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Valdecoxib: Assessment of COX-2 potency and selectivity

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Abbreviations: PGE2, Prostaglandin E2; TxB2, thromboxane B2; ELISA, enzyme linked Immunosorbant assay; NSAIDs, non-steroidal anti-inflammatory drugs

Abstract

The discovery of a second isoform of cyclooxygenase (COX) led to the search for compounds that could selectively inhibit COX-2 in humans, while sparing prostaglandin formation from COX-1. Celecoxib and rofecoxib were among the molecules developed from these efforts. We report here the pharmacological properties of a third selective COX-2 inhibitor, valdecoxib, which is the most potent and *in vitro* selective of the marketed COX-2 inhibitors that we have studied. Recombinant human COX-1 and COX-2 were used to screen for new highly potent and *in vitro* selective COX-2 inhibitors and compare kinetic mechanisms of binding and enzyme inhibition with other COX inhibitors. Valdecoxib potently inhibits recombinant COX-2, with an IC_{50} of 0.005 μM; this compares with IC_{50} values of 0.05 μM for celecoxib, 0.5 μM for rofecoxib and 2.1 μM for etoricoxib. Unique binding interactions of valdecoxib with COX-2 translate into a fast rate of inactivation of COX-2 110,000 M^{-1} sec^{-1} (compared to 7,000 M^{-1} sec^{-1} for rofecoxib and 80 M^{-1} sec^{-1} for etoricoxib). The overall saturation binding affinity for COX-2 of valdecoxib is 2.6 nM (compared to 1.6 nM for celecoxib, 51 nM for rofecoxib and 260 nM for etoricoxib), with a

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slow off rate ($t_{1/2}$ ~ 98 min). Valdecoxib inhibits COX-1 in a competitive fashion only at very high concentrations (IC_{50} = 150 uM). Collectively, these data provide a mechanistic basis for the potency and *in vitro* selectivity of valdecoxib for COX-2. Valdecoxib showed similar activity in the human whole blood COX assay (COX-2 IC_{50} = 0.24 uM; COX-1 IC_{50} = 21.9 uM). We also determined whether this *in vitro* potency and selectivity translated to significant potency *in vivo*. In rats valdecoxib demonstrated marked potency in acute and chronic models of inflammation (air pouch ED_{50} = 0.06 mg/kg; paw edema ED_{50} = 5.9 mg/kg; adjuvant arthritis ED_{50} = 0.03 mg/kg). In these same animals, COX-1 was spared at doses greater than 200 mg/kg. These data provide a basis for the observed potent anti-inflammatory activity of valdecoxib in humans.

Introduction

The mechanism of action of the non-steroidal anti-inflammatory drugs (NSAIDS), as well the side effects, are explained by inhibition of prostaglandin (PG) synthesis by cyclooxygenase (COX) (Vane, 1971). The recent finding of a second COX isoform (COX-2) provided the basis for the discovery of anti-inflammatory drugs with improved safety. COX-1 is expressed in most tissues and cells, and is abundant in the GI tract, kidney and platelets. Prostaglandins formed by this enzyme are important for normal physiological function in these tissues. The second isoform, COX-2, is prominently expressed in inflamed tissues, where it produces pro-inflammatory prostaglandins (Mitchell et al., 1993; Masferrer et al., 1994; Seibert et al., 1994 and Crofford, 1997) and to a lesser extent constitutively expressed in brain and kidney (Seibert et al., 1997). This suggested that COX-2 could provide a well-defined molecular target for rational drug development, with the hypothesis that specific inhibitors of this enzyme may achieve anti-inflammatory and analgesic efficacy without affecting production of physiological PGs (Needleman and Isakson, 1997).

Testing schemes were developed that relied on assessing the ability of compounds to selectively inhibit COX-2 over COX-1. Initially cells were utilized that expressed the proper isoform (for example: LPS stimulated fibroblasts for COX-2 or platelets for COX-1). Purified ovine COX-1 and COX-2 (Futaki et al., 1994) were also used in assays that

followed the production of PGE_2 by ELISA or radiometric methods. Later, recombinant enzymes, expressed in mammalian cells (Meade et al., 1993) or expressed in insect cells and purified (Barnett et al., 1994; Gierse et al., 1995) were utilized in screening assays. These *in vitro* assays provided a powerful means for assessing COX selectivity and potency and led to the discovery and clinical development of the first rationally designed COX-2 selective inhibitors, celecoxib (Penning et al., 1997) and rofecoxib (Black et al., 1999; Chan et al., 1999).

Recombinant enzymes have been useful for defining the kinetic mechanism of COX-2 selective inhibition and for structural studies of the molecular basis underlying this phenomenon. The results of these mechanistic experiments indicated that COX-2 selective inhibitors such as celecoxib weakly inhibit COX-1 in a competitive fashion, but potently inhibit COX-2 through a time-dependent, slowly reversible mechanism (Gierse et al., 1999). The potent and specific time-dependent mechanism confers selectivity for COX-2 (Copeland et al., 1994; Gierse et al., 1995; Ouellet and Percival, 1995). The specificity for COX-2 demonstrated by diaryl heterocyclic inhibitors such as celecoxib is based on their interaction with a unique side pocket in the COX-2 active site (Kurumbail et al., 1996); a single amino acid difference within the catalytic sites of the two isoforms provides a major contribution to COX-2 specificity (Gierse et al., 1996).

Despite advances in understanding the molecular and kinetic bases for specificity, methods for evaluating *in vitro* activity on COX isoforms vary widely, leading to considerable differences in reported selectivity (Jouzeau et al., 1997; Smith et al., 1994; Meade et al., 1993; Laneuville et al., 1994; Smith et al., 1995; Gierse et al., 1995; Chan et al., 1995; and Smith et al., 1997). This suggests the need for methods to clearly evaluate COX isoform specificity *in vivo*. The observation by Masferrer et al. (1994) that the inflamed rat air pouch produces PGs derived from COX-2, whereas gastric PG levels mirror COX-1 activity suggest a means for directly evaluating selectivity *in vivo*.

In this report we utilize *in vitro* and *in vivo* methodology to assess COX isoform selectivity, and describe the pharmacological activity of valdecoxib, a rationally designed COX-2 inhibitor (Talley et al., 2000) as well as the other marketed COX-2 selective inhibitors, celecoxib, rofecoxib (Black et al., 1999) and etoricoxib (Riendeau et al., 2001) (Figure 1).

