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Biological activity and characteristics of triamcinolone-acetonide formulated with the self-regulating drug carriers, Transfersomes®

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Abstract

Novel formulations of the halogenated corticosteroid, triamcinolone-acetonide, based on ultradeformable mixed lipid vesicles, Transfersomes®, are described. Their performance was tested in vivo using radioactive label measurements, to study the drug biodistribution, and murine ear edema, to determine the drug bioactivity. Sparse use of drug-loaded Transfersomes® on the skin ensures an almost exclusive delivery of triamcinolone-acetonide into the organ, thus arguably increasing the treatment safety. Delivery of triamcinolone-acetonide in the skin with ultradeformable vesicles prolongs the anti-inflammatory drug action several times compared to drug usage in a conventional crème or an ointment, the robustness of biological response for the former being at least identical to the latter. The required dose of Transfersome®-based triamcinolone-acetonide is also greatly reduced. The drug dose of 0.2 μg cm^{-2} suppresses 75% of arachidonic acid-induced murine ear edema for at least 48 h. In contrast, a conventional formulation of triamcinolone-acetonide requires a 10-fold higher drug dosage to achieve a similar effect. In either case, increasing the applied corticosteroid amount delays the onset of anti-edema action.

Keywords: Improved dermatic; Ultradeformable vesicle; Targeted delivery; Topical application; Transfersome®, triamcinolone-acetonide

1. Introduction

Approximately one-third of all dermatics contain corticosteroids. Such drugs are also widely used for systemic medication [1]. Unfortunately, all biogenic, natural (gluco)-corticosteroids exert only rather short and weak action in vivo. To improve the situation, synthetic (halogenated) corticosteroids were introduced. The synthetic derivatives are more potent than their natural kin and have a longer duration of action, but are burdened by increased risk of adverse effects [1]. This is true for dexamethasone, prednisolone, triamcinolone, their derivatives, and many related drugs.

Side effects are most probable when halogenated corticosteroids are repeatedly used on the skin. This often necessitates the discontinuation of a therapy performed with synthetic corticosteroids, mainly due to severe skin thinning. The drug-induced skin atrophy is due largely to the catabolic and anti-proliferative actions of corticosteroids that are also responsible for most of the drug's anti-inflammatory action.

It appears that a measure of a corticosteroid's potency as a glucocorticoid is the degree of inhibition of corticotropin secretion it produces [1]. Local corticotropin inhibition is also observed, and has prompted attempts to improve the local biodistribution and action of corticosteroids by galenic means. Most modern corticosteroid dermatics therefore contain substances that partition into the skin, and act as skin permeation enhancers [2]. Drug supersaturation also increases the (thermodynamic) activity of locally applied therapeutics activity [3] but is burdened with metastability.

Corticosteroid incorporation into lipid vesicles (liposomes) and topical administration of the resulting formulation has been claimed by some authors to increase the drug concentration in the skin [4–10], but other authors observed just the opposite [11]. Deviant results have also been reported for the therapeutic benefit of liposome-based corticosteroid dermatics [12]. This may explain why no corticosteroid product containing liposomes has reached the market.

We developed self-regulating drug carriers, so-called Transfersomes® ('carrying bodies', a trademark of IDEA AG), specifically to overcome the skin barrier. Such carriers

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now seem to fulfil the promises previously made in conjunction with liposomes [13–16].

Transfersomes® overcome the skin barrier by opening extracellular pathways between cells in the organ and then deforming to fit into such passages. In the process, Transfersomes® undergo a series of stress-dependent adjustments of the local carrier composition to minimise the resistance of motion through the otherwise confining channel [16]. This allows Transfersomes® to transport the drugs associated into and across the skin easily and very reproducibly. This happens at a rate substantially higher than that achieved by more conventional topical corticosteroid formulations and offers excellent means for controlling drug distribution in the skin [17].

In this paper, we present the results of biological tests with an intermediate potency synthetic corticosteroid, triamcinolone-acetonide, with a potency of 2 relative to hydrocortisone (which has a nominal potency of 1) [35]. We compare triamcinolone-acetonide in Transfersomes® with a commercial ointment and a crème, using a lower intrinsic drug concentration in the former type of formulation.

In the currently used commercial products based on a lotion, crème or an ointment, triamcinolone-acetonide is usually used at a concentration in the order of 0.1 wt.% [1]. To determine relative potency of our novel triamcinolone-acetonide formulation, we used different agent concentrations in appropriate carriers and then tested the apparent biological potency of the product. As a secondary question, the effect of Transfersome®-mediated delivery on drug biodistribution in the skin and on the anticipated risk/benefit ratio was assessed.

Topical usage of triamcinolone-acetonide with Transfersomes® was found to reduce the necessary drug dosage to the levels of 0.01 wt.%. Epicutaneous drug administration in these highly deformable carriers was also observed to prolong markedly the biological response time and to increase the reproducibility of the biological drug action. These results further illustrate the advantages and varied uses of the Transfersome® delivery system for triamcinolone-acetonide [18] and for other corticosteroids.

