# Comparison of Tissue Concentrations After Intramuscular and Topical Administration of Ketoprofen

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***Purpose.*** To assess whether topical ketoprofen, which has been reported to provide analgesic effects in clinical studies, reaches predictable tissue concentrations high enough to account for the reported analgesia. Intramuscular ketoprofen was used as positive control.

***Methods.*** Muscle and subcutaneous tissue concentrations were assessed by microdialysis. Plasma and tissue concentrations after intramuscular injection were described using a three-compartment population pharmacokinetic model. The prediction performance of the model was assessed by superimposing tissue concentrations of 12 subjects that did not participate in the present study.

***Results.*** Most dialysate concentrations after topical dosing of ketoprofen (100 mg) were below the quantification limit of 0.47 ng/ml. Plasma concentrations increased slowly and reached an apparent plateau of 7–40 ng/ml at 10–12h. No decline was observed up to 16 h. Tissue concentrations after intramuscular injection (100 mg) were about 10 times higher than those after topical dosing. Tissue concentrations measured in the majority of the 12 subjects that did not participate in the present study were found within the range of two-thirds of the predicted concentrations.

***Conclusion.*** Predictable and cyclooxygenase-inhibiting concentrations of ketoprofen were achieved in subcutaneous and muscle tissue after intramuscular but not after topical dosing. Thus, the tissue concentrations of ketoprofen after topical administration can hardly explain the reported clinical efficacy of topical ketoprofen.

**KEY WORDS:** microdialysis; ketoprofen; NSAIDs; topical administration; population pharmacokinetic model.

## INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are generally effective for the relief of pain and inflammation (1–3). However, in a significant number of patients, oral administration of NSAIDs can result in a number of adverse effects that affect, primarily, the gastrointestinal tract and the kidney (4–6). It has been reported that between 1.3% and 1.6% of regular NSAID users are at risk for gastrointestinal side effects requiring a hospital visit or hospitalization (7). One approach to reducing the side effects associated with oral NSAID use has been to apply the drug to the skin overlying affected joints and muscles. Despite having been approved by health authorities for the treatment of pain and inflammation, there are doubts that topical NSAIDs have any action other than as rubefacients (8). A systematic review of published controlled clinical studies with topically applied NSAIDs, however, revealed a number needed to treat (NNT) for acute pain of 3.9 and for chronic pain of 3.1 (9). That means that at least one patient in three who uses a topical NSAID will achieve a successful outcome. The NNT reported for topical NSAIDs is similar to the NNTs for oral analgesics in moderate or severe pain (10). As compared with other analgesics such as acetaminophen (NNT for 500 mg = 5.6) or tramadol (NNT for 100 mg = 4.8) the NNT for topical NSAIDs is relatively low (9). However, this positive result may be skewed by publication restricted to positive findings, as negative reports of efficacy are not usually published. From a pharmacological point of view, a specific analgesic effect of an NSAID can only be achieved when a sufficient drug concentration is achieved at the site of injury to inhibit cyclooxygenase activity and local prostaglandin release. In a recent study using microdialysis we have demonstrated that topically administered ibuprofen reached high concentrations in subcutaneous tissue with sufficient reliability. However, concentrations in muscle tissue were highly variable and projected therapeutic levels were only reached in half of the subjects (11). Similar results have been reported for topical diclofenac (12). Although other NSAIDs have not been evaluated for their ability to penetrate to deeper tissue such as muscle, the highly variable muscle penetration findings for ibuprofen and diclofenac are in contrast to their low NNT. Given this, there are at least two possible explanations for the observed similarity between the NNTs of topical and systemic NSAIDs: (i) the analgesic effect of topical NSAIDs is not caused by inhibition of cyclooxygenase activity or (ii) tissue concentrations after systemic NSAID administration are highly variable as well.

The present study was conducted to assess, in a crossover design, (1) local target tissue (dermal and muscle) and (2) plasma concentrations of ketoprofen following either topical or intramuscular administration of an equivalent dose of ketoprofen.

## METHODS

### Study Protocol

Eight healthy male volunteers (age range 24 to 28 years, weight 82.0 ± 8.3 kg, all subjects had normal weight) were included in the study after having given written informed consent. The study was conducted in accordance with the declaration of Helsinki and was approved by the local Ethics Committee.

