Regional Drug Delivery I: Permeability Characteristics of the Rat 6-Day-Old Air Pouch Model of Inflammation

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Purpose. To determine the permeability characteristics of the rat air pouch model of inflammation using permeability extremes within which the NSAIDs S[+] ibuprofen, piroxicam and diclofenac could be evaluated.

Methods. Permeability was calculated using concentration data obtained following intrapouch and intravenous administration of [^{3}H]-water, [^{14}C]-urea, [^{14}C]-inulin and [^{125}I]-albumin and compared to similar data obtained for the three NSAIDs.

Results. Similar permeability values (5–6.5 ml hr^{-1}) were obtained for the three NSAIDS which fell between the permeability extremes of the molecular weight markers [^{3}H]-water (9.7 ml hr^{-1}), [^{14}C]-urea (6.8 ml hr^{-1}), [^{14}C]-inulin (1.0 ml hr^{-1}) and [^{125}I]-albumin (0.6 ml hr^{-1}). Coadministration of equipotent anti-inflammatory doses of the NSAIDs did not affect local blood flow to the air pouch (as assessed by urea kinetics) but did reduced vascular permeability (as assessed by albumin flux into the pouch).

Conclusions. Comparison of the NSAIDs with the permeabilities of the molecular weight markers indicates that a perfusion rate limitation probably exists. Systemic absorption is complete over the first two hours following intrapouch administration of the NSAIDs, therefore albumin flux into the pouch is insufficient to materially affect the permeability of the NSAIDs. However, subsequently (post 5hr) albumin concentration in the pouch rises sufficiently to lower the effective flux of the NSAIDs.

KEY WORDS: permeability; rat air-pouch model; inflammation; non-steroidal anti-inflammatory drugs; ibuprofen; piroxicam; diclofenac.

INTRODUCTION

The usefulness of any drug delivery or targeting approach ultimately must be evaluated against some quantifiable measure of benefit within the body. Central to these quantitative issues are the pharmacokinetic features of the drug and the targeting system.

We have used the rat air pouch model of inflammation as an experimental system to assess the pharmacokinetic and pharmacodynamic advantages afforded by regional delivery for a series of non-steroidal anti-inflammatory drugs (NSAIDs) (1–3). One determinant of targeting is the permeability of the target site to drug, which is the subject of this report. Molecular weight markers, [^{3}H]-water, [^{14}C]-urea, [^{14}C]-inulin and [^{125}I]-albumin have been used to provide permeability extremes within which the NSAIDs can be evaluated. In order to characterise the pouch kinetics, NSAIDs and markers have been administered directly into the pouch and intravenously, with sampling at both sites. In addition, we have investigated the effect of NSAID treatment on the permeabilities of urea and albumin as measures of changes in local blood flow and vascular permeability in response to inflammation.

MATERIALS AND METHODS

Materials

S[+] ibuprofen, piroxicam and diclofenac were gifts from Boots Pharmaceuticals (Nottingham, United Kingdom), Pfizer Inc, (Sandwich, United Kingdom) and Ciba Geigy (Basel, Switzerland) respectively. [^{14}C]-urea (sp.act. 7.3 mCi mmol^{-1}) was obtained from Sigma Chemical Co. (Poole, Dorset, United Kingdom), [^{125}I]-albumin (sp.act. 1.02 mCi mg^{-1}) and [^{3}H]-water (sp.act. 100mCi ml^{-1}) from ICN Biomedicals Inc. (High Wycombe, United Kingdom) and [^{14}C]-inulin (sp.act 10mCi mmol^{-1}) from International Amersham (Aylesbury, United Kingdom). Microvette CB300 EDTA coated tubes were supplied by Sarstedt (Leicester, United Kingdom). Carrageenan (viscarin GP 109) was from Marine Colloids (Philadelphia, Pa USA); heparin from CP Pharmaceuticals (Wrexham, United Kingdom), other chemicals were analytical or hplc grade from BDH Chemicals Ltd (Poole, United Kingdom).

