Local Enhanced Topical Delivery (LETD) of Drugs: Does It Truly Exist?

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There is considerable uncertainty over whether and to what extent topically applied drugs can be delivered directly to anatomical sites beneath the skin, without prior entry into the systemic blood circulation. The *in vivo* studies reported in this work were designed to assess whether local enhanced topical delivery (LETD) can be achieved with piroxicam, a nonsteroidal antiinflammatory drug. Equivalent doses of tritium-labeled drug were administered by the i.v. or topical routes to male rats. The *topical* plasma profile reveals a maximum concentration (Cp_{max}) at 12 hr, compared to a typical, multiexponential decline in plasma concentration after i.v. dosing. All four muscles from the topically dosed shoulder exhibit two distinct peaks, the first at 4 hr and a later one at 12 hr (which coincides with the topical Cp_{max}). The contralateral muscles from the nondosed shoulder, in contrast, produce only a single peak at 12 hr after topical dosing. After the i.v. administration of piroxicam, the concentration–time profiles for each muscle closely parallel that seen for the i.v. plasma. Tissue-to-plasma ratios (T/P) show that the topical nondosed and the i.v. muscles are nearly constant over the entire time course of this study, indicating a pseudo-equilibrium between the plasma and those muscles. However, the early T/P ratios for the topically dosed muscles are markedly elevated and gradually decline to a constant value only after 12 hr, indicating that a similar pseudo-equilibrium is not established in this case. Thus, these results strongly imply that the topical administration of a drug can lead to LETD for tissues subjacent to the skin. Further, based on the elevated T/P ratios, these local enhanced drug levels cannot be solely attributed to entry from the systemic blood and suggest summarily that the cutaneous microvasculature is simply not an "infinite sink" for removal of all topically applied drugs.

KEY WORDS: local enhanced topical delivery; piroxicam; rat *in vivo* model; selective topical drug delivery.

INTRODUCTION

Drugs applied topically to the skin can be categorized according to their intended site of action. *Local enhanced topical delivery* (LETD) would refer to the situation where the desired target would be various subjacent structures such as the underlying muscle and synovium. Implicit in this definition of LETD is the premise that the local accumulation of drug does not occur solely as a consequence of topically applied drugs being first absorbed into the systemic blood, then distributed via the local vasculature. Although the phenomenon of LETD has not been firmly established, there are a number of examples in the literature which suggest that it does, indeed, occur (1–3,4). The true significance of LETD, if it exists, lies within the larger concept of *selective topical drug delivery*, or the differential disposition of drugs to either systemic or local sites. That is, the actuality of LETD would immediately conjure questions as to what biopharmaceutical factors determine or influence the extent to which topically applied drugs can be targeted locally or systemically.

While it is true that the inherently low permeability of skin, specifically the stratum corneum, still remains as the primary limitation for topical drug delivery, there has been substantial progress recently in understanding the properties of stratum corneum and how to modify or circumvent its poor transport characteristics (5,6). Thus, it is now sometimes possible, in the laboratory, to obtain reasonable permeability coefficients for many topically applied therapeutants using penetration enhancers such as oleic acid (7). With that in mind, other physiological features of the skin (e.g., metabolism, blood flow, etc.) would seem to be a logical choice as far as areas in need of greater understanding (and investigation) to probe the phenomena of LETD and selective topical drug delivery. Appropriately, a recent publication has pointed to the potential role of the cutaneous microvasculature in determining the disposition of topically applied agents (8).

The objective of these studies was to determine whether LETD can be achieved, on an acute basis (i.e., after a single dose), following the topical application of piroxicam to rats *in vivo*. As experimental points of comparison, several muscle concentration–time profiles and tissue-to-plasma (T/P) ratios were compared after i.v. and topical dosing. Importantly, the levels of drug as a function of time were also assessed in the contralateral nondosed muscles after topical administration.

