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Functional characterisation of novel analgesic product based on self-regulating drug carriers

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ABSTRACT

We compared a new formulation of ketoprofen (Diractin®) based on ultradeformable vesicle (Transfersome®) carriers with conventional topical gels with the drug (Gabrilen®; Togal® Mobil Gel; Fastum®). Depending on water concentration, between a few percent and >95% of ketoprofen in Diractin® is associated with the vesicles. The low free drug concentration on open skin (1–3%) minimises ketoprofen diffusion from Diractin® through the organ, keeping effective permeability coefficient for the product (even after increase to $\sim 3.5 \times 10^{-3}\,\mathrm{cm\,h^{-1}}$ at 24 h) below that of conventional gels ($\sim 0.3–2.1 \times 10^{-1}\,\mathrm{cm\,h^{-1}}$). The carrier's stress-responsiveness enables constriction crossing without vesicle breakdown. The carrier stiffening upon dilution, e.g. in tissues below the skin's diffusive barrier, helps avoiding the drug uptake in cutaneous blood capillaries. Diractin® therefore can deposit ketoprofen in deep subcutaneous tissues, which the drug from conventional gels reaches mainly via systemic circulation. *In vitro* efficacy of daily drug delivery through skin is ≤1.6% for conventional topical NSAID gels and merely ~0.05% for Diractin®. In contrast, *in vivo* ketoprofen transport by ultradeformable carriers through non-occluded skin into living pigs' subcutaneous muscles is 5–14× better than for conventional gels. Locally targeted drug transport by the self-regulating, ultradeformable vesicles is thus clearly non-diffusive and quite efficient.

1. Introduction

Pharmaceutical products applied on the skin in a conventional gel or patch rely on drug's concentration gradient (*chemical driving "force"*) to drive molecular diffusion (permeation) of a drug across the skin barrier. The latter is primarily located in the stratum corneum (sc) of the epidermis (Walters and Hadgraft, 1993; Cevc, 1997), and thinner than 20 μm.

A drug molecule that has permeated the stratum corneum may continue to diffuse deeper, into viable epidermis; here, its motion is negatively affected by much lower local drug concentration gradient (Kretsos et al., 2004). In the underlying dermis a drug also faces the danger of wash-out via cutaneous blood capillaries. Only the molecules that had crossed this deepest skin layer bypassing the secondary skin barrier of cutaneous clearance ultimately may reach subcutaneous tissues, including deep peripheral fatty and muscle layers.

Most NSAID molecules diffuse across the stratum corneum and into deeper skin regions rather well, using hydrophobic pathways in inter-cellular lipid matrix of the skin (Singh and Roberts, 1994a,b). One reason is suitable lipid–water partition coefficient of most NSAIDs (Hadgraft et al., 2000), but the small size of classical NSAIDs is helpful as well (Mitragotri et al., 1999).

Total quality and quantity of skin lipids therefore influences NSAID permeation across the primary skin barrier; the observed site dependency of topical NSAID absorption (Shah et al., 1996) reflects this.

According to Fick's first law of diffusion, the transepidermal flux of a topically applied NSAID from a gel or a patch is proportional to the barrier's permeability coefficient (P_{sc}) and the involved skin area (A) multiplied by the transport driving free NSAID concentration difference across the barrier ($\Delta c(t)$). The skin permeability coefficient, to a good approximation, is a product of NSAID's diffusion coefficient (D_{sc}) and partition coefficient (K_{sc}) in the stratum corneum divided by the skin barrier thickness (d_{sc}). (The former two parameters may be time dependent, leading to $P_{sc}(t)$.)

The diffusive flux ($J(t)$) through the skin of a topically applied NSAID from a gel or a patch is given by:

$$J(t)\big|_{\text{permeation}} = P_{sc}(t)\,\Delta c(t) = \left(\frac{K_{sc}(t)D_{sc}(t)}{d_{sc}}\right)\Delta c(t) \sim P_{sc}\Delta c(t) \quad (1)$$

Drug permeation enhancers, which are included in most currently available topical gels, sprays, or patches facilitate NSAID

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Table 1
Composition of the tested articles containing ketoprofen (KT)

Test article	KT concentration/distribution	Carriers concentration	Excipients (*manufacturers specifications*)
Gabrilen® gel	2.5%/, in solution	0	Carbomer, ethanol, 2-propanol, ammonia solution, purified water
Togal® Mobil Gel with Ketoprofen	2.5%/, in solution	0	Carbomer, ethanol, 2-propanol, ammonia solution, purified water
Fastum® gel	2.5%/, in solution	0	Carbomer, ethanol, methylparabene, propylparabene, lavender essence, diethanolamine, purified water
Diractin®	2.29%/, mainly carrier-associated[a]	~10%[a]	Carbomer, soy phosphatidylcholine, polysorbate 80, methylparabene, butylated hydroxytoluene, NaOH, Na_2HPO_4, NaH_2PO_4, Na edetate, benzylic alcohol, ethanol, purified water

[a] After non-occlusive application on the skin.

partitioning and/or diffusivity in the skin (Walters and Hadgraft, 1993; Cevc, 1997; El-Kattan et al., 2000; Wu et al., 2001; Ceschel et al., 2002).[1] Marketed conventional topical analgesics moreover employ drugs in their most soluble and/or best permeable form (Hadgraft et al., 2000; Ceschel et al., 2002) and at highest tolerable/achievable drug concentration (Ceschel et al., 2002). All these measures increase diffusive flux of NSAID-s across the skin (cf. Eq. (1)).

NSAIDs from a conventional gel or a patch on the skin distribute quite rapidly in the organ. If an NSAID can diffuse into cutaneous blood vessels as well as it crosses the surrounding tissue, the majority of free drug molecules is then cleared near skin surface (Kretsos and Kasting, 2005); the drug then reaches a depth of a few millimetres merely (Singh and Roberts, 1994b). This has been observed, or implied, for most topical NSAIDs (Monteiro-Riviere et al., 1993; Singh and Roberts, 1994b; Heyneman et al., 2000; Cevc and Blume, 2001; Higaki et al., 2002) as well as for many other topically applied amphiphatic drugs (Singh and Roberts, 1993; Boutsiouki et al., 2001; Morgan et al., 2003).

To overcome the problem, a drug should not pass easily and quickly through the skin and into cutaneous blood vessels. Suitable drug carriers can help to reach the goal.

First of all, a suitable carrier should load the drug effectively to protect it against undesired clearance in the skin. Second, the carrier should retain the drug long enough, on, in, and also below the skin barrier. An useful carrier should moreover be able to cross the stratum corneum independent of drug concentration. In contrast to the conventional carriers that cannot trespass the skin barrier, ultradeformable carriers can overcome the skin barrier obstacle and reach the goal in the following fashion.

An ultradeformable carrier loaded with NSAID molecules for targeted delivery into deep subcutaneous tissues is placed in an aqueous suspension on the skin without occlusion. The excess of applied water starts immediately to evaporate. When the *carrier's solubility limit* is reached, the ultradeformable and very hydrophilic aggregate begins to experience osmotic stress; "*hydrational driving force*" across the primary skin barrier in the stratum corneum thus builds-up (Cevc and Blume, 1992; Schätzlein and Cevc, 1998; Cevc and Gebauer, 2003). The large number of molecules in each carrier, which represents a migration unit, enlarges the force acting on the carriers in comparison with that acting on a single molecule (Cevc, 1996, 2002). The carrier's high adaptability and its hydroaffinity both allow and prompt the drug-loaded ultradeformable vesicles "pull" across the skin barrier along transepidermal hydration gradient (Cevc and Gebauer, 2003). The process continues until the carrier has reached viable epidermis, where water abounds. In this well hydrated skin region the hydrational "pull" on the carrier ceases. The carrier's bulkiness now hampers further spontaneous motion, as both the carrier's concentration gradient and diffusivity in living tissues are small. The carrier is also too big to enter into cutaneous (micro)vessels and be taken away by the blood.

