used to manufacture the original vesicle suspension to obtain phosphatidylcholine concentration in the range 0.5 to 5 wt.%. (Changes in total lipid concentration were found to have only a marginal, if any, effect on the outcome of experiments, due to the non-diffusive nature of Transfersome® mediated transport [15].) Formulations thus contained between 0.005 and 0.5 wt.% corticosteroid per milliliter of suspension, depending on the final drug and total lipid concentration. Liposomes comprised only SPC. The very deformable mixed lipid vesicles (Transfersomes®) contained an equimolar mixture of SPC and polysorbate.

2.3. Animals

NMRI mice for in vivo experiments were purchased from Charles-River (Germany). They were kept in a local facility, for up to 8 weeks, under standard laboratory conditions (a group of three to five mice of 8–12 weeks of age per suspended cage; standard chow; water ad libitum; 12-h light/dark regime), adhering to the ''Principles of Laboratory Animal Care'' (NIH publication #85-23, revised in 1985).

Outbred pig was killed in a local slaughterhouse on the day of experiment and afforded the skin for ex vivo experiments shortly thereafter.

2.4. Artificial barrier penetration experiments

To establish which kind of vesicles is able to cross pores smaller than the average aggregate size, two sets of experiments were done. In the first set, very deformable (Transfersome®) and simple (liposome) vesicles, loaded with the tested corticosteroid, were subjected to a low relative pressure (0.2 MPa) and thus pushed into a polycarbonate membrane perforated by 80-nm pores. In the other test situation, the flux driving pressure was increased to 0.9 MPa. The average vesicle size in either kind case was around 200 nm.

2.5. Skin penetration experiments

Cutaneous samples were excised from the skin of mice pretreated epicutaneously in vivo as is described in the following section. Subcutaneous fatty tissue was carefully removed with a scalpel. The outermost stratum corneum layers were collected sequentially with five Scotch tape-strippings and analysed individually. The remaining skin tissue was dissolved, destained, and used for radioactivity counting as has been described before [18].

To assess corticosteroid transport into porcine skin, ex vivo, full thickness skin samples (20 to 30 cm^2) were excised from a fresh cadaver (less than 12 h old) and then fixed on a wet substrate. Several test areas (1 cm^2) on a larger tissue were pre-marked with a water-resistant pen and treated further as detailed for in vivo experiments with mice, except in that the test formulation remained on the skin for 6 h at 32 °C.

2.6. Biodistribution studies

Biodistribution studies involved first the trimming of hair on upper animal's back with a pair of scissors on the day before an experiment. The appropriate amount of drug formulation (0.5 to 25 μl) was then applied epicutaneously on the pre-marked area (typically ~ 1 cm^2) with a positive displacement micropipette. The applied volume was spread as uniformly as possible over the selected skin surface, using the side of application micropipette tip, and allowed to dry. For each individual time point, 20 μl of the blood was collected from the preheated tail-vein of a mouse with a glass capillary. Eight hours after the test drug formulation application, mice ($n = 4$) were killed by heart puncture. Their dorsal skin was then excised and treated as is described in Ref. [18].

2.7. Pharmacodynamics

Pharmacodynamics of corticosteroids in mice is conveniently assessed by measuring suppression of the chemically induced ear oedema by a given, topically administered, test substance [21]. For this purpose, we first injected (10 μl/g body weight) a mixture containing 6-ml 0.9% NaCl, 1-ml Ketavet 100 (Parke-Davis, Berlin, Germany) and 0.25-ml Rompun (Bayer, Leverkusen, Germany) into animals peritoneum. We then smeared the given drug amount in a test formulation over the inner side of one ear of each fully anaesthetised test animal, leaving thereafter the site to dry. (When so stated, the ear was wiped free of the formulation on the skin surface with a cotton swab after 8 h.) At the specified time after the test drug administration, we applied the oedema inducing arachidonic acid (5 μl in 5-μl ethanol) to each ears. The ear that did not receive the test drug served

as negative control. We measured the change in ear thickness after local arachidonic acid administration in triplicate, on living non-anaesthesised animals, with a hand-calliper. The difference typically amounted to a factor of approximately 2. We assessed each challenge time-point independently. The test animals were sometimes reused, however, after a washout period of 2–3 weeks, with essentially the same result.

2.8. Statistics

Most of the reported data are the means of three independently measured values. The bars in illustrations represent the standard error of the mean. (When no range is given, only a single experiment was done.) Statistical significance was determined using Student's (paired) *t* test. It was deemed to be given when $P>0.05$. Data analysis was performed with the software package ORIGIN (Microcal, OR, USA).

3. Results

After initial pre-biological drug carrier adaptability tests, we compared differential ability of various radiolabelled drug formulations in murine skin penetration tests in vivo. Alternatively, the excised porcine skin barrier similarly has been studied ex vivo. Main study emphasis was on the temporal and dose dependency of in vivo effects, however, of hydrocortisone and dexamethasone administered on murine skin in different formulations.

3.1. Barrier penetration ability of corticosteroid loaded vesicles

When exposed to a low pressure (0.2 MPa) in front of a nano-porous barrier, lipid vesicles are only little stressed. We thus observed that all tested vesicle suspensions under such pressure flow orders of magnitude less rapidly than water or a suspension of mixed micelles through the semi-permeable, nano-porous barrier (Table 1). An approximately fivefold increase of driving pressure ensured the suspension of mixed-lipid, deformable vesicles to traverse the barrier at nearly the same rate as pure solvent or micelle suspension. In contrast, the trans-barrier flux of conventional liposomes suspension driven by comparable pressure remained relatively low (Table 1).

3.2. Corticosteroid delivery into skin from various formulations

As an example, we tested the skin crossing ability of dexamethasone applied with mixed-lipid vesicles or a conventional crème formulation. Epicutaneous drug application (0.04 μg dexamethasone cm^{-2}) for 8 h in the vesicles on murine skin produced nearly uniform drug distribution across the stratum corneum. The average value determined over five strips was approximately 3% (Fig. 1, left panel). The corresponding value for the crème was similar, 2.5%, but the decay with depth was markedly, although not statistically significantly, steeper. Between 20% and 40% of dexamethasone-derived radioactivity was found in the stripped murine skin 8 h after epidermal administration of the drug in the mixed-lipid vesicles, depending on experimental conditions. The value measured with dexamethasone crème was at least 50% lower (data not shown).