MATERIALS AND METHODS

Materials

Radiolabeled piroxicam (4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide) was obtained by tritium exchange according to the method of Boskin and Rogers (9). After repeated recrystallizations in ethanol, HPLC analysis demonstrated a purity of greater than 98%. The final specific activity of piroxicam was found to be 27 μCi/mg, and the tritium label was determined by mass spectroscopy and ^{13}C-NMR to be singly attached to the 8 position on the aromatic ring of the benzothiazine nucleus (10). Previous animal studies in rats, dogs, and rhesus monkeys found this tritium label to be relatively stable and useful in identifying specific metabolites of piroxicam (11). Tissue digestion and liquid scintillant solvents, Solvable and Formula-989, were purchased from New England Nuclear (Boston, MA). All other chemicals and solvents utilized were at least reagent grade. The animals were Sprague–Dawley (Charles River, Wilmington, MA) male rats, approximately 30 days old, received during the week of their intended use in these experiments. No restrictions were placed on their food and water intake prior to experimentation, but only rats weighing 125 g ± 10% were utilized. All of the animals in the

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topical group were dosed early in the day, then housed individually for the duration of the experiment to prevent any cross-contamination. In addition, efforts were made to observe the rats, particularly the first 12 hr after dosing, in order to detect evidence of oral ingestion by the animal or loss of material to the cages. Suspect animals were not included in the results.

Methods

The animals were dosed with 50 μg of ^{3}H-piroxicam by either the i.v. or the topical route. For the i.v. dose, the rat was first lightly anesthetized with ether while the jugular vein was surgically exposed on the neck. Drug, dissolved in 50μl of sterile normal saline, was injected as a bolus directly into this vein. The topical dose was applied onto a 1-cm^2 area of the animal's right shoulder. Prior to the application, the hair was clipped with animal shears, taking care not to injure the skin surface. The actual skin area was marked by means of a template and indelible ink to facilitate dosing uniformity. The topical piroxicam was administered in 10 mg of a Carbopol-based gel where the final concentration of drug was 0.5% (w/w). This amount of formulation was just sufficient to provide a thin film covering over the 1-cm^2 dosing area. This specific region on the shoulder was selected for two reasons: First, it is not readily accessible to the animal's mouth or paws; second, the muscles which lie beneath this site are well defined and can easily be isolated by conventional surgical procedures.

At preselected times after dosing (*viz.*, 2, 4, 6, 8, 12, 16, 24, and 48 hr), the rats were lightly anesthetized with ether before exsanguination by cardiac puncture. In general, 70 to 90% of their estimated total blood volume was removed by this method. After obtaining the blood sample, the dosed area on the right shoulder was wiped with ethanol prior to skin removal. The skin was removed by first making an incision near the hind quarter, and then pulling it toward and over the head of the animal. This process exposed three superficial muscle groups, the tricep, acromiotrapezius, and spinodeltoideus. The surfaces of these muscles were wiped with ethanol to check for contamination resulting from the removal of the skin. These three muscles were sequentially excised intact, dipped in ethanol, gently blotted dry, and weighed immediately in tared vials. As each muscle was removed, the surfaces of the remaining tissues were again wiped with ethanol. Last, a deeper muscle, the supraspinatus, lying immediately on top of the shoulder joint capsule, was similarly isolated. For those animals which were dosed topically, an identical procedure was utilized for the left or nondosed shoulder area. The entire process required no more than 10 min per side. With topical dosing, a minimum of eight animals per time point was utilized to generate the profiles, while at least four rats per point were employed for the i.v. portion of the study.

The muscles and blood were analyzed for tritium using a tissue digestion method. Each sample was incubated with 1.2 to 2 ml of Solvable for 3 to 5 hr at 50°C. Once the contents of the vial were completely solubilized, 12 ml of Formula 989 liquid scintillant cocktail was added. The samples were counted for 10 min on a Packard 2000CA liquid scintillation analyzer (Packard Instruments, Sterling, VA) which corrected for quenching and chemiluminesce. Blank and spiked tissue samples were processed in parallel as standards or controls. In addition, the 4-hr spinodeltoideus and acromiotrapezius muscles were exhaustively extracted and analyzed by HPLC to ascertain initially the relative extent of metabolism in those muscles after topical dosing.

