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Meloxican: Influence on Arachidonic Acid Metabolism

PART II. *IN VIVO* FINDINGS

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ABSTRACT. Meloxicam is a new nonsteroidal anti-inflammatory drug (NSAID) derived from enolic acid. Preclinical studies have indicated that meloxicam has potent anti-inflammatory activity, together with a good gastrointestinal and renal tolerability profile. This report summarizes studies undertaken to compare meloxicam to other NSAIDs in the inhibition of the inducible cyclooxygenase (COX-2) in inflamed areas (pleurisy of the rat, peritonitis of mice) and their influence on the activity of the constitutive cyclooxygenase (COX-1) in stomach, kidney, brain, and blood. In pleurisy of the rat, meloxicam was twice as potent as tenoxicam, 3 times as potent as flurbiprofen, 8 times as potent as diclofenac, and 20 times as potent as tenidap at inhibiting prostaglandin E_2 (PGE_2) biosynthesis. In the peritonitis model in mice, meloxicam was approximately twice as active as piroxicam, and more than 10 times as active as diclofenac in the suppression of PGE biosynthesis. Doses of meloxicam sufficient to inhibit PGE_2 biosynthesis in the pleural exudate and peritoneal exudate had no influence on leukotriene-B_4 (LTB_4) or leukotriene-C_4 (LTC_4) content. The effect of meloxicam on the PGE_2 content of rat gastric juice and rat urine was weaker than that of piroxicam or diclofenac. Meloxicam was a weaker inhibitor of the increased PGE_2 concentration in brain of rats and mice (induced by convulsant doses of pentetrazole) than piroxicam, diclofenac, or indomethacin. Meloxicam had a weaker effect on serum thromboxane-B_2 (TXB_2) concentration in rats than piroxicam or tenoxicam. The *in vivo* findings confirm the results of *in vitro* tests, conducted separately, showing that meloxicam preferentially inhibits COX-2 over COX-1. COX-2 is the inducible isoenzyme implicated in the inflammatory response, whereas COX-1 has cytoprotective effects in the gastric mucosa. Therefore, a preferential selectivity for one isoenzyme over another, as displayed by meloxicam, may have implications in the clinical setting in terms of a more favorable risk: benefit profile. BIOCHEM PHARMACOL 51;1:29–38, 1996.

KEY WORDS. meloxicam; prostaglandins; thromboxane; leucotrienes; *in vivo*

Meloxicam is a new NSAID† that has a distinctive pharmacodynamic and pharmacokinetic profile when compared to other, established NSAIDs. However, meloxicam, like piroxicam and tenoxicam, is derived from enolic acid. Meloxicam has shown potent anti-inflammatory activity in rats [1, 2], inhibiting local and systemic signs of adjuvant arthritis in the rat. High anti-inflammatory potency is coupled with low gastrointestinal toxicity and nephrotoxicity [1]. Anti-inflammatory, analgesic, and antipyretic actions of aspirin-like drugs are mediated through inhibition of PG synthesis [3]. In addition, adverse effects such as gastrointestinal toxicity, platelet aggregation, and renal toxicity associated with NSAID therapy are mediated through the same pathway [4]. Raz *et al.* [5] described an inhibition of the IL-induced expression of COX in fibroblasts by glucocorticoids as a novel mechanism of suppression of arachidonic acid metabolism. Recently, it has been established that two isoforms of COX exist which than can be distinguished by their sites of action as well as structurally [6]. COX-1 is the constitutive isoform that leads, among other things, to the production of prostacyclin, which is antithrombogenic and cytoprotective in the gastric mucosa [7]. It is thought that the undesirable effects of NSAIDs are related to their inhibition of COX-1 [8, 9]. COX-2 is the inducible isoform of COX and is implicated in the formation of inflammatory mediators. Consequently, inhibition of COX-2 by NSAIDs is responsible for their anti-inflammatory activity [9].

Several workers have explored the effects of various NSAIDs on COX-1 and COX-2 *in vitro*. For example, indomethacin, acetylsalicylic acid, and ibuprofen have shown less activity against COX-2 than COX-1 [8, 10]. In addition, the strongest inhibitors of COX-1, indomethacin and acetylsalicylic acid, have been suspected to cause the most gastrointestinal injury among other commonly prescribed NSAIDs in clinical practice [11, 12]. Variable selectivity between COX-1 and COX-2 shown by NSAIDs ranges from high selectivity for COX-1 (indomethacin) and equal selectivity (diclofenac) [8].

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† Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; RIA, radioimmunoassay; PG, prostaglandin; PGE_2, prostaglandin E_2; IL, interleukin; PGI_2, prostaglandin I_2; LTB_4, leukotriene-B_4; LTC_4, leukotriene-C_4; TXB_2, thromboxane-B_2; BW, body weight; PGF_1, prostaglandin F_1.

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This variation may be related to the diversity of risk/benefit profiles displayed between NSAIDs.

Several studies have attempted to elucidate the inhibitory effects of meloxicam compared to other, established NSAIDs on the activity of the constitutive COX-1 and the inducible COX-2 *in vitro* and *in vivo*.

A previous study has explored the inhibitory effects of NSAIDs on COX-1 and COX-2 found in intact cells (stimulated or nonstimulated guinea-pig peritoneal macrophages) or on cell-free enzyme preparations from bovine seminal vesicles (COX-1), bovine brain, and sheep placenta (COX-2).