Microdialysis probes (CMA 120, CMA, Stockholm, Sweden; molecular weight cut off 20 kDa, outer diameter 520 μm, membrane length 30 mm, shaft length 8 cm, outlet tubing 12 cm) were inserted into the subcutaneous tissue and into the medial vastus muscle of the same thigh as has been described previously (11). The sites of skin puncture were covered with a sterile transparent plaster (Tegaderm, 3M, Ontario, Canada) to prevent direct penetration of ketoprofen gel down the catheter. The microdialysis system was constantly per-

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fused with 0.9% saline at a flow rate of 2 μl/min using a microinfusion pump (CMA/100, CMA, Stockholm, Sweden). The volume of the dead space between microdialysis membrane and outlet of the tube was 12.3 μl causing a lag time between diffusion of drug molecules at the membrane and sampling of the dialysate of 6.16 min. Time points were corrected correspondingly.

A 20-min baseline dialysate sample was collected before drug administration. According to a randomized two-way cross-over design the subjects received either an intragluteal injection or a topical dose of 100 mg ketoprofen (Gabrilen® i.m. or 4g 2.5% Gabrilen® Gel, Kreussler, Wiesbaden, Germany). The washout period between the medications was at least 7 days. In three subjects dialysis was repeated following multiple applications of the gel (every 6 h for 4 days). The gel was administered onto the skin of the ventral thigh without occlusion or rubbing (area 20 × 30 cm) including the skin overlying the microdialysis membranes. Dialysate was sampled in 20–30 min intervals up to 8 h after drug administration. Because the skin was somewhat sticky at the site of gel application, it was cleaned with lukewarm water at the end of the dialysis period. Throughout the dialysis period the volunteers stayed in a supine position. The room temperature was kept between 24 and 25°C. Plasma samples were taken up to 12 or 16 h after i.m. and topical ketoprofen, respectively. All samples were stored at –20°C until assay.

### Calibration

To characterize the transfer rate of the probes, the *in vitro* and *in vivo* recovery (rec) of ketoprofen was assessed as previously described (11). That is, probes for *in vitro* calibration were placed into solutions at different concentrations of ketoprofen (1, 2, 20, 100, and 200 μg/ml) and were perfused with 0.9% saline at a flow rate of 2 μl/min. The concentrations in the dialysate ($C_{dialysate}$) were measured and the recovery was calculated as

$$\text{rec}(\%) = 100 \cdot \frac{C_{dialysate}}{C_{ketoprofen\text{-}solution}}.$$

*In vivo* recovery was determined in a pilot study on three subjects using the zero net flux method (13,14). Microdialysis probes were perfused with ketoprofen solutions at five different concentrations between 2 and 200 μg/ml, and the dialysate concentration was measured. The net difference between the perfusate and dialysate ketoprofen concentrations correlated linearly with the concentration of the perfusate, and the recovery was the slope of the regression line (14,15). Subsequently, free ketoprofen tissue concentrations were calculated by dividing the dialysate concentrations through the value of the *in vivo* recovery.

### Analysis of Ketoprofen Concentrations

Because it has been shown previously that pharmacokinetics of R- and S-ketoprofen are identical (16), analysis of plasma and dialysate concentrations was done for racemic ketoprofen. Racemic ketoprofen as analytical standard was obtained from Sigma (Deisenhofen, Germany). Plasma concentrations of ketoprofen after intramuscular administration were assayed by high-performance liquid chromatography (HPLC) (16). Plasma concentrations after topical administration and dialysate concentrations were assayed by means of HPLC coupled with tandem mass spectrometry (LC-MS/MS).

### LC-MS/MS Analysis

Aliquots (200 μl) of human plasma samples were mixed with 100 μL of internal standard solution (50 ng/ml ketoprofen-[$^2$H, $^{13}$C]). After solid-phase extraction the eluate was analyzed for ketoprofen. Calibration standards were prepared in human plasma and assayed at the beginning of each sequence. For control of interassay variation, spiked quality control standards in human plasma were measured in each run in randomized order among samples. Aliquots (20 μl) of dialysate samples were transferred to 0.5 ml polypropylene microtubes, and 50 μl of the internal standard solution (50 ng/ml ketoprofen-[$^2$H, $^{13}$C]) was added to each tube and vortex mixed.

#### *Chromatography*

HPLC analysis was done using a Merck Hitachi LiChroGraph® L-6000A pump (E. Merck, Darmstadt, Germany) connected to a Merck Hitachi L-7250 LaChrom® autosampler (E. Merck). Chromatographic separations were obtained under isocratic conditions using a reversed phase C8 column (Phenomenex® Luna™, 50 × 4.6 mm, 3 μm, Phenomenex® Ltd., Aschaffenburg, Germany) at a flow of 1 ml/min. The mobile phase consisted of acetonitrile and 0.1% acetic acid in water (65/35; v/v). For analysis, 35 μl of the dialysate sample and 40 μl of the human plasma sample were injected onto the LC-MS/MS. Ketoprofen and ketoprofen-[$^2$H, $^{13}$C] eluted after approximately 1.1 min.