RESULTS AND DISCUSSION

The average plasma concentration–time profiles obtained after topical and i.v. dosing are illustrated in Fig. 1. The i.v. profile corresponds quite well to that reported previously by Roskos and Boudinot (12), in which rats were given a similar dose of piroxicam, both exhibiting multiexponential declines in concentration as a function of time. After *topical* administration, the plasma reaches a maximum drug concentration at 12 hr, then decreases roughly in parallel with the i.v. curve. The concentration–time profiles for the muscles excised from the right shoulder area (i.e., topical dosage site) are shown in Fig. 2. In each muscle, the drug level after i.v. dosing was greatest at the 2-hr time point, implying that the maximum concentration occurs before the first sample. Further, the change in concentration appears to parallel that of the i.v. plasma, suggesting that the blood and muscles are in a state of pseudo-equilibrium. In contrast, two distinct peaks are seen in the spinodeltoideus, acromiotrapezius, supraspinatus, and spinodeltoideus muscles after topical application, one at 12 hr and an earlier one at 4 hr.

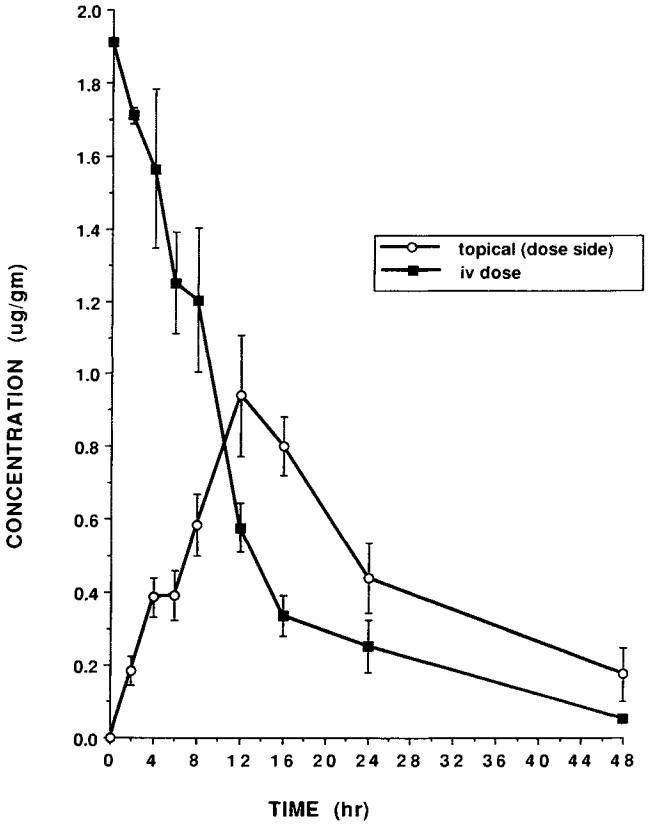

Fig. 1. Average plasma concentrations of piroxicam following i.v. and topical administration. Error bars represent the SE (for i.v., $n > 4$; for topical, $n > 8$).