We obtained a similar overall picture in ex vivo experiments with dexamethasone on porcine skin (Fig. 1, right panel). Six hours after an epicutaneous drug application in mixed lipid vesicles, between 11% and 13% of the drug-derived radioactivity was found in the skin; 13% were found in the first tape strip and 11% in the fifth strip. In contrast, the application of dexamethasone in a commercial crème accumulated 23% of the drug in the first tape strip and only 9% in the fifth strip (data not shown). After the treatment with crème, 18% of the radioactivity was found in the residual stripped, skin and 2% in the underlying fascia. The treatment with dexamethasone in very deformable vesicles led to an accumulation of 10% of the applied radioactivity in the residual skin and 8.5% in the fascia (data not shown). The skin treatment for 12 h with a dexamethasone crème formulation accumulated from 9% to 1% of the drug, respectively, in the outermost 10 tape-stripped layers of the stratum corneum. For the formulations comprising very deformable vesicles loaded with the drug, the accumulation was from 7% to 4%, respectively (data not shown). This is an appreciable increase in drug transport compared to a commercial crème. Similar improvement is also attested to by the fact that the total drug amount in the residual skin increases from 15% to 30% when formulations containing very deformable carriers are used instead of the commercial crème (data not shown).

Table 1
Capability (relative to that of water) of the corticosteroid-loaded very deformable vesicles, Transfersomes®, liposomes and micelles to penetrate through the pores approximately three times smaller than the penetrant size under influence of two different hydrostatic pressures[a]

Formulation	Low pressure (0.2 MPa)	High pressure (0.9 MPa)
Micelles	1.1 ± 0.1	1.1 ± 0.1
Liposomes	0.0001	0.001
Transfersomes	0.001	1 ± 0.1
Liposomes with hydrocortisone	0.0001	0.001
Transfersomes® with hydrocortisone	0.001	1 ± 0.1
Transfersomes® with dexamethasone	0.001	1 ± 0.1

[a] The artificial barrier consisted of a polycarbonate membrane perforated by pores of 100 nm diameter. Liposomes and Transfersomes were of comparable size. The quoted transport efficacy corresponds to the ratio of aggregate-to-water transport measured under identical conditions (by high pressure liquid chromatography and gravimetry, respectively).

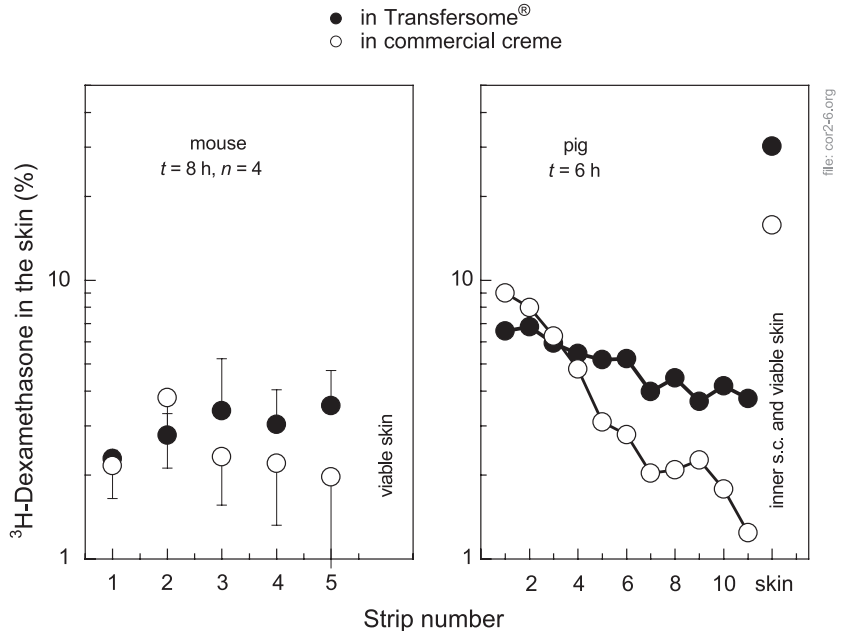

Fig. 1. Distribution of dexamethasone-derived radioactivity in the skin of mice (left panel) and one pig (right panel) following topical drug application in very deformable vesicles (Transfersomes®; closed symbols) or in commercial crème (open symbols).

We have also reanalysed our previously published data [18] with a focus on the therapeutic index. When a relatively high area-dose (10 μg cm^{-2}) is used, less than 10% of the hydrocortisone-derived radioactivity is still found in the skin 8 h after the application. At the same time point, 50% of the dexamethasone derived radioactivity is still found in the skin (Fig. 2), the remainder being recovered in the body [18]. Reducing the dosage level by 2 orders of magnitude increases the amount of hydrocortisone retained in the skin, as judged by radioactivity count, to nearly 100%. The radioactivity derived for dexamethasone increases to 75% (Fig. 2). The relative drug concentration in murine skin is much higher than in the blood (data not shown), despite the fact that the treated-to-total-skin area in mice is relatively small.

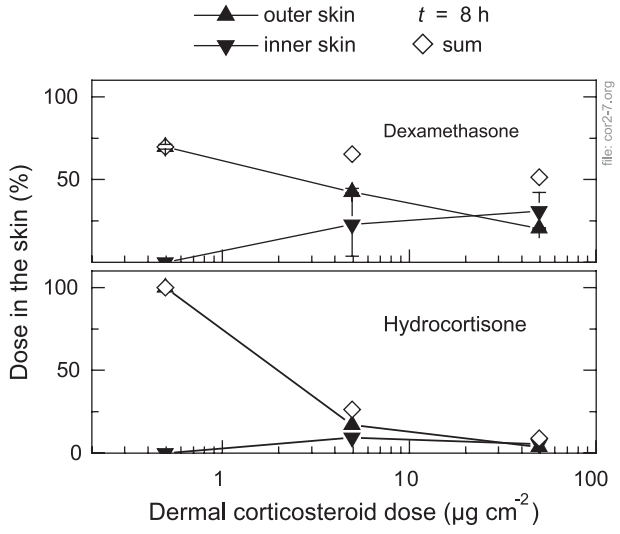

Fig. 2. Dose-per-area effect for hydrocortisone (lower panel) and dexamethasone (upper panel), applied on the skin in very deformable carriers, Transfersomes®, on intracutaneous drug concentration at $t = 8$ h, measured separately in the outer skin (the stratum corneum, up-arrow) and the viable skin tissue (down-arrow). The sum of both values is given by diamonds. (Straight lines merely should guide the eye.)

