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Meloxican: Influence on Arachidonic Acid Metabolism

PART 1. *IN VITRO* FINDINGS

G. Engelhardt, R. Bögel, Chr. Schnitzer and R. Utzmann*

DEPARTMENT OF PHARMACOLOGICAL RESEARCH,
DR. KARL THOMAE GMBH, D-88397 BIBERACH/RISS, GERMANY

ABSTRACT. Meloxicam is a new nonsteroidal anti-inflammatory drug (NSAID) derived from enolic acid. Meloxicam has shown potent anti-inflammatory activity in animal models together with low gastrointestinal and renal toxicity. Studies were undertaken to compare meloxicam to other NSAIDs in their ability to inhibit either constitutive cyclooxygenase (COX-1) or inducible cyclooxygenase (COX-2). COX-1 was isolated as a cell-free enzyme from bovine seminal vesicles or bovine brain or was present in nonstimulated macrophages derived from the guinea-pig peritoneum. COX-2 was induced in peritoneal macrophages stimulated by lipopolysaccharide (LPS) or isolated as a cell-free enzyme from sheep placenta. Of all NSAIDs tested, meloxicam was the most selective inhibitor of COX-2 in intact cells. In cell-free enzyme preparations, however, meloxicam showed the same activity against COX-1 and COX-2. All other NSAIDs tested were more potent inhibitors of COX-1 than of COX-2. The inducible cyclooxygenase COX-2 has been implicated in the mediation of the inflammatory reaction, whereas the products of the constitutive cyclooxygenase COX-1 have cytoprotective effects in the gastric mucosa, support microcirculation in the kidney, and are antithrombogenic. Therefore, differential inhibitory effects of NSAIDs on COX-1 and COX-2 may have a bearing on the risk-benefit profile displayed in clinical practice. Meloxicam shows a preferential inhibitory effect on COX-2 over COX-1, which may be directly related to the favorable tolerability profile with potent anti-inflammatory effects observed in animal studies. BIOCHEM PHARMACOL 51;1:21–28, 1996.

KEY WORDS. meloxicam; COX-1; COX-2; macrophage; isolated enzyme.

Meloxicam is a new NSAID† characterized by a unique pharmacodynamic and pharmacokinetic profile that allows plasma concentrations in the therapeutic range to be maintained with once-daily dosing. The anti-inflammatory potency of meloxicam in the rat is higher than that of well-established NSAIDs [1]. Meloxicam is not only a potent inhibitor of acute exudation in adjuvant arthritis in the rat but, also, an intense inhibitor of bone and cartilage destruction as well as of albumin/globulin ratio shift. Meloxicam inhibits the increase of the spleen weight and erythrocyte sedimentation rate of rats suffering from adjuvant arthritis [2]. In spite of the high anti-inflammatory potency shown in the rat, meloxicam has shown low gastrointestinal toxicity and nephrotoxicity [1].‡

In clinical studies, meloxicam has demonstrated reliable efficacy against both osteoarthritis and rheumatoid arthritis [3, 4].

Vane first established the now well-recognized observations that the anti-inflammatory, analgesic, and antipyretic actions and the common side-effect profile (gastrointestinal irritation, renal toxicity, and inhibition of platelet aggregation) of aspirin-like drugs are mediated through the inhibition of prostaglandin biosynthesis [5, 6].

In 1989 Raz *et al.* [7] described an inhibition of the IL-1-induced expression of COX in human fibroblasts by glucocorticoids as a novel mechanism of suppression of arachidonic acid metabolism. This mechanism is distinct from the influence of steroids on phospholipase A_2. Recently, it has been established that COX exists in two isoforms known as COX-1 and COX-2 [8]. The inducible isoform of COX (COX-2) has a shorter amino acid sequence that the constitutive enzyme (COX-1), and differences between the two are found in both the C- and N-termini [8]. COX-2 is induced in fibroblasts and macrophages by proinflammatory stimuli and cytokines [9].

The constitutive COX, COX-1, leads to the production of prostacyclin, which is antithrombogenic [10] and, in the gastric mucosa, cytoprotective [11]. The therapeutic anti-inflammatory actions of the NSAIDs are due to the inhibition of inducible COX-2. The undesirable adverse effects, irritation of stomach mucosa and nephrotoxicity, however, are due to inhibition of the constitutive enzyme COX-1 [12, 13].

