University of Alberta

Renal Effects of COX-2-Selective Inhibitors

by

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Dedication

This thesis is dedicated to my wife Nooshin and my daughter Sarah for their patience and support.

Abstract

We compared the effect of a selected group of NSAIDs with different inhibitory for cyclooxygenase-1 (COX-1) and COX-2 on urinary sodium and potassium excretion in rats. We also tested the hypothesis that the relative extent of renal effects of the three NSAIDs, rofecoxib, celecoxib, and meloxicam, is governed by their degree of kidney exposure as compared with plasma.

Chronic arthritis has been reported to impair glomerular function. In addition, both acute inflammation and chronic arthritis can alter the pharmacokinetics of some drugs. Since NSAIDs are mainly used in the treatment of inflammatory conditions, we studied the effect of inflammation on sodium and potassium excretion and pharmacokinetics of rofecoxib and meloxicam.

As compared to placebo, rofecoxib, celecoxib, diclofenac, and flurbiprofen significantly reduced the excretion rate of sodium and potassium. Meloxicam had no significant effect either on sodium and potassium excretion or urine flow rate. There was a significant correlation between the area under the plasma concentration-time curve from time of dosing to 24 h post-dose (AUC_{0-24}) of rofecoxib and the change in sodium and potassium excretion. The 24-h post-dose concentration of celecoxib was correlated to the change in sodium and potassium excretion. The ratios of kidney to plasma concentration were 2.14 ± 1.63, 3.61 ± 2.34, and 0.27 ± 0.10 for rofecoxib, celecoxib, and meloxicam, respectively.

Sodium and potassium excretion rates were not affected by inflammation. The AUC_{0-24} of rofecoxib, but not meloxicam, in inflamed rats was significantly higher as

compared with that of normal rats. The ratios of kidney/plasma concentrations were not significantly altered.

At the examined dosage levels, no relationship was found between reported COX-2/COX-1 selectivity and renal effects. The lower ratio of kidney to plasma concentration of meloxicam compared to rofecoxib and celecoxib indicates less distribution into kidneys for meloxicam. Inflammation altered kidney function demonstrated by an increase in blood urea nitrogen and plasma creatinine. However, inflammation does not influence the urinary electrolyte excretion. Since the pattern of kidney effect of the examined NSAIDs in inflamed rats is similar to that of previously reported healthy rats, one may conclude that inflammation does not exacerbate the adverse effect.

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List of Abbreviations

≈	Approximately
μg	Microgram
APPROVe	Adenomatous Polyp Prevention on Vioxx
ARF	Acute renal failure
AUC	Area under the plasma concentration-time curve
BUN	Blood urea nitrogen
CL	Clearance
CL_I'	Intrinsic clearance of free drug
CL_I	Intrinsic clearance
cm	Centimeter
C_{max}	Peak plasma level
Co	Cobalt
COX	Cyclooxygenase
Coxibs	COX-2-selective inhibitors
CV	Coefficient of variation
CYPs	Cytochrome P450s
ET-1	Endothelin-1
F	Systemic availability of drug
fu	Unbound fraction
Ge	Germanium
GFR	Glomerular filtration rate
GI	Gastrointestinal

h	Hour
HPLC	High performance liquid chromatography
IC_{50}	Inhibitor concentration that is required for 50% inhibition of an enzyme in vitro
IFN-α	Interferon-α
IL-1β	Interleukin-1β
INAA	Instrumental neutron activation analysis
kb	kilo-base pairs
kg	Kilogram
L	Liter
log P	Partition coefficient
M	Molar
mg	Milligram
MI	Myocardial infarction
min	Minute
ng	Nanogram
NO	Nitric oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
Pb	Lead
pg	Pico gram
PG	Prostaglandin
PGI_2	Prostacyclin
Q	Hepatic blood flow

RA	Rheumatoid arthritis
RPN	Renal papillary necrosis
S	Second
SD	Standard deviation
SE	Standard error
$t_{1/2}$	Half-life
TARGET	Therapeutic Arthritis Research and Gastrointestinal Event Trial
t_{max}	Time to reach peak plasma level
TNF-α	Tumor necrosis factor-α
TXA_2	Thromboxane A_2
UGT	UDT-glucuronosyltransferase
V_d	Volume of distribution
VIGOR	Vioxx Gastrointestinal Outcomes Research
V_{ss}	Steady-state volume of distribution
W	Weight
WBA	Human whole blood
WHMA	William Harvey human modified whole blood

Chapter 1

Introduction

1.1 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs are a heterogeneous group of compounds including aspirin, which irreversibly inhibits cyclooxygenase (COX), and several other classes of organic acids (1). Despite a wide range of pharmacokinetic characteristics, they have some common properties (2). Most NSAIDs are weak organic acids, well absorbed, highly protein-bound, and extensively metabolized. NSAIDs are one the most common classes of drugs and are considered as the first line for the treatment of rheumatoid disorders (3). The number of NSAID prescriptions in the US has been estimated to be 70 million annually; each year, $US6.8 billion is spent on NSAIDs all over the world (4).

1.1.1 History

The inhibitors of COX have been used by human since 1500 B.C. (5). In 1874, salicin, the ingredient of the common white willow, was used to reduce the symptoms related to rheumatic fever (6). Also, sodium salicylate, which had antipyretic and uricosuric effects, was used for the treatment of rheumatic fever and gout in 1875 (1). Felix Hoffman who was assigned by the pharmaceutical manufacturer, Bayer, to develop a more efficient form of salicylic acid, synthesized acetylsalicylic acid by acetylating of the hydroxyl group on the benzene ring of salicylic acid (6). The compound was introduced in 1899 by Heinrich Dreser, Bayer's chief pharmacologist, under the name of aspirin (1). It is believed that aspirin is originated from St Aspirinius, patron saint against headaches (6). However, *Spiraea*, the origin plant species of salicylic acid, is known for the basis of the name by some authors (1). In 1971, Vane proposed that the therapeutic effects of aspirin and other NSAIDs was the result of COX inhibition (6). After discovery of aspirin, many new compounds, that share similar therapeutic effects, have been introduced into clinical

medicine. The goal was to make compounds which were more efficient and had less side effects (2). Discovery of COX-2 in the early 1990 led to development of new compounds with more selectivity for COX-2 inhibition (5).

1.1.2 Cyclooxygenase Isoforms

COX-1 and COX-2 are two isoforms of COX enzyme (7). COX-1 is expressed constitutively in many tissues and is involved in many physiological functions such as regulation of platelet aggregation, gastric mucosa protection, and maintenance of renal function (8).

Inducible expression of COX-2 under inflammatory conditions has been documented in the literature (9). While growth factors and pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) increase the levels of COX-2, corticosteroids and anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 inhibit the expression of COX-2 (10). However, there are several studies reporting constitutive expression of COX-2 in mammalian brain (11), stomach (12), colon (13), and kidney (14).

A new isoform, COX-3, has been identified (15;16). Kis et al. (17) have detected COX-3 mRNA in different parts of rat brain. However, some authors believe that COX-3 does not exist as a distinctive enzyme in humans (18).

1.1.3 COX Structure

63% of amino acids of COX-1 and COX-2 are identical and 77% are similar (19). While human COX-1 derives from a 22-kilo-base pairs (kb) gene with an mRNA transcript at

2.8 kb, the human COX-2 gene is a small immediate early gene at 8.3 kb with a mRNA ≈ 4.5 kb (10).

Two enzymes, COX-1 and COX-2, form a lipophilic channel containing an active site, which binds to the substrate (19). Comparison of COX-1 and COX-2 shows that isoleucine at positions 434 and 523 in COX-1 is substituted for a smaller size amino acid, valine, in COX-2 (10). Exchanging valine at position 523 makes COX-2 active site more accessible to bulkier molecules compared to that of COX-1 (19). Since selective COX-2 inhibitors, such as celecoxib and rofecoxib, have a large molecule, they are not able to reach the active site of COX-1 (10).

Human COX-3 originates from COX-1 gene and expresses COX-3 mRNA transcript at 5.2 kb which is most present in cerebral cortex and heart (15).

1.1.4 Localization of COX isoforms in Major Organs

1.1.4.1 Brain

Although COX-2 is the predominant isoform of rat spinal cord, the amount of COX-1 and COX-2 mRNA are equal in human brain (10). Basic levels of COX-2 are present in brain and cerebral microvessels (20). Although COX-1 is present in brain neurons, it is mainly located in forebrain (10). The constitutive expression of COX-1 in macrophages and microglial cells and COX-2 in endothelial cells has been detected in rat brain (21). In addition, COX-2 is located in cortical pyramidal and is involved in control of cerebral blood flow (22).

1.1.4.2 Platelet

Although COX-1 is the main isoform of COX in platelets (10), the expression of COX-2 has been detected in the platelets of patient who had a bypass surgery (23).

1.1.4.3 Stomach

COX-1 is the main form of COX in the gastrointestinal (GI) tracts of the rat, dog, monkey, and human (24). Haworth et al. (25) have studied the expression of COX-1 and COX-2 in GI of rat. While COX-1 was present in the lamina propria, submucosal vascular endothelium and within the muscularis and muscularis mucosae of all regions of GI tract, its levels were less in stomach and large intestine compared to those of small intestine. COX-2 was present within the parasympathetic ganglia of the submucosa and muscularis of entire GI tract, while the highest expression of COX-2 was seen in the ileocaecal junction.

1.1.4.4 Kidney

In rats, basal levels of COX-1 is expressed in medullary collecting ducts and interstitial cells and, to a lesser degree, in the cortex of rat kidneys (26). In addition, COX-1 is present in papillary interstitial cells (27). COX-2 expression was seen in macula densa, adjacent cortical thick ascending limb in renal cortex (26;27) and papillary interstitial cells (27).

In human, COX-1 is present in the collecting ducts, renal vasculature and papillary interstitial, while COX-2 is expressed in podocytes and small blood vessels (27).

Recently, Adegboyega and Ololade (14) have shown the constitutive expression of COX-2 in normal human kidneys. COX-2 was present in the endothelial cells of arteries, arterioles, and glomeruli of the cortex. Also, COX-2 was detected in the cortical thick ascending limb of the loop of Henle, the endothelial lining of the vasa recta, and the collecting ducts.

1.1.5 Selectivity of NSAIDs for COX-1 and COX-2 inhibition

The potency to inhibit COX-1 and COX-2 varies among NSAIDs. Several assays, including purified recombinant enzymes, transfected cells, and whole-blood assays, have been developed to evaluate this potency (28).

Copeland et al. (29) used recombinant COX-1 and COX-2 enzymes to measure the selectivity of two compounds. Enzyme was incubated with various concentrations of NSAIDs. After selected incubation times, arachidonic acid was added and the enzyme activity was determined by the measurement of absorbance change of a known compound, N,N,N',N'-tetramethyl-p-phenylenediamine. Since enzyme activity did not replicate the protein binding and transcellular passing of drug, whole-cell systems were develop to measure NSAIDs' potency (19).

Mitchell et al. compared the cultured animal cells (intact or broken) and purified enzyme preparations (30). The potency of NSAIDs to inhibit COX isozymes were measured in bovine aortic endothelial cells for COX-1 and endotoxin-activated J774.2 macrophages for COX-2. Since enzyme preparations assay did not show the same activity for enzyme that were seen with cultured animal cells assay, they concluded that cultured animal cells would be a better predictor of NSAIDs' potency (30).

In whole blood assay (WBA), thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) were measured as the indexes of COX-1 and COX-2 activities, respectively (28). In order to induce COX-2, the whole blood had to be incubated with lipopolysaccharide for 24 h. Therefore, the time course of COX-1 and COX-2 assays was different. To address this concern, a modified form of WBA, the William Harvey modified assay (WHMA), was developed. In this assay, pre-stimulated human A-549 monocytic cells were used to measure COX-2 activity (19).

Performing WBA and WHMA, using human airway epithelial cells, Warner et al. (3) have reported COX-1 and COX-2 activities of various NSAIDs. Comparing the inhibitory concentration that is required for 50% inhibition of an enzyme in vitro (IC_{50}) and IC_{80} concentration values, IC_{80} provided values closer to the steady-state plasma concentrations of many examined NSAIDs. Therefore, they concluded that IC_{80} values were better estimates of COX-1 and COX-2 potency. Table 1-1 depicts the IC_{80} concentrations and COX-2-COX-1 ratios for some NSAIDs.

Table 1-1. Comparison of several NSAIDs with respect to the IC_{80} concentrations (3).

Compound	COX-1 IC_{80} (μM)	WBA-COX-2 IC_{80} (μM)	WHMA-COX-2 IC_{80} (μM)	COX-2-COX-1 selectivity (Using WBA)	COX-2-COX-1 selectivity (Using WHMA)
Aspirin	8	100	30	0.08	0.27
Diclofenac	1	0.27	0.23	3.70	4.35
Flurbiprofen	1	24	51	0.04	0.02
Ibuprofen	58	67	150	0.87	0.39
Indomethacin	0.46	5	2	0.09	0.23
Naproxen	110	260	330	0.42	0.33
Celecoxib	28	6	3	4.67	9.33
Etodolac	69	8	3	8.63	23.0
Meloxicam	22	7	2	3.14	11.0
Rofecoxib	100	6	5	16.7	20.0

1.1.6 NSAIDs Categories

NSAIDs are divided into two categories: traditional NSAIDs and COX-2-selective inhibitors (coxibs). However, occasionally aspirin has been considered as a separate class by some authors (31).

1.1.6.1 Traditional NSAIDs

'Traditional' NSAIDs are isoform nonselective, reversible, active site inhibitors of COX (31). NSAIDs, with a wide range of availability, are used more than any other therapeutic compounds (32). Inhibition of PGE_2 and PGI_2 (prostacyclin) production is the basis of their analgesic, and antipyretic, and anti-inflammatory effects (31). This class includes a range of compounds with a variety of chemical structures (Table 1-2).

Table 1-2. Chemical classification of NSAIDs (32).

Group	Example
Salicylates	Aspirin
Propionic acid derivatives	Ibuprofen, naproxen
Pyranocarboxylic acids	Etodolac
Acetic acids	Diclofenac
Indoleacetic acids	Indomethacin
Oxicams	Piroxicam
Pyrroloppyrrole	Ketorolac
Fenamates	Mefenamic acid

1.1.6.2 COX-2-Selective Inhibitors (Coxibs)

Coxibs were developed with the notion that they exert their anti-inflammatory effect with inhibition of COX-2 and have fewer side effects due to less potency for COX-1, which plays a housekeeping role in many tissues through the body (2). In this attempt, COX-2-selective compounds such as rofecoxib and celecoxib were introduced to the presented NSAIDs such as etodolac, meloxicam, and nimesulide that had shown selectivity for COX-2 (3). However, new studies have revealed the cardiovascular (33) and renal effects (34) associated with inhibition of COX-2.

Among this group of NSAIDs, selectivity for COX-2 is varied: celecoxib, meloxicam, and etodolac are considered as moderately COX-2 selective, while rofecoxib, valdecoxib, etoricoxib, and parecoxib have been categorized as highly COX-2 selective (33).

1.1.7 Mechanisms of Action of NSAIDs

NSAIDs inhibit COX activity leading to a decrease in the production of prostanoids from arachidonic acid (35).