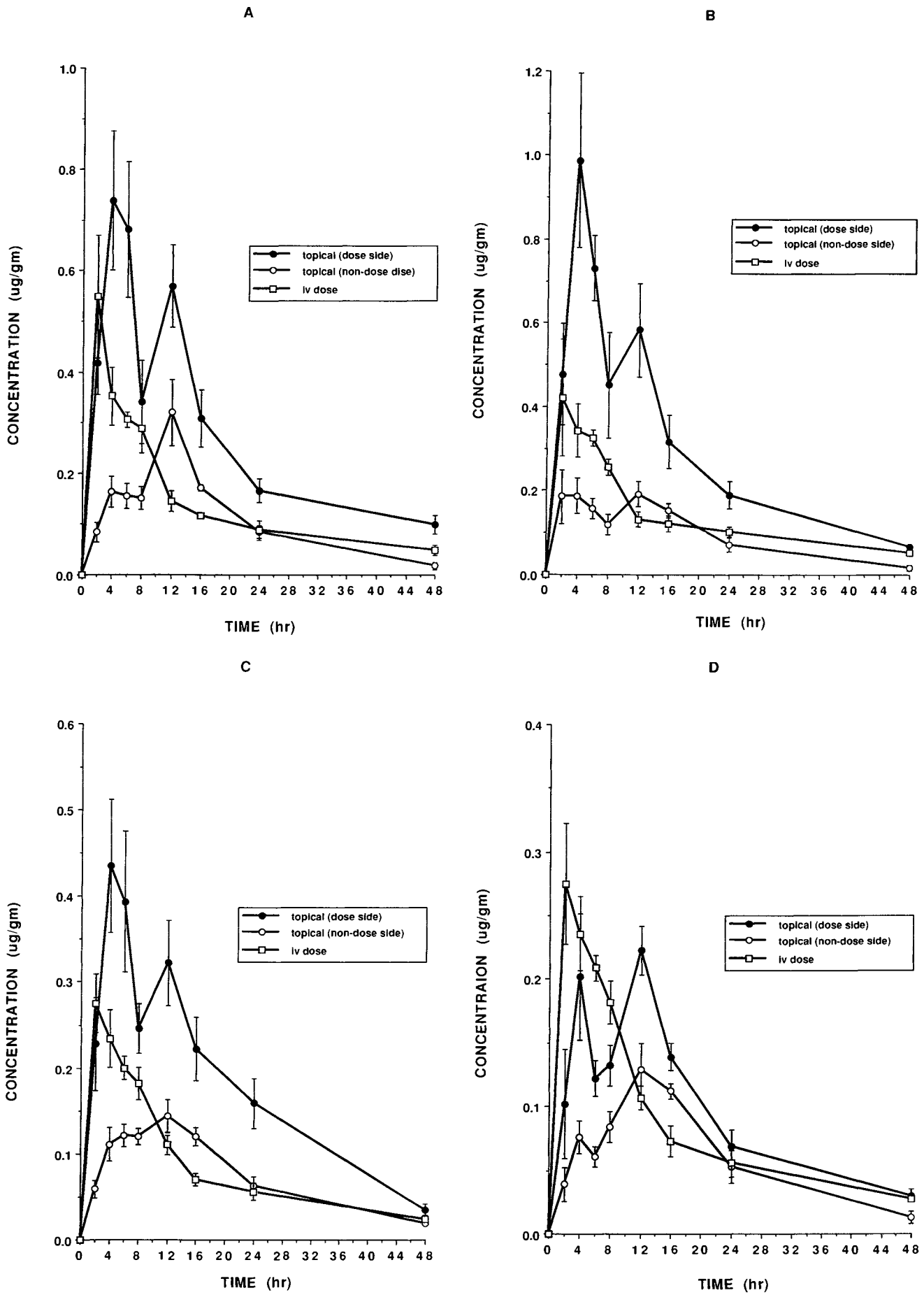

Fig. 2. Average muscle concentrations of piroxicam as a function of time following i.v. and topical administration including the results from the nondosed shoulder. Error bars represent SE. (A) Acromiotrapezius; (B) spinodeltoideus; (C) tricep; (D) supraspinatus.