The pharmacology of COX-1 and COX-2 is different [13]. Indomethacin, acetylsalicylic acid, and ibuprofen were found

* Corresponding author.

† Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; LPS, lipopolysaccharide; RIA, radioimmunoassay; PGE_2, prostaglandin E_2; IL-1, interleukin-1.

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to be less active against COX-2 than COX-1 [12, 14]. The strongest inhibitors of COX-1, indomethacin and acetylsalicylic acid, have been suspected to cause the most gastrointestinal injury among the commonly prescribed NSAIDs [15, 16]. The spectrum of selective activities of the standard NSAIDs against the two enzymes ranges from a high selectivity for inhibiting COX-1 (e.g. indomethacin) to equal potency for inhibiting COX-1 and COX-2 (e.g. diclofenac) [12]. This variable selectivity for COX-1 and COX-2 inhibition may explain the differences observed between side-effect profiles of NSAIDs at their anti-inflammatory doses.

Accordingly, we investigated the effect of meloxicam and other NSAIDs on COX-1 and COX-2 *in vitro* and *in vivo*. The aim of the study was to prove the hypothesis that the high anti-inflammatory potency and good gastrointestinal and renal tolerance of meloxicam observed in clinical studies to date may be explained by a preferential selectivity of meloxicam for inhibiting COX-2 over COX-1.

The *in vitro* effects of meloxicam and other established NSAIDs on COX-1 and COX-2 are presented here. The *in vivo* activity of these NSAIDs on COX within different tissues is described in a separate publication.*

MATERIALS

Compounds

Meloxicam was synthesized in the laboratories of Dr Karl Thomae GmbH. Piroxicam, tenoxicam, isoxicam, tenidap, diclofenac, indomethacin, naproxen, ketoprofen, ibuprofen, flurbiprofen, acetylsalicylic acid, and dexamethasone were used as reference substances.

Diclofenac (Ciba-Geigy, Basel, Switzerland), indomethacin (Agrar, Rome), acetylsalicylic acid (Bayer, Leverkusen, Germany), and dexamethasone (Roussel-Uclaf, France) were obtained from commercial sources. Piroxicam, tenoxicam, isoxicam, tenidap, naproxen, ketoprofen, ibuprofen, and flurbiprofen were synthesized in the laboratories of Dr Karl Thomae GmbH.

Animals

The animals used in the study were DHP guinea-pigs, bred in our own colony. The animals were kept in rooms with a 12-hr light-dark cycle at 21.5 ± 1.0 C and 60 ± 10% r.h. prior to the study. The guinea-pigs received Altromin MS 3022 (Altromin GmbH, Lage/Lippe) and water *ad libitum*.

METHODS

PGE_2 Generation in LPS-Stimulated Guinea-Pig Peritoneal Macrophages (COX-2 Activity)

INHIBITION OF COX DURING THE INDUCTION PHASE. PGE_2 generation by peritoneal macrophages *in vitro* was determined.

TABLE 1. Inhibition of COX activity of nonstimulated guinea pig peritoneal macrophages (COX-1) and of COX induced by LPS (COX-2); incubation of cells with test compound during 6-hr induction

Compound	COX-1			COX-2			Ratio
	Concentration range (mol/L)†	IC_{50} (mol/L) (95% confidence limits)	R.C.*	Concentration range (mol/L)	IC_{50} (mol/L) (95% confidence limits)	R.C.*	COX-2/COX-1
Meloxicam	1×10^{-9}–1×10^{-7}	$5.77\ (4.56–7.21) \times 10^{-9}$	32.5	1×10^{-10}–1×10^{-8}	$1.91\ (1.40–2.69) \times 10^{-9}$	28.8	0.33
Piroxicam	1×10^{-9}–1×10^{-6}	$5.27\ (3.58–7.41) \times 10^{-9}$	22.8	1×10^{-7}–1×10^{-7}	$1.75\ (1.49–2.02) \times 10^{-7}$	20.7	33
Tenoxicam	1×10^{-9}–1×10^{-7}	$2.01\ (0.58–17.9) \times 10^{-8}$	20.4	1×10^{-8}–1×10^{-5}	$3.22\ (2.09–4.89) \times 10^{-7}$	23.0	15
Tenidap	1×10^{-8}–1×10^{-5}	$3.93\ (2.99–5.19) \times 10^{-7}$	26.0	1×10^{-5}–1×10^{-4}	$4.78\ (3.92–5.97) \times 10^{-5}$	79.1	122
Diclofenac	3.2×10^{-10}–1×10^{-8}	$8.55\ (5.80–11.7) \times 10^{-10}$	29.3	1×10^{-9}–1×10^{-8}	$1.91\ (1.47–2.39) \times 10^{-9}$	49.9	2.2
Indomethacin	1×10^{-10}–1×10^{-8}	$2.10\ (1.26–3.13) \times 10^{-10}$	31.1	1×10^{-9}–1×10^{-7}	$6.39\ (4.97–8.04) \times 10^{-9}$	39.2	30
Flurbiprofen	1×10^{-9}–1×10^{-7}	$1.50\ (0.86–2.82) \times 10^{-8}$	24.9	1×10^{-9}–1×10^{-5}	$4.76\ (1.27–12.7) \times 10^{-6}$	10.0	317
Dexamethasone	1×10^{-6}–1×10^{-5}	$>1 \times 10^{-5}$	–	1×10^{-8}–1×10^{-6}	$4.12\ (2.61–7.47) \times 10^{-7}$	25.1	–