Methods

Materials

Arachidonic acid, supplied as the sodium salt, was obtained from NuChek Prep (Elysian,

MN); PGE_2 and thromboxane B2 (TxB_2) ELISA kits were obtained from Cayman Chemical (Ann Arbor, MI); celecoxib, valdecoxib, rofecoxib, etoricoxib, meloxicam and SC-560 were all prepared by Pharmacia Medicinal Chemistry Department; indomethacin, naproxen, ibuprofen, diclofenac, etodolac 6-MNA, piroxicam and all standard buffer reagents were obtained from Sigma, Chemical Co. (St. Louis, MO).

***In vitro* Potency and Selectivity**

Human recombinant enzyme assay. Compounds were evaluated for selectivity of inhibition *in vitro* using baculovirus-expressed recombinant human COX-1 and COX-2 enzymes as previously described (Gierse et al., 1995). For COX-2 selective inhibitors IC_{50}'s were generated from a mean of at least 4 separate determinations, NSAIDs for comparison purposes only were tested once. Inactivation rate constants were determined as previously described (Walker et al., 2001) by measuring oxygen consumption directly with a Clark style polarographic electrode. In addition kinetic constants were determined by measuring the cyclooxygenase activity indirectly by utilizing tetramethyl-p-phenylenediamine (TMPD) as a co-substrate with arachidonic acid by the method previously described (Gierse et al., 1999).

Radioligand binding assay. A direct binding assay that measures radiolabeled inhibitor

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binding to enzyme was developed to assess binding directly to COX-1 and COX-2 without the confounding influence of enzyme kinetics. The method is described in detail in (Hood et al., 2003) and is summarized here. COX-1 or COX-2 specific monoclonal antibodies at 10 µg/ml in 100 mM $NaHCO_3$, pH 8.2 were coated (100 µl/well) onto 96-well Immulon-2 microtiter plates (Dynex Technologies Inc., Chantilly, VA) by incubating overnight at room temperature in a humidified chamber. The coated plates were washed with Dulbecco's phosphate buffered saline (D-PBS), without $CaCl_2$ and $MgCl_2$, pH 7.4 (D-PBS; Life Technologies, Grand Island, NY) and then treated with a blocking reagent consisting of 10% skim milk in D-PBS (0.2 ml) for 90-120 min at 37º C to decrease nonspecific binding to the plate. The coated and blocked plates were washed, COX enzyme added at 20-35 µg/ml in 50 µl of binding buffer (100 mM Tris, 1 µM hemin, pH 8.0) and then incubated at room temperature for 60-120 min. Finally, these antibody captured enzyme coated plates were washed with D-PBS and aspirated to dryness immediately prior to the binding assay. To determine competitive binding with valdecoxib, various concentrations of the indicated compound were incubated with [3H]-valdecoxib and allowed to compete for the binding to COX-2 for 120 minutes. Ki values were determined from logit-log transformations of the binding data. For determination of dissociation rates, [3H]-compounds were incubated with murine COX-2 immobilized on a 96-well plate for 120 minutes before excess cold inhibitor (10 uM) was added to initiate the dissociation time course. At the indicated times the incubation was halted by aspiration and the remaining radiolabeled inhibitor was released

from the enzyme and counted. Specific binding of valdecoxib was 93%, celecoxib 73%, and rofecoxib 50%.

COX inhibition in human whole blood. The assay of Patrignani et al was used to assess COX inhibition in human whole blood (Patrignani et al., 1994). For COX-2 selective inhibitors IC_{50}'s were generated from a mean of at least 5 separate determinations, NSAIDs for comparison purposes only were tested once. To evaluate COX-1 mediated thromboxane B_2 (TxB_2) production, venous blood from healthy human donors was collected in tubes without anticoagulants and allowed to clot. Plasma (0.5 ml) was incubated in 96-well culture plates for 1 hour at 37^{o}C with compound suspended in dimethylsulfoxide (DMSO 0.4% final concentration) and the mixtures were incubated for 10 minutes at 37^{o}C. The reaction was stopped by cold centrifugation (4^{o}C) at 800 x g for 10 minutes to pellet the cells. The supernatants were recovered and diluted 1:200 in ELISA buffer for quantitation of TxB_2 by ELISA. Expected levels of TxB_2 formed in this assay are 50-100 ng/ml serum. Twelve concentrations of compound starting at 200 μM with 3 fold dilutions were examined in duplicate.

To evaluate compounds for COX-2 mediated PGE_2 production, venous blood from healthy human donors was collected in heparinized tubes. Blood (0.5 ml) was incubated in

96-well collection plates for 24 hours at 37^{o}C with 100 µg/ml lipopolysaccharide (LPS Sigma L-2630, Sigma Chemical Co, St. Louis, MO) and compound dissolved in DMSO (0.4 % final concentration of DMSO). The reaction was stopped by cold centrifugation (4 C) at 800 x g for 10 minutes to pellet the cells. Plasma supernatant (40 ul) was precipitated with 4 volumes of methanol (160 ul) and spun at 800 x g for 10 minutes. Supernatants were recovered and diluted 1:50 in ELISA buffer for quantitation of PGE_2 by ELISA. Expected PGE_2 levels in this assay are approximately 15 ng/ml for unstimulated and 50-150 ng/ml for simulated plasma. Twelve concentrations of compound starting at 200 µM with 3 fold dilutions were examined in duplicate. IC_{50} values were generated by fitting the data with a four parameter logistic regression fit then determining the point that intersects 50% of the difference between negative (unstimulated) and positive (stimulated with LPS) uninhibited controls (control IC_{50}).

