Percutaneous Absorption of Ketoprofen from Different Anatomical Sites in Man

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Purpose. The purpose of this study was to investigate the percutaneous absorption of ketoprofen applied topically to different anatomical sites on the body.

Methods. The study design was a randomized, four-way crossover in 24 healthy male subjects. One gram of ketoprofen 3% gel (30 mg dose) was applied every six hours for 25 doses over a 100 cm^2 of the back, arm, and knee. A 0.5 ml of ketoprofen solution (60 mg/ml) was applied to the back as a reference treatment. Plasma and urine samples were obtained for the assay of racemic ketoprofen and ketoprofen enantiomers (S and R), respectively.

Results. The relative bioavailabilities of ketoprofen gel were 0.90 ± 0.50, 1.08 ± 0.63, and 0.74 ± 0.38 when applied to the back, arm, and knee, respectively. The plasma ketoprofen Cmax for gel applied to the back and arm were similar ($p > 0.05$) but Cmax was lower when applied to the knee ($p < 0.05$). The time to Cmax ranged from 2.7 to 4.0 hours and was similar for gel treatments on the back and arm, but longer for the knee treatment. The fraction of dose excreted in urine as total S and R enantiomers ranged from 5.41 to 9.10%.

Conclusions. The percutaneous absorption of ketoprofen was similar when applied to either the back or arm but was lower when applied to the knee.

KEY WORDS: ketoprofen; nonsteroidal anti-inflammatory agent; topical application; percutaneous absorption; regional variation; pharmacokinetics; urinary excretion; enantiomers.

INTRODUCTION

Ketoprofen, a racemic benzoylpropionic acid, is a nonsteroidal anti-inflammatory drug which has anti-inflammatory and analgesic activity (1). Oral ketoprofen has been well tolerated with a low incidence of renal, hepatic, or toxic cutaneous reactions. A topical formulation of ketoprofen has been developed for the temporary relief of minor aches and pains of muscle and joints and to minimize gastrointestinal side effects after oral administration.

The percutaneous absorption of drugs from topical application is known to be influenced by differences in skin structure at various regions of the body, and changes in skin integrity which may occur with gender, age, race, presence of disease, and skin condition e.g., occlusion, abrasion, temperature etc. (2). Variation in drug permeation through the skin from different anatomical sites of body is known. The permeation of scopolamine (3) *in vitro* through epidermis isolated from various skin sites was observed to be highest from the post auricular skin and the *in vivo* permeation of [^{14}C]Cortisol (4) was forty-two times higher from scrotum compared to the ventral aspect of forearm in man. In this study, we investigated the percutaneous absorption of ketoprofen applied topically to different anatomical sites on the body. For this purpose, the skin on the back, arm, and knee were selected for drug application. Ketoprofen solution was applied topically to the back as a reference treatment for comparison of the absorption of ketoprofen from gel treatments.

MATERIALS AND METHODS

Subjects and Ethical Considerations

Twenty-four healthy male subjects entered the study. The study was approved by an appropriate institutional review board and all subjects gave written informed consent before participation in the trial. The subjects were 19 to 30 years of age (24.2 ± 3.6 years), and were within 10% of their ideal body weight (75.1 ± 8.9 kg). All subjects were judged to be healthy based on medical history, physical evaluation, and clinical laboratory testing, including complete hematology, blood chemistry, urinalysis, and 12-lead electrocardiogram. Subjects presenting with sunburn, scars, tattoos, or uneven skin tones at the sites of application were excluded. All subjects were free of other medication at least 2 weeks before and during the study.

Study Design and Procedures

The study design was a randomized, four-way complete crossover in which ketoprofen 30 mg (1 gm of 3% gel or 0.5 ml of 60 mg/ml solution) QID for 25 doses was applied on the skin as the following treatments:

A: ketoprofen solution on the skin above the latissimus dorsi muscle of the back.

B: ketoprofen gel on the skin above the latissimus dorsi muscle of the back.

C: ketoprofen gel on the skin above the biceps muscle of the arm.

D: ketoprofen gel on the skin above the insertion of the sartorius muscle of the inner knee joint.

Each study period was separated by at least a seven day drug free washout period.

Study Medication Application

A 100 cm^2 area of the skin at selected sites on the body was marked with a template and was used for the multiple applications. Prior to each application the drug remaining on the skin was washed off three times with a soft cloth immersed in warm water. After application the site was left uncovered for 30 minutes. The subjects were advised not to take a shower until 5 hours after the application was made.