* Regression coefficient.
† No of concentrations, 3–4; No of wells/concentration, 7–12.

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Leucocytes from guinea pigs (450–600 g) were obtained 24 hr after intraperitoneal injection of 10 mL/500 g BW of 2% oyster glycogen type II in sterile 0.9% saline by peritoneal lavage with 40 mL sterile 0.9% saline (containing 10 IU heparin/mL).

Cells were counted after adequate dilution by a Casy-1-counter. The percentage of monocytes in the total leucocyte population was determined by a smear stained by hemacolor (Diagnostica Merck). Viability of the cells was tested by trypan blue staining. Cells were washed, centrifuged, and then resuspended in sterile serum-free RPMI 1640 (Sigma), pH 7.4. A suspension of 27–30 × 10^6 cells/mL was then plated on plastic dishes (12 wells/dish), 1 mL/well. The monocytes were allowed to adhere for 2 hr at 37°C in 95% O_2 and 5% CO_2. Nonadherent cells were then removed by washing the wells 3 times with 1 mL serum-free RPMI 1640.

Adherent cells were incubated with different concentrations of the test substances in 1.0 mL RPMI 1640 containing 5% fetal calf serum, 50 units penicillin, 50 µg streptomycin/mL and 10 µg LPS (lipopolysaccharide from *S. typhimurium*)/mL at 37°C in 95% O_2 and 5% CO_2. Incubation with media alone acted as control. After 6 hr, the media were removed and, after the wells were washed 3 times with 1.0 mL RPMI 1640, the adherent cells were incubated with 1.0 mL/well of a solution of 32.84 µmol/L arachidonic acid in RPMI 1640 for 20 min at 37°C in 95% CO_2. The media were then collected and stored at −80°C for RIA.

PGE_2 contents were determined after adequate dilution by a commercial PGE_2 [^{125}J] RIA-kit NEK 020A. Bound radioactivity was measured by an LB 2104 Multi-Crystal-Counter (Berthold).

INHIBITION OF COX AFTER INDUCTION OF COX-2 BY LPS. The first steps of the procedure were as described above. Adherent cells were incubated in the medium described for 6 hr with 10 µg LPS. The culture media were then changed and one of the test substances was added for 30 min at 37°C in 95% O_2 and 5% CO_2. Arachidonic acid (32.84 µmol/L) was then added and the cells were incubated for an additional 20 min at 37°C in 95% O_2 and 5% CO_2. Thereafter, the media were collected and stored at −80°C for RIA.

PGE_2 GENERATION BY NONSTIMULATED GUINEA-PIG PERITONEAL MACROPHAGES (COX-1) ACTIVITY). The technique used was as described above (during the induction phase), except that adherent cells were incubated with or without test substances for 6 hr without addition of LPS.

Prostaglandin biosynthesis by a cell-free enzyme preparation from bull seminal vesicles, bovine brain or sheep placenta. COX-1 was isolated as microsome extract from bull seminal vesicles [17] or bovine brain and COX-2 from sheep placenta [18], according to Flower *et al.* [19], and stored in small amounts at −25°C.