3.3. Murine ear oedema suppression by topical corticosteroids

We tested the rate and efficiency of corticosteroid delivery into living skin with very deformable carriers for hydrocortisone and dexamethasone. An appropriate crème or a lotion applied on a comparable skin was used for comparison.

3.3.1. Hydrocortisone pharmacodynamics

Hydrocortisone applied epicutaneously in a commercially available creme or a lotion did not suppress the arachidonic acid-induced murine ear oedema immediately. The lag time for 50% suppression increased with the topically used drug dose from 2 to 4.5 h in the tested range between 2.5 and 10 μg cm^{-2}. In the middle-time period (for the three tested doses: 4–8; 4–8; 4–16 h) we observed a quasi-plateau in the measured suppression effect. The oedema suppression typically fell below 50% after approximately 7 h, when hydrocortisone was applied with a crème at doses between 2.5 and 5 μg cm^{-2}. For 10 μg cm^{-2} dose, the 50% activity time increased to 16 h.

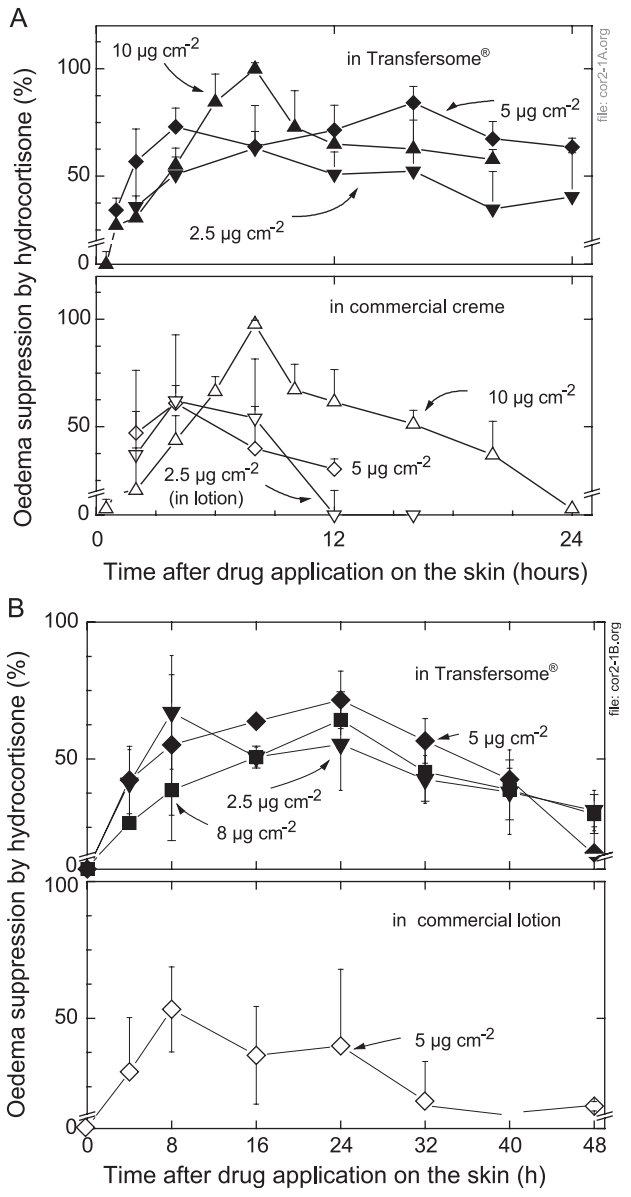

Fig. 3. (A) Suppression of arachidonic acid-induced ear oedema in mice by hydrocortisone applied on the skin in very deformable carriers (Transfersomes®; upper panel, closed symbols) or a commercial crème and lotion (lower panel, open symbols) for a period of 24 h. Mean values (up-triangles: 10 μg cm^{-2}; diamonds: 10 μg cm^{-2}; down-triangles: 2.5 μg cm^{-2}) and standard error of the mean (bars) are given. (B) Time dependency of suppression of the arachidonic acid-induced ear oedema in mice caused by an epicutaneous administration of hydrocortisone in very deformable carriers (Transfersomes®; upper panel, closed symbols) or a commercial lotion (lower panel, open symbols) for 2 days. Mean values and their standard error are given. [Squares: 2.5 μg cm^{-2}; for other symbols see legend to (A).] In most cases: $n=3$.

Hydrocortisone applied in a crème or a lotion had a barely observable oedema suppressing effect after 24 and 32 h, or less, respectively.

Applying hydrocortisone in very deformable vesicles to murine ears, and then challenging the treated sites with arachidonic acid at different times, resulted in 50% reduction of oedema within approximately 2 h (2.5 μg cm^{-2}) to 4 h (10 μg cm^{-2}) post drug application. The effect lasted for at least 17 h (Fig. 3A) and more often close to 48 h (Fig. 3B). For the vesicle-based formulations the plateau width (at approximately 68%) was nearly dose insensitive (4–16, 4–24, 4–20 h and three times 8–32 h for the first and second test series, respectively).

The standard deviation of the results obtained by treating the skin with hydrocortisone in very deformable vesicles was less than that observed with hydrocortisone crèmes. Specifically, we calculated the mean of relative error to be 0.378 and 0.161 (median: 0.153 and 0.142) for the drug in vesicles used at 10 and 5 μg cm^{-2} dose, respectively, and to be 0.838 (median: 0.264) for the crème at dose of 10 μg cm^{-2}.