2. Materials and methods

All substances used in the study were of pharmaceutical quality. Soybean phosphatidylcholine (SPC) was purchased from Nattermann Phospholipids-Aventis (Cologne, Germany) or from Lipoid KG (Ludwigshafen, Germany); in both cases, its purity exceeded 95%. The remaining components, which rendered carrier membranes more deformable, were obtained from Henkel (Düsseldorf, Germany). They were typically of the polysorbate type and are described in detail in Ref. [13].

The corticosteroid derivative triamcinolone-acetonide was purchased in pharmaceutical quality from SynoPharm (Hamburg, Germany). Injectable quality bi-distilled water was purchased from the local pharmacy, as were the commercial drug formulations used as experimental controls. Triamcinolone-acetonide in a lotion or a crème (Volon A® lotion or crème, respectively; Squibb-Hayden, Munich, Germany) or in a different crème (Delphicort; Lederle, Wolfratshausen, Germany) were used for the purpose.

2.1. Sample preparation

The formulations used in biodistribution studies were labelled with triturated corticosteroids purchased from Amersham or ICN. To prepare such formulations for use in animals, all lipids were dissolved in methanol/chloroform (1:1 v/v) in the appropriate amounts and a dry-mixed lipid film was prepared under vacuum (10 Pa; 12 h).

Formulations contained between 0.005 and 0.5 wt.% corticosteroids per ml of carrier suspension. The latter consisted chiefly of phosphatidylcholine (SPC) in the final concentration between 0.5 and 5 wt.%. Except when stated otherwise, the lipid was introduced as an ethanolic solution (SPC/EtOH = 1:1 w/v). SPC lipid was taken up in a buffer with pH = 6.5 and homogenised by sonication (titanium micro-tip, Heat Systems W 380, USA, 4 °C) or other mechanical means for the manufacture of human therapeutics to achieve the desired vesicle size. The final vesicle size was determined with photon correlation spectroscopy (ALV-5000 Laser, ALV-Laser Vertriebsgesellschaft, Langen, Germany) and was typically between 100 and 200 nm. In experimental situations, the lipid suspension was diluted appropriately, if required, maintaining pH around 6.5.

At least one of the carrier components was characterised by its membrane solubilising capacity, as is required by the basic rationale of the Transfersome® design [13]. Typically, polysorbate 80 was used, which was included in the formulation at 9:11 w/w ratio relative to SPC, except in one test series in which polysorbate concentration was doubled. This carrier substance was thus included in the final medication approximately 10 times below the lytic concentration, which ensured high carrier deformability without compromising the integrity of the Transfersome® vesicle [15]; the Transfersomes® must have these properties in order to maximise drug carrier transport across the stratum corneum [16].

2.2. Animal experiments

Animal trials were performed on 8–12-week-old NMRI mice, adhering to the ‘‘Principles of Laboratory Animal Care’’ (NIH publication #85-23, revised in 1985). The animals were kept under standard laboratory conditions (three to five animals per suspended cage; standard feed and water ad libitum; 12 h light/dark regime). A general anaesthesia was used to keep the test animals stress-free during the manipulations.

2.2.1. Drug localisation in the skin in vivo

The hair at the chosen skin site was trimmed to a length of 2 mm 1 day before the beginning of the experimental trials using a pair of scissors. The precise application site on the upper back was marked and the appropriate amount (0.5–25 μl) of the drug formulation was applied with a micropipette on the skin. The applied formulation was uniformly spread on the mouse skin (using the side of the micropipette tip) and allowed to dry. Tail blood (20 μl) was collected with a glass capillary. Eight hours after the application of the drug formulation, the animals were killed by heart puncture; the treated skin area was undermined, and then carefully excised. The outermost layers of the stratum corneum were removed and collected by five sequential tape-strippings (Tesa-Film; Baiersdorf, Hamburg, Germany). The residual skin tissue and other organ samples were then excised, de-stained, and used for radioactivity counting as described earlier [20].

2.2.2. Drug activity in vivo

All animal tests were done in mice. Biological action in mice is most often tested by measuring the suppression of the chemically induced edema by the topically administered corticosteroids. This is an established method for the purpose, which yields relevant results despite the known difference between human and murine skin properties [19].

In brief, we used a modification of the classical method, changed so as to avoid killing the animals for the purpose of measuring their ear thickness [19]. The test animals were first anaesthetised with an intraperitoneal injection of 10 μl per g body weight of a mixture containing 6 ml 0.9% NaCl, 1 ml Ketavet 100 (Parke-Davis, Berlin, FRG), and 0.25 ml Rompun (Bayer, Leverkusen, Germany). The appropriate amount of drug formulation was then applied to the inner side of one ear over an area of 0.8 cm^2 and left to dry. Afterwards, when so stated, the ear was cleaned of superficial formulation with a dry cotton swab. Each mouse was anaesthetised before the addition of arachidonic acid in ethanol (1:2 v/v, 10 μl) to induce ear edema, which typically just about more than doubled the ear thickness/weight. The arachidonic acid solution was applied to the same ear area as the drug formulation.