This study explores the influence of meloxicam on COX-2 activity in inflamed areas (pleurisy of the rat, peritonitis of mice) and on the activity of COX-1 in the stomach, kidney, brain, and blood.

MATERIALS

Compounds

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

Diclofenac (Ciba-Geigy, Basel, Switzerland), indomethacin (Agrar, Rome), acetylsalicylic acid (Bayer AG, Leverkusen, Germany), and dexamethasone (Roussel-Uclaf, France) were obtained from commercial sources.

Piroxicam, tenoxicam, tenidap, naproxen, and flurbiprofen were synthesized in the laboratories of Dr Karl Thomae GmbH.

Animals

The animals used were Chbb:THOM (SPF) rats and Chbb: NMRI (SPF) mice. The animals were kept in rooms with as 12-hr light-dark cycle at 21 ± 1.0°C and 60 ± 10% r.h. before the study. The animals were given Altromin-R™ (Altromin GmbH, Lage/Lippe), and tap water *ad libitum* throughout.

METHODS

Influence on Arachidonic Acid Metabolism in Inflamed Tissue

EFFECT ON PGE_2 CONTENT OF PLEURAL EXUDATE IN CARRAGEENAN-INDUCED PLEURISY IN THE RAT. Pleurisy was induced according to Vinegar *et al.* [13] in male rats weighing between 350 and 400 g by intrapleural injection of 0.5 mL of 0.4% sterile solution of carrageenan in 0.9% NaCl solution, under ether anesthesia on the right side of the mediastine between the 4th and 5th rib.

The animals were sacrificed 24 hr after administration of carrageenan, the pleural exudate collected, and the pleural cavity washed with 2.0 mL of 0.9% saline (containing 5 IU heparin/mL).

The concentration of PGE_2 in the pleural exudate was measured by RIA. The prostaglandin E_2 [^{125}J] RIA-Kit NEK-020 (NEN) was used. Tubes were counted in an LB 2104 Multi Crystal Counter (Berthold).

All test substances were triturated in 1% methylcellulose and administered by gavage (1.0 mL/100 g BW) once daily for 5 successive days. The last administration and the intrapleural injection of carrageenan were performed together. The number of animals was between 9 and 19 per dose group (for details see Table 1). Control animals ($n = 60$) received corresponding amounts of the vehicle.

EFFECT ON PGE_2 CONTENT OF PERITONEAL EXUDATE IN ZYMOSAN-INDUCED PERITONITIS IN MICE. Peritonitis was induced in female mice weighing between 30 and 35 g by intraperitoneal injection of 0.5 mL of 1% zymosan in sterile 0.9% NaCl solution. The animals were sacrificed 24 hr after administration of zymosan and the peritoneal exudate collected. Saline (2.0 mL of 0.9% containing 5 IU heparin) was injected intraperitoneally 15 min before this, to wash the peritoneal cavity.

The concentration of PGE_2 in peritoneal exudate was determined by RIA as described above.

All test substances were triturated in 1% methylcellulose and administered by gavage (0.2 mL/10 g BW) 30 min before and 8 hr after injection of zymosan. Between 6 to 9 animals were used per dose group (for details, see Table 2). Control animals ($n = 20$) received corresponding amounts of the vehicle.

INFLUENCE ON LTB_4 CONTENT IN PLEURITIC EXUDATE OF RATS. The procedure of this experiment was as described in thesection above (Effect on PGE_2 content of pleural exudate). Between 9 and 19 animals were used per dose group, between 15 and 18 per control group.

Instead of PGE_2 concentration, the concentration of LTB_4 in the pleural exudate was measured by RIA. The LTB_4 [^{3}H]

TABLE 1. Influence on PGE_2 content of pleural exudate of rats after oral administration (once daily for 5 days)

Compound	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	4	0.25–2.0	14–17	0.65 (0.54–0.78)	97.3
Piroxicam	4	0.5–4.0	14–16	0.85 (0.60–1.09)	59.1
Tenoxicam	4	0.5–4.0	14–19	1.32 (1.14–1.52)	72.2
Tenidap	5	2.0–32.0	9–14	12.8 (9.62–18.0)	54.4
Diclofenac	4	2.0–16.0	14–17	5.06 (3.72–6.63)	40.0
Naproxen	4	4.0–32.0	14–18	12.7 (9.74–16.6)	74.5
Flurbiprofen	3	1.0–4.0	7–14	2.18 (1.78–2.75)	107
Dexamethasone	2	0.05–0.1	16	<0.1	

PGE_2 content of exudate in 60 control rats: 2.30 ± 0.92 ng/exudate (MV ± SD); R.C. regression coefficient.

TABLE 2. Influence on PGE_2 content in peritoneal exudate of mice after oral administration (24.5 and 16.5 hr before exudate collection)

Compound	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	3	0.5–0.2	7–9	1.36 (1.15–1.70)	97.7
Piroxicam	3	1.0–4.0	7–8	2.87 (1.89–10.9)	42.9
Diclofenac	3	4.0–16.0	6–7	>16	
Naproxen	3	8.0–32.0	5–8	20.7 (14.9–45.8)	91.7
Acetylsalicylic acid	4	25–200	7–8	83.6 (68.1–105)	78.1

PGE_2 content of exudate in 26 control mice: 2.43 ± 1.02 ng/exudate (MV ± SD); R.C. regression coefficient.

