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Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations

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ABSTRACT

We studied skin occlusion effects *in vitro* and *in vivo* on local and systemic delivery of ketoprofen across the organ, using the drug in a conventional non-occlusive topical gel (Togal® Mobil-Gel), an occlusive tape (Mohrus®), and the new targeted analgesic (Diractin®), comprising ultradeformable, hydrophilic carriers in the form of a Transfersome® vesicle. *In vitro* occluded skin permeability to ketoprofen from the tape (0.086 cm h^{-1}) marginally *exceeds* the value for the drug from carriers in a gel (0.058 cm h^{-1}), which resembles conventional gel on open excised skin (0.057 cm h^{-1}); smallness of occlusion-induced permeation enhancement (∼1.5×) may be due to the high tested applied dose. In contrast, open skin permeability to the drug from the carriers *in vitro* is ∼15× *lower* (0.004 cm h^{-1}). The benefit of ketoprofen association with the carriers for targeted transcutaneous delivery only shows-up *in vivo* after an non-occlusive epicutaneous application: the area under the curve (AUC) in peripheral deep muscle for the carrier-based gel then exceeds AUC for conventional gel ∼35-fold. The AUC for occluded ultradeformable, hydrophilic carriers measured in living pigs is conversely ∼10× lower, being 1.4–2.2× below that of the tape that is inferior to non-occluded carriers formulation (normalised c_{max}: ∼200×). Occlusion thus disables ultradeformable, hydrophilic carriers by eliminating transcutaneous hydration gradient that normally drives the carriers across the skin. Compared with other non-steroidal anti-inflammatory agents (NSAIDs) for local usage, Diractin® is thus evidently well differentiated and innovative.

1. Introduction

Drug molecules normally rely on their concentration gradient (*chemical driving "force"*) to cross the skin barrier. The transport involves molecular diffusion (permeation) and is proportional to the gradient's magnitude and the barrier's permeability to the drug (Walters and Hadgraft, 1993; Cevc, 1997). Increasing either of the two enlarges transcutaneous drug flux and is therefore likely to improve efficacy of an epidermally applied drug.

Good pharmaceutical products designed for epicutaneous usage consequently contain the highest reasonably achievable drug concentration (Hadgraft et al., 2000). One or more skin permeation enhancers may be included as well, despite their skin irritation potential (Benson, 2005; Karande et al., 2005). To maintain optimum concentration of the drug as well as permeation enhancer(s) on the skin, the organ can furthermore be covered with an impermeable wrapping, e.g. in the form of a patch. The reason is that the organ's occlusion and hydration both enhance the skin's permeability to many molecules *in vivo*[1] as well as *in vitro* (Maibach and Hikima, 2006).

Several years ago we introduced a passive, drug concentration insensitive system for non-diffusive transcutaneous drug delivery (Cevc, 1995). The system contains aggregate vesicle (Transfersome®) with unusually high adaptability and surface hydrophilicity (Cevc and Gebauer, 2003; Cevc, 2004). These two desirable properties are a consequence of suitable amphipaths inclusion into the vesicle bilayer. The latter is very fluid, flexible, and conveniently permeable, owing to the shape-dependent and reversible vesicle components demixing (Cevc et al., 2003). Such ultradeformable vesicle therefore tends, and is capable, to follow hydration gradient ("directed osmotic stress") across the skin; why the vesicle is driven to water rather than the other way around is explained in detail elsewhere (Cevc and Gebauer, 2003).

We proposed that an application of sufficiently deformable and stable aggregate carriers in an aqueous suspension on open skin leads to the following sequence of events (Cevc, 2004). First, excess

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[1] The most notorious such enhancer is DMSO, which affects positively both partitioning and/or diffusivity in the skin but also irritates the organ and produces a smelly degradation product.

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water evaporates from the skin, increasing concentration of all non-volatile components on the organ. When the carrier vesicles reach their solubility limit the aggregates start to experience an attractive hydration gradient ("*hydrational driving force*" or hydrotaxis) across the skin barrier. This guides and drives the vesicles from relatively dry skin surface through the primary skin barrier (the *stratum corneum*) into relatively water-rich viable skin regions (Cevc et al., 2003). The large number of molecules in each vesicle, designed to migrate as an entity, magnifies the force on each carrier, which is a composite body, over that affecting single component molecules. The proviso is that the vesicle is not fragmented during the passage (Cevc, 2002).

The vesicle's high adaptability moreover lowers activation energy for trans-barrier transport (Cevc et al., 1996; van den Bergh et al., 1999; Honeywell-Nguyen et al., 2004). The corresponding osmotic pressure across the *stratum corneum* can therefore pull the deformable vesicles across the skin barrier efficiently (Cevc and Gebauer, 2003). The process continues until the vesicles reach the water-rich viable epidermis, where unidirectional osmotic "pull" ceases (Schätzlein and Cevc, 1998). The aggregates' bulkiness hampers further vesicle motion, as the aggregates' concentration gradient as well as their diffusivity are comparably small in a living tissue (Cevc, 2004). Any lipid bilayer vesicle is also too big to enter blood vessels in the skin (Allen et al., 1993).

The missing "pull" on the vesicles in the skin makes their diffusion-based re-distribution from outer into deeper skin regions unimportant. The deficiency is compensated by the "push" on the fore-running carriers exerted by the vesicles that are still in the dry part of the *stratum corneum*; intercellular fluid motion in living skin may be influential as well. The suitably applied and adaptable vesicle carriers consequently move across the skin and into body continuously as long as at least some vesicles are still left on the skin, where they continue to experience transepidermal hydration gradient (Cevc, 1996).

We have postulated and proven hydro-tactic transcutaneous motion for empty ultradeformable vesicles more than a decade ago (Cevc and Blume, 1992). To our knowledge, no similar study has been conducted with a water-soluble drug associated with an ultradeformable, epicutaneously applied carrier. This is regretful as numerous active pharmaceutical ingredients, especially non-steroidal anti-inflammatory agents (NSAIDs), were proposed to have therapeutic value after application on the skin with (Cevc and Blume, 2001; Brunner et al., 2005; Jain et al., 2005) or even without (Heyneman et al., 2000) vesicles.

Conventional topical NSAID gels, lotions, or patches contain various organic solvents (e.g. short-chain alcohols or DMSO) as drug permeation enhancers. Such excipients facilitate drug's partitioning and/or diffusivity in the skin (see footnote 1) (Walters and Hadgraft, 1993; Cevc, 1997; El-Kattan et al., 2000; Wu et al., 2001). Solvent evaporation from and diffusion into the skin can pose a problem to such products, by causing undesirable time dependency of the drug transport (Trottet et al., 2004). Most efficient skin permeation enhancers are also prone to irritate the skin (Tugwell et al., 2004).

Herein we describe the results of a comparative study of ketoprofen transport across open or occluded skin *in vitro* and *in vivo*. We compare the drug from ultradeformable vesicles contained in occluded or non-occluded Diractin® (a registered trademark of IDEA AG, previously known as IDEA-033) with ketoprofen from an open application of a conventional topical gel (Togal® Mobil-Gel, Togal Werke) or from a market leading occlusive patch of the drug (Mohrus® tape, Hisamitsu).