3.3.2. Hydrocortisone dose dependency

The average oedema suppression mediated by hydrocortisone from a crème was 38%, 44% and 55% at 12 h, and 19%, 29% and 49% at 24 h after application of 2.5, 5.0 and 10 μg cm^{-2} of the drug, respectively. The corresponding result obtained with similar hydrocortisone doses in very adaptable carriers after 24 h was 49%, 68.5% and 66%, respectively. Representing the data differently: with increasing hydrocortisone dose in a crème, the average oedema suppression in the plateau region changed from 58% to 51% and 65 ± 19%. For the 5 μg cm^{-2} dose in a lotion, the average oedema-

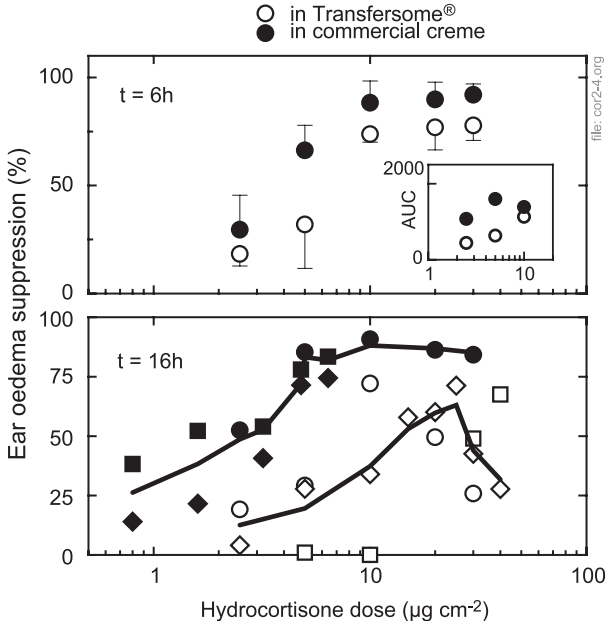

Fig. 4. Dose dependency of hydrocortisone-induced murine ear oedema suppression 6 h (upper panel) and 16 h (lower panel) after drug administration on the skin in very deformable carriers (Transfersomes®; closed symbols) or in commercial crème (open symbols). Different symbols correspond to various independent experiments. Lines represent the corresponding average values; $n=3$. Inset: Areas under the oedema suppression curve, calculated from the data given in Fig. 3A and B.

Table 2
AUC (in % h) calculated from the arachidonic acid-induced oedema suppression by different hydrocortisone and dexamethasone formulations applied on murine ears

Formulation	Dose (μg cm^{-2})	Hydrocortisone (% h)	Dose (μg cm^{-2})	Dexamethasone (% h)
Crème	2.5	434	0.45	(2363)[a]
	5	634	0.9	(3282)[a]
	10	1128	6	3038 (>3975)[a]
Transfersomes®	2.5	1375	0.4	(>3767)[a]
	5	1595	0.8	>5070 (>4645)[a]
	10	>1383		

The corresponding pharmacodynamic results are given in (Figs. 3, 5 and 6), respectively.
[a] Not wiped.

suppression amounted to merely 43 ± 9%. For the vesicle-based formulation, the average suppression with increasing dose was 54 ± 6%, 71 ± 7% or 71 ± 16% and around 55% (range: 54 ± 10%, 62 ± 8%, 50 ± 11%) in the first and second set of pharmacodynamic experiments, respectively.

The tested commercial crème-like hydrocortisone preparation was thus confirmed to be biologically reasonably active. According to the results of our mouse ear oedema suppression experiments, the 50% bioactivity level is reached after 6 h for the drug dose around 7 μg cm^{-2} (Fig. 4, upper panel). Interestingly, hydrocortisone in therapeutic practice is often used at doses approaching 200 μg cm^{-2}, although the anti-oedema effect in our experience reaches a plateau at approximately 10 μg cm^{-2} (Fig. 4, upper panel). We found that the anti-oedema effect of hydrocortisone in commercial crème even starts to decrease for doses above 20–30 μg cm^{-2}, when measured at a relatively late time point (t = 16 h). In contrast, the formulation of hydrocortisone in very deformable vesicles is 50% active at a dosage half that observed for the crème at t = 6 h and at a fourth of said dosage at t = 16 h (Fig. 4).

We calculated the area under the curve (AUC) for hydrocortisone in commercial crème to be 434% h, 634% h, and 1128% h for dosages of 2.5, 5, and 10 μg cm^{-2}, respectively. The AUC for the formulations containing very deformable vesicles is higher, 1375% h, 1595% h, and 1383% h, for the dosage levels of 2.5, 5, and 10 μg cm^{-2}, respectively. These data are summarised in Table 2.

3.3.3. Dexamethasone pharmacodynamics

Applying dexamethasone in a topical crème to a murine ear reduced the arachidonic acid-induced oedema to 50% within less than 4 h for a dose around 1 μg cm^{-2}, which is quasi-optimum. The effect lasted for approximately 40 h (Fig. 5). Lowering the applied dose to 0.4 μg cm^{-2} kept the lag time for 50% oedema suppression at 4 h but reduced