The rat air pouch model of inflammation used was as previously described (1). Experiments were carried out on day 6 when the reactivity of the air pouch to the irritant carrageenan is at a maximum (4). Immediately following this procedure compounds were administered as a bolus, either intravenously (IV) via the jugular vein, or into the air pouch (IP). The doses of markers administered by both routes were: [^{3}H]-water (0.6μCi) and [^{14}C]-urea (0.6μCi,10μM) in combination, [^{14}C]-inulin (2.0μCi,10μM) and [^{125}I]-albumin (0.1μCi,0.3μM) in combination, as solutions in phosphate buffered saline, pH 7.4) (1ml kg^{-1}). NSAIDs were administered as a solution either in 1:9 (v:v) polyethylene glycol 400 and polypropylene glycol mixture (S[+] ibuprofen, piroxicam) or 1:9 v:v ethanol and PBSA mixture (diclofenac). The following doses of NSAID were administered: S[+] ibuprofen, 5 to 20mg kg^{-1} (IV), 0.05 to 1mg kg^{-1} (IP); piroxicam, 0.025 to 0.5mg kg^{-1} (IV), 0.05 to 0.5mg kg^{-1} (IP); diclofenac, 10 to 20mg kg^{-1} (IV and IP). None of the NSAIDs showed any binding to the carrageenan as assessed using Centifree Micropartition System units as previously described (5). Serial samples (250μl) were collected from the carotid artery (n=8) and by direct sampling from the air pouch (n=8) into EDTA tubes. The carotid cannula was flushed with 0.1ml heparinised normal saline (10U ml^{-1}) after each blood sample. Samples were stored at −20°C until analysed by HPLC for ibuprofen, diclofenac and piroxicam (5). β-Emitting samples were counted on a LKB Wallac 1218

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Rackbeta liquid scintillation counter after addition of 4.5ml scintillation fluid (Optiphase "HiSafe"II, LKB Wallac); γ-emitting samples were counted on a LKB Wallac 1282 Compugram gamma counter.

Data Analysis

The apparent permeability of a compound traversing from the pouch into the systemic circulation, P_{app}, is defined by

$$\frac{dAa}{dt} = P_{app}\,(C_{pouch} - C_A) \quad (1)$$

where Aa(t) is the amount of compound absorbed systemically at time t following administration into the pouch, and C_{pouch} and C_A are the concentration of compound in the pouch and arterial blood respectively. When C_A is much lower than C_{pouch}, Eq. 1 reduces to

$$\frac{dAa}{dt} = P_{app} \cdot C_{pouch} \quad (2)$$

which upon integration yields

$$P_{app} = \frac{Aa(t)}{\int_0^t C_{pouch}dt} \quad (3)$$

In the case of inulin and albumin Eq. 3 was evaluated at 10 hr after instillation of compound into the pouch. For the remaining compounds a time t_m was selected when systemic absorption was essentially over and the condition $C_{pouch} > C_A$ still holds. Under these latter circumstances P_{app} is given by

$$P_{app} = \frac{F.\,Dose}{\int_0^{t_m} C_{pouch}dt} \quad (4)$$

where F is the fraction of IP dose absorbed systemically at time t_m.

Pharmacokinetic parameters were calculated from the plasma concentration-time data after IV administration in the standard manner. AUC in both pouch and plasma was calculated from experimental observations using the linear trapezoidal approximation. In the case of IV administration, where appropriate, extrapolation to infinity was carried out using the last concentration point divided by the terminal rate constant. Appropriate exponential equations were fitted to the pouch and plasma data using nonlinear regression analysis. The extent of systemic absorption with time following IP administration, Aa(t), was calculated using numerical deconvolution of plasma data following IP administration, with reference to plasma data following IV administration, assuming a step-wise input function (6).

RESULTS

Intrapouch Administration

Plots of the percent dose ml^{-1} remaining in the air pouch with time following IP bolus doses of the molecular weight markers and the three NSAIDs, S[+] ibuprofen, piroxicam and diclofenac are shown in Fig. 1. During the initial 2hr period pouch profiles for [^{3}H]-water, [^{14}C]-urea and [^{14}C]-inulin declined monoexponentially; the concentration of [^{125}I]-albumin fell only marginally during this period. The initial volumes of distribution within the pouch for water, urea and inulin were all around 5.3ml/250g, which equates to the volume of fluid placed into the pouch; that of albumin was slightly larger (6.4ml/250g). For the range of molecular weight markers studied, half-life increased with increasing molecular weight (Table I).