Our main conclusion is that occlusion increases transcutaneous drug diffusion, and thus arguably efficiency of topical drug delivery from conventional topical NSAID products; occlusion also helps free drug diffusion from Diractin(R) across the skin. In contrast, occlusion diminishes the carrier-mediated ketoprofen transport through the skin *in vitro* (where only diffusion is seen) as well as *in vivo* (where carrier transport prevails). This discrepancy proves fundamental difference between the conventional and the new carrier-based therapeutic systems.

2. Materials and methods

Test articles The modern ketoprofen formulation, Diractin® (IDEA AG, Munich, Germany) comprises ~2.3% sodium ketoprofen, associated with the highly adaptable carriers, the Transfersome(R) vesicles, carbomer, microbicides, and a very small amount of short-chain alcohols; the main carrier ingredients are soybean phosphatidylcholine and polysorbate. Togal® Mobil-Gel (Togal Werke, Munich, Germany) contains 2.5% dissolved sodium ketoprofen, ethanol, 2-propanol, carbomer, ammonia solution, and purified water; according to the manufacturer's recommendations, the product should be used without skin occlusion. To test also ketoprofen performance in a marketed product after occlusive use, we selected the first approved, and one of best established patch products, Mohrus® tape (Hisamitsu Pharmaceutical Co., Inc., Tosu, Japan); the product contains 20 mg ketoprofen (2%), menthol (>2%) and hydrogenated rosin glycerol ester (<20%) per patch (100 cm^2) comprising a rubber-based adhesive agent.

Drug diffusion in vitro was studied with a set of commercially available flow-through Franz-diffusion cells (Becker and Mager, 2000). In brief, a fixed volume of each tested product was applied on excised human abdominal skin. The latter had been sliced to thickness of ~450 μm and had an application area of $A = 0.1963\ cm^2$, which was left open or covered with an impermeable foil (i.e. was used under occlusion), as the case may be. To avoid finite dose effect, the applied volume was chosen to be rather high, 0.03 mL, corresponding to 0.687 mg or 3.50 mg cm^{-2} ketoprofen for Diractin® and 0.833 mg or 4.25 mg cm^{-2} ketoprofen for Togal® Mobil-Gel. The area dose tested *in vitro* was thus 7–8.5× above that we used in pre-clinical animal experiments (see next section). High applied dose per area consequently slowed down the tested products drying time on the skin by a similar factor of 7, at least, and in our impression by approximately one order of magnitude.

Receiver volume under the skin was flushed continuously with the phosphate-buffered saline (pH 7.4; flow rate: 0.2 mL h^{-1}). This washed the lower skin surface and provided total sampling volume of 4.8 mL over 24 h period. For each given time point, one test sample (0.2–1.6 mL, depending on the sampling period) was collected from each of the 5–6 Franz-cells employed and sampled in parallel. The samples were analysed individually with a validated HPLC assay, described further in the text, and averaged to yield a nominal transcutaneous flux value for every sampling period. By dividing the measured flux value, first, with the skin surface area (to get flux density, j) and, second, with the applied ketoprofen concentration (to get skin permeability coefficient, P) transcutaneous ketoprofen transport was characterised as a function of time (identified with the middle time point for each sampling time period). The area under flux versus time curve afforded information on the total transported drug quantity.

Drug diffusion in vivo was measured in neutered male hybrid piglets (~10 kg bodyweight) supplied by a local farm animal breeder, as is described in more detail in Radeck and Heller (1989). All the experiments were approved by the responsible committees and done in accordance with the appropriate European Community guidelines.

In brief, a catheter was first inserted into *V. jugularis* of each animal. One day later, the animal was anaesthetised (ketamine,

azaperone and propofol) and the drug in either Diractin® or Togal® Mobil-Gel was applied on the skin of its thigh. More specifically, 50 mg of the drug in either Diractin® or Togal® Mobil-Gel was spread uniformly over an area of 100 cm^2 to achieve ketoprofen area dose of 0.5 mg per cm^2 of the skin. (The dose applied pre-clinically *in vivo* was thus broadly in the range of the clinically used single doses of Diractin® or Togal® Mobil-Gel, different respective dosing recommendations (at least 50 mg versus 100 mg per knee; possible dose adjustment for other sites/areas) and frequency of daily application (b.i.d versus q.i.d.) neglecting.) To use 50 mg ketoprofen on occluded skin, 2.5 Mohrus® tapes were attached to the pig's leg, as occlusion is explicitly discouraged by the manufacturer of Togal® Mobil-Gel and of most other topical gel products.

We thus left the skin open in some and occluded it in the other experiments, as is specified further in the text. Before subcutaneous tissue sampling we always stripped the skin 30-times with an adhesive tape. (This removed the residual test material from the skin surface and the outer *stratum corneum* and simultaneously minimised danger of subcutaneous tissue samples contamination with the drug from the application site.) In the following, we excised the rest of the organ, the skin-free biopsies of subcutaneous fat, and the underlying muscle tissues. (The latter were sliced into one superficial (0–1.5 cm) and one deep muscle (1.5 – ≤3 cm) sample.) We finally froze the specimen at −20 °C and immediately stored them protected from light until the drug concentration was measured with a validated HPLC method as is described further in the text.

Shortly before each peripheral tissue sampling, blood samples were also taken through the fixed jugular catheter.

Terminal half-life of ketoprofen, $t_{1/2,a}$, for each tested tissue, was read-off directly from the linear part of logarithmic concentration versus time plot.

All statistical (paired *t*-test) and other calculations were performed with a commercial software package Origin (OriginLab, Inc., Northampton, MA, USA).

2.1. Drug concentration measurements

Ketoprofen in citrate plasma and phosphate-buffered saline samples was assessed with a previously described method (Radeck and Heller, 1989; DeGraves et al., 1996). In short, 500 μL of plasma or saline was mixed with an internal standard (25 μL fenoprofen) in 250 μL of 0.6 M H_2SO_4 and 4 mL of isooctane/isopropanol (95/5, v/v) mixture. The sample was centrifuged at 3000 rpm for 10 min, to collect the organic layer into a new test tube that was subsequently concentrated under dry nitrogen. The concentrated sample was mixed with 200 μL of eluent (sodium pH 3.2 acetate buffer/acetonitrile (50/50, v/v) for further HPLC analysis. To determine ketoprofen concentration in muscle tissues, 10–20 mg of the latter were first homogenised in 1 mL of phosphate buffer. The blend was further treated as citrate plasma. The eluent flowed (1 mL min^{-1}) through a Luna C18 pre-column and a Luna C18 150 mm × 4.6 mm analytical column at room temperature. All reagents and water were of HPLC quality. Ketoprofen peaks were detected by measuring UV light absorbance at 254 nm and were quantified based on the data calibration with an internal, analytical grade, fenoprofen standard.

