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Pharmacodynamic Comparison of Regional Drug Delivery for Non-steroidal Anti-inflammatory Drugs, Using the Rat Air-pouch Model of Inflammation

S. W. MARTIN, A. J. STEVENS, B. S. BRENNAN, M. ROWLAND AND J. B. HOUSTON

Department of Pharmacy, University of Manchester, Oxford Road, Manchester M13 9PL, UK

Abstract

The inhibition of prostaglandin E_2 (PGE_2) synthesis by *S*-(+)-ibuprofen and piroxicam have been assessed following intravenous and regional (intrapouch) drug delivery using the rat air-pouch model of inflammation. Anti-inflammatory response was defined as the decrease in the area under the exudate PGE_2 concentration-time curve between 3 and 10 h, following regional administration of the irritant carrageenan.

Dose-response studies indicated that bolus regional administration of *S*-(+)-ibuprofen increased potency 30-fold compared with systemic administration and could be further improved 10-fold by regional infusion, whereas regional administration of piroxicam showed no therapeutic advantage. Examination of the concentration-response using AUC revealed that for a given response, average pouch concentrations for *S*-(+)-ibuprofen during the PGE_2 inflammatory response (3 to 10 h) was similar, irrespective of route or mode of administration. In contrast, an advantage following systemic rather than regional administration was revealed for piroxicam, based on plasma concentration-response data, indicating a major systemic anti-inflammatory component for piroxicam but not for *S*-(+)-ibuprofen.

These observations stress the need to take account of both pharmacodynamics and pharmacokinetics when considering the potential advantages of regional administration.

A number of theoretical studies have examined the pharmacological benefits to be derived from site-specific drug delivery (Øie & Huang 1981; Hunt et al 1986; Boddy et al 1989). These studies, based upon evaluating the time integral of drug concentrations, have clearly defined the pharmacokinetic principles which underlie the benefits of drug targeting and have indicated the optimal conditions for target-organ-directed drug administration. Although the integral of drug concentration estimated by area under the concentration–time curve (AUC) at the site of action can be a useful predictor for principles governing drug delivery, it may not be an accurate estimate of drug effect. Due to the possible complex relationships between response and concentration, such as threshold concentration, all-or-none effects, desensitization and feedback control, the advantage of drug targeting may not be reflected in the AUC ratios alone (Campbell 1990).

We have recently established a rat air-pouch model of inflammation (Martin et al 1994) and quantified the pharmacokinetic advantage gained by regional drug delivery of *S*-(+)-ibuprofen and piroxicam (Stevens et al 1993) based on AUC measurements following air-pouch and intravenous administration. The drug targeting indexes, DTI (Hunt et al 1986), for *S*-(+)-ibuprofen and piroxicam in this rat model were 150 and 15, respectively (Stevens et al 1993). Having demonstrated this difference in pharmacokinetic advantage, the aim of the current study was to determine whether *S*-(+)-ibuprofen and piroxicam exhibit corresponding pharmacodynamic responses following regional air-pouch administration.

Correspondence: J. B. Houston, Department of Pharmacy, University of Manchester, Oxford Road, Manchester M13 9PL, UK.

Materials and Methods

Production of air-pouch

Male Sprague-Dawley rats, 200–250 g, were used throughout. Air-pouches (10 mL sterile air) were produced on the dorsal surface of rats using the method described by Martin et al (1994). On day 3, air pouches were reinflated with 6 mL sterile air and experiments were conducted on day 6 when the reactivity of the air-pouch to the irritant carrageenan is at a maximum (Sedgwick et al 1983).