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Ketoprofen Gel. One ml of air free 3% ketoprofen gel (specific gravity ≈ 1.0) was applied every six hours for 25 doses to the site using a 3 ml syringe (No. 9586, Becton Dickinson & Co., Rutherford, New Jersey 07070). The gel was spread over the designated surface area using fingers covered with a vinyl glove and gently rubbed for approximately 5 minutes.

Ketoprofen Solution. Ketoprofen solution, 60 mg/ml in acetone, was applied to the site using a 0.5 ml glass syringe by rotating the tip of the syringe and slowly pushing the plunger. The site of application was not rubbed to spread the solution.

Sampling. Serial blood samples were drawn by cubital venipuncture in chilled (0 to 4°C) vacutainer tubes containing potassium oxalate and sodium fluoride (No. 367725, Becton Dickinson & Co.) just before and 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, and 48 hours after the last (25^{th}) application. Additionally, trough blood samples were drawn prior to the morning dose (17^{th} and 21^{st} dose) on days 5 and 6 for each study period. Plasma was separated by refrigerated centrifugation (0 to 4°C, 2000 rpm for 10 minutes), transferred to a Sarstedt® polypropylene tube (Cat No. 60,541, Sarstedt, Arlington, Texas) and frozen at −70°C until analysis.

Urine was collected in plastic containers just prior to and from 7 AM to 1 PM on days 6 and 7 over a collection interval of 0 to 6 hour for all treatments. During the collection intervals, urine was stored in the refrigerator. At the end of a collection interval, the urine was mixed, the pH and volume recorded, and a 20 ml aliquot was saved in two polyethylene screw capped tube (16 mm × 100 mm) and frozen immediately. The tubes were stored at −20 to −30°C until analysis.

Assay

Analytical methods for racemic ketoprofen in plasma and ketoprofen enantiomers (S and R) in urine will be published separately. These plasma and urine assays are briefly described below.

Plasma Samples. One ml of plasma was combined with 0.1 ml of internal standard (tolmetin) solution and 0.5 ml of pH 1.8 phosphate were extracted with an organic solvent mixture composed of 1-butanol and methyl-t-butyl ether. The organic phase was transferred to a clean tube and the analytes were back extracted in 0.5 ml of ammonium acetate buffer, pH 6.1.

Separation was accomplished by reverse HPLC on a 5μ, 15 cm × 4.6 mm Cosmosil C-18 column using a mobile phase composed of 20% acetonitrile in 0.25 M ammonium acetate buffer, pH 5.0, at a flow rate of 1.8 ml/minute. Ketoprofen and the internal standard were detected by monitoring the column effluent for UV absorbance at 258 nm (tolmetin) and at 350 nm (ketoprofen) and ketoprofen was quantitated by means of calibration curves generated by weighted, linear least squares regression of the ketoprofen to internal standard peak height response ratios versus calibration standard concentrations. The lower limit of quantitation was 5 ng/ml of racemic ketoprofen and the method was linear through at least 100 ng/ml.

Urine Samples. One ml of urine was combined with 0.1 ml of naproxen internal standard solution and 0.1 ml of 1 N sodium hydroxide. After incubation at room temperature for 15 minutes, the mixture pH was adjusted with 0.1 ml of 50% phosphoric acid and the analytes were extracted with n-butyl chloride for 10 minutes. R and S ketoprofen were derivatized to form their respective (S)-α-methylbenzyl amide diastereomers using a modification of the procedure of Avgerinos and Hutt (5).

The S and R enantiomers of ketoprofen were chirally separated by capillary gas chromatography on a Hewlett Packard HP-1 fused silica capillary column and detected by selective ion monitoring of the stereoisomers at 210 m/z, and the internal standard naproxen at 185 m/z. The GC injector was maintained at 300°C and the oven temperature was programmed to ramp from 100°C to 170°C at 10°/minute followed by an increase from 170°C to 290°C at 20°/minute with a 2 minute hold at 170°C and a 1 minute 290°C. The carrier gas was helium at a flow rate of 1.5 ml/minute. System calibration was accomplished by weighted linear least squares regression analysis of the observed peak area ratios of ketoprofen isomer to internal standard. Using 1 ml of urine, the lower limit of quantitation was 50 ng/ml for each isomer. The linearity of the method was validated over a concentration range of 50 ng/ml through 375 ng/ml for each isomer.