3. Results

3.1. Skin occlusion effects on ketoprofen transport through skin in vitro

Results of *in vitro* drug diffusion studies are given for two different commercial preparations of ketoprofen in Fig. 1. The first

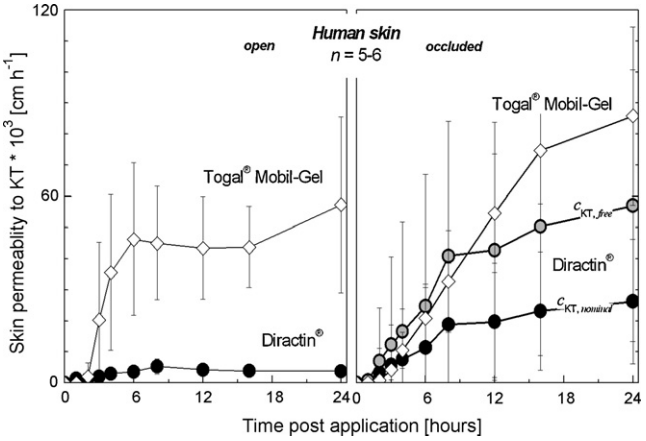

Fig. 1. Left: *in vitro* open skin permeability to ketoprofen (KT) applied superficially either in a conventional, solution-based gel (Togal® Mobil-Gel, diamonds) or associated with the ultradeformable vesicular carriers (Diractin®, bullets) *in vitro* (both $n = 5-6$). Right: results of similar experiments done with occluded skin *in vitro* (grey symbols give the results calculated with the free rather than nominal drug concentration).

data set pertains to the drug associated with ultradeformable vesicle carriers in Diractin®, and is nearly identical to that measured and reported in another study (Cevc et al., 2008), confirming good experimental reproducibility. The second data set relates to a conventional product, Togal® Mobil-Gel, comprising of the drug dissolved in a thickened hydro-alcoholic solution.

The apparent permeability values given in Table 1 were first calculated by dividing the experimental flux density with the nominal drug concentration in each tested formulation. The more realistic, "intrinsic" permeability coefficient value, $P_{sc,KT}$, was obtained by using the freely diffusable, non-bound drug concentration. According to Fig. 1 (right panel), the rate of ketoprofen diffusion across occluded skin increases with time. The apparent value for Diractin® always remains 2–3-times below that for a conventional topical gel, however (bullets in right panel of Fig. 1: $P_{sc,KT} = 0.022 \pm 0.003\ cm\ h^{-1} = 2.20 \times (1 \pm 0.15) \times 10^{-2}\ cm\ h^{-1}$ for $8 \leq t\ h^{-1} \leq 24$; Togal® Mobil-Gel: $P_{sc,KT} = 0.0717 \pm 0.0158\ cm\ h^{-1} = 7.17 \times (1 \pm 0.022) \times 10^{-2}\ cm\ h^{-1}$ for $12 \leq t\ h^{-1} \leq 24$; cf. Table 1 for the $t = 24$ h data). The discrepancy largely disappears (grey circles and diamonds in the right panel of Fig. 1) if the dissolved, "free", drug concentration is employed instead of the nominal drug concentration. For $8 \leq t\ h^{-1} \leq 24$ one then gets $P_{sc,KT} = 0.048 \pm 0.007\ cm\ h^{-1} = 4.78 \times (1 \pm 0.15) \times 10^{-2}\ cm\ h^{-1}$. The resulting skin permeability coefficient is 0.058 cm h^{-1} for Diractin® and 0.085 cm h^{-1} for Togal® Mobil-Gel (cf. Table 1).

Open skin is generally more difficult to overcome than occluded skin. Ketoprofen therefore diffuses through a non-occluded skin less well than through an occluded cutis (cf. Fig. 1, left versus right).

Time dependency of transcutaneous diffusion is broadly similar for conventional and the carrier-based ketoprofen formulations. The flux-derived open skin permeability for both such formulations starts low and then rises to a quasi-plateau after approximately 6 h. The increase is somewhat faster for Togal® Mobil-Gel than for Diractin® (cf. Fig. 1, left).

The maximum permeability of the drug diffusing from Togal® Mobil-Gel through an open skin is lower than for an occluded skin. With Togal® Mobil-Gel we measured permeability coefficient to be $P_{sc,KT} = 0.0471 \pm 0.0058\ cm\ h^{-1} = 4.71 \times (1 \pm 0.12) \times 10^{-2}\ cm\ h^{-1}$ for $6 \leq t\ h^{-1} \leq 24$ (see Table 1 for the $t = 24$ h values). The initial ($2 \leq t\ h^{-1} \leq 10$) differences between open and occluded Togal® Mobil-Gel applications *in vitro* are not statistically significant (cf. left and right panel in Fig. 1).

Table 1
Transcutaneous ketoprofen diffusion *in vitro* measured with various drug formulations applied on the skin in Franz-diffusion cells at $t = 24$ h

	Drug concentration		Cumulative transport	Permeability, $P_{sc,KT}$		$P_{sc,KT}$ ratio open/occluded
	Nominal ($mg\,mL^{-1}$)	Free ($mg\,mL^{-1}$)	% of applied	Apparent ($\times 10^{-2}\,cm\,h^{-1}$)	Intrinsic ($\times 10^{-2}\,cm\,h^{-1}$)	
Open skin						Apparent
Diractin®	22.9	(1.15)[a]	0.06	0.37 ± 0.07	(8.08)	(*7.1*)
Togal® Mobil-Gel	25.0	25.0	0.82	5.73 ± 2.83	5.73	*1.50*
Diractin®/Togal®		(0.05)[b]		*0.06*	(*1.41*)	
Occluded skin						Intrinsic
Diractin®	22.9	11.25[b]	0.36	2.62 ± 2.01	5.83	*0.72*
Togal® Mobil-Gel	25.0	25.0	0.87	8.58 ± 2.86	8.58	*1.50*
Diractin®/Togal®		*0.45*		*0.32*	*0.68*	

(Barrier) Permeability (coefficient) = Flux/Product concentration. Nomin. = nominal drug concentration. Free = dissolved drug concentration. (The derived values were calculated to third decimal point and then rounded.) Values printed in italic have no units.

[a] The estimated dissolved ketoprofen concentration in the product at the carrier solubility limit reached after partial product drying on the skin: lower limit for the free drug concentration in a Franz-cell used in this study.

[b] The free, i.e. dissolved, ketoprofen concentration measured independently (Cevc et al., 2008) with an original Diractin® preparation.

The apparent open skin permeability coefficient for ketoprofen from Diractin® is $P_{sc,KT} = 0.0037 \pm 0.0003\,cm\,h^{-1} = 3.69 \times (1 \pm 0.07) \times 10^{-3}\,cm\,h^{-1}$ for $8 \leq t\,h^{-1} \leq 24$. This value is approximately 15-times below that calculated for an occluded skin, which is 10-times greater than the corresponding $P_{sc,KT}$-ratio for Togal® Mobil-Gel and Mohrus® tape, being merely 1.5. The free ketoprofen concentration is thus evidently lowered in the presence of ultradeformable carriers, from ~50% on open skin to ~5% on occluded skin covered with Diractin® (cf. Eq. (1)).