***In vivo* Potency and Selectivity**

Rat air pouch model of inflammation. Air pouches were produced by subcutaneous injection of 20 ml of sterile air into the intrascapular area of the back of male Lewis rats (175 - 200 g) **(**Charles River Laboratories, Wilmington, MA), 6 animals per dose group. Pouches were allowed to develop for 1 day. Animals were fasted with free access to water. Compounds or vehicle were administered by gavage 2 hours prior to injection of 2 ml of a 1% suspension of

carrageenan (Sigma Chemical Co, St. Louis MO) dissolved in saline into the pouch. At 3 hours post-carrageenan injection, the pouch fluid was collected by lavage with 1 ml of cold heparin-saline. The fluid was centrifuged at 800 x g for 10 minutes at 4ºC, and the supernatants were collected for analysis of PGE_2 by ELISA. At the end of the 3 hours post-carrageenan injection, rats were anesthetized with a CO_2/O_2 gas and blood was collected by heart stick. The gastrointestinal tract was exposed and observed for lesions. Sections of stomach mucosa were dissected and immediately frozen for further prostaglandin analysis. Frozen tissues were processed by homogenization in 70% ethanol. After centrifugation, the supernatants were collected, dried under a stream of nitrogen, and re-suspended in ELISA buffer for PGE_2 determination by ELISA.

Acute carrageenan-induced edema and hyperalgesia in the rat. Paw edema was induced by injecting 0.1 ml of a 1% carrageenan saline solution into the hindpaw of male Sprague-Dawley rats (180-280 g), 6 animals per dose group. Prior to experiments, the rats were allowed free access to food and water. Compounds or vehicle (0.5% methylcellulose in water) were administered orally 1 hour before the carrageenan injection, and paw volume was measured by water displacement at intervals thereafter. Analgesic activity was assessed as inhibition of hyperalgesia produced in response to a thermal stimulus from a radiant heat source (high-intensity projector lamp bulb) positioned under a Plexiglas floor directly

beneath the hind paw. The withdrawal latency of the affected paw was compared with the contralateral (i.e., normal) paw and determined to the nearest 0.1 second with an electronic clock circuit and a microcomputer. Each point represents either the change in paw volume or withdrawal.

Rat adjuvant arthritis model. Dose-response curves for anti-inflammatory activity were established as described by Billingham (1983)Male Lewis rats (180-200 g), 6 animals per dose group, were injected with 1 mg of heat-killed mycobacterium buytricum (Difco Laboratories, Detroit, MI) suspended in light mineral oil (1 mg/0.025 ml) [subcutaneously in the plantar surface of the right hind paw (day 0)]. Control animals were injected with mineral oil only. Rats were assigned numbers and body weights were followed each week. Fourteen days after injection, the left (without mycobacterium injection) paw volume was measured by the plethysmometer (Model 7150; Ugo Basile, Italy). Rats were qualified for use in the assay by measuring the left paw volume. A paw volume of greater than 0.375 ml after subtracting the vehicle treated left paw volume (left paw volume from injected rat minus left paw volume from normal rat greater than or equal to 0.375 ml) was required for inclusion in the study. After the animals were regrouped, rats received either vehicle (0.5% methylcellulose/0.025% Tween-20, 1 ml/rat, B.I.D.) or compound (0.01 mg/kg to 10 mg/kg per day in 1 ml of vehicle, P.O. B.I.D). On day 22 (7 days after compound dosing) and the day 26 (11 days after compound dosing), left paw volume was measured. ED_{50} and ED_{80}

data were calculated based on the data from day 26.

Results

Assessment of COX inhibition *in vitro* and *in vivo*. Inhibition of COX isoforms *in vitro* by valdecoxib and several NSAIDs was assessed using recombinant human enzymes (Table 1, left panel). Consistent with previous reports, most NSAIDs appear to non-selectively inhibit both isoforms of COX. Valdecoxib and diclofenac are the most potent inhibitors of COX-2 in this setting, exhibiting IC_{50} values of 0.005, and 0.01 μM, respectively. In contrast to the NSAIDs evaluated, valdecoxib demonstrates clear potency and selectivity for COX-2, consistent with a previous report (Talley et al., 2000).

In an effort to more fully characterize the potency of COX-2 enzyme inhibition, kinetic parameters for time dependent inhibition were determined (Table 2). Time dependent parameters of K_I and k_{inact} obtained with valdecoxib for COX-2 are 35 μM and 3.8 sec^{-1} for the oxygen uptake assay (Table 2). The efficiency of compound inhibition for COX-2 can be more conveniently expressed as a function of the k_{inact}/K_I ($sec^{-1}/\mu M$). When expressed in this manner, valdecoxib and celecoxib have a similar efficiency for COX-2 inhibition, with valdecoxib having a faster rate of inactivation (k_{inact}). Consistent with the

standard endpoint recombinant enzyme assay, both rofecoxib and etoricoxib were less potent, primarily due to their slow rate of inactivation of COX-2.

In the two-step model of COX-2 inhibition, potency is largely due to time dependent inhibition, where the forward rate (k_{inact}) is vastly greater than the off rate (k-2), due to tight binding. Thus, the off rate cannot be directly determined from enzyme kinetic measurements. In order to assess the off rate of inhibitors from COX-2, a binding assay was utilized that directly measures the kinetics of binding of radiolabeled compounds to COX-2. Using this method it was found that valdecoxib had a slow dissociation rate from COX-2 ($t_{1/2}$ = 98 min, vs. 50 min for celecoxib and 17 min for rofecoxib). Competitive binding Ki's were also determined using the binding assay; the rank order of potency for binding was similar to that found with the enzyme inhibition assay, though quantitative differences in potency are apparent (Table 3).

Isoform specificity in human cells was also assessed using the whole blood assay of Patrignani et al (1994) as modified by Chan et al (1995) (Figure 2 and Table 1, center panel). In this analysis, some of the NSAIDs showed some selectivity for COX-2 (e.g. diclofenac, etodolac). However, there are several anomalies evident in these data; naproxen and the active metabolite of nabumetone were inactive on COX-2 despite their known anti-inflammatory activity in humans, whereas drugs that exhibited specificity for COX-2, such

as diclofenac and etodolac, are known to produce GI toxicity in patients with the same incidence as other NSAIDs (Physicians' Desk Reference 1998). Interestingly, only slight differences in potency for COX-2 inhibition were observed among the COX-2 selective inhibitors.

