Regional Drug Delivery II: Relationship Between Drug Targeting Index and Pharmacokinetic Parameters for Three Non-Steroidal Anti-Inflammatory Drugs Using the Rat Air Pouch Model of Inflammation

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Purpose. To quantify the advantage gained by direct administration to a target site for two non-steroidal anti-inflammatory drugs (NSAIDs) piroxicam and diclofenac in the rat air pouch model of inflammation. To derive a model relating drug targeting index (DTI) to the pharmacokinetic parameters of the target and systemic sites, and to compare predictions with observations.

Methods. DTI was calculated based on area under the concentration time curve at target (pouch) and systemic site (venous blood) following administration into and sampling from both sites. A model was derived relating DTI to systemic clearance, target permeability, plasma protein binding and fraction of the targeted dose that is systemically available.

Results. Both NSAIDs exhibited linear pharmacokinetics over the dose ranges studies. They differed primarily in total body clearance which was approximately 16 fold greater for diclofenac (213 ml hr^{-1} per 250 g) than piroxicam (13 ml hr^{-1} per 250 g). Observed DTIs (11, 114 and 276 for piroxicam, S[+]ibuprofen [studied previously] and diclofenac) were ranked in order of total body clearance but were approximately 7.5 fold lower than predicted (101, 700 and 2214 respectively).

Conclusions. The discrepancy was explained by the influx of the plasma binding protein, albumin, into the target site due to increased vascular permeability associated with the inflammatory response. The originally derived equation for DTI, which assumed only unbound drug diffuses across the target site, was modified to take into account the simultaneous flux of bound drug.

KEY WORDS: drug targeting index; regional administration; pharmacokinetics; rat air pouch model; inflammation; non-steroidal anti-inflammatory drugs; diclofenac; piroxicam; S[+]ibuprofen; albumin flux.

INTRODUCTION

One direct method of targeting is by regional drug administration. Although used in the treatment of localised malignant diseases, the actual benefits of this target-organ directed drug administration have often been disappointing compared with conventional intravenous or oral drug administration (1–3). In recent years several theoretical studies have appeared (4–6) which attempt to predict the benefit to be derived from regional drug administration, however, few have attempted to quantitatively evaluate the advantages and major determinants of site specific targeting *in vivo*. Regional drug delivery is a useful experimental procedure for investigating many of the kinetic determinants of site specific drug delivery (4). Drug is administered directly to the target site and both pharmacokinetics and response can be compared to those achieved following systemic administration. We have previously shown with S[+]ibuprofen that the rat air pouch model of inflammation is a suitable model to explore the quantitative issues surrounding regional drug delivery (7).

The current work extends our findings to two other non-steroidal anti-inflammatory drugs, piroxicam and diclofenac chosen for wide differences in systemic clearance, to allow an evaluation of the pharmacokinetic issues pertinent to the regional delivery of drugs that are otherwise distributed non-specifically throughout the body. The observed advantage of regional administration of these two compounds and S[+]ibuprofen were compared to theoretical expectations, to allow an assessment of the underlying assumptions used in developing theoretical relationships.

MATERIALS AND METHODS

Chemicals

Piroxicam and 6-methylpiroxicam were gifts from Pfizer (Sandwich, England); diclofenac, [^{14}C]-diclofenac (372 kBq/mg), and 2-(p-cyclohexen-1′-yl-phenyl) propionic acid were from Ciba Geigy (Basel, Switzerland). Carrageenan (viscarin GP 109) were obtained from Marine Colloids (Philadelphia, Pennsylvania USA), heparin from CP Pharmaceuticals (Wrexham, United Kingdom) and microvette CB300 EDTA coated tubes from Sarstedt (Leicester, England). Other chemicals were of analytical or hplc grade from BDH Chemicals Ltd (Poole, United Kingdom).

Air Pouch Production

Male Sprague-Dawley rats (200–250g) obtained from the Biological Services Unit, University of Manchester were used throughout. Air pouches were produced as described previously (8). Experiments were conducted on day 6 when the reactivity of the air pouch to the irritant carrageenan is maximal (7).

Bolus Dose Studies

On day 5, the jugular vein and carotid artery were cannulated and the animals left to recover overnight. On day 6, carrageenan (20 mg in 5ml phosphate buffered saline pH 7.4 (PBSA)) was injected into the pouch. Immediately, the NSAID was administered as a bolus (1ml/kg) either via the jugular vein (i.v. - piroxicam 0.5, 0.1, and 0.05mg/kg, di-

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clofenac 10 and 20mg/kg) or directly into the pouch (i.p. - piroxicam 0.5 and 0.05mg/kg, diclofenac 10 and 20mg/kg). Piroxicam was given as a solution in PEG 400 and propylene glycol mixture (9:1 by v/v) and diclofenac as a solution in ethanol and PBSA (1:9 v/v). Serial samples (250μl) were collected from the carotid arterial cannula (blood) (n = 10) and directly from the air pouch (n = 10) into EDTA coated tubes, over 30 hr for piroxicam and 4 hr for diclofenac. These samples were centrifuged to obtain plasma and cell-free exudate, respectively, and frozen at −20°C until analysed. Between blood samples, the arterial cannula was flushed with 0.1ml heparinized normal saline (2U/ml).

Chemical Analysis

Piroxicam

Piroxicam concentrations were determined by HPLC based on the method of Boudinot and Ibrahim (10). A Hypersil ODS (20cm × 4.6mm) column with uv detection at 360nm and an eluent of 45% methanol, 55% phosphate buffer pH8 at 1ml/min was employed. To 150μl of plasma or exudate was added: 50μl of 6-methylpiroxicam (5μg/ml) as internal standard and 200μl of 1M phosphate buffer pH2. Following vortex mixing 2ml of the extractant dichloromethane was added followed by rotary mixing for 10 min. After centrifugation, the aqueous layer was removed and disgarded and the organic layer placed into a clean tube and evaporated to dryness under nitrogen at 45°C. The sample was reconstituted in 120μl of eluent, transferred into a crimp top vial and placed in the autosampler of a Hewlett Packard 1090M LC Chemstation; 100μl was injected on to the column. The limit of detection was 10 ng/ml with intra and interday precision of less than 10% when measured at 50, 500 and 2,500 ng/ml.

Diclofenac

Diclofenac concentrations were determined by HPLC based on the method of Godbillon *et al.* (11). A Hypersil ODS (20cm × 4.6mm) column with uv detection at 282nm and an eluent of 55% methanol, 16% acetonitrile and 29% of 1% (by volume) acetic acid pumped at 1ml/min was employed. To 100μl of plasma or exudate was added: 50μl of 2-(p-cyclohexen-1′-yl-phenyl)propionic acid (50μg/ml) as internal standard and 250μl of 0.9M phosphoric acid. Following vortex mixing, 2ml of the extractant hexane/2-propanol (90:10 v/v) was added followed by rotary mixing for 10 min. The sample was then centrifuged and frozen. The organic layer was poured into a clean tube and evaporated to dryness under nitrogen at 45°C. The sample was reconstituted in 120μl of eluent and then processed for HPLC analysis as for the piroxicam assay. The limit of detection was 0.1 μg/ml with intra and interday precision of less than 10% when measured at 0.5, 5 and 10 μg/ml.