#### *Mass Spectrometry*

MS and MS/MS analyses were performed on a PE Sciex API 3000® (Toronto, Ontario, Canada) triple quadrupole mass spectrometer. The instrument was operated in atmospheric pressure chemical ionization (APCI) utilizing a heated nebulizer interface. For measurement of the carboxylic acid ketoprofen the negative ion mode was chosen. High-purity nitrogen was used as nebulizer, curtain, auxiliary, and collision gas. The heated nebulizer temperature was set at 500°C.

Selected reaction monitoring (SRM) was employed using nitrogen as the collision gas with collision energy of 10 eV. Precursor-to-product ion transitions of m/z 253 → m/z 209 for ketoprofen and m/z 257 → m/z 213 for the internal standard, both deriving from the loss of carbon dioxide, were used for the SRM with a dwell time of 200 ms.

Calibration standards were evaluated using a weighted linear regression (1/concentration) with theoretical concentrations of calibration standards and measured peak area ratios (peak area analyte/peak area analytical standard) by MacQuan (version 1.6, Perkin Elmer, Toronto, Canada, 1991-1998). Linearity of the calibration curve was proven from 0.473 ng/ml to 505 ng/ml in human plasma and from 2.53 ng/ml to 505 ng/ml in ultrafiltrated human plasma. The coefficient of correlation for all measured sequences was at least 0.9997 for human plasma and 0.9993 for ultrafiltrated plasma. For human plasma the intraday precision and relative error of the assay determined from the measured concentrations of the spiked quality control standards ranged from 0.7% to

4.1% and –7.0% to 3.1%, respectively, and the interday precision ranged from 2.2%–7.4% with a relative error of –1.6% to 4.9%. For ultrafiltrated plasma the intraday precision and relative error of the assay determined from the measured concentrations of the spiked quality control standards ranged from 1.0% to 4.4% and –4.3% to 1.5%, respectively, and the interday precision ranged from 4.8%–5.1% with a relative error of –3.1% to 4.6%.

### Data Analysis

Ketoprofen plasma and tissue concentrations after intramuscular injection were described by a conventional three-compartment pharmacokinetic model, with an additional dosing compartment (compartment 0). The model consisted of a system of differential equations:

$$dA0/dt = -ka \cdot A0$$

$$dA1/dt = ka \cdot A0 - CL \cdot C1 - CL12 \cdot C1 - CL13 \cdot C1 + CL12 \cdot C2 + CL13 \cdot C3$$

$$dA2/dt = CL12 \cdot C1 - CL12 \cdot C2$$

$$dA3/dt = CL13 \cdot C1 - CL13 \cdot C3 \quad (1)$$

with

$$A0 \text{ at time } 0 = \text{Dose}$$

$$A1, A2, A3 \text{ at time } 0 = 0$$

and

$$C1 = A1/V1,\ C2 = A2/V2 \cdot fu,\ C3 = A3/V3 \cdot fu$$

where *dA(0..3)/dt* describe the change over time of amount of drug in compartments 0 to 3. *A1* to *A3* denote the amount of drug in compartments 1 to 3, respectively; the first order input rate constant after intramuscular injection is denoted by *ka; CL* denotes the total body clearance; and *CL12* and *CL13* the intercompartmental clearances. Because free rather than protein-bound ketoprofen was measured in dialysate, a scaling factor for the tissue compartments was introduced, accounting for the free fraction of ketoprofen, f*u*.