The missing "pull" on a carrier *in* the skin keeps low the diffusion-based carrier re-distribution from the outer into deeper skin regions. The pull can be replaced by a "push", however, on the carriers that already crossed the barrier. Such push is exerted by the ultradeformable aggregates still sensing the hydration gradient in the stratum corneum. Suitably designed and applied carriers consequently continue to move into body interior so long as at least some of the "late-movers" remains subject to transepidermal hydration gradient, which may last for hours.

The ultradeformable carrier's hydration sensitivity (Cevc and Gebauer, 2003) and its unique driving force (Cevc et al., 2008b) create an unprecedented opportunity to control the depth of the carriers migration—and thus the range of the carrier-mediated drug delivery into peripheral tissues (Cevc et al., 2008a). Its basis is the unmatched, non-diffusive nature of transcutaneous transport mediated by carriers.

In this work we describe the main preclinical characterisation results obtained with a new ketoprofen formulation comprising Transfersome® (trademark of IDEA AG): IDEA-033 = Diractin® (trademark of IDEA AG). We provide information on the carrier adaptability, drug loading, and drug permeability through human skin *in vitro*.

Our main conclusions are that ketoprofen can be associated with the ultradeformable, stress-responsive vesicles. The latter can then overcome pores much narrower than their own diameter without an appreciable change in the vesicle size; ketoprofen loading onto/into the vesicle is also a useful countermeasure against the drug's undesired clearance in the skin through the blood capillaries. The ultradeformable ketoprofen carriers can therefore trespass the skin permeability and clearance barriers *in vivo*. The process is orders of magnitude more efficient than, and different from, the drug's diffusion from a topically applied conventional gel. This provides unmatched opportunity for improving therapy of inflamed or painful deep peripheral tissues by means of targeted ketoprofen delivery into such tissues, which would otherwise require whole body treatment and bring the danger of systemic adverse side effects.

2. Materials and methods

2.1. Test articles

The chemical components of Diractin® are listed in Table 1, and in greater detail in a recent patent (Cevc and Vierl, 2003).

[1] The most notorious of such enhancers is DMSO, which positively affects both partitioning and/or diffusivity in the skin, but is rather smelly and irritating to the organ.

The same table also provides compositional information, as taken from the respective product information leaflets, for the marketed comparator products. The latter included the conventional topical ketoprofen gels Gabrilen® gel (Kreussler Pharma GmbH, Wiesbaden, Germany), "Togal® Mobil Gel mit Ketoprofen" (Togal Werke, Munich, Germany), and Fastum® gel (Laboratorios Menarini S.A., Barcelona, Spain).

2.2. Carrier characterisation

Characterisation of the gel product containing the self-regulating mixed lipid vesicle (Transfersome®) aggregates, or its precursor suspension, relies on proprietary test assays of IDEA AG (for a basic description see Cevc, 1996). In brief, the key assay measures trans-barrier penetration flux ($J(t)$) of a suspension of the carriers/aggregates as a function of the flux driving pressure (Δp) applied over a filter with 4–5 times narrower pores than the average carrier diameter. Such flux for the deformable carrier suspension has a dual pressure-dependency, which can be parameterised with the following equation (Cevc et al., 2003):

$$J(t)\big|_{\text{penetration}} = P'(\Delta p)\Delta p(t) = P'_{\max} f(\Delta p)\Delta p = P'_{\max}\{1 - \text{erf}(a^*\Delta p)^{-0.5} + (0.25\pi a^*\Delta p)^{-0.5}\exp[-(a^*\Delta p)^{-1}]\}\Delta p. \quad (2)$$

erf is error-function and $P'_{\max}$ the maximum barrier penetrability, which can be determinable by filtering just the suspending medium or else the test suspension under sufficiently high driving pressure ($a^*\Delta p >> 1$). The stress- or pressure-dependent modulation of the tested suspension's ability to cross the barrier ($f(\Delta p)$ or the curly bracket in the second line of Eq. (2)) is thus described, in inverse pressure units, by only one adjustable parameter of the model: a^*.

We performed all penetrability tests at least in triplicate and used a large number of different driving pressures to maximise the resulting data quality. For every test suspension we have also confirmed that the average carrier size after narrow pore crossing was at least 3-times larger than the pore diameter. As we described elsewhere in greater detail (Cevc et al., 2002, 2003), we did this by comparing with photon correlation spectroscopy (ALV, Langen, Germany) the average drug carrier's size before and after the described filtration.

2.3. Carrier diffusion in vitro

Carrier diffusion *in vitro* was assessed by comparing the vesicles influx into microdialysis (cut-off: 3 MDa; Dermal Dialysis, Erlangen, Germany) and open-flow microperfusion (OFM, average pore size: 100 μm >> $2r_{\text{ves}} \sim 100$ nm; Joanneum Research Forschungsgesellschaft, Graz, Austria) capillaries *in vitro*. For the purpose, the capillaries were connected to a peristaltic pump (Gilson, Bad Camberg, Germany) and perfused with the Ringer solution at a constant flow-rate of 0.2 μL min^{-1} at room temperature. (The sampling time was 20 min per glass vial, with a 60 min interval between each change of the test vial.) During perfusion, each capillary was placed in a glass container containing different outer phases, being either various Transfersome® suspensions, prepared by 50-, 100-, or 200-times dilution of the original suspension with the Ringer solution (Fresenius, Germany), or a simple Ringer solution, serving as a negative control. The tested carrier concentrations were first increased and then decreased in the specified three steps. The sampled perfusate was weighed and stored immediately at 2–8 °C until analysis. This provided the drug/carrier concentration ratio in the perfusate as a function of the carrier's concentration in the outer phase and thus afforded information about the drug and the carrier filtration, or uptake. The employed experimental set-up thus mimicked potential clearance of the carriers via cutaneous microvasculature.

2.4. Drug–carrier association and release

Drug–carrier association and release were checked with a different assay. In brief, the ketoprofen-containing carrier vesicle suspension was filled into the donor side of a diffusion cell. The cell was separated from the >100-times more voluminous receiver by a filter membrane (cut-off: >10 MDa) that was highly permeable to the drug but impermeable to the carriers. This ensured that the drug concentration in the receiver volume mirrored the free drug concentration in donor, which we confirmed by doing similar measurements with the carrier-free drug solutions. Owing to ketoprofen concentration gradient between the drug-loaded carrier aggregates in donor and the receiving bulk, the drug is gradually discharged from the carriers through the filter into the receiver . We measured the drug concentration in the latter continuously with UV absorption spectrometer for several hours, and evaluated the results relying on the known extinction coefficient of ketoprofen. (This clarified that the drug association with the carrier vesicles persists for a long time.) The same data set also yielded information on the total degree of initial ketoprofen association with the carrier, which we calculated from the starting rate of the drug's diffusion across the filter using Eq. (1).