Determination of Albumin

Albumin in plasma and exudate was determined by radial immuno-diffusion (Binding Site Institute, Birmingham Research Park, Birmingham), following appropriate dilution. Ring area was assessed by first imaging the ring and then processing the image; unknown concentrations were calculated by reference to a calibration curve (140 – 1400 mg/L).

Protein Binding

Pooled plasma and 10-hr air pouch exudate, obtained from rats bearing air pouches who had undergone the same surgery and carrageenan treatment as those used for drug targeting assessment, were spiked separately with piroxicam (0.5–100μg/ml), or diclofenac (^{14}C and cold 0.01–100μg/ml) and allowed to equilibrate for 15 min at 37°C. Duplicates (1ml) were then placed into Centrifree Micropartition System units (Amicon, Stonehouse, Gloucestershire) and spun at 2000g for 30 min at 37°C using a fixed angle rotor. Plasma, exudate and corresponding ultrafiltrate (200μl plasma; 400μl exudate) were then analysed for piroxicam by HPLC and [^{14}C]-diclofenac by scintillation counting, and the percentage of unbound NSAID calculated.

Pharmacokinetic Analysis

The area under the concentration-time profile (AUC) was calculated from the observed measurements using the linear trapezoidal approximation, with extrapolation to time zero and during the terminal phase, to infinity. The disposition kinetics following i.v. bolus doses, where derived from the fit of a biexponential equation to the plasma data, in the standard manner (12).

The selective gain associated with direct targeting, the Drug Targeting Index (DTI), was calculated as the ratio of the dose-normalised AUC in the air pouch and plasma following direct pouch administration divided by the same ratio following i.v. administration (5). That is,

$$DTI = \frac{\left[\frac{[AUC/Dose]_{exudate}}{[AUC/Dose]_{plasma}}\right]_{i.p.}}{\left[\frac{[AUC/Dose]_{exudate}}{[AUC/Dose]_{plasma}}\right]_{i.v.}} \quad (1)$$

where i.p., and i.v. represents intrapouch and intravenous administration, respectively.

The observed DTI values were compared with those predicted by a model that assumes that only unbound drug permeates across the membrane separating the target tissue from the target vasculature, and that the system operates under linear pharmacokinetic conditions. The model and derivation are provided in Appendix 1. The predicted DTI is given by (Eq (24A), Appendix 1).

$$DTI = 1 + \left(\frac{1}{Q_T} + \frac{1}{fu \cdot P}\right)\frac{CL_T}{1 - f_T} \quad (2)$$

where Q_T is the target tissue blood flow, fu is the fraction of drug in plasma unbound, P is the permeability surface area product for unbound drug fluxing across the membrane separating the target tissue from the vasculature, CL_T is the total body clearance of the drug (the sum of the systemic and target clearances) and f_T is the fraction of the dose administered directly into the target that is eliminated there. That is, $1 - f_T$ is the fraction of the target dose that escapes into the systemic circulation.

RESULTS

Intravenous Administration. Plasma concentration-time profiles for piroxicam (Fig 1A) and diclofenac (Fig 2A) could best be described by a biexponential equation. Pertinent pharmacokinetic kinetic data are listed in Table I. There was no indication of dose dependency in plasma pharmacokinetics over the dose range 0.05–0.5mg/kg for piroxicam, and up to 20 mg/kg for diclofenac. Air pouch exudate concentrations of piroxicam (Fig 1A) could be monitored for up to 30 hr for all doses employed; the concentration peaked at 5 hr and then declined slowly. Plasma and air pouch exudate concentrations tended to converge becoming similar by 24 hr. Air pouch exudate diclofenac concentration reached a maximum at 0.75 hr, then declined and could be monitored for up to 4 hr. Plasma and air pouch concentrations tended to converge with time becoming similar by 3 hr (Fig 2A).

Intrapouch Administration. Fig. 1B and 2B shows the results after intrapouch administration. For both drugs, decay from the air pouch occurred very rapidly. The dose-normalised pouch AUCs were not significantly different ($p > 0.05$), between 0.05 and 0.5 mg/kg piroxicam, (28.5 ± 2.8(sem) vs 23.5 ± 2.3 hr.L^{-1} per 250g) and between 10 and 20 mg/kg diclofenac (9.5 ± 0.6 and 9.8 ± 1.2 hr L^{-1} per 250 g rat respectively), indicating linear air pouch kinetics. Appearance of piroxicam in plasma was rapid reaching a maximum at 3 hr, after which time the concentration declined in line with that obtained after the i.v. bolus. The air pouch exudate and plasma concentrations tended to converge with time becoming similar by 10 hr. Appearance of diclofenac in plasma was also rapid reaching a maximum at 0.3 hr followed by a decline parallel with that obtained after i.v. bolus administration.

DTI Values

AUC determinations were calculated to the last measurable concentration, which occurred at approximately 4 and 30 hr for diclofenac and piroxicam, respectively. Based on the mean dose-normalised AUC values over the dose ranges studied, the calculated DTI was 276 for diclofenac, 114 for S[+]ibuprofen and 11 for piroxicam (Table II).

Protein Binding. Table III summarises the degree of binding of the NSAIDs in plasma and pouch fluid. Both diclofenac and piroxicam were highly bound in plasma, independent of drug concentration over the range of interest and with no difference between values in control plasma and those in plasma taken from pouched rats at 10 hr post-carrageenen administration. The unbound fractions in plasma were 0.025 and 0.016 for piroxicam and diclofenac, respectively. Owing to the lack of sensitivity of the assay, it was not possible to determine the binding of ibuprofen in plasma. None of the three NSAIDs were bound to constituents of the phosphate buffered saline containing carrageenan placed in the air pouch.

Albumin Influx. The plasma albumin concentrations of 22.5 and 22.2 mg ml^{-1}, 1 min and 5 hr after carrageenan administration to pouched and cannulated rats, were lower than that of control rats, 28.5 mg ml^{-1}. Previous studies have shown that over a 10 hr period radiolabelled albumin progressively rises in concentration in the air pouch, following i.v. bolus administration of a tracer dose of albumin (13). Also, the pouch albumin concentration rose at a similar rate of approximately 2.5% of the plasma concentration per hr for the first two hr and at approximately 1.3% hr^{-1} thereafter such that by 10 hr the pouch albumin concentration had reached 15% of that in plasma in the presence of the NSAIDs (13).