Plasma and dialysate data from all subjects dosed intramuscularly were fitted simultaneously using NONMEM V 1.1 (NONMEM Project Group, UCSF, San Francisco, CA). Plasma data were assigned to compartment 1, muscle dialysate data to compartment 2, and dialysate data from subcutaneous tissue to compartment 3. The ketoprofen dose was defined to enter the system through compartment 0. An additive model was chosen to describe the residual error, denoted by $\varepsilon$, which is a normally distributed parameter with mean zero and variance $\sigma^2$. An additive model was preferred over proportional or mixed additive/proportional error models because the latter resulted in a consistent underestimation of the data, judged by visual inspection. Plasma total ketoprofen concentrations and dialysate-free ketoprofen concentrations were assigned separate residual errors, $\varepsilon_1$ and $\varepsilon_2$, respectively. The interindividual variability was assumed to be lognormally distributed and was described by parameters $\eta$ with mean zero and variance $\omega^2$. Interindividual variability was assigned in a stepwise fashion to each structural parameter of the pharmacokinetic model. The final model was selected on the basis of the NONMEM objective function that is minus twice the logarithm of the likelihood. If by introducing a parameter into the model the NONMEM objective function significantly decreased, this indicated that the fit was improved by the respective parameter, and it therefore remained part of the model, provided that the 95% confidence interval of the estimate of any parameter did not include zero. Statistical significance was judged using likelihood ratio test, with the number of degrees of freedom equal to the difference in the number of free parameters between full and reduced models ($\alpha$-level 0.05). The importance of the subject's covariates, weight and height, for clearances and volumes, and of the plasma albumin concentration for f*u,* were assessed analogously. Estimates of interindividual variance components were converted into percentage coefficients of variation (%CV) by taking their square root and multiplying it by 100. All NONMEM calculations were performed using first order conditional estimation method and "$\eta$-$\varepsilon$ interaction" to reduce the influence of model misspecification.

The population model was used to simulate free ketoprofen concentrations in muscle tissue after intramuscular in-

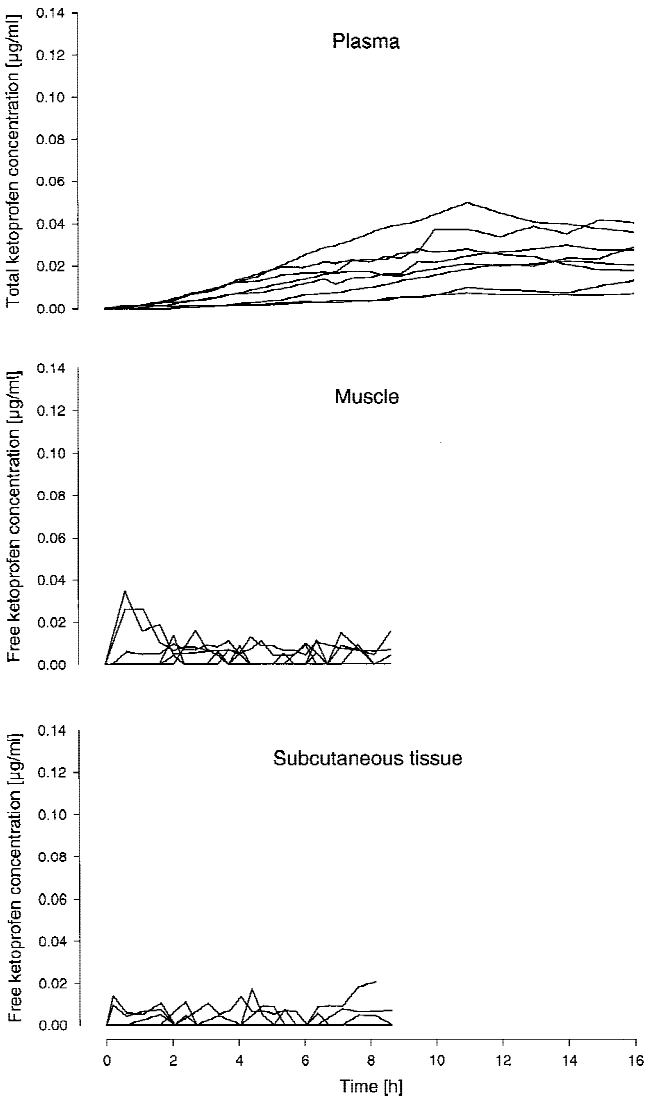


**Fig. 1.** Individual observed ketoprofen concentrations in plasma (total concentrations) and in muscle and subcutaneous tissue (free tissue concentrations) after topical administration of 100 mg ketoprofen gel onto a surface of 20 × 30 cm of the thigh.

jection of 50 mg ketoprofen (Orudis, Aventis Pharma, Germany) into the thigh muscle. Simulated tissue concentrations were compared with muscle dialysate concentrations obtained from 12 healthy volunteers who did not participate in the actual study.

## RESULTS

### *In Vitro* and *In Vivo* Recovery

*In vitro* recovery for ketoprofen was 60.5 ± 7.2% (mean ± SD), respectively. It was constant over a wide concentration range (1–200 μg/ml).