1.1.7.1 Arachidonic Acid Cascade

Phospholipids, present in cell membranes, are metabolized to arachidonic acid by phospholipase A_2 (Figure 1-1) (36). COX converts arachidonic acid to PGG_2 and consequently to PGH_2 (37). Depending on the type of tissue and stimuli, PGH_2 is further converted by individual PG synthases to five major prostanoids: PGD_2, PGE_2, prostacyclin, $PGF_{2\alpha}$, and TXA_2 (36) which bind to specific receptors termed DP, EP, IP, FP, and TP , respectively (31)

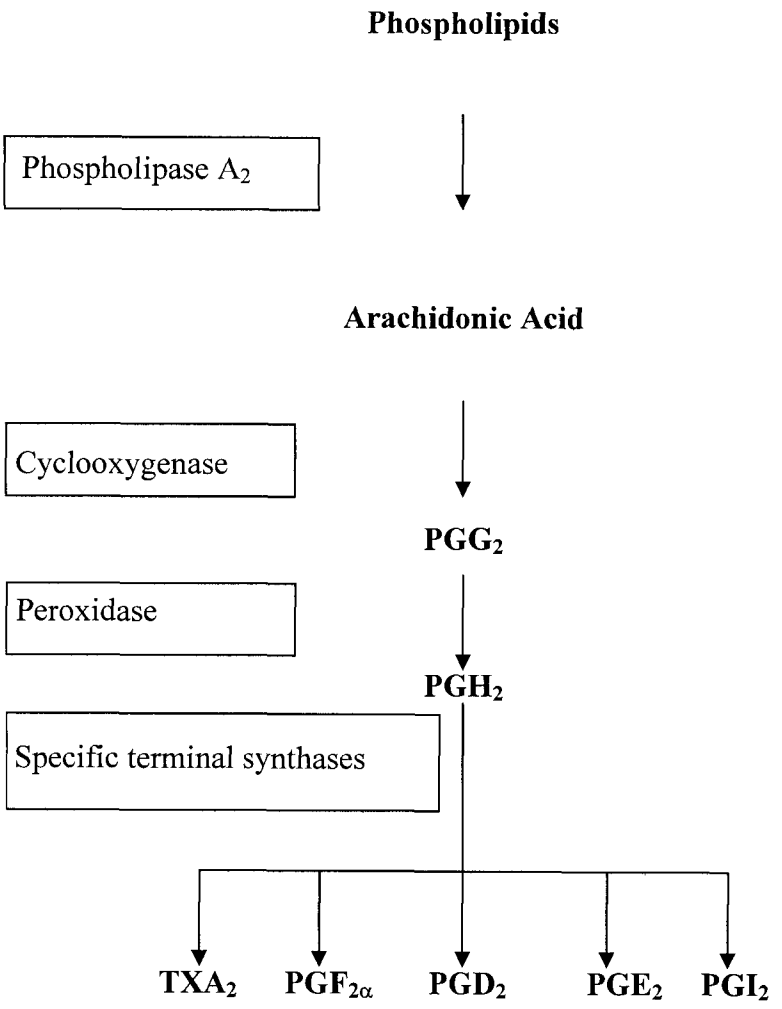

Figure 1-1. Biosynthesis of prostaglandin (36).

1.1.7.1.1 Thromboxane A_2

TXA_2, involved in platelet aggregation, vasoconstriction and bronchoconstriction (38), is formed by platelets, macrophages, and lung parenchyma (39). Since TXA_2 plays a role in some medical conditions such as asthma, myocardial ischemia, pulmonary hypertension, and thromboembolic disorders, many pharmaceutical companies have shown interest in developing TXA_2 receptor antagonists, TX synthase inhibitors and drugs having both action mechanisms (38).

1.1.7.1.2 Prostaglandin I_2 (Prostacyclin)

Prostacyclin, produced by vascular endothelium, blocks platelet aggregation (31) and has a vasodilatory effect (31;40). While COX-1 produces basic levels of prostacyclin, over expression of prostacyclin is contributable to COX-2 activity under stress conditions (41).

Prostacyclin, along with TXA_2, is involved in regulation of renal blood flow (42). Nielsen et al. showed that infusion of prostacyclin, which is a potent vasodilator, elevated the renal blood flow in healthy subjects (43).

1.1.7.1.3 Prostaglandin E_2

PGE_2, the main prostaglandin produced in the brain, plays a role in synaptic plasticity, neurogenesis, and fever sensing and signaling (44). Many inflammatory conditions of brain are associated with higher levels of PGE_2 (45).

PGE_2, which is predominantly present in kidney tubules such as medullary collecting tubule, the cortical collecting tubule, and the thin limb of Henle's loop, can be expressed tenfold in response to exogenous compound (46). Infusion of various doses of

PGE_2 increased urinary excretion of sodium and potassium in dogs (47). It also increased the secretion of renin from juxtaglomerular cells (48).

1.1.7.1.4 Prostaglandin D_2

PGD_2 is the major prostanoids is produce by mast cells (49). PGD_2 plays a role in bronchoconstriction demonstrated in allergic asthma (50). This prostanoid and histamine are involved in activating and recruiting eosinophils to the site of inflammation (51). PGD_2 exert its effect through two receptors: DP1 ad DP2 which are different in respect to their origin, signaling pathway, and pattern of expression (52). Although DP2 activation is involved in inflammation process observed in allergy, DP1 stimulation leads to improvement in asthma (51).

1.1.7.1.5 Prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$ is a smooth muscle contactor (53) that exert its effect by stimulating FP receptors. While $PGF_{2\alpha}$ produced by COX-1 demonstrates its action in luteolysis, TXA_2 as well as $PGF_{2\alpha}$, induced by COX-2, are involved in the last stages of parturition (31).

1.1.8 Therapeutic Effects of NSAIDs

NSAIDs exert their analgesic, and antipyretic, and anti-inflammatory effects by inhibition of COX (1). Inhibition of COX-1, within platelets, is a therapeutic goal to reduce the chance of thromboembolic events (10).

1.1.8.1 Analgesic Effect

'Clinical pain' is a complex phenomenon involving peripheral and central sensitization (54). It has been shown that COX-2 is involved in peripheral inflammatory pain by

facilitating transmitter release and activation of prostanoid receptors in the spinal cord (55). The basal levels of COX-2 are present in brain and spinal cord. However, many stimuli elevate the expression of COX-2, and consequently PGE_2, which depolarises the second-order neurons by opening a sodium ion channel. Moreover, PGE_2 facilitate release of neurotransmitter by binding to presynaptic EP1 receptors located on the primary afferent neurons (54). Furthermore, It has been shown that NSAIDs exert direct analgesic effect by activation of spinal glutamate and substance P receptors (56). The notion that non-selective NSAIDs and coxibs, but not selective COX-1 inhibitors, have analgesic effect, proves the role of COX-2 in pain signaling (54).

1.1.8.2 Antipyretic Effect

Elevated expression of some cytokines such as IL-1β, IL-6, interferon-α (IFN-α), IFN-β, and TNF-α, as a result of infection or inflammation, increases the production of PGE_2 in brain and consequently stimulates the hypothalamus to increase body temperature. NSAIDs inhibit fever by suppression of PGE_2 synthesis (1).

It has been suggested that the antipyretic effect of acetaminophen is explained by inhibition of COX-3 (57). Since therapeutic doses of acetaminophen yield to steady-state concentrations of 100 micro molar (μM), the concentration for inhibition of COX-3, but not COX-1 and COX-2, the authors concluded that acetaminophen exerts its antipyretic effect via blockage of COX-3.

1.1.8.3 Antithrombotic Effect

TXA_2, adenosine diphosphate, and fibrinogen, which cause platelets aggregation, are the compounds released by platelets as a result of adherence of their glycoprotein IIb

receptors to a ruptured plaque (58). Aspirin exert its antithrombotic effect by irreversible inhibition of COX-1 (10) through acetylation of the hydroxyl group of serine residue (59).

Although COX-1 is inhibited by other NSAIDs, the inhibition is competitive, reversible, and incomplete (70 to 90%) at therapeutic levels. Since platelets have a significant capacity to produce TXA_2, the level of inhibition by NSAIDs is not sufficient to obstruct platelet aggregation in vivo (60).

In 2001, Ouellet et al. (61) studied the effect of different NSAIDs, with different COX-1/COX-2 selectivity, on the extent of COX-1 inhibition by aspirin. They found that co-administration of NSAIDs with aspirin may alter the ability of aspirin to block COX-1 by competition over active site of COX-1. The compounds that had the lowest affinity for COX-1 (e.g. etoricoxib) had the lowest potency for interference with COX-1 inhibition. In contrast, diclofenac, which had the highest affinity to inhibit COX-1 among the tested NSAIDs, showed the maximum interference with the antagonism effect of aspirin on COX-1.

1.1.8.4 Anti-inflammatory Effect

The characteristics of inflammation are swelling, redness, heat and pain mediated by an increase in vascular permeability (62). In this process, many cell types such as neutrophils, macrophages, mast cells, lymphocytes, platelets, dendritic cells, endothelial cells, and fibroblasts are recruited to the site of inflammation (63). Also, cytokines, produced by macrophages and lymphocytes, are released locally (62).

Although inflammation leads to recovery in many cases, it may cause tissue destruction (64). It is believed that the tissue damage observed in many diseases such as

multiple sclerosis, Alzheimer disease, rheumatoid arthritis (RA), systemic lupus erythematosus, and Hashimoto's thyroiditis is accompanied by inflammation (63). NSAIDs block production of COX products, mostly PGE_2, and decrease the symptoms linked to arthritis (5). In addition to short term benefits such as reduction of the heat, redness, swelling, pain and damage to joints, bones and soft tissues, long-term use of NSAIDs has effect on neutrophil and lymphocyte functions (32).

1.1.9 Side Effects of NSAIDs

The use of NSAIDs is associated with two major side effects: renal and GI effects.

1.1.9.1 Renal Effects

The incidence of nephrotoxicity associated with the use of NSAIDs is estimated to be 1-5% of exposed patients (65). Acute renal failure (ARF), renal papillary necrosis (RPN), hyperkalemia, and sodium retention with hypertension or edema are the four major renal syndromes related to the use of NSAIDs (66).

1.1.9.1.1 Urinary System

The urinary system is composed of two kidneys, two ureters, the urinary bladder, and the urethra (67). The kidneys have a bean-shape structure and lie on the posterior wall of the abdomen behind the peritoneum on each side of the vertebral column (68). Each kidney is divided into three regions: the cortex, the medulla, and the renal pelvis (67). In addition to removal of waste products, the kidneys are involved in regulation of plasma volume, osmolarity, hydrogen, and ions including sodium, potassium, calcium, magnesium, chloride, bicarbonate, and phosphate (67). Each kidney has approximately 1.3 million nephrons (Figure 1-2) (69). Each nephron consists of the renal corpuscle, including

Bowman's capsule and glomerulus, and a tubule (67). Via afferent arteriole, the blood is filtrated through the renal corpuscle into the tubule, which consists of the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and collecting ducts (69). The remaining blood leaves the glomerulus through efferent arteriole and form a set of capillaries that supply the blood to the tubule (67). The glomerular filtrate passes the tube and forms the urine. The composition of the glomerular filtrate may change by removal of water and substance, tubular reabsorption, from the filtrate or secretion of substances into the filtrate, tubular secretion (69).

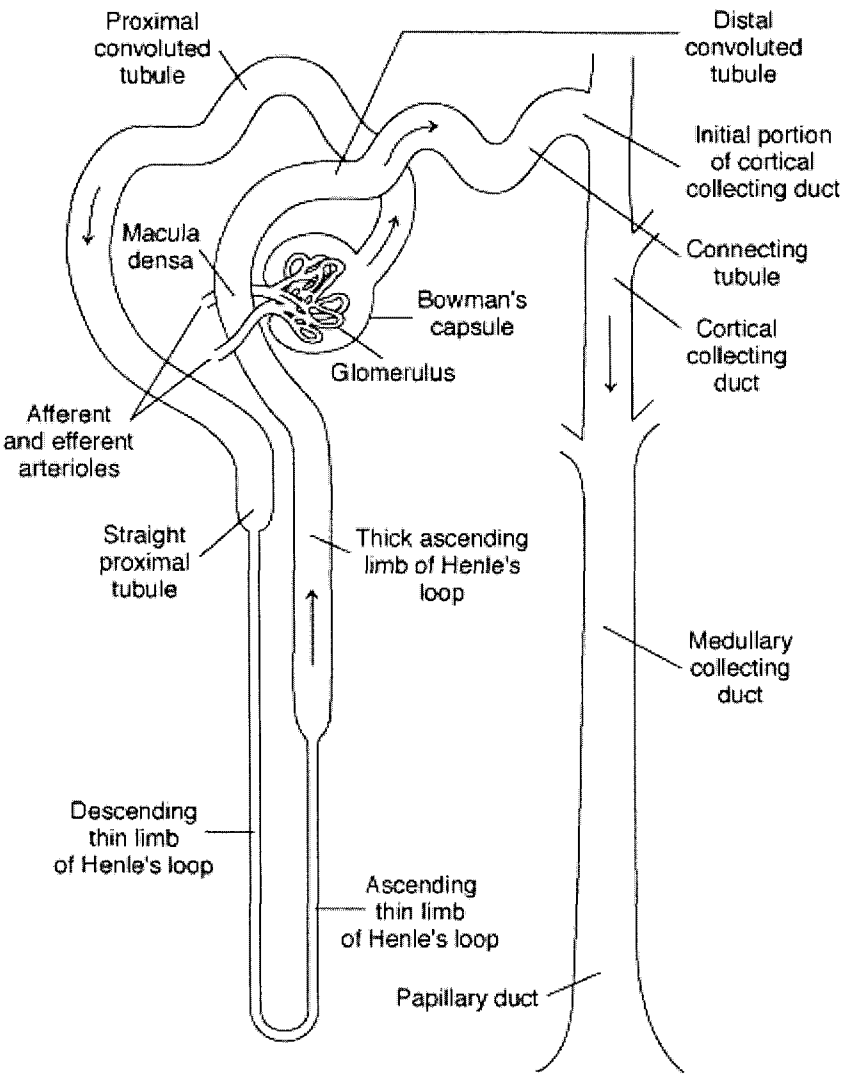

Figure 1-2. Structure of a nephron (68).

1.1.9.1.1.1 Sodium Balance

Although a large amount of sodium is filtered by nephron, 96-99% of the ion reabsorbs along renal tubule (69). Na-K-ATPase pumps, positioned in the basolateral membrane of the renal tubular epithelial cells, actively transport sodium from cell to interstitial tissue (68). Aldosterone released from the adrenal cortex increases the reabsorption of sodium by stimulating the synthesis of Na-K-ATPase pumps (67). The secretion of renin stimulates the release of aldosterone by activation of renin-angiotensin-aldosterone system (67).

1.1.9.1.1.2 Potassium Balance

Potassium undergoes reabsorption in the proximal tubules and secretion in the late distal tubules and collecting ducts (67;69). Aldosterone regulates the secretion of potassium by increasing the number of Na-K-ATPase pumps and potassium channels located in the late distal tubules and collecting ducts (67).

1.1.9.1.2 Acute Renal Failure

The development of ARF is reported to occur in patients at risk such as those with underlying volume depletion, renal insufficiency, congestive heart failure, diabetes, nephrosis, cirrhosis and old age (70). However, the higher risks ARF in elderly patients are suggested to be contributed to the concomitant diseases (34). ARF is diagnosed by the elevated values of blood urea nitrogen (BUN), creatinine, potassium, and body weight (71). Two different mechanisms could be responsible for the ARF associated with the use of NSAIDs: (1) interstitial nephritis (2) a decrease in renal blood flow due to inhibition of production of vasodilator PGs (72). The side effect appears to be exposure-dependent. It

has been shown that administration of NSAIDs with longer half-life increases the chance of renal impairment in elderly subjects (73). In another study, the higher doses of ibuprofen were associated with the higher degrees of renal dysfunction (71).

ARF associated with the use of diclofenac in two women is documented by Rossi et al. (74). Although one of the subjects had underlying diseases, no risk factor was reported in other subjects. Nevertheless, the renal function was improved in both cases when diclofenac was discontinued. Literature contains various reports indicating the ARF associated with the use of other NSAIDs such as ketorolac (75), indomethacin (76), naproxen (77), and ibuprofen (78). There are reports showing that the use of COX-2-selective inhibitors increases the chance of ARF. Graham (79) reported a case of ARF in a 57-year-old woman diagnosed with osteoporosis. ARF occurred when the dose of celecoxib was increased from 200 milligram (mg)/day to 400 mg/day. The levels of creatinine and BUN and blood pressure were increased. Also, the presence of edema was evident in the subject. Renal function became normal when celecoxib was stopped. In addition, ARF caused by rofecoxib is reported in a 49-year-old patient who had undergone renal transplantation (80), 23-year-old healthy woman (81), and 71-year-old woman (82). A case of ARF induced by valdecoxib is reported by Muhlfeld et al. (83).

1.1.9.1.3 Renal Papillary Necrosis

The mechanism of RPN induced by NSAIDs is explained by Kovacevic et al. (84). Under normal conditions, the vasoconstriction induced by angiotensin II, norepinephrine, and vasopressin, due to hypovolaemia, is neutralized by vasodilatory effect of renal PGs. Administration of NSAIDs, decreases the renal blood flow to the inner medulla, which is normally hypoxemic compared to the cortex. This could result in RPN.

