Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 360 (2008) 29–39
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Preclinical characterisation of NSAIDs in ultradeformable carriers or conventional topical gels

Gregor Cevc *, Stefan Mazgareanu, Matthias Rother

IDEA AG, Frankfurter Ring 193a, D-80807 Munich, Germany

Received 29 October 2007; received in revised form 23 January 2008; accepted 25 January 2008
Available online 6 February 2008

Abstract

We compared *in vivo* transport and biodistribution of ketoprofen applied on the skin in ultradeformable carriers (Diractin®) or a conventional topical gel (Gabrilen®) with oral drug (Oruvail®); for reference we used *in vitro* study data. The drug from Gabrilen® diffuses into body with low bioavailability (<10%) and limited regio-selectivity (AUC(deep muscle/plasma) ∼45/0.8 (t = 0–8 h), reaching maximum concentration in subcutaneous tissues and plasma at similar time (t_{max} ∼3–4 h). The apparent drug elimination half-life is then similar to oral ketoprofen ($t_{1/2,a}$ ∼2 h). In contrast, Diractin® containing ultradeformable carriers (Transfersome® vesicles) delivers the drug more efficiently (>50%) and more directly into peripheral muscles (AUC(deep muscle/plasma) ∼447/0.7 (652/1.4) for t = 0–8 (0–24) h; t_{max} ∼1 h), arguably in non-diffusive fashion. Ketoprofen from Diractin® moreover disappears from body periphery slower ($t_{1/2,a}$ ∼4–6 h), owing to sustained drug release from the carriers in target tissue. Final clearance always proceeds via plasma (t_{max} ∼4 h). Epicutaneous application of ketoprofen in conventional gels or the carrier-based formulation thus leads to different local accumulations and clearances. Ketoprofen from Diractin® achieves more desirable biodistribution and clearance, arguably due to spontaneous carrier-mediated drug transport across the skin, which ensures local and relatively long-lasting drug deposition into peripheral target tissues.

Keywords: Ketoprofen; Drug delivery via the skin; Ultradeformable lipid vesicle, Transfersome®; Targeted analgesic, Diractin®; Biodistribution

1. Introduction

Topical NSAIDs are usually applied on the skin in a conventional gel or a patch (Heyneman et al., 2000). The aim is to reach deep subcutaneous tissues for direct treatment (Lin et al., 2004; Vaile and Davis, 1998), i.e. sparing residual body from the drug's adverse side effects. The list of commercially available products includes topical preparations of ketoprofen, a broad activity NSAID suppressing cyclooxygenase as well as lipoxygenase activity.

According to originators information, ketoprofen availability in humans is ∼90% for all oral formulations, compared with an intravenous injection. For 75–200 mg single oral doses, the area under the curve is moreover dose proportional. Food intake with a fast-release tablet Oruvail® (Sanofi-Aventis) reduces peak concentration c_{max} by ∼50% and delays its appearance (t_{max} ∼1.2 h [range: 0.5–3 h] for fasting subjects; t_{max} ∼2.0 h [range: 0.75–3 h] for fed test people). Circadian changes in the drug absorption also lead to c_{max} fluctuations. Ketoprofen is >99% bound to plasma proteins, mainly to albumin. The drug half-life is $t_{1/2} = 2.1 \pm 1.2$ h for the fast and $t_{1/2} = 5.4 \pm 2.2$ for the slow release tablet. The difference is due exclusively to slower drug uptake in the latter case, as oral-dose clearance is in either case essentially the same: CL = 6.9 ± 0.8 and 6.8 ± 1.8 L h^{-1}, respectively. (Following an intravenous drug injection, ketoprofen elimination half-life is $t_{1/2} = 2.05 \pm 0.58$ h [mean ± S.D.].) After a single 200 mg (retarded) dose of Oruvail®, the plasma drug concentration declines slowly to an average value of 0.4 μg mL^{-1} at t = 24 h. By then, ∼80% of ketoprofen has been excreted in the urine, primarily as the glucuronide metabolite. The area under the curve in human blood is $AUC_{0-24\,h} = 32.1 \pm 7.2$ and 36.6 ± 8.1 μg h mL^{-1} in fasted and fed individuals, respectively.

Local delivery makes the drug pharmacokinetics more complex for at least two reasons: the difficulty of overcoming the primary, permeability barrier of the skin (Walters and Hadgraft, 1993) and subsequent cutaneous clearance (Singh and Roberts, 1994a,b; Hadgraft et al., 2000; Clough et al., 2002; Higaki et

* Corresponding author. Tel.: +49 89 324 633 0.
E-mail address: info@idea-ag.de (G. Cevc).

0378-5173/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2008.01.051

al., 2002; Monteiro-Riviere et al.,1993; Kretsos and Kasting, 2005; Cevc and Vierl, 2007). The problem of local clearance is gravest for the molecules with a high permeability coefficient in the skin, including all topical NSAIDs (Hadgraft et al., 2000), as such molecules can also enter blood vessels relatively easily.

For example, all marketed topical NSAIDs are cleared from the skin extensively, as witnessed by occasional gastrointestinal haemorrhage observed after their excessive usage (Figueras et al., 1994). The whole body reservoir may feed back the drug into peripheral organs. To ensure that a topically applied NSAID will act safely and directly in deep subcutaneous tissue, one must therefore control the drug's elimination from the organ. Ideally, one wishes minimum NSAID diffusion into superficial blood vessels, and through them into whole body, and maximum drug's propensity to reach deep tissue below the skin. Arguably, such opposite goals cannot be achieved by way of simple drug diffusion.

We introduced an approach to drug delivery (Cevc, 1996, 1997, 2002, 2004) that can overcome said problems. Its basis is an ultradeformable and very hydrophilic aggregate carrier, so called Transfersome®.[1] The aggregate is a vesicle loaded with the drug. The drug loaded ultradeformable vesicle was shown to penetrate the skin spontaneously and independent of concentration (Cevc and Gebauer, 2003) to carry its cargo through the primary and past the secondary skin barrier into deep subcutaneous tissue (Cevc and Blume, 2001). The proviso is preservation of the carrier's physical integrity, which has been confirmed in a special study (Cevc et al., 2002), and is also tackled less directly in this work.

Our closest previous publication (Cevc and Blume, 2001) dealt with a popular NSAID, diclofenac (MW = 296 Da; $pK_a = 4$ in water; pH-independent partitioning coefficients $\log P_{o/w} = 4.5$ [neutral] and 0.7 [ionic]), delivered through the skin of living rodents or pigs from a conventional gel (Voltaren® Emulgel®, Novartis) or with the ultradeformable carriers, the Transfersome® vesicles. We now also developed several carrier formulations comprising another NSAID, ketoprofen, of broadly similar molecular characteristics (MW = 254 Da; $pK_a = 4.4$ in water; pH-independent partitioning coefficients $\log P_{o/w} = 3.16$ [neutral] and -0.95 [ionic]), and with very attractive biological characteristics (Kantor, 1986).

The key pharmaceutical characteristic of our lead formulation are described in the accompanying paper (Cevc et al., submitted for publication-b) together with some basic *in vitro* drug transport data; the parallel paper also makes a preliminary *in vivo* comparison of ketoprofen in ultradeformable carriers and in conventional topical gels. Here we present a more extensive preclinical data set for both kinds of formulations. We mainly focus on the drug concentration in porcine peripheral tissues (→efficacy) and serum (→safety) after a single epicutaneous or an oral drug application. We conclude that ultradeformable vesicles loaded with the drug cross the skin on their own accord, as is explored in further detail in a separate paper (Cevc et al., submitted for publication-b). This allows the carriers to deliver ketoprofen efficiently and directly into deep peripheral tissues, such as targeted superficial and deep subcutaneous muscles. Local usage of ultradeformable carriers loaded with ketoprofen moreover prolongs the drug's residency time in such tissues.

