

## New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes

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### Abstract

Transfenac, a lotion-like formulation of diclofenac, is described. It consists of pharmaceutically acceptable ingredients and mediates the agent transport through intact skin and into the target tissues. Therapeutically meaningful drug concentrations in the target tissue are reached even when the administered drug dose in Transfenac is below 0.5 mg/kg body weight. Ultradeformable agent carriers, called Transfersomes, form the basis of Transfenac. These Transfersomes are proposed to cross the skin spontaneously under the influence of transepidermal water activity gradient (see [Biochim. Biophys. Acta 1104 (1992) 226]). Diclofenac association with ultradeformable carriers permits it to have a longer effect and to reach 10-times higher concentrations in the tissues under the skin in comparison with the drug from a commercial hydrogel. For example, Transfenac achieves intramuscular agent concentrations between 0.5 and 2 µg/g and 2 and 20 µg/g at  $t = 12$  h, depending on the tissue depth, when it is administered in the dose range 0.25–2 mg/kg of rat body weight. A much higher drug concentration in a hydrogel (1.25–10 mg/kg body weight) creates the drug level of only < 0.5 µg/g in the muscle. The drug concentration in the rat patella for these two types of formulation is between 1 µg/g and 5 µg/g or 0.4 µg/g, respectively. The relative advantage of diclofenac delivery by means of ultradeformable carriers increases with the treated muscle thickness and with decreasing drug dose, as seen in mice, rats and pigs; this can be explained by assuming that the drug associated with carriers is cleared less efficiently by the dermal capillary plexus. In pigs it suffices to use 0.3 mg of diclofenac in highly deformable vesicles per kg body weight, spread over an area of 25 cm<sup>2</sup>, to ensure therapeutic drug concentration in a 5-cm thick muscle specimen, collected under the agent application site. When the drug is used in a hydrogel at 8 times higher dose, the average intramuscular concentration is at least three times lower and subtherapeutic. This suggests that diclofenac in Transfersomes has the potential to replace combined oral/topical diclofenac administration; in humans. © 2001 Published by Elsevier Science B.V.

### 1. Introduction

Diclofenac is one of the most potent and commer-

cially successful non-steroidal anti-inflammatory agents. It has an annual turnover of over 1 billion US dollars [2] approximately 15% of which is achieved with the topical remedies. Around 25 mg of the drug in a body ensure good antiphlogistic and analgesic effects. This corresponds to a therapeutic diclofenac concentration in the plasma, or tissue to be treated, of approximately 0.5 µg/g tissue.

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The nearly perfect drug absorption in the gastrointestinal tract is partly offset by the first-pass hepatic biotransformation of  $\sim 50\%$ . The peak plasma concentration of diclofenac is close to  $1 \mu\text{g/ml}$ , for the daily dose between 100 and 150 mg, but this level is not maintained long. Approximately 30% of the orally administered diclofenac is excreted in the bile, owing to the amphipathic character of the weakly acidic drug. The remainder finally accumulates in the urine, with a terminal elimination half-time of approximately 1 h [1].

The chief disadvantage of oral diclofenac administration is not the insufficient bioavailability but rather the serious side effects of the drug. These adverse effects are mainly due to the poor agent specificity, resulting from the drug binding to certain (e.g., prostaglandin) receptors. The primary site of such adverse action is the gastrointestinal tract [28]. Orally administered diclofenac is therefore poorly tolerated and causes stomach ulcerations [32].

It would be desirable to reach the therapeutic drug concentration in the target tissue while simultaneously keeping the systemic and gastrointestinal agent concentrations as low as possible. Obviously, such a goal can only be achieved by delivering diclofenac into the body via the route other than the mouth.

For these reasons, topical injections or epicutaneous applications of special diclofenac formulations were introduced. The best known is Voltaren Emulgel,<sup>2</sup> developed specifically for the epicutaneous administration. Although the drug penetration depth of Emulgel is merely 3–4 mm [4], this formulation has reached nearly 15% of the total market to date. As Voltaren gel normally falls short of its therapeutic dose by the factor of 10, 1 cm under its administration site on the skin (see [3–5] for further discussion), this medication is typically combined with oral Voltaren to ensure good therapeutic effects.

The depth of diclofenac permeation below the skin is limited by the relatively good drug solubility in aqueous systems ( $> 1\%$ ) and fast clearance. Low skin permeability is also an obstacle. In Voltaren Emulgel, diethylammonium salt of the drug and several skin permeation enhancers are used to overcome the skin barrier [6]. This notwithstanding, the diffu-

sive flow of the drug monomers across the skin cannot match the drug elimination from the subcutaneous tissue: the drug spill-over into the blood circulation leaves too little drug in the target organs for adequate stand-alone therapy.

It is believed that only around 10% of diclofenac from the epicutaneously applied Voltaren Emulgel is biologically available. The other topical diclofenac formulations are less effective by the factor of 2 [7].

In an attempt to transport an amount of the drug that would be in the therapeutic concentration range across the skin, diclofenac was incorporated into phospholipid vesicles (liposomes) [11] without solving the problem. The probable reason for this is the inability of conventional liposomes to cross the skin barrier [8–10].

We have introduced special drug carriers, so-called Transfersomes<sup>3</sup> for the non-invasive delivery of pharmaceuticals across the skin. These ultradeformable drug carriers trespass the intact skin spontaneously, probably under the influence of the naturally occurring, but previously overlooked, transcutaneous hydration gradient. The 'moisture seeking' (hydrotaxis) of Transfersomes [13] permits the carrier to bring more than 50% [14–16,19] of the epicutaneously administered drug across the skin barrier. The process is controllable by formulation/administration parameters and provides a good basis for the topical as well as systemic therapy [16–18].

Here we report some of our results obtained using the formulation of diclofenac in ultradeformable vesicles, Transfenac. We argue that a single administration of the lotion-like Transfenac formulation on the normal skin creates therapeutically meaningful drug concentrations in the target tissues. Transfenac hence promises to become the remedy of first choice for the treatment of diseases of superficial tissues, such as muscles or joints.

## 2. Materials and methods

The drug-to-lipid ratio in the proprietary vehicles, called Transfersomes [20], used in this study was between 1/4 and 1/9. The precise value depended on the

<sup>2</sup> Voltaren Emulgel is the trademark of Novartis.

<sup>3</sup> Transfersome, 'carrying body', is a trademark of IDEA AG.

particular formulation type, but the former composition was commonly used. This ensured that the flexibility of the vesicle membrane was at least 5 times higher than that of standard phosphatidylcholine vesicles in fluid phase. This which was assessed as described in detail in [20,36]. In short, the conclusion was made by comparing the rate of enforced vesicle transport across a semi-permeable barrier with the pores  $\geq 3$  times smaller than the average vesicle diameter. The chief requirement for the efficacy of drug transfer across the skin permeability barrier was thus fulfilled [16,19].

Specifically, vesicles with the size in the range 100–200 nm were prepared from soy phosphatidylcholine (from Natterman Phospholipids (Cologne, Germany) or Lipoid (Ludwigshafen, Germany), with the standard deviation of size distribution typically around 30%. In brief, the formulation was prepared by suspending the lipids in an aqueous phase in which the drug was dissolved. Sufficiently vigorous stirring or other suitable kind of suspension homogenization (e.g., sonication) was used to diminish the average vesicle size in original suspension. Vesicles dimension was finally brought to the desired value by extruding the suspension through 100–200 nm pore filters.

The suspension pH value was between 6.8 and 7.4, in different experiments, but here only the data obtained with pH  $7.1 \pm 0.1$  are reported. For reference, the apparent dissociation constant of membrane bound drug is  $pK_a = 6.1$ . Under the conditions chosen, a proportion of the drug was thus non-ionized and the diclofenac-vesicle association was always appreciable. Based on the results of indirect partitioning measurements we estimate that approximately 75% of diclofenac in a typical starting suspension was membrane bound, owing to (partial) charge as well as to amphipathic character of the drug.