DISCUSSION

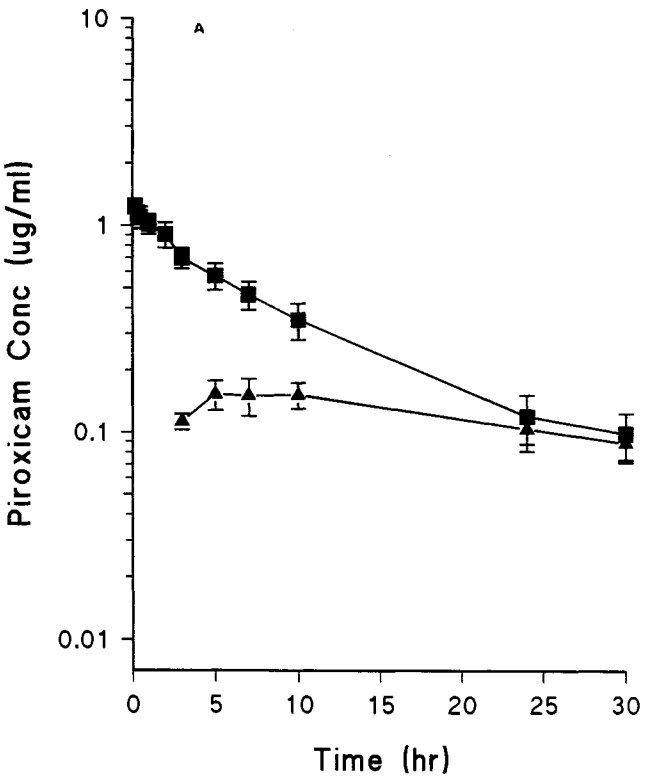
Plasma Kinetics of NSAIDs

The observed systemic clearance of piroxicam 13 ml hr^{-1} compares reasonably favourably with the value of 5 ml hr^{-1} for rat reported by Roskos and Boudinot (14), following 0.5mg/kg i.v. They described their data covering a time period of 96 hr by a triexponential equation with a terminal half-life of approximately 13 hr, which only became apparent after 30 hr. Our data covering 30 hr were best described by a biexponential equation with a terminal half-life of approximately 8 hr. The additional area and longer half-life tends to explain the lower systemic clearance and larger volume of distribution at steady state (125 ml vs 63 ml (Table I)) estimated by Roskos and Boudinot. Bearing this in mind, it appears that the presence of the air pouch does not markedly alter the pharmacokinetics of piroxicam.

Table I. Mean (±sem) Plasma Pharmacokinetic Parameters Obtained Following an i.v. Bolus Dose of Piroxicam and Diclofenac to Rats Bearing Six Day Air-Pouches[a]

Dose (mg/kg)	Clearance[b] (ml hr^{-1})	V_{ss}[b,c] (ml)	Initial t½ (hr)	Terminal t½ (hr)
Piroxicam				
0.05 (n = 5)	13 ± 2	115 ± 20	0.47 ± 0.10	7.1 ± 0.45
0.1 (n = 5)	10 ± 1.5	105 ± 13	1.08 ± 0.27	8.5 ± 1.10
0.5 (n = 5)	14 ± 2	150 ± 25	1.32 ± 0.19	8.3 ± 0.95
Diclofenac				
10 (n = 4)	213 ± 23	193 ± 40	0.11 ± 0.11	1.4 ± 0.24
20 (n = 5)	185 ± 28	105 ± 30	0.14 ± 0.01	1.3 ± 0.30

[a] Not statistically different ($P > 0.05$) by 1 way ANOVA.
[b] Normalised to a standard 250g rat.
[c] Volume of distribution at steady state.



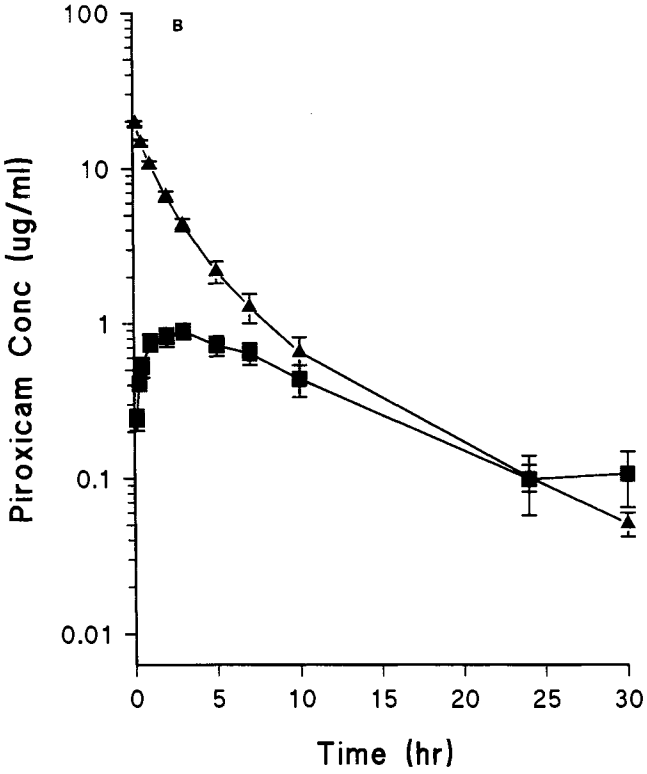
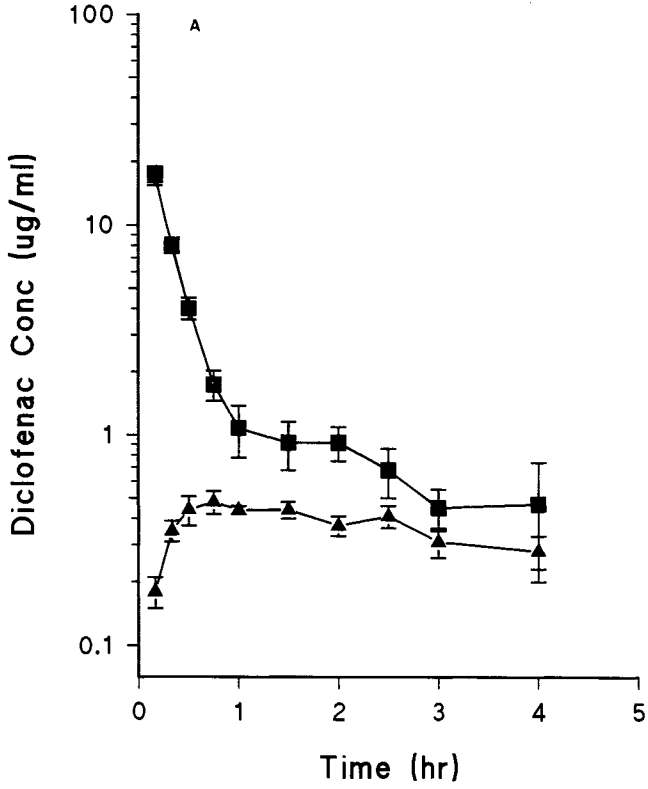


Fig. 1. Piroxicam: Semilogarithmic plots of mean ± sem plasma (■) (n = 5) and corresponding air pouch exudate (▲) (n = 5) concentration-time profiles of piroxicam obtained from rats bearing 6 day air pouches injected with 20mg carrageenan in 5ml PBSA following a 0.5mg/kg bolus dose of piroxicam administered A: intravenously; B: directly into the pouch.