3.2. Effects of skin occlusion on efficiency of transcutaneous ketoprofen delivery in vivo

Any drug molecule that has crossed the skin *in vivo* without degradation near the site of application must ultimately reach the systemic blood circulation. Potential differences in transcutaneous drug transport can therefore be unveiled by comparing time dependency of ketoprofen concentration in a (Franz-cell) receiver compartment with the time-course of the drug concentration changes in plasma.

Ketoprofen concentration in porcine plasma, which we determined as a function of time after a local application of either Togal® Mobil-Gel, Mohrus® tape or Diractin® on an open or occluded skin is given in the left and right panels of Fig. 2, respectively. The data for an open topical Togal® Mobil-Gel versus occlusive Mohrus® tape application reveals that skin coverage *increases ketoprofen diffusion in vivo* by approximately an order of magnitude; skin occlusion thus influences transcutaneous ketoprofen diffusion from conventional products both *in vivo* and *in vitro*.

In contrast, skin coverage *decreases* maximum rate of the *carrier-mediated ketoprofen transport* through the organ by half an order of magnitude. (The factor calculated from the maximum plasma concentrations originating from an epicutaneous Diractin® application is 6.4. The AUC based factor is 4.4.)

We also assessed ketoprofen distribution in peripheral muscles below the site of epidermal application for various test articles applied with or without occlusion. The results are given in Figs. 3 and 4 for the superficial and deep peripheral muscles, respectively, which underlay the site of the drug application.

Intramuscular ketoprofen concentrations show similar time and occlusion dependency as the drug concentrations in plasma after local ketoprofen usage. First, skin occlusion suppresses transcutaneous ketoprofen deposition from epicutaneously applied Diractin® into peripheral muscles; conversely, the drug transport from Mohrus® tape into similar tissues is higher than for an non-occluded Togal® Mobil-Gel (cf. Figs. 3 and 4). Second, covering the skin with an impermeable coating nearly abolishes depth-dependency of the drug concentration in peripheral muscles. The increase in systemic as well as peripheral drug concentrations is then nearly the same (7.1-fold versus 7.8–9.1-fold).

Ketoprofen concentrations achieved in peripheral muscles below the skin covered by a Mohrus® tape is around $0.7\,\mu g\,g^{-1}$. This is approximately one order of magnitude lower than the value reached by an open epicutaneous application of Diractin®. Occluding the latter brings both results close together, however. The intramuscular drug concentration reached by a non-occlusive Togal® Mobil-Gel application is one order of magnitude below that obtained with Mohrus® tape and two orders of magnitude below that achieved by non-occluded Diractin® (cf. Table 2). In other words, skin occlusion effectively abolishes ketoprofen transport from Diractin® through an occluded skin.

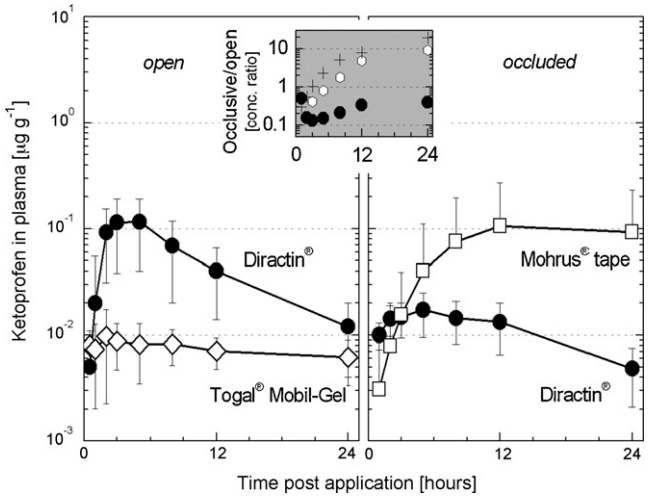

Fig. 2. Left: porcine skin permeability to ketoprofen *in vivo*, as reflected in the systemic drug concentrations measured after an epicutaneous, non-occlusive application of the drug in a solution-based, conventional topical gel, Togal® Mobil (diamonds, $n = 6$) or in the ultra-adaptable carriers present in Diractin® (bullets, $n = 6$). Right: the results measured with occluded skin to which the drug had been applied in a commercial patch, Mohrus® tape (boxes, $n = 6$) or in Diractin® (bullets, $n = 6$). Inset shows the ratio of ketoprofen concentrations in the animals treated with Diractin® on open or occluded skin (bullets) or with Togal® Mobil-Gel versus Mohrus® tape (open pentagons). Crosses give Mohrus®/Diractin® drug concentrations ratio.

Table 2
Relative efficiency of ketoprofen delivery from various products through porcine skin into deep peripheral muscles below application site *in vivo*

	c_{free} ($\mu g\,\mu L^{-1}$)	c_{max} ($\mu g\,g^{-1}$)	c_{max}/c_{free} ($\mu L\,g^{-1}$)	AUC_{0-24h} $\mu g\,g^{-1}\,h$	t_{max} h	$t_{1/2,a}$ h
Open skin						
Diractin®	*(1.1)*[a]	120	*108*	651.9	1	4
Togal® Mobil-Gel	25.0	3.5	*0.14*	18.4	3	2
Diractin®/Togal®	*(0.05)*[a]	*34.7*	*777*	*35.4*		
Occluded skin						
Diractin®	11.25	6.9	*0.6*	74.0	(3)	*7*
Mohrus® tape	20.00	9.6	*0.5*	164.4	(3 (24))	
Diractin®/Mohrus®		*0.72*	*1.3*	*0.45*		
Open versus occluded skin						
Diractin®/Diractin		*17.4*	*170*	*8.8*		
Togal®/Mohrus®		*0.4*	*0.3*	*0.1*		

c_{free} gives the nominal (for commercial products) or measured (for Diractin®) dissolved drug concentration. Values in a bracket are best estimates. Values in double bracket give the position of the tentative second peak. Values printed in italic were calculated from the 2–3 digit data points and then rounded to one digit; they also have no unit.
[a] See legend to Table 1 for an explanation; the given values were calculated from the non-rounded numbers.