Concentrations of ketoprofen in dialysate and perfusion medium correlated linearly over a wide concentration range (2–1500 μg/ml, $r = 0.997$, $P<0.001$). *In vivo* recoveries for muscle and subcutaneous tissue calculated on the basis of the slope of the regression line were 67.2 ± 10.3% and 58.9 ± 12.2%, respectively.

### Plasma and Tissue Concentrations After Topical Ketoprofen

Plasma and tissue concentration-time courses of ketoprofen after topical administration are shown in Fig. 1. Plasma concentrations slowly increased, reaching an apparent plateau of 0.007–0.05 μg/ml after approximately 10–12 h. No decline was observed during the observation period of 16 h. Most muscle and subcutaneous dialysate concentrations were below the quantification limit of 0.00047 μg/ml. Only in three subjects concentrations of about 0.006 and 0.004 μg/ml could be measured in muscle and subcutaneous tissue, respectively. In these subjects concentrations stayed relatively constant during the observation period of 8 h. In two subjects somewhat higher muscle concentrations of up to 0.026 and 0.035 μg/ml were observed during the first 90 min following administration of topical ketoprofen. Following repeated applications of ketoprofen gel (every 6 h for 4 days) subcutaneous and muscle concentrations were relative constant during the dialysis period of 8 h. Subcutaneous tissue concentrations ranged form 0.005–0.019 μg/ml, muscle concentrations ranged form 0.004–0.015 μg/ml.

### Plasma and Tissue Concentrations After Intramuscular Ketoprofen

Maximum free ketoprofen concentrations in muscle ranged from 0.026 to 0.124 μg/ml with a median of 0.055 μg/ml. In subcutaneous tissue, maximum free ketoprofen concentrations of 0.042 μg/ml (range: 0.022–0.102 μg/ml) were obtained. Free tissue concentrations were about 100 times lower than total plasma concentrations.

Individual measured and predicted ketoprofen plasma and tissue concentrations after intramuscular injection of ketoprofen are shown in Fig. 2. The parameters of the three-compartment population model are given in Table I. Measured ketoprofen muscle tissue concentrations from 12 volunteers who did not participate in the present study were superimposed to muscle tissue concentrations predicted by the pharmacokinetic model obtained from the present study. Fig. 3 shows that most of the observations were found within the 16.7 and 83.3 percentiles of the prediction, *i.e.,* within the range of two-thirds of the predicted concentrations.

## DISCUSSION

Direct measurement of ketoprofen tissue concentrations by means of microdialysis indicates that a single dose of topically administered ketoprofen (Gabrilen® Gel) reaches subcutaneous and muscle tissue in low concentrations and with low predictability. Although dermal absorption of ketoprofen might be different from other topical ketoprofen formulations, the current results support the generally held skepti-

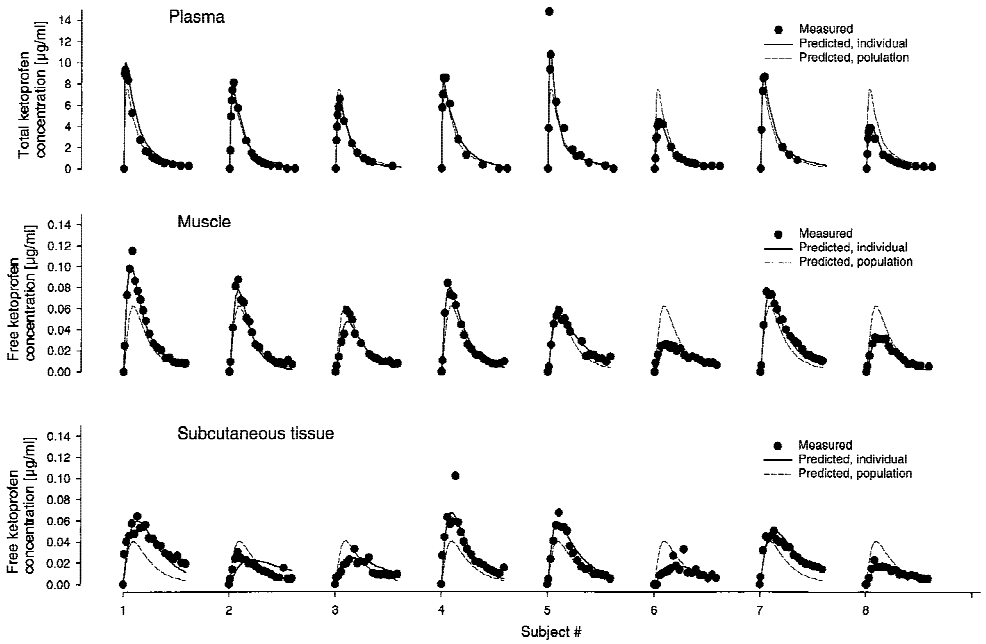


**Fig. 2.** Individual observed (dots) and predicted (lines) ketoprofen concentrations in plasma, muscle and subcutaneous tissue after intramuscular injection of 100 mg ketoprofen to eight healthy male volunteers. The dashed lines show the population central tendency of the concentration vs. time profile as estimated by NONMEM for the respective compartment.