The rate of prostaglandin synthesis was determined radiochemically under standardized conditions using the method of White and Glassman [20]. [1-^{14}C]-arachidonic acid (NEN), specific activity 1.9 GBq/mmol, was used as substrate. 50 µL of cofactor solution (0.3 mg reduced glutathione and 0.3 mg 1-epinephrine/mL in oxygen-free 0.1 M Tris buffer, pH 8.2) was prepared in small test tubes and 10 µL of enzyme extract added. When the batch had been mixed and allowed to stand on an ice bath for 15 min, 20 µL of the vehicle with and without the test substance and 20 µL of 30 µmol/L [1-^{14}C]-arachidonic acid was added. After intensive stirring, the batch was incubated at 37°C for 25 min. The enzymatic reaction was stopped by adding 5 µL of 2N HCl. To improve chromatographic separation, 5 µL of unlabeled PGE_2 (0.2 mg/mL) was added. The [1-^{14}C]-prostaglandins biosynthesized in this way were chromatographically separated from the [1-^{14}C]-arachidonic acid *via* silica gel columns. Arachidonic acid was eluted with hexane/dioxane/glacial acetic acid (70:30:1, V/V/V) and subsequently with glacial ethyl acetate/methanol (85:15, V/V). The remaining substrate and the product were measured in a liquid scintillation counter.

STATISTICAL ANALYSIS. IC_{50} values were calculated from the reduction in arachidonic acid conversion produced by different concentrations of the test substance compared with the control preparation, using linear regression analysis [21] with predefined confidence limits of 95% [22].

RESULTS

PGE_2 Generation in LPS-Stimulated Guinea-Pig Peritoneal Macrophages

INHIBITION OF COX DURING THE INDUCTION PHASE. Freshly prepared guinea-pig peritoneal macrophages showed only a weak COX activity (<0.7 ng PGE_2/7 × 10^6 macrophages/20 min). After incubation for 6 hr together with LPS, the activity was much higher. After exposure to LPS for 6 hr, macrophages released 40.3 ± 7.34 ng PGE_2/7 × 10^6 macrophages/20 min.

Meloxicam and diclofenac were the most potent inhibitors of COX-2 activity in experiments where macrophages were incubated together with different concentrations of test substances during the induction phase of COX-2 by LPS over 6 hr. Meloxicam was approximately 3 times more potent than indomethacin, 92 times more potent than piroxicam, 170 times more potent than tenoxicam, and 3500 times more potent than flurbiprofen (see Table 1 and fig. 1). Tenidap showed only weak activity under these conditions. However, in view of the variation in the dose-response curves derived, it was difficult to determine specific relative potencies of each drug studied.

INHIBITION OF COX AFTER INDUCTION OF COX-2 BY LPS. In these experiments, macrophages were incubated with the test substance for 30 min after preincubation with LPS for 6 hr and, therefore, after induction of COX-2. Under these conditions, the IC_{50} values of some NSAIDs were greater or less than those found after incubation of the cells with the test substances during induction of the enzyme (Table 2).

Meloxicam was a more potent inhibitor of COX-2 during the induction phase than after its induction was complete. The IC_{50} of meloxicam was 23 times higher, and for diclofenac 5 times higher, for inhibition of induced COX than that needed to inhibit the enzyme during induction.

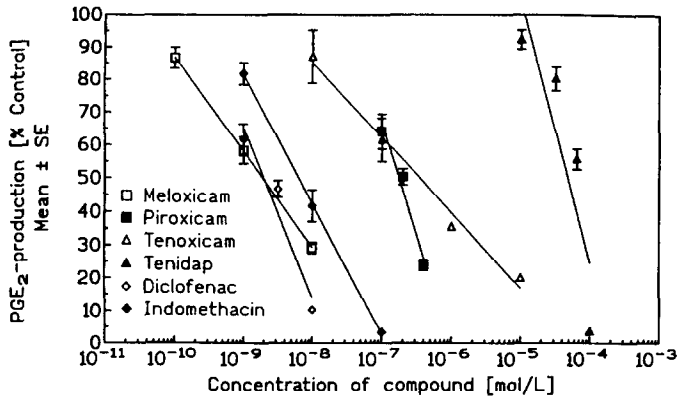

FIG. 1. Influence on PGE_2 production of guinea-pig peritoneal macrophages stimulated by LPS for 6 hr *in vitro*.

PGE$_2$ GENERATION IN NONSTIMULATED CELLS. In these assays nonstimulated cells were incubated with the test substance for 6 hr without adding LPS.