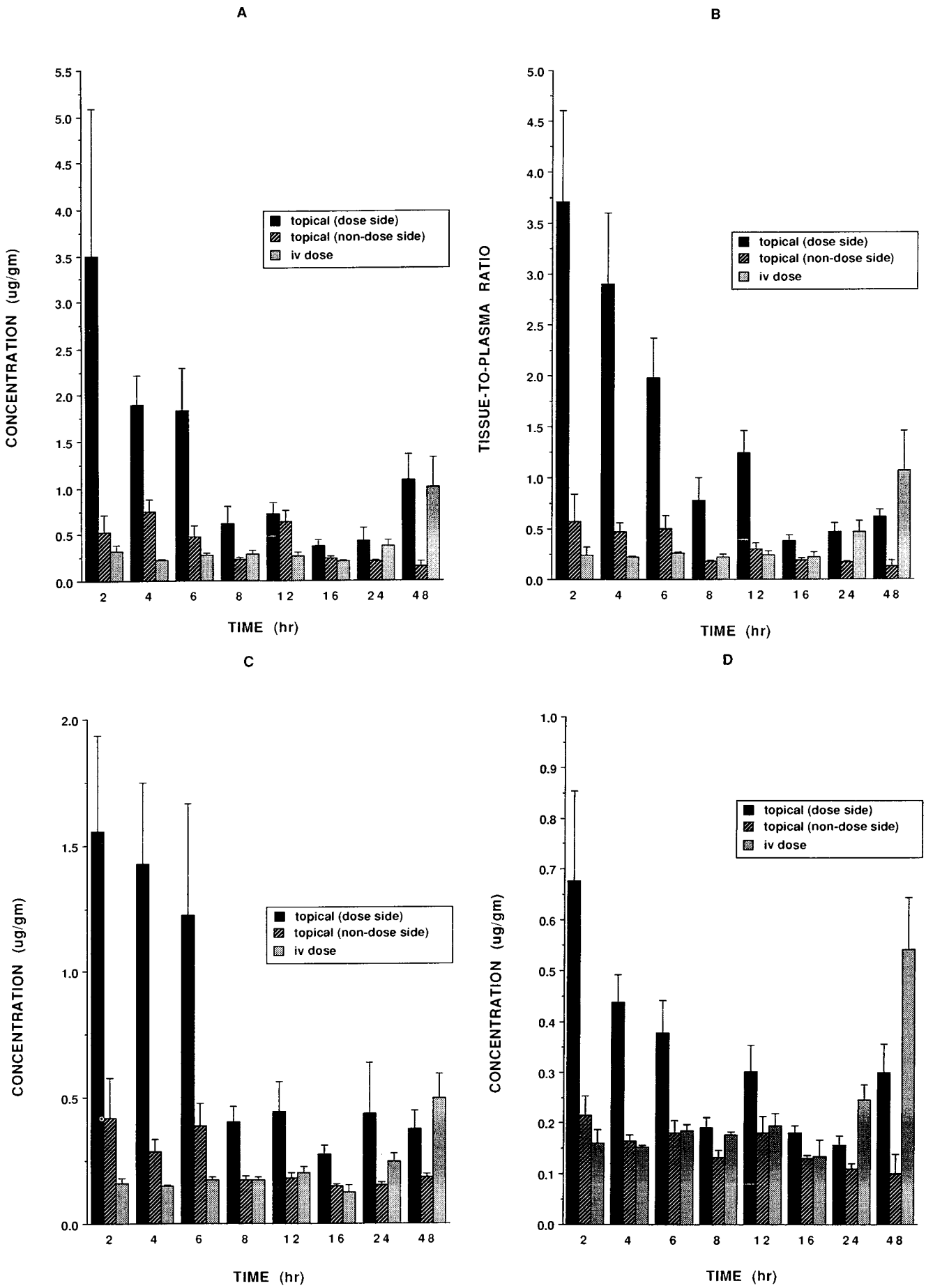

Fig. 3. Average tissue-to-plasma ratios for the dosed muscles following i.v. and topical administration including the results from the nondosed shoulder. Error bars represent SE. (A) Acromiotrapezius; (B) spinodeltoideus; (C) tricep; (D) supraspinatus.

The concentration at 12 hr corresponds to the time at which the maximum drug levels are observed in the topical plasma profile, suggesting that some drug is entering the muscle from the systemic blood. However, the presence of the earlier peak is not consistent with piroxicam derived from the systemic blood and insinuates that there is an alternate route, after topical dosing, for delivery directly to the muscles. This conclusion is further supported by the T/P ratios obtained for each muscle, and by comparison between the nondosed and the dosed shoulders, both of which are discussed below.