RIA-kit NEK-037 (NEN) was used. Tubes were counted in a Canberra Packard Tri-Carb 2660 Liquid Scintillation Spectrometer.

INFLUENCE ON LTC_4 CONTENT OF PERITONEAL EXUDATE IN ZYMOSAN-INDUCED PERITONITIS IN MICE. The procedure of this experiment was the same as described above (Effect on PGE_2 content of peritoneal exudate).

Ten animals were used per dose group, 40 animals in the control group. The concentration of LTC_4 in the peritoneal exudate was measured by RIA. The LTC_4 [^{3}H] RIA-kit NEK-030 (NEN) was used. Tubes were counted in a Canberra Packard Tri-Carb 2660 Liquid Scintillation Spectrometer.

Influence on COX in Different Tissues

EFFECT ON PGE_2 CONTENT OF GASTRIC JUICE OF THE RAT. Gastric juice of rats was collected according to Shay *et al.* [14] in male rats weighing between 135 and 165 g. After 16-hr fasting, pylorus ligature was conducted under ether anesthesia. When the abdominal wall was closed, 3.0 mL 0.9% saline/100 g BW was injected intraperitoneally. At the end of a collecting period of 6 hr, rats were sacrificed and the volume of gastric juice measured.

The concentration of PGE_2 in gastric juice was measured by RIA. The commercial prostaglandin E_2 [^{125}J] RIA-kit NEK-020 (NEN) was used. Tubes were counted in an LB 2104 Multi-Crystal-Counter (Berthold).

All test substances were suspended in 1% methylcellulose and administered by injection (1.0 mL/100 g BW) in the duodenum immediately after ligature of the pylorus. Between 9 and 20 animals were used per dose group, 173 in the control group. Control animals received corresponding amounts of the vehicle.

INFLUENCE ON PGE_2 AND 6-KETO-$PGF_{1\alpha}$ EXCRETION IN THE URINE OF WATER-LOADED RATS. Female rats weighing between 180 and 210 g with free access to food (Altromin standard diet for rats and mice R 8013; Altromin GmbH, Lage/Lippe) and tap water, until 2 hr before the beginning of the study, were loaded by stomach tube with 4.0 mL bidistilled water/100 g BW.

The test substances were administered by stomach tube as a trituration in 1% methylcellulose in a volume of 1.0 mL/100 g BW 10 min after the administration of water. Between 6 and 7 animals were used per dose group, 131 animals in the control group. Control animals received the corresponding volume of the vehicle. Urine excreted within 6 hr was collected in metabolic cages. Room temperature and humidity remained constant (21.5 ± 0.5°C; 60 ± 5% r.h.) during this experiment.

The PGE_2 and 6-keto-$PGF_{1\alpha}$ contents were determined after adequate dilution by RIA. The commercial PGE_2 [^{125}J] RIA kit NEK-020A and 6-keto-$PGF_{1\alpha}$ [^{125}J] RIA kit NEK-025A (NEN) were used. Bound radioactivity was measured by an LB 2104 Multi-Crystal-Counter (Berthold).

The amount of prostaglandins excreted in 6 hr per 100 g BW was calculated from the PGE_2 concentration and the 6-keto-$PGF_{1\alpha}$ concentration measured in the urine.

Influence on Prostaglandin Concentration in the Brain

INFLUENCE ON INCREASE OF PGE_2 CONCENTRATION IN THE BRAIN OF MICE INDUCED BY CONVULSANT DOSES OF PENTETRAZOLE. Male mice weighing 25–30 g, fasted for 16 hr, received pentetrazole in a dose of 100 mg/kg BW as a solution in 0.9% saline (0.1 mL/10 g BW) intraperitoneally.

Test substances were administered as a trituration in 1% methylcellulose (0.2 mL/10 g BW) by gavage 60 min before pentetrazole injection. Between 6 and 10 animals were used per dose group, 73 mice in the pentetrazole control group. Control animals received a corresponding volume of the vehicle.

The animals were killed by decapitation immediately following running off clonic seizures. Brains were immediately frozen in liquid nitrogen and samples stored at −28°C.

For extraction, the brain samples were homogenized in 5.0 mL ice-cold ethanol. The homogenates were centrifuged at 12,000 g for 20 min at 4°C. After adequate dilution, the supernatant was used for determination of PGE_2 by RIA. The commercial PGE_2 [^{125}J] RIA-kit NEK 0-020A (NEN) was used.

The measurement of the bound radioactivity was conducted using the LB 2104 Multi-Crystal-Counter (Berthold).

INFLUENCE ON INCREASE OF PGE_2 CONCENTRATION IN THE BRAIN OF RATS INDUCED BY CONVULSANT DOSES OF PENTETRAZOLE. Male rats weighing between 60 and 80 g, fasted for 16 hr, received pentetrazole in a dose of 100 mg/kg BW as a solution in 0.9% saline (0.2 mL/100 g BW) intraperitoneally.

Test substances were administered as a trituration in 1% methylcellulose (1.0 mL/100 g BW) by stomach tube 60 min

before (dexamethasone 180 min before) pentetrazole injection. Between 6 and 10 animals were used per dose group, 37 rats in the pentetrazole control group. Control animals received a corresponding dose of the vehicle.

The animals were killed by decapitation immediately following running off clonic seizures. Their brains were immediately frozen in liquid nitrogen and stored at −28°C.