Anti-inflammatory studies

On day 5 cannulae were introduced into the jugular vein and carotid artery of each rat and the animals were left overnight to recover. On day 6, the irritant carrageenan (20 mg in 5 mL phosphate-buffered saline, pH 7·4) was injected into the air pouch. Immediately afterwards, *S*-(+)-ibuprofen was administered as an intravenous bolus (5 or 20 mg kg^{-1}), air-pouch bolus (0·05–1 mg kg^{-1}), intravenous infusion (bolus 16·25 $\mu g\,kg^{-1}$ + infusion 60 $\mu g\,h^{-1}\,kg^{-1}$, or bolus 48·75 $\mu g\,kg^{-1}$ + infusion 180 $\mu g\,h^{-1}\,kg^{-1}$ for 10 h) or air-pouch infusion (bolus 0·2 $\mu g\,kg^{-1}$ + infusion 0·6 $\mu g\,h^{-1}\,kg^{-1}$ or bolus 3·25 $\mu g\,kg^{-1}$ + infusion 12 $\mu g\,h^{-1}\,kg^{-1}$ for 10 h). In a separate series of experiments, piroxicam was administered as an intravenous bolus (0·0025–0·2 mg kg^{-1}) or an air-pouch bolus (0·025–0·2 mg kg^{-1}). The vehicle for the bolus doses was a polyethylene glycol 400: polypropylene glycol mixture (PEG : PPG, 1 : 9 by volume) administered on a 1 mL kg^{-1} basis. The vehicle for the infusions was phosphate-buffered saline (pH 7·4).

Serial blood samples (250 μL) were collected via the carotid artery cannula (n = 8) and directly from the air-pouch (n = 8) into EDTA tubes, and the cannulae were flushed with 0·1 mL heparinized saline (10 units mL^{-1}). The samples were immediately spun and the plasma stored at −20°C to await HPLC analysis for ibuprofen and piroxicam. Pouch exudate prostaglandin E_2 (PGE_2) was determined by adding a 50-μL portion of exudate to 25 μM indomethacin (which halted any further eicosanoid synthesis), followed by the addition of 50 μL methoxyamine hydrochloride. The concentration of PGE_2 methyl oxime derivative was determined by RIA (Amersham RPA530). The lowest level of detection of PGE_2 was 8 pg mL^{-1}. Intra-assay variability at the mid range was 29 ± 0·7 pg per tube (CV = 2·3%, n = 5).

Pharmacokinetic analysis

The area under the curve was calculated using the linear trapezoidal rule from 3 to 10 h using Siphar (Simed, Creteil, France).

Chemicals

S-(+)-Ibuprofen and piroxicam were gifts from Boots Pharmaceuticals (Nottingham, UK) and Pfizer Inc. (Sandwich, UK), respectively. Sterile Acrodiscs were supplied by Gelman Sciences (Northampton, UK). Carrageenan (viscarin GP 109) was from Marine Colloids (Philadelphia, USA), heparin from CP Pharmaceuticals (Wrexham, UK). Microvette CB300 EDTA-coated tubes were from Sarstedt (Leicester, UK) and other chemicals were of analytical grade from BDH Chemicals Ltd (Poole, UK). Results are expressed throughout as the mean ± s.e.m.

Results

Inflammatory response to carrageenan

Previously we have characterized the exudate PGE_2 concentration–time course over 21 h following carrageenan administration (Martin et al 1994). After an initial lag of 3 h, PGE_2 concentration rose to a maximum at 15 h, then declined. Air-pouch administration of piroxicam (0·025–0·1 mg kg^{-1}) produced a dose-dependent inhibition of PGE_2 synthesis over 10 h with no change in the temporal profile (Fig. 1). Air-pouch administration of *S*-(+)-ibuprofen also decreased PGE_2 concentration in a dose-dependent manner. Larger systemic doses of *S*-(+)-ibuprofen (5 and 20 mg kg^{-1}) were required to achieve a similar reduction in PGE_2 concentration to that obtained by air-pouch administration (0·1 and 1 mg kg^{-1}) (Fig. 2), demonstrating an advantage of the latter over the systemic route. However, equivalent doses of piroxicam given by either route gave comparable reductions in PGE_2 concentration (data not shown).

Routes of administration effect for S-(+)-ibuprofen and piroxicam

The area under the concentration–time curve (AUC) for PGE_2 in air-pouch exudate between 3 and 10 h was used to assess the anti-inflammatory activity, since AUC_{3-10} has a lower coefficient of variation (CV 30%, n = 20) than that at any single time point, such as 10 h (CV 53%, n = 20).