Following IP administration, the three NSAIDs displayed biphasic efflux profiles with the majority of drug loss (65–91%) occurring during the initial phase. The initial volume of distribution tended to be larger for the NSAIDs than for the low molecular weight markers and closer to that for albumin (Table I). The profiles for NSAIDs were intermediate between water and urea (low molecular weight and freely permeable markers) and inulin and albumin (high molecular weight markers) and this is reflected in the half-life estimates.

Gain into the Systemic Circulation

Figure 2 shows semi-logarithmic pouch and plasma concentration-time profiles for the four molecular weight markers following IP administration. The magnitude of the plasma concentration-time profile decreased as the molecular weight increased. Uptake into the plasma was well defined for water, urea and inulin, resulting in maximum concentrations by 45 min, whilst plasma albumin concentration continued to rise showing no definite peak even by 10hr. During the first 5hr plasma concentrations of water and urea approached those in the pouch whereas those of inulin and

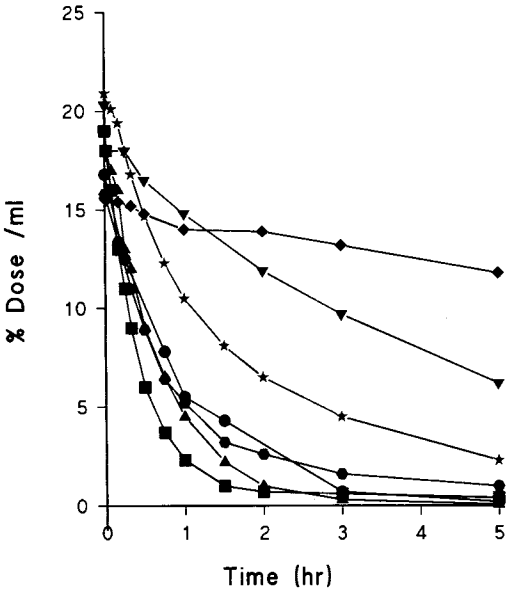

Fig. 1. Percentage of dose/ml remaining in the pouch fluid with time following intrapouch administration of markers [^{3}H]-water (■),[^{14}C]-urea (▲), [^{14}C]-inulin (▼), [^{125}I]-albumin (♦) and 3 NSAIDs, S[+] ibuprofen (●), piroxicam (★) and diclofenac (⬤). Each point represents the mean of between 4 and 8 animals.

Table I. Air Pouch Exudate Pharmacokinetic Parameters for Markers and NSAIDs Following Bolus Administration into the Air Pouch[a]

	Compound	Molecular weight	Initial volume (ml)	Initial half-life (hr)	Terminal half-life (hr)	Apparent permeability (ml hr^{-1})
Marker:	[^{3}H]-Water	20–22	5.1 ± 0.15	0.31 ± 0.02	—	9.7 ± 0.7
	[^{14}C]-Urea	62	5.2 ± 0.07	0.46 ± 0.03	—	6.8 ± 0.3
	[^{14}C]-Inulin	5000	5.2 ± 0.12	2.80 ± 0.26	—	1.0 ± 0.1
	[^{125}I]-Albumin	69000	6.4 ± 0.14	—[b]	—	0.59 ± 0.1
NSAIDs:	S[+]Ibuprofen	206	6.0 ± 0.2	0.56 ± 0.04	3.4 ± 0.41	6.5 ± 0.6
	Diclofenac	318	6.2 ± 0.2	0.26 ± 0.04	1.3 ± 0.20	6.1 ± 0.8
	Piroxicam	331	5.5 ± 0.5	0.63 ± 0.14	3.4 ± 0.67	5.1 ± 0.4

[a] Mean ± sem (n = 4–8), normalised to a 250g rat.
[b] Insufficient loss to allow determination of half-life.

albumin were always two orders of magnitude lower than pouch concentrations.

