JOURNAL OF APPLIED TOXICOLOGY
J. Appl. Toxicol. 2008; **28**: 829–838
Published online 14 March 2008 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jat.1342

Effect of inflammation on kidney function and pharmacokinetics of COX-2 selective nonsteroidal anti-inflammatory drugs rofecoxib and meloxicam

Sam Harirforoosh† and Fakhreddin Jamali*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

Received 22 June 2007; Revised 2 January 2008; Accepted 7 January 2008

ABSTRACT: Chronic arthritis adversely affects glomerular function and nonsteroidal anti-inflammatory drugs (NSAIDs) reduce electrolyte urinary excretion. In addition, both acute and chronic inflammations may alter clearance of drugs. We studied (a) the effects of inflammation on the renal function and pharmacokinetics of rofecoxib and meloxicam; (b) whether inflammation could exacerbate reduced electrolytes excretion changes observed with NSAIDs; and (c) the influence of inflammation on distribution of these drugs into the kidney. Single oral doses of rofecoxib (10 mg kg^{-1}), meloxicam (3 mg kg^{-1}) or placebo were administered to normal or pre-adjuvant arthritic rats. Blood and urine samples were collected for the measurement of plasma nitrite, BUN and creatinine. The urinary excretion of sodium and potassium was also determined. Nitrite, BUN and plasma creatinine were increased starting on day 9 in the groups with inflammation. Sodium and potassium excretion rates were not affected by inflammation. Meloxicam did not alter the electrolyte excretion in any of the groups. Rofecoxib significantly decreased sodium and potassium excretion in normal rats and potassium excretion in inflamed rats. Inflammation significantly increased plasma concentrations of rofecoxib, but not meloxicam. The ratios of the kidney:plasma concentrations were not significantly altered by inflammation following either drug. Inflammation altered kidney function, demonstrated by increases in BUN and plasma creatinine. However, it did not influence the urinary electrolytes excretion. Since we have observed similar patterns of the effect of NSAIDs on kidney under healthy and inflammatory conditions, one may conclude that inflammation does not exacerbate the adverse effect. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: inflammation; kidney; rat; pharmacokinetics; rofecoxib; meloxicam

Introduction

Rheumatoid arthritis (RA) is a prevalent autoimmune disease which targets joints and results in pain, inflammation and disability (Kvien, 2004; Ruderman, 2005). Decreased glomerular filtration rate (GFR), elevated urinary tubular enzyme levels and proteinuria are reported in patients with RA (Boers *et al*., 1990). Since RA patients are commonly on nonsteroidal anti-inflammatory drugs (NSAIDs) or disease-modifying agents, it is difficult to specifically identify the contribution of the disease or the drugs in the manifestation of these side effects. We have previously reported that NSAIDs, including celecoxib and rofecoxib, but not meloxicam, reduce sodium and potassium urinary excretion in healthy rats (Harirforoosh and Jamali, 2005). The reduced electrolytes excretion appears to be well correlated with the extent of the drug distribution in the kidney (Harirforoosh *et al*., 2006). It is, however, not known whether inflammation increases the extent of drug distribution in the kidney, thereby increasing the severity of the side effect. It has been reported that over-expression of the pro-inflammatory mediators increases the permeability of the blood–brain barrier (Didier *et al*., 2003).

Inflammation reduces clearance, hence it increases the circulating concentration of drugs that are efficiently cleared by hepatic anzymes (Piafsky *et al*., 1978; Monshouwer *et al*., 1996; Mayo *et al*., 2000; Sattari *et al*., 2003; Ling and Jamali, 2005). A reduced clearance is also expected to proportionally increase the drug concentration in the kidney and give rise to increased renal side effects.

The present work was carried out to examine: (1) whether in the absence of NSAIDs, inflammation causes reduced renal function; (2) if inflammation exacerbates the acute renal effect of rofecoxib and renders meloxican effective in diminishing the electrolytes urinary excretion; (3) whether inflammation influences the extent of distribution of selective cyclooxygenase-2 (COX-2) inhibitors into the kidney; and (4) the effect of inflammation on the pharmacokinetics of these drugs.

* Correspondence to: F. Jamali, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.
E-mail: fjamali@pharmacy.ualberta.ca
† Present address: Department of Pharmaceutical Sciences, College of Pharmacy, East Tennessee State University, Johnson City, TN 37614, USA.
Contract/grant sponsor: Canadian Arthritis Network (Canadian Network of Centres of Excellence).

We used a newly developed model of inflammation, pre-adjuvant arthritis (Pre-AA), as previously described (Ling and Jamali, 2005). The systemic signs of inflammation such as increased proinflammatory mediators concentrations and depressed drug metabolism emerge 6–8 days post-adjuvant injection when the animal is still agile and appears healthy. This allows a period of approximately one week (Pre-AA) to study the effect of inflammation on the desired factors in the absence of the pain and stress associated with the fully developed adjuvant arthritis (AA), which emerges approximately 14 days after injection of *Mycobacterium butyricum*.

Since the effect of NSAIDs on the electrolytes excretion appears following the first dose (Harirforoosh and Jamali, 2005), we carried out the study after oral administration of single doses.

Methods

Chemicals

Tablets of rofecoxib (Vioxx; Merck Frosst, QC, Canada) and meloxicam (Mobicox; Boehringer Ingelheim, ON, Canada) were obtained from a local pharmacy in Edmonton. Meloxicam powder was purchased from Unichem Laboratories Limited (Mumbai, India). All solvents were HPLC-grade and purchased from Caledon Laboratories Ltd (Georgetown, ON, Canada). Methyl cellulose 4000 and *ortho*-phosphoric acid were purchased from BDH pharmaceuticals (Toronto, ON, Canada). *Asperigillus* nitrate reductase, sodium nitrate, sodium nitrite, nicotinamide adenine dinucleotide phosphate, lactate dehydrogenase, flavin adenine dinucleotide, pyruvic acid, *N*-(1-naphthyl)ethylenediamine and sulfanilamide were purchased from Sigma Chemical Co. (St Louis, MO, USA). Hepes was purchased from FisherBiotech (Fair Lawn, NJ, USA). *Mycobacterium butyricum* was purchased from Difco Laboratories (Detroit, MI, USA). The immunoassay kit for measurement of TNF-α was purchased from Bio-Source International (Camarillo, CA, USA).

Dosage Selection and Administration

Tablets of Vioxx or Mobicox were crushed to make a fine powder, suspended in 0.5% methyl cellulose suspension and administered via gastric intubations. Doses were checked for their drug content using HPLC.