Data Analysis

The plasma concentration-time data for ketoprofen were analyzed by noncompartmental methods (6). The maximum plasma concentration (Cmax) and time to reach Cmax (tmax) were derived from the raw data. The area under the plasma concentration-time curve after the last dose (AUCss) was calculated by the linear trapezoidal rule over a 6 hour dosing interval. The apparent systemic clearance (CL/F) of ketoprofen was calculated as dose applied divided by AUCss. The terminal elimination rate constant (K) was obtained from the slope of natural log of terminal linear plasma concentration vs time data. The apparent half-life ($t_{1/2}$) was calculated as 0.693 divided by K. The relative bioavailability (F_{rel}) of ketoprofen gel applied to different anatomical sites on the body was determined as ratios of AUCss for ketoprofen gel treatments to solution.

The amount excreted in urine over a 6 hour dosing interval on days 6 and 7 for S and R enantiomers was calculated by multiplying the concentration of each isomer in urine by the volume of urine in the collection interval. The excretion rate during the dosing interval was obtained from the concentration of enantiomer in urine × the urine flow rate. The fraction of dose excreted in urine (fe, %) was obtained by dividing amount of each isomer excreted in urine over a 6 hour interval on days 6 and 7 by the dose applied to the skin.

Statistical Analysis

Statistical comparisons of the plasma and urinary pharmacokinetic parameters for different treatments were made by analysis of variance using SAS (version 6.0) (7). A p-value of $p < 0.05$ was used to determine significance. Multiple t-tests were used to determine the difference between treatments.

RESULTS AND DISCUSSION

All 24 subjects completed the study periods according to the protocol. Study medications were well tolerated by subjects and adverse events reported were mild and of a topical nature e.g., burning sensation, redness, erythema, papules, dryness at the application site.

Plasma Pharmacokinetics

The mean plasma racemic ketoprofen concentration-time plots after the last application of ketoprofen solution or gel applied to different anatomical sites are illustrated in Figure 1. Although all treatments were removed by washing after 6 hours of application, ketoprofen plasma concentrations were measurable up to 48 hour suggesting prolonged release of ketoprofen from subcutaneous tissue into the blood.

The pharmacokinetic parameters after topical application of ketoprofen from all treatments are shown in Table 1. In general, the peak mean plasma concentration was the highest for solution (154.4 ng/ml) and the lowest for gel applied to the knee (85.2 ng/ml). Multiple comparisons indicated that the Cmax for gel applied to the back and forearm were similar to the Cmax from solution treatment applied to the back ($p > 0.05$). The Cmax for gel application to the knee was lower compared to the Cmax from solution treatment or gel applied to the forearm ($p < 0.05$). Although the Cmax from gel treatment to the back was 25.6% higher than that from the knee, this difference was not significant ($p > 0.05$). The tmax after all topical applications ranged from 2.7 to 4.0 hrs. For gel treatments administered on the back and arm, the tmax was similar ($p > 0.05$) but tmax was longer for the knee ($p < 0.05$) compared to solution treatment on the back. Literature data after oral administration of a 50 mg dose of ketoprofen indicated that the Cmax is 4800 ± 1800 ng/ml at a tmax of 1.20 ± 0.76 hr in healthy male subjects (8). Compared to the oral data, the Cmax from topical application of ketoprofen is about 20 fold lower and the absorption of ketoprofen from topical application appears to be slower. The systemic concentrations of ketoprofen have also been found to be 100 fold lower compared to tissue concentrations below the application site in patients undergoing knee joint surgery (9). Topically applied ketoprofen thus provides high local concentration below the site of application but lower systemic exposure.

Steady-state areas under the plasma concentration-time curve (AUCss) for the gel applied to the back and arm were similar to solution treatment on the back ($p > 0.05$). AUCss however, was 30% lower for the gel applied to the knee compared to gel treatment to the arm or solution treatment to the back ($p < 0.05$). For gel treatment on the knee, the AUCss was 18.6% lower compared to gel treatment on the back but the difference was not significant ($p > 0.05$). The CV (%) for AUCss ranged from 49 to 59% for all treatments indicating high intersubject variability in the percutaneous absorption of ketoprofen. The relative bioavailabilities of ketoprofen gel were 0.90 ± 0.50, 1.08 ± 0.63, and 0.74 ± 0.38 when applied to the back, arm, and knee, respectively compared to solution treatment to the back. In addition, similar systemic exposure of ketoprofen from the gel and the solution when applied to the back indicated that formulation was