Intravenous Administration

Figures 3A and 3B show semi-logarithmic plasma concentration-time curves for the four molecular weight markers following IV administration. For all the markers the plasma data are best described by biexponential equations; pharmacokinetic parameters are listed in Table II. As expected, the estimated volumes of distribution at steady-state of urea and water were similar to total body water (7), that of inulin was smaller and closer to the extracellular volume (7) and that of albumin even smaller, at 0.07L kg^{-1}. Over the 10hr period of study, both urea and inulin are extensively eliminated, with the clearance of inulin (0.12L hr^{-1}), being as expected, equal to glomerular filtration rate (7). Both water and albumin are subject to only minor elimination over the study period.

Also shown in Figures 3A and 3B are the pouch profiles for the markers after IV administration. By 30 min, for both urea and water the concentrations in plasma and exudate were similar (Fig 3A); thereafter plasma and pouch concentrations mirrored each other. For inulin, plasma and pouch concentrations were equal by 1hr (Fig 3B) and thereafter declined in parallel. Albumin pouch concentrations showed

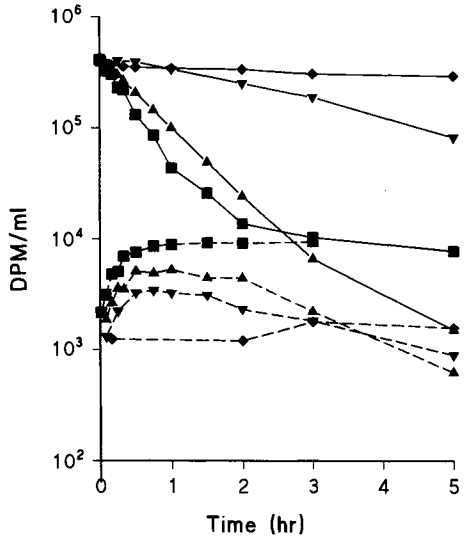

Fig. 2. Semilogarithmic plots of pouch and plasma concentrations (dpm/ml) versus time for the markers, following intrapouch administration; [^{3}H]-water (■), [^{14}C]-urea (▲), [^{14}C]-inulin (▼) and [^{125}I]-albumin (♦) in pouch fluid (solid lines) and plasma (dotted lines). Each point represents the mean of between 4 and 21 animals.

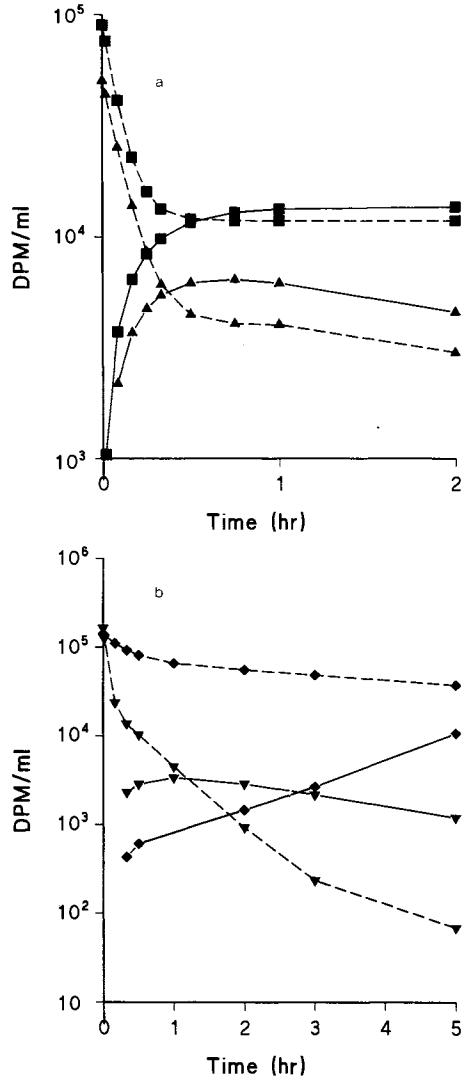

Fig. 3. Semilogarithmic plots of pouch and plasma concentrations (dpm/ml) versus time for [^{3}H]-water and [^{14}C]-urea (panel A) and [^{14}C]-inulin and [^{125}I]-albumin (panel B) following intravenous administration. Symbols and lines as in Fig. 2.