For rofecoxib, a single dose of 10 mg kg^{-1} was chosen based on its effect on the electrolytes excretion (Harirforoosh *et al*., 2006). Following repeated doses of a series of NSAIDs, we observed that the urinary effect appears following the first dose (Harirforoosh and Jamali, 2005). In addition, the recommended dose of rofecoxib for chronic treatment of rheumatoid arthritis is 25 mg once daily (Schwartz *et al*., 2002).The area under the plasma concentration–time curve $(AUC)_{0-24}$ following a single 25 mg dose of rofecoxib is reported to be 2941 ng h ml^{-1} for healthy young male volunteers (Depre *et al*., 2000). An oral dose of 10 mg kg^{-1} of rofecoxib to the rat yields an AUC_{0-24} of 2963 ng h ml^{-1} (Halpin *et al*., 2000). For meloxicam, a therapeutically equivalent dose to 10 mg kg^{-1} rofecoxib is 3 mg kg^{-1} (Canadian Pharmacists Association, 2003).

Experimental Design and Sample Collection

All experiments were performed on male Sprague–Dawley rats (270–300 g) and carried out within the guidelines of the Animal Care Committee of University of Alberta. In order to test the baseline urine volume, sodium and potassium excretion, urine samples were collected 0–8 h on day 0. In order to measure the baseline nitrite, TNF-α, total protein, plasma creatinine and BUN, 0.5 ml blood was collected via tail vein from anesthetized rats following the completion of the urine collection. The treatment protocol is presented in Tables 1 and 2. Owing to the large number of the animals required, the experiment was carried out using groups of four to eight rats consisting of one or two rats from each treatment group.

Meloxicam Study

As depicted in Table 1, after urine and blood collection, rats were randomly divided into three groups: one inflamed (MEL/INF, $n = 7$) and two control (PL/CONT, $n = 6$; and MEL/CONT, $n = 6$) groups. Inflamed animals received 0.2 ml of a 50 mg ml^{-1} of heat-killed *Mycobacterium butyricum* suspended in squalene into the tail base. The same volume of sterile normal saline was injected into the tail base of control rats. The animals were transferred to standard rat cages, then fed with standard commercial rat chow, and housed at ambient temperature and humidity with a 12 h light–dark cycle.

On day 9, blood samples were collected via the tail vein for measurement of inflammation markers. On day 12, a silastic catheter was inserted into the right jugular vein of rats anesthetized by halothane. Rats were then allowed to recover overnight. On day 13, the PL/CONT rats received methyl cellulose and MEL/CONT and MEL/INF animals received 3 mg kg^{-1} meloxicam orally via a gastric gavage. Plasma samples were collected at 0, 0.25, 1, 2, 4, 6, 8 and 24 h post-dosing. During the dosing day, urine was also collected up to 8 h. We have previously observed that the electrolytes excretion over 8 h urine collection reliably reflects that of over 24 h (Harirforoosh *et al*., 2006). Twenty-four hours post-meloxicam dosing, rats were anesthetized and blood sample were collected for determination of proinflammatory mediators by cardiac puncture. Animals were subsequently

DOI: 10.1002/jat

Table 1. Study protocol A: effect of inflammation and meloxicam

Day 0	1	9	13 (Post NSAID)
Baseline test of biomarkers and electrolytes	Induction of inflammation and group designation	Test of biomarkers	Dosing; test of biomarkers and electrolytes and pharmacokinetics
	Inflamed (MEL/INF) group received adjuvant:		MEL/INF received meloxicam
All animals		All groups	
	Control (CONT) groups received saline: PL/CONT, MEL/CONT		PL/CONT received placebo MEL/CONT received meloxicam

Table 2. Study protocol B: effect of inflammation and meloxicam

Day 0	1	9	13	14 (Post NSAID)
Baseline test of biomarkers and electrolytes	Induction of inflammation and group designation	Test of biomarkers	Test of biomarkers and electrolytes	Dosing with rofecoxib; test of biomarkers and electrolytes and pharmacokinetics
	Inflamed (ROF/INF) group received adjuvant:			
All animals		All groups	ROF/INF	All groups
	Control (ROF/CONT) group received saline:			

sacrificed and kidneys were excised stored at −70 °C until analyzed for drug concentration measurement.

Rofecoxib Study

After the baseline urine and blood collection, rats were randomly divided into two groups: inflamed (ROF/INF, $n = 6$) and control (ROF/CONT, $n = 7$; Table 2). Control and inflamed rats received 0.2 ml of either sterile normal saline or a solution of 50 mg ml^{-1} of heat-killed *Mycobacterium butyricum* suspended in squalene into the tail base, respectively. The animals were then handled as described under 'Meloxicam Study'.

On day 9, blood samples were collected via the tail vein for measurement of inflammation markers. On day 12, a silastic catheter was inserted into the right jugular vein of rats anesthetized by halothane. Rats were then allowed to recover overnight. On day 13, to assess the effect of inflammation on renal function in absence of any NSAID effect, blood and urine samples were collected from ROF/INF rats. On day 14, ROF/CONT and ROF/INF animals received 10 mg kg^{-1} rofecoxib via gastric gavage and blood samples were collected at 0, 0.25, 1, 2, 4, 6, 8 and 24 h. During the dosing day, urine was also collected up to 8 h. Rats were anesthetized and blood sample were collected 24 h post-rofecoxib dosing for determination of proinflammatory mediators by cardiac puncture. Animals were subsequently sacrificed and kidneys were excised stored at −70 °C until analyzed for drug concentration measurement.

Total Protein, BUN and Plasma Creatinine Analysis

Total protein, BUN, and plasma creatinine concentrations were determined with a VetTest 8008 analyzer (Idexx Laboratories Inc, Westbrook, MA, USA) as previously described (Sterczer *et al*., 1999; Ngure *et al*., 2000). The analyzer is a dry chemistry system that uses dry reagent slides. Briefly, the blood sample is placed on the slide containing several layers. After passing through the spreading and filtrating layers, the blood sample reacts with the reagent layer. Then, the reacted sample is collected on the indicator layer for analysis. Subsequently, an optical device is used to measure the intensity of the reaction.