2.5. Drug diffusion in vitro

To determine drug diffusion across the skin barrier *in vitro* we followed the regulatory authority recommendations. In brief, we inserted a small piece of excised human abdominal skin (thickness: ~500 μm; application area of 0.1963 cm^2) into a commercially available Franz-diffusion cell. 0.025 mL of the test product, corresponding to 0.573 mg for Diractin® or 0.625 mg ketoprofen for the conventional gels, were then applied on a non-occluded skin surface, to form the donor compartment. The underlying receptor volume was permanently flushed with the phosphate-buffered saline (pH 7.4; flow-rate: 0.2 mL h^{-1}), to wash the lower, dermal, skin barrier surface and to provide a total test sample volume of 4.8 mL. For each given time point, we collected one test sample (0.2–1.6 mL, depending on the sampling period) from each of the 6 employed Franz-cells that fulfilled a plausible tightness criterion. We analysed the resulting specimen individually and finally calculated an apparent transcutaneous flux value for each given sampling period, from the average of all independently measured data. To derive the skin permeability coefficient from such data we employed Eq. (1).

2.6. Animal experiments

Animal experiments were performed with neutered male hybrid piglets (approx. 8–12 kg bodyweight (BW)) supplied by a local farm animal breeder. They are described in adequate detail in separate publications (Cevc et al., 2008a,b). The research adhered to the Principles of Animal Care (NIH Publication #85-23, revised in 1985). In brief, the drug was applied on a non-occluded skin surface of the hind limb and left there for 1 h, to dry out macroscopically. The test animals were anaesthetised before muscle tissue sampling, by a dislocated i.m. injection of 10% ketamine (0.15 mL kg^{-1} BW) and azaperone (0.05 mL kg^{-1} BW) with subsequent intravenous infusion of propofol (as needed). The treated skin was stripped 30-times with an adhesive tape to remove the residual test material and the outer parts of the stratum corneum. To minimise further the sample

tissue contamination with the drug, the remaining skin tissue was excised with a scalpel and the skin-free biopsy of subcutaneous fat and the underlying muscles was collected with a single-use biopsy punch (diameter: 6 mm). All tissue samples were immediately frozen at −20 °C and stored protected from light until the drug concentration measurement.

2.7. Drug transport in vivo

Drug transport *in vivo* was assessed by determining ketoprofen concentrations in superficial (0–1.5 cm) and deep (1.5–<3 cm) muscle below an epicutaneous drug application site (sampled 1 h post-application), using the known applied drug quantity and the treated skin area as input parameters. However, this only provided the lower limit for the normalised rate of the drug transport (=flux density) across the skin. From the limiting value we thus derived the "true rate" of transcutaneous carrier transport by assuming >90% drug–carriers association during skin crossing, justified by results of our drug–carrier association and release experiments.

2.8. Drug concentration measurements

The drug concentration measurements relied on the high-pressure liquid chromatography (HPLC) with a UV detection unit. To determine ketoprofen concentration in fluid samples (*in vitro* receptor fluid, porcine plasma), the drug was first extracted from samples with a solid-phase method. Preparation of firm tissue samples involved tissue homogenisation followed by a solid-phase extraction and HPLC/UV analysis. The method showed linearity in 25–451 ng mL^{-1} range in the receptor fluid, in 9–358 ng mL^{-1} range in porcine plasma, and in ~4–336 ng g^{-1} range in pigs' tissue. The plasma measurements accuracy was 90.5% (5.9% coefficient of variation [CV]) for the lower limit of quantification and 102.9–111.0% (<2.3% CV) for the mid to high linear part of concentration range. Similar accuracy was also achieved for solid porcine tissues: 88.4% (6.3% CV) for the lower limit of quantification and 101.4–106.3% (<2.7% CV) for the mid to high linear concentrations range.

2.9. Carrier concentration measurements

The carrier concentration measurements relied on a nano-HPLC/UV method. To calibrate the outcome, the same carrier suspensions that were tested *in vitro* were also repeatedly subjected to direct concentration measurements. From the resulting data an average curve was derived that provided a reference for quantifying the carriers' concentration in the tested samples.

3. Results

Illustrative results of *in vitro* adaptability measurements, done with the carriers from the novel ketoprofen suspension gel, Diractin®, are given in Fig. 1. They reveal a non-linear relative increase of penetrability ("permeability") of the nano-porous filter, which simulates hydrophilic pathways through the stratum corneum, with the flux-driving pressure, Δp. The average vesicle size, measured with photon correlation spectroscopy, is nearly the same before and after the filter crossing, the lower the driving pressure and the vesicles transport, the more so (Fig. 3). The data taken together unambiguously prove the stress responsiveness as well as shape adaptability of the carrier vesicles in the tested formulation. They also vindicate the carrier's ability to maintain physical integrity during and after narrow pores passage, confirmed previously with the empty ultradeformable vesicles *in vitro* and *in vivo* (Cevc et al., 2002).

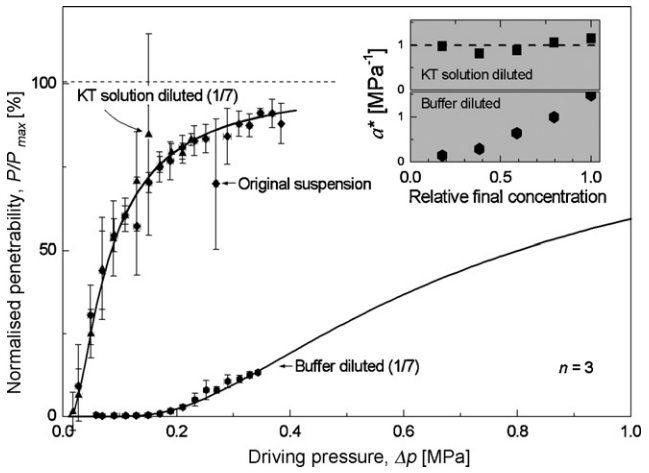

Fig. 1. Penetrability, normalised to similar suspension viscosity, of a nano-porous filter barrier ($r_v/r_{pore} > 4/1$) to different suspensions containing vesicle aggregates with a high ("Original suspension", "KT solution diluted") and low ("Buffer diluted") adaptability, a^*, calculated from Eq. (2), which was also used to generate the data-fitting curves. Inset shows the a^*-parameter values that were derived from the curves similar to those shown in the main figure body pertaining to the original suspension contained in Diractin® diluted either with a simple or ketoprofen (KT)-containing buffer (lower and upper inset, respectively).

The middle data sets (diamonds) in Fig. 1 shows, for example, that the carrier's adaptability in the original suspension, described in terms of parameter a^*, is approx. 5 MPa^{-1}. (Parameter a^* corresponds approximately to the inverse value of the pressure (Δp^*) that is needed to achieve ~57% of maximum possible trans-filter flux (J_{max}) or penetrability (P'_{max}) with the tested suspension (cf. Eq. (2)).) This parameter thus gauges the average aggregate adaptability that depends on the average aggregate bilayer flexibility as well enforced bilayer permeability (Cevc, 1996; Cevc et al., 2003). a^* is therefore formulation-dependent and thus formulation describing.

The carrier composition leaves adaptability parameter value nearly unchanged (see triangles in Fig. 1 and the upper inset). Conversely, a simple lowering of total amphiphat concentration, causing water concentration in the tested suspension to increase and vesicle composition to change, shifts the characteristic $f(\Delta p)$-curve (cf. Eq. (2)) towards higher driving pressures. The derived adaptability parameter value correspondingly decreases. For example, mixing the original carriers suspension with a simple phosphate buffer (pH 7.4) lowers a^*-value from 5 MPa^{-1} to <2 MPa^{-1} (see the lower curve and inset in Fig. 1). This indicates loss of water soluble, carrier-controlling components (including ketoprofen) from the mixed lipid aggregates in the final, diluted suspension.

