Penetration of a topically applied nonsteroidal anti-inflammatory drug into local tissues and synovial fluid of dogs

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Objective—To investigate penetration of a topically applied nonsteroidal anti-inflammatory drug (NSAID) into tissues and synovial fluid.

Animals—5 Greyhounds.

Procedure—Dogs were anesthetized and microdialysis probes placed in the dermis and gluteal muscle over each coxofemoral (hip) joint. Methylsalicylate (MeSA) was applied topically over the left hip joint. Dialysate and plasma (blood samples from the cephalic and femoral veins) were obtained during the subsequent 5 hours. Dogs were euthanatized, and tissue samples and synovial fluid were collected and analyzed for salicylic acid (SA) and MeSA by use of high-pressure liquid chromatography.

Results—SA and MeSA concentrations increased rapidly (< 30 minutes after application) in dialysate obtained from treated dermis. Salicylic acid also appeared in plasma within 30 minutes and reached a plateau concentration after 2 hours, although combined drug concentrations (SA plus MeSA) in plasma obtained from femoral vein samples were twice those measured in plasma obtained from the cephalic vein (SA only). Treated muscle had a progressive decrease in NSAID concentration with increasing depth (SA and MeSA), but it was significantly higher than the concentration in untreated muscle. Substantial amounts of SA and MeSA were also measured in synovial fluid of treated joints.

Conclusions and Clinical Relevance—Topically applied NSAIDs can penetrate deeply into tissues and synovial fluid. Local concentrations higher than circulating systemic concentrations are suggestive that direct diffusion and local blood redistribution are contributing to this effect. Systemic blood concentrations may be inadequate to describe regional kinetics of topically applied drugs. (*Am J Vet Res* 2005;66:1128–1132)

Topical application of **nonsteroidal anti-inflammatory drugs (NSAIDs)** has obvious advantages over oral administration, including avoiding hepatic first-pass metabolism and limiting gastrointestinal tract disturbances.[1] However, controversy still exists concerning the efficacy of topical NSAIDs in the treatment of musculoskeletal conditions,[2] although topical NSAIDs are generally accepted as therapeutically beneficial and are used extensively as over-the-counter medications for patients with soft tissue inflammation and arthritic pain.

Much of the controversy relates to the mechanisms by which topical NSAIDs penetrate into joints and deep tissues. Several studies have suggested that topical NSAIDs, including diclofenac[3] and felbinac,[4] achieve therapeutic concentrations in tissues and synovial fluid via systemic redistribution in the blood after local absorption by the skin. However, these findings, derived from comparing patterns of systemic-plasma versus tissue-drug concentrations, did not measure analyte concentration in plasma collected from regional draining vessels, which may have been much higher than those determined after dilution in the systemic circulation. Other studies[5–7] in humans and nonhuman animals have suggested that there may be direct penetration, with possible local enhancement of topical delivery as a result of the orientation of local vascular networks.[8,9]

Investigation of penetration of topically applied drugs is limited by the availability of suitable in vivo techniques. In vitro techniques, although useful, may not adequately account for metabolic processes, alterations in vascular flow, or penetration into joints or deep tissues.[7,10] For example, first-pass metabolism is evident when **methylsalicylate (MeSA)** is applied to skin, with esterases in the epidermis and dermis rapidly hydrolyzing the **salicylic acid (SA)** ester to SA,[11] which contributes to markedly different ratios of MeSA to SA when comparing in vivo and in vitro results.[7] Microdialysis is one technique that has permitted direct characterization of time-versus-concentration patterns for transdermal penetration of drugs.[7,12–14]

To overcome deficiencies of existing techniques for investigating penetration of topically applied drugs, we developed an experimental procedure in dogs that combined microdialysis and direct tissue concentrations to measure penetration of a commercial SA ester. This technique also permitted measurement of drug in plasma obtained from regional drainage vessels in addition to that in the systemic circulation to more accurately relate the time course and mechanism of penetration of topically applied drugs into deep tissues.