An important further finding of our study is that relative drug concentration in deep subcutaneous tissue increases approximately linearly with the applied drug/carrier dose per area. Conversely, the systemic drug concentration is one to two orders of magnitude lower and proportional to total applied product dose. The starting drug concentration on the skin thus arguably should not influence ketoprofen delivery with ultradeformable drug carriers through the organ, unlike in case of conventional NSAID topical gel usage.

2. Materials and methods

Test articles: The novel NSAID formulation, Diractin® (=IDEA-033) contains ~2.3% sodium ketoprofen, carbomer, methylparabene, and a small amount (<5%) of short chain alcohols (benzyl alcohol, ethanol, glycerol) in an adequately buffered aqueous suspension of highly adaptable carriers (with soybean phosphatidylcholine and polysorbate as their main ingredients). Butylhydroxytoluene and linalol are also present in formulation. All tested Diractin® formulations were manufactured by or on behalf of IDEA AG. Gabrilen® gel, according to manufacturer's specifications (Kreussler Pharma GmbH, Wiesbaden, Germany), contains 2.5% sodium ketoprofen, ethanol, 2-propanol, carbomer, ammonia solution and purified water.

The oral comparator was Ketoprofen-ratiopharm® 50 (ratiopharm GmbH, Ulm, Germany), containing 50 mg ketoprofen per tablet.

Carrier characterisation in Diractin®, or its precursor suspension, was done with the proprietary test assays of IDEA AG (for a brief description see ref. Cevc et al., 2003), confirming the product's compliance with specifications. The assays done for this study included the carrier deformability test, involving vesicle passage through an artificial semi-permeable barrier (with pores >4× narrower than the average vesicle diameter), and the carrier stability check (with the dynamic light scattering) after such passage. Each test was done at least in triplicate.

Drug diffusion experiments in vitro are described in greater detail in our accompanying publication (Cevc et al., submitted for publication-b). In brief, miniaturised Franz-diffusion cells were employed to measure the drug diffusion *in vitro* through microtomised human skin (thickness: 400 μm) following all the relevant authority recommendations (FDA, 1997).

Short-term animal experiments in vivo always involved neutered male hybrid piglets (~8–12 kg body weight) supplied by a local farm animal breeder, and generally adhered to the Principles of Animal Care (NIH Publication #85-23, revised in 1985). We used three to six animals per group in the experiments with oral drug, conventional topical gel products, or Diractin®. Total number of the animals investigated with each tested product is given in figure captions.

The test piglets were anaesthetised with ketamine (15 mg per kg body weight [BW]), azaperone (2 mg kg^{-1} BW) and propofol (30–50 mg kg^{-1} BW, according to anaesthetic effect),

[1] Transfersome® (=carrying body) is a registered trademark of IDEA AG.

after insertion of jugular vein catheter but before executing any further potentially painful procedure. We always used the animal's hind limb for testing, to minimise site-to-site variability effects (Shah et al., 1996). The hair at such site was trimmed short with an electric hair clipper, avoiding any skin damage, and washed with lukewarm water prior to the test product application. The designated application area (∼100 cm^2) was then marked with water-resistant ink. Either 2 mL of a conventional gel or 2.2 mL of a gel containing ultradeformable carriers were distributed uniformly over a non-occluded skin surface for the 50 mg ketoprofen dose per pig, corresponding to approximately 5 mg kg^{-1} BW. To achieve different doses we adjusted the applied gel volume per area, unless stated otherwise further in the text.

Before tissue sampling, the skin on the hind limb was stripped 20 times with adhesive tape to remove residual test material and the *stratum corneum*. The skin was then carefully excised. This opened way free to gather the skin-free biopsies of subcutaneous fat and the underlying muscles, with a biopsy punch (diameter: 5 mm; up to a depth of ∼3 cm). Each biopsy sample was separated into a specimen of subcutaneous fat tissue, the superficial (≤15 mm depth), and deep (15–30 mm depth) muscle tissue sample. The resulting wound was taken care of immediately after the sampling, under a prophylactic analgesia by s.c. injection of meloxicam. (For avoidance of doubt: every given time point relates to one sample taken from different part of the skin and muscle.) In parallel, blood samples were taken from the jugular vein catheter to gain sodium citrate plasma samples. Ketoprofen concentration in plasma and tissue samples was determined with a validated bioanalytical method described further in the text.

To determine residual product amount on the skin 24 h post the drug application, a circular piece of treated skin was carefully excised from the treated site. The epidermis including the stratum corneum was removed from the skin using the usual thermo-facilitated separation (∼60 °C, 30–90 s, depending on the sample size). Ketoprofen concentration of the stratum corneum and the dermis was again determined separately with the validated bioanalytical method.

All tissue and plasma samples were promptly frozen at −20 °C and stored protected from light. After the last biopsy had been collected, the test animals were killed by an intravenous injection of a mixture of embutramide (1 mg kg^{-1} BW), mebezonium iodide (0.25 mg kg^{-1} BW), and tetracaine (25 μg kg^{-1} BW).

Oral drug was given to the sedated test animals in a fashion that ensured good control over drug uptake. One 50 mg tablet per pig was placed into the oral cavity, before the swallowing reflex was induced. Swallowing of the tablet was visually controlled with a laryngoscope.

Long term animal experiments in vivo were done with Göttingen® Minipigs (average weight at start of experiment: 6–11 kg depending on gender; age at start of experiment, 4–5 months). Animals were dosed twice daily with Diractin® or the matching placebo at a fixed dosing volume per kg ratio of 0.317 mL kg^{-1} BW on a skin area of approximately 180 cm^2 onto the animals back. As the concentration of ketoprofen in Diractin® had to be maintained at nominally ∼2.3%, to ensure proper functionality of the formulation, Diractin® was mixed with matching placebo, giving the following three dosing groups: 7.26, 3.65, and 1.46 mg kg^{-1} BW per dose. The volume of applied placebo was equivalent to the volume applied in all three dosing groups.

Drug concentration measurements relied on high-pressure liquid chromatography (HPLC) with UV detection. The plasma sample preparation involved solid phase extraction prior to the HPLC/UV analysis. The tissue sample preparation procedure involved tissue homogenisation followed by solid phase extraction prior to the HPLC/UV analysis. The method showed linearity in 9–358 ng mL^{-1} range in pig plasma and from 4.4 to 335.6 ng mL^{-1} in pig muscle tissue. The accuracy measurements results in plasma were 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 concentrations in the linear range. For pig tissue, similar accuracy results were: 88.4% (6.3% CV) for the lower limit of quantification and 101.4–106.3% (<2.7% CV) for the mid to high concentrations in the linear range.

Drug and carrier transport in vivo: The primary data set consisted of the drug concentration values measured for plasma and various tissues at the given time after test article application. Total daily efficiency of ketoprofen transport through the skin was also determined directly by measuring residual drug amount on the skin at $t = 24$ h post-application; additional input was the known total applied drug quantity and the treated skin area. This afforded the lower limit for the normalised rate of drug transport across the skin (=flux density). To get an independent, but less direct, total transport estimate we furthermore calculated the area under the drug concentration *vs*. time curve (AUC) from the measured concentration *vs*. time data. From the total drug flux values we deduced effective rate of transcutaneous carrier transport as well, by assuming that >95% of the drug is associated with the carriers during skin crossing (Cevc et al., submitted for publication-b).

To estimate the drug's elimination rate independent of any model, we used computer to draw a straight line into the relevant part of the logarithmic drug concentration *vs*. time plot. We then read-off the apparent 50% elimination time, $t_{1/2,a}$, from such line, separately for each investigated tissue.

Averaging and data presentation was done with the commercial graphical software package Origin® (OriginLab Inc., Northampton, MA, USA), which we also used for significance testing (paired *t*-test). The results given in Figs. 1–4 provide the mean value (MV) ± standard deviation of the mean (S.D.). The results given in the text give mean value × (1 ± S.D./MV), unless specified otherwise.