When compared to the COX activity of macrophages stimulated by LPS, the enzyme activity of nonstimulated cells (COX-1 activity) after 6-hr incubation was weak; 6.69 ± 1.33 ng PGE_2 were released by approximately 7×10^6 cells over 20 min compared to 40.3 ± 7.34 ng $PGE_2/7 \times 10^6$ cells/20 min in LPS-stimulated cells.

Indomethacin was the most potent inhibitor of COX-1 activity in nonstimulated macrophages (Table 1 and Fig. 2). Meloxicam and piroxicam were more active than flurbiprofen (3 times), tenoxicam (4 times), and tenidap (70 times) under these conditions.

There were clear differences between the activity of the NSAIDs tested against the two types of COX (Table 2). Piroxicam, tenoxicam, tenidap, indomethacin, and flurbiprofen inhibited COX activity in the nonstimulated macrophages (COX-1 activity) more potently than in the LPS-stimulated macrophages (COX-2 activity). The inhibitory activity of diclofenac against the COX in non-stimulated cells was similar to that found in these cells after induction by LPS.

Meloxicam showed weaker effects against COX activity of nonstimulated cells than against LPS-stimulated cells.

PGE_2 Generation in Cell-Free Enzyme Preparations

INHIBITION OF COX ACTIVITY OF ENZYME PREPARATION FROM BULL SEMINAL VESICLES. In this preparation, COX-1 was responsible for the COX activity [7]. All NSAIDs tested in the cell-free system had a weaker activity against COX-1 activity than in intact cells.

Meloxicam, like tenoxicam and piroxicam, showed only a weak activity on the enzyme preparation from bovine seminal vesicles (Table 3).

Flurbiprofen was the most potent inhibitor of this isolated COX preparation. The compound was 45 times more active than meloxicam and twice as active as diclofenac in inhibiting COX activity.

TABLE 2. Effect of NSAIDs on COX-2 activity in guinea-pig peritoneal macrophages induced by LPS *in vitro*; comparison of the activity found with test compounds administered during the induction phase with that seen when compounds administered following enzyme induction

Compound	During induction†		After induction			Ratio after induction/ during induction
	IC_{50} (mol/L) (95% confidence limits)	R.C.*	Concentration (mol/L)‡	IC_{50} (mol/L) (95% confidence limits)	R.C.*	
Meloxicam	$1.91\ (1.40–2.69) \times 10^{-9}$	28.8	$1 \times 10^{-8}–1 \times 10^{-6}$	$4.47\ (3.61–5.49) \times 10^{-8}$	34.8	23
Piroxicam	$1.75\ (1.49–2.02) \times 10^{-7}$	20.7	$1 \times 10^{-8}–1 \times 10^{-6}$	$1.68\ (1.34–2.13) \times 10^{-7}$	31.0	1.0
Tenoxicam	$3.22\ (2.07–4.89) \times 10^{-7}$	23.0	$1 \times 10^{-8}–1 \times 10^{-6}$	$6.81\ (3.46–19.7) \times 10^{-7}$	21.8	2.1
Diclofenac	$1.91\ (1.47–2.39) \times 10^{-9}$	49.9	$1 \times 10^{-9}–1 \times 10^{-7}$	$1.04\ (0.73–1.48) \times 10^{-8}$	32.5	5.4
Flurbiprofen	$4.76\ (1.27–12.7) \times 10^{-6}$	10.0	$3.2 \times 10^{-7}–3.2 \times 10^{-6}$	$1.00\ (0.87–1.15) \times 10^{-6}$	63.8	0.2

* Regression coefficient.
† Data from Table 1.
‡ No. of concentrations, 3; No. of wells/concentration, 8.

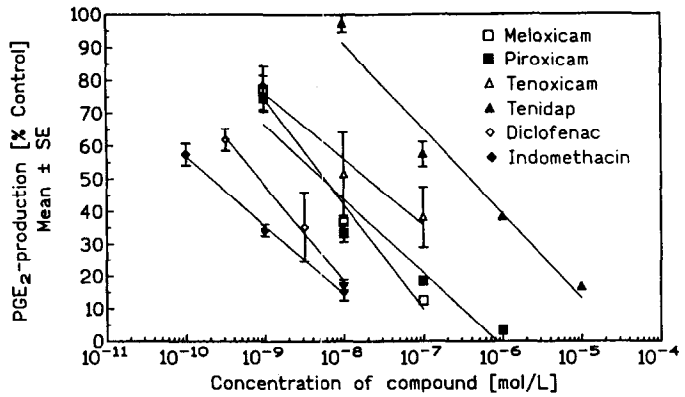

FIG. 2. Influence on PGE_2 production of nonstimulated guinea-pig peritoneal macrophages after 6 hr incubation *in vitro*.