Materials and Methods

Animals—Five adult male Greyhounds were used in the study. Dogs were 3 to 6 years of age and weighed 27 to 34 kg. All dogs were healthy and had not been receiving medica-

Received August 24, 2004.
Accepted November 19, 2004.
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Supported by the National Health and Medical Research Council of Australia. Support for Dr. Mills was provided by a University of Queensland Early Career Research Grant.
The authors thank Liisa Ahlstrom for technical support, Brett McWhinney for performing analyses of methylsalicylate and salicylic acid, and Dr. John Morton for assistance with statistical analysis.
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tions; values for routine indices of hematologic and serum biochemical analyses were within reference ranges. This study was approved by the Animal Ethics Committee of the University of Queensland (SVS/445/03/UQ).

Study protocol—For each experiment, dogs were anesthetized by IV administration of sodium pentobarbital[a] (30 mg/kg), intubated, and maintained on oxygen. Dogs were monitored continually throughout the anesthetic episode, including assessment of heart rate, respiration rate, and reaction to stimuli (ie, blink reflex and jaw tone), and additional pentobarbital was administered as necessary to maintain anesthesia. Intravenous administration of physiologic saline (0.9% NaCl) solution was initiated at a rate of 2.5 mL/kg/h, and a lumbosacral epidural of morphine[b] (0.2 mg/kg; diluted in saline solution and injected at a dosage of 0.3 mL/kg) was administered via a 22-gauge spinal needle[c] positioned by use of the loss-of-resistance technique. An 18-gauge central venous catheter[d] was inserted into the left femoral vein and advanced to a point immediately caudal to the junction of the external iliac vein and caudal vena cava (this distance had been measured in 3 Greyhounds that were dissected during preliminary studies; the distance was marked on the catheter prior to insertion). Each dog was then placed in sternal recumbency, and the coxofemoral (ie, hip) joints were fully abducted without obvious effort. Partial pressure of expired carbon dioxide and blood oxygen saturation (via pulse oximetry) were measured,[e] and rectal temperature was measured by use of a digital thermometer.[f] Skin over each hip joint was clipped, and 2 microdialysis probes[g] were inserted (1 into the dermis and 1 into the gluteal muscle) immediately over each joint. This was achieved by use of an 18-gauge, 32-mm catheter[h] that was tunneled through the dermis or muscle. Probes were inserted in a retrograde direction and were approximately 2 cm apart on the skin surface (point at which the catheter entered and exited through the skin). Catheter entry and exit holes in the skin were sealed with cyanoacrylate glue.[i] Correct placement and depth of each probe were measured ultrasonographically.[j] Skin blood flow over the thigh region 5 cm cranial to probe insertion sites was measured by use of laser Doppler.[k]

A syringe driver[l] was used to pump PBS solution (pH, 7.4) through each microdialysis probe at a rate of 1.8 µL/min. This technique was used to measure drug concentrations in the surrounding interstitial fluid. Samples of plasma (harvested from 5-mL blood samples collected from the cephalic and femoral veins) and dialysate (50 µL; collected from the microdialysis probes in the dermis and muscle overlying both hips) were collected at 30-minute intervals. Sixty minutes later, 5 g of a commercial MeSA cream[m] was applied topically to the skin immediately over the 2 microdialysis probes in the left hip (treated hip). The MeSA cream was applied to a contact region of 3 × 7 cm. This area was then covered with a transparent surgical adhesive dressing,[n] and investigators were careful to prevent the MeSA cream from contacting the catheter entry or exit points. Samples of plasma and dialysate and physiologic variables were obtained at 30-minute intervals for an additional 5 hours after application of the MeSA cream.

Five hours after application of the MeSA, an overdose of pentobarbital was administered, a cannula was inserted in the carotid artery, and each dog was exsanguinated. Incisions were made over both hips (treated and untreated) on each dog to permit collection of samples of skin, gluteal muscle (3 samples; 1 each at depths of 2, 5, and 15 mm below the skin surface), synovial fluid, joint capsule of the hip joint, and articular cartilage of the hip joint. Each sample was excised, blotted with tissue paper to remove extraneous blood, and then frozen at −20°C until analysis. All samples were analyzed for SA and MeSA concentrations by use of high-pressure liquid chromatography within 48 hours after collection, as described elsewhere.[7]