A steady-state concentration of *S*-(+)-ibuprofen in the pouch or plasma was maintained using bolus dose and infusion rates based on the studies of Stevens et al (1993). A dose-related (bolus + (infusion rate × infusion time)) reduction in PGE_2 levels was obtained following both routes of administration. On comparing these observations with bolus data for the two routes of administration, clear differences in mode and route of administration were observed for *S*-(+)-ibuprofen (Fig. 3A). A clear rank order of air-pouch infusion > air-pouch-bolus > intravenous infusion > intravenous bolus was evident with each dose–response curve differing approximately by a factor of 10. Thus a 1000-fold difference in dose was

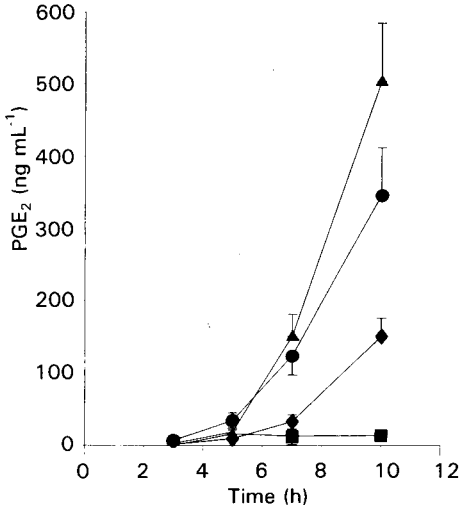

FIG. 1. The time course of air-pouch exudate PGE_2 formation following air-pouch administration of 0·2 (■), 0·05 (●) and 0·025 mg kg^{-1} (◆) piroxicam. Control (▲), mean ± s.e.m. (n = 6–9).

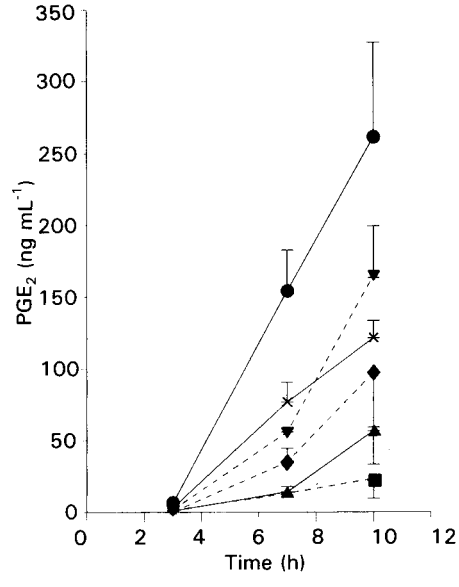

FIG. 2. The time course of air-pouch exudate PGE_2 formation following air pouch administration of 1 (■--■), 0·5 (◆--◆) and 0·1 mg kg^{-1} (▼--▼) *S*-(+)-ibuprofen (mean ± s.e.m.; n = 6–9), and following intravenous administration of 20 (▲—▲) and 5 mg kg^{-1} (×—×) *S*-(+)-ibuprofen (mean ± s.e.m.; n = 12–14). Controls ●.