Changes in mouse ear edema (relative to that of the untreated, challenged ear) were determined either by measuring the ear thickness with a microcaliper (our modified method) or by weighing the flexed ear area of the killed mouse (the original procedure [19]). We obtained similar results with both methods. All values are the means of at least three independently measured experiments and bars represent the standard deviation of the mean. The onset of edema suppression was defined as the time at which edema suppression first reached the level of 50%, or the weight decreased from a maximum around 30 mg (for a 6 mm tissue disc) to around 22 mg. The half-life for edema suppression was identified with the latest time point at which the observed suppression was $\geq 50\%$. Changes below 20% were found often to be insignificant and are therefore excluded from typical data presentation.

2.2.3. Adverse side effects in mice in vivo were assessed both locally and systemically

The first aim was reached by measuring changes in the thickness of the treated 1 cm^2 skin in a fold on the back of mice with a caliper and comparing the result with a comparably prepared, but untreated, remote site on the same animal. Additionally, the corresponding site on an animal treated with empty, negative control vesicles was used for comparison. For the second purpose, changes in body weight and gross appearance of the test animals were notified. The drug application was done at the same dosage (10 μg cm^{-2}) twice weekly with the drug in Transfersomes® or every day with the commercial crème. The applied dose was thus 10 times too high for the Transfersome®-based formulation and appropriate for Volon A®. Judging from the cumulative edema suppression data (cf. Fig. 4), the appropriate dose/application frequency ratio for the two formulations should have been 8–15 times lower rather than 3 times lower for Transfersomes®, to achieve "equipotency". The Transfersome®-treated group was thus deliberately overdosed.

Data analyses were performed with the software package ORIGIN® (Microcal, OR). Statistical significance was determined using the ANOVA test. The specific level of significance used is given in the text, being typically $P \geq 0.05$.

3. Results

The biodistribution and the pharmacokinetics of triamcinolone-acetonide in Transfersomes® administered in vivo directly to intact murine skin or injected intravenously in mice were compared in our previous study [17]; both drug applications gave similar distributions of the drug-derived radioactivity. In this study, we focused our interest on the in vivo effects of the administration of triamcinolone-acetonide in Transfersomes®, using mouse ear edema model for the purpose. In short, glucocorticosteroid delivery into the skin with ultradeformable carriers ensured a high degree of drug localisation, increased the apparent drug potency and prolonged duration of drug action, all these changes being advantageous for dermal therapy.

3.1. Ear edema suppression in mice

The rate and the efficacy of Transfersome®-mediated corticosteroid delivery in the skin were first tested for triamcinolone-acetonide in Transfersomes® in an animal model. The topical administration of a similar drug in a crème or lotion was used for reference. In general, the agent was found to act remarkably long compared with the duration of action of the other corticosteroids that we tested [18].

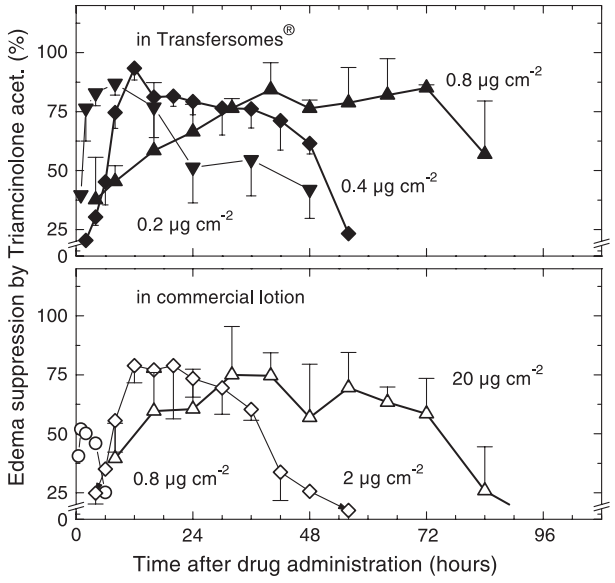

Fig. 1. Time dependency of the suppression of arachidonic acid-induced ear edema in mice caused by an epicutaneous administration of triamcinolone-acetonide in ultradeformable carriers (Transfersomes®; upper panel, closed symbols) or in a commercial lotion (lower panel, open symbols). Mean values and their standard error are given.

Increasing the drug dose per area generally slowed down and prolonged the biological action of topically applied triamcinolone-acetonide (see Fig. 1). When applied as a topical lotion, the onset of edema suppression was measured to be 1.5, 7.6 or 12.5 h for the dosages 0.8, 2, or 20 μg cm^{-2}, respectively (Fig. 1, lower panel). In contrast, when the agent was applied to the murine skin in a Transfersome® suspension, the onset of edema suppression was 1.5, 6.2 or 9 h for the dosages 0.2, 0.4, or 0.8 μg cm^{-2}, respectively (Fig. 1, upper panel). These times differed up to 20% between various equidose experiments but are similar when compared on the basis of quasi-equivalent action. The reverse dose dependence in temporal terms is a fairly general but seldom reported property of corticosteroids that is incompletely understood. We did not attempt to explore the origin of phenomenon due to our focus on modified delivery, rather than on drug properties and action.