Recovery of MeSA and SA by use of microdialysis probes—In vitro recovery of SA and MeSA was calculated as described elsewhere.[7] Briefly, probes were removed after the dogs were euthanatized and placed in solutions of 2% bovine serum albumin[o] buffer (pH, 7.4) that contained known concentrations of SA and MeSA. Recovery was used to adjust dialysate concentrations to those estimated to be found immediately outside the probe tip. Because in vitro recovery may differ from in vivo recovery,[10] an internal reference technique[15] that involved the use of ^{14}C-radiolabeled SA[p] was also performed to confirm in vivo recovery and monitor probe integrity. Limit of detection for the assay was 10 µg/L.

Data analysis—The **area under the curve (AUC)** was calculated by use of curve-fitting software[q] to measure SA and MeSA concentrations in samples of plasma and dialysate in each dog. The Student *t* test for paired data was then used to compare differences in AUC and in tissue concentrations of SA and MeSA, whereas physiologic variables for each dog were compared by use of a 1-way ANOVA. These analyses were performed by use of a statistical software package.[r] Significance was defined as values of $P < 0.05$.

Results

Physiologic variables—Mean ± SD values for rectal temperature (37.4 ± 0.9°C), blood oxygen saturation (98.5 ± 1.3%), and partial pressure of expired carbon dioxide (33.7 ± 3.2 mm Hg) were stable and not significantly different throughout the study. Skin blood flow remained relatively constant throughout the study (2.1 ± 0.3 mL/min/100 mg of tissue).

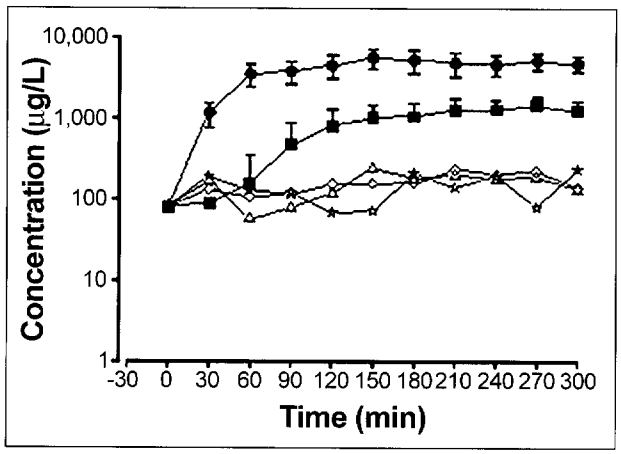

Figure 1—Mean ± SD concentrations of salicylic acid (SA) in dialysate collected from the dermis (solid circles) and gluteal muscle (open triangles) over the coxofemoral (ie, hip) joint, concentrations of MeSA in dialysate collected from the dermis (solid square) over the treated hip joint, and concentrations of SA in dialysate collected from the dermis (asterisk) and gluteal muscle (open diamonds) over the contralateral untreated hip joint of 5 Greyhounds after topical application of a commercial methylsalicylate (MeSA) cream to the left (treated) hip. Concentration of SA in dialysate collected from the dermis of the treated hip was significantly ($P = 0.010$) higher than the concentration in dialysate collected from the dermis of the untreated hip, whereas MeSA concentration was significantly ($P = 0.032$) higher in dialysate collected from the dermis of the treated hip, compared with the concentration in dialysate collected from the dermis of the untreated hip. Time 0 = Time of topical application of MeSA.