3. Results

3.1. Residual ketoprofen on the skin

To begin with, we inspected the residuum of ketoprofen from locally applied Diractin® on the skin 24 h post-application. Epidermal ketoprofen concentration was then 325× (1 ± 0.61) μg g^{-1} (median: ∼240 μg g^{-1}). The corre-

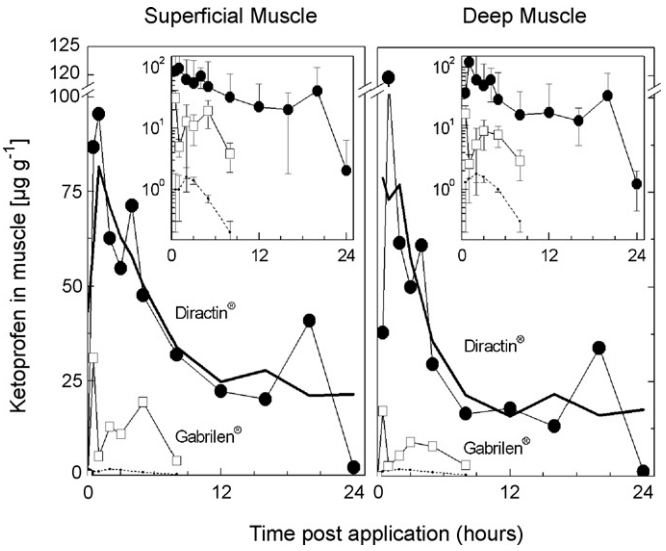

Fig. 1. Left panel: Temporal dependency of ketoprofen concentration in superficial porcine muscle tissue (0–15 mm below treatment site) after an epicutaneous application of 50 mg drug in Diractin® (bullet, $n = 4$–24) or Gabrilen® (box, $n = 6$). Lines should only guide the eye: the thick ones in the main panels give the result of three nearest neighbours averaging for Diractin®. The thin lines connect the original data for Gabrilen® or oral ketoprofen (KT). The small bullets with the dashed lines provide comparison with the results of an oral drug application ($n = 3$). Right panel: The related results measured in deep muscle tissue (15–30 mm below application site). Insets show the same data in a logarithmic plot, and include standard deviations of the mean.

sponding dermal value was $146 \times (1 \pm 0.46)\,\mu g\,g^{-1}$ (median: $\sim 116\,\mu g\,g^{-1}$). This corresponds to $0.33 \times (1 \pm 0.36)\,\mu g$ (median: $\sim 0.35\,\mu g$) and $3.22 \times (1 \pm 0.41)\,\mu g$ (median: $\sim 2.52\,\mu g$) total ketoprofen in the outer and inner skin region, respectively. The total extracted drug quantity was $210.42 \times (1 \pm 0.44)\,\mu g$ (median: $\sim 171\,\mu g$), corresponding to 0.43% of the applied dose.

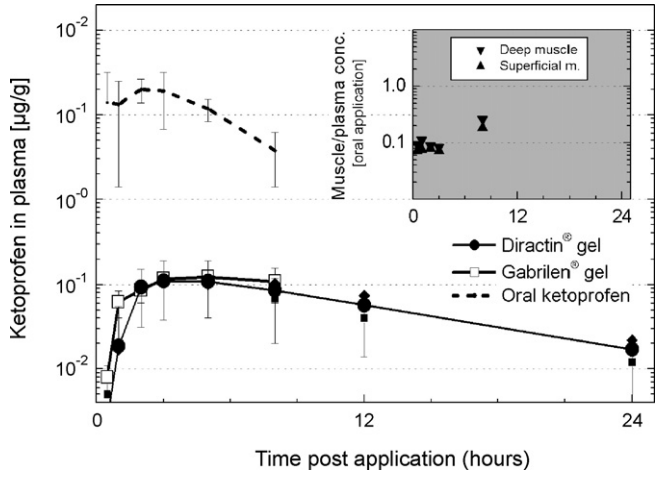

Fig. 2. Ketoprofen concentration in porcine plasma as a function of time following an oral application of 50 mg drug (Ketoprofen ratiopharm®, dots, $n = 3$) or an epicutaneous application of the equivalent drug quantity in Gabrilen® gel (squares, $n = 6$) or Diractin® (bullets, $n = 12$). Inset: the calculated relative concentration ratio of ketoprofen in muscle and plasma derived by combining the data shown in the figure and the inset.

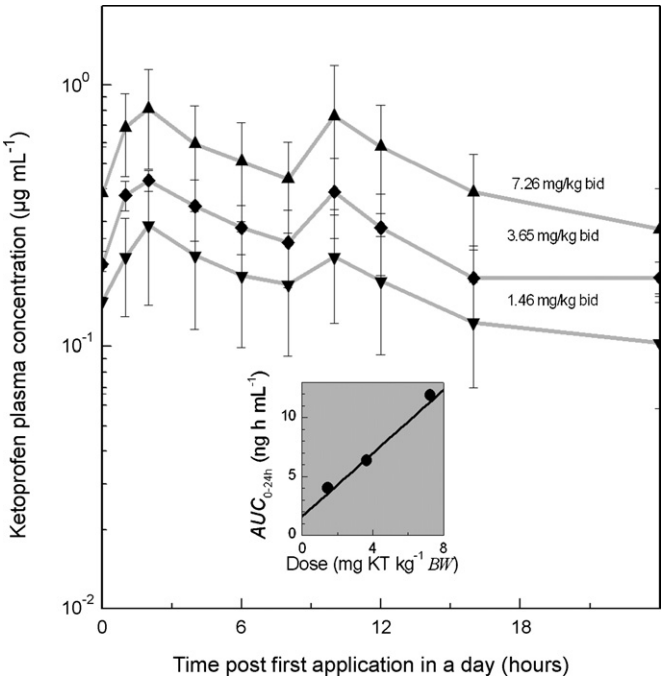

Fig. 3. Temporal and dose dependency of total ketoprofen concentration in plasma of the pigs treated epicutaneously with Diractin® 2× daily for 6 months (second test article application at $t = 8$ h). Inset: dose dependency of the area under the curve (bullets).

3.2. Transcutaneous drug transport

It is instructive to compare efficiency of ketoprofen transport through normal skin *in vitro* and *in vivo* for different drug formulations. We therefore combined our *in vitro* data, which are published in full detail in the accompanying paper (Cevc et al., submitted for publication-b), with the results of this study to get

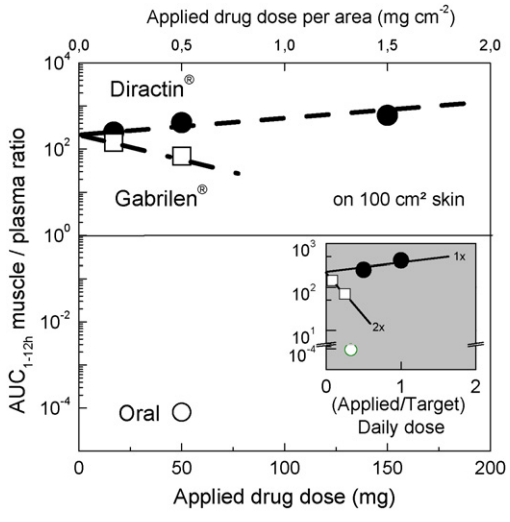

Fig. 4. Drug/carrier dose and dose per area effect on targetability of ketoprofen delivery into peripheral tissues, expressed in terms of cumulative muscle *vs.* plasma drug concentration ratio, AUC. (Symbols: experimental results (cf. Table 2); lines: linear regression fit to the data.) Inset: the same results adjusted for different required daily frequency of dosing, which is 2× higher for conventional gels (e.g. Gabrilen®, box) than for Diractin® (bullet).