**Table I.** Parameters of the Population Pharmacokinetic Model for Intramuscularly Injected Ketoprofen

| | Fixed effects: population central values (and % SEE) | Random effects, given as %CV |
|---|---|---|
| ka [$h^{-1}$] | 1.96 (10) | — |
| CL [$l \cdot h^{-1}$] | 7.01 (12) | 33 |
| V1 [l] | 3.38 (34) | 78 |
| CL12 [$l \cdot h^{-1}$] | 4.47 (30) | 56 |
| V2 [l] | 3.83 (28) | — |
| CL13 [$l \cdot h^{-1}$] | 1.52 (24) | 60 |
| V3 [l] | 3.48 (32) | — |
| f*u* | 0.014 (7) | — |

*Note.* As estimated by NONMEM from plasma and dialysate data using a standard three compartment model with an additional dosing compartment (Equation 1). Dashes indicate that the respective parameters were tested during model building but rejected from the final model. %CV: percent coefficient of variation. % SEE: percent standard error of the population parameter estimate.

cism that currently available commercial formulations of topical NSAIDs do not provide evidence of site-specific delivery to target tissue (*i.e.,* muscle or joint). Absorption appears to increase somewhat with repeated topical applications. However, tissue concentrations of ketoprofen were still considerably lower than those following intramuscular administration. However, there might be substance specific or formulation specific differences. Recently, we have reported that ibuprofen (Dolgit® Gel) reliably reaches high concentrations in subcutaneous tissue, but not muscle tissue, after topical administration (11). Fig. 4 compares the areas under the dialysate concentrations versus time curves (AUCs) obtained in the present ketoprofen study to those of the ibuprofen study. Ibuprofen concentrations in muscle were also highly variable and in only half of the subjects considered to be effective. However, in contrast to ketoprofen, subcutaneous ibuprofen concentrations after topical administration clearly exceeded those after oral dosing.

Compared with topical administration of ketoprofen, tissue concentrations after intramuscular dosing were about 10 times higher. Because with intramuscular ketoprofen we saw the descending segment of the plasma (and tissue) concentration versus time profile, the data could be described using a pharmacokinetic model. In analogous methodology to that used for cephalosporins (17), plasma and tissue concentrations were described with a compartmental model that regarded the tissue concentrations as an equivalent of the peripheral compartments. In contrast to the previous approach that fitted plasma concentrations first and adapted only the amplitude of the tissue concentrations in the subsequent fit, we fitted plasma and tissue concentrations in a single step. A two-compartment model described the data less well and resulted in different values of f*u* for muscle and subcutaneous tissue what would have had the consequence that f*u* cannot be interpreted as the free fraction of ketoprofen, as is commonly accepted (17). The mixed effects population approach was chosen in the present data-rich situation in an attempt to identify covariates for ketoprofen disposition because an analysis of previous data suggested a correlation between the subjects' body height and the central volume of distribution (not shown). However, judged by goodness-of-fit criteria those covariates were not justified by the data obtained in the