The test formulations contained between 4 and 10 mg diclofenac per millilitre of suspension. Depending on the dosage used, volumes between 3 and 75  $\mu\text{l}/\text{cm}^2$  were used. More details on the manufacturing, use, and characterization of Transfenac, together with the formulation stability data, will be published separately.

Diclofenac in a hydrogel (Voltaren Emulgel, a product of Ciba-Geigy) was purchased from the local pharmacy. It contained 11.6 mg/ml of diclofenac diethylamine salt, together with an undisclosed amount

of 2-propanol and propyleneglycol as the skin permeation enhancers.

The radioactive [ $^3\text{H}$ ]diclofenac, custom made by Amersham (specific activity 1.2 MBq/mg (33  $\mu\text{Ci}/\text{mg}$ ) dry mass), was added to pre-made Transfenac or diclofenac in a gel (Voltaren Emulgel) at least 3 days before the experiment to the final concentration of 1.8 MBq/ml; ( $\mu\text{Ci}/\text{ml}$ ); in one arm of the study, [ $^{14}\text{C}$ ]diclofenac, a gift of Ciba (Basel, Switzerland), was used similarly at specific radioactivity of 2 MBq/mg dry mass (1.1 MBq/ml), but with the label attached in the upper cyclic part of the drug molecule. Vertical axis label always identifies the marker used.

### 2.1. Animal experiments

Animal experiments were done on NMRI mice, Wistar rats, and common pigs. The animals were prepared for the drug administration by manually trimming the hair of mice and rats to the length of maximally 2 mm. In pigs the tentative application site was washed only, with luke warm water. Test material was applied topically with a precision, positive displacement glass pipette in the case of Transfersome suspension and with a spatula for hydrogel. (In the former case, the liquid was applied in several aliquots, allowing for sufficient drying time between individual applications to prevent suspension dripping.) Oral administration was done through a fine tube using the same Transfenac that was also used on the skin.

Stressful or painful manipulations were always carried out under general injection anaesthesia (10  $\mu\text{l}/\text{g}$  body weight containing mixture of 6 ml 0.9% NaCl, 1 ml Ketavet 100 (Parke-Davis, Berlin, Germany) and 0.25 ml Rompun (Bayer, Leverkusen, Germany) given i.p.), lasting approximately 30 min. The designated application site on the animals back or hind extremity was marked with a permanent pen and the drug was administered as described further in the text.

The blood samples ( $\sim 20 \mu\text{l}$ ) were taken at given intervals by tail puncture in rodents and through a soft-catheter located in one of the dorsal veins in the pigs. At the specified times, the animals were killed by a heart puncture (rodents) or by a lethal dose of anaesthetic agent (in pigs). The treated skin as well as all the other organs of interest were excised. The

tissue and organ samples were prepared and used for the diclofenac radioactivity counting as described in the following paragraphs and in our previous publications [21].

The blood and tissue samples were discoloured with 0.4 ml  $\text{H}_2\text{O}_2$  and 0.2 ml  $\text{HClO}_4$  at  $80^\circ\text{C}$  overnight. Subsequently, all probes were neutralized with 0.2 ml  $\text{CH}_3\text{COOH}$ . After the addition of 10 ml Aquasol-2 (NEN-DuPont, Dreieich, Germany), the total radioactivity of samples was determined with a beta-scintillation counter (Berthold, Wildbad, Germany).

#### 2.1.1. NMRI mice

NMRI mice (8–12 weeks old) were transferred into individual cages on the day of experiment and were otherwise kept under standard conditions (normal diet; water ad libitum; 12:12 h light/dark regime). They received the drug-loaded lipid suspension from a micropipette on the upper part of the back or on the hind leg(s). The suspension was left at the site of administration during the entire course of experiment, spread without occlusion over  $0.5\text{--}4\text{ cm}^2$ .

#### 2.1.2. Wistar rats

Wistar rats were treated similarly except in that the drug formulation was always administered on hind legs, covering the knee and the proximal part of the femur muscles, spread over an area of up to  $3\text{ cm}^2$ . After killing the animals under anaesthesia, the appropriate muscles were sliced in thin pieces by using a pair of sharp anatomical scissors.

#### 2.1.3. Pigs

Pigs had a weight of approximately 15 kg and were kept in separate boxes. They received one (at  $t=0$ ) or two doses (at  $t=0$  and, where stated,  $t=12\text{ h}$ ) of the labelled drug formulation on the first day of the experiment. The administration site was on one of the hind legs close to the knee and had an area of up to  $80\text{ cm}^2$ , for the highest dose used. Twenty-four hours after the first drug administration each animal was anaesthetized and a 1-cm thick sample was cut from the treated leg (data not shown). The wound was then taken care of surgically. On subsequent 5 days, that is, on days 2–6, the non-labelled drug in ultradeformable vesicles or in a hydrogel was administered on the contralateral leg without occlusion

once or twice daily, corresponding to the treatment on the day 1. On the day 7, the radioactive formulations were used on the same leg that was also treated on days 2–6. Twelve hours following the last drug administration, all animals were anaesthetized and killed.

Five sequential muscle samples (each  $\sim 1\text{ cm}$  thick) were collected under the drug administration site. Patella from the treated knee was also excised. A 1-cm thick piece of shoulder muscle was taken as the 'untreated muscle' reference sample.

#### 2.2. Biodistribution

To get the therapeutically relevant information on the biodistribution of diclofenac in the target tissues, muscle(s) under the application site were collected down to the bone and sliced to the samples representative of the depths of 0–0.25 cm, 0.25–0.5 cm, 0.5–0.75 cm, 0.75–1.0 cm, 1.0–1.25 cm and 1.25–1.5 cm for the 500-g rats; somewhat thinner sections were used for the smaller rats, but always covered all the tissue down to the bone. From the specific radioactivity in these slices the drug-related penetration profile of the radioactively labelled diclofenac was finally determined.

Other samples included at least two muscle samples from the sites close to the drug administration site (typically, superficial and deep calf and shank muscles), liver and bladder (with the residual urine).

#### 2.3. Data analysis

Data analysis was done with the software package ORIGIN (Microcal, OR, USA). Statistical significance was checked with Student's  $t$ -test and considered to be granted at  $P < 0.05$ . All results give the mean of all measured values  $\pm$  standard deviation.

### 3. Results

Biodistribution and some pharmacokinetic parameters of two Transfersomes-based suspensions, and of one commercial, hydrogel-based formulation of diclofenac were studied. This was done using mice, rats, and pigs. Ultradeformable vesicles were found to improve the regio-specificity and the efficacy of

agent delivery into the body. While it was possible to improve substantially the target-tissue/blood drug concentration ratio, therapeutic drug levels in the systemic blood circulation were also reached, when so desired, by applying a sufficient amount of the drug on intact skin in highly deformable drug carriers.

### 3.1. Biodistribution

The biodistribution of the [ $^3\text{H}$ ]diclofenac-derived radioactivity in mice is illustrated in Fig. 1.

Topical use of diclofenac is believed generally to improve the regio-specificity of therapy but is not always applicable. In this study the epicutaneously administered Transfenac and Voltaren Emulgel were seen to deliver an appreciable amount of the drug (around 1  $\mu\text{g/g}$ ) in the murine dorsum. The proviso was that the drug dose was greater than 2.5 mg/kg body weight (body weight), which is already an excessive agent amount.

The localization of diclofenac in the treated tissue is formulation as well as application-site dependent. Several drug doses were tested on the murine hind

legs to support the conjecture. The carrier-mediated drug delivery achieves up to one order of magnitude better performance than the use of current commercial drug-in-gel formulations. This is clear especially when the drug concentration in subcutaneous tissue vs. applied dose is compared (see also Section 4). The difference is greater for lower doses (see Fig. 1) and thicker tissues. For example, the relative drug concentration in the dorsal muscles treated with 9 mg of the drug per kg body weight in Transfersomes is approx. 1.9 higher than in the case of using gel-based formulation (6.7  $\mu\text{g/g}$  vs. 3.5  $\mu\text{g/g}$ ) whereas the concentration ratio deep in the hind legs of rats is close to 10, when the animals are treated with approx. 0.6 mg/kg body weight. The discrepancy in pigs is even more dramatic (see Section 4).