Table 1
Relative transcutaneous ketoprofen delivery efficiency, defined as the ratio of permeability coefficients measured with a conventional topical gel (Gabrilen®) and the carrier-based product (Diractin®) at the times given

	Relative skin permeability (*in vivo*: lower limit)		
In vitro (human skin)	$t = 4$ h	t_{max} [a]	$t = 16$–24 h
Diractin®/Gabrilen®	0.01	0.02	0.07
In vivo (porcine skin)	$t = 1$ h	$t = 5$ h[b]	
Diractin®/Gabrilen®	5.09	4.35	

[a] The maximum values and t_{max} were read from the smoothed curves given in Fig. 1 in Cevc et al. (submitted for publication-b).
[b] *In vivo*, the dominant maximum is observed 3–5 h post-application.

relative drug transport rates for Gabrilen® and Diractin® (cf. Table 1).

Differential factor between Diractin® and Gabrilen® exceeds the differential factor pertaining to various conventional gels 4–10 times. On the average, it amounts to an overall difference of 20–50 during the first 12 h post-application. Towards the end of observation period, i.e. for $t = 16$–24 h, the arithmetic mean ratio of conventional gel *vs*. Diractin® is ∼14. In contrast, the corresponding mean ratio determined with conventional gels (Gabrilen® *vs*. Togal® Mobil gel) is then around 1 (data not shown). This confirms the ∼12× *in vitro* differential between the conventional and the carrier-based products.

3.3. Drug transport into subcutaneous peripheral tissues

A full *in vivo* set of transport and biodistribution data must be explored to interpret and understand properly the differences between different NSAID formulations. Similar data are also needed to gauge the potential therapeutic benefit of various NSAID products for the local application on the skin.

We therefore measured with Diractin® and Gabrilen® the detailed temporal dependency of total ketoprofen concentration in superficial (depth: ≤15 mm) and deep (depth: 15 to ≤30 mm) muscles, as such tissues act as rather voluminous primary receiver compartments.

In our experience, ketoprofen uptake in the muscles below the non-occluded skin treated with ketoprofen is remarkably fast. Relatively high drug concentration is measured already after the first hour of application. An apparent primary concentration maximum is followed for conventional topical gel by the secondary increase of drug concentration in muscles (cf. Fig. 1 and Table 2).

Table 2
Pharmacokinetic characteristics of a single 50 mg ketoprofen dose in peripheral muscle tissue and plasma of the pigs treated with the drug orally and through non-occluded skin above the studied muscles

	Injected[a] i.m.	Oral[b]		Conventional topic gel	Carrier-based gel, e.c.
	Human	Human *Oruvail*®	Pig KT-ratiop	Pig Gabrilen®	Pig Diractin®
	50/100 (mg)	50 (mg)	50 (mg)	50 (mg)	50 (mg)
	n.a.	n.a.	$n = 6$	$n = 6$	$n = 4$–24[c]
Superficial muscle					
t_{max} (h)	*1*		2	5	1
c_{max} (μg mL^{-1})*	*55*		1.5	19 (31)	95
$t_{1/2,a}$ (h)**	*2*		2	(2)	6
$AUC_{0-8\,h}$ (μg h mL^{-1})			6.0	87	447
$AUC_{0-24\,h}$ (μg h mL^{-1})	*200*		6.3+	95+	847
Deep muscle					
t_{max} (h)			2	3	1
c_{max} (μg mL^{-1})*			1.7	9 (17)	120
$t_{1/2,a}$ (h)**			2	2	4
$AUC_{0-8\,h}$ (μg h mL^{-1})			7.6	45	356
$AUC_{0-24\,h}$ (μg h mL^{-1})			8.2+	51+	652
Plasma					
t_{max} (h)		*2*	2	4	4
c_{max} (μg mL^{-1})*		*2.4*	20	0.12	0.1
$t_{1/2,a}$ (h)**		*2.1*	2	2	6
$AUC_{0-8\,h}$ (μg h mL^{-1})			101	0.8	0.7
$AUC_{0-24\,h}$ (μg h mL^{-1})		*37*	108+		1.4
c_{max} BW (g^2 mL^{-1})		*88/273*++	200	∼1.2	∼1.0
AUC BW (g^2 h mL^{-1})		*2.2/2.6*++	∼1.3	∼0.01	∼0.01

KT-ratiop = Ketoprofen-ratiopharm® (Ratiopharm).
[a] Intramuscular injection data (italic) stem from ref. Tegeder et al. (2001) and are multiplied by 10^3, to allow for the drug binding effect: assuming $1/10^3$ free/bound ketoprofen concentration ratio (Borga and Borga, 1997).
[b] Human reference data (italic) for Oruvail® (Sanofi-Aventis) are taken from the product originators information.
[c] Smaller number for some of the late-time data points.
* c_{max} corresponds to the well defined concentration peak.
** $t_{1/2,a}$ read-off directly from the linear part of logarithmic concentration *vs*. time plot.
+ Based on extrapolation of the $t = 8$ h concentration value to 24 h using the given $t_{1/2,a}$.
++ Human oral drug results that are normalized with regard to the body weight refer to the fasted and fed subjects, respectively.

Table 3
Cumulative ketoprofen concentration (area under the concentration *vs.* time curve, AUC) in peripheral muscle tissue and plasma of pigs treated with a single application of various drug amounts on non-occluded skin surface or orally measured at $t = 8$ h

n	Ketoprofen Dose[a]		Muscle[b]		Carrier gel ratio	Plasma
	Total (mg)	Per Area ($\mu g\ cm^{-2}$)	Superficial ($\mu g\ h\ g^{-1}$)	Deep ($\mu g\ h\ g^{-1}$)		($\mu g\ h\ mL^{-1}$)
Ketoprofen-ratiopharm® 50, oral						
3	50		6	8		101
Diractin®, on the skin (= e.c.)						
4	17	170	147	97	**2**	0.4
4	50	500	278	266	**6**	0.7
4	50	1500	440	428	**(9)[c]**	0.7
Gabrilen® gel, on the skin (= e.c.)						
4	17	170	47	54		0.3
4	50	500	104	44		0.8

The horizontal box and the vertical shadow highlight depth independency and dose area dependency, respectively, observed with Diractin®.
[a]500 $\mu g\ cm^{-2} = 0.5\ mg\ cm^{-2}$ is the highest dose of a semisolid product per area that can be used in a single application; higher area doses require repeated applications over a period of time.
[b]Superficial: 15 mm below the skin; deep: 15 mm to ≤30 mm below the skin. *n* = number of test animals.
[c]Calculated based on the drug dose of 500 $\mu g\ cm^{-2}$.

The real drug concentration peak appears 3–4 h post-topical Gabrilen®, application, however,[2] the drug concentration at this peak being 1.5–2 times below the starting concentration value. After this clear peak, the measured intramuscular ketoprofen concentration, which originates from a conventional NSAID gel application, decreases quasi-exponentially with an apparent half-life of $t_{1/2,a}$ ~2 h in the superficial as well as deep muscles (cf. Table 2). The average drug concentration in superficial muscle for Gabrilen® is $\sim 14 \times (1 \pm 0.7)\ \mu g\ g^{-1}$ with a median value of $\sim 11\ \mu g\ g^{-1}$. In the deep muscle the drug concentration is somewhat, but insignificantly ($p = 0.21$), lower: $\sim 7.5 \times (1 \pm 0.7)\ \mu g\ g^{-1}$, median $\sim 6\ \mu g\ g^{-1}$. For comparison, the maximum total ketoprofen concentration arising from an oral drug uptake into superficial or deep muscles is $1.6 \times (1 \pm 0.44)$ or $1.8 \times (1 \pm 0.28)\ \mu g\ g^{-1}$, respectively.[3]

The results measured with the carrier-based formulation on porcine skin are different. Before the data averaging, a "primary" and "secondary" peak are "discernible" in some intra-muscular drug concentration *vs.* time curves. Averaging the data from several test series eliminates such "peak", however, demonstrating that they are due to statistical scattering. The averaged time *vs.* drug concentration curve, indeed, shows only a single, early peak at t_{max} ~1 h (cf. Fig. 1). The average intramuscular drug concentration at later points in time decreases for approximately 9–12 h. The decrease for the carrier-based formulation is quasi-exponential, with $t_{1/2,a}$ ~6 h and $t_{1/2,a}$ ~4 h in superficial and deep muscles, respectively.