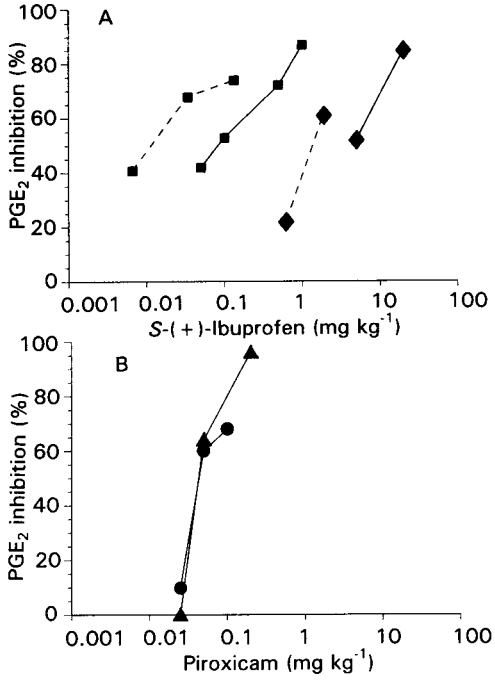

FIG. 3. Dose–response curves for NSAIDs. Response is defined as the percentage decrease of AUC_{3-10} for PGE_2 in air-pouch exudate in drug-treated animals relative to the respective control group (A) following bolus administration of *S*-(+)-ibuprofen via air-pouch (■—■) and intravenously (◆—◆) and infusion of *S*-(+)-ibuprofen via air-pouch (■---■) and intravenously (◆---◆), and (B) bolus administration of piroxicam via air-pouch (▲) and intravenously (●). Each point represents the mean of 6–14 animals.

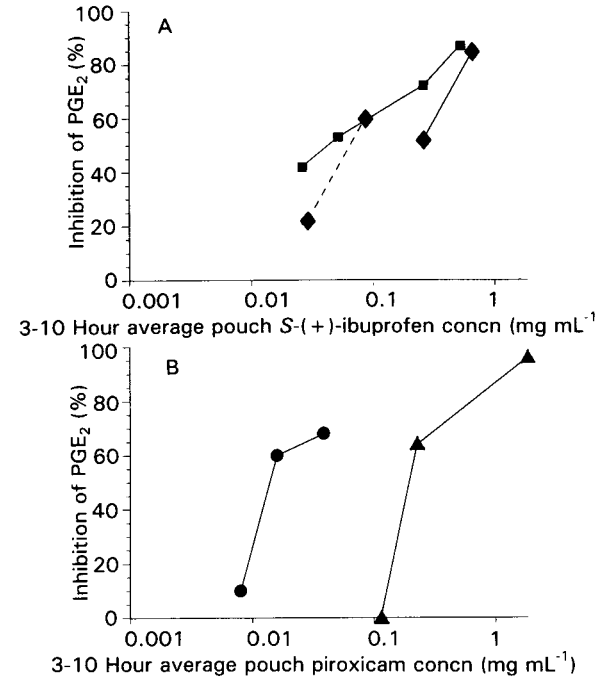

FIG. 4. Pouch concentration–response curves for (A) *S*-(+)-ibuprofen and (B) piroxicam. See Fig. 3 for definition of response. Concentration is given by the area under the curve (AUC) or NSAID pouch concentration between 3 and 10 h divided by the time interval of 7 h. Bolus administration of *S*-(+)-ibuprofen via air-pouch (■—■) and intravenously (◆—◆), and intravenous infusion of *S*-(+)-ibuprofen (◆---◆). Bolus administration of piroxicam air-pouch (▲) and intravenous (●).

required to give a 50% inhibition in PGE_2 response between air-pouch infusion and intravenous bolus administration of *S*-(+)-ibuprofen. Examination of the slope of the dose–response curves showed that piroxicam was 3 and 100 times more potent than *S*-(+)-ibuprofen following air-pouch and intravenous bolus administration, respectively, but no regional advantage was seen (Fig. 3B).

Air-pouch concentration-effect relationships for S-(+)-*ibuprofen and piroxicam*

The temporal profiles of air-pouch concentration for both drugs differ following air-pouch and intravenous administration (Stevens et al 1993). Therefore, the exposure of the air-pouch to the drugs was determined by the area under the exudate concentration–time profile between 3 and 10 h (AUC_{3-10}). The PGE_2 response was also determined over the same time period and compared with the average air-pouch concentration of drug, estimated by dividing the pouch AUC_{3-10} by the time period of 7 h (Fig. 4A).

Comparison of Fig. 3A with Fig. 4A shows that differences in route and mode of administration effects tend to disappear when based on the average pouch concentration of *S*-(+)-ibuprofen. Thus, for any given anti-inflammatory response, the average concentration of *S*-(+)-ibuprofen present in the pouch between 3 and 10 h was similar irrespective of route or mode of administration, despite temporal differences in pouch concentrations over the 10 h. In contrast, based upon average pouch concentration between 3 and 10 h, piroxicam was one-tenth as potent after air-pouch administration compared with intravenous administration (Fig. 4B).