The drug concentration in the blood is approximately 2–3 times lower at  $t=12$  h. In the murine hind legs treated with 2.25–9 mg/kg body weight of Transfenac the drug concentration is close to 5  $\mu\text{g/g}$ . Voltaren Emulgel in the same dose range only yields values between 0.4 and 1.3  $\mu\text{g/g}$ . This corresponds to a relative degree of regionalization of for Transfenac and of 1 for Emulgel.

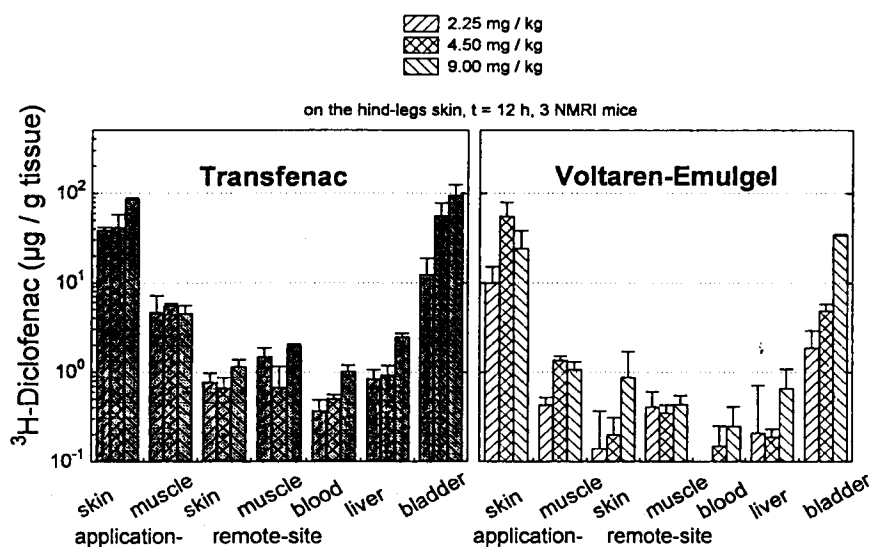


Fig. 1. (Left) Tissue concentration of diclofenac-derived  $^3\text{H}$ -radioactivity at  $t=12$  h after an epicutaneous application of 2.25 mg/kg body weight (right-dashed), 4.5 mg/kg body weight (hatched), and 9 mg/kg body weight (left-dashed) of the drug in ultra-deformable vesicles, Transfersomes, on the hind thigh of mice. (Right) Biodistribution of diclofenac-derived  $^3\text{H}$ -radioactivity 12 h after an application of 2.25 mg/kg body weight (right-dashed), 4.5 mg/kg body weight (hatched), and 9 mg/kg body weight (left-dashed) in the commercial hydrogel.

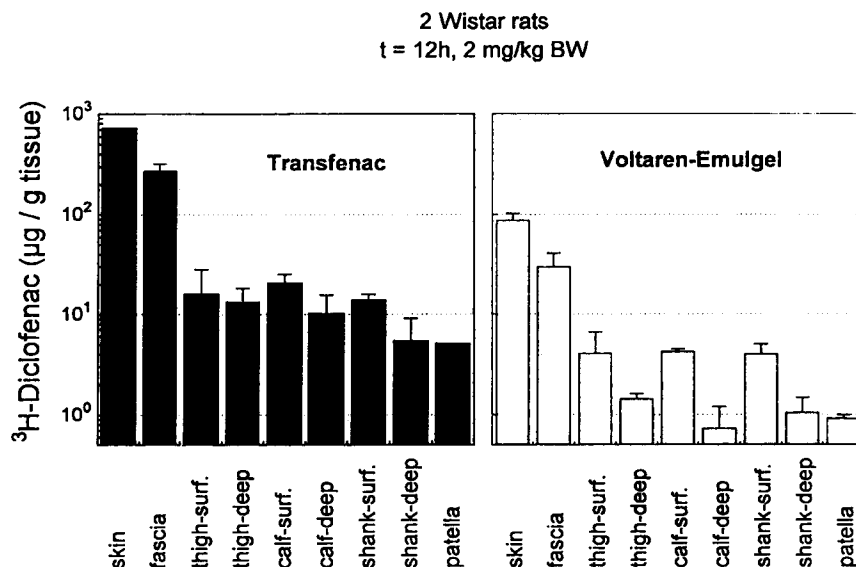


Fig. 2. [ $^3\text{H}$ ]Diclofenac biodistribution after the administration of 2 mg of the drug per kg body weight in highly deformable carriers, Transfersomes (left panel; black columns) or in a hydrogel (right panel; open columns) on rat hind legs.

The dorsal musculature of small rodents is typically only 2–3 mm thin. Mice are therefore unsuitable for predicting the penetration of diclofenac into more voluminous tissues. Hind extremities are better suited for such studies, since they can provide samples with a thickness of 5 mm (on a mouse) or up to 25 mm (on a rat).

Consequently, the characteristic of diclofenac bio-distribution in 'thick tissues' was studied with the radiolabelled drug formulations on the rat hind legs. The first set of corresponding biodistribution data is shown in Fig. 2. It demonstrates that the drug-derived radioactivity reaches the superficial muscle using a hydrogel as well as using the carrier-based formulation, but to a different extent. This is also illustrated in Fig. 3 which compares the drug distribution profiles in the tissues under the site of administration. When Voltaren Emulgel is used the average drug concentration in the muscle below the fatty layer is 0.8  $\mu\text{g/g}$  tissue, which is not much different from the agent concentration in the blood. Conversely, the average drug concentration in the muscle below the Transfenac-treated skin is 6.2  $\mu\text{g/g}$ , or more than 8 times higher.

A similar trend is observed when the administered drug dose is below 2 mg/kg body weight (Fig. 4 Figs. 5 and 7). For example, the average drug concentra-

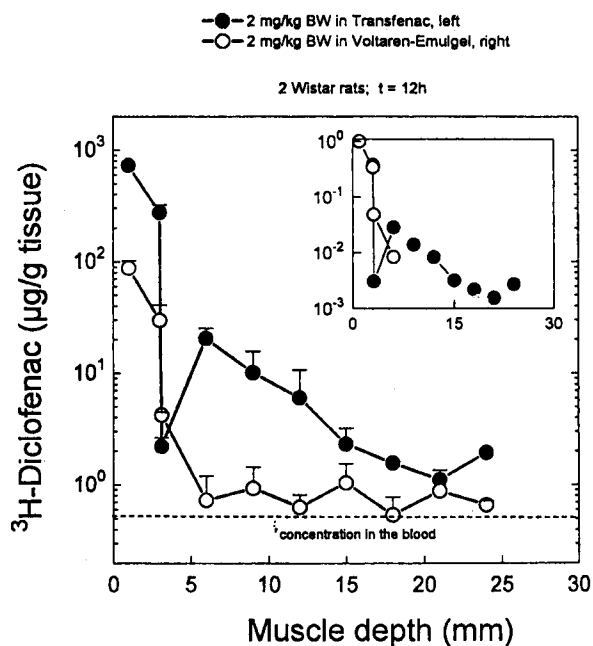


Fig. 3. [ $^3\text{H}$ ]Diclofenac penetration profile in the rat hind-leg muscles. Samples were taken 12 h after administering [ $^3\text{H}$ ]diclofenac in ultradeformable vesicles, Transfersomes (full symbols, left hind leg) or in a hydrogel (open symbols, right leg) on the skin at the dose of 2 mg/kg body weight. Diclofenac concentration in the blood is represented as a dashed line. Inset shows the normalized profile to the point where the intramuscular values become similar to those in the blood.

