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PHARMACOKINETIC AND LOCAL TISSUE DISPOSITION STUDIES OF NAPROXEN FOLLOWING TOPICAL AND SYSTEMIC ADMINISTRATION IN DOGS AND RATS

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ABSTRACT

The pharmacokinetic profiles of naproxen in blood and synovial fluid (SF) following topical and i.v. bolus administration in dogs, and the local tissue disposition of the drug following topical and oral administration in rats, were investigated to assess the feasibility of topical delivery of naproxen for local and systemic effects. The naproxen gel in poloxamer 407 (PF-127) was applied on the stifle joint of dogs, and serum and synovial fluid samples were collected. For local tissue disposition studies, the naproxen gel was applied on the dorsal skin in rats, and blood, skin, and muscle samples were taken at 3, 6, and 12 h postdose after removing the residual gel from the skin. Steady state serum concentrations occurred at ~20 h after topical doses and lasted for the next ~30 h in dogs. Similar SF–serum concentration ratios of naproxen were found between i.v. (0·61 ± 0·16) and topical (0·55 ± 0·14) routes of administration. Following the i.v. dose, the half-life of naproxen in SF (~60 h) was significantly longer than that in serum (~40 h). The bioavailability of naproxen in the topical gel was ~2% of the applied dose in dogs. A large accumulation of drug in the epidermis, dermis, and muscle tissue beneath the gel application site was found in rats. Isopropyl myristate (IPM) significantly increased the systemic absorption as well as the concentrations of naproxen in the underlying dermis and muscle tissues, but exerted little effect on the disposition of naproxen in the epidermis. ©1997 by John Wiley & Sons, Ltd.

KEY WORDS: naproxen; synovial fluid; bioavailability; tissue disposition; chemical enhancer

INTRODUCTION

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) widely used for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and acute pain associated with gout, surgery, and trauma. Anti-inflammatory

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activities of naproxen after topical application have been demonstrated by several authors[1,2] using animal models such as carrageenan-induced pleurisy and adjuvant-induced arthritis. Like many other NSAIDs, the common adverse effects of naproxen following the oral route of administration are gastrointestinal disturbances. Therefore, the delivery of NSAIDs through skin for both local and systemic effects has been investigated as an alternative therapeutic route to reduce the adverse effects associated with oral dose and to enhance the potential therapeutic value of these drugs.[3–5]

Recent studies have examined the local tissue disposition of NSAIDs following topical application and raised questions as to whether drugs found in the underlying tissues are delivered by direct penetration from the dose site or redistribution from the systemic circulation.[6–8] The absorption and underlying tissue disposition after topical dose are highly affected by physiological factors such as blood supply, tissue binding, and metabolism[6,9–11] as well as physicochemical properties of drugs such as lipophilicity, dissociation constant, and partition coefficient.[12,13] It is also known that vehicles used in the formulation can greatly influence the rate and extent of drug disposition in the skin.[14]

The present study was undertaken to determine the pharmacokinetic profiles of naproxen in the blood and synovial fluid following i.v. bolus and topical routes of administration in dogs. In addition, the absorption and disposition properties of naproxen into the systemic circulation, skin, and underlying muscle tissues were studied in rats after oral and topical routes of administration in the presence and absence of IPM.

MATERIALS AND METHODS

Chemicals

Naproxen and sodium naproxen (Sigma Chemical Co., St Louis, MO), poloxamer 407 (BASF Wyandotte Corporation, Parsippany, NJ), isopropyl myristate (Sigma Chemical Co., St. Louis, MO), acepromazine (Fermenta Animal Health Co., Kansas City, MO), ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, IA), xylazine (Miles Inc., Shawnee Mission, KS), HPLC grade acetonitrile and methanol (J. T. Baker Inc., Phillipsburg, NJ), and other chemicals were used as received from the suppliers.