Table II. Plasma Pharmacokinetic Parameters of Markers Following Intravenous Administration[a]

Marker	Initial t(½) (min)	Terminal t(½) (min)	Vss[c] (ml)	Cl[d] (L hr^{-1})
[^{3}H]-Water	2.6 ± 1.7	[b]	167 ± 15	[b]
[^{14}C]-Urea	4.1 ± 1.9	61.5 ± 14	210 ± 15	0.21 ± 0.04
[^{14}C]-Inulin	2.9 ± 1.2	216 ± 56	100 ± 40	0.12 ± 0.02
[^{125}I]-Albumin	34 ± 6.7	533 ± 106	17 ± 4	[b]

[a] Mean ± sem (n = 4–21), normalised to a 250g rat.
[b] Decay too slow to allow accurate estimates; values reach a virtual steady state after 60 min.
[c] Volume of distribution at steady state.
[d] Total systemic clearance, calculated from Dose/$AUC_{0-\infty}$.

a gradual and approximately linear rise over the experimental period (Fig 3B) reaching 20% of the plasma concentration by 5hr. Further experiments over a longer time period showed that this increase in pouch concentrations continues, reaching 50% of plasma albumin concentration by 10hr (Fig 5B).

Deconvolution of Plasma Data

The input parameters describing systemic absorption after IP administration are shown in Table III. Included with the four markers are estimates for the three NSAIDs, using the intravenous pharmacokinetic parameters reported previously by us (3, 5).

Water, urea and the three NSAIDs all showed similar and rapid kinetic profiles for systemic absorption which was over by 2hr; all were fully bioavailable, with the exception of diclofenac, which was only 50% bioavailable. Systemic absorption of inulin and albumin over 5hr was slow and reached only 36% and 8% respectively of the intrapouch dose. Since metabolism of diclofenac in the air pouch is unlikely to occur, one possible explanation is that diclofenac, the least soluble of the three NSAIDs studied, precipitated after intrapouch administration and incomplete redissolution occurred over the 4hr study period. To evaluate this aspect, the distribution of radioactivity at 3hr after IP administration of [^{14}C]-diclofenac (in the presence of cold drug) was investigated. Bioavailability was slightly higher in these experiments (65%) and the remaining dose could be accounted for in pouch exudate (8%) together with pouch lining and surrounding tissue (27%).

Table III. Parameters Describing Movement of Markers and NSAIDs Between Air Pouch and Systematic Circulation After Intrapouch Administration

		Time to 90% loss[a] (hr)	Systemic availability[b] (%)
Markers:	Water	1.4	100
	Urea	1.7	100
	Inulin	—	36
	Albumin	—	8
NSAIDs:	Ibuprofen	3.1	98
	Diclofenac	1.25	53
	Piroxicam	1.75	100

[a] Time taken for 90% of the total absorbed dose to be lost from the pouch exudate, calculated from deconvolution data.
[b] Percent of intrapouch dose absorbed systemically by 10hr, calculated from the plasma AUC following IP and IV administration.

Effect of NSAIDs Upon Plasma Kinetics and Flux into the Air Pouch of Urea and Albumin

Figures 4A and 4B show that the plasma kinetics of neither [^{14}C]-urea nor [^{125}I]-albumin was altered by the concurrent IV administration of any of the NSAIDs. There was no statistically significant effect over the following dose ranges; ibuprofen (5–20mg kg^{-1}), piroxicam (0.05–0.2mg kg^{-1}) and diclofenac (0.025–0.1mg kg^{-1}). The pouch concentration-time profiles for [^{14}C]-urea following IV administration in control and NSAID-treated animals were also similar (Fig 5A), with no statistically significant difference (by t-test) between the maximum concentration (approximately 0.1% of dose at 1hr), AUC or terminal half-life.

Following IV administration of [^{125}I]-albumin to control animals, pouch levels rose with time to approximately 50% of the plasma concentration by 10hr (Fig 5B). In contrast, in NSAID treated animals, pouch concentrations of [^{125}I]-albumin at 10hr only reached 15% of the plasma concentration.