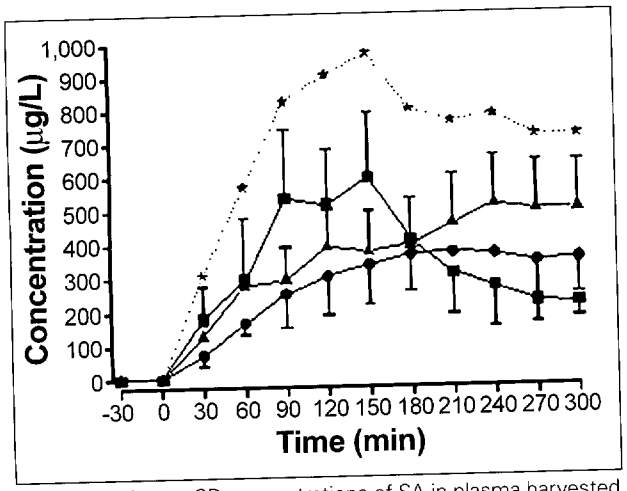

Figure 2—Mean ± SD concentrations of SA in plasma harvested from blood samples obtained from the cephalic vein (circles) and concentrations of SA (triangles) and MeSA (squares) in plasma harvested from blood samples obtained from the femoral vein of 5 Greyhounds after topical application of MeSA cream to the skin overlying the left hip joint. Total analyte concentrations (SA plus MeSA; asterisks) in plasma obtained from the femoral vein were significantly (P = 0.003) higher (area under the curve [AUC], 3,429 [h × µg]/L) than in plasma obtained from the cephalic vein (AUC, 1,348 [h × µg]/L). Time 0 = Time of topical application of MeSA.

Placement of microdialysis probes and in vitro and in vivo recovery—Ultrasonography confirmed that microdialysis probes in the dermis and muscle were at depths of 0.42 ± 0.04 mm and 19.5 ± 2.9 mm, respectively, below the skin surface. Analysis of in vitro recovery data revealed that there was a linear correlation between drug concentrations in the dialysate and the 2% bovine serum albumin solution over the range of concentrations for both SA and MeSA. Regression analysis was used to calculate a mean recovery of 35.3 ± 4.2% for SA and 29.1 ± 3.9% for MeSA. In vivo recovery was lower (27.3 ± 5.4%) for radiolabeled SA, although this would have been influenced by changing tissue concentrations of SA outside the microdialysis probe. In vitro recoveries were used to adjust dialysate SA concentrations for use in estimating corresponding tissue concentrations immediately outside the probe membrane.

Concentrations of SA and MeSA in dialysate and plasma—The time course of SA penetration into dialysate from the treated dermis was rapid, and the concentration was significantly (P = 0.010) higher than the dialysate concentration for the untreated dermis (**Figure 1**). Similarly, the MeSA concentration was significantly (P = 0.032) higher in dialysate from the treated dermis. However, SA concentrations in probes placed within the gluteal muscle were low and near the limit of assay detection. Concentrations of SA and MeSA were also detected in the regional and systemic plasma samples within 30 minutes after topical application of the MeSA cream, and they increased to a plateau concentration at approximately 180 minutes after application (**Figure 2**). The amount of SA measured in the plasma obtained from the femoral vein (AUC, 1,720 [h × µg]/L) was not significantly higher than that measured in plasma obtained from the cephalic vein (AUC, 1,348 [h × µg]/L), although the total amount of analyte (MeSA plus SA) in the local plasma (ie, plasma obtained from the femoral vein; AUC, 3,429 [h × µg]/L) was significantly (P = 0.003) higher that that in the systemic circulation (ie, plasma obtained from the cephalic vein) in which SA was detected but MeSA was not detected.

Concentrations of SA and MeSA in tissue and synovial fluid—We detected large amounts of SA and MeSA in skin from the treated hip with a progressive decrease in concentrations of both analytes with increasing tissue depth in the underlying muscle (**Table 1**). We detected SA but not MeSA in muscle of the contralateral untreated hip; however, similar concentrations of SA were found at each tissue depth. Substantial amounts of SA and MeSA were found in the synovial fluid collected from the treated hip, whereas no detectable concentration of SA or MeSA was measured in the synovial fluid from the untreated hip, the fibrous joint capsule from either hip, or articular cartilage from either hip.

Discussion

In the study reported here, we documented local direct penetration of a topically applied NSAID to deep tissues and synovial fluid. Penetration of topically applied SA formulations into underlying muscle has been reported elsewhere.[6,7,10,16] Similarly, diclofenac has been measured in synovial fluid collected from the knee joints of humans,[3,4] although the mechanism by which it got there is unclear.