The maximum duration of drug action following epicutaneous administration of the highest tested triamcinolone-acetonide dosage in Transfersomes® or in a lotion is ≥ 84 and 75 h, respectively. If a similarly low dose is used in the commercial lotion and in ultradeformable carriers (0.8 μg cm^{-2}) the maximum duration of drug action for the former is merely 2 h, that is, 40 times shorter than for the latter formulation.

Within the time window of 4–24 h after challenge to the murine ear, the average magnitude of the edema suppression is $60 \pm 23\%$ for a dosage of 2 μg cm^{-2}. The result is $43 \pm 19\%$ for a dosage of 20 μg cm^{-2} when the triamcinolone-acetonide is in either case applied as a topical lotion. The magnitude of the edema suppression in an identical time window for the tested triamcinolone-acetonide in Transfersomes® is approximately 10% higher, being $69 \pm 23\%$ for an applied dosage of 0.4 μg cm^{-2} and $52 \pm 13\%$ for an applied dosage of 0.8 μg cm^{-2}. The overall reproducibility of the results obtained with Transfersomes® is approximately 30% better than that of the commercially available lotion for the intermediate tested drug dose; also, the average single time reproducibility is 30% better for the Transfersomes®-based formulation (the specific results for the intermediate tested dose being $10 \pm 6\%$ and $7 \pm 3\%$, respectively). Averaged over a 4–48-h time interval, the average suppression levels are $56 \pm 22\%$ and $70 \pm 18\%$, respectively.

The observed differences between the equipotent treatments are statistically insignificant at the level of $P < 0.1$, when calculated for the first day or the entire observation period, but become significant at $P = 0.08$ during the second day of treatment. The differences between the results of equidose comparisons are highly significant ($P > 0.001$) at all times.

The results of the second test series given in Fig. 2 are very similar to those shown in Fig. 1, except in that the late time values are somewhat, but not significantly higher ($P < 0.1$). The data suggests that triamcinolone-acetonide after a single administration on the skin in Transfersomes® is active biologically for more than 96 h, even when the skin surface is wiped "clean" by cotton swab. The values in Fig. 2 also imply that the drug used at the dose of 20 μg cm^{-2} in a commercial crème (Delphicort) may be marginally more effective than the drug in a lotion (Volon A® Lotion). The respective average suppression values are $67 \pm 24\%$ and $54 \pm 20\%$ for the 24–96 h period. For the Transfersome®-

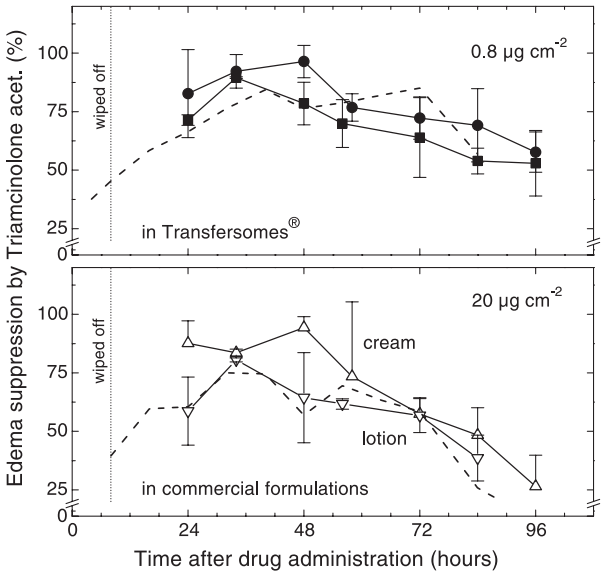

Fig. 2. Effect of residual drug and vehicle elimination from the skin surface at different times after a topical treatment with triamcinolone-acetonide in Transfersomes® (upper panel, closed symbols), in a commercial lotion, or a crème (open down-arrow and up-arrow, respectively, lower panel). Dashed lines give the corresponding results from Fig. 1 for comparison.

based formulations used at the drug dose of 0.8 μg cm^{-2}, the corresponding average values are 68 ± 13% and 78 ± 13% for two different experiments.

The results given in Fig. 3 support the abovementioned conclusions. The edema suppression mediated by two different types of Transfersomes® related with the outcome of the skin treatment with triamcinolone-acetonide in similarly large liposomes or a lotion reveals that the latter two, when used at an equal dosage, are at least 20 times less effective.

The observed gain in drug efficacy is not explicable by simple skin permeation enhancement, which is generally accepted to be negligible for polysorbate [2]. If permeation enhancement in our study was important, the results obtained with Transfersome® formulations with and without ethanol, which is a well established permeation enhancer [2], would have to be significantly different. Fig. 4 shows that this is not the case, however.