Since *in vitro* measurements of COX isoform selectivity may be unreliable predictors of *in vivo* activity, we directly assessed the effect of various doses of several NSAIDs and valdecoxib on PG content *in vivo* derived from either COX-1 (gastric mucosa) or COX-2 (inflamed air pouch). This provided a quantitative biochemical assessment of the specificity of inhibition of COX isoforms *in vivo*. As shown in Figure 3, valdecoxib dose-dependently decreased inflammatory PGE_2 production, with half maximal inhibition (ED_{50}) occurring at approximately 0.06 mg/kg; little inhibition of COX-1 derived gastric PG content was observed over a wide dose range. In contrast, NSAIDs showed no specificity for either COX isoform (meloxicam and nabumetone) or apparent COX-1 specificity (etodolac) in this *in vivo* assay. Quantitative comparisons of several NSAIDs derived from dose-response analyses are shown in Table 1, panel 3.

Activity in acute inflammation and hyperalgesia. *In vivo* potency and efficacy of valdecoxib was evaluated in a standard model of acute inflammation and pain. The injection of carrageenan into the rat paw caused marked increases in paw volume (edema) and thermal

hyperalgesia that were maximal within 1-3 hr. Prophylactic administration of either valdecoxib or naproxen produced sustained inhibition of edema and hyperalgesia (Figure 4). When administered prior to carrageenan injection, valdecoxib was as efficacious as naproxen in blocking the inflammatory pain response.

Anti-inflammatory activity in adjuvant-induced arthritis. Adjuvant-induced arthritis in Lewis rats was used as a model of chronic anti-inflammatory activity. Valdecoxib exhibited potent activity in this assay (ED_{50}= 0.03 mg/kg) (Table 5). This was equivalent in maximal efficacy to the standard NSAID indomethacin, and the steroid dexamethasone, as seen from the comparison of the respective time courses of treatment (Figure 5)

Discussion

Evidence is presented here that valdecoxib potently and selectively inhibits COX-2 *in vitro* and *in vivo*; and, in animals, possesses lower potential for inhibition of COX-1 as well as anti-inflammatory and analgesic activity comparable with NSAIDs. The evidence for potency and selectivity of valdecoxib *in vitro* was seen in the recombinant enzyme assay and was maintained in the human whole blood assay.

Factors underlying the high potency of valdecoxib for COX-2 were assessed mechanistically. Consistent with previous studies, the primary kinetic factors underlying the

potency and COX-2 selectivity of valdecoxib was time dependent inhibition of COX-2 and not COX-1 (Copeland et al., 1994). This was manifested by a very rapid rate of inactivation (k_{inact}) and observed rate of inactivation ($K_{observed}$) of COX-2 by valdecoxib. These results, coupled with the finding of a very slow off rate of binding, provide a mechanistic basis for the potency and specificity of valdecoxib for COX-2. The *in vitro* potency and selectivity of valdecoxib for COX-2 was confirmed *in vivo*, using biochemical measures of COX activity. Thus, dose-dependent inhibition of PG formation at sites of inflammation was observed (ED_{50} = 0.03-0.06 mg/kg) without significant effects on COX-1-dependent PG production in the stomach (ED_{50} > 200 mg/kg). These data are significant because it is now recognized that predictions of COX-2 selectivity based solely on *in vitro* results are suspect (Jouzeau et al., 1997) and require confirmation *in vivo* (Meade et al., 1993).

As would be expected of a potent COX-2 inhibitor, valdecoxib was as effective as indomethacin in both the acute carrageenan inflammation model (ED50 ~ 6 mpk) and in the adjuvant-induced arthritis model (ED_{50} ~ 0.03 mg/kg). The maximal effects seen with valdecoxib in these studies are similar to those produced by NSAIDs. Reports describing knockout animals, in which the COX-2 gene for has been deleted (Morham et al., 1995 and Dinchuk et al., 1995), along with increasing awareness of physiological roles for COX-2, have raised concern for adverse consequences of selective COX-2 inhibition (Richardson

and Emery, 1996). While the studies described here were not designed to evaluate adverse effects, the level of inhibition of COX-2 produced by valdecoxib in animal models is no different than already seen with drugs used extensively in patients.

It is believed that inhibition of PG synthesis by COX-1 is pivotal to the production of gastrointestinal injury by NSAIDs (Mitchell et al., 1993 and Seibert et al., 1995). In addition to the minimal effects on gastric PGs, valdecoxib produced no functional evidence of COX-1 inhibition in acute models that screen for NSAID-type GI toxicity. The absence of gastric or intestinal injury after single doses of valdecoxib in rodents (compared to indomethacin which produced clear gastric injury, peritonitis, and fibrosis in all animals) suggests that valdecoxib possesses lower potential for GI toxicity than a non-selective inhibitor.

Assays using human whole blood, which typically measure COX-1 inhibition in platelets and inhibition of COX-2 induced in monocytes with LPS, were developed to mitigate the uncertainty of *in vitro* systems. However, as shown here, these assays appear to suffer the same limitations seen with *in vitro* assays. Thus, inconsistencies noted between these results (Table 1) and the known clinical activity of several drugs (e.g., diclofenac, naproxen, nabumetone and meloxicam) suggest that this approach does not necessarily predict either selectivity or improved GI safety in humans.

I*n vivo* assessments of COX-2 selectivity are ultimately more relevant because they represent pharmacological activity in whole animals. PGs measured in experimentally induced inflammatory exudate and gastric extracts provide simultaneous measures of the activities of COX-2 and COX-1, respectively. *In vivo*, all NSAIDs appear non-selective with little or no separation of dose-response curves for inhibition of inflammation and gastric PGs. This is consistent with the well-recognized toxicity of these drugs in patients given ordinary clinical doses.

In conclusion, the exquisite anti-inflammatory potency of valdecoxib *in vivo* is due in large part to its ability to potently inhibit COX-2. By *in vitro* measurements, valdecoxib is a highly potent COX-2 inhibitor. Further enzyme kinetic analyses revealed that the potency of valdecoxib for COX-2 is due to its fast rate of inactivation of COX-2 and tightness of binding, manifested by a slow off rate. Valdecoxib's potency is maintained in animal models of acute inflammation and pain without apparent effect on COX-1 at maximal doses administered. Valdecoxib's efficacy in a chronic rat model of arthritis is reflective of its low clinical dose. Clinical evaluations of the effectiveness and side effects of valdecoxib have confirmed these conclusions.

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Footnotes:

The animal studies were approved by the Pfizer St. Louis Institutional Animal Care and Use Committee (IACUC). The animal care and use program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International

Legends for Figures

Figure 1. COX-2 selective inhibitors used in the current study.