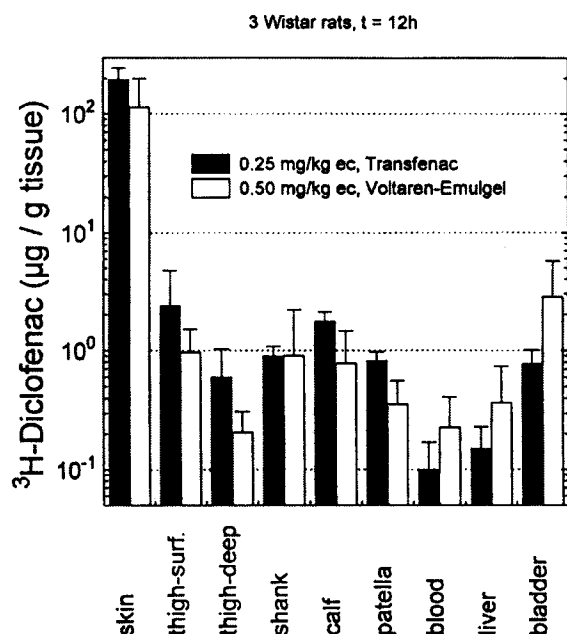


Fig. 4. Biodistribution of [ $^3\text{H}$ ]diclofenac-derived radioactivity after the topical administration of a small drug amount in very deformable vesicles, Transfersomes (black columns) or in a hydrogel (open columns) on the hind legs of Wistar rats.

tion in the superficial muscle treated with Transfenac is 14–20.5 mg/g and approximately half these values for the deeper muscles (data not shown). In the contralateral leg treated with Voltaren Emulgel the in-

tramuscular concentration is around 4 mg/g near the surface and only approximately 0.7 mg/g deep in the muscle. This latter value is very close to the corresponding blood concentration level of  $\sim 0.5$  mg/ml, which indicates the lack of drug delivery specificity.

The results illustrated in Fig. 4 stem from measurements with rats treated on one hind leg with either 0.25 mg/kg body weight of diclofenac in ultradeformable vesicles or, alternatively, with 0.5 mg to 0.6 mg of the drug per kg body weight in a commercial hydrogel. A similar drug distribution profile was obtained under the application site as with the higher drug doses but Transfenac was again more regio-specific than Voltaren Emulgel (see Fig. 4), especially deep under the treated skin; this is seen from the fact that the deep-muscle-to-blood drug concentration ratio for Transfenac is close to 6 while for the gel-like preparation a value near 1 is found. This difference in regio-specificity is commented on further in the text.

The relative drug concentration profile, normalized with regard to the surface concentration value, is similar near the skin surface for Transfenac and Voltaren Emulgel (see Fig. 3). Greater divergence is observed deep in the muscle (see insets to Figs. 3 and 5). We therefore infer that the mechanism of drug transport across the skin is different for the vesicle and gel-based diclofenac formulations, but the prin-

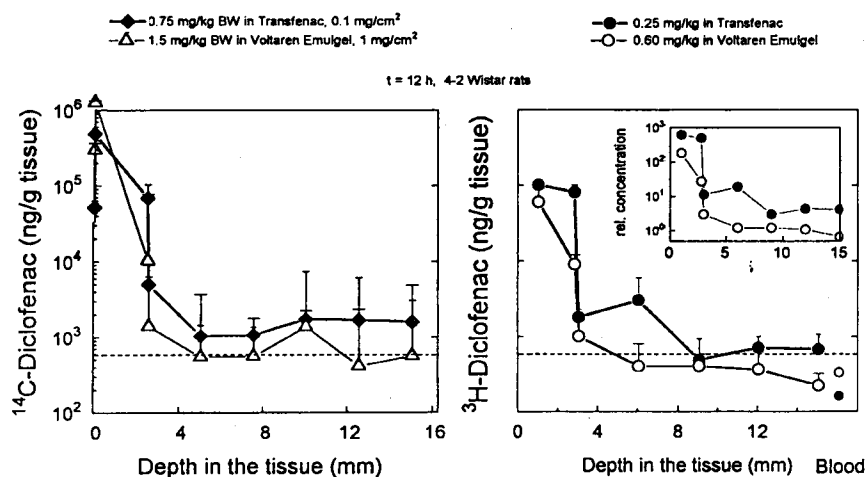


Fig. 5. Diclofenac distribution profile 12 h after an epicutaneous administration of the drug in ultradeformable vesicles (full symbols, 0.1 mg/cm $^2$ ) or in a hydrogel (open symbols, 1 mg/cm $^2$ ). (Left) 0.75 mg/kg body weight as  $^{14}\text{C}$ -labelled Transfenac or 1.5 mg/kg body weight as Voltaren Emulgel. (Right) 0.25 mg/kg body weight (labelled with  $^3\text{H}$ ) in deformable carriers or 0.6 mg/kg in Emulgel on one of the hind legs of four (left) or two (right) test rats. (Inset) The normalized drug distribution profile expressed as relative difference of local and systemic concentrations.

ciple of agent propagation through the muscle appears to be similar in either case. We believe that this difference is due to the fact that, in contrast to diclofenac molecules, Transfersomes are too large to enter into the cutaneous blood circulation. The drug from Transfersomes is consequently cleared much less efficiently by the intradermal capillary plexus allowing more drug to reach the deep subcutaneous tissues. The porcine data discussed later in this text indirectly support such conclusion.

For obvious reasons the drug concentration is always the highest at the skin surface. The average carrier-mediated diclofenac concentration in the skin ( $726.6 \pm 0.1 \mu\text{g/g}$ ), after a single epicutaneous administration of 2 mg diclofenac per kg body weight, is significantly higher than that resulting from a hydrogel administration ( $87.7 \pm 14.7 \mu\text{g/g}$ ). This is also true for the drug concentration in fascia (275.6 and  $29.8 \mu\text{g/g}$ ) or for the diclofenac level in the superficial muscle near the drug administration site (on average: 20.5 and  $4.2 \mu\text{g/g}$ ; see Figs. 2 and 3). The drug concentration in the rat patella, which is  $5 \mu\text{g/g}$  and  $0.9 \mu\text{g/g}$  for Transfenac and Voltaren Emulgel, respectively, depends strongly on the choice of drug vehicle as well. These conclusions can be drawn despite the fact that the thickness of and the drug concentration in vehicle was different for Transfenac and Emulgel, taken that the administration in

either case was done non-occlusively to mimic the therapeutic situation.

Similar differences are observed when a much smaller amount of diclofenac is used. Administering between 0.25 and 0.75 mg diclofenac per kg body weight (see Figs. 4 and 5) causes a 2-fold disparity between drug concentration deep under the skin when Transfenac or Voltaren Emulgel is used, in favour of the former, carrier-mediated delivery. (It should be noted that there is a large, and fairly constant, difference between the drug concentrations in the patella near the treated site mediated by Transfenac or Voltaren Emulgel; see Figs. 2 and 4.) The relative advantage of Transfenac over the drug in a hydrogel is greater deep in the tissue. Fig. 6, which shows the results from dose finding study, and therefore presents some gaps, illustrates this.

The above-mentioned observations suggest that ultradeformable vesicles not only improve the transport of diclofenac through the skin. They also carry a higher payload into the muscles under the application site. Near the bone, at a depth of 24 mm in the large and at 15 mm in the small rats, the carrier-mediated agent accumulation exceeds the serum values by a factor of 17.5 at  $t=8$  h and by a factor of 10–14 for the different kinds of formulations studied at  $t=12$  h. For the hydrogel-based formulation the muscle-to-blood concentration ratio near the skin is 3.8 at  $t=12$  h when the drug dose is 0.5 mg/kg body

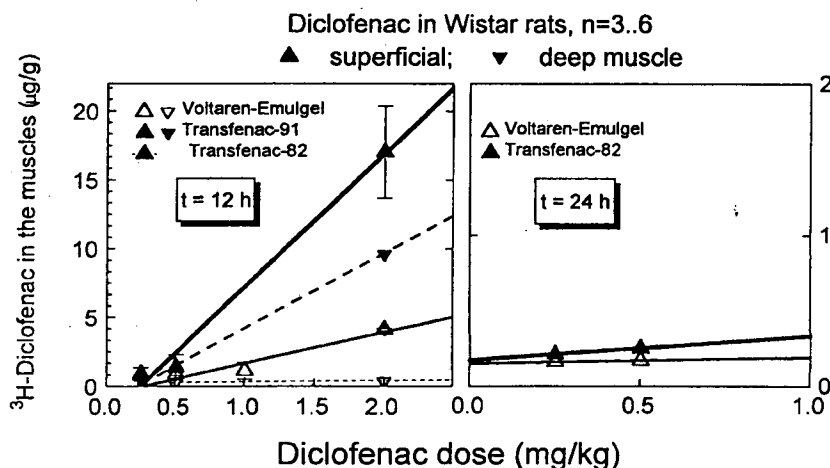


Fig. 6. Intramuscular drug concentration, deduced from the [ $^3\text{H}$ ]diclofenac-derived radioactivity data, at two different times after the administration of [ $^3\text{H}$ ]diclofenac in highly deformable vesicles (full symbols) or in a hydrogel (open symbols). Up-triangles pertain to the superficial, down-triangles to the deep muscle tissue.

weight. In the deeper muscle this advantage is lost. The regio-specificity also decreases with time until the treated-muscle/blood ratio falls below the insignificant level of 1.5 at  $t=24$  h, irrespective of the kind of drug formulation (data not shown).