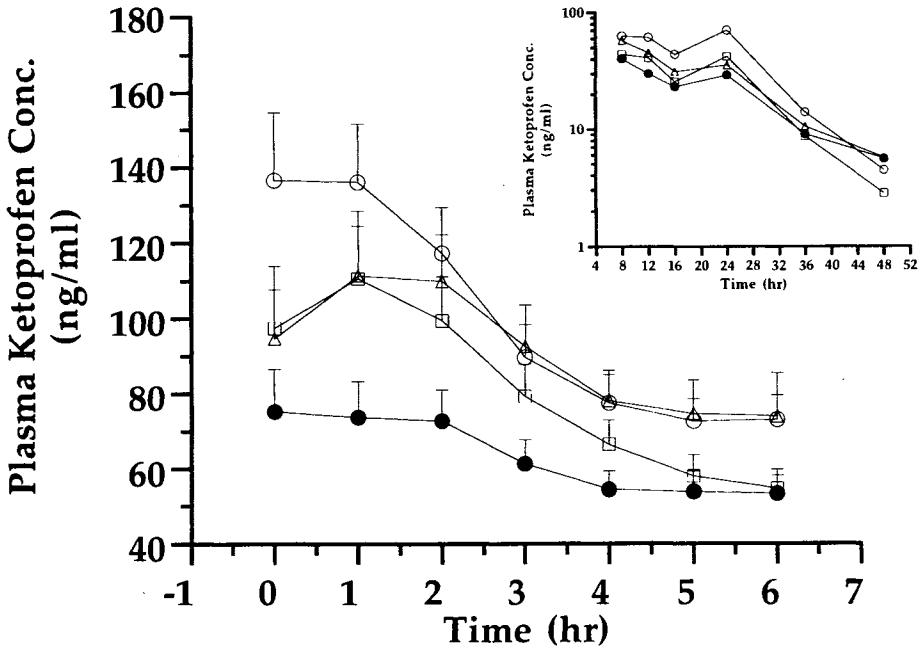

Fig. 1. Ketoprofen plasma concentrations (mean ± SE) observed over the dosing interval (6 hours) after the last dose in 24 healthy, male subjects during 4-way crossover treatment of 30 mg equivalent dose of either 3% gel on the back (□), arm (△), and knee (●) or solution (○) (60 mg/ml) on the back applied every six hours for 25 doses. All doses were removed from skin by washing at 6 hr after application. Decline in the plasma ketoprofen concentrations after 6 hours are shown in the inset figure as log concentration vs time plots.

Table I. Mean (CV, %) Plasma Ketoprofen Pharmacokinetics After Topical Application

Parameters	A. Solution (back) (n = 24)[a]	B. 3% Gel (back) (n = 24)	C. 3% Gel (arm) (n = 24)	D. 3% Gel (knee) (n = 24)
Cmax	154.39	119.58	119.52	85.19[b,c]
(ng/ml)	(60.68)	(78.30)	(57.71)	(64.66)
Tmax	2.83	3.00	2.67	4.04[b,c]
(hr)	(81.73)	(73.56)	(74.73)	(52.85)
AUCss	597.81	489.79	550.85	379.07[b,c]
(ng · hr/ml)	(48.71)	(59.13)	(55.86)	(53.20)
CL/F	61.01	80.08	69.21	99.81
(L/hr)	(42.56)	(48.64)	(47.77)	(55.92)
$t_{1/2}$	13.93	16.62	15.24	19.68[b]
(hr)	(51.97)	(57.50)	(53.18)	(86.32)
Frel	1.00	0.90	1.08	0.74
	(0.00)	(55.00)	(58.22)	(51.71)
Css(trough)	145.61[d]	103.76[e]	94.35[e]	68.09[e]
(ng/ml)	(63.33)	(76.33)	(78.28)	(66.08)

[a] Number of subjects.
[b] Significantly different from treatment A ($p < 0.05$).
[c] Significantly different from treatment C ($p < 0.05$).
[d] Average steady-state trough levels on days 5, 6, and 7.
[e] Average steady-state trough levels on days 6 and 7.

not rate-limiting in percutaneous absorption. Based on these results, the absorption of ketoprofen from topical gel treatments in this study was similar from the back and arm but lower from the knee. The decrease in absorption of ketoprofen from the knee may be due to alteration in the thickness and diffusivity of the stratum corneum in this region as indicated by Scheuplein et al. (10) for the regional variation in the permeability of water.