Pharmacokinetic studies in dogs

Intravenous bolus administration. Three female beagle dogs weighing $12 \pm 0{\cdot}8$ kg were used in this study. A dose of naproxen ($1\,mg\,kg^{-1}$), dissolved in a pH 7·4 phosphate buffer–alcohol solution ($10\,mg\,mL^{-1}$) was administered to each dog through the cephalic vein over a period of 20 s. At 0,

0·17, 0·5, 1·0, 2·0, 4·0, 12·0, 24·0, 36·0, 48·0, 72·0, and 96·0 h postdose, 3 mL blood was collected from the jugular vein using vacutainer tubes (Becton Dickinson, SST® tubes) and centrifuged for 10 min at 2500 rpm to separate the serum. The synovial fluid (50–100 μL) was collected from the joint cavity of the left hindleg using a 1 mL syringe with a 20-gauge needle at 0, 0·5, 4·0, 12·0, 24·0, 48·0, 72·0, and 96·0 h postdose. The serum and SF samples were stored at −20 °C and analyzed within 1 week after collection.

Topical administration. After 5 weeks of the washout period, the same three dogs were used in the topical dose study. One day prior to the experiment, the elbow and stifle joints of hindlegs were carefully shaved with an electric clipper (Oster, Model A-2). No visible signs of damage on the skin surface were observed. On the following day, a dose (5 mg naproxen/kg B.W.) of the 2% naproxen gel in PF-127 was uniformly and quickly applied over the shaved skin area (7 cm × 10 cm) of the right hindleg joint using a gloved finger without excessive pressure. A gauze pad with an occlusive lining was placed over the dosed area and secured with Elastikon tape until the area was completely cleansed with 50% alcohol at 36 h postdose. Three milliliter samples of blood were obtained from the jugular vein using vacutainer tubes at 0, 2, 4, 8, 12, 24, 36, 48, 72, and 96 h postdose. Using a syringe with a 20-gauge needle, 5–100 μL SF was collected from the left stifle joint at 0, 4, 12, 24, 48, 72, and 96 h postdose. The samples were processed similarly as described in the section of i.v. bolus dose.

The gel was prepared by the cold method described by Schmolka:[15] an appropriate amount of PF-127 was slowly added to pH 5 phthalate buffer (0·2 M), and the mixture was left in a refrigerator until becoming a clear solution. Naproxen dissolved in ethanol was then mixed into the cold solution, and the solution was incubated at room temperature until a clear gel was formed.

Skin disposition study in rats

Topical administration. Thirty male Sprague–Dawley rats (175–200 g) were equally divided into six groups of five rats each. Three groups were treated with the 1% naproxen gel, and the other three groups were treated with the gel containing 2% (w/w) IPM on the dorsal site (3 cm × 3 cm) for sampling at three different times (3, 6, and 12 h postdose). One day prior to dosing, the ventral and dorsal areas of the skin were carefully shaved with an electric clipper (Oster, Model A-2) under light anesthesia using the cocktail of acepromazine–xylazine–ketamine. No signs of skin damage were observed after shaving. Two hundred and fifty milligrams of the 1% naproxen gel in PF-127 was applied uniformly on the marked area of approximately 3 cm × 3 cm of the dorsal skin, which was then covered with a piece of Parafilm and secured by adhesive tape. At 3, 6, or 12 h postdose, the rats in each of the three groups were anesthetized

and sacrificed by exsanguination through cardiac puncture. Approximately 3 mL blood were collected by this method and subjected to assay for the quantitation of naproxen. The skin and muscle tissues were sampled at both nondosed ventral and dosed dorsal sites, and wet weights were measured. Prior to excising tissue samples, the dosed area of the skin was completely washed with wetted cotton in 50% ethanol. The procedure used was approved by the Animal Care and Use Committee of the University of Georgia.

Oral administration. Fifteen male Sprague–Dawley rats (175–200 g) were equally divided into three groups of five rats each for three sampling times. Rats were dosed with 2·74 mg naproxen sodium (equivalent to 2·5 mg of naproxen) in 1 mL physiological saline by an animal feeding needle (George Tiemann and Co.). Blood, skin, and muscle tissues were collected from each group of rats at 3, 6, and 12 h postdose and processed for the assay of naproxen in the same manner as described in the section of topical administration.