Measurement of drug concentrations in peripheral vasculature[16,17] has been used to suggest systemic redis-

Table 1—Mean ± SD concentrations of salicylic acid (SA), methylsalicylate (MeSA), and total analyte (SA plus MeSA) in samples of tissue and synovial fluid obtained from 5 Greyhounds after topical application of a commercial MeSA cream to the skin over the left (treated) coxofemoral (ie, hip) joint.

	Treated			Untreated		
Sample	SA	MeSA	Total	SA	MeSA	Total
Skin (µg/g)	38,330 ± 20,056*	143,500 ± 107,900*	182,300 ± 114,600*	0 ± 0	0 ± 0	0 ± 0
Muscle depth						
2 mm (µg/g)	3,975 ± 1,735*	5,923 ± 2,776†	9,896 ± 3,756‡	346 ± 330	0 ± 0	346 ± 330
5 mm (µg/g)	2,471 ± 1,163§	2,159 ± 1,434‖	4,639 ± 2,527¶	289 ± 263	0 ± 0	289 ± 263
15 mm (µg/g)	1,318 ± 573#	0 ± 0	1,318 ± 573	306 ± 252	0 ± 0	306 ± 252
Synovial fluid (µg/L)	256.4 ± 137.3**	284.2 ± 183.2††	539.8 ± 261.9‡‡	0 ± 0	0 ± 0	0 ± 0

Muscle depth represents the depth beneath the skin at which the sample was obtained.
–‡‡Within a row, value differs significantly (P < 0.001, †P = 0.005, ‡P = 0.002, §P = 0.004, ‖P = 0.028, ¶P = 0.013, #P = 0.017, **P = 0.006, ††P = 0.088, and ‡‡P = 0.033) from the corresponding value for the contralateral untreated hip.

tribution as the main source of drug penetration into deep tissues and joint fluid. However, application of pharmacokinetic principles to peripheral plasma drug residues, particularly when not accounting for dilution,[6] would be unlikely to apply to those concentrations found at local application sites.[7] To our knowledge, the study reported here is the first in which investigators measured direct regional vascular drug residues, revealing a 2-fold higher concentration of drug (SA and MeSA) in this regional drainage, compared with concentrations (SA only) in the systemic circulation. Concentrations of SA in muscle tissue samples obtained from the untreated hip were comparable to systemic plasma concentrations, confirming the existence of some systemic distribution of drugs shortly after topical application. More importantly, there was no evidence of SA in the contralateral (untreated) hip, yet both the applied ester (MeSA) and SA were clearly detected in synovial fluid obtained from the treated hip and plasma obtained from the regional vasculature.

Elucidating the exact mechanisms that govern the transdermal movement of drugs is a complex issue and beyond the scope of the study reported here; however, it has been reviewed elsewhere.[1] Studies[8,16] in rats have revealed that concentrations of NSAIDs in tissues peak at 2 to 4 hours and again at 10 hours after application, which reflects direct absorption and systemic delivery, respectively. Use of microdialysis in our study confirmed early (within 30 minutes after application) penetration of topically applied MeSA. Some evidence of early appearance of drug in muscle obtained from the treated hip was apparent, although these concentrations were approximately the limit of detection of the assay and may be unreliable.

Direct penetration of NSAIDs reportedly[18] is prominent to depths of only the first 3 to 4 mm of tissue, decreasing exponentially from this point in deeper tissue layers. Drug penetration did not necessarily correlate with depth of the microdialysis probe, which suggests that there perhaps was a combination of direct penetration and systemic blood redistribution. To overcome these difficulties, drug tissue residues were determined in samples obtained after drainage of systemic blood from each dog (ie, exsanguination) to limit any contamination of tissue samples by the blood. Analysis of these results revealed a gradual decrease in NSAID concentration as samples were obtained closer to the joint of the treated hip, compared with concentrations in the untreated hip in which SA concentrations were similar in the tissues irrespective of depth.

A drawback of the microdialysis technique is that the low flow rate of perfusate, which is necessary to permit equilibration with the interstitial fluid, resulted in limited volumes of dialysate for analysis at each collection point. The type of perfusate will also influence drug concentrations within the dialysate,[19] and although PBS solution may be adequate to use when assessing cutaneous penetration of SA and SA esters from the dermis,[6,7] it may be unsuitable for detecting drug bound to proteins within interstitial fluid and within muscle cells. Therefore, we were unable to assess the time course of drug penetration into the tissues beneath the site of application by use of the microdialysis technique and instead had to rely on concentrations measured in tissues obtained at the completion of the study. Additional studies are necessary to assess more suitable perfusates that can be used to investigate tissue drug concentrations in dogs.