During the second 12 h period post-Diractin® application the average intramuscular ketoprofen concentration declines less rapidly. (The apparent, abrupt fall in ketoprofen concentration measured between 20 and 24 h post-application is in our view just a statistical artefact.)

The area under the curve (AUC), or better to say the area under the lines that connect all the drug concentration data points measured during 0–8 h or 0–24 h post-tested product application, highlights functional differences between various formulations (cf. Table 2 for $t = 0$–8 h and Table 3 for the full data set). The respective drug concentration ratio is 2.7 or 6 for the carrier- and gel-based products, during first third of a day, and 27 or 30, during the whole day post application, as calculated from all the measured data pertaining to superficial or deep muscles, respectively (cf. Table 3). Such a high ratio for Diractin® suggests a high bioavailability of the drug applied on the skin in Transfersome® carriers, in agreement with the low measured residual drug amount on the skin at $t = 24$ h, which is in the range of a few percent. The fact that peripheral AUC for Diractin® multiplied by volume of the treated muscle and divided by the apparent half-life of the drug elimination corresponds to 0.68 of total applied dose (or 68% = AUC $V_m/t_{1/2,a}$/Dose 100% = 34 mg/50 mg 100%) supports credibility of the conclusion. For a conventional gel the same kind of estimate yields merely 3.3 mg, or 6.6%.

Elevating locally applied Diractin® dose on the skin raises peripheral drug concentration. Temporal profile of Diractin®-mediated intramuscular drug concentration is nearly unaffected by such an increase, however, except for the apparently faster

[2] It is possible, but unlikely, that this peak is a consequence of insufficient experimental resolution.
[3] This implies that relative free drug's concentration in plasma is at most 8% (ratio: ~11), but the real value is probably much smaller (<1%), as in case of oral ketoprofen usage.

drug concentration build-up in deep peripheral muscles by relatively higher doses per area (data not shown).

In contrast, the area under the lines that connect the intramuscular concentration data originating from a conventional gel Gabrilen® application is unaffected by the epicutaneously applied ketoprofen amount, or nearly so. Some, but inconsistent, Gabrilen® area dose dependency is detected merely in the peripheral muscles close to the treated skin surface (cf. Table 3).

Oral ketoprofen produces at least 10× lower intramuscular concentration of ketoprofen compared with epicutaneously applied ketoprofen formulations. A single oral dose of ketoprofen leads to the drug concentration peak at t_{max} ~2 h with quasi-exponential tail ($t_{1/2}$ ~2 × (1 ± 0.2) h). Some, if not most, of the orally applied drug found in peripheral muscles probably stems from the residual blood in such tissues.

3.4. Drug transport into blood

Ketoprofen concentration in plasma of the animals treated with Gabrilen® or Diractin® peaks at t_{max} ~4 h (cf. Fig. 2). c_{max} in plasma is aligned with the (secondary?) drug concentration peak in the muscles of gel treated animals. In contrast, the plasma drug concentration resulting from an oral drug attains the highest value earlier, already at t_{max} ~2 h.

Plasma concentration maximum for an oral drug is several orders of magnitude higher than the result of an epicutaneous NSAID application (cf. Fig. 2).

The systemic drug elimination time, read-off directly from the measured data set, is $t_{1/2,a}$ ~2 h for the tested oral ketoprofen product and 6 h for Diractin®.

The area under the plasma concentration curve is roughly proportional to the *total drug amount applied* on the skin (cf. Table 3). Repeated short-term local application of the drug, in the simplest possible picture, should therefore bring additional drug into all compartments. In other words: the added drug should simply "top-up" the pre-existing drug in the organ. This is suggested by smaller area under the curve for the pigs that received a single drug dose of 5 mg kg^{-1} BW on the skin ($AUC_{0-24\,h}$ = 1.4 μg h mL^{-1}) compared with the ~5× higher $AUC_{0-24\,h}$ for the pigs treated twice daily with 1.46, 3.65 and 7.26 mg kg^{-1} BW in Diractin® for 6 months (respective $AUC_{0-24\,h}$ = 4, 6.4, and 11.9 μg h mL^{-1}). The corresponding, less relevant, average daily drug concentrations were measured to be 0.19, 0.29 and 0.54 μg mL^{-1}, which is ~10× higher than the result of a single Diractin® application on the skin (0.05 μg mL^{-1}; cf. Fig. 3). For comparison: the area under the curve for the pigs' plasma, calculated by Bateman extrapolation, is $AUC_{0-24\,h}$ = 121 μg h mL^{-1}, after a single oral application of ~5 mg kg^{-1} of pig body weight.

The apparent systemic half-life of ketoprofen is moderately shortened for the lower two locally applied tested doses of Diractin® ($t_{1/2,a}$ ~4.5 h).

Based on compositional comparison and the results of *in vitro* permeation experiments done with several conventional topical ketoprofen gels or Diractin® we conclude that free drug concentration in the carrier-based product is approximately 10× lower than in a simple gel (Cevc et al., submitted for publication-b). The differential, and greater, ketoprofen transport from Diractin® must consequently rely on transport mechanism that is concentration independent but involves the drug-loaded carriers. The latter must also be able to move spontaneously through and below the skin, as this is the main functional difference between conventional gels (containing the free drug) and Diractin® (containing the drug associated with the carriers). Similarity of the results measured with various conventional ketoprofen gels supports such general conclusion.

4. Discussion

Diffusion of NSAID molecules through the skin is insufficient for achieving high drug concentration in the deep subcutaneous target tissues. Increasing epicutaneously applied drug amount in a conventional gel thus may elevate ketoprofen concentration in superficial muscle but also raises the drug concentration in rest of the body (see Table 3); conventional gel dose escalation consequently lowers peripheral *vs*. systemic drug concentration ratio (see the lower line in Fig. 4). Likewise, skin permeation enhancers may increase transcutaneous drug diffusion, but then also feed more drug into systemic blood circulation. In the worst realistic situation (Radermacher et al., 1991), either of these two measures can bring peripheral and systemic NSAID concentrations to a similar level, annihilating any benefit from local drug application.

The data given in Table 2 and Fig. 1 highlight on the problem. During the first third of the day following 50 mg (0.5 mg kg^{-1}) ketoprofen application in Gabrilen® on the skin, the average drug concentration shows a tendency (p = 0.21) to be ~50% higher in superficial muscle than in deep peripheral muscle (median value for the latter: ~8 μg mL^{-1}; range: 2.6–19 μg mL^{-1}). An injection of 100 mg of the drug (0.61 mg kg^{-1} BW) into human muscle produces median concentration of 55 μg mL^{-1} (range: 26–124 μg mL^{-1} (Tegeder et al., 2001)), calculated under the assumption that only 0.1% of ketoprofen in muscles is free, given that the protein content in such tissue is 4–5× higher than in the plasma where 0.5% of the drug is free (Borga and Borga, 1997).

The situation is different for the carrier-based formulation Diractin®. The product has approximately 50-times lower skin permeability than Gabrilen® *in vitro* (cf. Table 1). Despite this, the carrier-based formulation generates a depth independent (p = 0.74) drug concentration profile during first 8 h post-application with an average value rather close to that of an injection. More precisely, Diractin® gives raise to intramuscular concentration of 54× (1 ± 0.6) μg g^{-1} (median: 50 μg g^{-1}) in superficial and of 64× (1 ± 0.3) μg g^{-1} (median: 63 μg g^{-1}) in deep muscle, which is approximately 1/3 of the similarly measured dermal drug concentration. Both intramuscular results are close to the ketoprofen concentration measured after an i.m. injection in human volunteers of 50 mg of the drug (Tegeder et al., 2001).