Traditional NSAIDs such as ibuprofen (85), aspirin (86), naproxen (84), flurbiprofen (87), and indomethacin (88) are reported to produce RPN. However, the use of COX-2-selective inhibitors is associated with RPN as well. Akhund et al. (89) have reported a case of RPN related to celecoxib therapy in a 61-year-old woman diagnosed with RA. Hematuria and intermittent right flank pain due to RPN was stopped after discontinuation of celecoxib.

1.1.9.1.4 Hyperkalemia

PGI_2 stimulates the juxtaglomerular cells in the kidney to release renin (90). This leads to the secretion of aldosterone, which increases the potassium excretion (34). Therefore, the inhibition of PGI_2 production by NSAIDs may result in hyperkalemia (90). The presence of hyperkalemia caused by NSAIDs is more evident in patients who have underlying diseases such as renal dysfunction, cardiac failure, diabetes, or multiple myeloma or take potassium supplements, sparing diuretics, or angiotensin-converting enzyme inhibitors (65).

Alva and Kotian (91) have reported a case of hyperkalemia in a fifty year old male who was taking diclofenac for sixteen days. Hyperkalemia's presence was confirmed with bradycardia, hypotension, and electrocardiography and biochemical changes. Although many other NSAIDs such as naproxen (92), ibuprofen (93), and piroxicam (94) cause hyperkalemia, indomethacin is the major NSAIDs related to hyperkalemia, even in healthy subjects (65). This could be due to the direct effect of indomethacin on potassium uptake. Like traditional NSAIDs, COX-2-selective inhibitors may increase the risk of hyperkalemia in patients at risk (66).

1.1.9.1.5 Sodium Retention

The most frequent renal side effect of NSAIDs is sodium retention (65). It has been estimated that 25% of NSAIDs user may develop sodium retention (95) which may lead to edema and gain weight (96). Several mechanisms have been proposed to explain the effect of NSAIDs on sodium excretion. The reabsorption of sodium at the thick ascending limb of the loop of Henle is reduced by PGE_2 (34) through inhibitory effect on Na-K-2Cl cotransporter activity (97). NSAIDs decrease the production of PGE_2 which leads to an increases in the expression of Na-K-2Cl cotransporter (98). Also, a reduction in glomerular filtration rate (GFR) caused by NSAIDs may limit sodium excretion by kidney (66). In addition, a change in blood flow due to inhibition of prostacyclin production has a major effect on sodium excretion (99). It has been shown that the decrease in GFR caused by NSAIDs is related to inhibition of COX-1, while sodium excretion reduction is due to inhibition of COX-2 (100;101).

COX-2-selective inhibitors show similar side effects to traditional NSAIDs in terms of sodium excretion reduction (102). To compare the renal effects of rofecoxib and indomethacin, 15 patients 60 to 80 years of age in a randomized, three-period, single-dose crossover study were administered 250 mg rofecoxib, 75 mg indomethacin, or placebo (103). Sodium excretion was significantly less in rofecoxib and indomethacin groups compared to placebo.

A randomized double-blind study was conducted to assess renal function after administration of celecoxib, 200 mg twice a day; celecoxib, 400 mg twice a day; naproxen, 500 mg twice a day; or placebo for 7 days in healthy subjects restricted to salt

consumption (104). Celecoxib decreased sodium and potassium excretion similar to those of naproxen in subjects.

In a randomized, double blinded trial, 36 healthy older adults received rofecoxib (50 mg every day), indomethacin (50 mg three times a day), or placebo for 2 weeks (101). A transient reduction in sodium was observed in the rofecoxib and indomethacin groups.

A randomized, double blinded, crossover study was carried out to compare the renal effects of rofecoxib and celecoxib, and naproxen in 67 healthy elderly subjects on a sodium-replete diet (96). Subjects received daily 25 mg rofecoxib, 400 mg celecoxib, 1000 mg naproxen, or placebo for 28 days. Rofecoxib, celecoxib, and naproxen decreased sodium excretion by 17, 24, and 31%, respectively.

1.1.9.1.5.1 Cardiovascular Events Associated with the Use of NSAIDs

Literature contains conflicting evidences with respect to the cardiovascular effects of selective COX-2 inhibitors. The result of Vioxx Gastrointestinal Outcomes Research (VIGOR) study showed that the use of rofecoxib was associated with the higher rate of myocardial infarction (MI) compared with that of naproxen (105). Since the results of the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial (106) revealed that the chance of cardiovascular events increased among subjects who took rofecoxib more than 18 months, Merck & Co., Inc. withdrew its product, rofecoxib, from the market in 2004 (33). Moreover, a population-based retrospective cohort study among subjects aged 66 years and older was carried out to evaluate the influence of various NSAIDs on the risk for a first MI (107). Increased risk for MI was compared with those who had not used an NSAID. The results of study showed that patient who took low and high doses of

rofecoxib had higher odds of myocardial infarction. Co-administration of aspirin decreased the risk in patients who took lower doses of rofecoxib. This had been predicted earlier (33). The higher risks of rofecoxib for MI were contributed to the higher selectivity of rofecoxib for inhibition of COX-2. However, the results of Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), which assessed the gastrointestinal and cardiovascular safety of the COX-2 inhibitor lumiracoxib compared with two nonselective NSAIDs, naproxen and ibuprofen, among 18,325 patients age 50 years or older, did not reveal any significant differences between the lumiracoxib users and people who took ibuprofen or naproxen with respect to MI (108). It is of note that the COX-2-COX-1 selectivity ratio for lumiracoxib is 515 (109) as compared to 77 (3) and 1.42 (3) for rofecoxib and celecoxib, respectively, using the IC_{50} values obtained by WBA.

Some studies have proposed the mechanisms involved in cardiovascular events associated with the coxibs usage. COX-1 is present in platelet, whereas COX-2 is dominant form of COX enzyme in endothelial cells. TXA_2 and prostacyclin are the products of COX enzyme in platelet and endothelial cells, respectively (110). Since nonselective NSAIDs inhibit both TXA_2 and prostacyclin, the balance of TXA_2/PGI_2 does not change. In contrast, coxibs do not affect production of TXA^2 in platelets. Therefore, TXA_2/PGI_2 ratio is more toward prothrombotic event (111).

Davies and Jamali (33) have suggested that the physiochemical properties and pharmacokinetic parameters of rofecoxib may be involved in cardiovascular events associated with the drug.

It may be also possible that the cardiovascular events are explained by the renal dysfunction, particularly reduced sodium excretion, related to the use of NSAIDs. The selective inhibitors may increase sodium retention (34) and consequently blood pressure (112). The link between urinary sodium excretion and MI in 2937 mildly and moderately hypertensive patients has been reported by Alderman et al. (113) Since the side effects of other NSAIDs have not been studied in the long term studies such as APPROVe, caution should be taken in use of NSAIDs (33).

1.1.9.2 Gastrointestinal Effects of NSAIDs

The use of NSAIDs has been limited due to their GI toxicity (114). It has been reported that the 4-week incidence rate of gastric and duodenal ulcers among people who took NSAIDs to be 8 and 4%, respectively (115). The use of NSAIDs is associated with the upper GI events such as dyspepsia, heartburn, nausea and vomiting (116). High rates of prevalence of dyspepsia (19% of those with normal endoscopy and in 9% of those with abnormal endoscopic findings) was observed in arthritic patients who took NSAIDs for more than six weeks (117). Some large prospective outcome studies have reported the rate of serious upper GI complications such as major bleeding, perforation, and obstruction among arthritic patients who take NSAIDs to be 1–1.5% (114).

Two major mechanisms have been proposed to explain the GI toxicity of NSAIDs. It could be the result of local irritation and/or through inhibition of PGs (118). Most NSAIDs are week acids and exist in nonionized form in acidic pH of stomach. Therefore, they pass the cell membrane due to their lipophilic character. Since the pH of the cells in inside is different from that of outside, NSAIDs are ionized and trapped in the cells and consequently exert their toxicity (119). Since PGs are involved in secretion of

mucosal bicarbonate and mucus and maintaining mucosal blood flow of GI, inhibition of PGs by NSAIDs may lead to injury (120). It has been shown that the toxicity of NSAIDs is time and dose dependent. Using permeability test, as a marker of GI toxicity, Davies et al. reported that the elevated permeability of intestine lasted 12 and 36 h following administration of 10 and 20 mg/kg, respectively, indomethacin to rats (121). The GI permeability was higher in the rats treated with 20 mg/ kilogram (kg) compared with those of 10 mg/kg. The doses more than 20 mg/kg resulted in massive bleeding, ulceration, and death.

In order to decrease the GI toxicity of NSAIDs, the modified formulations of NSAIDs have been developed (122). For instance, a new enteric-coated formulation of naproxen decreased drug related GI complaints in arthritic patients, while two formulation revealed the equal efficacy (123). However, administration of the modified formulation may increase the toxicity by shifting the release site to the lower parts of intestine, where monitoring of GI tract is more difficult to perform (122;124).

The notion that coxibs do not inhibit COX-1, which protects GI from injury by production of PGs (2), provided a basis for the development of coxibs with less GI toxicity. It has been accepted that coxibs generally provide more GI tolerability over traditional NSAIDs (125). In one study, 8059 arthritic patients were treated with celecoxib, 400 mg twice per day, ibuprofen, 800 mg 3 times per day, or diclofenac, 75 mg twice per day (126). The results of six months of trial revealed that the rate of GI events was lower in patients who took celecoxib compared with other NSAIDs. Of note, celecoxib lost its advantage over diclofenac and ibuprofen when the trial continued for 12 or 15 months (127). In other study, rofecoxib, 50 mg daily, showed less GI toxicity than

naproxen, 500 mg twice daily, in arthritic patient who were older than 50 years (105). The number of GI events was more than two-fold higher in naproxen group.

1.1.10 Diclofenac

The chemical structure of diclofenac, 2-[(2,6-dichlorophenyl) amino] benzene acetic acid, is depicted in Figure 1-3 . Diclofenac is a faintly yellowish white to light beige, virtually odorless, slightly hygroscopic crystalline powder that is available as sodium or potassium salt (128). Since diclofenac is poorly soluble in water and has a high partition coefficient, it more used as sodium or potassium form (129). Diclofenac sodium is sparingly soluble in water and has a log P (partition coefficient) value of 13.4 at pH 7.4 (130). The partition coefficient value of diclofenac potassium which is soluble in water is calculated to be 15.45 at pH 5.2 (130).

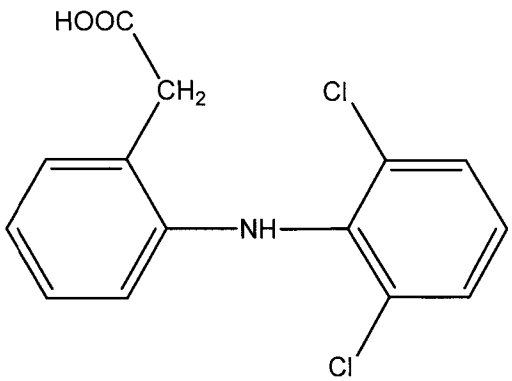

Figure 1-3. Chemical structure of diclofenac.

Diclofenac sodium is used in symptomatic treatment of RA, osteoarthritis, and ankylosing spondylitis in the United States (1). The recommended dose in the treatment of RA is 75-150 mg/day in divided doses. It is recommended that osteoarthritic patients take 75 mg/day dose that can be increased to 150 mg (131).

1.1.10.1 Pharmacokinetics of Diclofenac in the Rat

Diclofenac sodium is rapidly absorbed (132). The time to reach peak plasma level (t_{max}) is reported to be 10 minutes (min) and the half-life ($t_{1/2}$) of the drug is 16 h. Since the area under the plasma concentration-time curve (AUC) and peak plasma level (C_{max}) of diclofenac sodium increased proportionally when the dose was increased, Torres-Lopez et al. were able to report a linear pharmacokinetics after administration of 3 doses, 1, 3.2, or 10 mg/kg, to rats (132). Following oral administration of diclofenac, 3 mg/kg, to rats, the mean apparent clearance (CL) was calculated to be 1.3 L/h/kg (133). In the rat, biliary is the major root of excretion (134). Conjugates of the hydroxylated metabolites and the ester glucuronide of unchanged diclofenac are the major metabolites in the urine and bile of rats, respectively (135). In the bile, glucuronide conjugates of diclofenac sodium easily revert to the drug by alkaline hydrolysis (134). Glucuronidation take places by *UGT2B1* in rat (136). The mechanisms of hepatotoxicity caused by diclofenac have been studied by Kretz-Rommel and Boelsterli (137) using inhibitors of UDP-glucuronosyltransferase (UGT) or CYP2C subfamily. They have suggested that the acute liver cell injury is attributed to the formation of toxic metabolite(s) catalyzed by *CYP2C*. On the other hand, hepatitis may be caused by reactive metabolites formed by *UDPGT*.

1.1.10.2 Clinical Pharmacokinetics of Diclofenac

Diclofenac shows linear pharmacokinetics in doses of 25 to 150 mg in human (138). Although diclofenac is highly absorbed through the GI tract, the bioavailability of drug is about 60%, mostly attributable to first-pass metabolism (139). Food delays the absorption of drug (139). The t_{max} of drug, following a single oral dose of 50 mg, was 1.0 to 4.5 h (140). The volume of distribution (V_d) is reported to be 0.12 to 0.17 liter (L)/kg (141).

More than 99.7% of drug is bound to albumin in plasma (134). Diclofenac has a short $t_{1/2}$ of 1.2-3.5 h (138). The CL of drug was 236 mL/min, following intravenous administration of a solution containing 50 mg diclofenac sodium to young subjects (140). Diclofenac is metabolized by cytochrome P450s (CYPs) and UGTs enzymes. Glucuronidation occurs by UGT2B7 in human (136). Administration of diclofenac to healthy subjects 8-day pre-treated with digoxin increased the plasma concentrations of digoxin (138). Therefore, the blood levels of digoxin should be monitored in case of co-administration with diclofenac.

1.1.11 Flurbiprofen

Flurbiprofen, (±)-2-(2-fluoro-4 biphenylyl) propionic acid (Figure 1-4), is a member of propionic acid family (130). It is slightly soluble in water at pH 7.0 and readily soluble in most polar solvents and available as a white or slightly yellow crystalline powder (128). The log P value of flurbiprofen is reported to be 4.16 (142). Flurbiprofen is indicated for use in RA, degenerative joint disease, and ankylosing spondylitis (143).The recommended dose in the treatment of RA, osteoarthritis, and ankylosing spondylitis is 200 mg/day in divided doses. The maximum daily dosage is 300 mg/day (131).

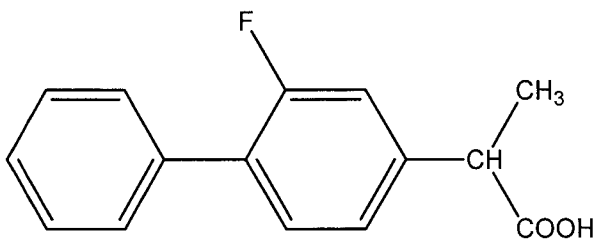

Figure 1-4. Chemical structure of flurbiprofen.

1.1.11.1 Pharmacokinetics of Flurbiprofen in the Rat

While a portion of flurbiprofen absorbs through the stomach, the absorption is complete and fast from small intestine of rats (144). The unbound fraction (fu) of drug is reported to be less than 0.5% (144).

Flurbiprofen presents in two enantiomeric, S and R, forms (130). Most of anti-inflammatory effect of drug is attributed to the S-enantiomer (145). Jamali et al. (146) who administered R-enantiomer to the rat and human subjects, observed two enantiomers in plasma and urine of rats, but not human. This suggested that R-enantiomer is bioinverted to S-enantiomer in the rat, while this inversion is negligible in human. Following intravenous administration of racemic flurbiprofen, 20 mg/kg to rats, the CL of R-enantiomer was higher than S-enantiomer, 2.34 ± 0.05 versus 0.90 ± 0.06 mL/min/kg, respectively (147). Terminal $t_{1/2}$ of R-enantiomer was not significantly different from that of S-enantiomer, 2.55 ± 0.79 versus 3.07 ± 1.21 h, respectively. Also, no significant difference was found when the V_d of R and S-enantiomer was compared, 0.32 ± 0.02 versus 0.22 ± 0.02 L/kg, respectively.