The above-mentioned result is not much – but significantly ( $P < 0.05$ ) – different from the value of 0.89 measured after a single peroral use of diclofenac (data not shown). Transfenac supersedes in absolute terms the results obtained with the diclofenac-loaded, topically administered gel at  $t=24$  h and even more so at  $t=12$  h (see Fig. 6). Similar observations are made for the joints near the gel administration site. The muscle-to-liver ratio is typically between 1 and 3 for Voltaren Emulgel and around 6 for Transfenac.

The drug concentration in the 'treated patella' is greater than  $2 \mu\text{g/g}$  tissue (data not shown) and close to  $1 \mu\text{g/g}$  tissue (Fig. 4) when the administered Transfenac dose is 0.5 or 0.25 mg/kg body weight, respectively. Experimental scattering is quite large but the average concentration measured 12 h after applying 0.5–1 mg of diclofenac per kg body weight in a gel is significantly lower, between  $0.36 \mu\text{g/g}$  (Fig. 4) and  $\sim 1 \mu\text{g/g}$  (data not shown).

The results of our experiments with pigs, that have a skin permeability barrier similar to that of humans [22], support the results obtained with rats. When the average drug concentration is calculated for a 5-cm thick muscle under the treated site, the following results are obtained. Local administration of Transfenac gives rise to a therapeutically meaningful drug concentration in the muscle, and sometimes even in the patella, under the drug application site. A twice daily administration for 6 consecutive days of 0.075 mg of diclofenac per kg body weight, followed by a single treatment with the detectable radioactive drug, gives concentrations of  $0.433 \mu\text{g/g}$  in the muscle and  $0.088 \mu\text{g/g}$  in the patella, 12 h after the second [ $^3\text{H}$ ]Transfenac application. Skin treatment with a single dose of 0.15 mg/kg body weight at  $t=-12$  h raises these levels to  $0.811 \mu\text{g/g}$  in the muscle and to  $0.327 \mu\text{g/g}$  in the patella (see Fig. 7). For a dose of  $2 \times 0.150$  mg/kg body weight, the value in the muscle is  $2 \mu\text{g/g}$  and in the patella  $0.163 \mu\text{g/g}$ . An untreated muscle contains 0.039, 0.031 and  $0.047 \mu\text{g/g}$  for the three increasing doses, respectively. A 4- to 8-fold higher dose ( $< 1.2$  mg/kg body weight) of diclofenac in a hydrogel yields a drug concentration of  $0.133$

$\mu\text{g/g}$  in the treated and  $0.021 \mu\text{g/g}$  in the untreated muscle. In the latter case, the proximal patella does not contain any drug within the experimental error. If the topical administration of diclofenac in a gel is complemented with an oral drug uptake of 1.2 mg/kg body weight on the day 1, the drug concentration measured in the treated muscle is  $0.32 \mu\text{g/g}$  while in the untreated one  $0.1 \mu\text{g/g}$  are found.

These results indicate that even with a smaller drug content in ultradeformable carriers there is approximately one order of magnitude higher diclofenac concentration in the target tissue than using the administration of commercial Voltaren Emulgel (see Fig. 7).

In topical human therapy the painful regions are often treated two or three times per day with a diclofenac-loaded hydrogel. The poor success of such palliative therapy must be compensated with the oral uptake of diclofenac. Transfenac provides a solution to this problem as it generates a sustained drug release automatically.

In our experience, Transfenac only needs to be used once or twice daily on the skin to ensure a good therapeutic effect. Results measured with the

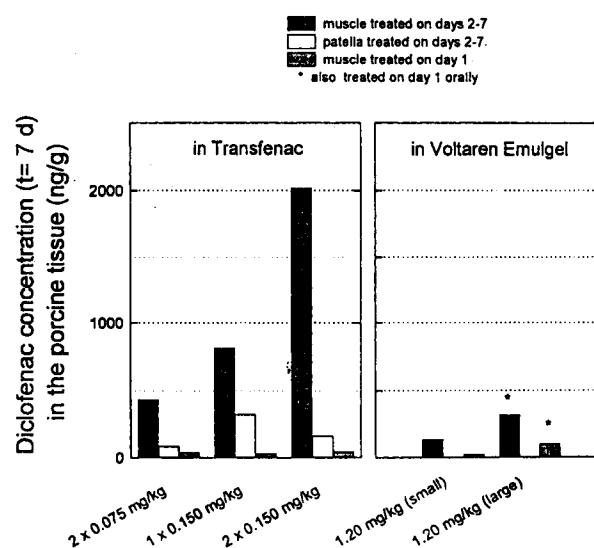


Fig. 7. The average radioactivity-derived drug concentration in individual pigs tested as described in the text with the drug in ultradeformable vesicles, Transfersomes (left panel) or in a hydrogel (right panel). Treated muscles (5-cm thick sample, black columns) and their proximal patella (open columns) are compared with the untreated muscles (grey columns).

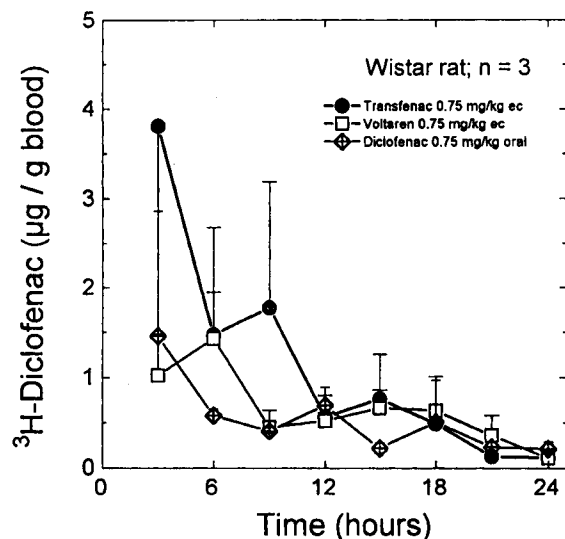


Fig. 8. The pharmacokinetics of [ $^3\text{H}$ ]diclofenac appearance and elimination from rat blood circulation after an epicutaneous administration of 0.75 mg of drug/kg body weight in highly deformable carriers (Transfersomes, closed circles), in a hydrogel (open squares) or through the mouth (crossed diamonds).

mice treated once or twice daily with a relatively high dose of diclofenac imply the above-mentioned conclusion (data not shown). Related lower dose treatment of pigs for 6 days is also compatible with this conjecture (see Fig. 7).

### 3.2. Pharmacokinetics

The drug from a hydrogel or Transfenac administered on the skin is cleared from the blood circulation with a half-time of  $\geq 4$  h (see Fig. 8). This is longer than the half-time value of Voltaren in humans,  $t_{1/2} = 2$  h.

## 4. Discussion

The relative merits of diclofenac in ultradeformable vesicles are best judged by comparing the results gathered with Transfenac with those obtained using conventional formulations and/or administrations of the drug.