3.2. Dose dependency

We used the mouse ear edema model to determine the dose dependency of drug action for the Transfersome® formulation versus commercial crème and lotion. Triamcinolone-acetonide in commercial crème exerted a strong biological effect when dosed at approximately 8 μg cm^{-2}. The 50% efficacy level was reached near 2 μg cm^{-2} (Fig. 4). In contrast, the Transfersome® formulation of such drug was 50% active at a dosage that was 10 times lower (0.2 μg cm^{-2}) than that required for the crème (1.6 μg cm^{-2}) or the slightly less potent lotion (2.6 μg cm^{-2}; cf. Fig. 4). These estimates pertain to the results measured 8 h after the skin challenge with arachidonic acid. Experiments performed at 1 h after the challenge showed a similar dose dependency for the Transfersome® formulations, reaching a maximum activity of 20%, and a less pronounced, but comparable effect to that observed with the lotion in the 8-h trials (data not shown). Trials after 16 h led to qualitatively similar results with an increase in the edema suppression values to approximately 80% (data not shown).

We also calculated the area under the curve (AUC) for the edema suppression, to assess cumulative triamcinolone-acetonide activity (see Fig. 5). For commercial lotions, the

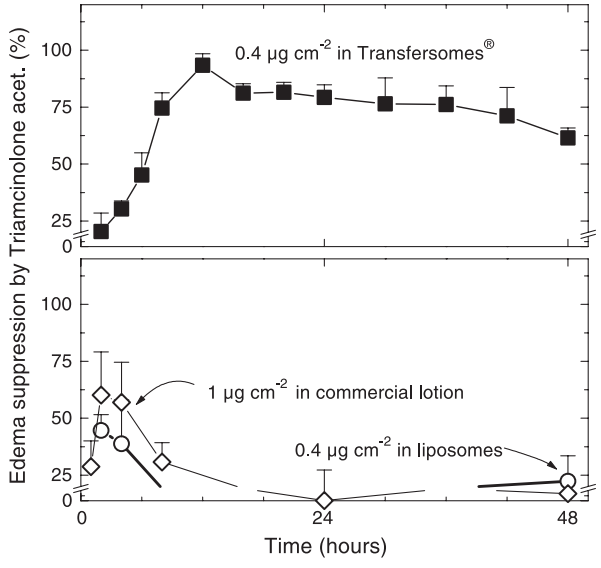

Fig. 3. Vehicle sensitivity of murine ear edema suppression by triamcinolone-acetonide in Transfersomes® (upper panel), in a commercial lotion (lower panel: open diamonds), or in conventional liposomes with the size comparable to that of Transfersomes® (lower panel: circles) as a function of time after an epicutaneous drug administration.

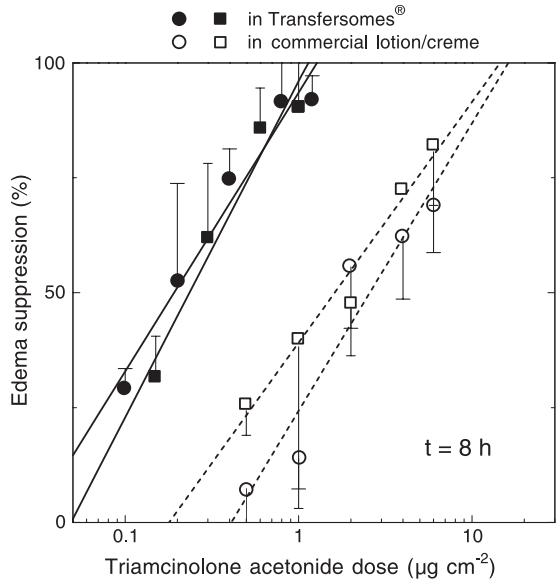

Fig. 4. Dose dependency of triamcinolone-acetonide-induced murine ear edema suppression 8 h after the drug administration on the skin in Transfersomes® containing two different polysorbate concentrations (closed symbols), in a lotion (open circle) or in a crème (open boxes). Straight lines give the results of quasi-linear fit for each individual data set.

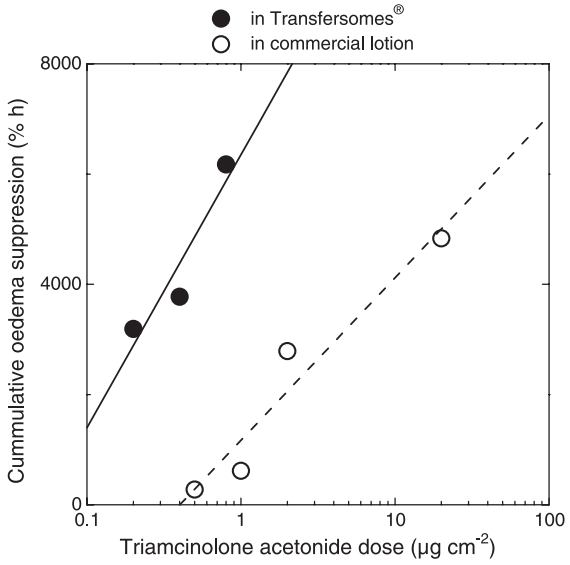

Fig. 5. Cumulative dose response to an epicutaneous administration of triamcinolone-acetonide in Transfersome® (closed circles) or in lotion (open circles) based on murine ear edema suppression effects.

resulting values are 275, 2789, and 4834 h% for dosages of 0.8, 2, and 20 μg cm^{-2}, respectively. The AUC's characterising Transfersome® formulations are much higher, having values of 3100, 3682, and 6174 h% for the dosages of 0.2, 0.4, and 0.8 μg cm^{-2}, respectively.