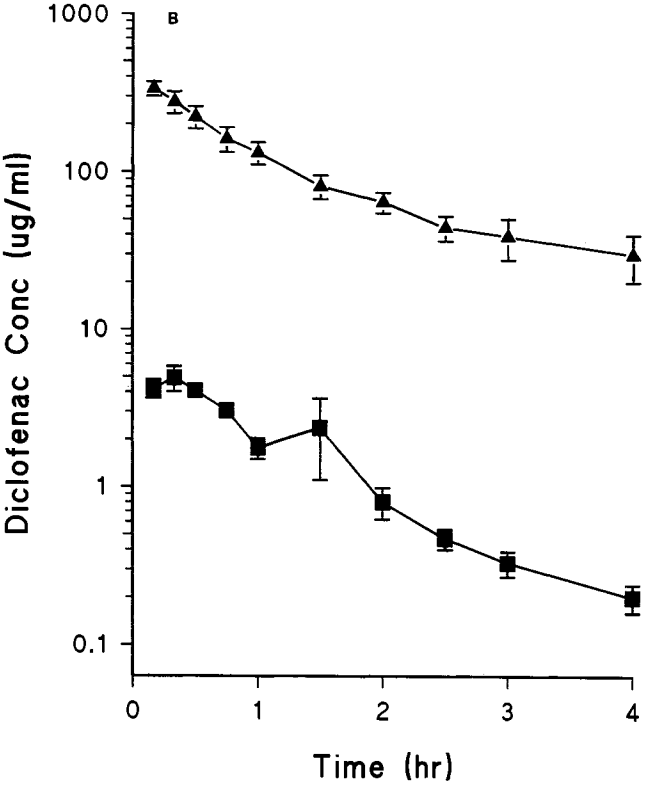


Fig. 2. Diclofenac: Semilogarithmic plots of mean ± sem plasma (■) (n = 4) and corresponding air pouch exudate (▲) (n = 4) concentration-time profiles of diclofenac obtained from rats bearing 6 day air pouches injected with 20mg carrageenan in 5ml PBSA following a 10mg/kg bolus dose of diclofenac administered. A: intravenously; B: directly into the pouch.

Table II. Dose-Normalised AUCs from Mean Plasma and Air Pouch Exudate Concentration-Time Profiles Following Intravenous and Intrapouch Bolus Administration, together with Exudate-to-Plasma AUC Ratios and Drug Targeting Index

Areas[a]	Route	Diclofenac	S[+]ibuprofen[b]	Piroxicam
Exudate AUC/Dose (hr L^{-1}kg)	intrapouch	41.0	38.9	85.8
Plasma AUC/Dose (hr L^{-1}kg)	intrapouch	1.07	4.12	22.1
Exudate AUC/Dose[c] (hr L^{-1}kg)	intravenous	0.15	0.39	7.3
Plasma AUC/Dose (hr L^{-1}kg)	intravenous	1.08	4.73	20.3
AUC Ratio Exudate:Plasma	intrapouch	38.3	9.44	3.9
AUC Ratio Exudate:Plasma	intravenous	0.14	0.08	0.36
Drug Targeting Index[d]		276	114	11.0

[a] Dose normalised area under concentration-time profiles diclofenac (0–4 hr), S[+]ibuprofen (0–10 hr), piroxicam (0–30 hr) based on total drug concentration.
[b] From Stevens et al (7).
[c] AUC normalised for the systemically available dose (13).
[d] Calculated using Eq 1.

The i.v. disposition kinetics of diclofenac in the rat remains largely undefined. Peris-Ribera *et al* (15) obtained a systemic clearance of 250 ml hr^{-1}, which compares favourably with our value of 213 ml hr^{-1}. They concluded that enterohepatic recirculation was mainly responsible for the second phase of diclofenac disposition following i.v. administration. As a large majority (70–90%) of the total AUC is incorporated in the first phase, the events in the second phase have little influence on total AUC and therefore on the estimate of systemic clearance. Overall, as with piroxicam and S[+]ibuprofen (5) the presence of the air pouch appears not to markedly alter the pharmacokinetics of diclofenac.

In comparison, the total systemic clearance of diclofenac was approximately four times that of S[+]ibuprofen (5) and 16 times that of piroxicam (Table I). The difference between the volumes of distribution at steady state was smaller (approximately 2 fold), indicating that the differences in terminal half-lives among the NSAIDs is primarily a reflection of differences in systemic clearance.

Air Pouch Exudate Kinetics of NSAIDs

Injection of carrageenan initiates an acute inflammatory reaction which alters the nature of the air pouch over the experimental time period (8, 16). As this may affect drug transport between plasma and air pouch exudate, caution was exercised in extrapolating AUC beyond the last measurable concentration, particularly following i.v. administration. The effect of such factors on AUC is less following intrapouch administration as the majority of the AUC, associated with systemic absorption of drug, is complete within 2 hr (13) and the contribution of systemically recirculating NSAID on the pouch AUC is very small.

Plasma Binding

The NSAIDs are bound primarily to albumin. For both diclofenac and piroxicam the surgical technique produced no obvious change in plasma protein binding, in keeping with the relatively small difference in plasma albumin concentration between control and surgically treated rats. The reported 2.6 per cent unbound piroxicam in rat plasma (1–10mg/L) (14) agrees well with the 3.1 per cent determined by us (Table III). There appears to be no previous reports of diclofenac binding in rat plasma for comparison. Although we were unable to determine the unbound fraction of ibuprofen in plasma, it is likely that the quoted value of 1.1 per cent (17) reasonably applies to the present study.

Predicted Versus Observed DTI

As originally defined, DTI refers to the ratio of drug delivered to the desired target site and toxicity site when the targeting system is used, to that when the drug alone is administered systemically (5). We believe that the use of plasma as a surrogate for the toxicity site is reasonable in the

Table III. Protein Binding of NSAIDs

NSAID		Fraction unbound X100			
		0.1 mg/L	1 mg/L	5 mg/L	10 mg/L
Piroxicam	Plasma from control rats[a]	—	2.8	—	2.5
	Plasma from pouch at 10 hr	—	4.2	2.7	2.4
	Exudate from pouched rats at 10 hr	—	12.3	10.0	10.8
Ibuprofen	Plasma from control rats[b]	—	1.2	—	1.1
Diclofenac	Plasma from control rats	1.98	1.55	—	1.65
	Plasma from pouched rats at 10 hr	1.54	1.47	1.65	1.78
	Exudate from pouched rats at 10 hr	3.62	3.94	—	3.98

[a] From Roskos and Boudinot (14).
[b] From Satterwhite and Boudinot (17).

case of the NSAIDs, as these drugs distribute relatively non-specifically, and the ratio of exposure of drug at the toxicity site (kidney and intestine) to that of plasma should therefore be independent of whether drug is administered directly into the pouch or systemically. We also believe, in the absence of any studies on the influence of temporal profile on the DTI for the NSAIDs, that AUC is a reasonable, and common measure of exposure of a site to drug.