Figure 2. Human whole blood assay. Compounds were evaluated for their ability to inhibit either COX-1 from platelets or COX-2 from LPS simulated whole blood as described in materials and methods. Twelve concentrations of compound starting at 200 μM with 3 fold dilutions were examined in duplicate. Curves were generated from a 4-parameter log fit of the data.

Figure 3. Rat air pouch model of inflammation and gastrointestinal PGE2 production. Dose-response curves for valdecoxib, etodolac, nabumetone and meloxicam were determined as described in materials and methods. Valdecoxib shows no inhibition of gastric PGE2 at maximally efficacious concentrations and higher, while the etodolac, nabumatone and B

Figure 4. Carrageenan-induced inflammation and the measurement of hyperalgesia (withdrawal latency) and edema (paw volume). Reduction in hyperalgesia (panel A) and edema (panel B) for either vehicle, squares; naproxen (10 mg/kg), circles or valdecoxib, triangles (10 mg/kg) according to materials and methods. Efficacy of valdecoxib equals naproxen.

Figure 5. Rat adjuvant arthritis model of chronic inflammation. Either indomethacin (2 mg/kg), triangles; valdecoxib (1 mg/kg) diamonds; or dexamethasone (0.1 mg/kg), squares were dosed BID for 11 days in animals according to materials and methods. Maximum efficacy of valdecoxib equaled the NSAID indomethacin and dexamethasone.

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Table 1

Potency and selectivity *In vitro* and *In vivo*

	Human Recombinant Enzymes uM (+/- SD)		Human Whole Blood uM (+/- SD)		Rat Air Pouch (mpk)	
	COX-1	COX-2	COX-1	COX-2	COX-1[b]	COX-2[c]
COX-2 Selective:						
Valdecoxib	**150 (32)**	**0.005 (0.002)**	**21.9 (3.5)**	**0.2 (0.05)**	**>200**	**0.06**
Celecoxib	**15 (3.4)**	**0.04 (0.013)**	**8.3 (1.4)**	**0.3 (0.06)**	**>200**	**0.2**
Rofecoxib	**>1000**	**0.5 (0.5)**	**7.0 (3.33)**	**0.2 (0.06)**	**>200**	**1.1**
Etoricoxib	**>1000**	**5 (4.3)**	**11.7 (4.7)**	**0.5 (0.08)**	**50**	**1.5**
NSAIDS:						
Indomethacin	**0.1**	**1.0**	**0.3**	**0.1**	**0.1**	**0.2**
Naproxen	**32**	**235**	**9.2**	**>100**	**0.1**	**1.3**
Ibuprofen	**38**	**117**	**3.6**	**10.4**	**0.2**	**2.5**
Diclofenac	**0.03**	**0.01**	**0.2**	**0.02**	**0.3**	**0.8**
Etodolac	**>100**	**54**	**14**	**0.3**	**0.2**	**5.1**
Nabumetone (6-MNA)	**82**	**>1000**	**>50**	**>50**	**14**	**22**
Piroxicam	**680**	**660**	**3.7**	**3.5**	**0.1**	**0.9**
Meloxicam	**1300**	**1700**	**9.5**	**0.3**	**0.7**	**1.0**
SC-560	**0.005**	**40**	**0.01**	**0.4**	**0.3**	**12.9**

[b] Inhibition of Gastric PGs

[c] Inhibition of Air Pouch PGs

Table 2

Rapid and time-dependent inhibition of COX-2 by valdecoxib

	K_i	k_{inact}	$K_{Observed}$
	(uM)	*(sec^{-1})*	*(M^{-1} sec^{-1})*
Valdecoxib	***35***	**3.8**	**110,000**
Celecoxib	**3** *3.5*	**0.4** *0.2*	**130,000** *57,000*
Rofecoxib	**NM** *29*	**NM** *0.04*	**7,000** *1,300*
Etoricoxib	**NM** *430*	**NM** *0.06*	**850** *130*

Italics= TMPD

NM = Not Measurable

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Table 3

Competitive binding to COX-2

	Ki (nM)
Celecoxib	**1.6 +/- 0.2**
Valdecoxib	**2.6 +/- 0.4**
Rofecoxib	**51 +/- 9**
Etoricoxib	**260 +/- 40**

Table 4

Potency: *in vivo* hyperalgesia

	ED50 (mpk)	
	Edema	**Hyperalgesia**
Valdecoxib	**5.9**	**14**
Celecoxib	**7**	**35**
Rofecoxib	**100**	**17**
Etoricoxib	**9**	**37**
Indomethacin	**1.2**	**ND**
Diclofenac	**2.1**	**ND**
Naproxen	**1.6**	**ND**
Piroxicam	**2.4**	**ND**

Table 5

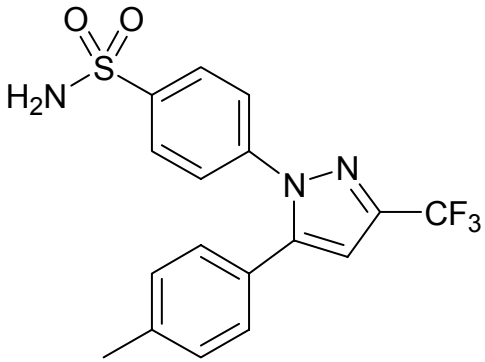
In vivo Efficacy

	Rat Adjuvant Arthritis			Human Clinical
	ED_{50} (mpk)	ED_{80} (mpk)	C_{max} Plasma @ ED_{80} (uM)	C_{Max} Plasma @ Single Dose (uM)
COX-2 Inhibitors				
Valdecoxib	**0.03**	**0.09**	**0.1**	**0.51 @ 10 mg**
Celecoxib	**0.47**	**1.0**	**1.1**	**1.8 @ 200 mg[1]**
Rofecoxib	**0.5**	**1.7**	**0.636-1.2**	**0.67 @ 25 mg**
Etoricoxib	**0.7**	**2.5**	**ND**	**10@ 120 mg[2]**
NSAIDs:				
Indomethacin	**0.7**	**2.0**		**11.2 @ 50 mg[1]**
Diclofenac	**0.05**	**0.43**		**4.4 @ 50 mg[1]**
Naproxen	**0.9**	**3.9**		**420 @ 500 mg[1]**
Piroxicam	**0.2**	**1**		**6.0 @ 20 mg[1]**