Quantitation of naproxen in serum, SF, and tissue

Naproxen in the serum and SF was quantified using a modified fluorometric HPLC method developed in our laboratory:[16] serum (200 μL) and SF (50 μL) samples after treating with acetonitrile (600 μL for plasma and 150 μL for SF) to precipitate proteins were centrifuged at 2500 rpm for 10 min. Using a microsyringe, 50 μL of the clear supernatant was directly injected onto the HPLC column for quantitation. The drug was eluted on the reverse phase C_{18} column with the mobile phase consisting of acetonitrile (47% v/v) and pH 2·5 hydrochloric acid buffer (0·04 M).

For the skin disposition study, the epidermis was separated from the dermal layer using the steam method developed in our laboratory. After loosening the dermal–epidermal junction by placing the excised full skin on a metal screen above a hot water bath for 3 min, the two layers were carefully parted using dissection forceps. The temperature of the vapor was maintained at $\sim 70\,^{\circ}C$ during the process.

The concentrations of naproxen in the epidermis, dermis, and muscle tissues were measured as follows: each of the weighed tissue samples was placed in a glass test tube (1·2 cm × 7·5 cm) and homogenized in 0·5 mL pH 2·5 hydrochloric acid buffer using a Tissue Tearor (model 985-370, Biospec Products, Racine, WI). One milliliter of acetonitrile was added dropwise to the tissue homogenate to precipitate proteins. After centrifugation at 3500 rpm for 10 min, the clear supernatant was directly injected onto the column for quantitation for naproxen as described.

Pharmacokinetic analysis

The plasma and SF concentration–time profiles of naproxen in dogs were analyzed using the RSTRIP polyexponential curve stripping program (MicroMath Inc., Salt Lake City, UT). The bioavailability of naproxen from the gel was calculated by comparing the area under the concentration–time curve for topical dose (AUC_t, 0–96 h) and that for i.v. dose (AUC_{iv}, 0–96 h). The Student *t*-test was used for the test of statistical difference. *p* values of less than 0·05 were considered significant.

RESULTS AND DISCUSSION

Following topical administration, drugs that reach the dermis can be either systemically absorbed or continue to diffuse into underlying tissues. The relative extent of the systemic absorption and local tissue disposition will determine the effectiveness of topical medications. Due to the lack of definitive guidelines for the development and optimization of topical formulations, drug permeability across the skin has often been regarded as the primary criterion for the efficiency of topical delivery without proper assessment of drug disposition in local tissues. In this study, the local tissue disposition and systemic absorption of naproxen were determined following topical, i.v., and oral routes of administration in animals to evaluate the feasibility of topical delivery of naproxen for local and systemic effects.

Serum and SF profiles of naproxen in dogs

The concentration–time (*c–t*) profiles of naproxen in serum and SF after i.v. bolus and topical administration in dogs are shown in Figures 1 and 2, respectively. The serum *c–t* profiles obtained from the i.v. bolus dose in three dogs exhibited two exponential phases with a relatively long elimination half-life of $39{\cdot}4 \pm 9{\cdot}70$ h which is in close agreement with the value of $35{\cdot}0 \pm 11{\cdot}6$ h found in beagle dogs by Runkel *et al.*[17] Figure 2 shows that the administration of the topical gel resulted in long sustained *c–t* profiles in serum, reaching the steady state concentration at $\sim$20 h postdose and lasting for the next $\sim$30 h. Based on the *c–t* data, the serum half-life and the bioavailability of naproxen in the topical gel were estimated to be $61{\cdot}2 \pm 12{\cdot}8$ h and $2{\cdot}0 \pm 0{\cdot}3\%$ of the applied dose, respectively. The longer half-life and low bioavailability of the topical naproxen were probably, in part, due to the large drug accumulation in the skin as shown in the skin disposition study using rats in this report. The significant reservoir function of the skin was first discussed explicitly by Vickers[9] who reported that the skin blanching effect of corticosteroids was repeatedly observed for as long as 14 d postdose by reoccluding the original site of drug application. However, the reservoir capacities of the skin were found to