The most commonly cited equation that allows prediction of DTI from pharmacokinetic parameters, is

$$DTI = 1 + CL_T/[Q_T (1 - E_T)] \quad (3)$$

where CL_T is total body clearance, Q_T is target blood flow and E_T is extraction ratio of the drug at the target site. This equation, which has been derived from both mass balance (5) and compartmental (6) considerations, is meant to apply to the ideal targeting system in which all drug is first delivered to the target site, prior to recirculation. However, careful analysis indicate that its use is essentially restricted to target site arterial administration. The situation is somewhat different when administration is directly into the target site, rather than into the artery feeding it. Here one needs to take into account both the permeability surface area product of unbound drug (P) transversing the membrane separating the target site from capillary blood, and the fraction of the locally applied drug that is eliminated there before reaching the systemic circulation, f_T. These two additional considerations have been taken into account in Eq (2), derived assuming that only unbound drug diffuses across the membrane and that the pharmacokinetics of the drug are linear, which is so for the three NSAIDs, over the ranges of doses studied.

The distinction between f_T and the extraction ratio of drug at the target site, E_T, can be appreciated by considering a drug with a low value of P that is readily eliminated at the target site. Because of its low permeability, when administered directly into the target much may be eliminated before it permeates into the systemic circulation. That is, f_T would be high and correspondingly, $(1 - f_T)$ low. In contrast, when given systemically, the value of E_T would be low, because of the low permeability. Within the context of the model, E_T is related to f_T by the expression, $E_T = [1/fu\,P + 1/Q_T] \cdot f_T$, from which it is seen that when the effective permeability $(fu \cdot P)$ of the drug is much greater than target blood flow, drug distribution becomes perfusion rate limited, $f_T \simeq E_T$, and Eq (2) reduced to Eq (3). That is, the DTI following placement of drug directly into the target site is then the same as that achieved following target arterial administration. In contrast, when the effective permeability is low, such that $Q_T \gg fu \cdot P$, Eq (2), reduces to

$$DTI = 1 + \frac{CL_T}{fu \cdot P(1 - f_T)} \quad (4)$$

in which case, the lower the effective permeability the higher the DTI, which can be much greater than that achievable following target arterial administration.

To predict DTI using Eq (2), estimates of Q_T, fu, P and f_T are required. All three NSAIDs are completely absorbed from the pouch (13), indicating no pouch metabolism, so that f_T (and E_T) is zero. Values of fu have been obtained for piroxicam and diclofenac and are available for ibuprofen (Table III), but not those for Q_T and P. Initially, the intention was to estimate blood flow to the pouch using γ-labelled microspheres (18), but this proved not possible due to the pouch vasculature being very highly disseminated, making it extremely difficult to identify those arteries perfusing the pouch from those not involved. Notwithstanding these difficulties, as shown in a companion paper (13), use can be made of the equation.

$$\left[\frac{Dose}{AUC_{exudate}}\right]_{i.p.} = \left(\frac{fu_T \cdot P \cdot Q_T}{fuP + Q_T}\right)\frac{1}{(1 - f_T)} \quad (5)$$

where $AUC_{exudate}$ is the AUC in the pouch after intrapouch administration, after correction for the contribution made by the return of systemically absorbed drug, and fu_T is the fraction of drug unbound in the exudate. Substituting Eq (5), into Eq (2), yields a predicted DTI given by

$$DTI = 1 + \left[\frac{AUC_{exudate}}{Dose}\right]_{i.p.}\frac{fu_T \cdot CL_T}{fu\,(1 - f_T)^2} \quad (6)$$

The values of $[Dose/AUC_{exudate}]_{i.p.}$ are 5, 6.5 and 6 ml hr^{-1} per 250 g rat for piroxicam, S[+]ibuprofen and diclofenac respectively (13). Furthermore, over the majority of the permeability study period, 2 hr, signified by the time for essentially complete absorption of systemically available NSAIDs following intrapouch administration (13), with little efflux of systemic albumin into the pouch and no binding of the drugs to the carrageenan solution initially placed there, binding of the NSAIDs was assumed to be negligible ($fu_T \sim 1$). Substituting the appropriate values for fu (Table III) and CL_T (piroxicam and diclofenac, Table I; ibuprofen 50 ml hr^{-1} per 250 g rat (5) for each NSAID into Eq (6) yields predicted DTI values of 101, 700 and 2214 for piroxicam, S[+]ibuprofen and diclofenac, respectively. Although the trend is the same, with increasing DTI in line with increasing total clearance, these predictions are approximately 7.5-fold higher than the experimental value of 11, 115 and 276, respectively (Table II).

We believe that the discrepancy between observed and predicted DTI values lies in the influx of albumin into the pouch, the plasma binding protein for the NSAIDs. Strict comparison between observation and prediction is complicated, however, by the anticipated continual fall in the value of fu_T, associated with the rising pouch concentration of albumin during the 4 to 30 hr period over which the AUC assessments were made. Initially, we thought that the discrepancy could be accommodated by retaining the existing model, in which only unbound drug fluxes between the vasculature and the pouch, but now taking into account the increasing binding within the pouch. (Eq (10B), Appendix 2). Certainly, a rising albumin concentration in the pouch towards that in plasma would explain the observed approach at later times of the concentration of the NSAIDs in the pouch, to that in plasma, after i.v. administration (e.g. Fig 1 and 2). However, this modification failed to describe the extensive and rapid rise in the pouch concentration of any of the NSAIDs. These observations could only be accommodated by incorporating a flux for both bound drug (associated with albumin) and unbound drug (see Eq (9B), Appendix 2), when much better agreement between observation and prediction