### 4.1. Oral

A single oral diclofenac application at the dose of

$\sim 5$  mg/kg body weight in a guinea-pig model, according to the literature [3], gives  $\geq 10$  times higher plasma and 14 times higher urine concentration than the diclofenac gel application on the intact animal skin. The corresponding blood value falls from 1.5 to 0.5  $\mu\text{g}/\text{ml}$  over a period of 8 h. In humans treated chronically with a dose of approximately 2 mg/kg per day orally, the drug concentration in the blood is around 1  $\mu\text{g}/\text{ml}$ , according to the manufacturers' specifications. In our experiments with mice, a single oral application of 9 mg/kg body weight created serum drug concentration of approximately 1  $\mu\text{g}/\text{ml}$  at  $t = 12$  h (see Fig. 1, right panel). Our data thus fall in the same range as previously published results measured in different animal species.

### 4.2. Skin

The transepidermal flux of diclofenac in vitro has been calculated by Roberts and Singh [4] to be between 0.09  $\mu\text{g}/\text{h}$  per  $\text{cm}^2$  and 0.36  $\mu\text{g}/\text{h}$  per  $\text{cm}^2$ , depending on agent's ionization state. The ionized diclofenac passes through the rat skin in a Franz-cell less readily ( $\log P = 3.57 \times 10^{-4}$  cm/h) than drug in the protonated form, that is, at low pH ( $\log P = 1.82 \times 10^{-2}$  cm/h). The highest reported flux is approximately 0.5  $\mu\text{g}/\text{h}$  per  $\text{cm}^2$ .

The in vivo penetration studies described in this work suggest that the carrier-mediated flux of agent through the intact rodent skin in situ is at least 10  $\mu\text{g}/\text{h}$  per  $\text{cm}^2$ , or 30–100 times higher than that generated by a more conventional drug formulation [4]. The upper limit estimate is 30  $\mu\text{g}/\text{h}$  per  $\text{cm}^2$ . Such good performance of the vesicle-based drug formulation is in our opinion due to the good capability of ultradeformable vesicles to cross the skin and thus to carry agents into the body.

Lipid-diclofenac combinations have been tested before in the rat model. In a study by a Japanese group [11], 15 mg/kg body weight of diclofenac (7-fold the dose normally taken orally by human patients) was applied on the shaved rat dorsum alone or in combination with a large amount of hydrogenated soya phospholipids (1 mg/ $\text{cm}^2$ ). This resulted in intradermal drug concentrations between 12  $\mu\text{g}/\text{g}$  and up to 60  $\mu\text{g}/\text{g}$  of the skin tissue, depending on the total lipid concentration used (0.1–1 wt%; data not shown). The relative bioavailability of the drug from

such formulations was rather low, however, close to approximately 5% for the solution and 25% for the lipid-supplemented formulations over a period of 7 h [11]. In the experiments reported in this work, the carrier-mediated drug concentration in rat skin was 726  $\mu\text{g/g}$ , when a 7.5-fold lower diclofenac dose was used. Maffei and colleagues also reported that the application of liposomal diclofenac resulted in localization of the drug at the site of application on rats with slow systemic availability; however, with the application of ultrasound pulsed drug systemic levels could be achieved [12].

#### 4.3. Blood and urine

A single application of  $\sim 10$ ,  $\sim 3.5$  or  $\sim 1.3$  mg/kg body weight diclofenac, in a commercial cream, onto the shaved but otherwise intact guinea-pig skin reportedly causes drug concentration in the blood to reach 0.1, 0.02 and 0.005  $\mu\text{g/ml}$ , for the three respective doses [3]. The corresponding published values for the urine are approximately 45, 21 and 5  $\mu\text{g/ml}$ , respectively [3]. In our experiments with the commercial diclofenac gel used on the murine hind legs ( $0.03 \pm 0.01$ )  $\mu\text{g/ml}$  were detected in the blood and ( $1.88 \pm 1.03$ )  $\mu\text{g/g}$  in the bladder plus residual urine, when the drug was applied at the dose of 2.25 mg/kg. This documents the compatibility of results between our and previous studies.

Conversely, an epicutaneous application of Transfenac under the same experimental conditions gives rise to a drug concentration of ( $0.37 \pm 0.12$ )  $\mu\text{g/ml}$ . This is 10 times higher than that of a hydrogel application. Comparable drug concentration is also found in the urine of test animals. Both results suggest that the drug delivered by means of ultradeformable vesicles penetrates the skin better and is eliminated more slowly from the skin than when a more conventional formulation of this drug is used for the reason discussed later. While the rate of drug liberation and biotransformation remains to be investigated we believe that, ultimately, all the drug is released from highly deformable carriers that have crossed the skin.

#### 4.4. Muscle

Six applications of  $2 \times 1.6$  mg diclofenac per day in

a gel on the guinea-pig back, according to the literature, result in the intramuscular drug concentrations under the application site of approximately 0.24  $\mu\text{g/g}$  [3]. In the distal muscle 0.07  $\mu\text{g/g}$  is found. This is 55% and 87% lower than in the plasma, which contains approximately 0.515  $\mu\text{g}$  of diclofenac per millilitre of sample [3].

Singh and Roberts [4] provided the data on the permeation of diclofenac from several aqueous drug preparations on the skin in vivo and in vitro. These authors have estimated the maximum reach of agent permeation through the rat skin not to exceed 4 mm. When diclofenac is applied on Wistar rat backs as a solution, the concentration of this drug deep in the muscle is hence nearly the same as in the plasma. The corresponding local drug concentration decrease, according to the same authors [4], is approximately 1/10 per millimetre. This corresponds to a relative concentration of approximately 10% in the dermis, near less than 1% in the subcutis, around 0.2% in the superficial and close to 0.015% in the deep muscle tissue.

We find that a single application of diclofenac in a hydrogel on the rat hind leg only creates an intramuscular drug concentration of ( $0.25 \pm 0.2$ )  $\mu\text{g/g}$  when the administered dose is close to 1 mg/kg body weight (data not shown). Raising this dose to 2 mg/kg body weight increases the intramuscular drug concentration to ( $0.5 \pm 0.3$ )  $\mu\text{g/g}$ , when a spillover from the contralateral leg treated with Transfenac is possible, and to approximately 0.3  $\mu\text{g/g}$  in other cases. The intramuscular concentration is then similar to that measured in the blood, when the drug is used in a hydrogel. It is also roughly comparable to the value reported by Singh and Roberts [4]. Using a small amount of Transfenac (0.25 mg/kg body weight) on the skin gives rise to a significantly lower serum drug level around 0.1  $\mu\text{g/ml}$  (see Fig. 4).

Penetration profiles achieved with Transfenac in the rat model are much closer to therapists desires. The formulation of diclofenac in ultradeformable vesicles gets into and across the skin approximately ten times more efficiently than the drug from a hydrogel administered at an intermediate dose (2 mg/kg body weight). In Fig. 3 the drug penetration profile in the latter situation is seen to extend throughout the entire hind leg, to a total depth of 25 mm, when Transfenac is used (see Fig. 2). Diclofenac gel brings

the majority of the drug to the depth of merely 3–4 mm.

Drug accumulation in the hind legs of rats treated epicutaneously with Transfenac ( $\sim 2$  mg/kg body weight) is not specific to this kind of animal: the results obtained in murine (see Fig. 1, left panel) or rat (see Fig. 2) hind-leg muscles are semi-quantitatively similar. Extrapolation to bigger animal species, including humans, is therefore reasonable and is justified by the results of experiments on pigs.