Extraction of PGE_2 and measurement by RIA was conducted following the procedure described in the section above.

EFFECT ON THROMBOXANE B_2 CONTENT OF RAT SERUM. Male rats weighing between 120 and 150 g, fasted for 16 hr, received the test substance *via* a stomach tube as a trituration in 1% methylcellulose (1.0 mL/kg BW). Between 5 and 12 animals were used per dose group, 42 rats in the control group. Control animals received a corresponding volume of the vehicle.

Blood samples were taken 3 hr after substance administration by puncturing the retroorbital venous plexus. Blood was left to coagulate at 37°C for 30 min. The serum was centrifuged at 3000 g twice for 3 min.

The determination of the TXB_2 concentration in the serum was carried out using an RIA after appropriate dilution. The commercial TXB_2 [^{125}J] RIA-kit NEK-024 (NEN) was used. Measurement of the bound radioactivity was performed with the LB 2104 (Multi-Crystal-Counter (Berthold).

Statistical Analysis

IC_{50} values were calculated from the reduction in arachidonic acid metabolites produced by different doses of the test substance and compared with those calculated from the control group using a linear regression analysis [15] with 95% confidence limits [16].

RESULTS

Influence on Arachidonic Acid Metabolism in Inflamed Tissue

EFFECT ON PGE_2 CONTENT OF PLEURAL EXUDATE IN CARRAGEENAN-INDUCED PLEURISY IN THE RAT. Meloxicam is twice as potent as tenoxicam as an inhibitor of PGE_2 in rat pleuritic exudate (Table 1). Piroxicam inhibited PGE_2 biosynthesis to the same order of magnitude as meloxicam, but the slope of the meloxicam dose-response curve was somewhat steeper than that of piroxicam (see Fig. 1).

Tenidap is only a weak inhibitor of PGE_2 formation in the pleural exudate of the rat. The activity of tenidap in this model was the same as naproxen.

In contrast to the results of *in vitro* studies conducted in a cell-free system on isolated COX-1, meloxicam, piroxicam, and tenoxicam are more potent inhibitors of PGE_2 formation in the pleuritic exudate than flurbiprofen, diclofenac, or naproxen.

Under these experimental conditions, dexamethasone is a very potent inhibitor of prostaglandin biosynthesis.

EFFECT OF PGE_2 CONTENT ON PERITONEAL EXUDATE IN ZYMOSAN-INDUCED PERITONITIS IN MICE. Meloxicam is a powerful inhibitor of PGE_2 formation in the inflamed area (Table 2). In the suppression of PGE_2 biosynthesis in the zymosan-induced peritoneal exudate of mice, meloxicam is approximately twice as active as piroxicam and more than 10 times as active as diclofenac or naproxen. The slope of the meloxicam dose-response curve is steeper than that of piroxicam. Therefore, exact estimation of relative potency could not be deduced by comparing ID_{50} values.

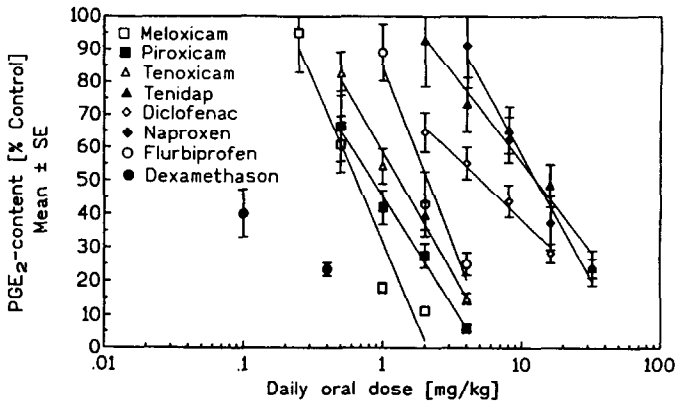

FIG. 1. Influence of NSAIDs on PGE_2 content of pleural exudate of rats after oral administration (once daily for 5 days).

INFLUENCE ON LTB_4 CONTENT IN PLEURITIC EXUDATE OF RATS. Doses of meloxicam and tenoxicam sufficient to inhibit PGE_2 biosynthesis in the pleura of the rat after stimulation by carrageenan have no influence on the LTB_4 content of the pleural exudate (Table 3).

In contrast to these findings, diclofenac and, to a larger extent, indomethacin and tenidap, at concentrations that inhibit PGE_2 biosynthesis, increase the LTB_4 content in the pleuritic exudate of the rat in a dose-dependent manner.

INFLUENCE ON LTC_4 CONTENT OF PERITONEAL EXUDATE IN ZYMOSAN-INDUCED PERITONITIS IN MICE. Table 4 summarizes the results of these experiments.

TABLE 3. Influence on LTB_4 content in pleuritic exudate of rats after oral administration (once daily for 5 successive days)

Substance	Dose (mg/kg/day)	*n*	LTB_4 content (ng/exudate) MV	SD	%
Control	—	18	1.948	0.173	—
Meloxicam	1.0	16	1.993	0.480	+2.3
	2.0	18	1.863	0.371	−4.4
Tenoxicam	2.0	16	2.025	0.232	+4.0
	4.0	19	2.172	0.313	+11.5
Diclofenac	2.0	15	1.952	0.481	+0.2
	8.0	18	2.308*	0.492	+18.5
Indomethacin	1.0	14	2.339*	0.619	+20.1
	2.0	16	3.145†	1.113	+61.4
Control	—	15	1.662	0.396	—
Tenidap	2.0	9	1.560	0.215	−6.1
	4.0	9	2.494†	0.449	+50.1
	8.0	12	3.184†	0.303	+91.6

* $P = <0.05$ compared to control by Student's *t*-test.
† $P = <0.001$ compared to control by Student's *t*-test.