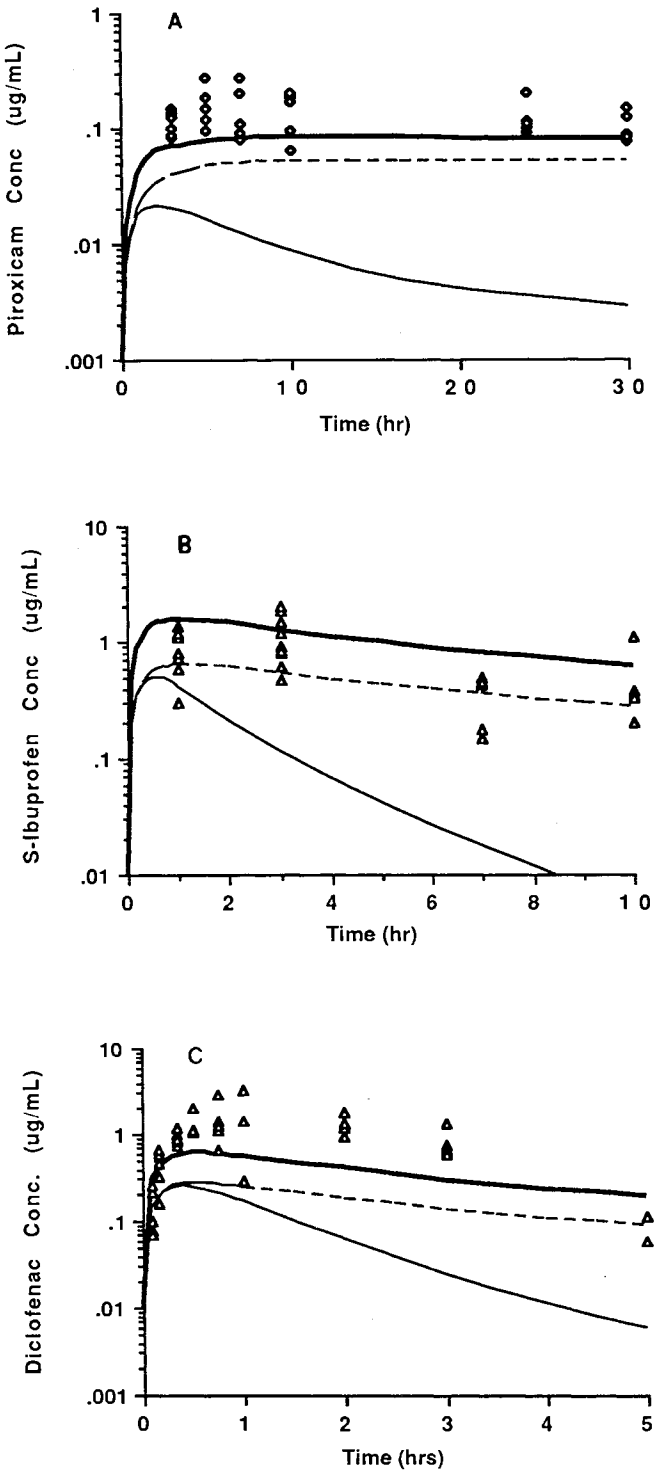


Fig. 3. Observed (▲) and predicted pouch concentrations after i.v. bolus dose of piroxicam (0.5 mg/kg, Panel A), S[+]ibuprofen (20 mg/kg, Panel B) and diclofenac (20 mg/kg Panel C). Prediction based on the assumption that either both unbound and bound drug flux across the membrane between pouch and vasculature (—) or only unbound drug fluxes or there is either no binding in the pouch, $fu_T = 1$ (—) or binding occurs due to influxing albumin (---). See Appendix 2 for details of the models.

was achieved. In taking into account the flux of albumin, the observed rise in pouch albumin with time was used. In practice, pouch albumin concentration is expected to rise asymptotically towards a limiting value, the maximum of which is the plasma albumin concentration. In the air pouch preparation, this rise may be a complex function of time as the permeability of the pouch membrane is continuously changing during and after the inflammatory process.

The flux of bound as well as unbound drug between plasma and target site diminishes the value of DTI, compared to that expected if only unbound drug is permeable. It can be readily shown by extension of the derivation in Appendix 1, that if Pb is the permeability of the protein (and hence bound drug), then the DTI is given by

$$DTI = 1 + \left(\frac{1}{Q_T} + \frac{1}{fuP + (1-fu)\cdot Pb}\right)\frac{CL_T}{(1-f_T)} \quad (7)$$

where the term $[fuP + (1-fu)Pb]$ may be regarded as the total effective permeability of the drug, P_{eff}. Assuming that the influx of the protein is a first-order process, with a fractional rate constant (k) of 0.015 hr^{-1} (the fractional rate of rise of albumin in the pouch), the permeability of the pouch membrane to bound drug, Pb is 0.075 ml hr^{-1} ($k \cdot Vp$ (5 ml)). Then for fu = 0.01, P = 5 ml hr^{-1} per 250 g rat, it is seen that the flux of binding protein increases the effective permeability of the drug by a factor of approximately two. That is, from 0.05 ml hr^{-1}, when only unbound drug permeates, to 0.125 ml hr^{-1} when both unbound and bound drug flux. This increase in total effective permeability reduces the DTI by a corresponding factor of 2.5, when distribution across the pouch membrane is permeability rate-limited. The DTI would be reduced still further if the permeability of the membrane to binding proteins is even greater, as occurs for albumin across the inflamed site in the absence of NSAIDs (13). However, the impact of the distribution of binding protein on DTI will be diminished as overall distribution becomes perfusion-rate limited.

APPENDIX 1

This appendix concerns the development of a physiologically-based model for DTI following direct administration into the target organ.

Consider a target site (T) of volume V_T perfused by blood, at flow rate Q, and from which drug is eliminated with an associated intrinsic clearance, CL_{int}. Assuming that only unbound drug permeates the membrance separating blood from the target tissue, by passive diffusion, and that both target site and blood within it act as well stirred compartments, then the corresponding rate equations, for a system operating under linear conditions, are:

$$\text{Target Site } V_T\frac{dC_T}{dt} = fu\cdot P\cdot C_{out,T} - fu_T\cdot(P + CL_{int})\cdot C_T \quad (1A)$$

$$\text{Target Blood } V_B\frac{dC_B}{dt} = Q_T\cdot C_{in} + P(fu_T\cdot C_T - fu\cdot C_{out,T}) - Q_T\cdot C_{out,T} \quad (2A)$$

where C_T, C_{in}, $C_{out,T}$ are the concentrations in the target

tissue, the inflowing (arterial) blood and outflowing (venous) blood respectively; fu, fu_T are the fractions of drug in blood and tissue respective, V_B is the volume of blood within the tissue and P is the permeability surface area product of unbound diffusing drug. Further, let the target blood recirculate to the rest of the body with a volume of distribution V_R and systemic clearance CL_S. Then

$$V_R \frac{dC_{in}}{dt} = Q_T \cdot C_{out,T} - (CL_S + Q_T)C_{in} \qquad (3A)$$

Consider the placement of a bolus dose of drug first into the systemic circulation ($Dose_s$) and then directly into the target tissue ($Dose_T$)