Nitrite Assay

Nitrite was measured in plasma of all rats using a method reported by Grisham *et al*. (1996). Briefly, 100 µl of plasma was incubated with *Asperigillus* nitrate reductase and treated with flavine adenine dinucleotide and nicotinamide adenine dinucleotide phosphate dehydrogenase to reduce all nitrate to nitrite. Then, lactate dehydrogenase and pyruvic acid were added. Finally, the samples were treated with the Griess reagent, and the absorbance was measured at 540 nm using a Powerwave × 340 plate reader (Bio-Tek Instruments, Fisher Scientific). Standard curves, constructed using standard solutions of sodium

DOI: 10.1002/jat

nitrate and sodium nitrite, were linear over the range of 6.25–200 μM (CV < 20%).

Tumor Necrosis Factor-α Analysis

Tumor necrosis factor-α (TNF-α) was determined using an immunoassay kit (rat TNF-α enzyme-linked immunosorbent assay), according to the manufacturer's instructions. Standard curves were used to determine the amount of TNF-α in plasma. The minimum detectable concentration was less than 4 pg ml^{-1} (CV < 5.2%).

Rofecoxib Assay

Rofecoxib concentrations were determined in plasma and kidney using a validated reverse-phase HPLC method (Jamali and Sattari, 2000) with some modifications. Briefly, a 100 μl aliquot of various concentrations (50–10 000 ng ml^{-1}) of rofecoxib standard solution was added to 100 μl of blank rat plasma. After addition of 100 μl of ketoprofen solution (1 μg ml^{-1}) as internal standard and 100 μl of acetate buffer (pH = 4.5), plasma samples were extracted with 6 ml of ethyl acetate. After vortex mixing and centrifuging the result, the organic layer was separated and evaporated to dryness. The residue was reconstituted into 175 μl of mobile phase, water–acetonitrile–acetic acid–triethylamine (72:28:0.1:0.05), and a 150 μL aliquot injected into the column.

In order to determine rofecoxib concentrations in the rat kidney, 2 volumes of HPLC-grade water were added to the weighed tissue samples and the mixture was homogenized using a Brinkmann Polytron homogenizer (model PT10/35, Instruments Co., Switzerland). Standard curve samples were prepared by adding 100 μl of various concentrations of rofecoxib standard solutions to 100 μl of blank homogenized kidney tissue mixture to make the final concentrations of 25–5000 ng ml^{-1}. Aliquots of 100 μl of 1 μg ml^{-1} internal standard solution, 100 μl of acetate buffer (pH = 4.5) and 6 ml ethyl acetate were added to each tube. The tubes were vortex-mixed for 90 s and centrifuged at 2500 *g* for 3 min. The organic layer was transferred to a tube and evaporated to dryness. The residue was dissolved in 175 μl of mobile phase and an aliquot of 150 μl was injected into HPLC.

The minimum quantifiable concentrations were 50 ng ml^{-1} in plasma (CV < 7.2%) and 50 ng g^{-1} in kidney homogenate (CV < 20.8%).

Meloxicam Assay

A validated reverse-HPLC method (Velpandian *et al*., 2000) with some modifications was used to determine the concentration of meloxicam in plasma and kidney. Stock solutions were prepared by dissolving meloxicam (100 μg ml^{-1}) and piroxicam as an internal standard (100 μg ml^{-1}) in methanol. A 100 μl aliquot of blank rat plasma was spiked with various concentrations of meloxicam stock solutions to make the final concentrations of 50–100 000 ng ml^{-1}. Then, 200 μl of 2 μg ml^{-1} piroxicam solution, 200 μl of 0.6 M H_2SO_4 and 2 ml of chloroform were added to each tube. The tubes were vortex-mixed (2 min) and centrifuged for 2 min. A 1 ml aliquot of the lower (organic) layer was removed and evaporated to dryness. The residue was dissolved in 100 μl of mobile phase and a 50 μl aliquot injected into the HPLC.

For determination of meloxicam in the kidney, 2 volumes of HPLC-grade water were added to the weighed kidney samples. The mixture was homogenized for 45 s. Standards curve samples were prepared by adding 100 μl of various concentrations of meloxicam stock solutions to 100 μl of homogenized mixture to make the final concentrations of 50–100 000 ng l^{-1}. A 100 μl aliquot of 5 μg ml^{-1} piroxicam solution, 200 μl of 0.6 M H_2SO_4, and 2 ml of chloroform were added to each tube. After vortex mixing and centrifuging of resultant, 1 ml of the lower (organic) layer was removed and evaporated to dryness. The residue was dissolved in 100 μl of mobile phase and a 50 μl aliquot injected into the HPLC.

The minimum quantifiable concentrations were 500 ng ml^{-1} in plasma (CV < 5.9%) and 500 ng g^{-1} in kidney homogenate (CV < 14.5%).

Electrolytes Analysis

Urine samples were analyzed for sodium and potassium using instrumental neutron activation analysis as previously described in details (Harirforoosh *et al*., 2006).

Data Treatment and Statistical Analysis

Urinary electrolytes excretion (μmol min^{-1}) was calculated from: $C_{X*}\ V_X/T$, where C_X is the concentration of urine electrolyte (μmol ml^{-1}), V_X is the urine volume (ml) and T is the urine collection time (min). Urine flow rate (ml h^{-1}) was estimated from V_X/T, where V_X is the urine volume (ml) and T is the urine collection time (h). The non-compartment model contained in WinNonlin, version 4.1 (Pharsight Corporation, CA, USA) was used to estimate pharmacokinetic parameters. Pharmacokinetic parameters, kidney/plasma concentration ratios and body weights estimated for Pre-AA groups were compared with those of respective controls using the two-tailed Student *t*-test. For the biomarkers and renal excretion data comparisons were made between the baseline and post-treatment values with the two-way ANOVA using the PROC MIXED procedure of SAS (SAS Institute Inc.,

DOI: 10.1002/jat

Table 3. The effect of inflammation on the body weight of normal (CONT) rats treated with vehicle or inflamed (INF) rats treated with *Mycobacterium butyricum*. Rats were treated with single doses of placebo (PL), meloxicam (MEL) or rofecoxib (ROF) on the final day (n = 6–7/group)

	Body weight, g				
Day	PL/CONT	MEL/CONT	MEL/INF	ROF/CONT	ROF/INF
Baseline	268 ± 2.58	273 ± 9.83	262 ± 3.36	265 ± 5.00	268 ± 2.58
Day 9	337 ± 8.16	331 ± 8.61	284 ± 14.6*	339 ± 6.27	298 ± 21.8*
Post NSAID	362 ± 18.9	346 ± 13.6	302 ± 22.0*	354 ± 13.0	323 ± 19.9*

Values are expressed as mean ± standard deviation; * significantly different from control.