TABLE 4. Influence on LTC_4 content of peritoneal exudate in zymosan-induced peritonitis in mice

Substance	Dose (mg/kg/day)	n	LTB_4 content (ng/exudate) MV	SD	%
Control	—	40	0.962	0.396	—
Meloxicam	0.5	10	0.981	0.466	+2.0
	1.0	10	1.090	0.287	+13.3
	2.0	10	1.115	0.645	+15.9
Tenidap	0.5	10	1.328*	0.590	+38.0
	1.0	10	1.410†	1.002	+46.6
	2.0	10	1.796†	0.643	+86.7

* $P = <0.05$ compared to control by Student's *t*-test.
† $P = <0.001$ compared to control by Student's *t*-test.

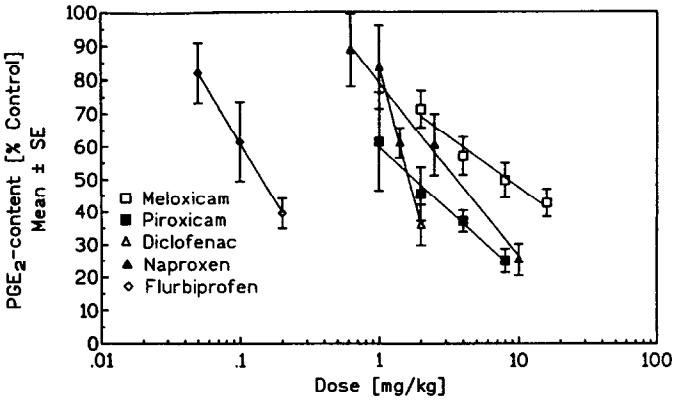

FIG. 2. Influence of NSAIDs on PGE_2 content of gastric juice of SHAY rats 6 hr after intraduodenal administration.

Doses of meloxicam that suppress PG biosynthesis in the peritoneal exudate of the mice did not influence the LTC_4 content of the exudate in a statistically significant manner. Conversely, tenidap caused a dose-dependent increase in LTC_4 concentration in the peritoneal exudate.

Influence on COX in Different Tissues

EFFECT ON PGE_2 CONTENT OF GASTRIC JUICE OF THE RAT. All NSAIDs tested lowered the PGE_2 content of gastric juice in the rat in a dose-dependent manner (Table 5), although the slopes of the dose-response curves differ between treatments (see Fig. 2).

Meloxicam is only a weak inhibitor of PGE_2 biosynthesis in the rat gastric mucosa. However, piroxicam and diclofenac cause equal effects at lower doses than meloxicam and consequently are more potent inhibitors of PG biosynthesis in the stomach.

Of all NSAIDs tested in the rat gastric mucosa, flurbiprofen is the most potent inhibitor of PG biosynthesis.

INFLUENCE ON PGE_2 AND 6-KETO-$PGF_{1\alpha}$ EXCRETION IN URINE OF WATER-LOADED RATS. The excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ in urine of rats is inhibited by all NSAIDs tested in a dose-dependent manner (Table 6 and 7). The slope of the dose-response curves differs between the various compounds.

As an inhibitor of renal PGE_2 excretion, the potency of meloxicam is similar to that of diclofenac. Tenoxicam and tenidap are nearly 3 times and piroxicam 8 times as potent as meloxicam. However, the slope of the tenidap dose-response curve is less steep than that of meloxicam.

The various drugs have a different influence on the excretion of 6-keto-$PGF_{1\alpha}$ in rat urine compared to PGE_2-excretion. Higher doses of most compounds (except diclofenac) are required to reduce 6-keto-$PGF_{1\alpha}$ urinary excretion by 50% compared to those required for suppression of PGE_2 excretion.

Meloxicam and indomethacin display similar potencies as inhibitors of renal 6-keto-$PGF_{1\alpha}$ excretion. Piroxicam is 3 and diclofenac 4 times more potent than meloxicam. Tenidap was a weaker inhibitor of renal 6-keto-$PGF_{1\alpha}$ excretion in the rat than meloxicam.

Influence on PG Concentration in Brain

INFLUENCE ON INCREASE OF PGE_2 CONCENTRATION IN THE BRAIN OF MICE INDUCED BY CONVULSANT DOSES OF PENTETRAZOLE. As described in the literature [17] under physiological conditions found here, only trace amounts of PGE_2 are present in mouse brain. After administration of a convulsant dose of pentetrazole, a rapid rise in PGE_2 concentration in the mouse brain was seen.

All NSAIDs tested suppressed the rise of PGE_2 concentration in brain provoked by pentetrazole, in a dose-dependent manner. Under these experimental conditions, meloxicam has similar potency to tenidap or tenoxicam (Table 8). Diclofenac is 3 times and piroxicam and indomethacin 13 times more potent than meloxicam.