(a) Input into systemic circulation Integration of Eq(1A)–Eq(3A), between times zero and infinity yields

$$0 = fu \cdot P \int_0^\infty C_{out,T}dt - fu_T(P + CL_{int}) \int_0^\infty C_T dt \qquad (4A)$$

$$0 = Q_T \int_0^\infty C_{in}dt + fu_T \cdot P \int_0^\infty C_T dt - (fu \cdot P + Q_T) \int_0^\infty C_{out,T}\, dt \qquad (5A)$$

$$Dose_s = Q_T \int_0^\infty C_{out,T} \cdot dt - (CL_s + Q_T) \int_0^\infty C_{in}dt \qquad (6A)$$

which upon appropriate substitution and rearrangement gives

$$R_s = \left[\frac{\int_0^\infty C_T dt}{\int_0^\infty C_{in}dt}\right] = \frac{fu \cdot Q_T \cdot P}{fu_T[P \cdot Q_T + (fu \cdot P + Q_T)\, CL_{int}]} \qquad (7A)$$

Now, the extraction ratio of drug across the target site, E_T ;given by:

$$E_T = \frac{\int_0^\infty C_{in}dt - \int_0^\infty C_{out,T_t}dt}{\int_0^\infty C_{in}dt} \qquad (8A)$$

or

$$1 - E_T = \frac{\int_0^\infty C_{out,T}dt}{\int_0^\infty C_{in}dt} \qquad (9A)$$

which, when substituted into Eq (5A) and expressing $\int_0^\infty C_T dt$ in terms $\int_0^\infty C_{out,T}dt$, given by Eq (4A), gives the relationship

$$1 - E_T = \frac{Q_T(P + CL_{int})}{P \cdot Q_T + (fu \cdot P + Q_T) \cdot CL_{int}} \qquad (10A)$$

or

$$E_T = \frac{fu \cdot P \cdot CL_{int}}{P \cdot Q_T + (fu \cdot P + Q_T)\, CL_{int}} \qquad (11A)$$

(b) Direct input into target tissue

Here the corresponding integral equations are:

$$Dose_T = fu \cdot P \int_0^\infty C_{out,T}\, dt - fu_T\,(P + CL_{int}) \int_0^\infty C_T\, dt \qquad (12A)$$

$$0 = Q_T \int_0^\infty C_{in}dt + fu_T \cdot P \int_0^\infty C_T\, dt - (fu \cdot P + Q_T) \int_0^\infty C_{out,T}\, dt \qquad (13A)$$

$$0 = Q_T \int_0^\infty C_{out,T}\, dt - (CL_s + Q_T) \int_0^\infty C_{in}dt \qquad (14A)$$

which on appropriate substitution and rearrangement gives

$$R_T = \left[\frac{\int_0^\infty C_T\, dt}{\int_0^\infty C_{in}dt}\right]_T = \frac{fu \cdot P \cdot Q_T + (fu \cdot P + Q_T)CL_s}{fu_T \cdot P \cdot Q_T} \qquad (15A)$$

Further, let f_T be the fraction of the dose placed directly into the target that is eliminated before entering the systemic circulation. Then $(1 - f_T)$ is the fraction of the applied target dose entering the systemic circulation.

To establish the relationship between E_T and f_T consider the situation of direct input into the target without recirculation ($C_{in} = 0$). Then:

$$1 - f_T = \frac{Q_T \int_0^\infty C_{out,T}\, dt}{Dose_T} \qquad (16A)$$

whilst from Eq (13A)

$$\int_0^\infty C_T\, dt = \left[\frac{fu \cdot P + Q_T}{fu_T \cdot P}\right] \int_0^\infty C_{out,T}\, dt \qquad (17A)$$

which when substituted into Eq (12A) and collecting terms provides

$$Dose_T = \left[\frac{Q_T \cdot P + (fu \cdot P + Q_T)\, CL_{int}}{P}\right] \int_0^\infty C_{out,T}\, dt \qquad (18A)$$

And, comparison of Eq (18A) with Eq (16A) gives

$$\frac{1}{1 - f_T} = \frac{Q_T \cdot P + (fu \cdot P + Q_T)CL_{int}}{Q_T \cdot P} \qquad (19A)$$

or

$$f_T = \frac{(fu \cdot P + Q_T)CL_{int}}{Q_T \cdot P + (fu \cdot P + Q_T)CL_{int}} \qquad (20A)$$

So that comparison between Eq (11A) ;and Eq (20A) indicates that

$$E_T = \left[\frac{fu \cdot P}{fu \cdot P + Q_T}\right] f_T \qquad (21A)$$

Now, the drug targeting index, DTI is defined by

$$DTI = \frac{R_T}{R_S} = \frac{\left[\dfrac{fu \cdot P \cdot Q_T + (fu \cdot P + Q_T)CL_s}{fu_T \cdot P \cdot Q_T}\right]}{\left[\dfrac{fu \cdot Q_T \cdot P}{fu_T[P \cdot Q_T + (fu \cdot P + Q_T)\, CL_{int}]}\right]} \quad (22A)$$

$$= \frac{fu \cdot P \cdot Q_T + (fu \cdot P + Q_T)CL_s}{fu \cdot P \cdot Q_T (1 - f_T)}$$

However,

$$CL_T = CL_s + Q_T \cdot E_T \quad (23A)$$

where CL_T is the total body clearance of drug. Whereupon, substituting for CL_s in Eq (22A) by Eq (23A), expressing E_T in terms of (Eq (21A) and collecting terms gives

$$DTI = 1 + \left(\frac{1}{Q_T} + \frac{1}{fu \cdot P}\right)\frac{CL_T}{(1 - f_T)} \quad (24A)$$

That is, Eq (2) in the main text.

APPENDIX 2

This appendix deals with the influence of albumin flux into the air pouch on the events within it, given the observed biexponential plasma profile after i.v. bolus administration.

Estimation of Albumin and Drug Binding in the Air Pouch Exudate with Time

Consider the binding of drug to a single site on albumin under nonsaturating conditions. Then, the equation defining the relationship between the fraction of drug unbound in plasma, fu and total plasma proein concentration, P_{T_P} is:

$$Ka = \frac{(1 - fu)}{fu \cdot P_{T_P}} \quad (1B)$$

or

$$fu = \frac{1}{1 + Ka\, P_{T_P}} \quad (2B)$$

where Ka is the association constant of the drug-protein complex and 1-fu is the fraction of drug bound to albumin.

Now the influx of the albumin into the pouch increases linearly with time (Fig 3), characterised by

$$P_{T_T}(t) = \theta \cdot P_{T_P} \cdot t \quad (3B)$$

where P_{T_T} is the albumin concentration in the pouch at time t, P_{T_P} is the albumin concentration in plasma (which remains relatively constant), and θ is the fractional rate of rise of albumin in the pouch.