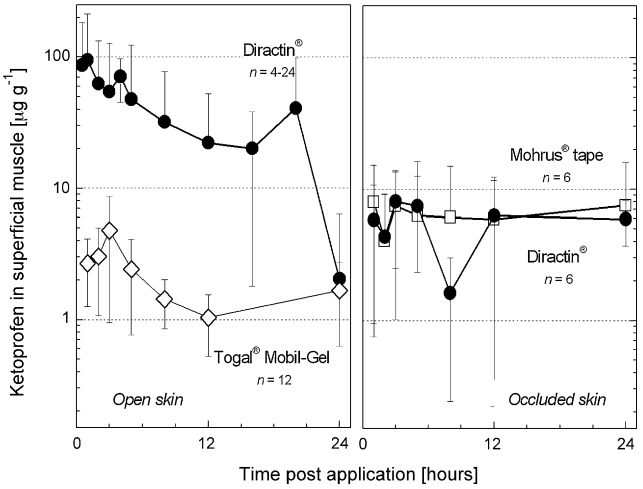

Fig. 3. Left: delivery of ketoprofen through open skin (100 cm^2) covered uniformly with a conventional topical gel (Togal® Mobil-Gel, diamonds) or a product based on ultradeformable carrier vesicles (Diractin®, bullets) into superficial peripheral muscles (0–1.5 below the skin). Right: results measured in equivalent fashion with 50 mg of ketoprofen in a commercial occlusive patch, Mohrus® tape (boxes) or occluded Diractin® (bullets).

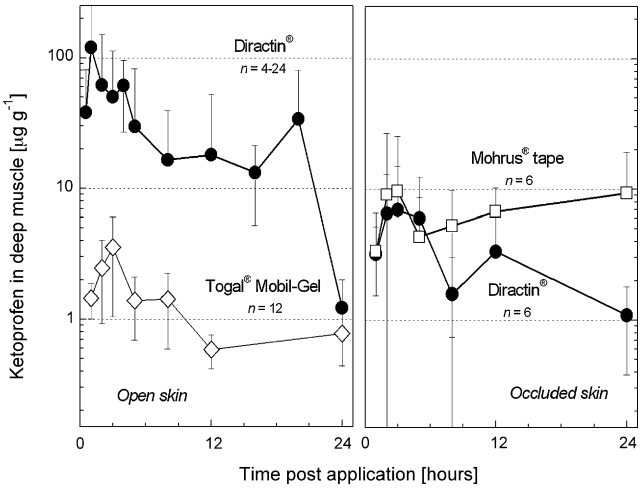

Fig. 4. Left: transcutaneous ketoprofen deposition into deep peripheral muscle (>1.5 cm below the skin surface) from a conventional topical gel (Togal® Mobil-Gel, diamonds) or a novel product comprising ultradeformable vesicles (Diractin®, bullets), applied in either case on an open skin surface. Right: the corresponding results for occluded skin covered with Mohrus® tape (boxes) or Diractin® (bullets). For more details see legend to Fig. 3.

3.3. Skin occlusion effect on kinetics of locally applied ketoprofen distribution

Experimental data scattering obscures quantitative effect of skin occlusion. The precise time dependency of ketoprofen deposition into, and the drug's clearance from, subcutaneous muscles thus remains uncertain to date. But no doubt exists that the bulk of the drug that has crossed an occluded skin barrier appears in the underlying peripheral tissues and blood later than the drug delivered through an open skin (cf. Table 2). It is moreover clear that the skin occlusion prolongs apparent "terminal elimination half-life", arguably by ensuring more continuous transcutaneous drug transport from the covered formulation.

To assess relative importance of the latter phenomenon we compared ketoprofen delivery across the skin from a commercial, Japanese market leading patch, Mohrus® tape, with Diractin® applied under occlusion. Figs. 2–4 reveal that the drug from occlusive patch distributes itself through a body with broadly similar pharmacokinetics as ketoprofen from the carrier-based occluded product. In either case, ketoprofen concentration in plasma increases gradually over many hours, followed by a rather slow drug clearance. However, the drug from the patch reaches approximately 6-times higher peak plasma concentration than ketoprofen from an epicutaneously applied and occluded ultradeformable carriers' suspension. This is also at least qualitatively expectable as the free drug concentration in Diractin® is nearly 2-times lower than in Mohrus® tape (cf. Table 2).

4. Discussion

The skin permeability coefficients given in Fig. 1, $P_{sc} = j_{permeation}\,\Delta c^{-1}$, were derived from experimental flux density data according to the Fick's first law of diffusion:

$$j_{p,permeation} = P_{sc,p}\,\Delta c_p \equiv P'_{sc,p}\,\Delta \Pi_p \qquad (1)$$

j_p represents the permeant's ("p") flux density (J_p/A) across the skin, $P_{sc\,p}$ the permeant's diffusion coefficient in the organ, and Δc_p is the transcutaneous permeant concentration gradient (any of which can be time-dependent); when Eq. (1) is used to describe the given drug transport, "p" becomes "d" or, more specifically, "KT". In the equivalent second expression on the right, the permeant's concentration is replaced by the *exogenous* transepidermal osmotic pressure difference, $\Delta \Pi_p$, caused by the permeant's, e.g. ketoprofen, concentration difference across the barrier, $\Delta \Pi_{KT} \propto \Delta c_{KT}$.

Occluded skin permeability to the drug is similar for all the systems tested in this study under quasi-steady-state conditions. This proves the validity of Eq. (1) for describing the underlying

diffusive transport. Eq. (1) thus allows the measured time-course of the drug flux to be interpreted as a sign of progressive skin barrier deterioration under occlusion with time.

Eq. (1) cannot explain occlusion-dependent lowering of the carrier-mediated drug transport across the skin, however. To highlight such phenomenon, one must therefore generalise the equation by including at least one term describing the drug-independent transcutaneous agent transport. One kind of such transport is powered by the endogenous trans-barrier osmotic pressure difference, $\Delta\Pi_{\text{endogenous}}$, which in the first approximation stems from – and is proportional to – the naturally occurring water activity difference across the skin barrier: $\Delta\Pi_{\text{endogenous}} \sim \Delta\Pi_{\text{w,endo}} \propto \Delta a_{\text{w,sc}}$ (Cevc and Gebauer, 2003). The correspondingly generalised steady-state transcutaneous flux density equation then reads:

$$j_{\text{total}} \approx P'_{\text{sc,KT}}\,\Delta\Pi_{\text{KT}} + P''_{\text{sc,Tfs}}\,\Delta\Pi_{\text{w,endo}} \quad (2)$$

Potential contributions of $P'_{\text{sc,Tfs}}\Delta\Pi_{\text{Tfs}}$ and $P''_{\text{sc,KT}}\Delta\Pi_{w,\text{endo}}$ are negligibly small, due to smallness of $P'_{\text{sc,Tfs}}$ and $\Delta\Pi_{\text{Tfs}}$ or $P''_{\text{sc,KT}}$, respectively.[2]

The first terms in Eqs. (1) and (2) are the same: they both describe simple transcutaneous diffusion of the dissolved, and thus free, drug molecules (in our study ketoprofen). The second term in Eq. (2) accounts for the skin penetration by ultradeformable carriers ("Tfs"). Eq. (2) thus clarifies that drug permeation through the skin is driven by exogenous osmosis, i.e. by the applied drug's concentration gradient. The skin penetration by the drug-loaded carriers is conversely driven by endogenous "osmosis", i.e. by the naturally occurring (negative) water concentration gradient across the skin.[3] Covering the skin can help maintain $\Delta\Pi_{\text{KT}}$ but brings $\Delta\Pi_{\text{w,endo}}$ down to zero.