An up-concentration of the carrier suspension has the opposite effect (cf. Fig. 2), as the drug and other water soluble amphiphilic molecules are now pushed on and into the carriers. The phenomenon could, but in a properly designed formulation does not, affect the carrier adaptability, $a^* \sim$ constant (data not shown), if care is taken to maintain the effective carrier composition.

Partial carrier drying on skin surface, consequently, enhances ketoprofen–carrier association. In a putative suspension with 50 wt% of the carrier and the drug, we calculate that the efficiency of ketoprofen–carrier association exceeds >95%, which is much above the ~55% measured with the original preparation (cf. Fig. 2). The free drug concentration in a semi-dry preparation simulating Diractin® situation on the skin is therefore ≤5%! The experimentally determined 90% drug release-time for the original suspension should therefore decrease with the carrier concentration, and vice versa.

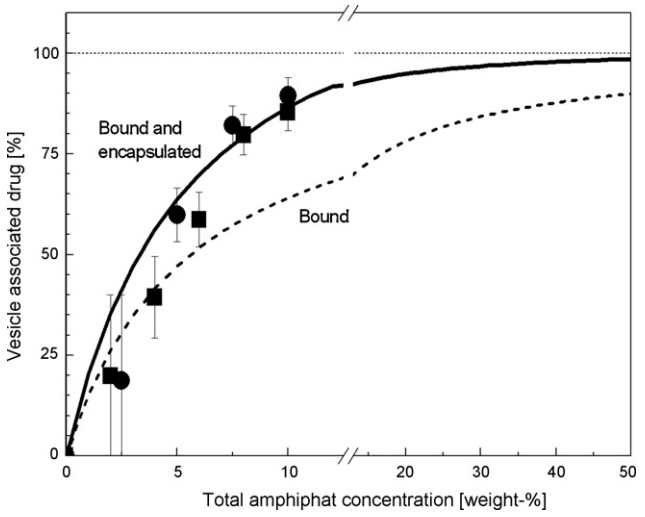

Fig. 2. Ketoprofen association with the mixed lipid vesicles comprising phosphatidylcholine and polysorbate as a function of total amphiphat concentration, which approaches total carrier weight with increasing concentration. The curve was calculated without adjustable parameters within the framework of the model that allows for the drug binding to and encapsulation into the carrier vesicles, using an independently measured "binding constant" and vesicle size information as input parameters.

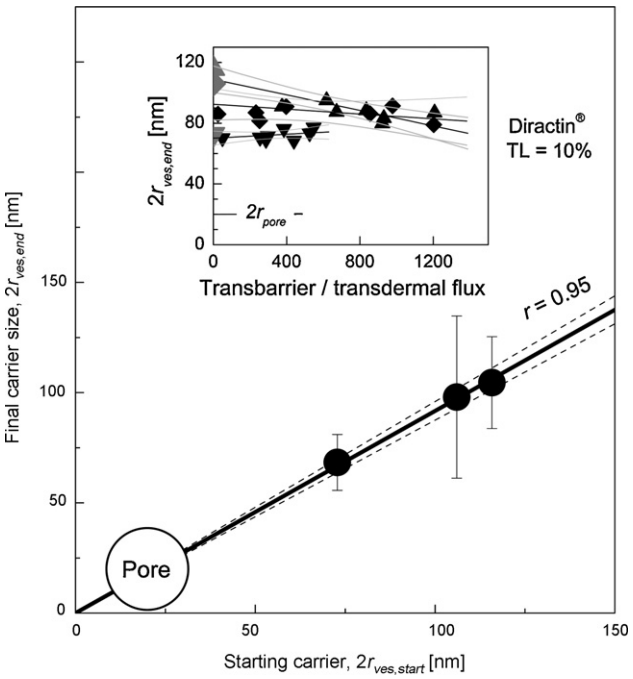

Fig. 3. Size (in)dependency of the vesicles used in Diractin® after enforced passage through narrow pores, measured with different test formulations. ($r_{ves,start}$ = starting vesicle radius; $r_{ves,end}$ = final vesicle radius after the crossing of a pore with radius r_{pore}). The average vesicle size is moderately sensitive to the shear-stress experienced by a vesicle inside a pore, but remains essentially the same in case of practically important, slow carrier motion through a pore. (Full line: linear regression fit; dashed lines: 95% confidence range; error bars: 95% confidence range of the linear extrapolation made with the data shown in the inset for relative flux ~0.) Inset: the original, measured data.

Drying-promoted drug association with the carrier also diminishes Diractin® sensitivity to the average vesicle size in the formulation. This notwithstanding, it is important to maintain the selected, relatively large, carrier size, as this is necessary to overcome the secondary skin barrier. Fulfilment of such requirement for Diractin® is corroborated by the data given in Fig. 3: the starting and final average vesicle sizes, measured before and after filtration, are not significantly different. The proviso is that vesicle transport rate is as small (rel. rate ~0) as during skin crossing. The same data moreover vindicate the claim that the carriers from Diractin® trespass narrow pores intact: the good correlation (r = 0.95) between the starting and final vesicle sizes shown in Fig. 3 proves this. The result only changes when flow-rate through pores – and thus the shear-force acting on a vesicle (Cevc et al., 2003) – is much increased. Some vesicles are then broken, the bigger their starting size the more so.

Insertion of various semi-permeable capillaries, flushed with a buffer, into solutions or suspensions with different ketoprofen and carrier concentrations creates drug and carrier concentration gradient across the capillary wall. Ketoprofen molecules and carriers then strive to enter the capillary volume, along their respective concentration gradients. The final drug and carrier concentrations are therefore finally equilibrated throughout the system, when the drug and the carrier size are smaller than the average pore size in capillary wall. Fig. 4 confirms that. If such condition is not met, however, no transport into the capillary is possible. The size-dependent exclusion from the capillaries with a constraining cut-off, i.e. with pores narrower than the average aggregate diameter, leads to the carrier filtration, as is also illustrated in Fig. 4.

We performed similar experiments with non-occluded skin *in vitro*. Ketoprofen molecules then diffused spontaneously from the tested conventional gels through the stratum corneum into epidermis and dermis, the latter two together with the washing buffer forming the receiver compartment (cf. Fig. 5, right panel).

The following check of the total transported drug amount revealed that the (free) drug permeation, i.e. diffusion from ultradeformable carriers through the skin, is rather inefficient; low cumulative amount of transported ketoprofen in the receiver compartment corroborates the conclusion (cf. Fig. 5, right panel and Table 2). We repeated experiments with several conventional topical NSAID formulations and always found that ~0.6–1.6% of the total applied drug had crossed the skin within 24 h, which is in the range of previously published values for similar formulations (see lower part of Table 2). For the carrier-based gel, the absolute value was much lower ~0.05%, corresponding to <0.1% in terms of the free drug concentration.

Reassuringly, the skin permeability to ketoprofen from Diractin® appears to be less variable than for the two closely related commercial comparators, Gabrilen® and Fastum® gel, as judged from relatively good reproducibility of the experiments with the former,

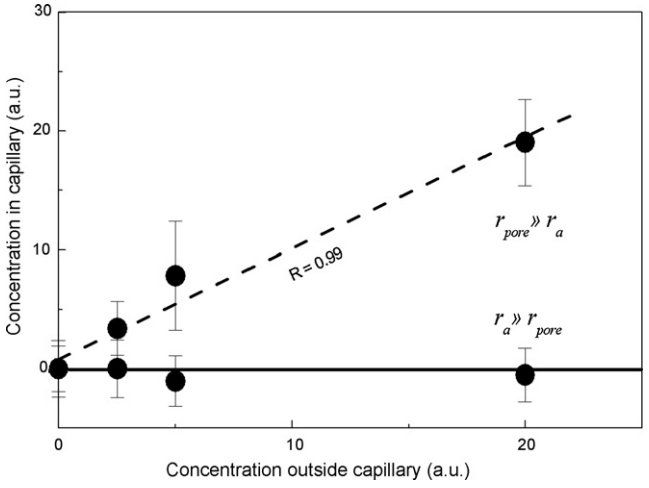

Fig. 4. The size-dependent filtration of the ultradeformable vesicular carriers by different nano-porous capillaries, simulating blood vessels *in vitro*. r_a = aggregate or agent radius; r_{pore}: pore radius.