Six metabolites have been detected in rats. 2-(2-fluoro-4'-hydroxy-4-biphenylyl)propionic acid, 2-(i-fluoro-3',4'-dihydroxy-4-biphenylyl)propionic acid, and 2-(2-fluoro-3'-hydroxy-4'-methoxy-4-biphenylyl)propionic acid are the three major metabolites of drug. The renal root is the major basis of excretion.

1.1.11.2 Clinical Pharmacokinetics of Flurbiprofen

Flurbiprofen has a short elimination $t_{1/2}$ (3-6 hours) and rapid and complete absorption. It follows a linear pharmacokinetics at therapeutic doses (148). The fu of flurbiprofen in human serum is 0.022% (149). After administration of 100 mg racemic flurbiprofen to

the healthy fasted subjects, The R-enantiomer demonstrated a larger V_d, 8.41 ± 3.0 versus 7.23 ± 1.9 L, and CL, 1.47 ± 0.5 versus 1.23 ± 0.34 L/h, when compared with S-enantiomer. However, no significant difference was observed when the $t_{1/2}$ of enantiomers was compared, 4.18 ± 1.3 versus 4.2 ± 1.2 h for R and S-enantiomers, respectively (146). Glucuronidation is the major pathway in metabolism of drug (148). Unchanged form and metabolites are mainly excreted by kidney (139). Since the binding site of flurbiprofen to albumin is different from that of warfarin, no significant interaction is reported between these two drugs (148).

1.1.12 Rofecoxib

Rofecoxib, 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone (Figure 1-5) (128), is a white to off-white to light yellow powder possessing melting point of 204-208 °C (150). It is sparingly soluble in acetone, slightly soluble in methanol and isopropyl acetate, very slightly soluble in ethanol, practically insoluble in octanol, and insoluble in water (128) (128). The log P value for rofecoxib is calculated to be 1.635 (150).

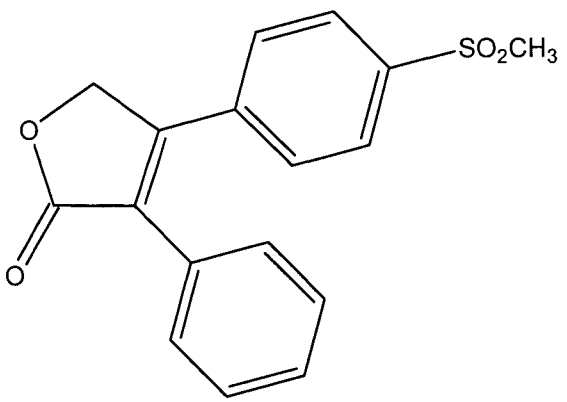

Figure 1-5. Chemical structure of rofecoxib.

Rofecoxib is advocated in the treatment of the signs and symptoms of osteoarthritis and RA and relief of pain (150). The recommended starting daily dose in

treatment of the osteoarthritis is 12.5 mg, which may be increased to 25 mg once a daily (131). The recommended daily dose in treatment of RA is reported to be 25 mg (96). The recommended initial daily dose in relief of acute pain is 50 mg, but this dose should be limited to a short period (131). In September 2004, Merck & Co., Inc voluntarily pulled out the drug from market because of the increased risk of cardiovascular events (151).

1.1.12.1 Pharmacokinetics of Rofecoxib in the Rat

Halpin and at el. (152), who administered intravenous and oral doses of rofecoxib to rats, reported that rofecoxib showed a high and rapid absorption at examined oral doses. The $t_{1/2}$ of drug ranged from 4 to 6 hours. Although AUC was increased proportional to administered intravenous doses, this relationship was not linear after oral doses. Increasing the dose from 5 to 10 mg/kg did not yield to an increase in AUC, suggesting of the low solubility of drug at higher doses. The fu value of the drug was 7%. Following oral administration of rofecoxib, 5 mg/kg, to two rats, the oral CL was reported to be between 29 and 34 mL/min/kg (153). The mean V_d of drug was 6.2 L/kg.

The drug is mainly metabolized in the liver (152). The major metabolite is identified as 5-hydroxyrofecoxib-*O*-β-D-glucuronide. Baillie et al. (154) have observed a secondary plasma concentration peak following administration of rofecoxib indicating enterohepatic recirculation of drug. Following intravenous administration of radiolabeled rofecoxib, radioactivity was observed in many tissues in 5 minutes (152). The concentration of drug was 2-fold higher in kidney compared to that of plasma at 5 and 30 minutes and 2 and 24 h post-dose.

1.1.12.2 Clinical Pharmacokinetics of Rofecoxib

Rofecoxib is well absorbed through the human gastrointestinal tract (150). The apparent V_d and CL of drug are reported to be 86 L and 7.3 L/h, respectively, following a 25 mg oral dose (155). Following administration of 12.5 mg rofecoxib to healthy subjects, the C_{max} and the t_{max} were 147 ± 34 μg/L and 2.4 ± 1.0, respectively. The $t_{1/2}$ of drug was calculated to be 9.0 ± 2.7 h (156). Depre et al. (157) who administered single and multiple doses of 25, 100, 250, or 375 mg rofecoxib to 31 healthy human subjects, observed linear kinetics within the 25 to 100 mg dosage range. For doses more that 100 mg, the relationship between dose and AUC was not linear that was speculated to be due the low solubility of drug in aqueous media. Rofecoxib is highly bound to the plasma proteins 87% (150) and mainly metabolized by cytosolic enzymes to the cis-dihydro and trans-dihydro derivatives (158). CYP plays a minor role in metabolism of rofecoxib (150).

Rofecoxib did not have any effect on pharmacokinetics of methotrexate in the patient diagnosed with RA (159). The effect of different doses of rofecoxib on the pharmacokinetics of warfarin was studied by Schwartz et al. (160). They observed no change in the pharmacokinetics of active enantiomer, S(-) warfarin. However, rofecoxib increased the AUC of less active enantiomer, R(+) warfarin. It has been shown that R(+) warfarin, but not S(-) warfarin, is metabolized by CYP1A2. Since Rofecoxib inhibited CYP1A2, they suggested that elevated concentrations of R(+) warfarin was attributed to inhibition of enzyme. Rofecoxib did not influence the pharmacokinetics digoxin (161) or prednisone (162) in healthy subjects.

1.1.13 Celecoxib

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (Figure 1-6), is a diaryl-substituted pyrazole with a molecular weight of 381.38 (128). The log P value of celecoxib is reported to be 3.68, indicating high affinity of drug for hydrophobic environments (163).

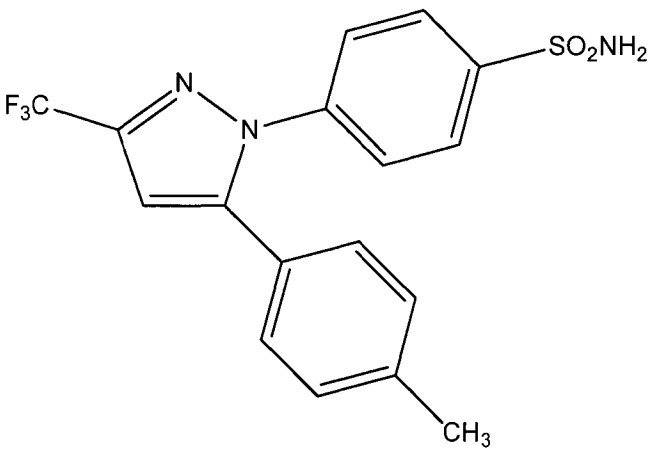

Figure 1-6. Chemical structure of celecoxib.

Celecoxib is indicated for relief of the signs and symptoms of osteoarthritis and RA in adult. At equivalent therapeutic doses, naproxen and ibuprofen have shown more efficacy than celecoxib in relief of dental pain (164). The recommended daily dose of celecoxib to relief osteoarthritis is 200 mg/day. The recommended daily dose of celecoxib for the relief of RA is 100 mg twice a day that may be increased to 200 mg twice a day (131).

1.1.13.1 Pharmacokinetics of Celecoxib in the Rat

After oral administration of celecoxib, 5 mg/kg, to the normal rats, the $t_{1/2}$ of drug was reported to be 3.1 ± 0.8 h (165). Celecoxib had a large V_d (5.5 ± 0.5 L/kg) and low extraction ratio. The CL of drug was 10 ± 1.7 mL/min/kg. The AUC of drug after

intravenous, intraperitoneal, and oral administration of drug is reported to be 9.3 ± 1.8, 6.6 ± 1.7, and, 4.6 ± 2.2 μ.h/mL, respectively. The involvement of first pass CL was confirmed by lower values of AUC after intraperitoneal and oral administration compared with intravenous. Given that the difference between the AUC of intraperitoneal and oral administration was not significant, the authors were not confident if gut had any effect on metabolism of drug. Although the binding of celecoxib to plasma proteins is reported to be linear over the concentration range of 0.1 to 3.0 μg/mL, the binding was decreased at 10 μg/mL due to the saturation of protein binding sites (166).

Paulson et al. (167) have reported a gender difference in the pharmacokinetics of celecoxib. Intravenous administration of celecoxib resulted in a shorter $t_{1/2}$, higher CL, and lower AUC in males as compared with females. This can be explained with the sex-specific expression of rat *CYP2C* and *CYP3A* genes.

1.1.13.2 Clinical Pharmacokinetics of Celecoxib

Paulson et al. (168) have categorized celecoxib as a drug with low solubility and high permeability. Therefore, it is expected that administration of drug with food improve the systemic availability of drug as a consequence of delaying in gastric empting. However, the 10% increase in AUC of drug observed in fed conditions was not considered to be clinically significant. They suggested that celecoxib could be absorbed through out the human GI tract. This would allow celecoxib to achieve complete absorption even in fasted conditions. Linear pharmacokinetics of celecoxib, within the range of 100 to 800 mg, was reported by McAdam et al. (169). Celecoxib is highly bound to the plasma proteins (170). The elimination $t_{1/2}$ of drug is reported to be between 11.2-15.6 h. Following oral administration of celecoxib, the apparent V_d and CL of drug are reported

to be 400 L and 30 L/h, respectively (170). Celecoxib is extensively metabolized. The first step in metabolism of celecoxib is hydroxylation of methyl group. Then, hydroxyl group is changed to carboxylic acid group. This moiety is a substrate for conjugation with glucuronide. Only 2.5% of drug is excreted unchanged (171).

The kinetics of methotrexate was not influenced when celecoxib was co-administered in patients with RA (172).

It is shown that celecoxib increases the international normalized ratio in a patient who received celecoxib along with warfarin, indicating elevation in anticoagulant effects of warfarin (173). However, Karim et al. (174) did not detect any significant change in pharmacokinetics of warfarin and prothrombin times of healthy patient who were on celecoxib and warfarin.

1.1.14 Meloxicam

Meloxicam, 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide (Figure 1-7), is a pastel yellow solid, practically insoluble in water (128). Meloxicam is present as an anion in the pH more than 4 and a zwitterion in the pH range 1 to 4 (175). The log P of meloxicam is reported to be 0.1 at pH 7.4 (128).

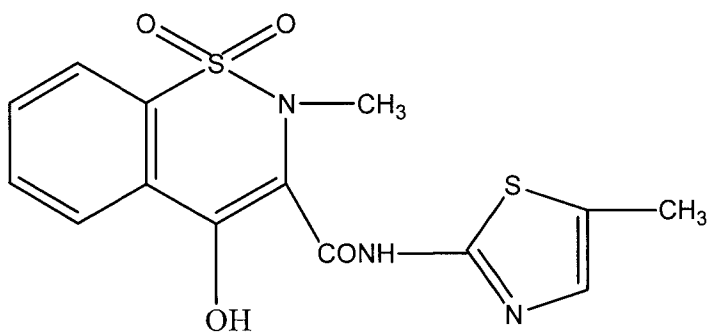

Figure 1-7. Chemical structure of meloxicam.

Meloxicam is used in treatment of RA, osteoarthritis, and ankylosing spondylitis (175). The usual recommended dosage of meloxicam for treatment of osteoarthritis is 7.5, which can be increased to 30 mg/day. 15 mg/day is taken by patients to relief the signs of RA (131).

1.1.14.1 Pharmacokinetics of Meloxicam in the Rat

The oral absorption and protein binding of meloxicam are reported to be 95 and 99%, respectively (176). After intravenous administration of meloxicam to rats, some gender-specific differences in pharmacokinetic parameters of drug were found. Elimination $t_{1/2}$ was 13.4 hr and 36.8 h for male and female rats, respectively. The female $AUC_{0-\infty}$ values were three times higher than those of males. The CL values were 0.015 L/hr/kg and 0.005 L/hr/kg for male and female rats, respectively. This can be explained by the metabolic pathway of meloxicam. 5'-hydroxymethyl derivative and a 5'-carboxy derivative are the major metabolites of meloxicam in rat and human (177). While CYP2C9 is the enzyme which largely produces 5'-hydroxymethyl derivative in human, *CYP2C7* and *CYP2C11* are two enzymes which perform the same function in the rat (176). The lack of *CYP2C11* in female rats may explain sex-specific differences in meloxicam pharmacokinetics. The steady-state volume of distribution (V_{ss}) of the male rats was not significantly different from that of the female rats, 0.257 versus 0.244 L/kg, respectively (176).

1.1.14.2 Clinical Pharmacokinetics of Meloxicam

Bioavailability of meloxicam after oral dose was 89% (175). Linear kinetics were observed after oral administration of 7.5-30 mg meloxicam to healthy subjects (178). The V_{ss} of meloxicam following intravenous dose was 0.2 L/kg (179). The elimination $t_{1/2}$ and

CL of drug are reported to be 20 h and 7-8 ml/min, respectively. Meloxicam is strongly bound to plasma protein (99.1-99.7%) (179). In addition to CYP2C9, which plays the major role, CYP3A4 is involved in the metabolism of meloxicam in human (180). DS-AC 2 SE, AF-UH, UH-AC 110 SE, and BI-BO 8032 NA are 4 inactive metabolites of meloxicam that are produced by oxidation of the 5-methyl moiety of thiazolyl ring or oxidative cleavage of the benzothiazine ring (175).

Co-administration of meloxicam with cimetidine, antacid, aspirin, beta-acetyldigoxin, methotrexate, warfarin or forusemide has not revealed any significant interaction (181).

1.2 Thesis Rationale

The use of traditional NSAIDs has been associated with the renal side effects such as ARF, RPN, hyperkalemia, and sodium retention (66). Although the development of coxibs have introduced the NSAIDs with less GI side effects to the market (125), there is no evidence to support the notion that coxibs have fewer renal effects (66). The degree of renal dysfunction associated with the use of NSAIDs may be correlated to the COX-2/COX-1 selectivity or pharmacokinetic parameters of NSAIDs.

It has been shown that patients with RA develop glomerular dysfunction characterizes by a reduction in GFR and creatinine CL throughout the disease course (182). Since selective COX-2 inhibitors are used by many arthritic patients to reduce pain and inflammation, patients may be more susceptible to kidney dysfunction induced by NSAIDs. Furthermore, inflammation is reported to alter pharmacokinetics of many drugs cleared by the liver (165). The effect of inflammation on CL of drugs is attributed to elevation of plasma protein binding or reduction in intrinsic hepatic metabolism (183).

Therefore, inflammation may change the pharmacokinetic profile of NSAIDs taken by arthritic patients.

1.3 Hypotheses

1. COX-2-selective inhibitors have renal side effects similar to the traditional NSAIDs.
2. COX-2/COX-1 selectivity is related to the degree of renal dysfunction associated with the use of selective COX-2 inhibitors.
3. The extent of renal side effect produced by selective COX-2 inhibitors is linked to the accumulation of drug in the kidney.
4. There is an association between pharmacokinetics and the extent of renal dysfunction.
5. Inflammation changes the kidney function.
6. Inflammation influences the pharmacokinetics of rofecoxib.
7. The distribution of drug into kidney is altered by inflammation.

1.4 Objectives

1. To evaluate the renal effects of selective COX-2 inhibitors.
2. To evaluate the correlation between renal dysfunction associated with selective COX-2 inhibitors and COX-2/COX-1 selectivity or degree of renal accumulation.
3. To evaluate the effect of inflammation on kidney function.
4. To evaluate the consequences of inflammation on Pharmacokinetics of rofecoxib and meloxicam.
5. To evaluate the degree of drug distributing into the kidney in presence or absence of inflammation.