Plasma concentration–effect relationships for S-(+)-*ibuprofen and piroxicam*

The PGE_2 response determined over the time period of 3 to 10 h was compared with the average systemic concentration of drug, estimated by dividing the systemic AUC_{3-10} by the time period of 7 h. Similar response–relationships were observed for plasma concentration and dose since the volume of distribution of both drugs is dose–independent (Stevens et al 1993). Thus, the advantage of regional administration was only apparent for *S*-(+)-ibuprofen.

Discussion

We have previously characterized the acute inflammatory reaction to carrageenan in the 6-day-old rat air-pouch model, by investigating the anti-inflammatory effects of *S*-(+)-ibuprofen upon a variety of inflammatory processes (Martin et al 1994). The level of PGE_2 was found to be the most sensitive to *S*-(+)-ibuprofen and was therefore chosen as a surrogate measure of anti-inflammatory activity. Although the cellular origin of PGE_2 in pouch exudate has not been identified, it is believed to originate from the pouch lining and invading leucocytes (Sedgwick & Lees 1986; Omata et al 1991).

It has often been assumed that intra-arterial or regional administration of drugs offers advantages over systemic administration by increasing drug delivery to the target site and thereby appreciably reducing the dose needed to produce an effect. Hunt et al (1986) proposed a drug targeting index, DTI, defined as the expected ratio of drug

delivered to response and toxicity sites when the drug-carrier combination is used, divided by the same ratio when free drug is administered intravenously, as a quantitative measure of the advantage associated with regional administration or targeting. Using the air-pouch model, Stevens et al (1993) found that the DTI for *S*-(+)-ibuprofen was 150, whereas a much smaller advantage was observed with piroxicam (DTI = 15). This regional advantage for piroxicam suggests that there would be little practical benefit in using this method of administration. Dose–response studies reported here support these claims, since a substantial benefit was achieved with regional administration of *S*-(+)-ibuprofen, whereas piroxicam given regionally showed no therapeutic advantage. The advantage obtained by infusion of *S*-(+)-ibuprofen over bolus administration was similar when given intravenously or via the air-pouch (10-fold when based upon dose). The difference between bolus and infusion response arises because approximately 90% of a bolus dose leaves the pouch during the first 3 h, which is before the inflammatory reaction as measured by PGE_2; thus continuous infusion delivers a similar amount of drug to the pouch over the 3- to 10-h period using approximately one-tenth of a bolus dose. Drug present in the pouch during the first 3 h does not appear to have any effect upon the resulting inflammatory reaction, as indicated by similar concentration–response relationships for infusion and bolus administration, based on pouch concentrations from 3 to 10 h.

Pharmacokinetic studies of air-pouch kinetics of *S*-(+)-ibuprofen and piroxicam indicate that the air-pouch does not differentiate markedly between these two drugs (Martin et al 1993; Stevens et al 1993). Hence, the difference in benefits from regional administration between *S*-(+)-ibuprofen and piroxicam cannot be explained by differences in air-pouch kinetics. However, differences in route and modes of administration seen for *S*-(+)-ibuprofen when based upon dose can be explained by the drug's pharmacokinetics. When both the pharmacokinetics and mode of administration are known, average pouch concentration of *S*-(+)-ibuprofen can be predicted and therefore a given anti-inflammatory effect can be obtained.

In the case of piroxicam, when potency was based upon average pouch concentration, direct air-pouch administration was less potent than intravenous administration, indicating that PGE_2 levels may also be influenced by an additional anti-inflammatory component outside the pouch. As the concentration–effect relationships for piroxicam indicate systemic levels to be better related to effect than pouch concentrations, the primary anti-inflammatory site of action of piroxicam may be systemic. Differences in the anti-inflammatory site of action between piroxicam and *S*-(+)-ibuprofen may be explained by differences in the multifactorial action of non-steroidal anti-inflammatory drugs on components of the inflammatory process. These drugs have been shown to inhibit the migration of polymorphonuclear leucocytes (PMN) into the air-pouch (Martin et al 1994; Sedgwick et al 1984); they also alter PMN function by reducing pro-inflammatory effects such as the surface expression of receptors for complement components and adherence molecules, superoxide anion generation, aggregation and chemotaxis (Huy et al 1986; Crowell & Van Epps 1990; Ottonello et al 1992; Villanueva et al 1992).