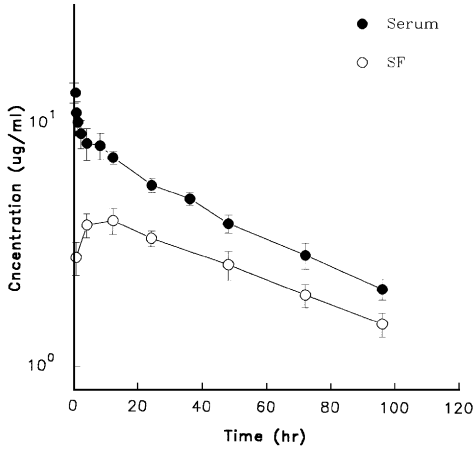

Figure 1. Naproxen concentrations in serum (●) and SF (○) following an i.v. bolus dose ($1\,mg\,kg^{-1}$) in beagle dogs (data shown as mean ± SD, $n = 3$)

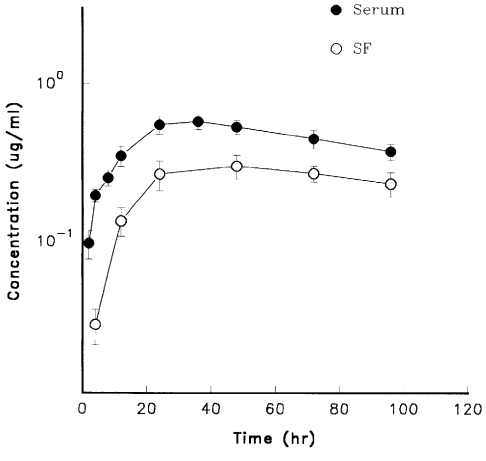

Figure 2. Naproxen concentrations in serum (●) and SF (○) following a topical dose ($5\,mg\,kg^{-1}$) in beagle dogs (data shown as mean ± SD, $n = 3$)

vary widely for different drugs, and those substances with lower diffusivities in the stratum corneum exhibited a more prominent skin depot formation.[18]

Since the synovial tissue corresponds to the biophase in arthritic inflammation, the naproxen concentrations in SF might serve as a better indicator of the drug's therapeutic efficacy. In dogs, the peak SF concentration appeared at $8{\cdot}20 \pm 1{\cdot}58$ h following the i.v. bolus dose, which indicates that SF represents a slowly accessible and kinetically separable compartment. In addition, the synovial half-life of naproxen ($59{\cdot}6 \pm 9{\cdot}53$ h) was significantly longer than the

serum half-life ($39{\cdot}4 \pm 9{\cdot}70$ h). The slower elimination of naproxen from SF than from the serum observed in this study is consistent with the previous studies which reported that the synovial half-life of naproxen in human was longer than its plasma half-life.[19,20] As shown in Figures 1 and 2, the SF concentrations of naproxen were much lower than the serum concentrations, and thus the so-called 'equilibration time',[21] which occurs when the SF concentration becomes equal to the serum concentration, was not observed for naproxen. Wallis and Simkin[21] reported that the 'equilibration time' was well correlated ($r > 0{\cdot}93$) with serum half-lives of various antirheumatic drugs. The mean SF–serum concentration ratio of naproxen for the i.v. dose was $0{\cdot}61 \pm 0{\cdot}16$ which was in close agreement with the ratio of $0{\cdot}55 \pm 0{\cdot}14$ obtained for the topical dose. Lower drug concentrations in SF than in the blood have also been reported for several NSAIDs;[22,23] this was attributed to lower protein binding of the drugs in SF than in the blood. According to Bauer *et al.*,[24] the protein concentrations in SF are consistently less than those found in the blood due to the small permeability of the synovial microvascular endothelium to blood proteins and continuous clearing of proteins back to the blood stream by lymphatics.