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Chapter 2[1]

Effect of Nonsteroidal Anti-inflammatory Drugs with Varying Extent of COX-2/COX-1 Selectivity on Urinary Sodium and Potassium Excretion in the Rat[2]

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[1] **This chapter is dealt with two hypotheses that COX-2-selective inhibitors have renal side effects similar to the traditional NSAIDs and COX-2/COX-1 selectivity is related to the degree of renal dysfunction associated with the use of selective COX-2 inhibitors.**

[2] **A version of this chapter has been published. Harirforoosh S, Jamali F. Can J Physiol Phramacol 2005; 83:85-90.**

2.1 Introduction

Cyclooxygenase (COX) is the enzyme that converts arachidonic acid to prostaglandin H_2 (PGH_2), which produces prostanoid including PGs, PGI_2 (prostacyclin), and thromboxanes (TXs) (1). Two isoforms of COX have been identified: COX-1 and COX-2 (2). COX-1 is present in platelets (3) and many other tissues including kidney, stomach, and endothelium (4). COX-1 produces a class of PGs that are involved in the normal physiological functions such as gastric mucosal protection and blood clotting (3). COX-2 is induced by inflammatory conditions, growth factors, and cytokines (4). It is primarily responsible for prostanoid synthesis that mediates the propagation of inflammation, pain, and fever. COX-2 is also expressed constitutively in few organs including the brain and kidney (3). COX-2 is present in macula densa, thick ascending limbs, and papillary interstitial cells of rat kidneys and in glomerular podocytes and small blood vessels of human kidneys (5). A recent study in human has detected COX-2 in the macula densa of patients with congestive heart failure and patients with Bartter-like syndrome (6).

It has been shown that COX-2-selective inhibitors cause less gastrointestinal (GI) side effects compared to that of non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) (7). However, they are expected to demonstrate as much renal side effects as other NSAIDs. The use of NSAIDs is associated with several renal side effects including interference with fluid and electrolyte homeostasis, acute renal failure, interstitial nephritis, nephritic syndrome, and renal papillary necrosis (5).

The literature contains some studies that compare COX-2-selective NSAIDs with other drugs in its class. In one study, patients who took celecoxib experienced less renal toxicity than those who received other NSAIDs (8). It has been reported that rofecoxib

had more renal side effects than celecoxib or nonselective NSAIDs (9). Since rofecoxib is the more COX-2-selective than other examined NSAIDs, the greater observed potency may be attributed to its high selectivity. However, it has been shown that both celecoxib and rofecoxib cause renal side effects comparable with the traditional NSAIDs (10).

In the present study, we compared the effect of a selection of therapeutically equivalent doses of NSAIDs (i.e., rofecoxib, celecoxib, meloxicam, diclofenac, and flurbiprofen) with a wide range of COX-2 selectivities, on sodium and potassium excretion as well as urine flow rate.

2.2 Methods

2.2.1 Chemicals

Tablets of rofecoxib (Vioxx®; Merck Frosst, QC, Canada), meloxicam (Mobicox®; Boehringer Ingelheim, ON, Canada), and capsules of celecoxib (Celebrex®; Searle, MI, USA) were obtained from a local pharmacy in Edmonton. Diclofenac and flurbiprofen were purchased from Sigma (St. Louis, MO, USA). Methyl cellulose 4000 was purchased from BDH pharmaceuticals (Toronto, ON, Canada).

2.2.2 Animals

All experiments were performed on male Sprague-Dawley rats (320-350 g) and carried out within the guidelines of the Animal Care Committee of University of Alberta. Rats were fed with normal sodium chloride diet (0.4% NaCl). Rats were deprived of food but had free access to tap water for the 8-h duration of the experiment and housed at ambient temperature and humidity with a 12-h light-dark cycle.

2.2.3 Dosage Forms and Administration

Tablets of Vioxx or Mobicox were crushed into a fine powder. Crushed tablets of Vioxx or Mobicox, the content of Celebrex capsules, diclofenac powder, and flurbiprofen powder were suspended in 0.5% methyl cellulose suspension and administered by oral gavage.

2.2.4 Selection of NSAIDs and Dosage Regime

As a measure of COX-2/COX-1 activity, we used data reported by Warner et al. (11). The authors have reported both COX-1 and COX-2 inhibitory concentrations at 50% and 80% levels, using 2 different methods (human whole blood assay and human modified whole blood assay). We used all reported COX-2/COX-1 activities by these authors to examine the possibility of a significant correlation between the latter and the urinary excretion of electrolytes.

The doses in this study were chosen to produce sufficient changes in electrolytes excretion to form a basis for comparison among different NSAIDs. The recommended dose of rofecoxib for chronic treatment of rheumatoid arthritis (RA) is 25 mg once daily (12). The area under the plasma concentration-time curve from time of dosing to 24 h post-dose (AUC_{0-24}) following a single 25 mg dose of rofecoxib is reported to be 2941 ng.h/mL for healthy young male volunteers (13). Among rats, an oral dose of 10 mg/kg of rofecoxib yields an AUC_{0-24} of 2963 ng.h/mL (14). Hence, we began our study with 10 mg/kg of rofecoxib. Although we could detect a significant decrease of 58% in sodium excretion as compared with placebo, the change for potassium excretion was not significant in the treated rats with rofecoxib (Figure 2-1). Therefore, we increased the

dose of rofecoxib to 30 mg/kg. Other NSAIDs were administered in doses therapeutically equivalent to that of 30 mg/kg rofecoxib (15).

To test the baseline urine volume rate and sodium and potassium excretion, rats were transferred to metabolic cages and urine samples were collected at 0 to 8 h on day 0. On day 1, 2, 3, and 4, animals received rofecoxib (30 mg/kg, n=8), celecoxib (120 mg/kg, n=6), meloxicam (9 mg/kg, n=6), diclofenac (30 mg/kg, n=3), or flurbiprofen (125 mg/kg, n=6). Control groups received 0.5% methyl cellulose as vehicle. Urine was collected up to 8 h after each dosing. Renal function was assessed by measurement of urinary sodium and potassium excretion using a NOVA Stat Profile Plus 9 analyzer (NOVA Biomedical, Waltham, MA, USA). The origin of measurement was based on the electrical charge produced by exchange of sodium or potassium ions through the membrane which were selective to specific electrolyte.

2.2.5 Data Treatment and Statistical Analysis

Data are expressed as mean ± standard error (SE). The 0-8 h urine flow rate, urinary sodium and potassium excretion, and the means of the latter indices for 1-4 days were calculated. The values calculated for the treatment groups were compared with those of respective controls using the two-tailed Student's t test. For comparison between more than 2 means analyses of variances followed by Duncan New Multiple Range test were used. The association between cyclooxygenase selectivity and sodium or potassium excretion was evaluated using linear regression analysis. Statistical significance was set at $p < 0.05$. Urinary electrolyte excretion (μmol/min/100g) was calculated from: $C_X * V_X * 100 / T * W$, where C_X is the concentration of urine electrolyte (mmol/L), V_X is the urine volume (mL), T is the urine collection time (min), and W is the weight of each rat (g).

Urine flow rate (mL/h/100g) was estimated from: $V_X * 100 / T * W$, where V_X is the urine volume (mL), T is the urine collection time (h), and W is the weight of each rat (g).

2.3 Results

The 4-day mean sodium and potassium excretion of control groups ranged from 0.41 ± 0.03 to 0.48 ± 0.04 and 0.62 ± 0.03 to 0.72 ± 0.06 μmol/min/100g, respectively (Figures 2-2 and 2-3).

The mean 1-4 day sodium excretion was significantly less in rats treated with rofecoxib ($p < 0.0011$), celecoxib ($p < 0.0001$), diclofenac ($p < 0.0001$) and flurbiprofen ($P < 0.0001$) as compared with respective control groups (Figure 2-2). Administration of meloxicam did not reduce sodium excretion significantly. As shown in Table 2-1 for individual days, there was a significant reduction from baseline in sodium excretion at least on the first day of treatment with rofecoxib, celecoxib, diclofenac and flurbiprofen.

The urinary potassium excretion was significantly reduced following rofecoxib ($p < 0.05$), celecoxib ($p < 0.01$), diclofenac ($p < 0.0001$), and flurbiprofen ($p < 0.0001$) treatment (Figure 2-3). Treatment with meloxicam did not change potassium excretion significantly. For individual days, potassium excretion was significantly decreased from baseline, at least at one point, after administration of diclofenac or flurbiprofen. However, the daily fluctuation in the electrolyte excretion was not significant following rofecoxib, celecoxib administration. Meloxicam did not change potassium excretion values from the baseline on any specific day (Table 2-1).

The average of 1-4 days urine flow rate values was significantly lower in rofecoxib and flurbiprofen groups compared with their respective control group. The differences did not reach significance for celecoxib, meloxicam, and diclofenac groups

(Figure 2-4). For individual days, the difference from baseline was significant in diclofenac group only on the fourth day of treatment (Table 2-1).

Correlation coefficient values for COX-2/COX-1 selectivity using data generated by Warner et al. (11), using two different methods, did not exceed 0.41 ($p > 0.49$). In addition, we did not find a correlation between COX-1 or COX-2 potency reported by Warner et al. (11) and percentage change in electrolytes excretion.

2.4 Discussion

The renal effects of NSAIDs are most commonly characterized by decreases in sodium and potassium excretion or renal perfusion (16). In healthy elderly subjects on a controlled intake of sodium, administration of 50 mg of rofecoxib once daily or 50 mg of indomethacin three times daily decreased urinary sodium excretion during the first 72 h of treatment compared with baseline. However, the glomerular filtration rate (GFR) decreased only in the indomethacin group. Given the known renal effects of NSAIDs, it seems likely that COX-2 inhibition causes acute sodium retention, whereas the decline in GFR is attributable to the blockade of COX-1 (17). Moreover, the expression of COX-2 in thick ascending limb cells containing Na-K-ATPase suggests a role for the enzyme in regulation of sodium in the rat kidney (18). Hyperkalemia due to the suppression of the renin–aldosterone axis is linked to the use of NSAIDs. In addition, a decrease in GFR may reduce potassium excretion (19). Accordingly, inhibition of both COX-1 and COX-2 can lead to decreases in sodium and potassium excretion. The severity of the decrease, therefore, may be governed by the degree of inhibition and the extent of drug in the site of action.

Several studies have evaluated the kidney effects of selective COX-2 inhibitors. Since the design and focus of each study has been different, particularly with respect to the renal effect, it is difficult to compare the result from one study to another. Svendsen et al. (20) compared the effects of a 2-week treatment with etodolac and ibuprofen on renal function of healthy subjects. They did not find any significant difference among treated groups in sodium or potassium excretion. Dilger et al. (21) have examined the effects of 200 mg twice daily celecoxib and diclofenac 75 mg twice daily on renal function of young and elderly subjects. They reported a transient decrease in sodium excretion in young subjects on the third day of treatment with both celecoxib and diclofenac. Schwartz et al. (12) reported a significant decrease in urinary sodium excretion on the first day of treatment with rofecoxib 25 mg daily, celecoxib 200 mg twice daily, and naproxen 500 mg twice daily in elderly subjects. One reason for the inconsistency between these studies could be the time of urine collection. The renal effects NSAIDs are best observed during the first 48 h of the commencement of the therapy. Hence, in this study, we compared the time course of the kidney effects of a range of NSAIDs with different degrees of COX-2 selectivity. We found a significant overall decrease in sodium and potassium excretion in rats treated with rofecoxib, celecoxib, diclofenac, or flurbiprofen as compared with their respective control groups during the first 4 days of NSAID therapy (Figures 2-2 and 2-3). As shown in Table 2-1, sodium excretion was decreased starting from the first day and continued throughout the treatment for diclofenac and flurbiprofen. The effect of celecoxib was greatest on the first day of treatment, but it was reduced to an insignificant reduction until the fourth day. Rofecoxib's effect was only significant on the first day. For potassium excretion,

although the overall effect was significant in the rofecoxib, celecoxib, diclofenac, and flurbiprofen groups, when the individual day values were considered, the significance of treatment effect was lost for rofecoxib and celecoxib groups (Table 2-1).

We did not detect any significant change in sodium or potassium excretion in rats treated with meloxicam even though a relatively high dosage regimen was used. We do not have an equivocal explanation for this unique behavior of meloxicam. One plausible explanation for our finding may be low concentrations of meloxicam in the kidney as compared with other NSAIDs. Accumulation in the kidneys is believed to be responsible for renal toxicity of other classes of drugs such as aminoglycosides antibiotics. A significant association has been observed between aminoglycosides concentrations in the kidneys and reduced renal function (22;23). It has been suggested that aminoglycosides are taken up by renal proximal tubular cells through a receptor-mediated indocytosis (24). The authors have demonstrated that blockage of these receptors with other substrates and (or) their peptide fragments such as megalin might be useful in preventing aminoglycoside-induced nephrotoxicity. They demonstrated that megalin substrates such as cytochrome *c* not only prevent gentamicin-induced nephrotoxicity, they reduce accumulation of gentamicin in the renal cortex, probably through decreasing gentamicin binding of to megalin. Consequently, urinary excretion of N-acetyl-β-D-glucosaminidase, a marker of renal tubular damage, was significantly reduced. Since meloxicam is not unique from other NSAIDs with regard to its cyclooxygenase activities, it is plausible to suggest that a lack of sufficient accumulation in the kidneys may explain its lack of significant effect on the excretion of the examined electrolytes.

In this study, we used a series of NSAIDs with a range of COX-2 selectivities. At the dosage range used, we found no significant correlation between urinary electrolytes excretion and COX-2/COX-1 selectivity. Similarly, among the three examined selective COX-2 inhibitors, the degree of selectivity does not appear to be governed by the renal effect following administration of the selected doses. Indeed, celecoxib, which has a COX-2/COX-1 selectivity of 1.43 based on 50% inhibitory concentrations, significantly reduced both sodium and potassium excretion, but meloxicam with a COX-2/COX-1 selectivity of 2.70 did not. The observed lack of association between COX-2/COX-1 selectivity or COX-2 potency and urinary electrolytes excretion, however, may not be conclusive since it was made at only one dose level for each NSAID. It is, therefore, unclear whether the doses used were within the ascending or plateau phase of the dose-effect curve. Nevertheless, the fact that meloxicam, even at relatively high dose, did not influence urinary electrolytes excretion is worthy of noting and presents a potentially important therapeutic observation.

In summary, our data suggest that NSAIDs have a transient and time-dependent effect on urinary excretion of electrolytes that may be independent of COX-2/COX-1 selectivity. In addition, meloxicam does not affect sodium or potassium excretion rates in the rat.

Table 2-1. Change from baseline in sodium excretion, potassium excretion, and urine flow rate/100g bodyweight after administration of rofecoxib (30 mg/kg, n=8), celecoxib (120 mg/kg, n=6), meloxicam (9 mg/kg, n=6), diclofenac (30 mg/kg, n=3), or flurbiprofen (125 mg/kg, n=6).