3.3. Adverse side effects

In our earlier work [17], we presented typical biodistribution data for the various Transfersome®-based corticosteroids. Here, we re-analyse the triamcinolone-acetonide related data with a focus on the anticipated risk/benefit ratio.

When a relatively high area–dose (~ 40 μg cm^{-2}) is used, approximately 20% of the triamcinolone-acetonide derived radioactivity are retained in the skin 8 h after application (Fig. 6, left panel); the remainder is recovered in the body [20]. Reducing the dosage level by approximately one order of magnitude increases the amount of skin-retained triamcinolone-acetonide based radioactivity to 90%. The extrapolated 100% limit is reached for the epicutaneous drug dose of 1 μg cm^{-2}, which we have shown will ensure a proper function in the murine ear edema. The inner/outer skin ratio for triamcinolone-acetonide correspondingly changes with the administered dose. The drug concentration in the murine skin is much higher than in the blood (Fig. 6, right panel) despite the fact that we treated a relatively large proportion of the total skin area in mice.

An improved risk/benefit ratio is also indicated by treating the test mice with triamcinolone-acetonide in Transfersomes® or in a crème so as to achieve an approximately equipotent effect. The results are given in Table 1 and demonstrate rather clearly the diminished danger of local as well as systemic side effects of the drug in ultradeformable carriers. In addition to having lost weight, the animals

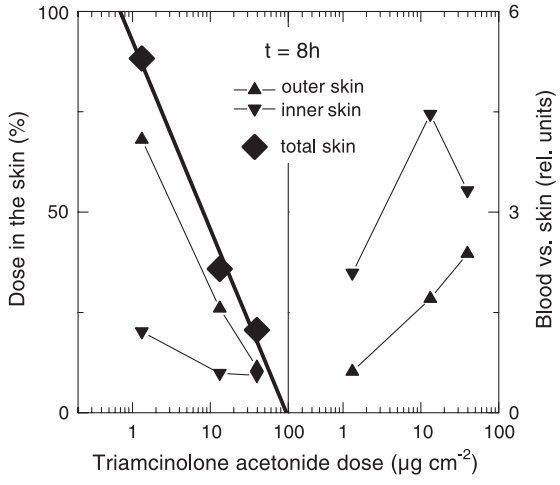

Fig. 6. Effect of changing the applied dose per area of triamcinolone-acetonide in Transfersomes® on the skin on the drug retention in the organ and its main regions (left panel). The corresponding local versus systemic drug concentration ratio is given in the right panel. The result of the linear extrapolation is shown in the left panel as a straight line.

Table 1
Adverse side effect of approximately equipotent topical treatment with triamcinolone-acetonide (TAC) on the skin for 6 weeks in ultradeformable vesicles (Tfs) or in Volon A crème

Formulation	TAC dose μg cm^{-2}	Change (%)		
		Treated/ remote	Treated/ control	Body weight
TAC Tfs	10	−2	−18	3
Volon A crème	10	−146	−67	−19
Empty Tfs	0	11	0	0

treated with Volon A® crème also generally were in poorer shape.

4. Discussion

Owing to their widespread use, corticosteroids have been incorporated into many modern drug delivery systems. For example, Mezei et al. [4–6] as well as several other groups [7–12] published a series of papers in which the advantages of liposomal corticosteroids were praised. In a 1993 review, Schaefer-Korting reiterated some of these thoughts [20]. To date, no liposome-based corticosteroidal dermatic has actually been commercialised, however. One possible reason for this is the lack of consistency in the alleged therapeutic benefits of liposomal corticosteroids. It is clear that at least some of the lipid aggregates have a beneficial effect on the risk profile of topicals. It is unclear, however, whether this reflects improved drug retention in the skin or else is a consequence of the less direct effects of carrier components upon the organ.

Phosphatidylcholine has been reported to improve indirectly the bioavailability of the epidermally applied corticosteroids [21]; a thin lipid film in close contact with the skin was proposed to promote stratum corneum hydration and also to create an environment into which corticosteroids can partition before their subsequent uptake. Lipids from the vesicles in such scenarios were postulated to take the role of skin permeation enhancers [16,22,23], which explains why the experiments were often done with occluded skin.

We currently know of no direct evidence for the skin crossing by any corticosteroid-loaded liposome: the published data normally refer simply to the drugs applied with such carriers or, even less directly, to the biological action of the drug. For example, Jacobs et al. [21] reported the degree of human skin blanching, as assessed by the vasoconstriction test, to increase upon concomitant treatment with phospholipids and different corticosteroids applied separately in commercial formulations. More relevantly, Fresta and Publisi [11] measured 6 and 1.3 times stronger skin reflection in the 4–8 h time period after the skin treatment with triamcinolone-acetonide in phospholipid- and skin lipid-based formulations, respectively. Their observation needs further verification to exclude contribution/interference from the light-reflecting lipid suspension at the skin surface,

which in our experience, is a big problem in these kinds of studies. Corticosteroid association with liposomes was inferred [5–10] to improve the drug activity by acting as a selective drug delivery system, and was concluded by some researchers to depend more on the vesicle charge than on size or the presence of membrane stiffening agents [12]. Korting et al. [24] have also concluded that liposome encapsulation moderately improves the activity of betamethasone diproprionate against atopic eczema but not against psoriasis vulgaris.