Cary, NC, USA). Statistical significance was set at $P < 0.05$. All data are presented as mean ± standard deviation.

Results

Emergence of Inflammation

Figure 1 depicts the serum concentration of the measured pro-inflammatory mediators over the experiment period. No significant change from baseline was observed in either serum nitrate or TNF-α concentrations in control groups. The adjuvant injection, however, elevated the nitrite serum concentration on day 9 and days 13 or 14 in all Pre-AA groups regardless of the treatment involved. Serum TNF-α concentrations were also elevated by inflammation. The effect, however, reached a significant level only on day 9 in MEL/INF and on days 13 or 14 in MEL/INF and ROF/INF rats.

The mean body weight gain was significantly less in the group with inflammation than controls on day 9 and days 13 or 14 (Table 3).

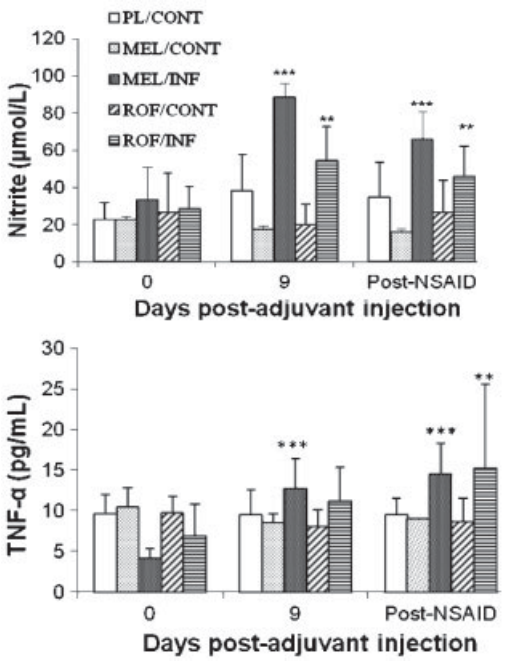

Figure 1. Serum inflammatory mediators concentrations 0 and 9 days after injection of vehicle (PL/CONT, MEL/CONT and ROF/CONT) or killed *Mycobacterium butyricum* (MEL/INF and ROF/INF) and on day 13 or 14 following a single oral dose of placebo (PL/CONT), meloxicam (MEL/CONT and MEL/INF) or rofecoxib (ROF/CONT and ROF/INF) (n = 6–7/group). Values are expressed as mean ± standard deviation; significantly different from baseline, ** $p < 0.01$, *** $p < 0.001$

Effect of Inflammation on Kidney Function

The effect of inflammation on the renal parameters is depicted in Tables 4 and 5. Compared with the baseline values, mean BUN was significantly increased on day 13 in MEL/INF group and on days 9 and 13 in ROF/INF group. Plasma creatinine concentration was significantly elevated in all groups with inflammation on day 9 and days 13 or 14 post-adjuvant injection. Inflammation had no significant effect on the total plasma protein concentration, the urinary flow rate and the electrolyte excretion.

Effect of NSAIDs on Urinary Electrolytes Excretion and Urine Flow Rate

Single doses of meloxicam had no significant effect on the electrolytes excretion (Table 4). Rofecoxib, on the other hand, significantly decreased the sodium excretion rate in both the normal group and in individuals with inflammation (Table 5). The urinary excretion of potassium was also reduced by rofecoxib but reached significance only in the normal group (Table 5). Neither NSAID influenced urinary flow rate.

Effect of Inflammation on Pharmacokinetics on NSAIDs

Inflammation resulted in a significant increase in rofecoxib plasma concentration (Fig. 2), as reflected by a

DOI: 10.1002/jat

Table 4. The effect of inflammation and a single dose of meloxicam on the renal parameters of normal rats (PL/CONT and MEL/CONT) treated with vehicle or inflamed rats (MEL/INF) treated with *Mycobacterium butyricum*. Rats were treated with single doses of placebo (PL/CONT) or meloxicam (MEL/INF and MEL/CONT) on day 13 (n = 6–7/group)

		Group		
Parameter	Time	PL/CONT	MEL/CONT	MEL/INF
Plasma				
BUN, mmol l^{-1}	Baseline	3.84 ± 0.93	4.24 ± 0.91	4.22 ± 1.06
	Day 9	4.57 ± 1.05	4.79 ± 0.66	4.61 ± 0.88
	Post NSAID	5.58 ± 0.69	5.33 ± 1.78	10.6 ± 9.3***
Creatinine, μmol l^{-1}	Baseline	15.2 ± 3.43	26.5 ± 2.07	20.6 ± 2.88
	Day 9	18.8 ± 3.42	29.2 ± 2.0	24.3 ± 2.43*
	Post NSAID	21.7 ± 3.67	28.0 ± 5.4	39.4 ± 34.2*
Total protein, g l^{-1}	Baseline	51.8 ± 1.64	52.5 ± 1.64	52.3 ± 1.11
	Day 9	55.0 ± 1.67	54.7 ± 1.75	52.9 ± 1.95
	Post NSAID	51.8 ± 4.75	53.8 ± 2.93	55.4 ± 0.89
Urine				
Flow rate, ml h^{-1}	Baseline	0.75 ± 0.11	0.54 ± 0.17	0.59 ± 0.30
	Post NSAID	0.92 ± 0.4	0.63 ± 0.16	0.65 ± 0.26
Sodium excretion rate, μmol min^{-1}	Baseline	1.83 ± 0.29	1.19 ± 0.53	0.89 ± 0.37
	Post NSAID	1.37 ± 0.46	0.82 ± 0.50	0.76 ± 0.37
Potassium excretion rate, μmol min^{-1}	Baseline	2.81 ± 0.87	2.08 ± 0.55	1.81 ± 0.64
	Post NSAID	2.10 ± 0.72	2.27 ± 0.78	1.41 ± 0.46

Data are mean ± standard deviation; significantly different from baseline, * $P < 0.05$, *** $P < 0.001$.