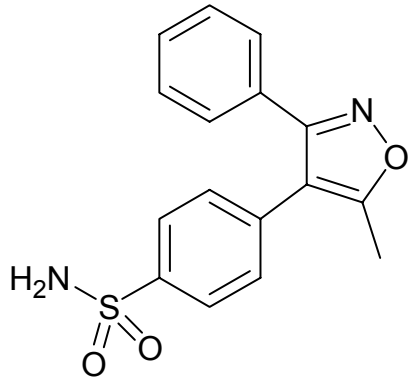
[1] Physicians Desk Reference

[2] Drugs 62(18):2637-2651 (2002)

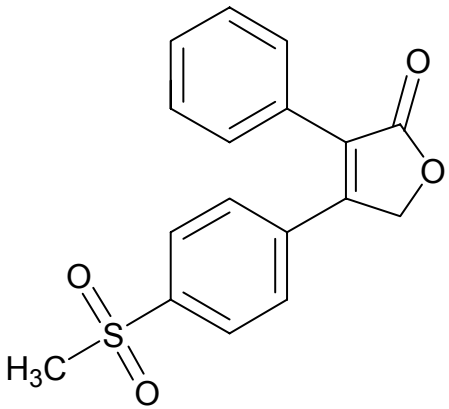
ND Not determined



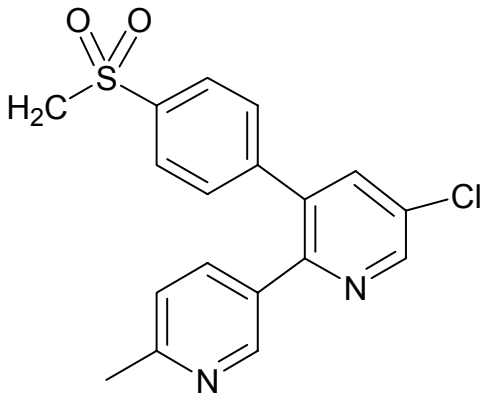
Celecoxib



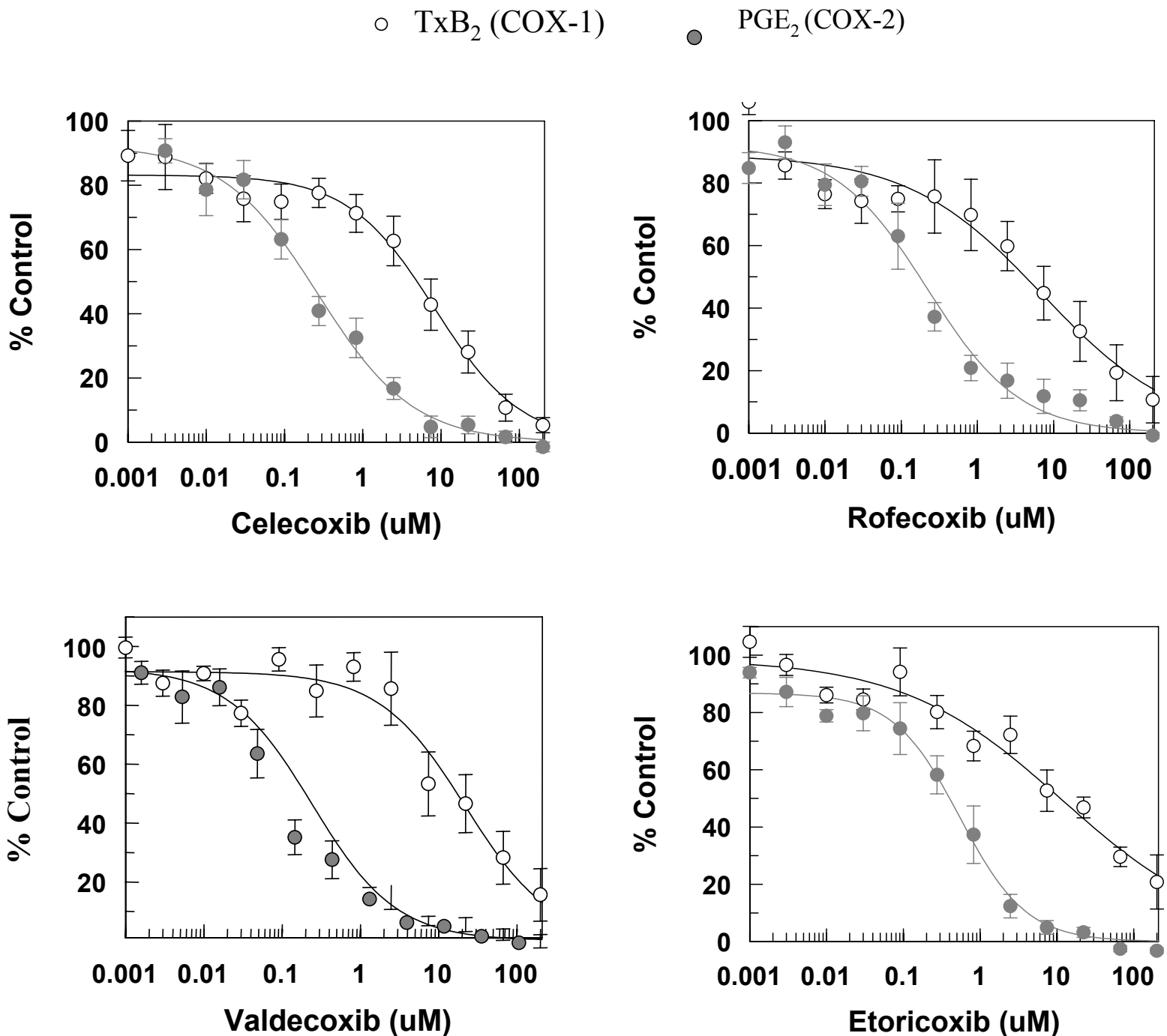
Valdecoxib

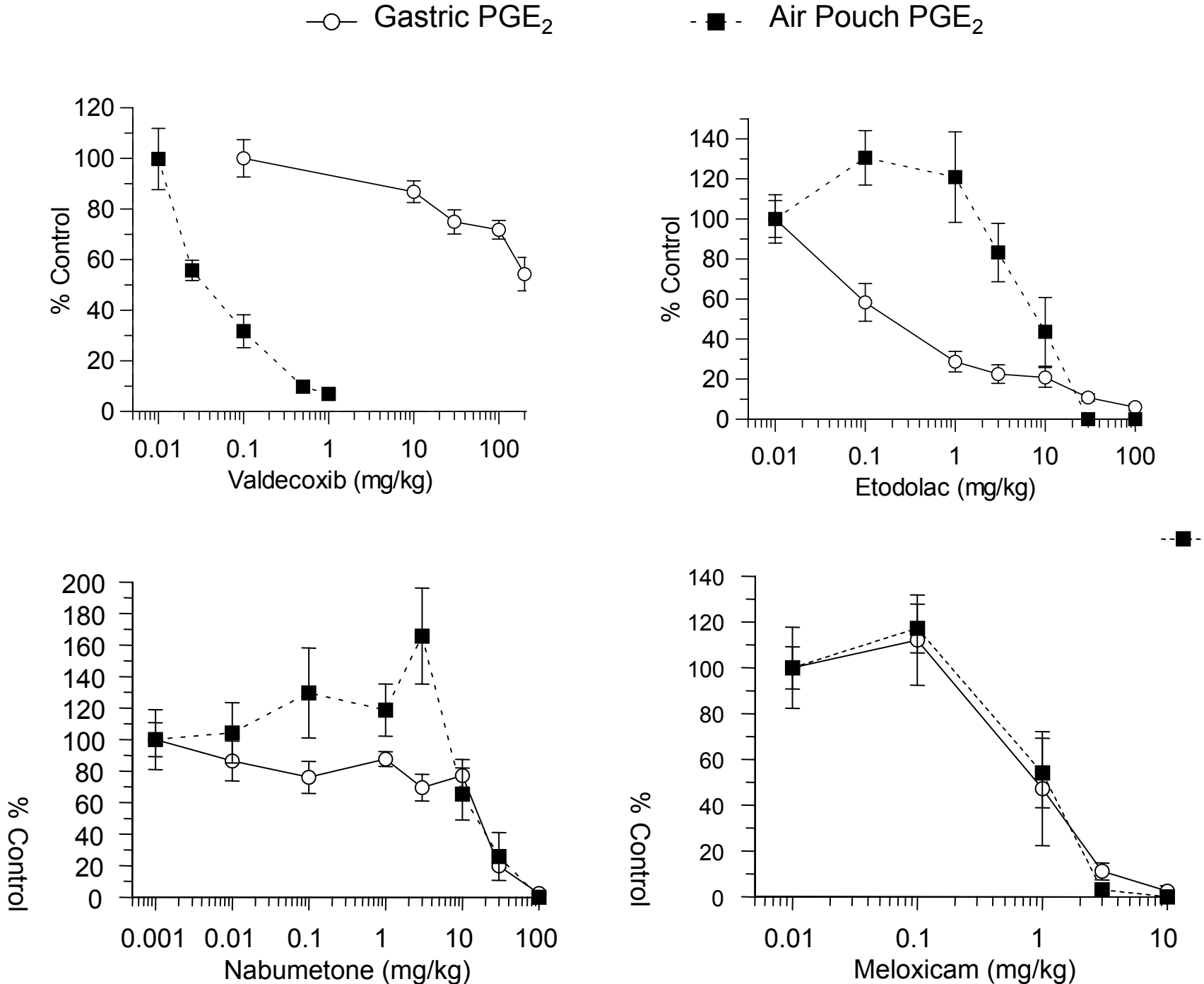


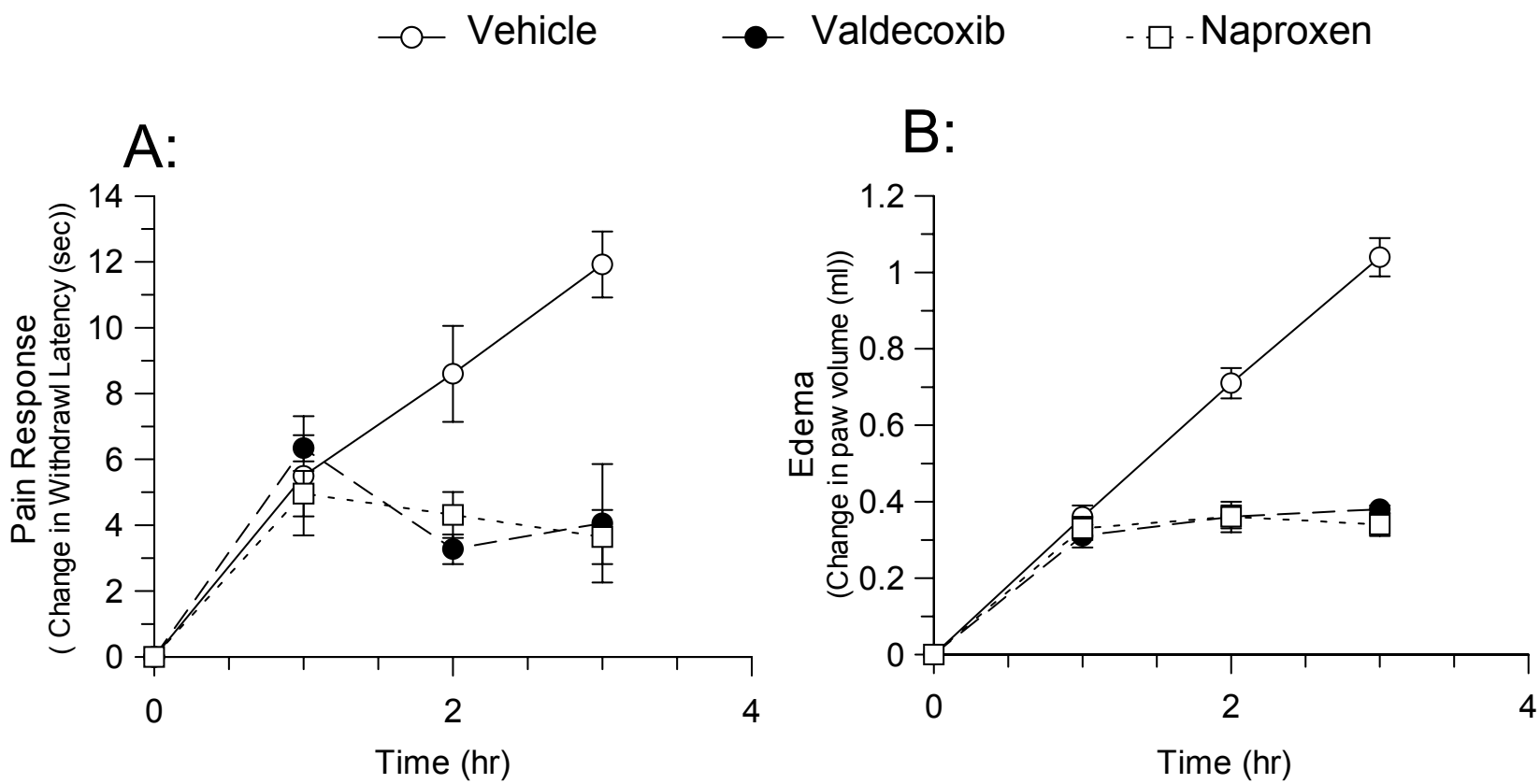
Rofecoxib