TABLE 5. Influence on PGE_2 content of gastric juice of SHAY rats 6 hr after intraduodenal administration of the test substance

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg) (95% confidence limits)
Meloxicam	4	2.0–16.0	10–20	8.38 (5.59–16.9)
Piroxicam	4	1.0–8.0	9–10	1.77 (0.52–2.94)
Diclofenac	3	1.0–2.0	10–14	1.64 (1.44–2.05)
Naproxen	3	0.625–10.0	9–10	3.56 (2.29–6.23)
Flurbiprofen	3	0.05–0.2	10	0.14 (0.10–0.28)
Acetylsalicylic acid	4	25–200	10–20	79.0 (47.3–207)

PGE_2 content of gastric juice of 173 control rats: 2.03 ± 0.88 ng PGE_2/100 g BW × 6 hr (MV ± SD).

TABLE 6. Influence of PGE_2 excretion in urine of water-loaded rats within 6 hr after oral administration

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	5	0.5–16.0	6–7	1.85 (1.05–2.78)	1.57
Piroxicam	3	0.125–2.0	6–7	0.24 (0.10–0.41)	1.38
Tenoxicam	4	0.125–2.0	6–7	0.61 (0.40–0.94)	1.78
Tenidap	5	0.063–16.0	6–7	0.64 (0.23–1.50)	0.84
Diclofenac	3	1.0–4.0	6–7	1.86 (1.23–2.55)	1.94
Indomethacin	3	1.0–4.0	6–7	0.96 (0.59–1.53)	2.61
Acetylsalicylic acid	3	25–100	6–7	46.7 (31.0–64.4)	3.86
Dipyrone	3	100–200	6	149 (127–182)	9.03
Acetaminophen	3	200–800	6–7	437 (334–584)	5.71

PGE_2 excretion of 131 control rats: 4.058 ± 1.653 ng PGE_2/100 g BW × 6 hr (MV ± SD); R.C. regression coefficient.

INFLUENCE ON INCREASE OF PGE_2 CONCENTRATION IN THE BRAIN OF RATS INDUCED BY CONVULSANT DOSES OF PENTETRAZOLE. In normal rats under physiological conditions, we found only very small amounts of PGE_2 in the brain tissue. Following administration of the convulsant dose of pentetrazole, a considerable rise of PGE_2 concentration in rat brain occurred.

As seen in mice, all NSAIDs tested suppressed the convulsion-induced rise in PGE_2 concentration in brain tissue in a dose-dependent manner.

Under these experimental conditions, the potency of meloxicam in the rat is similar to that of tenidap (Table 9). Tenoxicam is 3 times, piroxicam 10 times, and diclofenac more than 20 times more potent than meloxicam.

Dexamethasone, even at the high dose of 4.0 mg/kg, has no effect on pentetrazole-induced PGE_2 in rat brain.

EFFECT ON THROMBOXANE B_2 CONTENT OF RAT SERUM. Under the *ex vivo* conditions chosen, a dose-dependent decrease in thromboxane B_2 concentration can be demonstrated after coagulation of the blood for meloxicam and all other NSAIDs tested.

The potency of meloxicam is similar to that of indomethacin and tenidap (Table 10).

Meloxicam has a weaker effect on TXB_2 in the rat serum than piroxicam or tenoxicam. The doses required to obtain the same effect are approximately 5 times higher for meloxicam than for piroxicam.

Under the same experimental conditions, acetylsalicylic acid (an irreversible inhibitor of platelet COX) still shows approximately 1/14 of the potency of meloxicam. An accurate comparison of the potency of the basis of ID_{50} is not possible because of the clearly steeper dose-response curve for acetylsalicylic acid.

DISCUSSION

The new NSAID meloxicam differs in its pharmacodynamic profile and its tolerability profile when compared to NSAIDs currently in therapeutic use [1]. The recent discovery of the existence of two isoforms of cyclooxygenase known as COX-1 and COX-2 has resulted in further elucidation of the mechanisms of action of NSAIDs. An inducible cyclooxygenase, COX-2 [4, 18–22] produces mediators of inflammation [23], and a constitutive cyclooxygenase (COX-1) has a cytoprotective effect on the gastric mucosa [8, 9, 24]. 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.

Previous *in vitro* studies* have shown that meloxicam pref-

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TABLE 7. Influence on 6-keto-$PGF_{1\alpha}$ excretion in urine of water-loaded rats within 6 hr after oral administration

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	5	1.0–16.0	7	2.59 (1.75–3.59)	2.41
Piroxicam	3	0.125–2.0	7	0.77 (0.51–1.35)	2.57
Tenoxicam	4	0.125–2.0	6–7	1.24 (0.90–1.97)	2.04
Tenidap	3	1.0–16.0	6–7	7.31 (3.95–21.8)	1.73
Diclofenac	3	0.125–2.0	6–7	0.69 (0.43–1.12)	2.24
Indomethacin	4	0.5–4.0	6–7	2.13 (1.55–3.40)	2.37
Acetylsalicylic acid	4	6.25–50	6–7	32.2 (25.2–46.1)	2.95
Dipyrone	4	12.5–200	6–7	104 (42.6–560)	1.08
Acetaminophen	4	100–800	6–7	327 (215–537)	2.30

6-keto-$PGF_{1\alpha}$ excretion of 131 control rats: 6.589 ± 1.600 ng/100 g BW × 6 hr (MV ± SD); R.C., regression coefficient.