INHIBITION OF COX ACTIVITY OF ENZYME PREPARATION FROM BOVINE BRAIN. The results obtained in this test are presented in Table 3.

COX isolated from bovine brain showed a lower level of activity than that from bovine seminal vesicles. It was, however, inhibited by the NSAIDs to the same extent as the enzyme isolated from bovine seminal vesicles.

Meloxicam, even in this enzyme preparation, is a more potent inhibitor of prostaglandin biosynthesis than piroxicam or tenoxicam. The rank order of potency of the other NSAIDs as inhibitors of prostaglandin biosynthesis by the enzyme isolated from bovine brain is similar to that found in enzymes from seminal vesicles.

INHIBITION OF COX ACTIVITY OF ENZYME PREPARATION FROM SHEEP PLACENTA. The results obtained in this test are compiled in Table 4. The COX isozyme isolated from sheep placenta should essentially be a COX-2 [18].

Diclofenac was the most potent inhibitor of COX isolated from sheep placenta. Meloxicam was more active than piroxicam or tenoxicam in this setting.

There were clear differences between the activity of the NSAIDs against the COX isolated from sheep placenta and the COX preparation derived from bovine seminal vesicles (Table 4). Piroxicam, tenoxicam, diclofenac, indomethacin, and flurbiprofen inhibited the activity of COX isolated from bovine vesicles more potently than that isolated from sheep placenta.

Only meloxicam showed the same effects against the COX activity isolated from bovine seminal vesicles and sheep placenta.

DISCUSSION

The new NSAID meloxicam differs in its pharmacodynamic profile and in its tolerability profile compared to NSAIDs currently in therapeutic use [1]. Recently, data regarding the existence of two isoforms of COX known as COX-1 and COX-2 has been published. An inducible cyclooxygenase, COX-2 [7, 23–27], produces mediators of inflammation [28] and a constitutive COX (COX-1) has a cytoprotective effect on the gastric mucosa [12, 13, 29]. These observations have led us to investigate the differential effects of NSAIDs on COX-1 and COX-2. Furthermore, we wished to determine if any of the differential effects could be related to meloxicam's favorable tolerability profile compared to that of NSAIDs currently in therapeutic use.

This study investigated the effects of various NSAIDs on COX-1 and COX-2 in *in vitro* models using intact cells (stimulated or nonstimulated guinea-pig peritoneal macrophages) or in cell-free enzyme systems derived from bovine seminal vesicles or brain and sheep placenta. These models were specifically chosen to allow the individual effects of each agent on COX-1 or COX-2 to be differentiated, because in each model only one of the COX isoforms was assumed to be present. We used peritoneal macrophages from guinea pigs as intact cell system provided with COX. Provocation with intraperitoneal administration of oyster glycogen produced a large number of nonactivated cells that were characterized by a relatively low, but constant, basic activity of constitutive COX. Within 6 hr, stimulation with LPS produced a much greater COX activity. We assume that the activity of the nonstimulated guinea-pig macrophages, like the basal activity of macrophages of other species [17, 23], is due to COX-1, and the activity found after a 6-hr stimulation with LPS is most likely to be due to COX-2. COX-2 induction in this model was confirmed by our own finding that dexamethasone, which does not inhibit the activity of either the constitutive COX-1 or the already induced COX-2 when incubated with LPS during the induction phase, was able to inhibit enzyme activity.

Meloxicam was a potent inhibitor of COX-2 in the LPS-stimulated guinea-pig peritoneal macrophages. When meloxicam was incubated with nonstimulated cells containing only COX-1, it showed a lower potency. In comparison with results for the other NSAIDs, meloxicam manifested the best relation between effectiveness against COX-2 and effectiveness against COX-1 activity of the macrophages. Meloxicam preferentially inhibited COX-2. Indomethacin, piroxicam, tenoxicam, and tenidap preferentially inhibited COX-1.