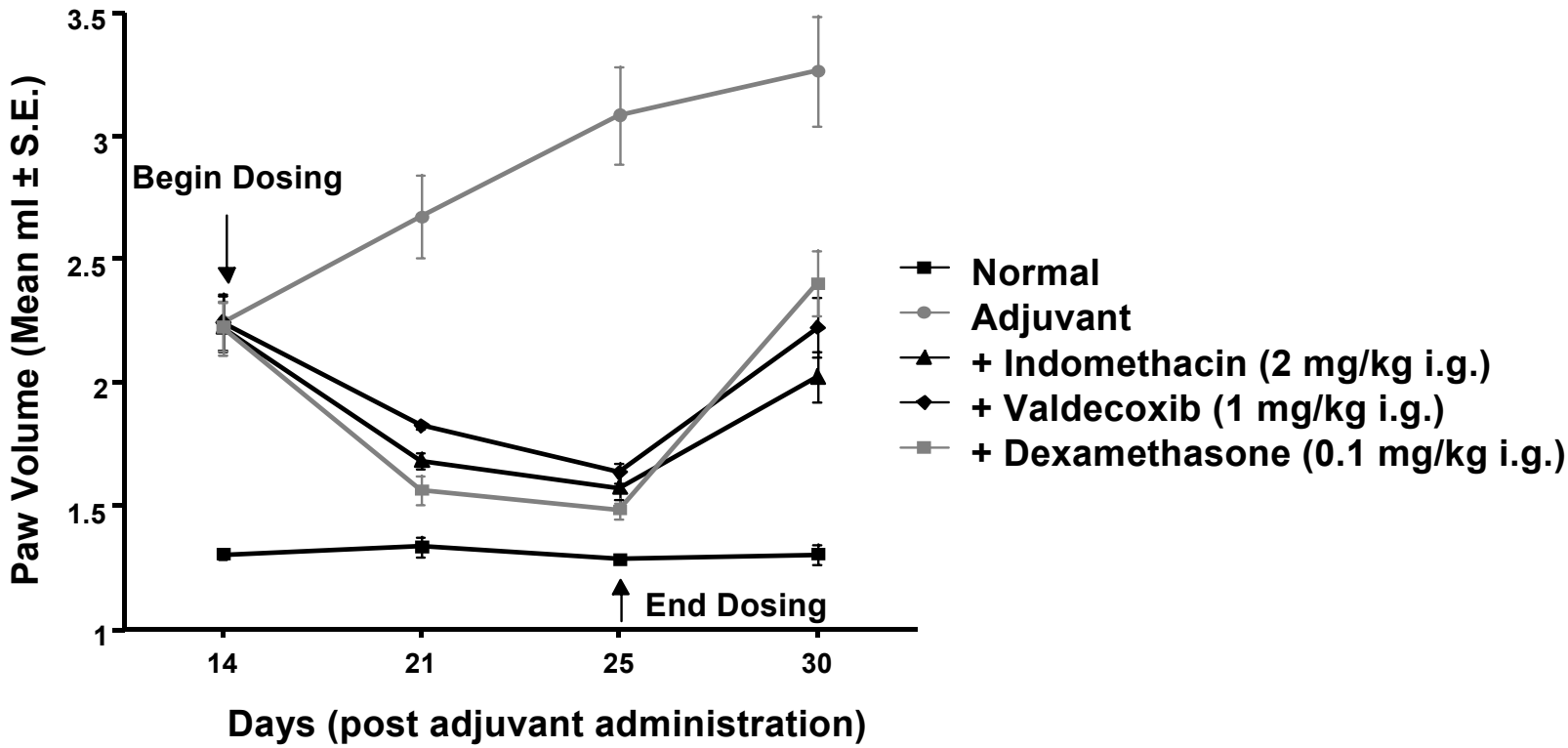


Etoricoxib


TxB2 (COX-1)
PGE2 (COX-2)
% Control
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Celecoxib (uM)
% Contol
Rofecoxib (uM)
% Control
Valdecoxib (uM)
% Control
Etoricoxib (uM)


Gastric PGE2
Air Pouch PGE2
% Control
Valdecoxib (mg/kg)
Etodolac (mg/kg)
Nabumetone (mg/kg)
Meloxicam (mg/kg)


Vehicle
Valdecoxib
Naproxen
A:
Pain Response
(Change in Withdrawl Latency (sec))
14
12
10
8
6
4
2
0
0
2
4
Time (hr)
B:
Edema
(Change in paw volume (ml))
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
Time (hr)


Paw Volume (Mean ml ± S.E.)
3.5
3
2.5
2
1.5
1
Begin Dosing
End Dosing
14
21
25
30
Days (post adjuvant administration)
Normal
Adjuvant
+ Indomethacin (2 mg/kg i.g.)
+ Valdecoxib (1 mg/kg i.g.)
+ Dexamethasone (0.1 mg/kg i.g.)