Occlusion-induced suppression of transcutaneous drug transport is thus diagnostic of the carrier-mediated ketoprofen delivery in the absence of occlusion! In contrast, an occlusion-induced increase of ketoprofen transport across the skin is indicative of the drug permeation, and reflects the better preservation of Δc_{KT}, and thus $\Delta\Pi_{\text{KT}}$ (longer "duty period"), and/or the enlarged $P_{\text{sc,KT}}$ or $P'_{\text{sc,KT}}$ values. Both phenomena can contribute to the apparent superiority of Mohrus® tape over the labelled, non-occlusive Togal® Mobil-Gel. (Analysis of the results given in Figs. 1 and 2 in terms of Eqs. (1) or (2) suggests that for ketoprofen the higher skin permeability is probably more important. Otherwise, covering the drug solution on the skin would merely double the skin permeability coefficient over that measured without occlusion.)

It is impossible to assign a significant proportion of the observed permeability changes with time to a variation of the drug's transcutaneous concentration or osmotic pressure gradient; total ketoprofen amount delivered over 24 h (<2% of the applied amount, cf. Table 1) is just too small for that! Skin permeability to ketoprofen therefore arguably increases with duration of occlusion.

In the tested area dose and time range, skin occlusion enhances the drug diffusion across the skin *in vitro* 1.5 times. This is relatively small and insignificant enlargement compared with experimental error (cf. Figs. 1–3). The calculated increase is also at the low end of the expected range (≤3-times (Brunner et al., 2005) to ∼2-times (Maibach and Hikima, 2006)) and less than the ∼2-fold of the increase reported earlier for the lipophilic drug estradiol applied on the skin with "deformable vesicles" (El Maghraby et al., 1999). This, and similarity of occluded Diractin® versus conventional products data, suggests that the drug permeation enhancing effect of alcohols (Trottet et al., 2004) must be small in Togal® Mobil-Gel. Our best explanation for relatively weak occlusion effect on ketoprofen diffusion across the skin, as determined in this study, is the high applied product dose *in vitro* that mimics partial Togal® Mobil-Gel occlusion.

If one takes the apparent difference between ketoprofen transport from Togal® Mobil-Gel through an occluded or open skin for real, one could interpret it as a sign of protracted skin permeation enhancement by occlusion. The relatively slow onset of observed permeation enhancement in the occluded skin could then also explain the smallness of skin covering effect, in terms of total transported drug amount within 24 h period (cf. Table 1). Such notion is consistent with the broadly similar differences measured soon after epicutaneous applications of Gabrilen® or Fastum® gel, both relative to each other and in comparison with Togal® Mobil-Gel (data not shown; Cevc et al., 2008).

Skin occlusion moreover delays the build-up of ketoprofen concentration in plasma: in pigs the time needed to reach its maximum is prolonged by a factor of ∼2, i.e. from between 3 and 5 h for Togal® Mobil-Gel to ∼12 h for Mohrus® tape and from 3 to 5 h for Diractin® (cf. Fig. 2). Such protraction could result from a slower or less lasting drug permeation through the open skin barrier.

The same phenomenon is evident in time dependency of the covered versus open "skin permeability to ketoprofen" ratio (cf. Fig. 2 inset). More specifically, the tested skin occlusion produces relative values greater than 1 (or a maximum for Mohrus® tape versus Togal® Mobil-Gel) and relative values below 1 (and a minimum for the occluded versus open Diractin®), respectively. The time-course of relative plasma drug concentrations originating from Mohrus® tape and Diractin® under occlusion is qualitatively similar to the former ratio (see crosses in inset to Fig. 2). The diffusive drug transport through an occluded skin thus seems to involve similar working principle for all the tested products, at least some time after an epidermal application.

In contrast, skin occlusion affects oppositely transcutaneous flux of the dissolved ketoprofen from Togal® Mobil-Gel and Mohrus® tape or the drug delivery with ultradeformable carriers in Diractin®. This is explicable in terms of Eq. (2), which predicts that an elimination of transcutaneous hydration gradient, $\Delta a_{\text{w,endo}} \rightarrow 0$, should bring the carrier-mediated, non-diffusive, transcutaneous transport of the drug to a stand-still. In other words: the second term in Eq. (2) identifies hydration gradient over an non-occluded *stratum corneum* as the source of superficially applied hydrophilic carrier motion—together with its payload—across the skin. Such gradient is responsible for most of the ketoprofen transport from Diractin® through the skin. Quantitative analysis of complete Diractin® data set with Eq. (2) moreover reveals that ketoprofen diffusion through an open skin barrier from Diractin® must be very small, in the range of merely a few percent. The low apparent skin permeability coefficient measured with the product *in vitro* (∼5%) is consistent with the conclusion.

Conventional topical NSAID products (e.g. Togal® Mobil-Gel, Gabrilen® gel; Fastum®, Mohrus® tape, etc.) are known to rely merely on drug's diffusion, which brings the active pharmaceutical ingredient through skin barrier and beyond. Such products consequently yield the best results on an occluded skin, due to lack of evaporation (which preserves original driving force by preventing

[2] For the drug in solution one can write: $J_{\text{d,permeation}} + J_{\text{d,penetration}} = P'_{\text{sc,d}}|\Delta\Pi_{\text{d}}| + P'_{\text{sc,d}}|\Delta\Pi_{w,\text{endo}}| \approx P_{\text{sc,d}}\Delta c_{\text{d}}$,[8] after having taken into account the different directions of $\Delta\Pi_{\text{d}}$ and $\Delta\Pi_{w,\text{endo}}$ or $\Delta a_{w,\text{sc}}$. Similar expression can be written for empty carriers, simply by replacing "d" with "Tfs" in all indices. In Diractin® some of the drug is in solution and some associated with their carriers. The index can then either relate to the drug "d" = "KT" or to the carriers with their associated drug "Tfs". For Diractin® one must therefore write two equations and add them together: $J_{\text{total}} = J_{\text{d,permeation}} + J_{\text{d,penetration}} + J_{\text{Tfs,permeation}} + J_{\text{Tfs,penetration}}$. In normal practice, the first and the last term are sufficient, as both the drug penetration as well as vesicle permeation through the skin are negligibly small.

[3] $\Delta\Pi_{w,\text{endo}}$ Transcutaneous water efflux driven by $\Delta\Pi_{w,\text{endo}}$ cannot preclude the carrier influx across the skin so long as water loss from the treated skin surface is bigger than transcutaneous water efflux.

drug precipitation) but also owing to simultaneous lowering of the skin's diffusive barrier (which may also be supported by maceration). The experimental data illustrated in Figs. 1 and 2 vindicate these implications.