Comparing our data with those of other similar studies, it should be pointed out that there are differences, in part, in the experimental techniques used. For instance, Mitchell *et al.* [12] incubated the cells with the test substance for a brief period (30 min) after induction of COX-2. Therefore, in this procedure, they tested inhibition of the induced enzyme's activity. In one part of our study we deviated from this procedure and allowed the test substances to act on the macrophages during the entire 6 hr of the induction phase. Consequently, this procedure also records any inhibition of COX-2 induction.

Some of our IC_{50} values for the inhibition of the COX-2 activity of macrophages differ, depending on whether the NSAIDs were present during the induction phase or after COX-2 induction. The fact that meloxicam, contrary to the other NSAIDs, is more effective when present during the induction phase than when applied after induction, could indicate that meloxicam inhibits induction of COX-2.

TABLE 3. **Inhibition of prostaglandin biosynthesis in a cell-free enzyme preparation from bovine seminal vesicles and bovine brain *in vitro***

Compound	Bovine seminal vesicle			Bovine brain		
	Concentration range (mol/L)†	IC_{50} (mol/L) (95% confidence limits)	R.C.*	Concentration range (mol/L)†	IC_{50} (mol/L) (95% confidence limits)	R.C.*
Meloxicam	1.25×10^{-6}–2.0×10^{-5}	$3.96\ (3.75–4.17) \times 10^{-6}$	61.6	6.25×10^{-7}–1.0×10^{-5}	$3.16\ (2.94–3.41) \times 10^{-6}$	49.9
Piroxicam	1.0×10^{-6}–1.6×10^{-5}	$9.10\ (7.55–11.4) \times 10^{-6}$	43.8	1.25×10^{-6}–4.0×10^{-5}	$1.15\ (1.03–1.29) \times 10^{-5}$	46.2
Tenoxicam	1.25×10^{-6}–2.0×10^{-5}	$5.09\ (4.88–5.32) \times 10^{-6}$	62.3	1.25×10^{-6}–2.0×10^{-5}	$8.97\ (8.17–9.92) \times 10^{-6}$	49.1
Tenidap	5.0×10^{-8}–8.0×10^{-7}	$2.53\ (2.45–2.62) \times 10^{-7}$	57.3	5.0×10^{-8}–8.0×10^{-7}	$1.97\ (1.74–2.18) \times 10^{-7}$	55.6
Diclofenac	5.0×10^{-8}–4.0×10^{-7}	$1.44\ (1.36–1.66) \times 10^{7}$	63.9	2.5×10^{-8}–4.0×10^{-7}	$9.58\ (7.33–12.5) \times 10^{-8}$	57.4
Indomethacin	5.0×10^{-8}–8.0×10^{-7}	$2.16\ (2.01–2.33) \times 10^{-7}$	53.4	1.0×10^{-7}–8.0×10^{-7}	$4.74\ (4.06–5.71) \times 10^{-7}$	60.4
Naproxen	6.25×10^{-7}–5.0×10^{-6}	$2.99\ (2.71–3.12) \times 10^{-6}$	68.5	6.25×10^{-7}–5.0×10^{-6}	$1.19\ (0.97–1.41) \times 10^{-6}$	58.3
Ketoprofen	5.0×10^{-8}–8.0×10^{-7}	$4.94\ (4.37–5.52) \times 10^{-7}$	63.0	5.0×10^{-8}–8.0×10^{-7}	$1.50\ (1.40–1.61) \times 10^{-7}$	54.3
Flurbiprofen	3.13×10^{-8}–2.5×10^{-7}	$8.85\ (8.27–9.49) \times 10^{-8}$	83.7	2.5×10^{-8}–2.0×10^{-7}	$8.99\ (8.16–9.98) \times 10^{-8}$	66.7
Ibuprofen	2.0×10^{-6}–2.0×10^{-5}	$1.22\ (1.09–1.35) \times 10^{-5}$	52.1	2.0×10^{-6}–2.0×10^{-5}	$3.96\ (3.12–4.83) \times 10^{-6}$	55.8
Acetylsalicylic acid	1.25×10^{-4}–1.0×10^{-3}	$3.42\ (3.14–3.72) \times 10^{-4}$	63.7	1.25×10^{-4}–1.0×10^{-3}	$3.83\ (3.56–4.13) \times 10^{-4}$	58.0

* Regression coefficient.

† No of concentrations, 3–6; No of wells/concentration, 5–11.