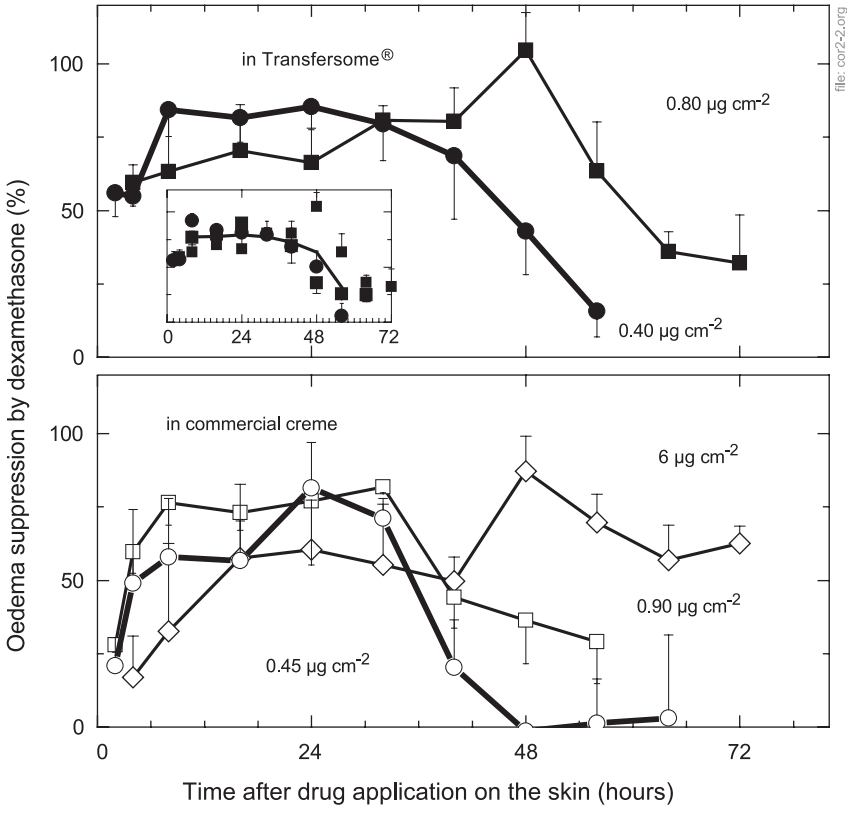

Fig. 5. Suppression of arachidonic acid-induced ear oedema in mice by dexamethasone used on the skin in very deformable carriers (Transfersomes®; upper panel, closed symbols) or in a commercial lotion (lower panel, open symbols) during a 3-day period. Mean values (n = 3; diamonds: 6 μg cm^{-2}; squares: 0.8–0.9 μg cm^{-2}; diamonds: around 0.4 μg cm^{-2}) and standard error of the mean (bars) are given.

the 50% protection time to approximately 35 h. Increasing the applied dosage in a crème to 6 μg cm^{-2} retarded the initial anti-oedema action of the drug; 50% inhibition was now reached not before 14 h (Fig. 5). In this case, however, the biological protection, as reflected in the at least 50% suppression of the arachidonic acid-induced oedema, persisted for at least 72 h.

At the first measured time point, which was 4 h after dexamethasone application in very deformable vesicles to murine ear, the arachidonic acid-induced oedema was already reduced by at least 50%. Similar result was obtained for both tested drug doses (0.8 and 0.4 μg cm^{-2}). The effect lasted for at least 45 h at the low dose (0.4 μg cm^{-2}) and for at least 52 h, or longer, at the higher applied dosage (Fig. 5). We did not investigate the temporal dependence of the effects caused by 6 μg dexamethasone cm^{-2} for the vesicle-based formulations. The reason is that this high dose already appears to be in the saturation range (cf. Fig. 7).

Removing the crème applied at the drug dose of 6 μg cm^{-2} by mechanical abrasion (wiping the ear with a cotton-swab) mimics consequences of natural elimination, such as arises from contacts with the ambient. In practice, this also resembles the effect of using a lower dosage of dexamethasone on the skin. The 50% oedema suppression level for the skin "cleaned" after 8 h is reached after 13 h and lasts for approximately 18 h. When dexamethasone is applied on the skin in very deformable vesicles at drug dose of 0.8 μg cm^{-2}, and the ear is wiped clean 8 h later, the 50% level of oedema suppression is achieved after 4 h. The effect remains at this level or higher for at least 96 h (Fig. 6).

The standard error of the mean for dexamethasone in very deformable vesicles is significantly lower (mean: 0.197; median: 0.223) than the relative scattering detected with dexamethasone creme (mean: 0.774; median: 0.753) at the tested dose.

3.3.4. Dexamethasone dose dependency

The average suppression of oedema by dexamethasone applied in a crème for the 24 h period was 73%. This is somewhat lower than the average value of 79% for the drug application with very deformable vesicles, but neither the measured absolute difference nor the calculated standard error deviation is statistically significant.

A commercial dexamethasone crème achieves 50% oedema suppression 16 h after application of the drug doses between 0.2 and 0.4 μg cm^{-2}, depending on the experiment (Fig. 7, lower panel). If the observation period is shortened to 6 h, the applied drug amount must be increased to 0.6 μg cm^{-2} barely to reach similar suppression (Fig. 7, upper panel). Dexamethasone in very deformable vesicles ensures

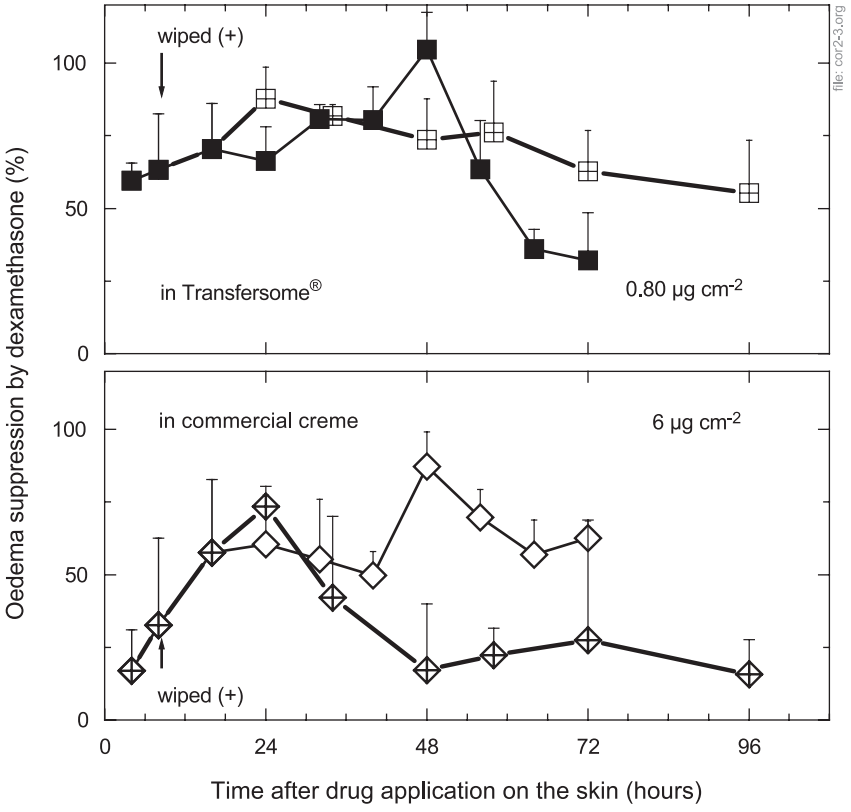

Fig. 6. Effect of the residual drug and vehicle elimination from the skin surface at different times after a topical treatment with dexamethasone in very deformable carriers (Transfersomes®; upper panel: 0.8 μg cm^{-2}; crossed symbols, after wiping) or in a commercial crème (lower panel: 0.8 μg cm^{-2}; crossed symbols, after wiping). $n = 3–4$.