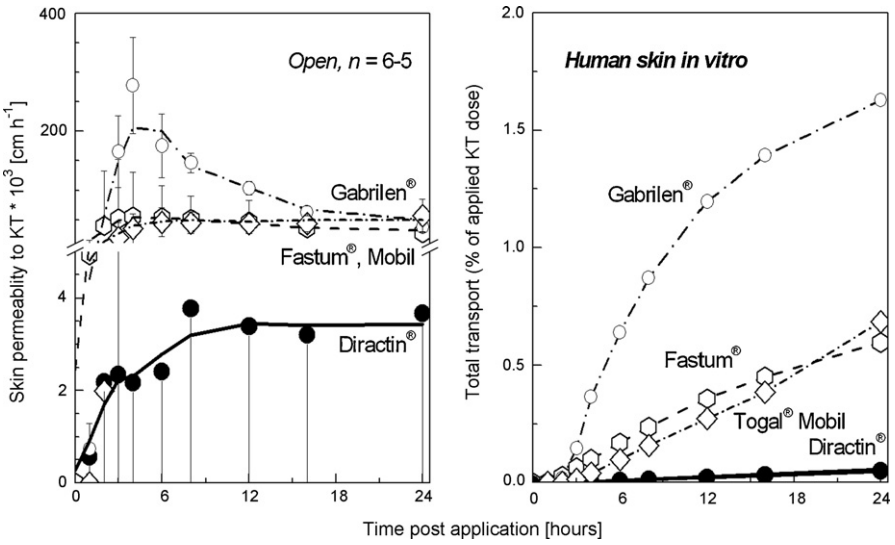

Fig. 5. *Left panel*: Human skin permeability to ketoprofen (KT) measured *in vitro* with two commercial formulations (Gabrilen® gel and Fastum® gel) and a product (Diractin®) based on ultradeformable carrier (Transfersome®). Symbols give the average and standard deviation of 6–5 independent measurements. Curves for conventional gels give the result of three nearest neighbour averaging. For Diractin®, the mean of two such averages is shown, pertaining to two independent experiments (same symbol size = same experiment). *Right panel*: Cumulative amount of ketoprofen ("total transport") in the receiver compartment under the skin "covered" with the tested formulations without occlusion. The results are derived from the same data set as given in the left panel, but are corrected for the different *nominal* drug concentrations in each formulation.

Table 2
Transcutaneous ketoprofen diffusion measured *in vitro* with various drug formulations applied on skin in non-occluded Franz-diffusion cells

	Prod. conc. (mg mL^{-1})	Cumulative amount[a]		Trans-barrier (at t = 24 h)	
		Receiver (μg cm^{-2})	Normalised (% of applied)	Flux density (μg cm^{-2} h^{-1})	Permeability[b] ($\times 10^{-3}$ cm h^{-1})
Original data					
Diractin®	22.9	0.08	0.049	0.084 × (1 ± 1.14)	3.67 (3.46)[c]
Diractin®	22.9	0.09	0.057	0.085 × (1 ± 0.19)	3.69 (3.46)[c]
Fastum® gel	25.0	0.69	0.598	0.703 × (1 ± 0.66)	28.10 (53.70)[c]
Togal® Mobil gel	25.0	1.40	0.686	1.432 × (1 ± 0.44)	57.27 (49.53)[c]
Gabrilen® gel	25.0	0.92	1.628	0.939 × (1 ± 0.19)	37.55 (206.28)[c]
Reference data					
H_1 "Gabrilen®" gel[d]	25.0	27.3 ± 8.5			∼2.2
H_2 Orudis	25.0	9		0.2	(∼8)
H_3 Ketum® gel	25.0		∼8	5.1 ± 2.4	(202)
H_4 Var. emulsions	0.1			∼1.5	∼15
H_5 Hydrogel	10.0			∼2.4	(240)
H_6 O/W emulsion	10.0			∼1	(100)
H_7 PC emulsion	10.0			∼4.5	(450)
R Microemulsions	30.0			8.6	(286)
M_1 Drug solution	71.8			91.1 ± 14.9	1269 ± 208
M_2 Oleo-hydrogel	30.0	4.7		29.6 ± 2.7	9871 ± 897
M_3 K-plaster	30.0	0.9		8.0 ± 0.5	2663 ± 160
M_4 K-gel	30.0	1.3		2.7 ± 0.2	887 ± 70

H_1: heat-separated human skin; 32 °C, 666 μL cm^{-2} (Bock et al., 2004), H_2: full thickness human skin; up to ∼30 °C, 3 μL cm^{-2} (Loden et al., 2004), H_3: dermatomed human skin (thickness: 400 μm); 32 °C, 4.8 μL cm^{-2} (Vincent et al., 1999), H_4: heat-separated pre-treated, occluded, human skin, pH 2.2; 566 μL cm^{-2} (Jaeckle et al., 2003), H_5: heat-separated, occluded, human skin, pH 7.4; 200 mg cm^{-2} (Paolino et al., 2005), H_6 and H_7: heat-separated, occluded, human skin, pH 7.4; particle size ∼30 nm, 200 mg cm^{-2} (Paolino et al., 2005), R: Full thickness dorsal rat skin; the product contained 6% oleic acid; 37 °C, 1130 μL cm^{-2} (Rhee et al., 2001), M_1: full thickness dorsal mouse skin; drug solution in 1/1 water/EtOH, 37 °C, 3888 μL cm^{-2} (Kommuru et al., 2000), M_2: full thickness murine skin; pH 6.0, 37 °C, 14 μL cm^{-2} (Rhee et al., 1999), M_3: full thickness murine skin; commercial product of P Company Korea; 37 °C, 14 μL cm^{-2} (Rhee et al., 1999), M_4: full thickness murine skin; commercial product of I Company Korea; 37 °C, 14 μL cm^{-2} (Rhee et al., 1999).

[a] Measured over 24 h permeation period in two independent experimental series (first Diractin® and Gabrilen®; second Diractin® and Fastum®; third Togal® Mobigel).

[b] (Barrier) Permeability coefficient = Flux/Product concentration (calculated from averaged curves). For reference data: dosing assumption 1 μL = 1 mg of formulation. Values in brackets calculated by GC.

[c] Maximum value (in brackets) derived from the (three nearest neighbours) averaged curves, in the case of Diractin® using both data sets together.

[d] Gabrilen® is now being sold as Phardol®.

carrier-based formulation compared with conventional topical gels (see the large and small bullets in Fig. 6).

The skin permeability coefficient for ketoprofen[2], derived from the data given in Fig. 5, is time-dependent (cf. Figs. 5 and 6). It increases during the first 3–4 h post-application for the conventional gels and over 6–8 h for the carrier-based formulation. After an epicutaneous Diractin® application the calculated permeability value settles finally around ∼3.5 × 10^{-3} cm h^{-1}.

In case of an epicutaneous Fastum® gel application, the skin permeability coefficient for ketoprofen remains fairly constant during 3 < t (h) < 12 time period, at around

[2] Permeability = flux/area/concentration gradient.