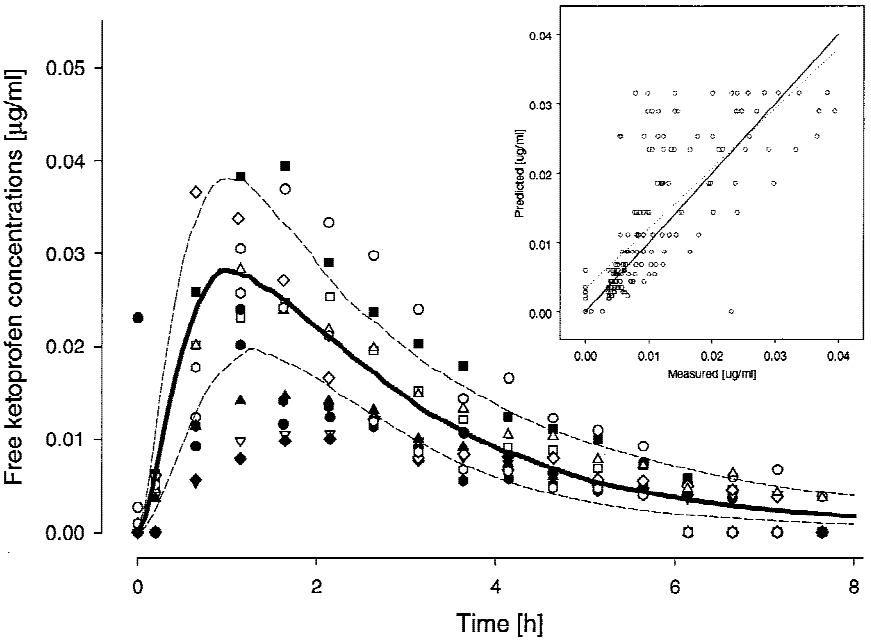


**Fig. 3.** Observed individual ketoprofen concentrations in muscle tissue from 12 subjects who did not participate in the present study, after intramuscular injection of 50 mg ketoprofen. Note that a different ketoprofen formulation and site of intramuscular administration was used in these subjects. Data from each individual are indicated by separate symbols. The thick line shows the population central tendency predicted by the pharmacokinetic model obtained in the present study. The dashed lines denote the 16.6 and 83.3 percentiles, between which two-thirds of the predicted data can be found. The insert shows a plot of predicted versus measured concentrations, with a line of identity (line) and a regression line (dotted line).

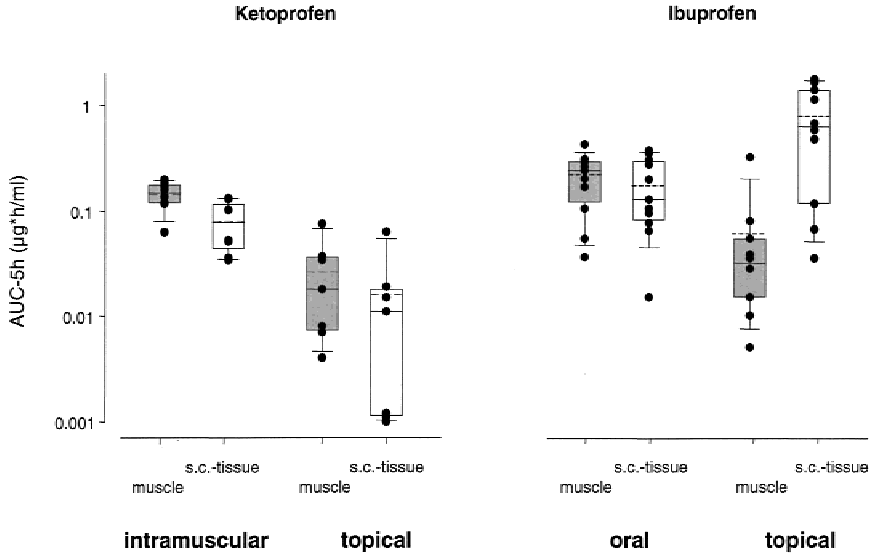


**Fig. 4.** Areas under the dialysate concentrations versus time curves (AUC) for muscle and subcutaneous tissue up to 5h after administration of 100 mg ketoprofen either intramuscularly or topically and 800 mg ibuprofen either orally or topically. The box shows the interquartile range, the line and dashed line show median and mean, respectively, the end of the whiskers show 5 and 95 percentiles, and the dots show individual values.

relative homogenous group of healthy volunteers. The modeling was subsequently preserved because reanalyzing of the data using a two-stage approach was not expected to provide additional useful information or better fits. The definite proof for the model quality was the demonstration of its prediction performance.

Superimposing intramuscular concentration data of 12 subjects who did not participate in the present study over those predicted by the population model showed that the prediction was of acceptable goodness considering that different formulations and sites of intramuscular administration were used in the studies. Plasma and tissue concentrations of ketoprofen after topical administration were not included in the fit because the dialysate concentration versus time data have to be considered as a result of both input from skin and distribution from plasma (analogously to the i.m. data). Dialysate was sampled directly below the skin area of drug administration. This would have required a much more complicated pharmacokinetic model. An attempt to fit the data to such a model failed because (i) in contrast to the data after i.m. injection, we did not see a descending segment of the plasma concentration versus time curves, and (ii) the dialysate data after topical administration were noisy and mostly below the quantification limit.