Disposition of naproxen in rat skin

Table 1 shows the naproxen concentrations in the epidermis, dermis, and muscle tissues of the rats at 3, 6, and 12 h after the topical and oral routes of administration. The data indicate that the majority of the penetrated drug is retained in the skin following the topical dose. The naproxen concentrations in the epidermis at these sampling times remain nearly the same, suggesting that

Table 1. Naproxen concentrations in skin tissues following administration (2·5 mg naproxen/rat) of topical gel (2% IPM) and oral solution at 3, 6, and 12 h postdose

	μg naproxen/g tissue					
	3 h		6 h		12 h	
Tissue	Topical	Oral	Topical	Oral	Topical	Oral
Epidermis	181 (±33·1)	0·78 (±0·13)	160 (±42·8)	0·29 (±0·03)	168 (±67·5)	0·15 (±0·03)
Dermis	40·6 (±10·2)	0·90 (±0·19)	34·4 (±5·06)	0·68 (±0·15)	28·0 (±4·28)	0·18 (±0·03)
Muscle	0·83 (±0·21)	17·0 (±8·43)	0·24 (±0·04)	5·30 (±2·92)	0·55 (±0·14)	4·01 (±2·22)
Blood	0·23 (±0·09)	52·5 (±9·70)	0·38 (±0·12)	25·3 (±7·15)	1·21 (±0·30)	13·4 (±5·05)
M–S ratio	3·61	0·32	0·63	0·21	0·45	0·30

the epidermis is saturated with the drug. However, an appreciable amount of naproxen ($0{\cdot}53 \pm 0{\cdot}24\,\mu g/g$ tissue) was found in the 3 h muscle sample. The initially high muscle–serum concentration ratio (M–S) of 3·6 is indicative of direct permeation of naproxen into the muscle tissues after the topical administration. However, at 6 and 12 h postdose, drug concentrations in the muscle have substantially declined despite increased serum concentration, resulting in the reduced M–S ratios of 0·63 and 0·45, respectively. The large decrease in naproxen concentrations in the muscle samples could be attributed to the rapid removal of drug by the systemic circulation. When an equal dose of naproxen was administered by oral intubation, drug concentrations in the blood and muscles were significantly increased while naproxen in the epidermis and dermis was greatly reduced as compared to the topical dose as shown in Table 1. Despite lower naproxen concentrations found in the muscle and blood after the topical dose, the M–S ratios were significantly higher than those of the oral dose. This result thus demonstrates the possibility of targeting drugs by topical delivery to local tissues without producing high serum concentrations. McNeill *et al.*[25] measured the muscle tissue–plasma concentration ratios (T–P) of piroxicam following i.v. and topical routes of administration in rats. They found that the T–P ratios remained essentially the same over time after i.v. administration, while the T–P ratios for the topical dose continually decreased. However, the T–P ratios of the topical dose were significantly higher than those of the i.v. dose.

Despite numerous studies reporting the activities of various chemical enhancers on percutaneous absorption, the potential effects of these chemical enhancers on the disposition of drugs in local tissue have been rarely investigated. Behl *et al.*[14] found that the effect of vehicles on the skin disposition was quite different from that on the transdermal absorption of drugs, indicating that increased absorption does not necessarily represent higher drug concentrations in the local target tissue. In this study, the effects of IPM on the local tissue disposition and systemic absorption of topical naproxen were investigated in rats. Figure 3(a)–(c) illustrates that IPM in the gel significantly enhanced the naproxen concentrations in the muscle ($\sim 60\%$) and dermis ($\sim 40\%$) as well as in the blood ($\sim 90\%$) as compared to the control gel without IPM at each of the three sampling points. On the other hand, the change in drug concentrations in the epidermis after IPM treatment shown in Figure 3(D) was not statistically significant ($p > 0{\cdot}05$). Based on the *in vitro* data found in our laboratory which showed the significant enhancing effect of IPM on the naproxen diffusivity through the skin by disrupting the lipid barrier,[26] it is suggested that IPM changed the dermal and muscle tissue disposition of naproxen by increasing the drug diffusivity through the epidermis without significantly enhancing the drug accumulation in the epidermis.