TABLE 8. Influence on pentetrazole-induced increase of PGE_2 concentration in brain of mice after oral administration

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	3	0.5–2.0	7–8	1.32 (1.18–1.50)	93.2
Piroxicam	3	0.05–0.2	6–7	0.10 (0.084–0.12)	74.7
Tenoxicam	3	0.5–2.0	7–8	1.02 (0.90–1.15)	117.3
Tenidap	3	0.5–2.0	7–10	1.41 (1.18–1.81)	89.6
Diclofenac	3	0.25–1.0	6	0.35 (0.25–0.43)	85.2
Indomethacin	3	0.05–0.2	10	0.10 (0.093–0.11)	153
Acetylsalicylic acid	4	25–200	8–10	68.7 (63.0–74.8)	98.5
Dipyrone	3	12.5–50	8–9	31.3 (28.4–35.0)	119
Acetaminophen	3	50–200	7–8	108 (97.0–122)	130

PGE_2 concentration in brain of 43 normal mice: 0.86 ± 0.13 ng/g WW (MV ± SD); following pentetrazole in 73 mice: 40.77 ± 7.23 ng/g WW (MV ± SD); R.C., regression coefficient.

erentially inhibits COX-2 over COX-1. The studies reported here have compared the effects of meloxicam and other NSAIDs on COX *in vivo*.

Two models of the inflammatory process were used: pleurisy of the rat and peritonitis in mice. COX-2 is responsible for the prostaglandin found in rat pleural exudate in the carrageenan-induced inflammation model used here [25]. Evidence for this is provided by our observation that PGE_2 biosynthesis is inhibited by very low doses of dexamethasone in the carrageenan rat pleurisy model. In the rat pleural exudate, meloxicam, piroxicam, and tenoxicam were more potent inhibitors of PGE_2 formation than flurbiprofen, diclofenac, or naproxen. In line with *in vitro* findings, tenidap was a weak inhibitor of PGE_2 formation, requiring 20 times the concentration necessary for inhibition by meloxicam. Meloxicam also showed a marked inhibitory effect on COX-2 in the mouse peritonitis model, showing the greatest potency, by far, of all the tested NSAIDs.

The effect of the NSAIDs on other inflammatory mediators was also investigated *in vivo* because, in addition to the consequences of inhibiting COX directly, COX inhibition by NSAID enhances the formation of other inflammatory products of lipooxygenase [26]. In this study, the effect of meloxicam and other NSAIDs on LTC_4 and LTB_4 were investigated. Significantly, at concentrations that reduced PGE_2 formation in both rat pleural exudate and mouse peritoneal exudate, meloxicam did not affect LTB_4 concentration or LTC_4 concentration. However, both diclofenac and indomethacin caused an increase in LTB_4 concentration under the same conditions. Tenidap caused an increase in LTC_4 and LTB_4. In previous studies, tenidap did not increase lipooxygenase activity, but inhibited the production of LTB_4 by polymorphic neutrophils derived from rheumatoid arthritis patients, *ex vivo* [27]. The differentiation displayed by meloxicam in terms of not enhancing LTB_4 and LTC_4 concentrations while inhibiting PGE_2 formation has favorable implications, so that the risk of inducing bronchospasm during treatment with meloxicam may be lower than with indomethacin, diclofenac, or tenidap.

Finally, we studied the effect of meloxicam and other NSAIDs on prostaglandin formation in various noninflamed tissue. Ulcerogenicity in the stomach is a dose-limiting adverse effect for all NSAIDs currently in therapeutic use. Except for the direct effect on the gastric mucosa of acetylsalicylic acid, the ulcerogenicity of the NSAIDs in the stomach is essentially a systemic effect that also correlates with plasma levels in man [28, 29]. Although other mechanisms are being investigated, the inhibition of PGI_2 and PGE_2 biosynthesis that protects the gastric mucosa [30] does play an essential role in the pathogenesis of NSAID-induced gastric ulcers. Stable prostaglandin derivatives provide good protection against NSAID-induced

TABLE 9. Influence of administered drugs on increase in PGE_2 concentration in the brain of rats induced by a convulsant dose of pentetrazole

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	3	1.0–4.0	6–9	1.87 (1.73–2.02)	130
Piroxicam	3	0.1–0.4	9–10	0.173 (0.157–0.188)	132
Tenoxicam	3	0.25–1.0	8–9	0.561 (0.513–0.617)	123
Tenidap	3	1.0–4.0	9–10	2.00 (1.88–2.13)	148
Diclofenac	3	0.05–0.20	8–10	0.078 (0.070–0.085)	125
Indomethacin	3	0.1–1.0	9–10	0.361 (0.307–0.425)	73
Acetylsalicylic acid	3	25–100	9–10	69.7 (58.1–89.7)	95
Dipyrone	3	12.5–50	9–10	39.7 (28.7–91.4)	56
Acetaminophen	3	50–200	8–9	94.3 (79.9–112)	88
Dexamethasone	1	4.0	8	>4.0	

PGE_2 concentration in brain of 27 normal rats: 0.26 ± 0.07 ng PGE_2/g WW (MV ± SD); PGE_2 concentration following pentetrazole in 37 rats: 15.71 ± 3.21 ng PGE_2/g WW; (MV ± SD); R.C., regression coefficient.