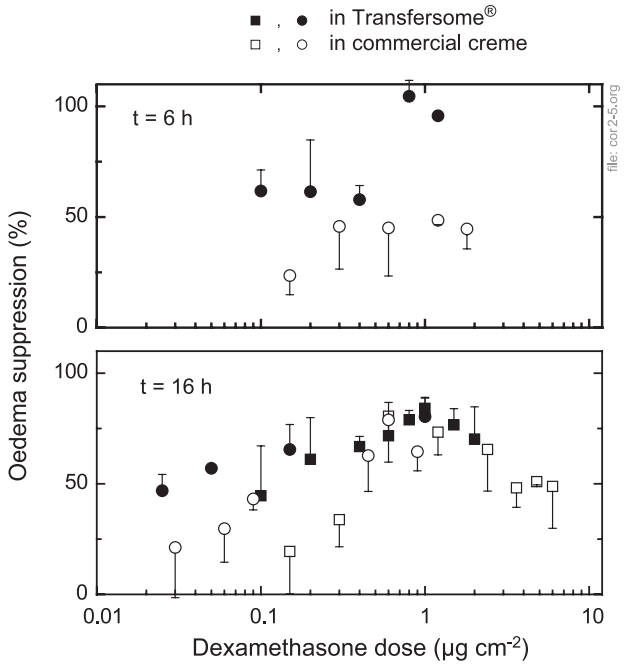

Fig. 7. Dose dependency of dexamethasone-induced murine ear oedema suppression 6 h (upper panel) and 16 h (lower panel) after epicutaneous drug administration in very deformable vesicles (Transfersomes®; closed symbols) or in commercial crème (open symbols). Different symbols correspond to independent experiments. $n=3$.

comparable oedema suppression at much lower dose range—between 0.003 and 0.15 μg cm^{-2}—after 16-h drug action. Oedema suppression measured after only 6 h reveals no clear dose dependency (cf. Fig. 7). There are also difficulties in obtaining reliable values for the higher drug doses, due to the inverse dose versus speed of action curve especially for the crème (with increasing dosage there is a slowing of the biological action). No doubt exists, however, that at all time points for all tested doses the effect of drug applied on the skin with very deformable vesicles greatly exceeds the effect of the drug from a commercial crème.

Experiments performed at 1 h after drug administration had similar dose dependency for the formulations with very deformable vesicles, reaching a maximum activity of 20%, and a less pronounced, but comparable effect to that observed with the crème in the 8-h trials (data not shown).

The AUC for dexamethasone in the tested commercial crème is calculated to be 2363% h, 3282% h, and >3975 (range: 2905;5044)% h for dosages of 0.45, 0.9, and 6 μg cm^{-2}, respectively. These values were calculated for maximum times reaching 52, 62 and 72 h, respectively. Wiping the ear after 8 h reduces the 6 μg cm^{-2} AUC value pertaining to the 72-h observation to 3038 (range: 1035;4005)% h. The AUCs measured for the formulations containing very deformable vesicles have values of 3767% h and 4645 (3810;5480)% h at 72 h for the much lower dosages of 0.4 and 0.8 μg cm^{-2}, respectively. If the ear is wiped 8 h after very deformable vesicles with the drug (0.8 μg cm^{-2}) application, the AUC is 5070 (range: 4194;5947)% h at 72 h (cf. Table 2). This shows a lower sensitivity of the vesicle-based formulation to mechanical abrasion in comparison to commercial crème.

4. Discussion

Several research groups have published papers on topical corticosteroid delivery by means of liposomes [7–12]. In spite of initial enthusiasm on liposomal corticosteroid dermatics [22], no corresponding drug has been commercialised to date. One possible reason for this is the lack of consistency in the alleged therapeutic advantages of liposomal corticosteroid dermatics. It is unclear whether or not the improvement in the therapeutic index of liposome-based drugs observed in some studies is due to an improved drug retention in the skin or else is a consequence of the less direct effects of liposome components on the organ [23]. Phosphatidylcholine, for example, has been reported to improve indirectly the bioavailability of epicutaneously applied corticosteroids [23]. It is even possible that some of positive observations are experimental artefacts [15] caused by the neglect of lipid deposit on the skin surface.

No doubt exists, however, that at least some corticosteroids reach greater depth in the skin when the drug is applied with certain [7–9], e.g. skin-derived [10], liposomes. Specifically, Fresta reported an ~11 times and ~19 times higher drug concentration in the dermis when dipalmitoylphosphatidylcholine and skin-lipid liposomes were used for 0.75–3 h, respectively [10]. This observation has an unclear meaning, however, as the applied drug dose in Fresta-study was 1–2 orders of magnitude above the commonly used dose (75 μg cm^{-2} when measuring biodistribution in pre-frozen skin samples and 500 μg cm^{-2} in skin blanching studies in vivo). Lack of difference between fluid phase skin-lipid and gel-phase phosphatidylcholine vesicles, which are known to have very different skin penetration ability [29], is also perplexing. It is therefore probable that Fresta did not measure direct drug diffusion but rather observed drug transport modulated by liposomes and possibly and enhancement by an unidentified lipid-derived skin permeation enhancer. This may explain the discrepancy between Fresta's and other groups findings [11,12,24]. For example, Kim et al. [11] reported that hydrocortisone absorption in the skin from a liposome gel is lowered compared with the conventional ointment formulation. The same researchers also reported an absorption peak at 45 min with a trend towards prolongation of drug permeation.

The biodistribution and the pharmacokinetics of directly or intravenously administered hydrocortisone, dexamethasone, and triamcinolone-acetonide in very deformable vesicles were compared in our previous study [18]. Both kinds of drug application gave a similar biodistribution of the drug-derived radioactivity.