In contrast, the ultradeformable and hydrophilic carrier vesicles in Diractin® rely on a non-diffusive, hydration driven locomotion to cross semi-permeable barriers, such as the skin. We first proposed the concept (Cevc and Blume, 1992) which was later confirmed by others (El Maghraby et al., 1999; Honeywell-Nguyen et al., 2003) working with empty, highly fluid mixed amphiphat vesicles. Additional support came from the subsequent, our data inspired, theoretical studies of deformable vesicle transport (Gompper and Kroll, 1995). We subsequently corroborated the conclusion that empty ultradeformable vesicles can cross even narrow pores much smaller than their own diameter (Cevc et al., 2003), driven by sufficiently large hydration pressure (Cevc and Gebauer, 2003), without significant fragmentation (Cevc et al., 2002). We have moreover shown the resulting rate of carrier transport to decrease logarithmically with water activity gradient across the barrier (Cevc and Gebauer, 2003). Diminishing trans-barrier hydration gradient below some characteristic, aggregate-deformability dependent, threshold must therefore bring the transcutaneous transport of ultradeformable vesicles to a stand-still, in agreement with Eq. (2).

The data illustrated in Fig. 2, which pertain to the drug-loaded vesicles, are compatible with the conclusions made in previous paragraph for the empty vesicles. The concept of hydration-driven transcutaneous transport consequently should also apply to the drug-loaded vesicles investigated in this work. Hydrotaxis may thus be concluded to drive transcutaneous transport of water-soluble drugs associated with ultradeformable carriers; such "endogenous hydrotaxis" acting on the sufficiently deformable and stable vesicles can therefore replace the need for transcutaneous "chemical push" on the drug itself.

But why is the transcutaneous drug transport from Diractin® apparently not affected by occlusion *in vivo* as it is *in vitro* (cf. Fig. 1)? The answer is that it probably is. Skin occlusion can enhance simple diffusion, as is evident in Franz-cells experiments. But the latter are insensitive to a non-diffusive carrier transport, despite its potentially large significance. In contrast, when a carrier-based product is tested *in vivo*, the carrier-mediated ketoprofen transport dwarfs drug's diffusion, making *in vivo* test insensitive to ketoprofen diffusion from Diractin® and explaining the observed "anomaly".

The assertion is supported by further experiments described in this study. Perhaps the best resulting proof is proportionality of ketoprofen diffusion rate and the dissolved drug concentration measured in Franz-cells. The ratio of the apparent *in vitro* skin permeability to the drug from Diractin® and Togal® Mobil-Gel is 0.06 (cf. Table 1). The value is nearly the same as the corresponding relative unbound drug concentration: 0.05.[4]

On the other hand, transcutaneous concentration gradient of the carrier or of the drug evidently does not influence ketoprofen transport across the skin in the presence of ultradeformable hydrophilic carriers (cf. Eq. (2) and Table 1). If any of these two gradients did play a role, ketoprofen transport by the carriers should be comparable and always as sensitive to occlusion as the free drug's diffusion across the skin; ketoprofen from Diractin® should then cross occluded skin better than non-occluded organ. This should hold true *in vivo* as well as *in vitro*. It indeed does so for a conventional Togal® Mobil-Gel. The drug's deposition with ultradeformable carriers through an intact skin into a body should then

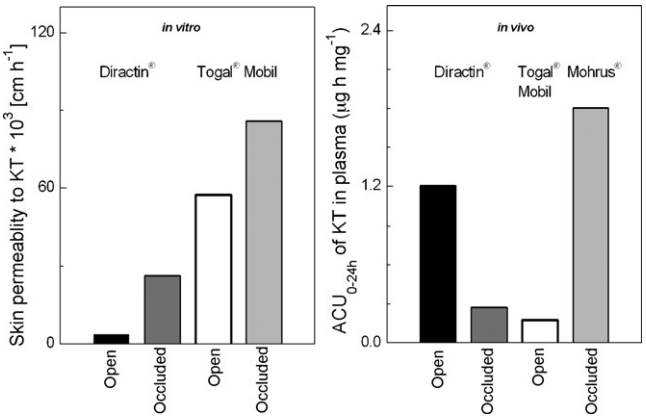

Fig. 5. Relative efficiency of transcutaneous ketoprofen delivery from conventional topical products, Togal® Mobil-Gel/Mohrus® tape (white or light grey) or the new product, Diractin® (black or dark grey), which comprises ultradeformable vesicular carrier (Transfersome®), measured over 24 h *in vitro* (human skin, left panel) and *in vivo* (porcine plasma, right panel). Results pertaining to open skin are shown in black and white and those measured with occluded skin in dark grey or grey.

be inferior, or at best comparable, to a topical ketoprofen delivery with a conventional Togal® Mobil-Gel—none of which is observed (cf. Figs. 1 and 2).

For example, the area under the drug concentration in plasma versus time curve is higher for Diractin® (AUC = 1.204 μg h g^{-1}) than for Togal® Mobil-Gel (AUC = 0.175 μg h g^{-1}). This is despite the fact that the dissolved drug concentration in the former product is at least 2× (and after partial drying 30×) below that in the gel. The former AUC value is also much higher than the result measured with Diractin® under occlusion (AUC = 0.271 μg h g^{-1}) notwithstanding the fact that any impermeable cover helps to keep the drug in solution.

5. Conclusions

Occlusion of the skin covered with various ketoprofen products for local application affects the drug transport through the organ. It also affects the drug biodistribution. First of all, an impermeable cover promotes and maintains the free drug diffusion *in vitro* and *in vivo*; the resulting higher skin permeation coefficient and longer apparent terminal elimination half-life of the drug are both beneficial. Approximately 10-times higher efficacy of transcutaneous ketoprofen delivery from an occlusive conventional patch (Mohrus® tape) compared with a conventional topical gel (Togal® Mobil-Gel) is a consequence of this.

Occlusion of the new ketoprofen product (Diractin®), which comprises ultradeformable, drug-loaded, vesicular carriers, also increases the inherently lower transcutaneous drug transport *in vitro* from the vesicles—but diminishes the drug amount delivered across the skin of a living mammal >10-fold; this is seen in peripheral porcine muscles tissues as well as in systemic blood circulation.

The corresponding quantitative differences are summarised in the last two lines in Table 1 (for muscles). They are also graphically represented in Fig. 5 (for plasma compartment) to illustrate the opposite effects of skin occlusion on Diractin® and on conventional products (Togal® Mobil-Gel; Mohrus® tape), based on the dissolved drug.

Experimental data presented in this work thus prove beyond reasonable doubt that ketoprofen transport through a non-occluded skin barrier with ultradeformable vesicle (Transfersome®) is powered by an endogenous hydrotaxis. If water evaporation from skin surface is prevented by occlusion, the transport-driving endogenous osmotic gradient across the

[4] The precise value, which we calculated from the drug partitioning versus carrier concentration data, depends on final degree of sample concentration after a partial product dehydration on skin surface.

skin, which exerts a "hydration force" on the drug-loaded vesicles, falls to zero. Lack of the carrier driving force is detrimental to non-diffusive carrier motion across the skin, and thus practically abolishes the carrier-mediated transcutaneous ketoprofen transport. Conversely, skin occlusion that preserves the initial dissolved drug concentration and may lower diffusive skin barrier is desirable for and helpful to conventional topical drug products.