The significant (p < 0.0005) differences between Diractin® and conventional gels discussed in previous paragraphs confirm that that the carrier-based product relies on a different mode of drug deposition, seemingly resembling an intramuscular injec-

tion more than a diffusion from a topical gel. The conclusion is supported by the well differentiated time dependencies. On the one hand, the drug from the ultradeformable vesicles in Diractin® compared with a conventional topical gel is consistently delivered faster (t_{max} = 1 h *vs.* t_{max} = 3.5 h) and reaches a higher concentration in peripheral tissues. On the other hand, the drug delivered into subcutaneous muscles with ultradeformable carriers remains in such tissues longer ($t_{1/2,a}$ = 4–6 h) than the drug that has diffused there from a conventional formulation ($t_{1/2,a}$ = 2 h).[4]

The high carrier-mediated intramuscular ketoprofen concentration thus corroborates the concept of direct, diffusion independent, transport of the drug mediated by ultradeformable carrier across the skin and beyond. During skin crossing the drug must be protected somehow from the local clearance that inevitably dissipates free drug concentration. This is tantamount to saying that the drug applied with an ultradeformable carrier on the skin can reach deep subcutaneous tissues loaded on or into the vesicles, which must have crossed the skin intact.[5] If this were not the case, not only the drug's deposition into but also drug's clearance from muscles below an application site would have to be similar to that of an injected free drug. If so, the apparent drug elimination half-life for Diractin® would have to be ∼2 h, as after an i.m. injection of ketoprofen (Tegeder et al., 2001), rather than >4–6 h as is observed (cf. Table 2). The carrier-caused prolongation of local drug residency time is also notable, as is similarity of the drug elimination time measured with Diractin® ($t_{1/2,a}$ = 4–6 h) or with the sustained release oral capsule (Oruvail®, Sanofi-Aventis, $t_{1/2}$ = 5.4 h, manufacturers specification). Ketoprofen thus appears to be released rather slowly from the drug-loaded carriers that have reached deep subcutaneous tissue. This indirectly proves that the drug from Diractin® is transported through and deep below the skin associated with physically intact carriers.

Other researchers have tried to control and improve transdermal NSAID delivery. Simple skin permeation enhancement, e.g. by using sono-phoresis (Cagnie et al., 2003) or ionto-phoresis (Panus et al., 1999), is insufficient for the purpose. Phospholipid organogels (Dowling et al., 2004), conventional liposomes (Valenta et al., 2000; Maestrelli et al., 2005) or nanoparticles (Ricci et al., 2005), but also ultradeformable vesicles (Cevc and Blume, 2001) have been tested with a similar idea in mind. Only seldom, if ever, the resulting effect on NSAID delivery into deep subcutaneous tissues was really beneficial (Cevc and Blume, 2001). Non-deformable colloids on the skin, which cannot cross the barrier intact (Cevc et al., 2002) typically lower the dissolved NSAID concentration and thus hamper drug diffusion through the skin (cf. Table 1), thus diminishing a potential beneficial effect of skin (semi)occlusion by at least some colloid formulations.

Particles or vesicles that can cross the skin but do not maintain integrity during the process loose their "cargo" right below the barrier. Such "carriers" are therefore unable to prevent local clearance, and drug distribution in peripheral tissues with or without the colloidal vehicle is remains essentially the same. Formulations containing such non-deformable carriers are also prone to yield similar results *in vivo* and *in vitro*. The prerequisite for significantly improved local drug delivery via the skin is the carrier's ability to cross the barrier *and* to bring the drug molecules past the cutaneous capillary plexus. This explains superiority of the colloidal carriers (Jain et al., 2005; Godin and Touitou, 2003) that are at least remotely related to the ultradeformable carriers (Cevc, 2004; Cevc et al., submitted for publication-b) contained in Diractin®.

For example, in the most recently published study dealing with one kind of reportedly elastic vesicle, diclofenac was delivered into rat muscles to concentration levels between 2.2 ± 0.14 and 5.3 ± 0.22 μg g^{-1}, 12 h post-epicutaneous application (Jain et al., 2005). A similar dose of commercial hydrogel tested under comparable conditions on the skin of rats afforded drug concentrations of just between 0.41 ± 0.07 and 1.1 ± 0.09 μg g^{-1} (Jain et al., 2005). The reported vesicle-dependent improvement is thus appreciable (5.4× or 4.8×, respectively). Relative improvement is less than that measured by us in rats and pigs (∼20×) treated with fully functional diclofenac-loaded Transfersome® vesicles (Cevc and Blume, 2001).

Here we report a similar gain (10–30×) for ketoprofen in Transfersome® on porcine skin. Our corresponding absolute ketoprofen concentration is also ≥5× higher (cf. Fig. 1) than that measured previously with diclofenac in rats (Jain et al., 2005). Whilst molecular differences between ketoprofen and diclofenac could be invoked to explain the difference, we rather believe that the main reason is the refined Transfersome® composition in Diractin®. To an extent, this also applies to our previous formulations of diclofenac in ultradeformable carriers ("Transfenac"; Cevc and Blume, 2001). Indeed, the latter also excel with relatively long residency time and 10× higher drug concentrations in subcutaneous tissue compared with the drug from commercial hydrogel (Voltaren® Emulgel®, Novartis). As in case of ketoprofen in ultradeformable carriers, the Transfersome® directed diclofenac delivery into peripheral tissues improves with decreasing drug dose per body weight and with increasing treated muscle thickness. Finally, when using ultradeformable carriers on skin, intramuscular diclofenac concentration is also nearly depth independent (Cevc and Blume, 2001), as is confirmed in Table 3 for ketoprofen as well.

We made semi-quantitatively similar observations in mice, rats and pigs treated with diclofenac in Transfersome® vesicles on the skin (after body weight normalisation) (Cevc and Blume, 2001). Our conclusions pertaining to ultradeformable vesicles comprised in Diractin® should therefore be rather generally valid, the notoriously large site-to-site variability reported for conventional topical gels (Shah et al., 1996) notwithstanding.

[4] Ketoprofen disappears more slowly from the plasma of humans treated on the skin for two weeks, with a comparable drug dose in conventional gel ($t_{1/2} \sim 17.1 \pm 9.1$ h (Flouvat et al., 1989)).

[5] Theoretical possibility of carrier disintegration in the skin and reformation below the barrier is of no real importance as too little free drug is found there to allow for the loss recovery and then lead to a meaningful amount of drug transport in the depth below application site.

Relative bioavailability of ketoprofen from a topical gel applied on human skin reportedly varies between 1.08 ± 0.63, 0.90 ± 0.50 and 0.74 ± 0.38, respectively, when the arm, back, and knee are studied (Shah et al., 1996). This probably reflects more than just skin permeability variation. Monteiro-Riviere et al. (1993) found out, for example, that the sites on porcine body periphery that are perfused by direct cutaneous vasculature (e.g. in ventro-caudal region) have a higher probability of local entry of transepidermally absorbed piroxicam into systemic blood circulation. In contrast, the sites perfused by musculocutaneous arteries retain a larger fraction of the absorbed drug within deep local tissues (Monteiro-Riviere et al., 1993). Local clearance diminution by using ultradeformable carriers could therefore minimise site-to-site variability, in addition to making any product based on such carriers insensitive to skin permeability changes. These inferences are indirectly supported by our earlier findings with diclofenac in Transfersome® (Cevc and Blume, 2001).

Relative contribution of perfusion to NSAID concentration in deep peripheral tissues is a function of relative dose (especially the drug dose per body weight) as well as of skin permeability, tissue exchange rate, and plasma clearance ratios for the chosen NSAID. However, the drug concentration build-up in peripheral target tissues, which originates from a conventional topical gel, also involves at least some secondary drug re-distribution from plasma into muscles. This is particularly likely for the products with a relatively high transcutaneous diffusion rate delivering the drug into extended but slim subcutaneous tissues. Multiple, 3–4 daily, drug applications, which are the current gold standard in field of conventional topical NSAID products, increase relative importance of perfusion as well. Several independent studies came to similar conclusion (Radermacher et al., 1991; Singh and Roberts, 1994a; Flouvat et al., 1989).