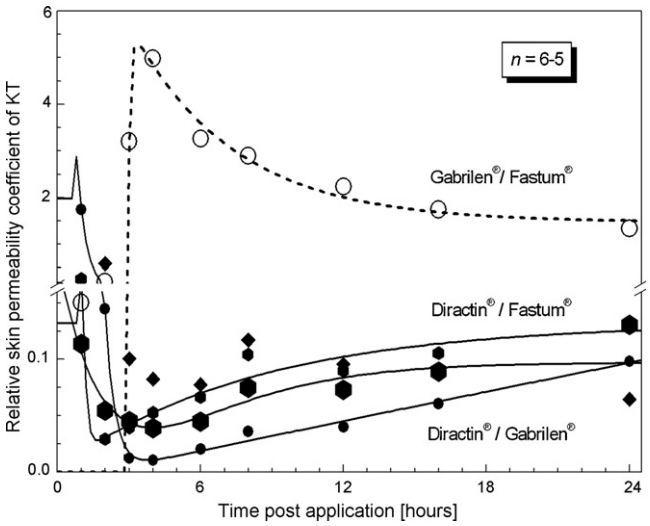

Fig. 6. The time-dependency of the apparent skin permeability coefficient for ketoprofen from conventional gels (Gabrilen® vs. Fastum® gel, open circles) or for the formulation containing ultradeformable vesicle, Transfersome®, carriers (Diractin® vs. Fastum®, closed diamonds of two different sizes for two separate experiments; Diractin® vs. Gabrilen®, small bullets). The fitted data were modelled by assuming several additive exponential permeability changes, plus a constant final value, and then assigning suitable values to the model parameters.

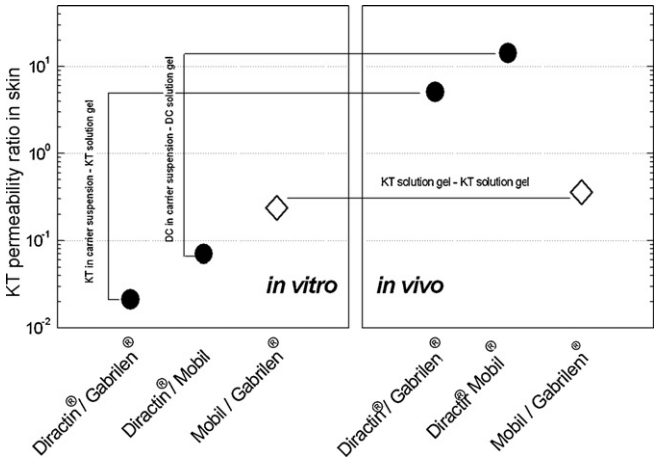

Fig. 7. Relative permeability of ketoprofen (KT) in human split thickness skin *in vitro* (at $t = 24$ h) and in porcine skin *in vivo* (at $t = 1$ h), as determined from the corresponding drug concentrations in superficial subcutaneous muscles.

5×10^{-2} cm h^{-1} sc in Psc is an index. The Psc but then decreases marginally during the following 12 h, to a final value of $\sim 3 \times 10^{-2}$ cm h^{-1}. Following an epicutaneous Gabrilen® gel application, the skin permeability coefficient or ketoprofen reaches a peak after 4–6 h at around 2×10^{-1} cm h^{-1}. The final drug permeability is $\sim 3.8 \times 10^{-2}$ cm h^{-1} at $t = 24$ h, and thus practically the same as for the Fastum® gel.

To highlight temporal the skin permeability or epicutaneous drug concentration changes, which influenced our *in vitro* ketoprofen transport measurements, we derived several relative permeability ratios given as function of time in Fig. 6.[3]

The time-dependency of ketoprofen transport through the skin is *qualitatively* different for the drug diffusing through the skin from a solution- or a vesicle-based gel. According to Fick's law (see Eq. (1)), or its generalisation (Cevc, 2003), the observed difference can only reflect the time-dependent skin permeability or (free) drug concentration changes on the skin.

The permeability coefficient ratio calculated for Gabrilen® gel shows a pronounced *maximum* (open symbols in Fig. 6). We believe that this is caused by the strong initial effect of the skin permeation enhancers included in Gabrilen® on $P(t)$ function. As enhancers are gradually diluted-out or else evaporate from skin surface, the difference between various tested conventional ketoprofen gels finally vanishes. Fastum® gel exhibits a smaller maximum, if any (cf. Fig. 5), but also starts with relatively low initial permeability coefficient value. The Togal® Mobil Gel with ketoprofen behaves similarly to Fastum®, and is characterised by the slowest skin permeability increase with time.

The uppermost curve in Fig. 6 illustrates the concept comparing ketoprofen diffusion and skin permeation enhancement by Gabrilen® with that of Fastum®, all in relative terms: after a lag-time of approximately 3 h, relative skin permeability first rapidly ($t_{in} \sim 0.1$ h) reaches a steep *maximum* that disappears with time ($t_{out} \sim 3$ h). The values given in Fig. 6 should not be taken too strictly, however, due to limited resolution of the existing data.

The calculated time-dependency of Diractin®/Fastum® apparent permeability ratio, to the contrary, shows a *minimum* in apparent skin permeability to ketoprofen ($t_{in} \sim 2$ h; $t_{out} \sim 2$–5 h; cf. Fig. 6). This minimum has different, and more complex, origin than the maximum observed with conventional topical ketoprofen gels. We believe that the starting negative slope is a consequence of gradual Diractin® drying on the skin. This increases total amphiphat concentration on the organ and, in parallel and consequently, diminishes the free drug concentration there, as is shown more directly in Fig. 2. The second, increasing part of the curve is indicative of the treated skin barrier decrease, which can have several reasons. First, one must realise that Diractin® contains less alcohol than either Fastum® or Gabrilen® gel; second, one must consider that the observed increase commences when the alcohol-induced permeability increase for conventional gels is already in decline; third, one should keep in mind the mechanism of skin barrier entering and crossing by an ultradeformable carrier. (This mechanism, arguably, involves widening of inter-cellular junctions to create sufficiently broad *hydrophilic* pathways in the skin, which then can accommodate the deformed vesicle (Schätzlein and Cevc, 1998; Cevc et al., 2003; Van den Bergh et al., 1999a,b)). It further stands to reason that the dissolved drug molecules can diffuse along the newly opened pathway and then contribute to overall efficacy of transcutaneous ketoprofen transport.

As for conventional gels, we have also modelled the resulting time-dependency of skin permeability for the drug from Diractin®. This revealed the drying time to be <3 h and the general hydrophilic pore broadening time to be of the order of 5 h.

It is close to impossible to conduct absolutely reliable skin permeability measurements *in vivo* for variety of reasons. We therefore reverted to the short-term (up to 1 h) muscle biodistribution data, taken from a wider data set (Cevc et al., 2008a,b), to estimate living skin permeability to ketoprofen and penetrability to the drug-loaded carriers at least semi-quantitatively. The resulting lower limits for *in vivo* permeability coefficient and its ratios are given in Fig. 7. Both are strikingly different for the conventional and the carrier-based formulations *in vitro* and *in vivo* (see bold numbers in Table 3); the intramuscular drug concentrations measured at later time points (Cevc et al., 2008a,b) confirm this discrepancy. In contrast, the ratios calculated for various conventional gels compared *in vitro* and *in vivo* are always similar.

[3] We repeated experiments with Diractin® twice: once together with Gabrilen® and once in the course of the Fastum® gel experiments; the results from both data sets are included in Figs. 5 and 6, and shown as different size bullets.