The apparent systemic clearance (CL/F) of ketoprofen after topical application ranged from 61.0 to 99.8 L/hr. Based on literature data for CL of ketoprofen (11) of 5.10 ± 1.14 L/hr the percutaneous absorption is estimated to be 5 to 8% which agrees with the results of Flouvat et al. for topical absorption of ketoprofen (12). The mean terminal elimination half-lives for ketoprofen varied between 14 and 20 hours and were similar for gel treatments as compared to the solution ($p > 0.05$). The elimination half-life of ketoprofen after intravenous (11) and oral (8) administrations is reported to be 1.5 to 2 hours. The longer $t_{1/2}$ after topical application is a result of prolonged absorption of ketoprofen from subcutaneous tissue thus limiting its disappearance from the body (flip-flop kinetics). The plasma trough concentrations reached steady-state on days 5 and 6 of dosing for solution and gel treatments, respectively (Table 1).

Table II. Mean (CV, %) Urinary Excretion of R and S Enantiomers After Topical Application of Racemate Ketoprofen

	A. Solution (back) (n = 24)[a]		B. 3% Gel (back) (n = 24)		C. 3% Gel (arm) (n = 24)		D. 3% Gel (knee) (n = 23)	
Parameters	S	R	S	R	S	R	S	R
Day 6 amount	1.508	1.242	1.107	0.886	0.992	0.826	0.728	0.615
excreted (mg)	(51.06)	(50.73)	(54.92)	(52.61)	(49.04)	(47.90)	(56.70)	(55.53)
Day 7 amount	1.516	1.233	1.168[c]	0.944[b]	1.186[c]	0.979[b]	0.911[c,e]	0.727[b,d]
excreted (mg)	(44.05)	(44.34)	(67.79)	(67.84)	(54.86)	(50.46)	(52.54)	(50.16)
Day 6 excretion	4.189	3.452	3.074	2.460	2.756	2.295	2.022	1.734
rate (mg/min)	(51.06)	(50.73)	(54.92)	(52.61)	(49.04)	(47.90)	(56.85)	(55.46)
Day 7 excretion	4.212	3.425	3.245	2.622	3.295	2.719	2.481	2.135
rate (mg/min)	(44.05)	(44.34)	(67.79)	(67.84)	(54.86)	(50.46)	(52.13)	(45.93)
Day 6	5.03	4.142	3.69	2.95	3.31	2.75	2.43	2.049
fe(%)	(51.06)	(50.73)	(54.92)	(52.61)	(49.04)	(47.90)	(56.70)	(55.53)
Day 7	5.05	4.110	3.89	3.15	3.96	3.26	2.98	2.425
fe(%)	(44.05)	(44.34)	(67.79)	(67.84)	(54.86)	(50.46)	(53.26)	(50.16)

[a] Number of subjects.
[b] Significantly different from treatment A, R enantiomer.
[c] Significantly different from treatment A, S enantiomer.
[d] Significantly different from treatment C, R enantiomer.
[e] Significantly different from treatment C, S enantiomer.

Urinary Excretion

The urine data for subject 8 for treatment D was excluded from analysis since his pre-dose urine sample indicated the presence of ketoprofen. Ketoprofen is extensively metabolized to acyl-glucuronide conjugates and excreted primarily in the urine (13). The urinary excretion of ketoprofen enantiomers (S and R) is shown in Table 2. For solution and gel treatments on the back, the amounts of R and S isomers excreted in the urine on days 6 and 7 were similar ($p > 0.05$), but for gel treatments on the arm and knee the amounts excreted in urine on day 6 were lower by 15 to 23% for both isomers compared to day 7 ($p < 0.05$). The amount excreted, the excretion rate, and the fraction of dose excreted in urine on day 7 was lower for gel treatments compared to solution treatment for the S and R isomers ($p < 0.05$). The fraction of the dose excreted in urine (R and S combined) on day 7 for the solution treatment was 9.1% and that for gel treatments ranged from 5.4 to 7.2%, supporting the above estimation of F based on plasma data. For R ketoprofen, urinary excretion was 16 to 20% lower than that for S ketoprofen, and was independent of treatments, suggesting urinary excretion favors the S isomer. Similar stereoselectivity in urinary excretion of conjugated ketoprofen in man with a mean S:R ratio of 1.2 has been noted by Jamali et al (14). Renal clearance of ketoprofen was estimated to be 11.12 to 13.37 ml/min which is in agreement with literature value (15).