In this contribution, we provide complementary information, suggesting that corticosteroids enter the skin best when

they are applied in rather deformable lipid vesicles. Why is this the case?

The data given in Table 1 suggest that sufficiently high driving pressure prompts complex, very deformable vesicles, but not simple liposomes, to start flowing through a barrier with pores significantly smaller than the average vesicle diameter. The passage is arguably enabled by the stress-dependent vesicle deformation in front of or in the core of a pore. No comparable flux is seen for the simple liposome suspensions. The difference is indicative of a unique, self-regulating mixed-lipid vesicle response to non-uniform stress near or in a narrow pore [13,15].

When a complex, very deformable vesicle is forced into a biological orifice, such as an inter-corneocyte constriction in the stratum corneum, the aggregate components redistribute nonuniformly, making the carrier locally more deformable. The vesicle thus adjusts itself to the inhomogeneous surrounding stress: the components that tolerate such a stress better are concentrated while the less tolerant substances are depleted from the most deformed sites. A very deformable vesicle can therefore adapt its form to inter-corneocyte pathway shape and therefore pass through the orifice relatively easily [27,28]. The very deformable agent carriers consequently cross the skin barrier through the hydrophilic intercellular pathways [26] that are too narrow to be penetrated by the less adaptable lipid aggregates, such as liposomes. Data given in Table 1 together with pharmacodynamic results corroborate there conclusions.

The water activity gradient in the skin points from the surface into the skin and falls rather rapidly through the outermost layers of the organ [27]. The rate and the efficacy of the resulting hydrotactic motion of very deformable vesicles are therefore independent of the applied drug concentration. This permits the selected corticosteroid concentration to be lowered to the level fixed by the intrinsic drug activity and not by the need to maximise the transepidermal drug transport by keeping the superficial drug concentration high. Corticosteroid formulations with an unusually low agent content are therefore feasible and useful the very deformable Transfersome® carriers. Resulting therapeutic products are thus expected to have excellent bio-tolerance, also due to the fact that they contain no skin permeation enhancers.

Once they have reached the wet, viable epidermis, the very deformable vescicles loaded with corticosteroids experience no further inward water activity gradient [15,16]. Consequently, any spontaneous carrier motion ceases in this skin region. The viable skin hence acts as a local reservoir for the carrier-mediated drug. The reservoir is partially identical to the site of desired biological action, and not subject to the further limitations on the diffusion through the stratum corneum. Our data, reported in Fig. 2, support the hypothesis.

The intracutaneous fate and the activity of the carrier-transported corticosteroids depend upon agent solubility in this compartment. Drugs such as hydrocortisone (low lipophilicity) have a tendency to leave the carrier and the diffuse into the surrounding hydrophilic environment. Therefore, these agents do not have a long biological half-life in comparison to the more lipophilic drugs. A reference to the difference in biological half-lives observed with hydrocortisone (the half-life for elimination is 1.5 h) or with the longer circulating dexamethasone (elimination half-life in mice between 1.5 and 3 h). These data must be analysed with care, however, as they are based upon radiolabel distribution data.

Hydrocortisone is a steroid hormone secreted by the adrenal cortex and was approved as a drug by FDA in 1951. The commercial drug forms include the unchanged hormone and different salts thereof (acetate, cypionate, sodium phosphate, butyrate, valerate, and sodium succinate), which are being used in a variety of galenic formulations. Wohlrab and Lasch described an improvement of hydrocortisone penetration into the skin after drug application in liposomes in a series of publications [7–9]. The small sample size in our biodistribution studies and other experimental differences preclude quantitative comparison of our and Lasch's findings, but use of vesicles on the skin seems to produce generally more favourable corticosteroid distribution in outer skin layers.

Locally absorbed hydrocortisone starts to be metabolised in the skin. Systemic hydrocortisone is quickly distributed into the kidneys, intestines, muscle, and liver, and is mainly metabolised in the latter organ, to inactive metabolites [1]. All inactive hydrocortisone metabolites, as well as a small portion of unchanged drug, are excreted in urine. The circulating drug is mainly bound to plasma proteins, only the unbound portion of a dose being active.

Dexamethasone (approved by FDA in 1958) and its derivatives are synthetic glucocorticoids used as anti-inflammatory or immunosuppressive agents. Dexamethasone is available as oral, parenteral, topical (spray), and ophthalmic dosage forms. Dexamethasone from topical preparations metabolised in situ (the skin, the eye) whereas the systemic drug is metabolised in the liver to inactive metabolites. These inactive metabolites, as well as a small portion of unchanged drug, are excreted in urine.

The degree of drug retention in the skin is likely to be much higher in humans, with more favourable treated-skin to total body-weight ratio, than on mice. The therapeutic dose per area of the synthetic corticosteroid dexamethasone in very deformable vesicle formulations is at least 10 times lower than that required for the use with commercial preparation. This allows us to postulate that most of the drug will be confined to human skin and that the danger of systemic side effects will be reduced.

Both hydrocortisone and dexamethasone administered in very deformable vesicles suppress ear oedema more rapidly than the corresponding topical crèmes or lotions. Moreover, the drug's biological effect persists longer if very deformable carriers are used. Another advantage is the reduced dosage of the corticosteroid necessary to reach certain therapeutic

effect, i.e. the increased relative drug potency effect. We have calculated that the maximally used daily dose could be reduced to 1 mg for dexamethasone and to below 20 mg for hydrocortisone. The latter upper limit is comparable to the natural production of hydrocortisone in humans (12 to 30 mg/day [1]). Such dosage therefore should be well tolerated in adults and infants, especially as the majority of the drug remains in the skin (cf. Fig. 2).

Equivalent dose for dexamethasone and hydrocortisone used in conventional formulations is 0.5 and 25 mg [2], respectively, yielding relative potency factor of 50 based on the results of vasoconstriction studies. We determined the equivalent dose ratio for these drugs in very deformable carriers, using murine ear oedema suppression test, to be around 60 (cf. Figs. 4 and 7), which gives credence to our assay.