Topically applied free NSAID molecules consequently profit from skin occlusion. In contrast, NSAIDs associated with the hydrophilic, ultradeformable carriers loose activity on occluded skin. Such is the case with the most modern and targeted NSAID products Diractin®, that comprises the ultradeformable Transfersome® carriers. This corroborates the fundamental difference between conventional topical products and the locally used drug formulations based on Transfersome® technology.

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References

Allen, T.M., Hansen, C.B., Guo, L.S.S., 1993. Subcutaneous administration of liposomes: a comparison with the intravenous and intraperitoneal routes of injection. Biochim. Biophys. Acta 1150, 9–16.

Benson, H.A.E., 2005. Transdermal drug delivery: penetration enhancement techniques. Curr. Drug Deliv. 2, 23–33.

Becker, T., Mager, D., 2000. In vitro skin penetration with split thickness skin using a new flow through diffusion cell. In: Poster at the 21st Congress of the International Federation of the Societies of Cosmetic Chemistry (IFSCC), Berlin, September 11–14.

Brunner, M., Dehghanyar, P., Seigfried, B., Martin, W., Menke, G., Müller, M., 2005. Favourable dermal penetration of diclofenac after administration to the skin using a novel spray gel formulation. Br. J. Clin. Pharmacol. 60, 573–577.

Cevc, G., 1995. Material transport across permeability barriers by means of lipid vesicles, in: Hoff, A.V., (series Ed.), Lipowsky, R., Sackmann, E., (volume Eds.), Handbook of Biological Physics, vol. I, North-Holland (Chapter 9, pp. 465–490).

Cevc, G., 1996. Lipid suspensions on the skin. Permeation enhancement, vesicle penetration and transdermal drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 13, 257–388.

Cevc, G., 1997. Drug delivery across the skin. Exp. Opin. Invest. Drugs 6, 1887–1937.

Cevc, G., 2002. Transfersomes®—innovative transdermal drug carriers. In: Rathbone, M., Roberts, M., Hadgraft, J. (Eds.), Modified Release Drug Delivery Technology. Marcel Dekker, New York, pp. 533–546.

Cevc, G., 2004. Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 56, 675–711.

Cevc, G., Blume, G., 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim. Biophys. Acta 1104, 226–232.

Cevc, G., Blume, G., 2001. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim. Biophys. Acta 1514, 191–205.

Cevc, G., Blume, G., Schätzlein, A., Gebauer, D., Paul, A., 1996. The skin: a pathway for the systemic treatment with patches and lipid-based agent carriers. Adv. Drug Deliv. Rev. 18, 349–378.

Cevc, G., Gebauer, D., 2003. Hydration driven transport of deformable lipid vesicles through fine pores and the skin barrier. Biophys. J. 84, 1010–1024.

Cevc, G., Schätzlein, A., Richardsen, H., 2002. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers intact. Evidence from double label CLSM experiments and direct size measurements. Biochim. Biophys. Acta 1564, 21–30.

Cevc, G., Schätzlein, A., Richardsen, H., Vierl, U., 2003. Overcoming semi-permeable barriers, such as the skin, with ultradeformable mixed lipid vesicles, Transfersomes®, liposomes or mixed lipid micelles. Langmuir 19, 10753–10763.

Cevc, G., Vierl, U., Mazgareanu, S., 2008. Targeted delivery of nonsteroidal anti-inflammatory agent into deep peripheral tissues. 1. Functional characterisation of a novel therapeutic product based on self-regulating drug carriers. Int. J. Pharm. in the press.

DeGraves, F.J., Riddell, M.G., Schumacher, J., 1996. Ketoprofen concentrations in plasma and milk after intravenous administration in dairy cattle. Am. J. Vet. Res. 57, 1031–1033.

El-Kattan, A.F., Asbill, C.S., Kim, N., Michniak, B.B., 2000. Effect of formulation variables on the percutaneous permeation of ketoprofen from gel formulations. Drug Deliv. 7, 147–153.

El Maghraby, G.M., Williams, A.C., Barry, B.W., 1999. Skin delivery of oestradiol from deformable and traditional liposomes: mechanistic studies. J. Pharm. Pharmacol. 51, 1123–1134.

Gompper, G., Kroll, D.M., 1995. Driven transport of fluid vesicles through narrow pores. Phys. Rev. E 52, 4198–4208.

Hadgraft, J., de Plessis, J., Goosen, C., 2000. The selection of non-steroidal anti-inflammatory agents for dermal delivery. Int. J. Pharm. 207, 31–37.

Heyneman, C.A., Lawless-Liday, C., Wall, G.C., 2000. Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs 60, 555–574.

Honeywell-Nguyen, P.L., Wouter Groenink, H.W., de Graaff, A.M., Bouwstra, J.A., 2003. The in vivo transport of elastic vesicles into human skin: effects of occlusion, volume and duration of application. J. Control. Release 90, 243–255.

Honeywell-Nguyen, P.L., Gooris, G.S., Bouwstra, J.A., 2004. Quantitative assessment of the transport of elastic and rigid vesicle components and a model drug from these vesicle formulations into human skin in vivo. J. Invest. Dermatol. 123, 902–910.

Jain, S., Jain, N., Bhadra, D., Tiwary, A.K., Jain, N.K., 2005. Transdermal delivery of an analgesic agent using elastic liposomes: preparation, characterization and performance evaluation. Curr. Drug Deliv. 2, 223–233.

Karande, P., Jain, A., Ergun, K., Kispersky, V., Mitragotri, S., 2005. Design principles of chemical penetration enhancers for transdermal drug delivery. Proc. Natl. Acad. Sci. U.S.A. 102, 4688–4693.

Maibach, T., Hikima, H., 2006. Skin penetration flux and lag-time of steroids across hydrated and dehydrated human skin in vitro. Biol. Pharm. Bull. 29, 2270–2273.

Radeck, W., Heller, M., 1989. Improved method for the determination of ketoprofen in plasma by high-performance liquid chromatography. J. Chromatogr. 497, 367–370.

Schätzlein, A., Cevc, G., 1998. 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, 583–592.

Trottet, L., Merly, C., Mirza, M., Hadgraft, J., Davis, A.F., 2004. Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride. Int. J. Pharm. 274, 213–219.

Tugwell, P.S., Wells, G.A., Shainhouse, J.Z., 2004. Equivalence study of a topical diclofenac solution (Pennsaid) compared with oral diclofenac in symptomatic treatment of osteoarthritis of the knee: a randomized controlled trial. J. Rheumatol. 31, 2002–2012.

van den Bergh, B.A.I., Bouwstra, J.A., Junginger, H.E., Wertz, P.W., 1999. Elasticity of vesicles affects hairless mouse skin structure and permeability. J. Control. Release 62, 367–379.

Walters, K.A., Hadgraft, J., 1993. Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York.

Wu, P.C., Chang, J.S., Huang, Y.B., Chai, C.Y., Tsai, Y.H., 2001. Evaluation of percutaneous absorption and skin irritation of ketoprofen through rat skin: in vitro and in vivo study. Int. J. Pharm. 222, 225–235.