#### 4.5. Joint

Radermacher and colleagues have applied diclofenac onto one knee and a placebo gel onto the other knee of humans [5]. The resulting drug accumulation in the synovial fluid was then concluded to stem mainly from the systemic blood supply. High relative drug concentrations in the smaller animal joints (3  $\mu\text{g/g}$ ), or in the synovial fluid of the repeatedly treated human finger and wrist ( $\sim 0.8$   $\mu\text{g/ml}$ ) [3], are thus likely to be due to the short diffusion distances in such tissue specimen. The data reported by Radermacher should hence be compared with the result of our measurements with the rat patella, which contains ( $5.05 \pm 0.07$ )  $\mu\text{g}$  of diclofenac per gram tissue, after a single epicutaneous Transfenac application. Our value exceeds by the factor of 5 the concentrations in the systemically treated (distal) knee ( $0.92 \pm 0.08$ )  $\mu\text{g/g}$  or muscle ( $\sim 0.75$   $\mu\text{g/g}$ ) of the same test rat. In the treated knee the Transfenac-derived drug concentration is also one order of magnitude higher than in the blood. This excludes the possibility of significant drug influx from the systemic blood circulation into the treated knee as the reason for the high drug concentration in the joint.

Similarly favourable results are obtained in pig patella after the topical administration of Transfenac, the specific drug concentration being between 0.09 and 0.36  $\mu\text{g/g}$ .

#### 4.6. Alternative formulations

Diclofenac to date was chiefly employed orally, even for the treatment of the well-localized pathological states and despite the systemic side effects of the drug. The introduction of Voltaren Emulgel was

therefore important and successful commercially but alternative formulations are now being sought.

For example, steroidal and non-steroidal anti-inflammatory drugs, including diclofenac, were formulated in a submicron emulsion. The latter consisted of approximately 100 nm large oil droplets [23] and were tested in the somewhat questionable [24] carrageenan-induced paw edema rat model. In such test diclofenac in submicron emulsion proved to be 40% more active than standard Voltaren Emulgel.

Diclofenac hydroxyethylpyrrolidine (DHEP) was incorporated in a plaster [35], containing 180 mg DHEP in an auto-adhesive medicated gauze pad of standard dimensions ( $10 \times 15$   $\text{cm}^2$ ). The steady-state measurements with such pad on animals indicated the sustained release (12 h) of the drug [7] with an estimated absorbed dose of 5–10 mg per application; this corresponds to a bioavailability of 2.5–5.5% [7] which is not sufficient for the successful treatment of all kind of diseases sensitive to nonsteroidal anti-inflammatory agents [25,35].

DHEP plasters were used twice daily (at 08:00 and 20:00 h) for 4 consecutive days by eight volunteers suffering from monolateral knee joint effusion [25]. Plasters were left on the affected area until the following application or for 12 h the 5th day. Venous blood samples were collected immediately before the first dose and 1, 4 and 8 h after the ninth application. Synovial fluid (SF) from the affected knee joint was collected at the beginning of the study and at 4 h after the ninth DHEP plaster application. Unchanged diclofenac was measured in plasma and SF by means of a gas liquid chromatography–mass spectrometry technique. Low but durable levels of diclofenac were obtained at each collection after dosage in plasma. Diclofenac was detectable at somewhat higher concentration in SF at the fourth hour of the ninth application [25].

Impeding the skin barrier increases the propensity for the drug permeation across the skin. This was demonstrated in a study in which a total of 120 patients, with moderate to severe pain, due to localized rheumatic or traumatic conditions, were randomly allocated to receive ultrasonic sessions three times weekly for 4 weeks. Voltaren Emulgel or regular gel was used as coupling medium [26]. The physician's overall assessment of therapeutic efficacy revealed that a satisfactory result was achieved in

86% of Voltaren Emulgel-treated patients compared with 76% of patients receiving regular gel ( $P < 0.05$ ). Tolerability was good or excellent in over 95% of patients in both treatment groups [26].

#### 4.7. Adverse side effects of the drug

The side effects of diclofenac, especially in the gastrointestinal tract, are often serious. For the period between 1983 and 1991, for example, 98 adverse drug reactions to topical nonsteroidal anti-inflammatory agents were reported by the Spanish pharmacovigilance organization [28]. Eleven general reactions, such as duodenal ulcer, gastrointestinal bleeding, diarrhoea, dyspnoea, facial oedema, aggravation of bronchospasm, and angioedema were described. Most commonly gastrointestinal (39%) and cutaneous (20%) adverse events were reported. The ratio of gastrointestinal to non-gastrointestinal reactions to diclofenac was higher in the case of slow-release enteric-coating diclofenac than in the case of plain diclofenac ( $P = 0.037$ ). Four cases of upper gastrointestinal haemorrhage, resulting from the cutaneous application of diclofenac (Voltaren Emulgel), were also reported by Zimmerman and colleagues [32]. Three of the patients had used the medication three times daily for at least 2 weeks over a large area before the onset of bleeding. In two cases a peptic ulcer was identified retrospectively, and the haemorrhage was massive enough to necessitate blood transfusions. Therefore, diclofenac should be used prudently in patients with a history of peptic ulcer [32].

In our expectation Transfenac, when administered directly onto a diseased site in a small but sufficient quantity, will cause less side effects, if any, than the currently available diclofenac formulations. This should partly be due to the more favourable drug biodistribution from the carriers that concentrate the agent in the tissue to be treated. Phospholipids, and especially phosphatidylcholine, are also known to improve the safety of their co-applied agents, when the latter are surfactant-like [33]. Moreover, the co-applied lipids are likely to minimize the danger of allergic contact dermatitis that may be induced by topical diclofenac [30].

We therefore expect that the toxicity and the side effects of diclofenac in vivo will be diminished in the presence of ultradeformable vesicles. Such an in-

crease in the agent safety was already documented for lidocaine in corresponding carriers [31].

#### 4.8. Transfenac and carrier safety

When applied on the intact skin, phospholipid suspensions are not detrimental to the skin. On the contrary, certain phospholipid preparations even seem to improve the hydration (and thus the optical appearance) of aged skin. Phospholipid suspensions are also non-irritating to the skin, at least up to the degree of 20% degradation [29].

Transfenac will be applied in a quantity ( $\leq 50$  mg) that will not exceed the total amount of this drug used currently parenterally ( $\leq 75$  mg/injection) or orally ( $\leq 150$  mg/day); the daily dose of Transfenac for humans should actually be lower:  $\leq 25$  mg and probably even  $\leq 10$  mg. Total amount of the phospholipid placed on the skin in the form of Transfenac, consequently, is unlikely to exceed 1 g/day in most cases. This is only 20% of the phospholipid quantity that is infused daily in the form of Lipofundin or any other comparable parenteral formulation of such lipid. It is also less than 20% of the natural variability of phosphatidylcholine concentration in the plasma for average healthy subjects. Based on these data, Transfenac is expected to be a very safe product.

### 5. Conclusions

In animal experiments, the diclofenac lotion based on ultradeformable vesicles, Transfenac, has proven to be superior even to the best commercial diclofenac gel for the topical administration. When used in a reasonable dose range in mice, Transfenac was shown to be at least 5 times more potent and significantly more site-specific than the 'competing' topical diclofenac formulations. The relative improvement is affected by the skin/muscle-weight ratio, which is 1/8 for mice and makes the murine model suboptimal for the prediction of therapeutic efficacy of epicutaneously applied diclofenac. In rats, a single epicutaneous application of 2 mg of diclofenac per kg body weight in highly deformable carriers produced at least 4 times higher drug concentration in the treated muscles than a drug-loaded hydrogel.

Agent concentration in distal muscles or in serum

is lowered by using a smaller agent amount, but more so with Transfenac than with the diclofenac loaded hydrogel. The effect is more pronounced for the large than for the small animals.

Diclofenac from ultradeformable vesicles can penetrate deep into the soft tissue under the drug application site. This is not observed when the drug is used in a gel (see Figs. 4–7), unless special treatment, such as sonophoresis [34], is used. The reach of the carrier-associated agent may therefore exceed by more than one order of magnitude the maximum agent penetration from the commercial Diclofenac-loaded gel. Ultradeformable vesicles even can bring a therapeutic quantity of diclofenac into the treated knee with reasonable reproducibility.

The relative advantage of using Transfenac instead of the more conventional diclofenac gel increases in the low dose range. This trend seems to increase with decreasing number of the drug administrations. Both are likely to be due, at least partly, to the sustained release of diclofenac from the carriers deposited into the subcutaneous tissue.