NSAID	Baseline	Day 1	Day 2	Day 3	Day 4
Sodium excretion (μmol/min/100g)					
Rofecoxib	0.42 ± 0.04	0.22 ± 0.05*	0.29 ± 0.05	0.30 ± 0.04	0.30 ± 0.05
Celecoxib	0.38 ± 0.02	0.13 ± 0.02*	0.31 ± 0.05	0.27 ± 0.10	0.20 ± 0.05*
Meloxicam	0.41 ± 0.03	0.27 ± 0.06	0.54 ± 0.04	0.54 ± 0.07	0.32 ± 0.06
Diclofenac	0.45 ± 0.08	0.13 ± 0.05*	0.05 ± 0.03*	0.13 ± 0.04*	0.05 ± 0.02*
Flurbiprofen	0.46 ± 0.07	0.17 ± 0.02*	0.09 ± 0.05*	0.14 ± 0.07*	0.03 ± 0.02*
Potassium excretion (μmol/min/100g)					
Rofecoxib	0.68 ± 0.13	0.62 ± 0.11	0.48 ± 0.05	0.58 ± 0.06	0.53 ± 0.05
Celecoxib	0.64 ± 0.04	0.48 ± 0.09	0.51 ± 0.08	0.56 ± 0.19	0.45 ± 0.12
Meloxicam	0.66 ± 0.06	0.51 ± 0.12	0.67 ± 0.04	0.81 ± 0.11	0.44 ± 0.04
Diclofenac	0.70 ± 0.06	0.43 ± 0.05	0.33 ± 0.01*	0.25 ± 0.09*	0.09 ± 0.06*
Flurbiprofen	0.71 ± 0.08	0.53 ± 0.08	0.33 ± 0.05*	0.30 ± 0.07*	0.18 ± 0.05*
Urine flow rate (mL/h/100g)					
Rofecoxib	0.19 ± 0.02	0.17 ± 0.02	0.13 ± 0.03	0.18 ± 0.02	0.16 ± 0.02
Celecoxib	0.19 ± 0.01	0.12 ± 0.02	0.19 ± 0.03	0.12 ± 0.03	0.14 ± 0.04
Meloxicam	0.22 ± 0.02	0.12 ± 0.03	0.23 ± 0.02	0.24 ± 0.04	0.16 ± 0.02
Diclofenac	0.24 ± 0.08	0.14 ± 0.04	0.17 ± 0.06	0.22 ± 0.04	0.05 ± 0.01*
Flurbiprofen	0.21 ± 0.03	0.16 ± 0.02	0.19 ± 0.04	0.19 ± 0.05	0.18 ± 0.04

Note: The values are expressed as mean ± standard error.

*Significantly different from baseline ($p < 0.05$).

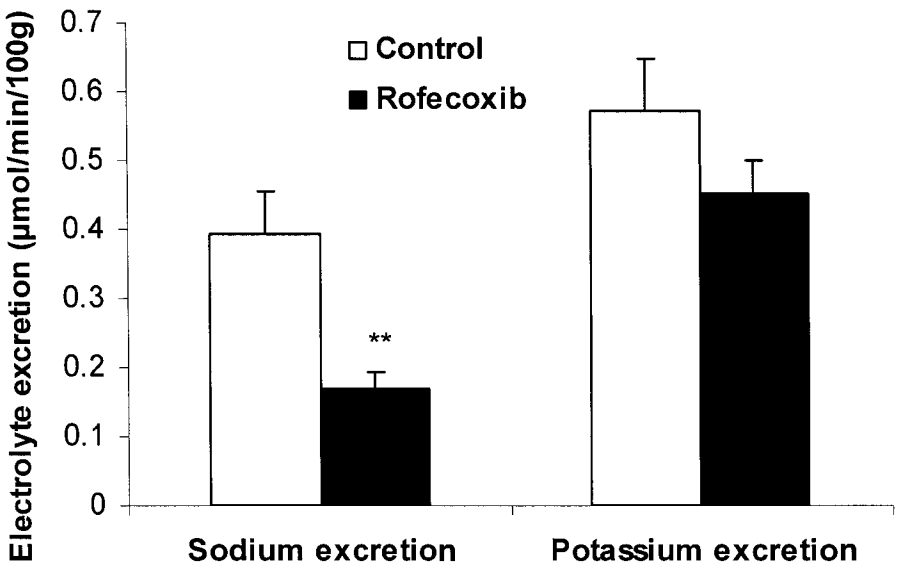

Figure 2-1. The effect of low dose of rofecoxib (10 mg/kg) on sodium and potassium excretion /100g bodyweight.

The values are expressed as mean ± standard error.

Values represent the average of measurements of days 1-4 (n=5).

**Significantly different from control ($p < 0.01$).

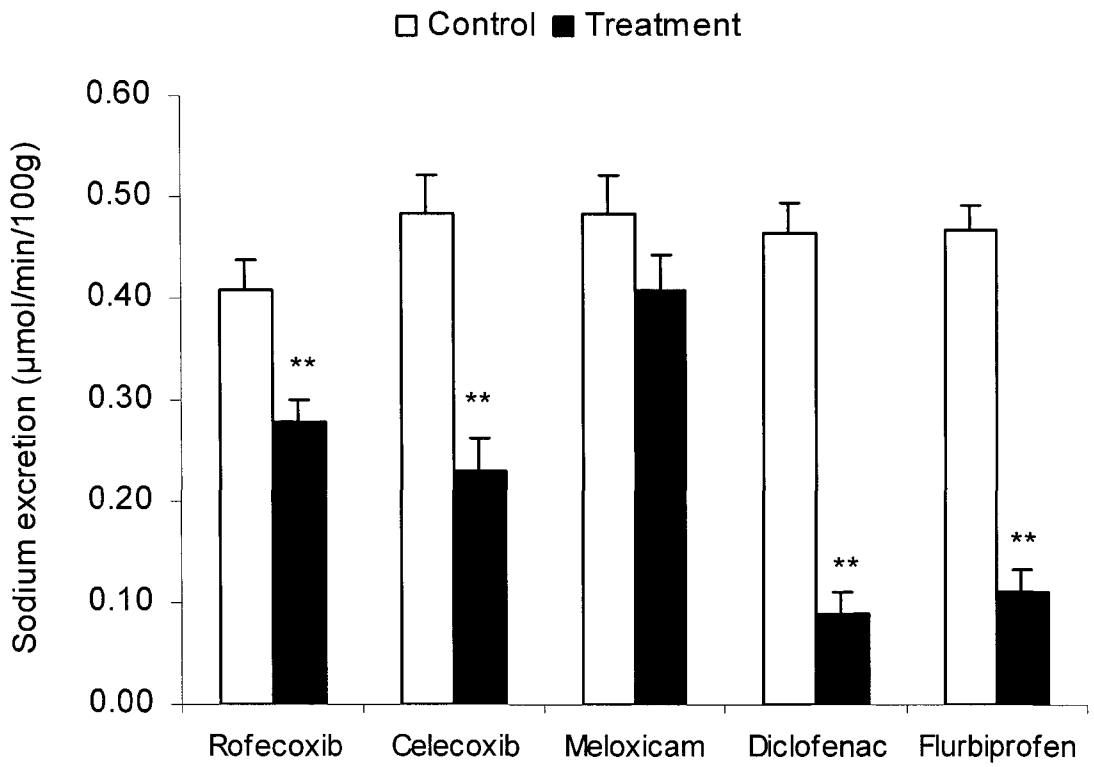

Figure 2-2. The effect of rofecoxib (30 mg/kg, n=8), celecoxib (120 mg/kg, n=6), meloxicam (9 mg/kg, n=6), diclofenac (30 mg/kg, n=3), or flurbiprofen (125 mg/kg, n=6) on sodium excretion/100g bodyweight.

The values are expressed as mean ± standard error.

Values represent the average of measurements of days 1-4.

**Significantly different from control ($p < 0.01$).

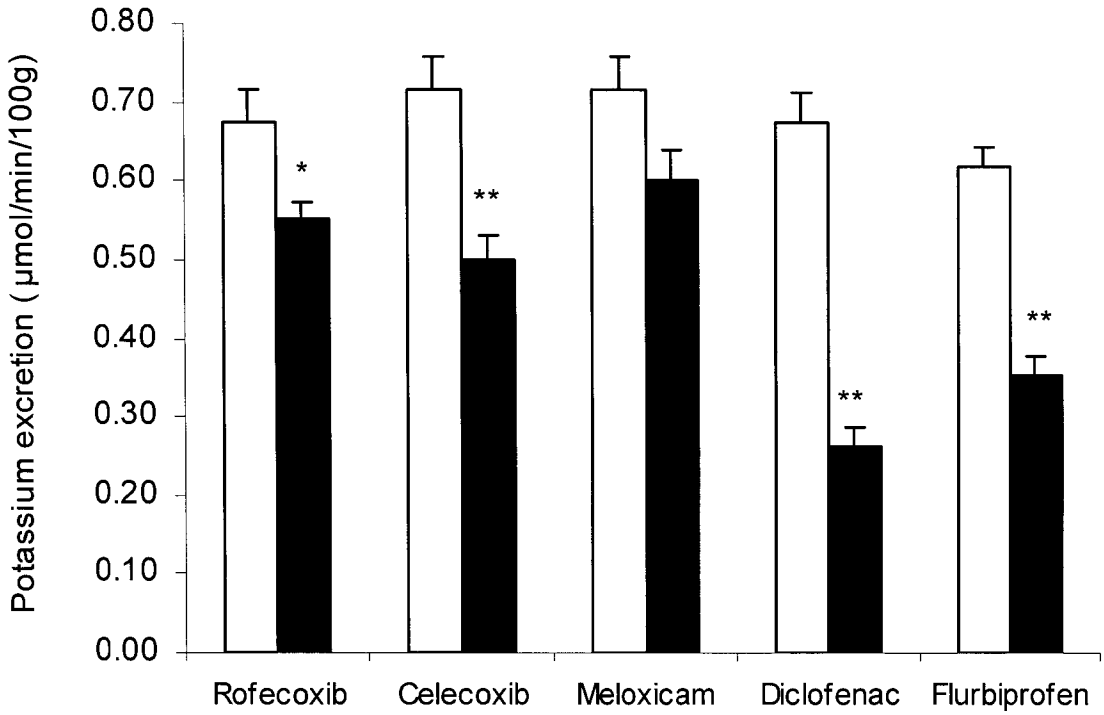

Figure 2-3. The effect of rofecoxib (30 mg/kg, n=8), celecoxib (120 mg/kg, n=6), meloxicam (9 mg/kg, n=6), diclofenac (30 mg/kg, n=3), or flurbiprofen (125 mg/kg, n=6) on potassium excretion/100g bodyweight.

The values are expressed as mean ± standard error.

Values represent the average of measurements of days 1-4.

*Significantly different from control ($p < 0.05$).

**Significantly different from control ($p < 0.01$).

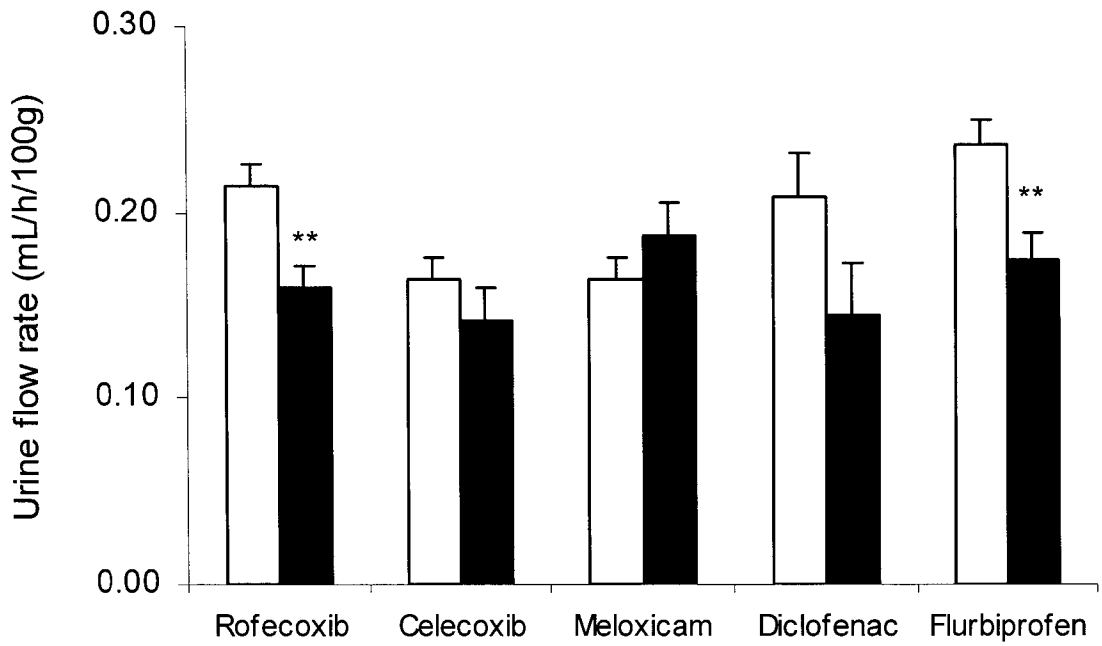

Figure 2-4. The effect of rofecoxib (30 mg/kg, n=8), celecoxib (120 mg/kg, n=6), meloxicam (9 mg/kg, n=6), diclofenac (30 mg/kg, n=3), or flurbiprofen (125 mg/kg, n=6) on urine flow rate/100g bodyweight.

The values are expressed as mean ± standard error.

Values represent the average of measurements of days 1-4.

**Significantly different from control ($p < 0.01$).

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Chapter 3[3]

The Extent of Renal Effects of COX-2-selective Inhibitors Is Pharmacokinetic-dependent

[3] **This chapter is dealt with the hypothesis that there is an association between the pharmacokinetics of COX-2-selective inhibitors and the extent of renal dysfunction.**

3.1 INTRODUCTION

At least two isoforms of cyclooxygenase (COX), COX-1 and COX-2, have been identified and one being discussed (1). Nonsteroidal anti-inflammatory drugs (NSAIDs) have different selectivity to inhibit two enzymes (2). The selective COX-2 inhibitors such as celecoxib, rofecoxib, meloxicam, and valdecoxib, demonstrate less gastrointestinal (GI) side effects than non-selective NSAIDs (3;4), and have no effect on platelet function at therapeutic doses (5). Other side effects of NSAIDs include sodium and potassium retention secondary to renal effects (6;7) as well as hypertension (8). The latter side effects appeared to be linked (8;9). This is in line with the reported association between reduced urinary sodium excretion and myocardial infarction (MI) observed in 2937 mildly and moderately hypertensive patients (10). There exists a controversy regarding a potential differences between selective COX-2 and other NSAIDs in causing electrolytes retention (6). However, after reviewing five human studies, Sandhu Heyneman (11) concluded that the selective COX-2-inhibitors, celecoxib and rofecoxib, may decrease sodium and potassium excretion, creatinine clearance, and glomerular filtration rate (GFR) with the same degree as those of other NSAIDs. We have also shown that, in the rat, selective COX-2 inhibitors, rofecoxib and celecoxib, but not meloxicam, as well as non-selective NSAIDs, diclofenac and flurbiprofen, cause reduction in sodium and potassium excretion rate, independent of their COX selectivity (7). In the latter work, we speculated that the observed difference among NSAIDs in reducing electrolytes excretion may be explained by differences in the pharmacokinetics of the drugs. In this study, we report the results of our attempts to correlate the renal effect with pharmacokinetics of three selective COX-2 NSAIDs.

3.2 METHODS

3.2.1 Chemicals

Tablets of rofecoxib (Vioxx®; Merck Frosst, QC, Canada) and meloxicam (Mobicox®; Boehringer Ingelheim, ON, Canada) and capsules of celecoxib (Celebrex®; Pharmacia Canada Inc., Mississauga, Canada) were purchased from a local pharmacy in Edmonton. Racemate ibuprofen was obtained as a gift from Upjohn Canada (Don Mills, Canada). Piroxicam and ketoprofen powders were obtained from Sigma (St. Louis, MO, USA). Meloxicam powder was purchased from Unichem Laboratories Limited (Mumbai, India). Isooctane (Assurance grade) was purchased from Merck KGaA (Darmstadt, Germany). All other solvents were high performance liquid chromatography (HPLC) grade and purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Methyl cellulose 4000 was purchased from BDH pharmaceuticals (Toronto, ON, Canada). All other chemicals were of analytical or HPLC grade.

3.2.2 Drugs, Dosage Forms and Route of Administration

Tablets of Vioxx or Mobicox were crushed to make a fine powder. Crushed tablets or the content of celebrex capsules were suspended in an aqueous solution containing methylcellulose 0.5%, and administered *via* gastric intubations. The drug content of the formulations was confirmed using HPLC.

In the previous study (7), we reported that a dose of 10 mg/kg rofecoxib was sufficient to produce changes in sodium excretion rate on the day after treatment compared to that of baseline. Therefore, we chose that dose and administered celecoxib and meloxicam in doses therapeutically equivalent to that of 10 mg/kg rofecoxib (12).

3.2.3 Experimental Design and Sample Collection

All experiments were performed on male Sprague-Dawley rats (330-360 g) and carried out within the guidelines of the Animal Care Committee of the University of Alberta.

On day 0, under halothane anesthesia, a polyethylene cannula was inserted into the right jugular vein of all rats. Rats were allowed to recover overnight and fed with normal sodium chloride diet (0.4% NaCl). They were housed in ambient temperature and humidity with a 12 h light-dark cycle.