Using the mouse ear edema model with our modifications of the measurement procedure, we were able to observe a marked difference between triamcinolone-acetonide and other corticosteroids tested; further results will be published separately. The biological action of TAC lasts much longer than, for example, that of hydrocortisone. The half-life for edema suppression of the latter drug is only 7–16 h [17], while triamcinolone-acetonide has a dose-dependent half-life for edema suppression of 30–90 h, depending on the applied dosage (cf. Fig. 1).

The results were generally found to be well reproducible in the present study, despite some temporal as well as maximum drug effect variability. Illustrative data are given in Fig. 7, which compares the outcome of three different experiments with the drug in Transfersomes® and of two test series with the drug in a lotion. A direct correlation of two original data sets is made in the inset to this figure, whereas in the main graph, the result of time-axis re-normalisation,

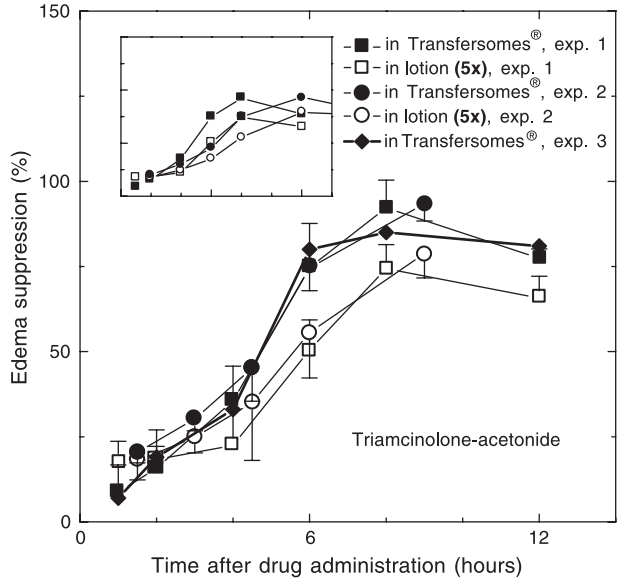

Fig. 7. Robustness of the murine ear edema suppression test with triamcinolone-acetonide as a function of time after the drug administration on the skin in Transfersomes® (closed symbols, single dose) or in commercial lotion (open symbols, five-fold dose). The inset shows the original data from two experiments; the main figure compares these data after the time axis re-normalisation for one data set (see main text body for details) including a third set of data measured with the drug in ultradeformable carriers. The inset has the same scale division as the main figure panel.

which aligned the first edema-suppression peak values, is given. This shows that the main biological assay used in this study is quite robust. The average standard deviation, however, is typically 7–50% lower for the Transfersome® group in comparison with commercial products.

In contrast, we believe that the vasoconstriction (skin blanching) test is not well suited for the testing of carrier-based dermatics. In our opinion, this is due to the inability of relatively large carriers to cross the walls of intact blood capillaries [25,26]. Drug carriers that have reached the viable epidermis can therefore only reach the blood through the lymphatic system [16,26,28,29], which hampers the ability of the carrier-associated drug to induce a strong local vasoconstriction. In contrast, dissolved drugs or small agent aggregates from a topical lotion partition readily into blood capillaries and are not unduly dissipated through the lymphatic system [29]. Monomeric agents, moreover, are prone to exert a much stronger local skin-blanching effect and to create more pronounced pallor than carrier-associated agents. These actions (rapid transfer into the skin and slow transfer into the blood capillaries) explain the faster onset of the desired edema-suppression as well as the retardation of the less desirable vasoconstriction observed with Transfersome® formulations of triamcinolone-acetonide. To our mind, the latter is actually a sign of the drug spillover into the blood circulation (see Fig. 6). One should not take this too seriously, however, as it is well known that the corticosteroid dose needed to induce peripheral vasoconstriction may be different from that required for an intradermal therapeutic effect. FDA and other regulatory agencies in the guidelines therefore caution researchers not to rely on blanching effect without individual test validation.

The abovementioned problem notwithstanding, the human skin-blanching test can give results with a meaning similar to that of the outcome of murine-ear edema-suppression test [18].

One should realise, that the drug intravasation, which precedes or accompanies any significant agent-dependent vasoconstriction, is not required for therapeutical corticosteroid action. In fact, corticosteroid intravasation is highly undesirable, as it unavoidably increases the danger of the systemic side effects. In contrast, keeping the drug in the cutaneous or subcutaneous tissue minimises the drug burden in remote tissues. Monomeric drugs, which cross the walls of the blood capillaries and then cause vasoconstriction, therefore bring with them a much higher chance of adverse side effects.