Diclofenac lotion comprising ultradeformable vesicles, Transfersomes, combines the safety of the conventional diclofenac formulations for the topical administration with a high or even improved efficacy. The latter matches that of the best available oral preparations of such drug. This suggests that the simple-to-use, topical Transfenac formulations have the potential to replace oral therapy with diclofenac. We believe that such novel formulations will compete successfully with the combined oral/topical treatment of rheumatoid disease.

## 6. Unlinked References

[27]

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## References

- [1] J.I.F. Reynolds (Ed.), *The Extra Pharmacopoeia* (Martindale), Pharmaceutical Press, London, 1993.
- [2] P.A. Todd, E.M. Sorkin, Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, *Drugs* 35 (1988) 244–285.
- [3] W. Riess, K. Schmid, L. Botta, K. Kobayashi, J. Moppert, W. Schneider, A. Sioufi, A. Strusberg, M. Tomasi, Die perkutane Resorption von Diclofenac Arzeim, *Forsch. Drug Res.* 36 (1986) 1092–1096.
- [4] P. Singh, M.S. Roberts, Skin permeability and local tissue concentrations of non-steroidal anti-inflammatory drugs after topical application, *J. Pharmacol. Exp. Ther.* 268 (1994) 144–151.
- [5] J. Radermacher, D. Jentsch, M.A. Scholl, T. Lustinetz, J.C. Frolich, Diclofenac concentration in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease, *Br. J. Clin. Pharmacol.* 31 (1991) 537–541.
- [6] Voltaren Emulgel, company brochure, Ciba, Basel, 1993.
- [7] A. Assandri, S. Canali, C. Giachetti, Local tolerability and pharmacokinetic profile of a new transdermal delivery system, diclofenac hydroxyethylpyrrolidine plaster, *Drugs Exp. Clin. Res.* 19 (1993) 89–95.
- [8] H. Schreier, J. Bouwstra, Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery, *J. Controlled Release* 30 (1994) 1–15.
- [9] S. Zellmer, W. Pfeil, J. Lasch, Interaction of phosphatidylcholine liposomes with the human stratum corneum, *Biochim. Biophys. Acta* 1237 (1995) 176–182.
- [10] A.C. Lauer, L.M. Lieb, C. Ramachandran, G. Flynn, N.D. Weiner, Transfollicular drug delivery, *Pharm. Res.* 12 (1995) 179–186.
- [11] T. Nishihata, K. Kotera, Y. Nakano, M. Yamazaki, Rat percutaneous transport of diclofenac and influence of hydrogenated soya phospholipids, *Chem. Pharm. Bull.* 35 (1987) 3807–3812.
- [12] R. Maffei-Facino, M. Carini, G. Aldini, L. Saibene, A. Macciocchi, Antioxidant profile of nimesulide, indomethacin and diclofenac in phosphatidylcholine liposomes (PCL) as membrane model, *Int. J. Tissue React.* 15 (1993) 225–234.
- [13] G. Cevc, G. Blume, Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force, *Biochim. Biophys. Acta* 1104 (1992) 226–231.
- [14] G. Cevc, Dermal insulin, in: M. Berger, F.A. Gries (Eds.), *Frontiers in Insulin Pharmacology*, Georg Thieme, Stuttgart, 1993, pp. 161 ff.
- [15] G. Cevc, A. Schätzlein, G. Blume, Transdermal drug carriers: basic properties, optimization and transfer-efficiency in the case of epicutaneously applied peptides, *J. Controlled Release* 36 (1995) 3–16.
- [16] G. Cevc, G. Blume, A. Schätzlein, D. Gebauer, The skin: a pathway for the systemic treatment with patches and lipid-based agent carriers, *Adv. Drug Deliv. Rev.* 18 (1996) 349–378.

- [17] G. Cevc, G. Blume, A. Schätzlein, Transfersomes mediated transepidermal delivery improves the regio-specificity and biological activity of corticosteroids in vivo, *J. Controlled Release* 45 (1996) 211–226.
- [18] G. Cevc, Lipid suspensions on the skin. Permeation enhancement, vesicle penetration and transdermal drug delivery, *Crit. Rev. Ther. Drug Deliv. Systems* 13 (1996) 257–388.
- [19] G. Cevc, Material transport across permeability barriers by means of lipid vesicles, in: R. Lipowsky, E. Sackmann (Eds.), *Handbook of Physics of Biological Systems*, Vol. 1, Elsevier, Amsterdam, 1995, pp. 441–464.
- [20] G. Cevc, European patent 91 114 163.8-2114, 1991.
- [21] A. Schätzlein, G. Cevc, Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes), *Br. J. Dermatol.* 138 (1998) 583–592.
- [22] K.A. Walters, J. Hadgraft (Eds.), *Pharmaceutical Skin Penetration Enhancement*, Marcel Dekker, New York, 1993, pp. 383–408.
- [23] D.I. Friedman, J.S. Schwarz, M. Weisspapir, Submicron emulsion vehicle for enhanced transdermal delivery of steroidal and nonsteroidal antiinflammatory drugs, *J. Pharm. Sci.* 84 (1995) 324–329.
- [24] Y. Hiramatsu, S. Akita, P.A. Salamin, R. Maier, Assessment of topical non-steroidal anti-inflammatory drugs in animal models, *Arzneimittelforschung* 40 (1990) 1117–1124.
- [25] G. Gallacchi, R. Marcolongo, Pharmacokinetics of diclofenac hydroxyethylpyrrolidine (DHEP) plasters in patients with monolateral knee joint effusion, *Drugs Exp. Clin. Res.* 19 (1993) 95–97.
- [26] T. el Hadidi, A. el Garf, Double-blind study comparing the use of Voltaren Emulgel versus regular gel during ultrasonic sessions in the treatment of localized traumatic and rheumatic painful conditions, *J. Int. Med. Res.* 19 (1991) 219–227.
- [27] M.A. Yorek, in: G. Cevc (Ed.), *Phospholipids Handbook*, Marcel Dekker, New York, 1993, p. 759.
- [28] A. Figueras, D. Capella, J.M. Castel, J.R. Laorte, Spontaneous reporting of adverse drug reactions to non-steroidal anti-inflammatory drugs. A report from the Spanish System of Pharmacovigilance, including an early analysis of topical and enteric-coated formulations, *Eur. J. Clin. Pharmacol.* 47 (1994) 297–303.
- [29] M.J. Parnham, A. Wendel, *Phospholipids and Liposomes*, Scientific Publication No. 2, Natterman Phospholipid GmbH, Cologne, 1992.
- [30] M. Gebhardt, A. Reuter, B. Knopf, Allergic contact dermatitis from topical diclofenac, *Contact Dermatitis* 30 (1994) 183–184.
- [31] P. Gonzalez, M.E. Planas, L. Rodriguez, S. Sanchez, G. Cevc, Noninvasive, percutaneous induction of topical analgesia by a new type of drug carriers and prolo..gation of the local pain-insensitivity by analgesic liposomes, *Anesth. Analg.* 95 (1992) 615–621.
- [32] J. Zimmerman, J. Siguencia, E. Tsvang, Upper gastrointestinal hemorrhage associated with cutaneous application of diclofenac gel, *Am. J. Gastroenterol.* 90 (1995) 2032–2034.
- [33] G.P. Martin, C. Marriott, Membrane damage by bile salts: the protective function of phospholipids, *J. Pharm. Pharmacol.* 31 (1981) 754–759.
- [34] S.P. Vyas, R. Singh, R.K. Asati, Liposomally encapsulated diclofenac for sonophoresis induced systemic delivery, *J. Microencapsul.* 12 (1995) 149–154.
- [35] M. Rosenthal, I. Bahous, A controlled clinical study on the new topical dosage form of DHEP plasters in patients suffering from localized inflammatory diseases, *Drugs Exp. Clin. Res.* 19 (1993) 99–105.
- [36] G. Cevc, D. Gebauer, J. Stieber, A. Schätzlein, G. Blume, Ultraflexible vesicles, Transfersomes, have an extremely low permeation resistance and transport therapeutic amounts of insulin across the intact mammalian skin, *Biochim. Biophys. Acta* 1368 (1998) 201–215.