Table 5. The effect of a single dose of rofecoxib on the renal parameters of normal rats (ROF/CONT) treated with vehicle and inflamed rats (ROF/INF) treated with *Mycobacterium butyricum*. Rats were treated with single doses of rofecoxib on day 14 (n = 6–7/group)

		Group	
Parameter	Time	ROF/CONT	ROF/INF
Plasma			
BUN, mmol l^{-1}	Baseline	4.78 ± 0.81	4.48 ± 0.83
	Day 9	4.42 ± 0.66	6.22 ± 0.63***
	Day 13		8.04 ± 0.78*
	Post NSAID	7.69 ± 0.82	7.33 ± 2.79
Creatinine, μmol l^{-1}	Baseline	26.4 ± 2.88	14.33 ± 3.78
	Day 9	26.9 ± 3.98	28.8 ± 3.43***
	Day 13		32.2 ± 4.96*
	Post NSAID	34.2 ± 5.85	48.2 ± 32.7***
Total protein, g l^{-1}	Baseline	54.3 ± 2.14	53.5 ± 4.18
	Day 9	53.0 ± 2.16	50.8 ± 1.60
	Day 13		56.8 ± 2.32
	Post NSAID	55.2 ± 3.43	52.7 ± 2.58
Urine			
Flow rate, ml h^{-1}	Baseline	0.61 ± 0.13	0.44 ± 0.21
	Day 13		0.71 ± 0.20
	Post NSAID	0.55 ± 0.23	0.31 ± 0.13
Sodium excretion rate, μmol min^{-1}	Baseline	1.62 ± 0.95	1.38 ± 0.41
	Day 13		1.57 ± 0.61
	Post NSAID	0.98 ± 0.56*	0.62 ± 0.41*
Potassium excretion rate, μmol min^{-1}	Baseline	2.55 ± 0.95	1.58 ± 0.61
	Day 13		2.18 ± 0.80
	Post NSAID	1.63 ± 0.82*	1.08 ± 0.53

Data are mean ± standard deviation; significantly different from baseline, * $P < 0.05$, *** $P < 0.001$.

significant elevation of AUC_{0-24} and a corresponding decrease in the oral clearance of the drug (Table 6). The peak concentration time (t_{max}) was also significantly delayed by inflammation ($P < 0.014$; Table 6). There was no significant difference in any other measured pharmacokinetic indices between the control rats and those with inflammation.

Since the plasma concentrations of meloxicam did not become log–linear within the 24 h sample collection period, we were not able to estimate $t_{1/2}$, which is needed

DOI: 10.1002/jat

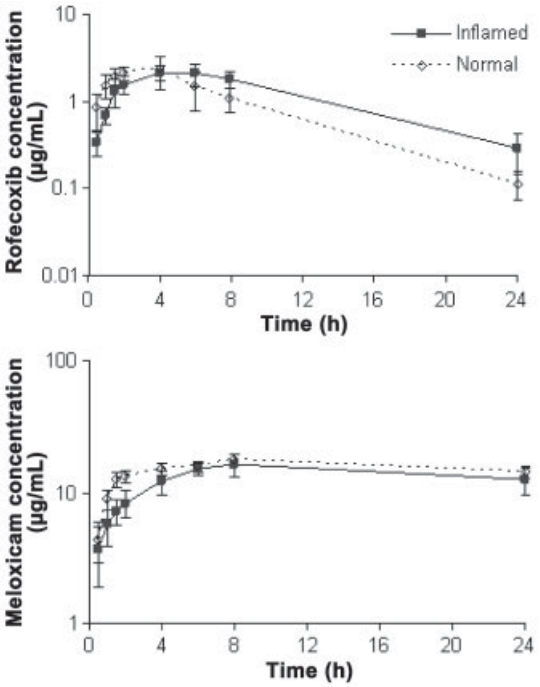

Figure 2. Plasma concentration-time profile of rofecoxib (10 mg kg^{-1}, p.o.) and meloxicam (3 mg kg^{-1}, p.o.) in the absence and presence of inflammation (n = 6–7/group). Values are expressed as mean ± standard deviation

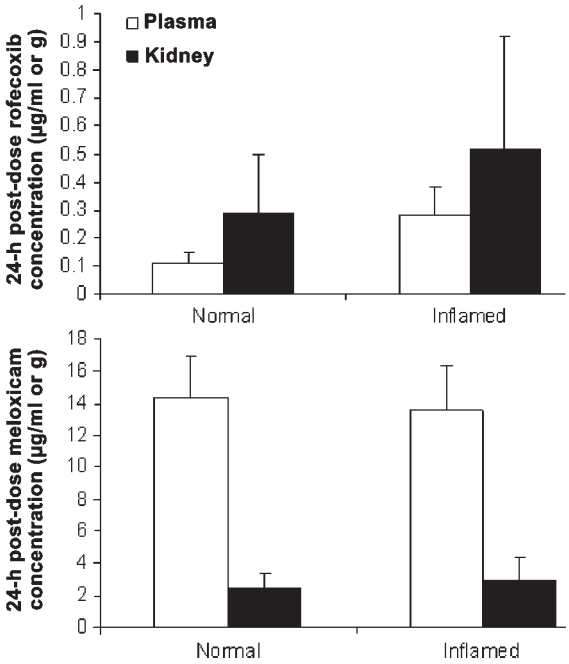

Figure 3. Twenty-four-hour post-dose concentrations of rofecoxib (10 mg kg^{-1}, p.o.) and meloxicam (3 mg kg^{-1}, p.o.) in plasma and kidney of normal and inflamed rats following a oral dose (n = 5–7/group). Values are expressed as mean ± standard deviation

for calculation of V_d and CL/F. None of the measured pharmacokinetic indices (AUC_{0-24}, t_{max} and C_{max}), however was influenced by inflammation (Fig. 2, Table 6).

Effect of Inflammation on the Distribution of Rofecoxib and Meloxicam into the Kidney

Inflammation did not alter kidney–plasma rofecoxib concentration ratio as the rise in the concentration in the kidney was proportional to that in plasma (Fig. 3). The kidney to plasma concentration ratio, however, remained unchanged between the groups ($P > 0.79$).

Inflammation had no effect on the plasma and kidney concentrations of meloxicam measure 24 h post single doses (Fig. 3).