From these relationships, it follows that

$$fu_T(t) = \frac{1}{1 + \left(\dfrac{1 - fu}{fu}\right)\theta \cdot t} \quad (4B)$$

Events in Air Pouch Exudate

Consider the events in the air pouch exudate following an i.v. bolus of drug, and with systemic disposition kinetics characterised by a biexponential equation ($C = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}$) and only unbound drug fluxing between the exudate and the vasculature. Then, in the exudate,

$$V_T \frac{dC_T}{dt} = P\,(Cu - Cu_T) + Pb \cdot Cb \quad (5b)$$

where Cu_T and C_T are the unbound and total drug concentrations in the pouch exudate, Cu and Cb is the unbound and bound drug concentrations in plasma, V_T are the volume of the pouh fluid and P is the permeability surface area product of the pouch membrane to unbound drug and bound drug, respectively,

Now let Pb be equal to the permeability of the pouch membrane to albumin, P_{alb}, given by

$$P_{alb} = \frac{Rate\ of\ influx\ of\ albumin}{Plasma\ albumin\ concentration} = \frac{V_T \cdot dP_{T_T}/dt}{P_{T_P}} \quad (6B)$$

which, by reference to Eq (3B), results in

$$Pb = V_T \cdot \theta \quad (7B)$$

Substituting Eq (7B) into Eq (6B), expressing concentration in terms of total drug in pouch and plasma and collecting terms then gives

$$\frac{dCb_T}{dt} = \left[\frac{fu \cdot P}{V_T} + (1 - fu) \cdot \theta\right] \cdot C - fu_T P \cdot C_T \quad (8B)$$

which, on further substituting for fu_T ;by Eq (4B) and C by $C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}$, provides

$$\frac{dC_T}{dt} = \left[\frac{fu \cdot P}{V_T} + (1 - fu) \cdot \theta\right](C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}) - \frac{fu \cdot P}{V_T\,[fu + (1 - fu) \cdot \theta \cdot t]} \quad (9B)$$

which upon integration gives C_T with time. All parameters in Eq (9B) are known or experimentally determined. Thus V_T = 5 ml (16), θ = 0.025 hr^{-1} for the first two hr and 0.013 hr^{-1} for the next eight hr for all drugs studied (fig 3), P is 6, 6.5 and 5 ml hr^{-1} per 250g rat for diclofenac, S-ibuprofen and piroxicam respectively (16) and the parameters C_1, λ_1 C_2, λ_2 characterising the plasma disposition kinetics of the drug where obtained by fitting of the biexponential equation to the i.v. plasma concentration-time profiles.

Equation (9B) allows for flux of unbound drug and movement of bound drug into the pouch. The reduced model with flux of unbound drug only, but binding of drug to albumin entering the pouch, is given by

$$\frac{dC_T}{dt} = \frac{fu \cdot P}{V_T}\left[(C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}) - \frac{C_T}{fu + (1 - fu)\theta \cdot t}\right] \quad (10B)$$

This is readily seen from Eq (9B) by ignoring the flux of bound drug.

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REFERENCES

1. H-S. G. Chen and J. F. Gross. Intra-arterial infusion of anticancer drugs: Theoretic aspects of drug delivery and review of responses. *Cancer. Treat. Rev.* **64**:31–40 (1980).
2. W. W. Eckman, C. S. Patlak and J. D. Fenstermacher. A critical evaluation of the principles governing the advantages of intra-arterial infusions. *J. Pharmacokinet. Biopharm.* **2**:257–284 (1974).
3. E. Tomlinson. Theory and practice of site-specific drug delivery. *Adv. Drug. Deliv. Rev.* **1**:87–198 (1987).
4. A. Boddy and L. J. Aarons. Pharmacokinetic and pharmacodynamic aspects of site-specific drug delivery. *Advanced Drug Delivery Reviews* **3**:155–163 (1989).
5. C. A. Hunt, R. D. Macgregor and R. A. Siegel. Engineering targeted in vivo drug delivery I. The physiological and physiochemical principles governing opportunities and limitations. *Pharm. Res.* **3**:333–344 (1986).
6. U. G. Ericksson and T. N. Tozer. Pharmacokinetic evaluation of regional drug delivery. *Acta Pharm. Jugosl.* **37**: 331–344 (1987).
7. A. J. Stevens, S. W. Martin, B. S. Brennan, M. Rowland and J. B. Houston. Experimental determination of a drug targeting index for S(+) ibuprofen using the rat air pouch model of inflammation. *J. Drug Targeting.* **2**:333–340 (1994).
8. S. W. Martin, A. J. Stevens, B. S. Brennan, D. Davies, M. Rowland and J. B. Houston. The six-day-old rat air pouch model of inflammation: Characterisation of the inflammatory response to carrageenan. *J. Pharmacol. Toxicol. Methods.* **32**:139–148 (1994).
9. S. W. Martin, A. J. Stevens, B. S. Brennan, M. Rowland and J. B. Houston. Pharmacodynamic comparison of region drug delivery for nonsteroidal anti-inflammatory drugs using the air pouch of inflammation. *J. Pharm. Pharmac.* **47**:458–461, (1995).
10. F. D. Boudinot and S. S. Ibrahim. High performance liquid chromatographic assay for piroxicam in human plasma. *J. Chromatogr.* **430**:424–428 (1988).
11. J. Godbillon, S. Gauron and J. P. Metayer. High performance liquid chromatographic determination of diclofenac and its monohydroxylated metabolites in biological fluids. *J. Chromatogr.* **338**:151–159 (1985).
12. M. Gibaldi and D. Perrier. *Pharmacokinetics*. 2nd Edition. Marcel Dekker, New York.
13. S. W. Martin, A. J. Stevens, B. S. Brennan, M. Ries, L. Gifford, M. Rowland and J. B. Houston. Regional Drug Delivery I: Permeability characteristics of the Rat 6-day-old air pouch model of inflammation. *Pharm. Res.* **12**:1980–1986 (1995).
14. L. K. Roskos and F. D. Boudinot. Effects of dose and sex on the pharmacokinetics of piroxicam in the rat. *J. Biopharm. Drug Dispos.* **11**:215–225 (1990).
15. J. E. Peris-Ribera, F. Tores-Molina, M. C. Garcia-Carbonell, J. C. Astorina and J. M. Pla-Delfina. Pharmacokinetics and bioavailability of diclofenac in the rat. *J. Pharmacokin. Biopharm.* **19**:647–665 (1991).
16. P. Hambleton and P. Miller. Studies on carrageenan air pouch inflammation in the rat. *Br. J. Exp. Path.* **70**:425–433 (1989).
17. J. H. Satterwhite and F. D. Boudinot. Effect of age and dose on the pharmacokinetics of ibuprofen in the rat. *Drug Met. Dispos. Biol. Fate. Chem.* **20**: 61–67 (1991).
18. J. R. Hales. Radioactive microspheres technique for studies of the circulation. *Clin. Exp. Pharmacol. Physiol.* **1**:31–46 (1974).