TABLE 4. **Comparison of IC_{50} values for NSAIDs against COX activity in bovine seminal vesicles and in sheep placenta**

Compound	I Bovine seminal vesicle†		II Sheep placenta			Ratio II/I
	IC_{50} (mol/L) (95% confidence limits)	R.C.*	Concentration (mol/L)‡	IC_{50} (mol/L) (95% confidence limits)	R.C.*	
Meloxicam	$3.96\ (3.75–4.17) \times 10^{-6}$	61.6	2.0×10^{-6}–8.0×10^{-6}	$6.03\ (4.97–8.76) \times 10^{-6}$	62.3	1.5
Piroxicam	$9.10\ (7.55–11.4) \times 10^{-6}$	43.8	1.0×10^{-5}–1.6×10^{-4}	$7.48\ (6.48–8.26) \times 10^{-5}$	51.0	8.2
Tenoxicam	$5.09\ (4.88–5.32) \times 10^{-6}$	62.3	1.0×10^{-5}–1.6×10^{-4}	$8.89\ (7.91–10.1) \times 10^{-5}$	44.8	17.5
Diclofenac	$1.44\ (1.36–1.66) \times 10^{-7}$	63.9	2.0×10^{-7}–2.0×10^{-6}	$7.33\ (6.81–7.94) \times 10^{-7}$	71.8	5.1
Indomethacin	$2.16\ (2.01–2.33) \times 10^{-7}$	53.4	2.5×10^{-6}–4.0×10^{-5}	$1.50\ (1.33–1.72) \times 10^{-5}$	51.2	69.4
Flurbiprofen	$8.85\ (8.27–9.49) \times 10^{-8}$	83.7	2.0×10^{-7}–2.0×10^{-6}	$9.93\ (9.14–10.5) \times 10^{-7}$	47.9	11.2

* Regression coefficient.

† Data from Table 1.

‡ No. of concentrations, 3; No. of wells/concentration, 8.

The test procedure where the test substance is added early and is present during the induction phase of COX-2 is probably closer to the conditions in the intact organism than a test procedure that only records COX-2 inhibition after induction. During inflammation, mediator-producing cells do not mature synchronously; in different cells various stages from the beginning to the completion of enzyme induction are to be found. There is no doubt that it makes more sense to inhibit the enzyme induction than to inhibit the activity of the induced enzyme.

COX-1 was isolated from bovine seminal vesicles. Meloxicam showed a much lower potency against COX isolated from the two bovine sources compared to diclofenac, indomethacin, or tenidap. Meloxicam inhibited COX from both sources to a similar extent, whereas diclofenac and indomethacin showed differential effects, both in our study and also that conducted by Flower and Vane [30]. Conversely, paracetamol [31, 32] and dipyrone [31] are considerably more effective against COX isolated from the central nervous system (CNS) than COX-1 isolated from the periphery. Possibly the COX isolated from bovine brain is not identical with COX-1. In brain, the expression of COX-2 is inducible [33–35].

The COX preparation isolated from sheep placenta is essentially a COX-2 [18]. Meloxicam was a potent inhibitor of COX-2 in the enzyme preparation isolated from sheep placenta. Diclofenac showed the greatest potency against COX-2 of all NSAIDs tested here. However, all NSAIDs, apart from meloxicam, displayed lower IC_{50} values for COX-1 compared to their corresponding values for inhibition of COX-2.

Other *in vitro* studies investigating established NSAIDs have also shown markedly different and frequently weaker activity against inducible COX-2 compared to constitutive COX-1 [12, 36, 37]. The relative concentrations effective against COX-1 and COX-2 vary according to the origin of COX-2 (isolated enzyme, various cell types, various species) and the system inducing COX-2 production [12, 36–38].

The most apparent differences have been identified between results obtained from isolated enzyme preparations and intact cell preparations. The observation that NSAIDs are more effective against COX in intact cells than in isolated enzyme preparations conforms to the findings of Mitchell *et al.* [12]. Results from intact cells, rather than cell-free enzyme preparations, are probably better related to the *in vivo* situation. The results from intact cells are essential as a bridge between *in vitro* to *in vivo* data.

Of all NSAIDs tested in intact cells, meloxicam was the most selective inhibitor of COX-2. Preferential activity against COX-2 and weaker activity against COX-1 may be implicated in the characteristic pharmacological profile displayed by meloxicam in animals and man: potent anti-inflammatory activity with low gastrointestinal toxicity.

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