Discussion

It has been shown that the levels of nitric oxide (Mayo *et al*., 2000) and TNF-α (Arend and Dayer, 1990) increase in RA patients. Indeed, Mayo *et al*. (2000) have demonstrated that nitrite concentration is a predictive marker of the disease severity in RA. In this study, the significant increases in serum concentrations of nitrite and TNF-α

Table 6. The pharmacokinetic indices of rofecoxib (10 mg kg^{-1}, p.o.) and meloxicam (3 mg kg^{-1}, p.o.) in the absence and presence of inflammation

	Rofecoxib		Meloxicam	
Parameter	Normal (n = 7)	Inflamed (n = 6)	Normal (n = 6)	Inflamed (n = 7)
AUC_{0-24} (µg h ml^{-1})	21.60 ± 3.4	30.51 ± 4.03*	367 ± 44	326 ± 82
C_{max} (µg ml^{-1})	2.60 ± 0.52	2.41 ± 0.37	19.70 ± 3.75	16.85 ± 3.96
t_{max} (h)	2.7 ± 1.2	5.6 ± 1.7*	6.70 ± 1.6	6.8 ± 1.8
CL/F (ml h kg^{-1})	470 ± 75	331± 42*	ND	ND
V_d/F (l kg^{-1})	3.9 ± 0.77	3.9 ± 0.76	ND	ND
$t^1/_2$ (h)	5.2 ± 0.9	6.5 ± 2.6	ND	ND

Values are expressed as mean ± standard deviation; * significantly different from normal rats.

DOI: 10.1002/jat

observed on day 9 or days 13 or 14 post-adjuvant injection (Fig. 1) confirms the presence of inflammation without full emergence of adjuvant arthritis and its associated pain, as reported by Ling and Jamali (2005) for the Pre-AA rat model of inflammation. Another characteristic of Pre-AA is a less body weight gain during the period of study, as our data indicate (Table 3).

The present data suggest that, indeed, inflammation, at least as it is manifested in the Pre-AA, is associated with reduced kidney function as indicated by increases in both plasma BUN and creatinine concentrations independent of the NSAID use (Table 5). Both plasma creatinine (Perrone *et al*., 1992) and BUN (Schnellmann, 2001) reflect the glomerular filtration rate. Our observation in Pre-AA rat model is consistent with that reported for the full adjuvant arthritis (DiJoseph *et al*., 1993). This lends support to the suggestion (Ling and Jamali, 2005) that these types of experiments can be carried out before the development of the full adjuvant arthritis and its associated pain and stress.

As expected (Harirforoosh *et al*., 2006), single doses of rofecoxib decreased sodium and potassium excretions in healthy rats (Table 5). Expression of COX-2 in the thick ascending limb cells containing Na-K-ATPase proposes a role for the enzyme in regulation of sodium in rat kidney (Vio *et al*., 1997; Ferreri *et al*., 1999; Roig *et al*., 2002). A decrease in GFR has also been linked to the inhibition of COX-1 since it has been observed following administration of indomethacin to healthy elderly subjects (Catella-Lawson *et al*., 1999). NSAIDs may change potassium balance by suppression of the renin–aldosterone axis and/or a reduction in GFR (Breyer and Harris, 2001). The NSAIDs' effect on the electrolytes excretion in both humans (Harirforoosh and Jamali, 2005; Harirforoosh *et al*., 2006) and rats appears shortly after the therapy commencement, even following the first dose (Harirforoosh and Jamali, 2005). For subsequent studies, therefore, we limited the treatment to a single dose (Harirforoosh *et al*., 2006).

Interestingly, inflammation did not exacerbate the effect of rofecoxib on electrolyte excretion (Table 5), despite its diminishing effect on renal function measured as increased plasma creatinine and BUN. We cannot rule out the possibility of a more extensive effect upon prolonged exposure to the NSAID in more severely afflicted forms of inflammation.

The present data confirm our previous observation (Harirforoosh and Jamali, 2005) that meloxicam does not influence electrolyte urinary excretion in healthy rats following a single dose (Table 4). Indeed, rofecoxib, celecoxib, diclofenac and flurbiprofen but not meloxicam significantly reduce the electrolyte excretion (Harirforoosh and Jamali, 2005). We have subsequently reported (Harirforoosh *et al*., 2006) that the effect of rofecoxib and celecoxib on electrolyte excretion is associated with kidney to plasma concentration ratios of greater than the unity. For meloxicam, on the other hand, a relatively small amount of the drug is found in the kidneys 24 h post-dose. One of the objectives of the present study was to investigate whether inflammation alters the extent of the drug distribution into the kidney, thereby, causing a significant effect on the electrolyte excretion. It has previously been shown that expression of pro-inflammatory cytokines such as TNF-α, may change the permeability of the blood–brain barrier (de Vries *et al*., 1996). Inflammation, at least in the form of Pre-AA, does not appear to render meloxicam effective in reducing the electrolytes excretion (Table 4). Our data on rofecoxib and meloxicam also suggest that inflammation does not alter relative distribution of these drugs into the kidneys (Fig. 3). Pharmacokinetics of both rofecoxib and meloxicam are linear following therapeutic doses (Davies and Skjodt, 1999), i.e. proportional elevation of concentration with increases in dose. An altered kidney drug concentration caused by inflammation, therefore, is expected to be detectable following single doses. We measured the kidney drug concentrations 24 h post-dose, allowing the attainment of pharmacokinetic pseudo-equilibrium phase, when the absorption and distribution phases are expected to be completed.

We have also shown that inflammation alters the pharmacokinetics of rofecoxib but not meloxicam (Fig. 2, Table 6). It has been reported that inflammatory conditions alter the clearance of drugs which are efficiently metabolized in the liver (Laethem *et al*., 1994; Piquette-Miller and Jamali, 1995; Guirguis *et al*., 2001). We calculated the hepatic extraction ratio of rofecoxib in the rat using data following intravenous doses by Halpin *et al*. (2000) to be 0.50–0.55. Rofecoxib, therefore, can be considered as an intermediate extraction ratio dug in the rat; hence, its clearance depends on the liver blood flow, plasma unbound drug fraction and intrinsic clearance (Emami *et al*., 1998). It is known that inflammation does not influence the liver blood flow (Walker *et al*., 1986). In addition, inflammation results in elevated α_1-acid glycoprotein (Piafsky *et al*., 1978), but reduced albumin concentration in plasma (Moshage *et al*., 1987). Since rofecoxib is mainly bound to plasma albumin (Warner *et al*., 2006), an elevated α_1-acid glycoprotein concentration is not expected to slow down its clearance. Moreover, Pre-AA and other inflammatory conditions are associated with reduced cytochrome P450 (CYP) enzyme activity (Chen *et al*., 1994) or content (Ling and Jamali, 2005). The inhibitory effect of inflammatory cytokines such as TNF-α, interleukin-1α (IL-1α), interferon-γ and IL-6 on activity of CYP and glucuronosyl transferase enzymes has been previously reported (Monshouwer *et al*., 1996). It is, therefore, reasonable to suggest that the increased rofecoxib plasma concentration in the rats with inflammation (Fig. 2, Table 6) is likely due to decrease in the intrinsic clearance of the drug.