The relative drug potency and local drug action thus changes in the opposite direction as the half-life of both tested drugs (cf. Fig. 8). So does the typically used drug concentration in conventional products. (The commercial dose range is 0.03–0.1% for dexamethasone- and 0.25–2.5% (mainly 1%) for hydrocortisone-containing commercial preparations.) All these values are far above the concentrations useful in the corresponding vesicle-based formulations, which are estimated to be below 0.01 wt.% and around 0.1 wt.%, respectively.

The dose per area could be reduced from conventional dosages by up to a factor of 10 by utilising formulations containing corticosteroids in very deformable carriers. This should reduce the intracutaneous corticosteroid concentration to the range of 1 μg/g (for high potency drugs) to 10 μg/g (for low potency drugs). As a result, drug concentrations very close to the 0.25 μg/g concentration of corticosteroids in normal human blood would be reached.

It stands to reason that drug delivery mediated by very deformable carrier shall reduce the side effects that are often observed with conventional corticosteroid therapy. For example, the naturally occurring corticosteroid, hydrocortisone, does not cause a significant skin atrophy even after long time use; when used on a large area, however, it can lead to various undesired systemic side effects, however.

Unfortunately, the currently available hydrocortisone in crème/ointment/lotion formulations has too low potency for an effective therapy of most common, but severe, dermatological diseases. Raising the concentration of epicutaneously applied hydrocortisone has no beneficial effects, probably due to saturation of the bioactivity of the drug at dosages exceeding 10 μg cm^{-2} (see Fig. 4). In this context, it is worth noting that *absolute potency* of hydrocortisone in very deformable carriers appears to exceed that of the drug in commercial crèmes or lotions. By comparing the average pharmacodynamic effects in "plateau region" (cf. Fig. 3A and B), we conclude that the increase over crème- and lotion-like formulations amounts to approximately 13% and 28%, respectively. From the direct dose–effect study done after 6 (16) h (cf. Fig. 4), the increase is estimated to be 22% (50%). If one uses the 24-h average for comparison, the increase is 60% (cf. Fig. 3A and B).

The standard deviations in experiments with very deformable drug carriers are smaller than the variability in tests with conventional formulations. This suggests that corticosteroids delivered with the more novel carriers have more reliable action, probably due to improved target specificity of such vesicle-based formulations. One can even speculate that the use of very low dosages of corticosteroids

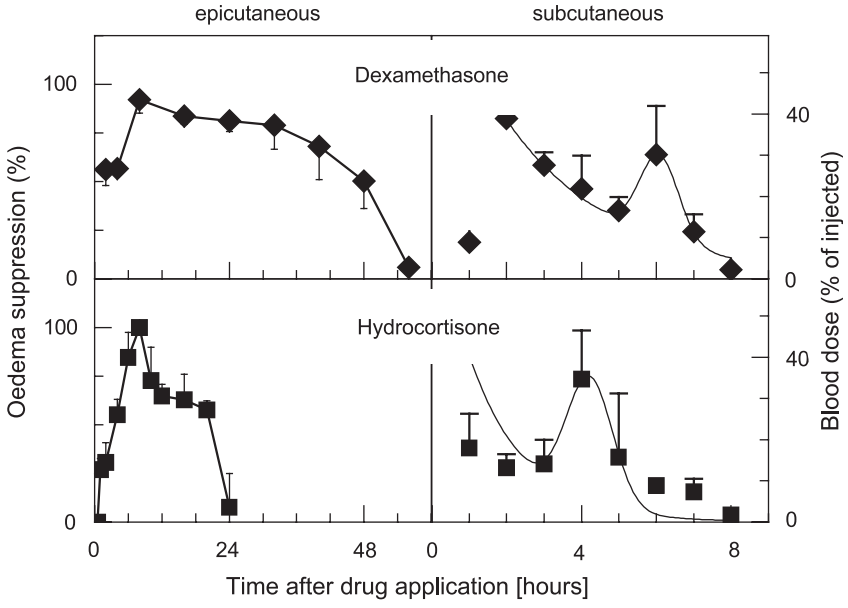

Fig. 8. Comparison of drug pharmacokinetics (right panel) with the time dependence of drug-induced ear oedema suppression (left panel) for hydrocortisone (upper half) and dexamethasone (lower half), all measured in mice ($n = 4–5$).

in very deformable carriers could allow preferential delivery of the drug into hyperactive cells in the skin, which take up particulate material most avidly. This would divert drug's action from the normal to the inflammatory cells, and thus could improve the therapeutic index of the corticosteroids delivered in such a way.

Rapid drug transfer into the skin and slow subsequence drug transfer into the blood capillaries, which are indirectly documented in Fig. 8, explain the faster onset of the desired oedema-suppression the retarded begin of the less desirable vasoconstriction, observed with the formulations containing very deformable vesicles is another consequence of this. Indeed, vasoconstriction is the first sign of drug spillover into blood circulation.

It is therefore important, and reassuring, that our value for the equivalent dose ratio for hydrocortisone and dexamethasone is realistic. We determined this ratio in murine-ear oedema-suppression tests to be around 60. Such value is rather close to the published ratio of $0.5/25 = 50$ stemming from vasoconstriction measurements in humans [2]. Murine ear oedema suppression data thus appear to be relevant for planning human clinical trials.

5. Conclusions

In this publication we have supplied conclusive evidence for the efficacy and therapeutic value of corticosteroids hydrocortisone and dexamethasone formulations based on very deformable carriers. The resulting advantages are multiple and include a faster onset of anti-oedema effects; longer action times; bioactivity that is unaffected by the mechanical drug abrasion; and, most importantly, the ability to drastically (at least by a factor of 10) reduce the necessary dosage that ensures therapeutic, and perhaps better, effects. Use of the described ultra-adaptable carriers, Transfersomes®, therefore might reduce the amount of systemic side effects and allow use of dexamethasone in situations where such drug previously was not considered due to the risks of its side effects. Similar carriers will potentially also allow use of the naturally occuring and well tolerated hydrocortisone in situations where it was not previously employed due to its relatively low biological potency. Last but not least, application of very deformable vesicles introduces a new method of use for therapeutically treating skin diseases, while offering unprecedented opportunities for modern, well-controlled, topical skin medication with the reduced frequency of application.

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