DISCUSSION

Theoretical studies indicate that the major determinants of the gain associated with drug targeting are: systemic availability following target organ administration, permeability and vascular perfusion of the target site, and systemic drug clearance (5, 8–10). We (3,5) have clearly demonstrated in the same air pouch model as currently used, the importance of systemic clearance in drug targeting. For the three NSAIDs, piroxicam, ibuprofen and diclofenac, in the present study we have concentrated on the factors determining the permeability of these NSAIDs in the air pouch system. By using a range of molecular weight markers, to provide extremes, it would appear that NSAID transfer is predominantly perfusion rather than permeability rate-limited.

Estimation of apparent permeability (P_{app}) using Eq.4 assumes that over the majority of the time period when absorption occurs, pouch concentrations are much greater than that in the arterial circulation. This is equivalent to the blood

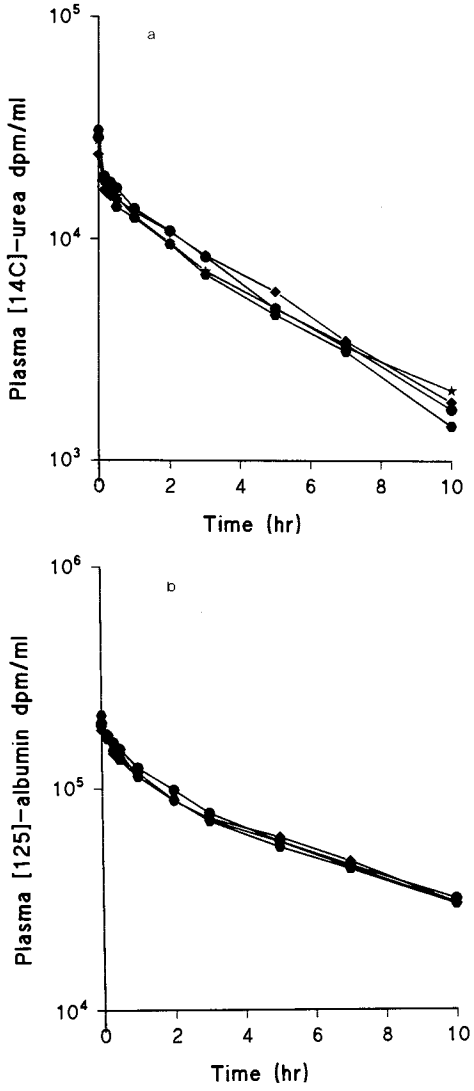

Fig. 4. Plasma kinetics of [^{14}C]-urea (panel A) and [^{125}I]-albumin (panel B) following intravenous administration alone (♦) and together with equipotent anti-inflammatory doses of ibuprofen (●), piroxicam (★) and diclofenac (⬤). Each point represents the mean of between 4 and 8 animals.

perfusing the pouch operating in a non-recirculatory mode. For radiolabelled water and urea, with systemic absorption after IP administration complete by 1hr and pouch concentrations remaining above systemic concentrations for this time, the use of $AUC_{pouch\ (0-1hr)}$ is reasonable. For the NSAIDs, the appropriate AUC period was 0–2hr. For inulin and albumin, the condition of complete absorption was not met during the 10hr study period, and then application of Eq.2 was more appropriate. The analysis shows a definite graduation of P_{app} from water and urea, through the NSAIDs, to inulin and albumin, in line with increasing molecular weight (Table III).

The pouch is a complex structure with many factors influencing the value of P_{app}. As a first approximation, assuming that only unbound drug permeates across the membrane separating pouch fluid from the vasculature, P_{app} may be shown to be given by (Appendix, Eq.7A)

$$P_{app} = \frac{Q_T \cdot fu_T \cdot P}{Q_T + fu \cdot P} \quad (5)$$

where P is the permeability of unbound drug, fu and fu_T are the fractions of compound unbound in plasma and pouch fluid respectively and Q_T is the blood flow rate. This vascular supply perfuses the tissues surrounding the pouch. Examination of this equation shows that for compounds not bound ($fu = fu_T = 1$) and highly permeable ($P >> Q_T$), P_{app} is a measure of, and sensitive to changes in, blood flow. Whereas, for poorly permeable compounds, which are not bound, P_{app} is a reasonably good measure of P. The molecular weight markers were chosen as they are not bound