Conventional topical ketoprofen products lead to low systemic drug concentration only when they are used at a relatively low drug dose; otherwise perfusion and systemic effects become important. Illustrative values for humans receiving ~6 mg ketoprofen per kg body weight are: $c_{max} = 0.144 \times (1 \pm 0.63)\ \mu g\ mL^{-1}$; ~2.6% of applied dose (Flouvat et al., 1989); $\sim 1.6\ mg\ kg^{-1}$ body weight: $c_{max} \sim 0.119\ \mu g\ mL^{-1}$; bioavailability 5–8% (Tegeder et al., 2001). These values resemble our ketoprofen results. Specifically for the dose of ~5 mg ketoprofen per kg body weight in Gabrilen® gives plasma concentration of $c_{max} = 0.123 \times (1 \pm 0.211)\ \mu g\ mL^{-1}$. For the same total and area dose ($0.5\ mg\ cm^{-2}$, cf. Fig. 2) of Diractin® we obtain $c_{max} = 0.116 \times (1 \times 0.72)\ \mu g\ mL^{-1}$. Identical oral drug dose (50 mg) brings plasma ketoprofen concentration to 167× higher level of $20.1 \times (1 \pm 0.68)\ \mu g\ mL^{-1}$. For comparison, an injection of 50 mg ketoprofen ($0.61\ mg\ kg^{-1}$ BW) into a thigh gives maximum plasma concentration of $\sim 7\ \mu g\ mL^{-1}$ at $t_{max} \sim 0.5$ h (Tegeder et al., 2001), assuming $1/10^3$ free/bound drug ratio (Borga and Borga, 1997).

The position of plasma concentration maximum was better defined in our experiments with pigs (t_{max} ~4 ± 0.5 h) than in earlier independent human studies (16.5 ± 14.9 h; Flouvat et al., 1989). Our porcine data are rather close to the outcome of a recent human study (t_{max} ~2.67–4.04 h), however, which moreover found a long "tail", i.e. "slow" final clearance of the gel-derived drug (Tegeder et al., 2001).

In our experience, ketoprofen elimination from the blood of piglets or humans treated with the oral drug is similarly fast, with $t_{1/2}$ ~2 h (Kantor, 1986); this is not surprising given that pharmacokinetic parameters of ketoprofen are known to scale with body weight (Lees et al., 2004).

In contrast to the situation observed with Diractin®, ketoprofen is cleared from porcine muscles treated epicutaneously with a conventional topical ketoprofen gel with an apparent half-life of $t_{1/2}$ ~2 h. This value is similar to the half-life of intramuscularly injected ketoprofen (Tegeder et al., 2001), and supports the concept of secondary drug redistribution and final clearance via plasma for conventional gels. The short peripheral elimination time of the drug from the conventional gel also explains why such products must be applied a number of times per day, typically 4×, to exert some therapeutic effect.

The high initial total muscle/plasma drug concentration ratio of ~100 (for a 50 mg total drug dose) for Diractin® is inexplicable by drug redistribution ("perfusion"). Even at its peak the total drug concentration in plasma is ~20 lower than in the treated muscles. We therefore exclude the possibility that ketoprofen from Diractin® on the skin experiences a similar flip-flop phenomenon as the drug from a topically applied conventional gel in humans (Flouvat et al., 1989) or rodents (Singh and Roberts, 1994a),[6] which can also lead to two intramuscular drug concentration peaks. We rather believe that the early, first drug concentration peak (cf. Fig. 1) is a consequence of an unusually fast initial drug transport through the high-permeability/limited-capacity pathways in the skin (Cevc and Vierl, 2007) with ultradeformable carriers. It is moreover possible that the penetrability of such pathways is positively influenced by NSAID loaded carriers on skin surface.

Another reason for improved ketoprofen deposition in peripheral tissues by ultradeformable carriers is the prolonged drug retention in, and by, such carriers, after they have crossed the skin barrier. Direct support of such notion is our observation that the drug clearance rate after intramuscular injection is 2–3 times shorter (Tegeder et al., 2001) than after an epicutaneous Diractin® application.

The different nature of ketoprofen delivery into peripheral tissues from the gels containing the dissolved drug or from Diractin®, which comprises the drug in association with ultradeformable carriers, is evident in Fig. 4. The figure also highlights the dissimilar area dose dependency of peripheral *vs*. systemic ketoprofen concentrations derived from Diractin® or a conventional topical gel on the skin: whereas raising the drug dose per area improves targeting of the former it deteriorates the local drug delivery of the latter. Fig. 4 thus indirectly but unambiguously proves that majority of ultradeformable carriers

[6] The concentration of compounds in tissues below a dermally treated site in anaesthetised rats according to Singh and Roberts (1994a) peaks twice: first, at 2–4 h and, second, at around 10 h. The presence of the first peak has been attributed to direct penetration and the re-supply from systemic blood was made responsible for the second peak.

(Transfersome® vesicles) can overcome the skin barrier intact (Cevc et al., 2002) and still loaded with the drug that is partly adsorbed to and partly encapsulated into the carriers. If the Transfersome® carrier did not protect the drug the latter would be cleared through cutaneous blood vessels and then could not reach deep subcutaneous muscles to reach there a high local concentration!

5. Conclusions

Despite its suitability (Kantor, 1986), the dissolved ketoprofen in conventional topical gels diffuses through the skin *in vitro* relatively inefficiently (after allowance for the ∼7x higher dose used in vitro: Gabrilen® ∼17%; Togal® Mobil Gel and Fastum®: ∼5% (Cevc et al., submitted for publication-b); see also Fig. 1) during 24 h period. The drug from ultradeformable carriers in a gel is even 10× less prone to cross the skin *in vitro* (0.4% (Cevc et al., submitted for publication-b); see also Table 1 and Fig. 1).

Ketoprofen applied epidermally on pigs in a conventional gel crosses the organ but is cleared from underlying muscles quite rapidly. Its half-life in pigs ($t_{1/2}$ ∼2 h, cf. Table 2) consequently resembles that of the drug injected intramuscularly (Tegeder et al., 2001), and is identical to the value measured in plasma of healthy young volunteers who took the rapidly absorbed oral drug (Oruvail®; Sanofi-Aventis information). We moreover estimate the drug bioavailability from a conventional gel to be only ∼7% in pigs, which is only little higher than to our result measured with human skin *in vitro* (∼5%; vide supra). Conversely, the drug applied on the living pig skin with the ultradeformable carriers in Diractin® disappears from subcutaneous peripheral tissues 2–3 times more slowly ($t_{1/2,a}$ ∼4–6 h, cf. Table 2), and from the skin nearly completely (>99%), having at least 10× higher estimated local bioavailability (∼70%). The latter value thus exceeds the corresponding *in vitro* bioavailability by a factor of ∼200! Such a vast *in vitro–in vivo* difference observed with Diractin® contrasts the similarity of ketoprofen *in vitro* and *in vivo* transport from conventional topical gels and proves fundamental differences between the former and the latter kind of product.

Systemic bioavailability of ketoprofen from Diractin® applied epicutaneously on non-occluded skin is proportional to the total applied drug dose and generally low (∼1/100 of oral drug).

Escalating conventional topical gel dose raises systemic *vs.* local ketoprofen concentration ratio, which precludes proper drug targeting into peripheral tissues with such kind of a product (cf. Fig. 4). In contrast, increasing the drug and carrier dose per area improves ketoprofen targeting into superficial as well as deep muscles below the site of epicutaneous Diractin® application, compared with systemic drug concentration. The difference illustrates and corroborates the conclusion that ketoprofen transport with ultradeformable carriers through the skin *in vivo* is not diffusive but rather depends on skin penetration by the carriers. In contrast, diffusion is responsible for the drug transport from conventional topical gels (cf. Fig. 4).