It is not clear how much free ketoprofen in muscle or subcutaneous tissue is needed to inhibit cyclooxygenase activity and prostaglandin release, hence to produce clinical efficacy. The $IC_{50}$ data for cyclooxygenase-2 inhibition pub-

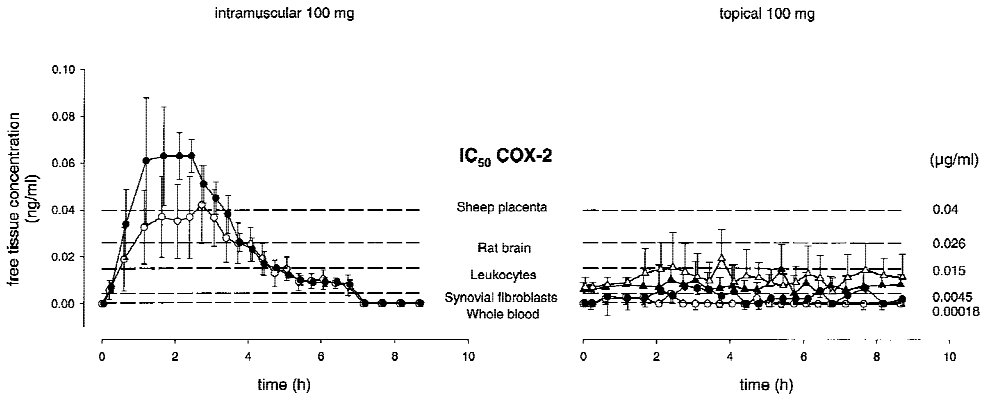


**Fig. 5.** Time courses of muscle (●) and subcutaneous (○) tissue concentrations (median ± semiinterquartile range) after a single dose of 100 mg of intramuscular (left) and topical (right) ketoprofen. Time courses of muscle (▲) and subcutaneous (△) tissue concentrations after repeated topical administrations of ketoprofen (100 mg every 6 h for 4 days; right figure). The lines indicate the in vitro $IC_{50}$ values for cyclooxygenase-2 inhibition found in the literature (sheep placenta (18), rat brain (19), leucocytes, synovial fibroblasts (20) and whole blood assay (18)). $IC_{50}$ data for S-ketoprofen were doubled because our tissue concentrations apply to racemic ketoprofen. $IC_{50}$ values for total (free and protein bound) ketoprofen were multiplied by the free ketoprofen fraction found in tissue and plasma $fu = 0.014$.

lished from a variety of in vitro models show significant variability; the values reported for S-ketoprofen range from 0.024 μM (whole blood assay) to 5.3 μM for COX-2 inhibition in sheep placenta (18). The reason for such high variability is not known but might be due to differences in the free fraction of ketoprofen or the accessibility of the cycylooxygenases, which might be better with single cells than with tissue fragments. It is not known which of these models most accurately predicts *in vivo* performance and hence clinical efficacy. Because *in vivo* data are not available, we compared the present muscle and subcutaneous ketoprofen concentrations following intramuscular and topical administration with the *in vitro* $IC_{50}$ values found in the literature to estimate the potential clinical outcome for both routes of ketoprofen administration (Fig. 5). Except for the whole blood assay the in vitro $IC_{50}$ values in Fig. 5 are considerably higher than the presently found tissue ketoprofen concentrations after a single topical dose of 100 mg, supporting the notion that a single dose of topical ketoprofen as used in the present study may not provide clinical evidence of efficacy. Although tissue concentrations following repeated topical doses are somewhat higher, it remains questionable whether tissue cyclooxygenase activity is efficiently inhibited. In contrast to the concentrations achieved with the topical route, median tissue concentrations following intramuscular ketoprofen exceeded the *in vitro* $IC_{50}$ values from Fig. 5, suggesting that a single dose of 100 mg intramuscular ketoprofen would result in an effective and reproducible inhibition of cyclooxygenase activity in muscle and subcutaneous tissue leading to clinical efficacy.

In summary, we have found that reproducible and cyclooxygenase-inhibiting concentrations of ketoprofen in subcutaneous and muscle tissue can be expected after a single intramuscular but not after a single topical dose of ketoprofen. With repeated topical administrations tissue concentrations of ketoprofen were somewhat higher but still considerably lower than those achieved with a single intramuscular dose. Thus, the tissue concentrations found following topical administration of ketoprofen gel can hardly explain the reported clinical efficacy of topical ketoprofen.

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