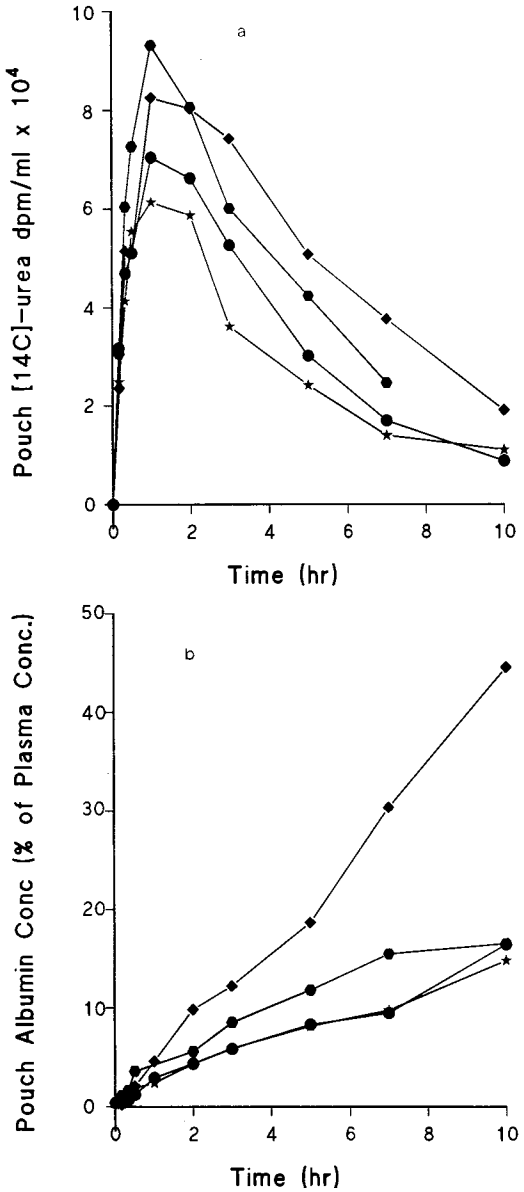

Fig. 5. Effect of NSAID upon the flux into the pouch of [^{14}C]-urea (panel A) and [^{125}I]-albumin (panel B) following intravenous administration alone (♦) and together with equipotent anti-inflammatory doses of ibuprofen (●), piroxicam (★) and diclofenac (⬤). Each point represents the mean of between 4 and 8 animals.

within body fluids and cover the range of permeabilities of interest.

Although the air pouch model has been used to study changes in vascular permeability to plasma protein under various inflammatory conditions (11,12), the general permeability characteristics of the 6-day-old air pouch membrane have not been previously well characterised. Tritiated water was used as a perfusion rate marker since it moves freely through the aqueous pores and channels of membranes and so its tissue distribution kinetics can be considered to be blood flow dependant. Therefore, the apparent permeability of the pouch to tritiated water of 9.8ml h^{-1} is probably an estimate of air pouch blood flow. Urea was used as an alternative marker to water since its permeability is also thought to be perfusion rate-limited. Urea displays the same distribution kinetics as water in the perfused liver and lung (13,14) and occupies total body water space when administered to animals (15). However, the apparent pouch permeability of urea (6.8ml h^{-1}) is less than that of water, and similar to the values obtained for the NSAIDs. This difference in apparent permeability between urea and water appears related to a difference in diffusion, due to differences in molecular size. The diffusion coefficient of labelled urea is approximately half that of labelled water, such that urea has the lower intra-tissue diffusivity (16,17). This suggests that the permeabilities of the pouch to urea depends upon both blood flow and intra-tissue diffusion. During the 6 day development of the air pouch the membrane surrounding the air cavity becomes a well organised continuous cellular lining. The pouch lining develops into a deep layer of densely arranged laminated fibrous connective tissue, interspersed with aggregations of mononuclear phagocytes and blood vessels (4,18,19).

After IP administration, a small fraction (8%) of albumin appeared in the vasculature over the 10hr study period. As the concentration of albumin in pouch fluid dropped by approximately the same degree, the volume of pouch fluid appears to remain constant over the experimental period. The small fraction of albumin that entered the systemic circulation probably did so via lymphatic drainage.