On day 1, rats were transferred into the metabolic cages at approximately 9:00 A.M. and urine samples were collected during the 0-8 h period.

On day 2, the animals received rofecoxib (10 mg/kg, n = 13), celecoxib (40 mg/kg, n = 7), meloxicam (3 mg/kg, n = 7), or placebo (methylcellulose 0.5%) (n = 6). Serial blood samples were collected at 0.5, 1, 1.5, 2, 4, 6, 8, (12 for rofecoxib only), and 24 h post-dose. Dosing was done approximately at 9:00 A.M. and urine was collected for 8 h post-dose.

On day 3, rats were sacrificed and kidneys were excised and stored at -70° C until analyzed. The renal function was assessed by the measurement of urinary sodium and potassium excretion rates.

3.2.4 Rofecoxib Assay

Rofecoxib concentrations were determined in plasma and kidney using a validated reverse-phase HPLC method (13) with some modifications. Briefly, a 100 μL aliquot of various concentrations (50-10000 ng/mL) of rofecoxib standard solution was added to 100 μL of blank rat plasma. After addition of 100 μL of ketoprofen solution (1 μg/mL) 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 resultant, 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. The minimum detectable concentration of rofecoxib in plasma was 50 ng/mL and the coefficient of variation (CV) was 7.2%.

In order to determine rofecoxib concentrations in the rat kidney, two 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 mixture to make the final concentrations of 25-5000 ng/mL. 100 μL of 1 μg/mL 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 seconds 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 detectable concentration of rofecoxib in kidney was 50 ng/g and the CV was 20.8% (Table 3-1).

3.2.5 Celecoxib Assay

Plasma samples were assayed for celecoxib by the method of Guirguis et al. (14). Two calibration curves of low and high range concentrations were prepared. 100 μL of various concentrations of celecoxib solution (50-1000 and 1000-100,000 ng/mL) were mixed with 100 μL of blank rat plasma. Then, 100 μL of 20 μg/mL ibuprofen solution, 200 μL

of 0.6 M H_2SO_4 and 5 mL isooctane-iso-propanol (95:5) were added to the test tubes. The tubes were vortex-mixed for 30 seconds and centrifuged at 2500 g for 3 min. The organic layer was transferred into a clean tube and evaporated to dryness. The residue was dissolved in 200 μL of mobile phase and a 150 μL aliquot injected into the HPLC. The minimum detectable concentration of celecoxib in plasma was 50 ng/mL and the CV was 7.8%.

The above assay was modified to analyze kidney samples. Two volumes of water were added to the weighed kidney samples. The mixture was homogenized. To the test tubes containing 100 μL of homogenized mixture, 100 μL of 20 μg/mL ibuprofen solution, 200 μL of 0.6 M H_2SO_4 and 5 mL isooctane:iso-propanol (95:5) were added. The tubes were vortex-mixed for 30 seconds and centrifuged at 2500 g for 3 min. The organic layer was transferred into a clean tube and evaporated to dryness. The residue was dissolved in 200 μL of mobile phase and a 150 μL aliquot injected into the HPLC. The minimum detectable concentration of celecoxib in kidney was 100 ng/g and the CV was 15.5% (Table 3-2).

3.2.6 Meloxicam Assay

A validated reverse-HPLC method (15) 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) and piroxicam as an internal standard (100 μg/mL) in methanol. 100 μL of blank rat plasma was spiked with various concentrations of meloxicam stock solutions to make the final concentrations of 50-100,000 ng/mL. Then, 200 μL of 2 μg/mL 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

minutes. 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 detectable concentration of meloxicam in plasma was 0.5 μg/mL and the CV was 5.9%.

For determination of meloxicam in the kidney, two volumes of HPLC grade-water were added to the weighed kidney samples. The mixture was homogenized for 45 seconds. 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. 100 μL of 5 μg/mL 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 detectable concentration of meloxicam in kidney was 500 ng/g and the CV was 14.5% (Table 3-3).

3.2.7 Neutron Activation Analysis of Urine Samples

Urine samples were analyzed for sodium and potassium using instrumental neutron activation analysis (INAA) at the University of Alberta SLOWPOKE Nuclear Reactor Facility. Calibration was performed using standard solutions of sodium chloride and potassium chloride. Sample and standards, measuring 250 μL, were irradiated in batches at a nominal thermal neutron flux of 5×10^{11} n cm^{-2} s^{-1} for 10 minutes in one of the inner irradiation sites of the reactor. Following a decay period of approximately 24 h the activated samples were individually counted in a 10 centimeter (cm) Pb cave for 480

seconds (s) at a sample-to-detector distance of 2 cm using a 41.4% relative efficiency Princeton Gamma-Tech hyperpure Ge detector with a full-width half-maximum of 1.76 keV for the 1332.5 keV full energy peak of ^{60}Co. The Ge detector was connected to a PC-based Aptec multichannel analyzer card. Elemental analysis was performed by the semi-absolute method of activation analysis (16). Sodium was quantified using the 1368.6 keV gamma-ray emission of ^{24}Na ($T½ = 14.959$ h) produced *via* the neutron reaction $^{23}Na(n,\gamma)^{24}Na$, while potassium was determined *via* the 1524.7 keV gamma-ray emission of ^{42}K ($T½ = 12.36$ h) produced *via* the neutron reaction $^{41}K(n,\gamma)^{42}K$. Spectrometer busies were generally kept below ~10%, however, a Tennelec TC 813 pulse generator was used to correct for pulse pile-up effects (17).

3.2.8 Data Treatment and Statistical Analysis

Urinary electrolyte excretion rate (μmol/min/100g) were calculated from: $C_X * V_X * 100 / T * W$, where C_X is the concentration of urine electrolyte (mmol/L), V_X is the urine volume (mL), T is the urine collection time (min), and W is the weight of each rat (g). Urine flow rate (mL/h/100g) was estimated from: $V_X * 100 / T * W$, where V_X is the urine volume (mL), T is the urine collection time (h), and W is the weight of each rat (g). The values calculated for the baseline were compared with those of after treatment using the paired Student's t-test. The non-compartment model contained in WinNonlin, version 4.1 (Pharsight Corporation, California, USA) was used to estimate pharmacokinetic parameters. Linear regression analysis was performed to examine the relationship of kidney parameters and pharmacokinetic properties of rofecoxib, celecoxib, and meloxicam. Statistical significance was set at $p < 0.05$. Data are expressed as mean ± SD.

3.3 RESULTS

The pairs comparison (baseline vs. treatment values) indicated that treatment with rofecoxib significantly decreased sodium (from 0.33 ± 0.14 to 0.21 ± 0.11 μmol/min/100g, $p < 0.009$) and potassium (from 0.59 ± 0.19 to 0.39 ± 0.20 μmol/min/100g, $p < 0.02$) excretion rates (Figure 3-1). Similarly, sodium and potassium excretion rates were significantly reduced in rats treated with celecoxib as compared with baseline values (from 0.08 ± 0.07 to 0.04 ± 0.04 μmol/min/100g, $p < 0.032$ and from 0.42 ± 0.18 to 0.18 ± 0.14 μmol/min/100g, $p < 0.006$, respectively (Figure 3-2). Meloxicam, on the other hand, significantly influence neither sodium (0.49 ± 0.17 vs. 0.38 ± 0.13 μmol/min/100g, $p > 0.3$) nor potassium (0.55 ± 0.20 vs. 0.53 ± 0.15 μmol/min/100g, $p > 0.7$) excretion (Figure 3-3). No significant change in sodium and potassium excretion was observed in the control group that received methyl cellulose solution as placebo, $p > 0.078$ and $p > 0.86$, respectively (Figure 3-4).

Figures 3-5, 3-6, and 3-7 depict plasma concentration-time curves of rofecoxib, celecoxib, and meloxicam, respectively, following oral administration. Table 3-4 depicts the analysis of pharmacokinetic data for rofecoxib, celecoxib, and meloxicam.

We found substantially higher ratios of 24-h post-dose kidney over plasma concentrations for rofecoxib and celecoxib group compared to that of meloxicam group (Figures 3-8, 3-9, and 3-10, respectively). The ratios were 2.14 ± 1.63, 3.61 ± 2.34, and 0.27 ± 0.10 for rofecoxib, celecoxib, and meloxicam, respectively.

A significant correlation was found between the area under the plasma concentration-time curve from time of dosing to 24 h post-dose (AUC_{0-24}) of rofecoxib and the degree of sodium and potassium excretion change from baseline, $r = -0.65$ and $r =$

-0.82, respectively (Figures 3-11 and 3-12). Although the 24-h post-dose plasma concentration was not correlated with the sodium excretion ($p > 0.11$, $r = -0.45$) (Figure 3-13), there was a significant correlation between those values and urinary potassium excretion ($p < 0.03$, $r = -0.61$) (Figure 3-14).

For celecoxib, significant correlations between AUC_{0-24} of celecoxib and urinary sodium excretion change from baseline ($p < 0.05$, $r = -0.80$) (Figure 3-15) were found. Such a correlation was not significant when AUC_{0-24} of celecoxib and urinary potassium excretion change from baseline were compared ($p > 0.12$, $r = -0.60$) (Figure 3-16). There were strong significant correlations between 24-h post-dose plasma concentrations of celecoxib and the degree of sodium and potassium excretion change from baseline, $p < 0.04$, $r = -0.80$ and $p < 0.004$, $r = -0.90$, respectively (Figures 3-17 and 3-18).

There was no significant correlation between AUC_{0-24} of meloxicam and the degree of sodium and potassium excretion change from baseline, $r = 0.46$, $p > 0.29$ and $r = 0.18$, $p > 0.69$, respectively (Figures 3-19 and 3-20). Neither did the 24-h post-dose meloxicam plasma concentrations demonstrated a significant correlation with the degree of sodium or potassium excretion rates, $r = 0.26$, $p > 0.57$ and $r = 0.02$, $p > 0.96$, respectively (Figures 3-21 and 3-22).

3.4 DISCUSSION

In a recent study, we compared the effect of various NSAIDs on the urinary excretion of sodium and potassium in the rat (7). We found that rofecoxib, celecoxib, diclofenac, and flurbiprofen, but not meloxicam, significantly reduced electrolyte excretion. The effect, however, appeared to be independent of the COX-2 selectivity of the NSAIDs. For example, celecoxib and meloxicam possess close COX-2/COX-1 selectivity (18) but only

celecoxib exhibits significant renal effects. In this study, we confirmed our previous finding (7) that among the tested selective COX-2 inhibitors, rofecoxib and celecoxib, but not meloxicam, significantly decreased sodium and potassium excretion. A reduction in urinary electrolytes excretion due to treatment with NSAIDs has been shown in several other studies (19-21). Although the baseline values for sodium excretion reported in this study were varied among four groups (0.08 ± 0.07 to 0.49 ± 0.17 μmol/min/100g), these values were within the ranges reported by other authors ($0.07 \pm 0.01 - 0.53 \pm 0.09$ μmol/min/100g) (22;23).

Several studies have suggested that both COX-1 and COX-2 are localized in the kidney of different species. COX-1 is distributed in the kidney of various mammalian species in relatively similar fashions. They are found in the renal vasculature, collecting ducts and papillary interstitial cells (24). Interestingly COX-2, that initially thought to be absent under healthy condition and be only expressed in response to inflammatory stimulation, is also found in healthy tissues (1). COX-2 is present in macula densa, thick ascending limbs, and papillary interstitial cells in rats and in glomerular podocytes and small blood vessels of human (25). A more recent study in humans has detected COX-2 in the macula densa of patients with congestive heart failure as well as those with Bartter-like syndrome (26). Vio et al. (27) have reported the expression of COX-2 in thick ascending limb cells of normal rat kidney. This is important in NaCl reabsorption mediated by Na-K-2Cl cotransporter and basolateral Na-K-ATPase. It has been shown that prostaglandin E_2 inhibits Na-K-ATPase and consequently NaCl reabsorption (28). Accordingly, selective COX-2 inhibitors could alter sodium balance through the blockage of prostaglandin E_2 (PGE_2) production in thick ascending limb, where COX-1 isozyme is

absent (27). Catella-Lawson et al. (29) have shown the involvement of COX-1 in regulation of GFR. They administered repeated doses of rofecoxib or indomethacin to healthy elderly subjects noticed significant reductions in the urinary sodium excretion within the first 72 h of both treatments. Since authors detected a reduction GFR but only after administration of indomethacin, and not rofecoxib, they suggested the effect on the GFR to be through the inhibition of COX-1 pathway.

Indomethacin caused a reversible hyperkalemia in a young woman with glomerulonephritis (30). Since hyperkalemia was accompanied by a decrease in PGE_2, renin, and aldosterone, it was suggested that indomethacin exerted its effect by inhibition of renin-angiotensin-aldosterone system. The use of COX-2 selective inhibitors also has been associated with hyperkalemia. Braden et al. (31) reported hyperkalemia in four oedematous patients who diagnosed with acute renal failure caused by rofecoxib or celecoxib administration. The hyporeninaemic hypoaldostronism was observed in those patient was contributed to the effect of COX-2-selctive inhibitors in suppression of renin secretion. Furthermore, a reduction in potassium excretion was suggested to be secondary to reduced GFR which is known to be associated with NSAIDs therapy (32). This could be the result of inhibitory effect of NSAIDs on the production of vasodilatory prostaglandins, which increase the renal blood flow (33).

The observation that the renal effect of NSAIDs was independent of COX-2 selectivity (7) prompted us to hypothesize that the exposure of the kidneys to the drug also plays a role in this process. A comparison of three COX-2 selective NSAIDs revealed that rofecoxib and celecoxib, which reduce the electrolytes urinary excretion, are found in the kidney tissues approximately 2-3 folds greater than in plasma (Figures 3-8

and 3-9). On the other hand, meloxicam, which did not significantly influence the electrolytes excretion, was found in the kidney several fold less than in plasma (Figure 3-10). This observation lends support to the notion that the extent of exposure of the affected organ may play a role in the process. As shown in Figure 3-10, kidney/plasma concentration ratio of meloxicam is lower than other examined NSAIDs, whereas the kidney concentration of the drug is higher than others. This may be explained with the lower molar potency of meloxicam for COX-2 inhibition. The IC_{50} for inhibition of COX-2 for meloxicam, rofecoxib, and celecoxib are reported to be 2.1, 0.84, and 0.83 μM, respectively (18). Therefore, a higher concentration of meloxicam is needed to exert the same degree of effect. This emphasizes the importance of our finding that the extent of distribution of meloxicam in the kidney relative to the plasma concentration is much lower than that of rofecoxib and celecoxib. If meloxicam had followed the same pattern of distribution, as rofecoxib and celecoxib did, the concentration of drug in the kidney could had been several time higher, consequently it might have exerted the renal effects.

Exposure-dependent nephrotoxicity has been shown to be involved in some drugs of other classes such as cyclosporine A (34;35) and aminoglycosides (36-38). Moutabarrik et al. (34), who found nephrotoxicity of cyclosporine A to be associated with the drug concentration in the kidney, concluded that the accumulation of drug in the tubular cells of kidney caused disruption and consequently death of cells. Furthermore, Perez et al. (35) found cytotoxicity after exposing the kidney proximal tubule epithelial cells to cyclosporin A. Co-treatment with cilastatin, an inhibitor of transcellular transport of cyclosporin A, reduced the side effects presumably by decreasing the accumulation of cyclosporin A in the tubular cells. Schentag et al. (38) showed that the higher

concentration of gentamicin in the deep tissue compartment is associated with the higher degrees of decreased creatinine clearance. For both cyclosporine A and aminoglycosides, therefore, the links between plasma concentrations during the post-pseudoequilibrium phase and nephrotoxicity have been established. Indeed, this is the basis of therapeutic drug monitoring for these drugs. Similarly, we found some significant correlations between post-pseudoequilibrium plasma drug concentrations (24-h post-dose) and reduction in excretion of at least one electrolyte for both rofecoxib and celecoxib (Figures 3-11 to 3-18).

The 24-h post-dose concentration-effect relationship may be explained by assuming that the latter reflects the tissue concentration at the post-pseudoequilibrium hence, is a marker of the concentration in the affected tissue, similar to the aminoglycosides case (36-38). Indeed, there was no significant correlation between the observed urinary electrolyte excretions and 8-h post-dose plasma drug concentrations.