Table 3
Relative ketoprofen delivery efficiency from various products *in vitro* or *in vivo*[a]

	Skin permeability relative value/(*in vivo*: lower limit)			Cumulative normalised concentration	
	$t = 1$ h	Maximum	$t = 24$ h	$t = 24$ h	Mean
In vitro, open					
Diractin®/Gabrilen®	1.74	0.02	0.10	0.04	0.05
Diractin®/Fastum®	0.07	0.06	0.13	0.09	0.09
Diractin®/Togal® Mobil gel n.a.	0.07	0.06		0.08	0.07
Fastum®/Gabrilen®	20.24	0.26	0.75	0.37	0.46
Fastum®/Togal® Mobil gel	n.a.	1.11	0.49	0.87	0.82
In vivo					
Diractin®/Gabrilen®	5.09				
Diractin®/Togal® Mobil gel	14.23				
Togal® Mobil gel/Gabrilen®	0.36				

KT *in vivo*/KT *in vitro*	Mean	$t = 1$ h/(maximum; $t = 24$ h)
In carriers/in gel 1	**151**	242; 60
In carriers/in gel 2	**422**	678; 167
In gel 1/in gel 2	**1**	1.4; 0.6

[a] (Non-rounded) Starting values taken from Table 2.

Total efficiency of transcutaneous ketoprofen transport measured with pigs *in vivo* (cf. Table 3) reflects the different mechanism of ketoprofen delivery from epicutaneously administered ultradeformable carriers (Diractin®) or conventional gels (Gabrilen®, Togal® Mobil Gel). The drug concentration in the superficial muscle tissue, which acts as a voluminous primary receiver compartment, is ≥5–10× higher for the carrier-based formulation compared with Gabrilen® gel during the first hours after epicutaneous application (see also Cevc et al., 2008a,b).[4]

Ketoprofen concentration measured in deeper muscles below the drug-treated skin does not change significantly with the epicutaneously applied drug amount in a conventional gel. Applying more drug molecules on the skin in Transfersome® carriers raises the peripheral drug concentration markedly, however. This supports our notion that the drug is transported in differently fashion from the conventional gels and the formulations based on ultradeformable carriers.

4. Discussion

Results given in Table 2 leave no doubt that ketoprofen delivery through the skin from a conventional gel (Gabrilen® gel; Fastum® gel; Mobil Gel) or the gel (Diractin®) containing ultradeformable carrier (Transfersome®) are different. To comprehend the difference, a clear picture of what happens to the various formulations *on and in* the skin is needed.

Fate of diverse colloids after an epicutaneous application was reviewed in detail (Cevc, 2004). For example, it is clear that small, compact aggregates (micelles) are good skin cleansing agents whereas solid or gelled composites form topical (drug) depots. Large and compact particles or relatively stiff vesicles, such as conventional gel-phase liposomes, are therefore confined to skin surface or to the outermost skin barrier layers (Lasch and Bouwstra, 1995; Van den Bergh et al., 1999a; Cevc, 2003). This explains why such particles cannot directly mediate transcutaneous drug delivery (Cevc, 1996). The more elastic "fluid liposomes"[5] can penetrate to the end of stratum corneum (Van den Bergh et al., 1999a,b; Honeywell-Nguyen et al., 2004) and most deformable and sufficiently stable vesicles even cross the skin intact (Cevc, 1996; Cevc et al., 2003). Such vesicles are consequently suitable for non-invasive intra- (Fesq et al., 2003; Cevc and Blume, 2004) as well as transdermal (Cevc and Blume, 2001; Cevc et al., 1998; Cevc, 2003) drug delivery.

The sigmoidal penetrability vs. driving force curve, as given in Fig. 1, is diagnostic of the high and variable vesicle deformability (Cevc et al., 2003). The carriers in Diractin® are thus demonstrably unusually adaptable and can adjust their properties to ambient stress. We have earlier explained this ability with the aggregates' bilayer capacity to self-regulate, especially by adjusting local composition to the pressure-induced vesicle shape transformations (Cevc, 1995). The latter can be imposed on the carrier by any kind of an anisotropic external force, or simply by a pore in the skin barrier.

Less deformable vesicles yield lower adaptability parameter values than proper Transfersome® carriers (Cevc, 1995; Cevc et al., 2003). Simple conventional liposomes, for example, are 10–100× less elastic (Cevc et al., 2003), have correspondingly lower a^*-value, and therefore rather break in (Frisken et al., 2000) than adjust their shape to a narrow pore. The need to use relatively high driving pressure to push the buffer-diluted carriers from Diractin® through a semi-permeable barrier with pores >4× narrower than the average carrier diameter is thus a sign of diminished vesicle adaptability in the diluted preparation compared to the original carriers from Diractin®. The conclusion is vindicated by lack of similar deterioration – actually, by nearly complete preservation of the original vesicle adaptability – after the vesicles dilution with a buffer comprising enough ketoprofen and polysorbate to preserve the original carrier composition.

Total carrier concentration effect on the free drug concentration (Fig. 2) is more trivial. It mirrors progressive binding of the drug to the vesicle bilayer surfaces with increasing carrier, and thus diminishing solvent, concentration. The starting increase of total vesicle-encapsulated fluid volume plays only a smaller role.

The data in Fig. 4 confirm that the carriers diluted from Diractin® cannot enter artificial capillaries with a 3 MDa molecular cut-off (see the horizontal line in Fig. 4). The non-fenestrated blood capillaries, such as those found in the skin, have an even more restrictive cut-off (~10 kDa ≪ 3 MDa), ensuring the vesicles to stay outside the blood capillaries. The ultradeformable carriers thus face a similar situation in a living skin tissue as other large particles that, are confined to the extra-capillary space. Subcutaneously injected liposomes, for example, do not cross blood capillary walls and

[4] In the study which will be published separately, we discovered that the difference grows with time for superficial muscle, but is more constant at all times in deep muscles below the skin (Cevc et al., 2008a).

[5] The term is sometimes used as a synonym for Transfersome® vesicles.

are consequently cleared from the site of injection only via lymphatic capillaries (Allen et al., 1993) which offer large fenestrations suitable for intravasation. Small entities avoiding blood capillary cut-off, in contrast, easily diffuse into cutaneous blood vasculature. This allows, first, equilibration of the free drug concentration between the outside and inside of a capillary and, second, rapid drug wash-out from the site of application (see the upper line in Fig. 4). Dramatic reduction in concentration of a locally applied drug (Morgan et al., 2003) and its diversion (Boutsiouki et al., 2001) into systemic blood circulation (Monteiro-Riviere et al., 1993; Singh and Roberts, 1994a; Higaki et al., 2002) are two practically important consequences of this.

Fig. 4 thus highlights the central problem of all previous attempts to improve drug targeting into deep peripheral tissue by merely enhancing NSAID diffusion through the primary skin barrier. After some time, in the best case, the skin permeation enhancement can help bringing the peripheral and systemic NSAID concentrations to a similar level, differential partitioning effects excluded. In the worst case, the drug is fed predominantly into the systemic blood circulation.

The conclusions made in previous paragraph apply to all conventional methods for improving topical drug delivery, including use of better diffusing drugs, prodrugs, ion-pairs or supersaturated drug solutions, eutectic systems or complexation, modification of drug transport by better stratum corneum hydration, or action of chemical enhancers on stratum corneum structure and components (Benson, 2005).