When a Transfersome® is forced into an orifice, such as an inter-corneocyte constriction in the stratum corneum, its components redistribute non-uniformly [14]. This permits the aggregate to accommodate to the inhomogeneous surrounding stress: the components that tolerate such a stress better are concentrated while the others are depleted from the most deformed sites. The protruding part of each carrier thus becomes highly deformable, thus permitting a Trans-

fersome® to adjust its form to the shape of the potential pathway and to cross the otherwise non-passable constrictions in the skin. The ultradeformable agent carriers consequently overcome the skin barrier through the hydrophilic inter-cellular channels [26] that are too narrow to be penetrated by other kind of lipid aggregates.

We are convinced that Transfersomes® are driven across the skin by the water activity gradient across the stratum corneum [27]. Such a gradient falls rather steeply with the depth in the skin [30] and points from the surface into the organ depth. The rate and the efficacy of the resulting hydrotactic Transfersome® motion are therefore independent of the applied drug concentration. This permits the latter to be lowered to the level fixed by the intrinsic activity of the drug and not by the need to maximise the transepidermal agent flow by keeping the superficial drug concentration high. Formulations of corticosteroids in Transfersomes® can thus be applied topically with an unprecedented low agent content. The fact that Transfersome® suspensions, unlike many standard topical drug formulations, contain no harmful skin permeation enhancers should also contribute favourably to the drug tolerance on the skin.

Corticosteroid-loaded Transfersomes® experience no further inward water activity gradient when they have reached the wet, viable epidermis [31]. Consequently, any appreciable spontaneous carrier motion ceases in this skin region. The viable skin hence acts as a local reservoir filled with the carrier-associated drug. This reservoir is partially identical to the site of the desired biological action and not subject to the further limitation on the diffusion through the stratum corneum. The relative drug accumulation in the skin (Fig. 6) supports our hypothesis, being always $\gg$10% and thus several orders of magnitude above the 0.001% reported in Ref. [11].

The fate and the activity of the carrier-transported corticosteroids in the skin depend on the agent solubility in the tissue. Triamcinolone-acetonide has a weak propensity to leave the carrier, due to its relatively high lipophilicity. The drug is therefore ideally suited for use in Transfersomes®. Such a conclusion is indirectly supported by the long biological half-life of the drug in Transfersomes® ($t_{1/2} \geq 6$ h).

Suitably optimised Transfersomes® excel in several respects over other vehicles for the intracutaneous drug delivery. Triamcinolone-acetonide from Transfersomes® is biologically active at doses at least one order of magnitude lower than those commonly used with commercial topical lotions/crèmes. In contrast, the relative potency of simple liposome-based formulations is not significantly better than that of commercial products.

Triamcinolone-acetonide in Transfersomes® suppresses arachidonic acid-induced skin edema longer than the marketed products for topical treatment. The retardation of anti-edema action, which would be seen when the applied drug dose was increased, is particularly important in the low dose range. It is also more pronounced for drugs, such as triamcinolone-acetonide, that have slow exchange rates, and allows the formulation of very low dosed formulations that retain a biological activity over a longer period of time without the subsequent systemic side effects frequent with topical lotions.

We expect that triamcinolone-acetonide in Transfersome® carrier systems will be used to the maximal daily dose limit of 1 mg. Based upon the said dosage considerations and our calculations, we infer that the intracutaneous corticosteroid concentration will probably be below 1 μg g^{-1}.

There should consequently be little intolerance of the dosage, even for the synthetic corticosteroid, such as triamcinolone-acetonide, as the relative degree of drug retention in the skin is likely to be much higher in humans with a more favourable treated-skin to total body-weight ratio, than in mice. With respect to the low therapeutic dose per area of triamcinolone-acetonide in Transfersome® formulations (at least 10 times less dosage required than for lotion based formulations), we expect that the danger of systemic side effects also will be negligible.

Another advantage of using triamcinolone-acetonide in Transfersomes® could be that the Transfersome® formulations are more reliable in their action than current topical crèmes and lotions. This is attested to by the relatively small standard deviations in the experiments with Transfersomes® versus commercially available crèmes.

In this paper, we have supplied conclusive evidence for the efficacy and therapeutic value of a new formulation of the synthetic corticosteroid triamcinolone-acetonide in ultradeformable Transfersomes®. With Transfersomes®, we thus introduced a new mechanism for therapeutically treating the skin disease. We expect multiple advantages of such treatment, including a faster onset of anti-edema effects; longer times of action, a biological action that is unaffected by mechanical abrasion, and most importantly, the ability to drastically (by at least a factor of 10) reduce the necessary dosage needed to achieve therapeutic effects. This will increase the risk/benefit ratio and potentially allow the use of triamcinolone-acetonide or a related drug in Transfersomes® in situations in which such drugs were not previously considered due to the danger of adverse side effects. The use of Transfersomes® on the skin therefore offers unprecedented opportunities for well-controlled and modern topical medication, not just for corticosteroids, but also for a variety of other low molecular weight and macromolecular therapeutics [32]. The bio-efficacy results reported in this work, moreover, relate to the recent work by Dr. Bouwstra, who also uses elastic vesicles comprising a phospholipid and a surfactant [33,34].

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