Drug penetration into deep tissues may be facilitated by a phenomenon of local enhanced topical delivery[8] whereby the local vasculature creates a convective force that carries topically applied drugs down into the underlying tissues before the drugs are distributed into the systemic circulation.[9] Indeed, it has been suggested[20] that MeSA can enhance its own penetration into deep tissues by increasing local blood flow. Gluteal musculature in dogs, similar to that in other species, is highly vascular and may contribute to deep penetration of SA and MeSA. One difference that may be evident in humans for the penetration of joints by NSAIDs is that many of the studies[3,4,21] have used the knee when investigating synovial concentrations of topically applied NSAIDs, and the relatively low amounts of muscle associated with this joint may alter the pattern of direct drug penetration observed in the dogs reported here. Furthermore, the validity of these evaluations of NSAID penetration into synovial fluid may be questioned because the inflammatory response associated with rheumatoid arthritis[3,4] or piercing of the synovial membrane[21,22] may enhance the ability of NSAIDs to penetrate the synovium.[23,24]

Concentrations of SA in synovial fluid obtained from the treated hip joint (256.4 ± 137.3 μg/L) were substantially less than those (130 to 150 μg/mL) that can inhibit the production of prostaglandin E, a primary inflammatory mediator, by 50% in inflammatory exudate.[25] This is consistent with anecdotal evidence of suitable efficacy of topically applied NSAIDs for patients with soft tissue inflammation but inconclusive results for patients with arthropathies.[2] Repeated application increases the bioavailability of topically applied MeSA over time.[26] However, variability in tissue penetration for other NSAIDs appears to be related to the stratum corneum barrier, with similar penetration achieved once this barrier is removed.[27] It would therefore be useful to repeat the study reported here with other NSAIDs in various vehicles to determine concentrations of drug that penetrate to deep tissues and joints.

Analysis of results of the study reported here revealed that topically applied NSAIDs can directly penetrate to deep tissues and synovial fluid. Concentrations in local blood were higher than concentrations in the systemic circulation, which would suggest that direct diffusion and local blood redistribution must be contributing to this effect. This study also documented that systemic blood concentrations may be inadequate to describe the regional kinetics of topically applied drugs.

a. Nembutal (60 mg/mL), Merial Australia, Parramatta, Australia.
b. Morphine sulphate injection (5 mg/mL), Sigma Pharmaceuticals, Sydney, Australia.
c. Terumo spinal needle, Terumo Corp, Tokyo, Japan.

d. Central line kit, Omega Spectramed, Swindon, UK.
e. Propaq CS vital signs monitor, Welch Allyn, Beaverton, Ore.
f. BD digital thermometer, Becton, Dickenson and Co, Franklin Lakes, NJ.
g. CMA microdialysis probe, 20 kd MWCO polycarbonate, CMA Microdialysis, Stockholm, Sweden.
h. Surflo, Terumo Corp, Tokyo, Japan.
i. Super glue, UHU GmbH, Buhl, Germany.
j. SONOS 5500, 12-5 MHz probe, HDI-5000, Phillips, Corona, Calif.
k. BLF21D laser Doppler flow meter, Transonics Inc, New York, NY.
l. Graseby MS-16a syringe driver, Smiths Medical, Bundall, Australia.
m. Dencorub pain-relieving cream (20% methylsalicylate), Carter Wallace, Brookvale, Australia.
n. Opsite, Smith & Nephew, North Ryde, Australia.
o. Bovine serum albumin, fraction V (B3009), Sigma Chemical Co, St Louis, Mo.
p. Hydrocortisone (^{14}C), American Radiolabelled Chemicals Inc, St Louis, Mo.
q. GraphPad Prism, version 4.00 for Windows, GraphPad Software, San Diego, Calif.
r. Stata, version 8.2, StataCorp, College Station, Tex.

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