TABLE 10. Effects on serum TXB_2 concentration of rats 3 hr after oral administration of the test substance

Substance	No. of doses	Dose range (mg/kg)	No. of animals/dose	ID_{50} (mg/kg/day) (95% confidence limits)	R.C.
Meloxicam	5	0.25–4.0	6–8	0.47 (0.13–0.86)	42.2
Piroxicam	4	0.0625–0.5	5–8	0.088 (0.010–0.152)	48.6
Tenoxicam	4	0.0625–1.0	8	0.19 (0.12–0.29)	60.9
Tenidap	3	0.125–2.0	8	0.37 (0.13–0.77)	55.9
Indomethacin	3	0.5–2.0	7–12	0.70 (0.19–1.10)	56.5
Acetylsalicylic acid	3	5–20	7–8	7.64 (4.60–10.2)	87.5
Dipyrone	4	25–200	6–10	61.2 (43.0–85.1)	78.4

TXB_2 serum concentration of 42 control rats: 150.3 ± 58.9 ng TXB_2/mL (MV ± SD); R.C., regression coefficient.

gastric ulcers in animal and man [31, 32]. In the rat, meloxicam influenced intragastric PGE_2 formation much less than diclofenac, piroxicam, or flurbiprofen. This result explains the good gastric tolerance [1] of meloxicam in the rat. In a previous study, flurbiprofen showed a more marked inhibition of PG biosynthesis in the gastric mucosa than in inflammatory exudate [33]. Recent studies for CGP 28237 and etodolac [34–36] have shown a relatively weak effect on PG biosynthesis in the gastric mucosa.

The effect of the NSAIDs on intrarenal PG synthesis was investigated in rats. In this model, excretion of intact nonmetabolized PGE_2 and 6-keto-$PGF_{1\alpha}$ were measured. Excretion of intact nonmetabolized PGE_2 is a measure for COX-1-induced production of intrarenal PGE_2 [37]. Urinary 6-keto-$PGF_{1\alpha}$ is a stable metabolite of prostacyclin, a product of COX-1, and its source is partly from the glomerulus and partly from the periphery [38].

Meloxicam has a weaker effect on urinary PGE_2 excretion in the rat than either piroxicam or tenoxicam. Tenidap has a stronger inhibitory effect on intrarenal PGE_2 formation than on 6-keto-$PGF_{1\alpha}$. Because PGE_2 is important in the maintenance of kidney microcirculation, inhibition of PGE_2 formation may adversely affect kidney physiology. Previous studies have observed that anti-inflammatory doses of NSAIDs cause a dose-dependent decrease in urinary PG excretion in rats [39] and have also noted a correlation with the anti-exudative effects in acute models of paw edema [39]. Additionally, some NSAIDs have demonstrated restricted renal excretion of PG at normal therapeutic doses in healthy volunteers [37, 40–43]. Disturbance of water and electrolyte excretion can be a direct consequence of inhibition of urinary PG biosynthesis [44–47].

In the rat and mouse brain, meloxicam showed a much weaker effect on convulsant-induced increase of PGE_2 formation than piroxicam, diclofenac, or indomethacin. In this model, dexamethasone did not inhibit the increase in PGE_2 concentration and, together with the very early increase in PGE_2 observed after anticonvulsant stimulation and previous observations that thromboxane concentrations also increase [17], these findings suggest that constitutive COX-1 activity is responsible for the rapid increase in PG biosynthesis in the brain. Additionally, COX-2 in the rat brain reaches its maximum only 2 hr after an inducing stimulus has been given [48] and, consequently, COX-2 would not be expected to have caused the rapid rise in PG concentration observed here. PG concentrations found in the normal brain are very low.

Interestingly, the difference in the effect of meloxicam and piroxicam on COX-1 mediated PGE_2 formation in the rat brain was much greater than that observed in our *in vitro* studies using cell-free systems from bovine brain.* This may be an indication that meloxicam reached a very low concentration in the rat brain following a single oral dose.

Meloxicam showed only a weak effect on TXB_2 content of rat serum following blood coagulation. COX-1 is the cyclooxygenase responsible for formation of TXB_2 in the platelet aggregation cascade mediating the clotting process. Consequently, the results are in agreement with the weak effects of meloxicam on COX-1 inhibition observed *in vivo*.

Therefore, in this series of studies conducted on intact animals and *in vitro** with meloxicam compared to established NSAIDs in therapeutic use, we have shown differential inhibitory effects on the two isoforms of COX responsible for mediating various pathways of prostaglandin synthesis. Meloxicam is a potent inhibitor of PG biosynthesis under inflammatory conditions (i.e. *via* inhibition of COX-2) but is a weak inhibitor of PG biosynthesis in various tissues under normal physiological conditions (*via* inhibition of COX-1).

Earlier workers have observed that anti-inflammatory potency does not reflect the degree of gastric intolerance associated with a particular agent, thus, suggesting differential inhibitory effects of NSAIDs on PG biosynthesis [30, 33, 34]. The elucidation of two separate isoforms found under different physiological circumstances has provided a possible mechanism for this observation [24]. We have shown that meloxicam, a new NSAID, has differential effects on these two isoforms, COX-1 and COX-2, which are different from those of established NSAIDs. Furthermore, these differential effects seem to reflect clinical observations so that meloxicam's favorable safety profile and high anti-inflammatory effects may be equated to the strong inhibitory effect of meloxicam on COX-2, relative to a weaker inhibitory effect on COX-1.

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