Diractin® consequently employs a well differentiated and novel mechanism of action to achieve efficient transcutaneous drug transport, local drug accumulation in deep peripheral tissue below the skin application site, and sustained drug release. This involves drug concentration-independent (cf. Tables 1 and 2), hydration driven (Cevc and Gebauer, 2003; Cevc et al., submitted for publication-a) skin penetration by the carrier. Preservation of the carrier integrity and loading during skin crossing (Cevc et al., submitted for publication-b) as well as the carriers ability to bypass cutaneous blood vessels (Cevc et al., submitted for publication-b) are needed for the directed (cf. Fig. 4) and sustained (cf. Fig. 1) drug release into peripheral target tissue.

Porcine data are relevant for humans. The similarity of porcine and human skin barrier (Meyer, 1996; Vardaxis et al., 1997), semblance of oral NSAID biodistribution and clearance in different species (Lees et al., 2004), and the agreement between the pharmacokinetic characteristics we determined for pigs (cf. Figs. 1 and 2) and the published human ketoprofen pharmacokinetic data (cf. Table 2) support this statement. We therefore conclude that Diractin® represents a truly innovative product with pharmacokinetic features more similar to a local injection than to a topical application of a conventional NSAID gel.

Acknowledgements

We are thankful to N. Daberkow-Nitsche, J. Füner and I. Susetzky for their help with the animal experiments.

References

Borga, O., Borga, B., 1997. Serum protein binding of nonsteroidal antiinflammatory drugs: a comparative study. J. Pharmacokinet. Biopharm. 25, 63–77.

Cagnie, B., Vinck, E., Rimbaut, S., Vanderstraeten, G., 2003. Phonophoresis versus topical application of ketoprofen: comparison between tissue and plasma levels. Phys. Ther. 83, 707–712.

Cevc, G., 1996. Lipid suspensions on the skin. Permeation enhancement, vesicle penetration and transdermal drug delivery. Crit. Rev. Therap. 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. M. 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., 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., Gebauer, D., 2003. Hydration driven transport of deformable lipid vesicles through fine pores and the skin barrier. Biophys. J. 84, 1010–1024.

Cevc, G., Mazgareanu, S., Rother, M. Vierl, U. Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations, Int. J. Pharm., submitted for publication.

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 semipermeable barriers, such as the skin, with ultradeformable mixed lipid vesicles, Transfersomes®, liposomes or mixed lipid micelles. Langmuir 19, 10753–10763.

Cevc, G., Vierl, U., 2007. Spatial distribution of cutaneous microvasculature, its depth profile, and local drug clearance after topical drug application on the skin. J. Contr. Rel. 118, 18–26.

Cevc, G., Vierl, U., Mazgareanu, S., 2008. Functional characterisation of novel analgesic product based on self-regulating drug carriers. Int. J. Pharm. 360, 18–28.

Clough, G.F., Boutsiouki, P., Church, M.K., Michel, C.C., 2002. Effects of blood flow on the in vivo recovery of a small diffusible molecule by microdialysis in human skin. J. Pharm. Exp. Ther. 302, 681–686.

Dowling, T.C., Arjomand, M., Lin, E.T., Allen Jr., L.V., McPherson, M.L., 2004. Relative bioavailability of ketoprofen 20% in a poloxamer-lecithin organogel. Am. J. Health Syst. Pharm. 61, 2541–2544.

FDA (Food and Drug Administration) CDER, 1997. Guidance for industry. Nonsterile semisolid dosage forms. Scale-up and post-approval changes. Chemistry, manufacturing and controls; in vitro release testing and in vivo bioequivalence documentation. http://www.fda.gov/cder/guidance.htm.

Figueras, A., Capella, D., Castel, J.M., Laorte, J.R., 1994. Spontaneous reporting of adverse drug reactions to non-steroidal anti-inflammatory drugs. A report from the Spanish System of Pharmacovigilance, including an early analysis of topical and enteric-coated formulations. Eur. J. Clin. Pharmacol. 47, 297–303.

Flouvat, B., Roux, A., Delhotal-Landes, B., 1989. Pharmacokinetics of ketoprofen in man after repeated percutaneous administration. Arzneimittelforschung 39, 812–815.

Godin, B., Touitou, E., 2003. Ethosomes: new prospects in transdermal delivery. Crit. Rev. Therap. Drug Carrier Syst. 20, 63–102.

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.

Higaki, K., Asai, M., Suyama, T., Nakayama, K., Ogawara, K., Kimura, T., 2002. Estimation of intradermal disposition kinetics of drugs: II. Factors determining penetration of drugs from viable skin to muscular layer. Int. J. Pharm. 239, 129–141.

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.

Kantor, T.G., 1986. Ketoprofen: a review of its pharmacologic and clinical properties. Pharmacotherapy 6, 93–103.

Kretsos, K., Kasting, G.B., 2005. Dermal capillary clearance: physiology and modelling. Skin Pharmacol. Physiol. 18, 55–74.

Lees, P., Giraudel, J., Landoni, M.F., Toutain, P.L., 2004. PK/PD integration and PK/PD modelling of nonsteroidal anti-inflammatory drugs: principles and applications in veterinary pharmacology. J. Vet. Pharmacol. Ther. 27, 491–502.

Lin, J., Zhang, W., Jones, A., Doherty, M., 2004. Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials. Br. Med. J. 329, 324–330.

Maestrelli, F., Gonzalez-Rodriguez, M.L., Rabasco, A.M., Mura, P., 2005. Preparation and characterisation of liposomes encapsulating ketoprofen–cyclodextrin complexes for transdermal drug delivery. Int. J. Pharm. 298, 55–67.

Meyer, W., 1996. Bemerkungen zur Eignung der Schweinehaut als biologisches Modell für die Haut des Menschen. Hautarzt 47, 178–182.

Monteiro-Riviere, N.A., Inman, A.O., Riviere, J.E., McNeil, S.C., 1993. Topical penetration of piroxicam is dependent on the distribution of the local cutaneous vasculature. Pharm. Res. 10, 1326–1331.

Panus, P.C., Ferslew, K.E., Tober-Meyer, B., Kao, R.L., 1999. Ketoprofen tissue permeation in swine following cathodic iontophoresis. Phys. Ther. 79, 40–49.

Radermacher, J., Jentsch, D., Scholl, M.A., Lustinetz, T., Frolich, J.C., 1991. Diclofenac concentration in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br. J. Clin. Pharmacol. 31, 537–541.

Ricci, M., Puglia, C., Bonina, F., Di Giovanni, C., Giovagnoli, S., Rossi, C., 2005. Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. J. Pharm. Sci. 94, 1149–1159.

Shah, A.K., Wei, G., Lanman, R.C., Bhargava, V.O., Weir, S.J., 1996. Percutaneous absorption of ketoprofen from different anatomical sites in man. Pharm. Res. 13, 168–172.

Singh, P., Roberts, M.S., 1994a. Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. J. Pharmacol. Exp. Ther. 268, 144–151.

Singh, P., Roberts, M.S., 1994b. Dermal and underlying tissue pharmacokinetics of lidocaine after topical application. J. Pharm. Sci. 83, 774–782.

Tegeder, I., Lötsch, J., Kinzig-Schippers, M., Sörgel, F., Kelm, G.R., Meller, S.T., Geisslinger, G., 2001. Comparison of tissue concentrations after intramuscular and topical administration of ketoprofen. Pharm. Res. 18, 980–986.

Vaile, J.H., Davis, P., 1998. Topical NSAIDs for musculoskeletal conditions: a review of the literature. Drugs 56, 783–799.

Valenta, C., Wanka, M., Heidlas, J., 2000. Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. J. Contr. Rel. 63, 165–173.

Vardaxis, N.J., Brans, T.A., Boon, M.E., Kreis, R.W., Marres, L.M., 1997. Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. J. Anat. 190, 601–611.

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