In conclusion, the topical administration of naproxen in the PF-127 gel resulted in long sustained *c–t* profiles in serum and SF in dogs. The extensive

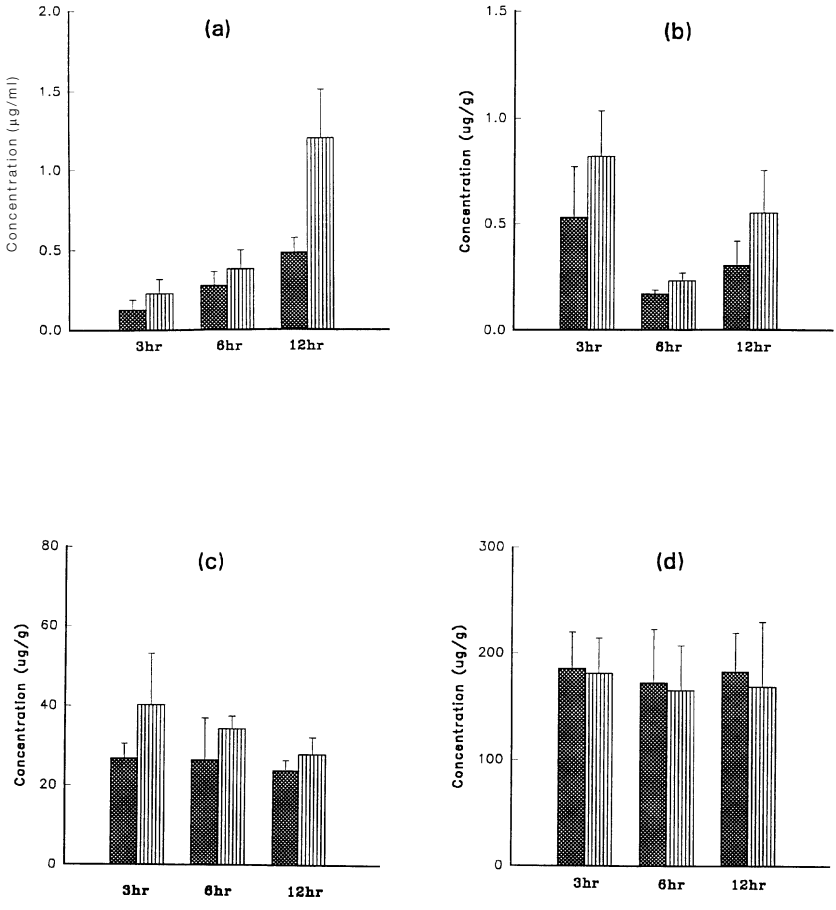

Figure 3. Naproxen concentrations in (a) serum, (b) muscle, (c) dermis and (d) epidermis following a topical dose ($2{\cdot}5\,mg\,kg^{-1}$) with (▥) and without (▨) IPM in rats (data shown as mean $\pm$ SD, $n = 5$)

reservoir function of the skin was indicated by the continuous steady state for the next 12 h after removal of the remaining gel from the skin. Naproxen concentrations in SF were much lower, reached the peak many hours later and declined more slowly as compared to serum concentrations, suggesting the presence of a significant diffusion barrier between the blood and SF. A large accumulation of naproxen in the skin and muscle tissues following a topical dose in rats could explain the low systemic availability and longer half-life of the topical naproxen found in dogs. IPM increased the naproxen concentrations in the dermis, muscle, and systemic circulation, but it had little effect on

the epidermal concentrations of naproxen. The low systemic absorption and large accumulation in the skin suggested the potential therapeutic value of topically delivered naproxen for rheumatoid arthritis and other topical inflammatory conditions.

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