The rates at which plasma proteins extravasate are low (20) and differ greatly between tissues. Skin capillaries have a low permeability compared to other tissues (21,22) and therefore a low rate of transfer of plasma proteins into the rat air pouch would be expected. The flux of albumin into the air pouch over the initial two hours is insufficient to affect the permeability estimates for the NSAIDs as their absorption from the pouch is essentially complete over this time period. The observation that the permeabilities of the NSAIDs are less than that of [^{3}H]-water or urea is probably a consequence of the lower intra-tissue diffusivity of the NSAIDs. However, at later times, the concentration of albumin in the pouch is sufficiently high to lower the effective permeability of the NSAIDs. Thus there is a slowing of the rate of removal of NSAIDs from the pouch at later times and producing a second phase in the time profile.

Changes in local blood flow and vascular permeability may occur at the site of inflammation (23) which in turn, may alter drug distribution between plasma and the inflammatory site. The effect of NSAIDs upon this acute anti-inflammatory response was investigated. Following IV co-injection of labelled urea and albumin, to simultaneously measure changes in blood flow and vascular permeability, only changes in vascular permeability in the presence of a NSAID were observed. NSAIDs at equipotent anti-inflammatory doses, as assessed by the degree of inhibition of prostaglandin E_2 synthesis in the air pouch (2), reduced the influx of albumin into the pouch thereby reducing the effects of protein binding in pouch fluid. However, the flux of urea into the pouch was unaltered in the presence of anti-inflammatory doses of NSAIDs, therefore the rate of perfusion would not be expected to alter during the experimental inflammation.

In conclusion only minor differences were observed in the air pouch permeability to piroxicam, ibuprofen and diclofenac based on exudate disappearance time profiles and numerical deconvolution of the plasma concentration-time data. Hence, the kinetics of systemic absorption was essentially the same for these NSAIDs, indicating that the air pouch does not differentiate between these compounds.

APPENDIX

Consider a target site (T) of volume V_T perfused by blood at flow rate Q_T and from which drug is eliminated with associated intrinsic clearance, CL_{int}. Then, assuming that only unbound drug can permeate the membrane, separating the blood from the tissue, by passive diffusion and that both tissue and blood within it act as well stirred compartments, the corresponding rate equations for events in tissue and target blood are:-

Target Tissue

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

Blood in target

$$V_B \frac{dC_{out\,T}}{dt} = Q_T \cdot C_{in\,T} + P\,(fu_T \cdot C_T - fu \cdot C_{out\,T}) - Q_T \cdot C_{out\,T} \qquad (2A)$$

where fu and fu_T are the fractions of drug unbound in tissue and target blood respectively, C_T is the total target drug concentration and $C_{in\,T}$, $C_{out\,T}$ are the target input and outflow blood concentrations. When a dose D is placed into the target tissue, operating in a non-recirculating mode, ($C_{in\,T}=0$), integration of Eqs. 1A and 2A between $t=0$ and $t=\infty$, yields

$$D = fu.P \int_0^\infty C_{out\,T}\, dt - fu_T\,(P + CL_{int}) \int_0^\infty C_T\, dt \qquad (3A)$$

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

Now defining f_T as the fraction of the administered target dose that is eliminated within the target, it follows that the fraction of the dose escaping the target ($1-f_T$) is given by:-

$$1 - f_T = \frac{Q_T \int_0^\infty C_{out\,T}\, dt}{D} \qquad (5A)$$

which when substituting $\int_0^\infty C_{out_T}$ by $\int_0^\infty C_T dt$ from Eq. 4A and rearranging, yields:–

$$\frac{D}{\int_0^\infty C_T\,dt} = \left(\frac{Q_T \cdot fu_T \cdot P}{Q_T + fu \cdot P}\right)\frac{1}{1 - f_T} \qquad (6A)$$

Comparison of Eq 6A with Eq 5, in the text, indicates that

$$P_{app} = \frac{Dose\ (1-f_T)}{\int_0^\infty C_T\,dt} = \frac{Q_T \cdot fu_T \cdot P}{Q_T + fu \cdot P} \qquad (7A)$$

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