CONCLUSIONS

In this study, ketoprofen absorption from 3% gel applied topically on the back and arm was similar, but absorption was lower from the knee when compared to solution treatment on the back. Based on plasma ketoprofen concentrations, absorption of ketoprofen was slow and prolonged with a systemic bioavailability of 5 to 8% suggesting systemic exposure from topical application will be less. The variability in the plasma ketoprofen concentrations was high and independent of the site or formulation used. The amount of R and S ketoprofen enantiomers excreted in the urine on day 7 ranged from 5.41 to 9.10% of the topically applied dose of racemate ketoprofen and consistent with plasma results. The small degree of stereoselectivity in urinary excretion for S ketoprofen may not be of clinical significance. Based on these results ketoprofen 3% gel appears to be a suitable topical dosage form for further clinical testing.

REFERENCES

1. T. Kantor. Ketoprofen: A review of its pharmacological and clinical properties. *Pharmacotherapy*. 6:93–103 (1986).
2. G. L. Flynn, E. E. Linn, T. Kurihara-Bergstrom, S. K. Govil, and S. E. Hou. Parameters of skin condition and function. In A. F. Kydonieus and B. Berner (eds.), Transdermal Delivery of Drugs (Volume II), CRC Press, Inc., Boca Raton, Florida, 1987, pp. 3–17.
3. J. E. Shaw, M. E. Prevo, and A. A. Amkraut. Testing of controlled-release transdermal dosage forms: Product development and clinical trials. *Arch. Dermatol.* 123:1548–1556 (1987).
4. R. J. Feldman and H. I. Maibach. Regional variation in percutaneous penetration of [^{14}C] cortisol in man. *J. Invest. Dermatol.* 48:181–183 (1967).
5. A. Avgerinos and A. Hutt. Determination of the enantiomeric composition of ibuprofen in human plasma by high-performance liquid chromatography. *J. Chromatogr.* 415:75–83 (1987).
6. M. Gibaldi and D. Perrier. Pharmacokinetics (second edition), Marcel Dekker, New York, 1982.
7. SAS, Version 6.0, Cary, NC: SAS Institute Inc., 1990.
8. R. A. Upton, R. L. Williams, T. W. Guentert, J. N. Buskin, and S. Riegelman. Ketoprofen pharmacokinetics and bioavailability based on an improved sensitive and specific assay. *Eur. J. Clin. Pharmacol.* 20:127–133 (1981).
9. R. Ballerini, A. Casini, M. Chinol, C. Mannucci, L. Giaccai, and M. Salvi. Study on the absorption of ketoprofen topically administered in man: Comparison between tissue and plasma levels. *Int. J. Clin. Pharm. Res.* 6:69–72 (1986).
10. R. J. Scheuplein and I. H. Blank. Permeability of the skin. *Physiol. Rev.* 51:702–747 (1971).
11. D. Debruyne, B. H. deLigny, J. Ryckelynck, F. Albessard, and M. Moulin. Clinical pharmacokinetics of ketoprofen after single intravenous administration as a bolus or infusion. *Clin. Pharmacokin.* 12:214–221 (1987).
12. B. Flouvat, A. Roux, and B. Delhotal-Landes. Pharmacokinetics of ketoprofen in man after repeated percutaneous administration. *Drug Res.* 39:812–815 (1989).
13. R. A. Upton, J. N. Buskin, R. L. Williams, N. H. G. Holford, and S. Riegelman. Negligible excretion of unchanged ketoprofen, naproxen or probenecid in urine. *J. Pharm. Sci.* 69:1254–1257 (1980).
14. F. Jamali, A. S. Russell, R. T. Foster, and C. Lemko. Ketoprofen pharmacokinetics in humans: Evidence of enantiomeric inversion and lack of interaction. *J. Pharm. Sci.* 79:460–461 (1990).
15. T. Ishizaki, T. Sasaki, T. Suganima, Y. Horai, K. Chiba, M. Watanabe, W. Asuke, and H. Hoshi. Pharmacokinetics of ketoprofen following single oral, intramuscular and rectal doses and after repeated oral administration. *Eur. J. Clin. Pharmacol.* 18:407–414 (1980).