Ketoprofen permeability through artificial lipid bilayers, which simulate the stratum corneum, correlates well ($r = 0.9868$) with the drug's permeability in heat-separated epidermis (Jaeckle et al., 2003). Increasing the skin lipid's fluidity and/or bilayers permeability thus promotes transcutaneous ketoprofen diffusion. Ceschel et al. (2002) have tested the phenomenon by studying ketoprofen transport through porcine skin *in vitro*.

For the purpose, they have used different saturated drug solutions supplemented with various co-solvents. The latter did not influence ketoprofen diffusion into the stratum corneum but affected the drug's partitioning into the skin. (In particular, the partition coefficient, and thus the permeability coefficient, of ketoprofen is inversely proportional to the drug's solubility in the matrix.) The co-solvents that increase ketoprofen solubility also decrease the drug's partitioning into the skin, and vice versa. Both factors together may enlarge the skin permeability coefficient for ketoprofen above that of the formulations containing fewer or less effective solvent molecules (cf. Table 2).

The information provided in previous paragraphs lends credence to our interpretation of the data given in Figs. 5 and 6: the non-monotonous and extremely formulation-sensitive time-dependency of skin permeability to ketoprofen (cf. Fig. 6) mirrors ketoprofen solubility and/or skin permeability changes. We modelled such changes with a simple mathematical formula and obtained good results, which are helpful to study and describe the drug uptake through skin *in vivo* (Cevc et al., 2008a,b).

Simply put: the initial relative increase and subsequent decrease of ketoprofen diffusion through the skin covered with Gabrilen® gel is most probably due to the transient effects of alcohol on the skin permeability barrier, as were observed by Trottet et al. (2004) with several loperamide formulations. The initial permeability increase is in either case a sign of progressive alcohol partitioning into the lipidic skin matrix. The ensuing permeability decrease is due to alcohol disappearance from the skin, either by way of evaporation or wash-out into the receiver fluid.

On the other hand, the initial, apparent carrier-mediated ketoprofen permeability decrease, which we observed with Diractin®, is arguably caused by decreasing free drug concentration during the product drying on the skin, as this promotes ketoprofen–carrier association (cf. Figs. 2 and 6). Subsequent, approximately 10-times smaller, drug transport increase could be due to the carriers-caused skin permeability barrier decrease, as observed with some other locally applied elastic vesicles (Van den Bergh et al., 1999a). A combination of both phenomena can account for the entire time-dependency of transcutaneous ketoprofen transport from Diractin® observed *in vitro*. In particular, the initial 26- to 95-fold apparent permeability decrease is a sign of the increased drug–carrier association, from the original 55% to the final ~96–98% (cf. Fig. 2). Ketoprofen diffusion through a non-occluded split human skin *in vitro* from the ultradeformable vesicles contained in Diractin®, in the extreme at $t \sim 4$ h, consequently *must* amount to ≤4% of the value measured with the conventional gels under similar conditions (cf. Fig. 6).

With a few unclear exceptions (Valenta et al., 2000), direct or indirect NSAID association with different lipid formulations, such as phospholipid organogels (Dowling et al., 2004) or conventional liposomes (Nishihata et al., 1987; Maestrelli et al., 2005), as well as other (nano)particles (Ricci et al., 2005) was found to diminish the apparent rate of drug diffusion across the skin. This is another manifestation of the free drugs concentration decrease, as illustrated for Diractin® in Fig. 5. Inability of the previously tested ketoprofen loaded particulate "carriers" to reproduce the second part of the permeability vs. time curve measured with Diractin® (cf. Fig. 6) suggests that the particulates that are far deformable than the Transfersome® vesicles cannot penetrate the stratum corneum. Such particles therefore cannot increase the width/number/penetrability of transport pathways in cutaneous barrier.

Relatively slow onset of drug diffusion from Diractin® gel (cf. Fig. 5), and the initial *decrease* of apparent skin permeability (cf. Fig. 6), proves that the product does not fluidise or extract skin lipids appreciably; if it did, the skin permeability coefficient would have to get higher (Karande et al., 2005) rather than lower shortly after an application (cf. Fig. 6). Time-dependency of ketoprofen permeability increase would then also have to be similar for Diractin® and Gabrilen®, as the latter has arguably such an effect on skin lipids. In reality, the only skin modifying effect of the carriers implied by our results is the secondary increase of *in vitro* skin permeability. The observed carrier effect on skin barrier is 10-times smaller than the permeation enhancement by the alcohols included in conventional topical NSAID formulations, and might be due to amphiphats/vesicles effect(s) on skin barrier.

5. Conclusions

Ketoprofen molecules associate with the mixed amphiphat vesicles used in Diractin® as carriers. The drug binding to the carrier depends on several factors, especially on total amphiphat concentration. Upon non-occlusive epicutaneous application of Diractin®, this concentration increase upon, partial suspension drying. As a result, more than 95% of the total applied drug amount is either bound to or encapsulated in to the carrier vesicles present on an open skin surface.

Ketoprofen carriers used in the Diractin® product are very deformable and capable of overcoming, without irreversibly changing their size, constrictions in a nano-porous barrier. This is true also when the pores have several times smaller diameter than the drug carriers. The flux vs. driving pressure curve, which characterises pore penetration by the carrier vesicles, confirms the self-regulating ability (stress responsiveness) of the carriers. This ability is lost upon strong dilution of Diractin® *in vitro* and for the same reason arguably in a living tissue.

Ketoprofen from a topically applied conventional gel crosses the skin as free drug, i.e. in the dissolved form, *in vitro* as well as *in vivo*. The drug's diffusion is relatively inefficient *in vitro* (0.7–1.6%) and arguably *in vivo*. In contrast, the drug from the ultradeformable carriers is 10× less prone to cross the skin *in vitro*, but stands at least one order of magnitude higher chance to overcome the skin barrier *in vivo*. The difference can be traced back to the carrier–drug association and the carrier-mediated drug transport across the organ. The initially small apparent skin permeability coefficient for ketoprofen from Diractin® is therefore explained easy: the low drug permeability simply mirrors the low free drug concentration (<5%) in presence of the partially dried ultradeformable carriers on a non-occluded skin.

Scrutiny of ketoprofen transport data from different formulations on skin *in vitro* suggests that the carriers employed in Diractin®, over time, can activate/widen hydrophilic pathways in the skin for the carriers. The final rate of *diffusive* drug transport across the skin *permeability barrier* from the vesicles is therefore also increased, but approximately 10-times less than by the alcohols included in conventional topical NSAID gel products. The resulting net effect on ketoprofen diffusion from Diractin® is always small, however, mainly due to low free drug concentration in the partially dehydrated product on open skin.

In vitro/*in vivo* results comparison furthermore implies that the ultradeformable carriers from Diractin® unusually improve ketoprofen transport through a normal skin. The resulting gain in local drug delivery in living pigs can reach two orders of magnitude, on the average. The best explanation for the extra contribution to the carrier-mediated drug transport is the spontaneous skin *penetrability barrier* crossing by the drug-loaded ultradeformable vesicles. Ketoprofen delivery through a living skin with such carrier vesicles therefore can dramatically increase the drug's concentration in deep subcutaneous tissue, which is practically impossible with the free drug. The carrier's ability to bypass cutaneous capillaries, which normally act as a local sink, plays a key role in this. If this were not the case, most of the epicutaneously applied drug quantity would be cleared near the very skin surface.

Ketoprofen transport studies yield comparable results *in vitro* and *in vivo*, when the drug transport is based on diffusion. This is the case when the drug is applied on skin in a conventional gel. In contrast, Franz-cell experiments are useless for testing the formulations that contain carriers capable of non-diffusive penetration of skin barrier. The *in vitro* and *in vivo* results for the latter kind of formulation can differ by two orders of magnitude.

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