The T/P ratios, calculated for the muscles from the right shoulder area after i.v. and topical dosing, are plotted as a function of time in Fig. 3. The T/P ratios remain essentially constant with i.v. administration, suggesting a pseudo-equilibrium between the plasma and the dosed muscles, findings which are consistent with piroxicam tissue levels being a consequence of absorption from the systemic blood. This is not the case, though, when piroxicam is applied topically. The T/P ratios for the 2-, 4-, and 6-hr dosed-muscle samples are from 5- to 15-fold greater ($P < 0.05$) than the analogous i.v. ratios. Further, the topical T/P ratios continually decrease with time, at least out to 8 hr. A pseudo-equilibrium, therefore, is not established between the right shoulder muscles and the plasma after topical application, notably at the early times postdose. This pattern is consistent with the suggestion above, namely, that the drug present in the topically dosed muscles cannot be explained merely by its appearance from the general circulation. A reasonable hypothesis to explain these enhanced drug levels in the muscle, therefore, is that material is being transported to the underlying tissue from the skin surface, by some other physiological process, without prior passage into the systemic blood. Comparison of the topical muscle concentration–time profiles between the dosed and the nondosed sites further substantiates this hypothesis.

The muscle concentration–time profiles from the dosed and nondosed shoulder are shown in Fig. 2, while the respective T/P ratios are depicted in Fig. 3 (the nondosed shoulder area was processed and analyzed only after topical administration, and not after i.v. dosing). As shown by the concentration curves, the nondosed muscle levels are three- to fivefold lower than those observed directly under the dosed site, and the overall shape of their profiles resembles that of the topical plasma, with a single peak at 12 hr. The T/P ratios for the muscles removed from the nondosed site, like that seen after i.v. dosing, are essentially constant again, implying a pseudo-equilibrium between the plasma and the nondosed muscle. Thus, the profiles obtained for the nondosed tissues can be attributed to piroxicam which is derived from the general blood. The marked difference in the concentration–time profiles and the T/P ratios for the dosed and nondosed sites undeniably supports the hypothesis that topical administration can lead to enhanced local drug concentrations in subjacent tissues such as the underlying muscles.

In conclusion, this study was designed to investigate the phenomenon of LETD after topical dosing, in the rat *in vivo*. The experiments were conducted in such a manner to minimize any artifactual contamination of the tissue samples by the surgical isolation procedure. Ethanol swipes and rinses of the various muscles during the surgical procedure did, in fact, indicate that such surface contamination was not a concern. Strategic placement of the topical dose and continual observation ensured that substantial material was not lost to the sides of the housing cage or ingested. Complete exsanguination of the animals prior to the isolation of the muscles reduced any possibility of blood contamination. Preliminary HPLC analysis suggested that the radioactivity represented primarily intact drug (i.e., >80%), at least in the earlier time points. Further, separate *in vitro* permeability experiments (data not shown) indicated that the biphasic pattern of the muscle profiles is not a consequence of dosage form release or performance. In essence, we feel that these are well-designed, well-controlled studies with findings that can be explained only by the local enhanced topical delivery (LETD) of topical piroxicam from the surface of the skin, without prior entry into the general circulation. It is particularly heuristic to realize that the enhanced appearance of piroxicam in the subjacent muscles after topical dosing is impossible to explain on the basis of mass balance and diffusional calculations (13). Accordingly, there must be a "convective" physiological force which can transport significant amounts of mass in relatively short periods of time. Our bias is that the cutaneous microvasculature may contribute, at least in part, to this deduced convective process.

While these data have been gathered with rats, and extrapolation to man is clearly speculative, these studies unequivocally demonstrate, in principle, the phenomenon of LETD. There are a large number of variables that need to be probed in order to define fully the determinants (and mechanism) of LETD and, eventually, selective (i.e., systemic vs local) topical delivery. These variables include surface area, permeability, physiochemical and pharmacological properties of drug and vehicle, cutaneous metabolism, cutaneous blood flow, and distribution, in addition to the host of factors which modulate systemic clearance. Once the influence of these factors is established, the overall significance and impact of local enhanced drug delivery can be fully appreciated.

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