We have also noticed that inflammation causes a significant delay in the attainment of the peak rofecoxib

DOI: 10.1002/jat

concentration (Fig. 2). This may be due to a delayed gastric empting caused by inflammation. Indeed, administration of proinflammatory cytokines results in significant delayed gastric emptying in mice. It has also been shown that induction of inflammation by intraperitoneal injection of acetic acid blocks gastric empting via activation of neurokinin receptors and release of calcitonin gene-related peptides (Julia and Bueno, 1997).

We cannot extrapolate our observation of increased rofecoxib concentration in the inflamed rat to humans since the absolute bioavailability of drug in human is not known (Davies *et al*., 2003). To the best of our knowledge, the only set of human bioavailability data available has been generated following radio-labeled drug and suggests 93% exposure following oral doses (Halpin *et al*., 2002).

For meloxicam, using published data (Aghazadeh-Habashi and Jamali, 2003), we calculated the hepatic extraction ratio; it was less than 0.1. This places meloxicam in the list of low-extraction drugs. Pharmacokinetics of these drugs are not expected to be influenced by inflammation (Guirguis *et al*., 2001). Not surprisingly, inflammation did not affect the systemic exposure of meloxicam (Fig. 2, Table 6). Meloxicam is a low-extraction drug (99% bioavailable) in humans as well (Busch *et al*., 1995). Therefore, it is reasonable to extrapolate our observation in the rat to humans and suggest that inflammation is unlikely to influence human pharmacokinetics of meloxicam.

In this study, the lack of exacerbating effect of inflammation on the ability of rofecoxib to reduce electrolytes urinary excretion was observed following single doses. The effect of repeated NSAID administration remained to be examined. Although the previous reports indicate that the effect is maximal shortly after the commencement of the therapy (Stichtenoth and Frolich, 2000) and even after single doses (Harirforoosh and Jamali, 2005; Harirforoosh *et al*., 2006), a more prolonged treatment period will be required to examine the potential augmentation of the NSAID effect on the GFR.

In summary, inflammation in the form of Pre-AA produces altered renal function measured in plasma BUN and creatinine concentrations without any effect on the urinary sodium and potassium excretion. Inflammation does not exacerbate the diminishing effect of rofecoxib on the electrolytes urinary excretion. Oral clearance of rofecoxib, but not meloxicam is decreased by inflammation. This reflects the difference between the two drugs in their degree of first-pass hepatic metabolism in the rat. The extent of distribution of rofecoxib and meloxicam into the kidney is not influenced by inflammation. However, for rofecoxib, a trend for higher concentration in the kidney of inflamed rats is observed, reflecting a proportionally greater concentration in plasma.

Acknowledgments—We thank Dr John Duke and Mr Russell Ambeault of University of Alberta SLOWPOKE Reactor Facility and Mr Greg Olson from the Surgical–Medical Research Institute of University of Alberta for their technical assistance. This study was supported by a grant from the Canadian Arthritis Network (Canadian Network of Centres of Excellence).

References

Aghazadeh-Habashi A, Jamali F. Pharmacokinetic study of meloxicam in the rat. In *Proceedings of the Canadian Society for Pharmaceutical Sciences 6th Annual Symposium*; pp. 53–54.

Arend WP, Dayer JM. 1990. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum*. **33**: 305–315.

Boers M, Dijkmans BA, Breedveld FC, Camps JA, Chang PC, Van BP, Pauwels EK, Cats A. 1990. Subclinical renal dysfunction in rheumatoid arthritis. *Arthritis Rheum*. **33**: 95–101.

Breyer MD, Harris RC. 2001. Cyclooxygenase 2 and the kidney. *Curr. Opin. Nephrol. Hypertens*. **10**: 89–98.

Busch U, Heinzel G, Narjes H. 1995. The effect of cholestyramine on the pharmacokinetics of meloxicam, a new non-steroidal anti-inflammatory drug (NSAID), in man. *Eur. J. Clin. Pharmac*. **48**: 269–272.

Canadian Pharmacists Association. 2003. *Compendium of Pharmaceutical and Specialties. The Canadian Drug Reference for Health Professionals*. Ottawa.

Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, Fitzgerald GA. 1999. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J. Pharmac. Exp. Ther*. **289**: 735–741.

Chen YL, Le V, Leneveu A, Dreyfus F, Stheneur A, Florentin I, De SM, Giroud JP, Flouvat B, Chauvelot-Moachon L. 1994. Acute-phase response, interleukin-6, and alteration of cyclosporine pharmacokinetics. *Clin. Pharmac. Ther*. **55**: 649–660.

Davies NM, Skjodt NM. 1999. Clinical pharmacokinetics of meloxicam. A cyclo-oxygenase-2 preferential nonsteroidal anti-inflammatory drug. *Clin. Pharmacokinet*. **36**: 115–126.

Davies NM, Teng XW, Skjodt NM. 2003. Pharmacokinetics of rofecoxib: a specific cyclo-oxygenase-2 inhibitor. *Clin. Pharmacokinet*. **42**: 545–556.

Depre M, Ehrich E, Van HA, De L, I, Dallob A, Wong P, Porras A, Gertz BJ, De Schepper PJ. 2000. Pharmacokinetics, COX-2 specificity, and tolerability of supratherapeutic doses of rofecoxib in humans. *Eur. J Clin. Pharmac*. **56**: 167–174.

de Vries HE, Blom-Roosemalen MC, van OM, de Boer AG, van Berkel TJ, Breimer DD, Kuiper J. 1996. The influence of cytokines on the integrity of the blood–brain barrier *in vitro*. *J. Neuroimmunol*. **64**: 37–43.

Didier N, Romero IA, Creminon C, Wijkhuisen A, Grassi J, Mabondzo A. 2003. Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. *J. Neurochem*. **86**: 246–254.

DiJoseph JF, Walter TL, Hartman DA, Carlson RP. 1993. Kidney dysfunction in the arthritic rat. *Int. J. Immunopharmac*. **15**: 933–941.

Emami J, Pasutto FM, Jamali F. 1998. Effect of experimental diabetes mellitus and arthritis on the pharmacokinetics of hydroxychloroquine enantiomers in rats. *Pharm. Res*. **15**: 897–903.

Ferreri NR, An SJ, McGiff JC. 1999. Cyclooxygenase-2 expression and function in the medullary thick ascending limb. *Am. J. Physiol*. **277**: F360–F368.