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References

Boddy, A., Aarons, L., Petrak, K. (1989) Efficiency of drug targeting: steady-state considerations using a three-compartmental model. Pharm. Res. 6: 367–372

Campbell, D. B. (1990) The use of kinetic-dynamic interactions in the evaluation of drugs. Psychopharmacology 100: 433–450

Crowell, R. E., Van Epps, D. E. (1990) Nonsteroidal anti-inflammatory agents inhibit upregulation of CD11b, CD11c, and CD35 in neutrophils stimulated by formyl-methionine-leucine-phenylalanine. Inflammation 14: 163–171

Hunt, C. A., MacGregor, R. D., Siegel, R. A. (1986) Engineering targeted in vivo drug delivery. I. The physiological and physicochemical principles governing opportunities and limitations. Pharm. Res. 3: 333–344

Huy, D. P., Roch-Arveiller, M., Lenoir, M., Muntaner, O., Thuret, A., Giroud, J. P. (1986) In vitro and in vivo effects of piroxicam on rat polymorphonuclear responsiveness after induction of two acute non-specific inflammatory reactions. Agents Actions 18: 366–371

Martin, S. W., Stevens, A. J., Houston, J. B. (1993) Permeability characteristics of NSAIDs in the rat air pouch model of inflammation. In: Hadgraft, J., Kellaway, I., Parr, G. (eds) Proc. Sec. Ann. UKAPS Conference, STATS, Cardiff, p. 35

Martin, S. W., Stevens, A. J., Brennan, B. S., Davies, D. E., Rowland, M., Houston, J. B. (1994) The 6-day-old rat air pouch model of inflammation: characterisation of the inflammatory response to carrageenan. J. Pharmacol. Toxicol. 32: 139–147

Øie, S., Huang, J.-D. (1981) Influence of administration route on drug delivery to a target organ. J. Pharm. Sci. 70: 1344–1347

Omata, M., Watanabe, M., Hirasawa, N., Tsurufuji, S., Mue, S., Ohuchi, K. (1991) A role of peripheral leukocytes in vascular permeability and edema formation in air pouch type allergic inflammation in rats. J. Pharmacobiodyn. 14: 267–275

Ottonello, L., Pastorino, G., Dapino, P., Beretta, A., Dallegri, F. (1992) Ibuprofen inhibits adhesion-dependent neutrophil response by a glycoprotein-unrelated mechanism. Drugs Exp. Clin. Res. 18: 23–27

Sedgwick, A. D., Lees, P. (1986) Studies of eicosanoid production in the air pouch model of synovial inflammation. Agents Actions 18: 429–438

Sedgwick, A. D., Sin, Y. M., Edwards, C. W., Willoughby, D. A. (1983) Increased inflammatory reactivity in newly formed lining tissue. J. Pathol. 141: 483–495

Sedgwick, A. D., Sin, Y. M., Mackay, A. R., Al-Duaij, A., Willoughby, D. A. (1984) Studies into the mode of action of non-steroidal anti-inflammatory drugs using a model of facsimile synovium. J. Pharm. Pharmacol. 36: 171–174

Stevens, A. J., Martin, S. W., Houston, J. B. (1993) Determination of a drug targeting index for two non-steroidal anti-inflammatory drugs when delivered regional to the rat air pouch model of inflammation. Eur. J. Drug. Metab. Pharmacokinet. 18: 100

Villanueva, M., Heckenberger, R., Palmér, M., Schrör, K. (1992) Stereospecific and non-stereospecific effects of ibuprofen on human platelet and polymorphonuclear leukocyte functions. Agents Actions 37(Suppl.): 162–170