Grisham MB, Johnson GG, Lancaster JR, Jr. 1996. Quantitation of nitrate and nitrite in extracellular fluids. *Meth. Enzymol*. **268**: 237–246.

Guirguis MS, Sattari S, Jamali F. 2001. Pharmacokinetics of celecoxib in the presence and absence of interferon-induced acute inflammation in the rat: application of a novel HPLC assay. *J. Pharm. Pharm. Sci*. **4**: 1–6.

Halpin RA, Geer LA, Zhang KE, Marks TM, Dean DC, Jones AN, Melillo D, Doss G, Vyas KP. 2000. The absorption, distribution, metabolism and excretion of rofecoxib, a potent and selective cyclooxygenase-2 inhibitor, in rats and dogs. *Drug Metab. Dispos*. **28**: 1244–1254.

Halpin RA, Porras AG, Geer LA, Davis MR, Cui D, Doss GA, Woolf E, Musson D, Matthews C, Mazenko R, Schwartz JI, Lasseter KC, Vyas KP, Baillie TA. 2002. The disposition and metabolism of rofecoxib, a potent and selective cyclooxygenase-2 inhibitor, in human subjects. *Drug Metab. Dispos.* **30**: 684–693.

Harirforoosh S, Jamali F. 2005. Effect of nonsteroidal anti-inflammatory drugs with varying extent of COX-2/COX-1 selectivity on urinary sodium and potassium excretion in the rat. *Can. J. Physiol. Pharmac.* **83**: 85–90.

Harirforoosh S, Aghazadeh-Habashi A, Jamali F. 2006. Extent of renal effect of cyclo-oxygenase-2-selective inhibitors is pharmacokinetic dependent. *Clin. Exp. Pharmac. Physiol.* **33**: 917–924.

Jamali F, Sattari S. 2000. High performance liquid chromatographic determination of cyclooxygenase II inhibitor rofecoxib in rat and human plasma. *J. Pharm. Pharm. Sci.* **3**: 312–317.

Julia V, Bueno L. 1997. Tachykininergic mediation of viscerosensitive responses to acute inflammation in rats: role of CGRP. *Am. J. Physiol* **272**: G141–G146.

Kvien TK. 2004. Epidemiology and burden of illness of rheumatoid arthritis. *Pharmacoeconomics* **22**: 1–12.

Laethem ME, Belpaire FM, Wijnant P, Rosseel MT, Bogaert MG. 1994. Influence of endotoxin on the stereoselective pharmacokinetics of oxprenolol, propranolol, and verapamil in the rat. *Chirality* **6**: 405–410.

Ling S, Jamali F. 2005. Effect of early phase adjuvant arthritis on hepatic CYP enzymes and pharmacokinetics of verapamil: an alternative approach to the use of an animal model of inflammation for pharmacokinetic studies. *Drug Metab. Dispos.* **33**: 579–586.

Mayo PR, Skeith K, Russell AS, Jamali F. 2000. Decreased dromotropic response to verapamil despite pronounced increased drug concentration in rheumatoid arthritis. *Br. J. Clin. Pharmac.* **50**: 605–613.

Monshouwer M, Witkamp RF, Nujmeijer SM, Van Amsterdam JG, van Miert AS. 1996. Suppression of cytochrome. *Toxicol. Appl. Pharmac.* **137**: 237–244.

Moshage HJ, Janssen JA, Franssen JH, Hafkenscheid JC, Yap SH. 1987. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J. Clin. Invest.* **79**: 1635–1641.

Ngure RM, Gateri LM, Ngotho JM, Ndung'u JM. 2000. Application of the Vettest 8008 system for the biochemical analysis of vervet monkey plasma. *Vet. Rec.* **146**: 612–613.

Perrone RD, Madias NE, Levey AS. 1992. Serum creatinine as an index of renal function: new insights into old concepts. *Clin. Chem.* **38**: 1933–1953.

Piafsky KM, Borga O, Odar-Cederlof I, Johansson C, Sjoqvist F. 1978. Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma alpha1 acid glycoprotein. *New Engl. J. Med.* **299**: 1435–1439.

Piquette-Miller M, Jamali F. 1995. Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab. Dispos.* **23**: 240–245.

Roig F, Llinas MT, Lopez R, Salazar FJ. 2002. Role of cyclooxygenase-2 in the prolonged regulation of renal function. *Hypertension* **40**: 721–728.

Ruderman EM. 2005. Current and future pharmaceutical therapy for rheumatoid arthritis. *Curr. Pharm. Des.* **11**: 671–684.

Sattari S, Dryden WF, Eliot LA, Jamali F. 2003. Despite increased plasma concentration, inflammation reduces potency of calcium channel antagonists due to lower binding to the rat heart. *Br. J. Pharmac.* **139**: 945–954.

Schnellmann RG. 2001. Toxic responses of the kidney. In *Casarett & Doull's Toxicology*, Klaassen CD (ed.). McGraw-Hill: New York; 502.

Schwartz JI, Vandormael K, Malice MP, Kalyani RN, Lasseter KC, Holmes GB, Gertz BJ, Gottesdiener KM, Laurenzi M, Redfern KJ, Brune K. 2002. Comparison of rofecoxib, celecoxib, and naproxen on renal function in elderly subjects receiving a normal-salt diet. *Clin. Pharmac. Ther.* **72**: 50–61.

Sterczer A, Meyer HP, Boswijk HC, Rothuizen J. 1999. Evaluation of ammonia measurements in dogs with two analysers for use in veterinary practice. *Vet. Rec.* **144**: 523–526.

Stichtenoth DO, Frolich JC. 2000. COX-2 and the kidneys. *Curr. Pharm. Des.* **6**: 1737–1753.

Velpandian T, Jaiswal J, Bhardwaj RK, Gupta SK. 2000. Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples. *J. Chromatogr. B Biomed. Sci. Applic.* **738**: 431–436.

Vio CP, Cespedes C, Gallardo P, Masferrer JL. 1997. Renal identification of cyclooxygenase-2 in a subset of thick ascending limb cells. *Hypertension* **30**: 687–692.

Walker KA, Barber HE, Hawksworth GM. 1986. Mechanism responsible for altered propranolol disposition in adjuvant-induced arthritis in the rat. *Drug Metab. Dispos.* **14**: 482–486.

Warner TD, Vojnovic I, Bishop-Bailey D, Mitchell JA. 2006. Influence of plasma protein on the potencies of inhibitors of cyclooxygenase-1 and -2. *FASEB J.* **20**: 542–544.