Meloxicam did not influence the electrolyte urinary excretion. Since the drug is an effective anti-inflammatory through inhibition of COX pathways, its lack of renal effect (Figure 3-3) may be explained by its limited distribution into the kidney (Figure 3-10). Indeed, no associations were found between the plasma drug concentrations and the urinary sodium or potassium excretions (Figure 3-19 to 3-22). This implies that in addition to be a COX inhibitor, an NSAID should have a relatively high extent of distribution into the kidney to influence the renal function.

Several explanations may be offered for the difference in distribution among the examined NSAIDs. Like other NSAIDs (39), rofecoxib, celecoxib, and meloxicam have a high degree of protein binding. Their bindings to protein have been reported to be 93, 91-

98, and 99% in rat, for rofecoxib, celecoxib, and meloxicam, respectively (40-42). There is also possibility of saturation of binding in higher concentration. Therefore, in absence of confirming data one cannot unequivocally rule out the involvement of protein binding in the observed different distribution of these drugs. Another explanation for the difference in the distribution of these drugs is the extent of ionization in biological media. Seedher and Bhatia (43) examined the solubility of rofecoxib, celecoxib, and meloxicam in the wide pH ranges. They observed the pH has only a limited influence on the solubility of rofecoxib and celecoxib. In contrast, aqueous solubility, hence, polarity of meloxicam increases with elevation of pH. This may limit the permeability of the drug into the cells and results in less kidney/plasma concentration ratio for meloxicam as compared with other two NSAIDs.

Davies and Jamali (1) have suggested that the physiochemical properties and pharmacokinetic parameters of rofecoxib may be involved in the cardiovascular events associated with the drug. The present data suggest that the renal effects of rofecoxib and celecoxib is related to their pharmacokinetics. It is worthy to note that some other effects of NSAIDs have been shown also to be pharmacokinetic-dependent. Both the analgesic (44;45) and anti-inflammatory (46;47) effects of some NSAIDs significantly correlate with their plasma concentration. Vakily et al. (48) have shown the higher concentrations of tiaprofenic acid in the effect compartment increases the intestinal permeability, suggesting that there is a relationship between the concentration and gastrointestinal toxicity.

In agreement with our study, Halpin et al. (40) have reported higher concentrations of rofecoxib in the kidney compared to that of plasma after intravenous

administration of rofecoxib at 2 mg/kg to the rat. However, we noticed that the range of the pharmacokinetic data, following oral administration of 10 mg/kg rofecoxib, in our experiment is different from what reported by the authors. For example, AUC_{0-24} and peak plasma level (C_{max}) are reported to be 2963 ng.h/mL and 400 ng/mL, respectively, whereas, our data indicate that the AUC_{0-24} and C_{max} values to be 29800 ng.h/mL and 3180 ng.h/mL, respectively. We cannot explain this discrepancy. Nevertheless, the C_{max} values observed by Sattari and Jamali (13), 640-1200 ng/mL, following oral administration of 5 mg/kg rofecoxib, fall between two sets of data.

In this study, animals were deprived of food since the night before and during the urine collection period after the administration of the drug. The latter was done to avoid contamination of urine with food and the potential increase in electrolytes content. To limit the period of food deprivation, we chose to collect urine for only 8 h. Our preliminary data indicated that the 8 h collection period was sufficient to reflect the change in electrolyte excretion post-NSAID-dose.

The interpretation of relations depicted in Figures 3-15 and 3-16 for celecoxib regarding AUC vs. electrolyte excretion needs extra attention, as they are each based on only 5 data points. This was because we did not collect serial blood samples in all animals to construct AUCs. However, we had more number of data points to compare 24-h celecoxib plasma concentration vs. electrolytes excretion (Figures 3-17 and 3-18).

As shown in Tables 3-1, 3-2, and 3-3, the error of our experiment to measure the concentration of the drugs in the kidney is relatively high. Therefore, these data should be considered with caution.

In summary, single doses of rofecoxib and celecoxib are associated with renal side effects as demonstrated by decreased sodium and potassium excretions. This effect is well correlated with the drug concentration in the systemic circulation. Accumulation of the examined drugs in the kidney tissue may play a role to producing the adverse effect.

Table 3-1. Coefficient of variation and accuracy for the rofecoxib assay in the rat kidney (n=3).

Added concentration (ng/g)	Measured concentration (ng/g)	CV*(%)	Accuracy**(%)
50	45.6	19.0	91.2
100	93.5	8.3	93.5
250	257.3	14.5	102.9
500	564.3	20.8	112.9
1000	1021.4	8.0	102.1
5000	4975.2	1.1	99.5

*Expressed as [(standard deviation of measured concentration)/(mean of measured concentration)]x100

** Expressed as [(mean of measured concentration)/(added concentration)]x100

Table 3-2. Coefficient of variation and accuracy for the celecoxib assay in the rat kidney (n=3).

Added concentration (ng/g)	Measured concentration (ng/g)	CV*(%)	Accuracy**(%)
100	88.0	3.6	86.0
250	113.7	9.3	42.8
500	329.6	11.9	69.7
1000	1145.5	3.9	111.7
1000	767.2	15.5	76.7
2500	2132.6	5.0	85.3
5000	3941.4	3.3	78.8
10000	8448.3	8.0	84.5
25000	21704.8	5.9	86.8
50000	44064.5	4.1	88.1
100000	102932.3	1.8	102.9

*Expressed as [(standard deviation of measured concentration)/(mean of measured concentration)]x100

** Expressed as [(mean of measured concentration)/(added concentration)]x100

Table 3-3. Coefficient of variation and accuracy for the meloxicam assay in the rat kidney (n=3).

Added concentration (ng/g)	Measured concentration (ng/g)	CV*(%)	Accuracy**(%)
500	665.3	12.0	133.1
1000	1208.7	11.4	120.9
5000	5516.8	10.4	110.3
10000	10997.0	14.5	110.0
50000	53930.0	12.1	107.9
100000	100827.5	6.9	100.8

*Expressed as [(standard deviation of measured concentration)/(mean of measured concentration)]x100

** Expressed as [(mean of measured concentration)/(added concentration)]x100

Table 3-4. Pharmacokinetic parameters of rofecoxib (10 mg/kg), celecoxib (40 mg/kg), or meloxicam (3 mg/kg) following oral administration of a single dose.

	Drug		
Parameter	**Rofecoxib (n=13)**	**Celecoxib (n=5)**	**Meloxicam (n=7)**
AUC_{0-24} (µg.h/mL)	29.8 ± 10.3	59.1 ± 18.0	319 ± 124
C_{max} (µg/mL)	3.18 ± 1.73	4.58 ± 1.34	18.3 ± 6.47
t_{max} (h)	3.38 ± 1.43	6.40 ± 1.67	4.75 ± 3.75
$t_{1/2}$ (h)	7.42 ± 4.48	5.59 ± 1.34	74.4 ± 9.74*

Note: The values are expressed as mean ± standard deviation

***Apparent t1/2**

*Expressed as [(standard deviation of measured concentration)/(mean measured concentration)]x100

** Expressed as [(mean measured concentration)/(added concentration)]x100

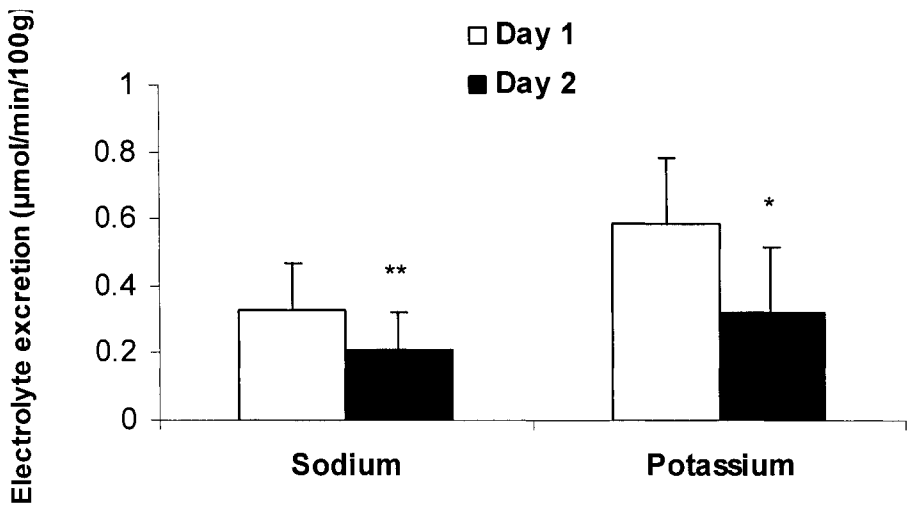

Figure 3-1. Electrolyte excretion/100g bodyweight after administration of rofecoxib (10 mg/kg, n=13).

*Significantly different from baseline ($P < 0.05$).

**Significantly different from baseline ($P < 0.01$).

The values are expressed as mean ± standard deviation.

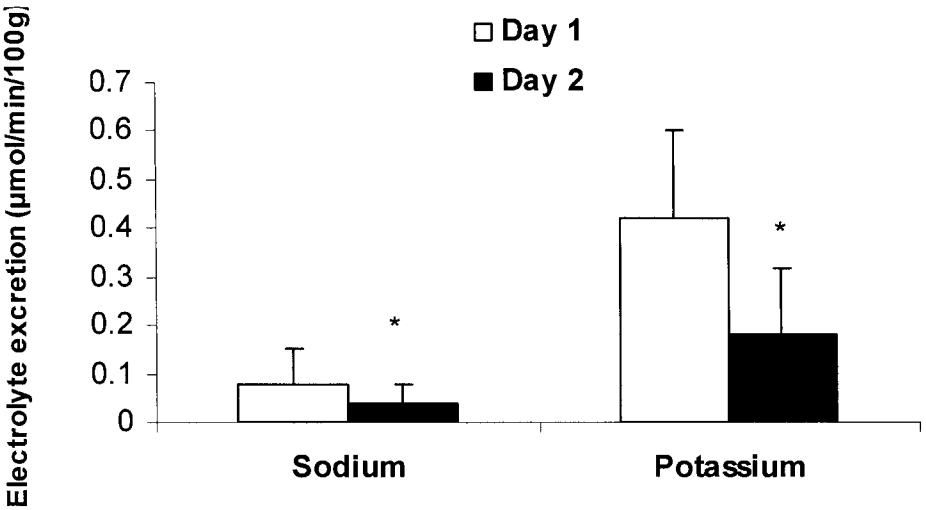

Figure 3-2. Electrolyte excretion/100g bodyweight after administration of celecoxib (40 mg/kg, n=7).

*Significantly different from baseline ($P < 0.05$).

**Significantly different from baseline ($P < 0.01$).

The values are expressed as mean ± standard deviation.

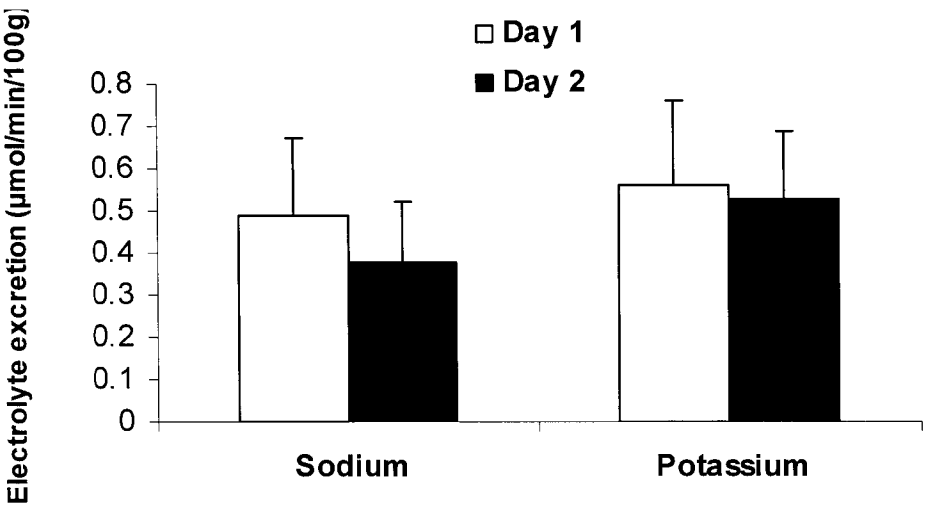

Figure 3-3. Electrolyte excretion/100g bodyweight after administration of meloxicam (3 mg/kg, n=7).

*Significantly different from baseline ($P < 0.05$).

**Significantly different from baseline ($P < 0.01$).

The values are expressed as mean ± standard deviation.

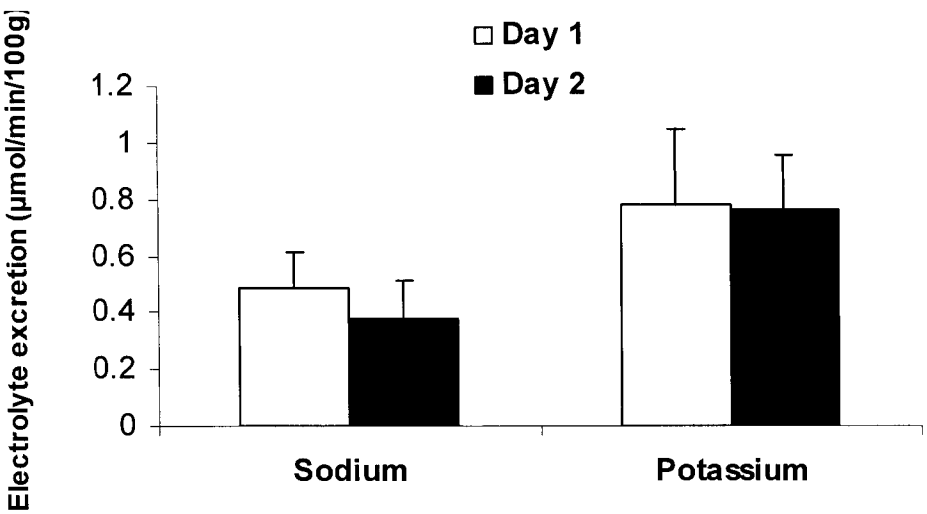

Figure 3-4. Electrolyte excretion/100g bodyweight after administration of placebo (n=6).

*Significantly different from baseline ($P < 0.05$).

**Significantly different from baseline ($P < 0.01$).

The values are expressed as mean ± standard deviation.

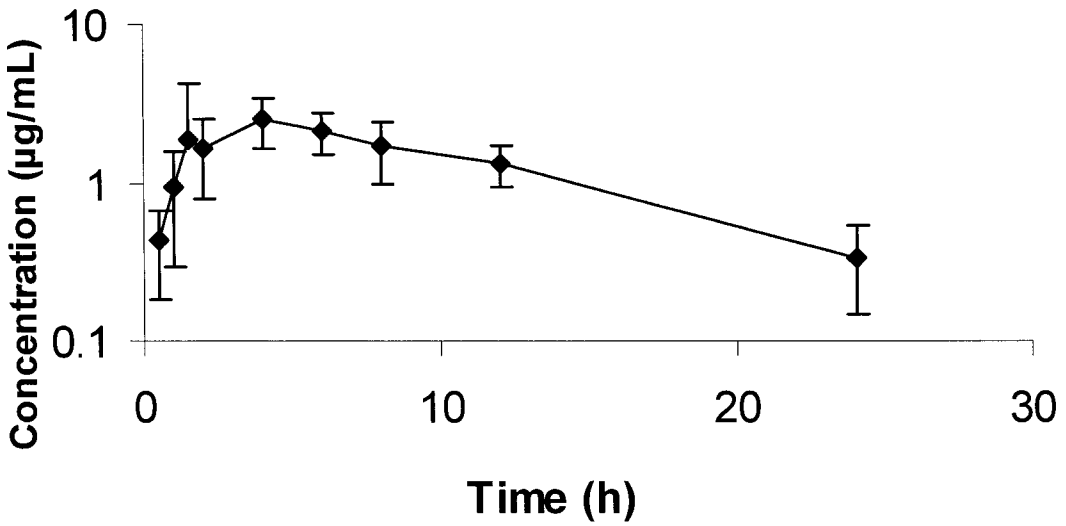

Figure 3-5. Plasma concentration-time profile of rofecoxib in the rat following a 10 mg/kg oral dose (n=13).

The values are expressed as mean ± standard deviation.

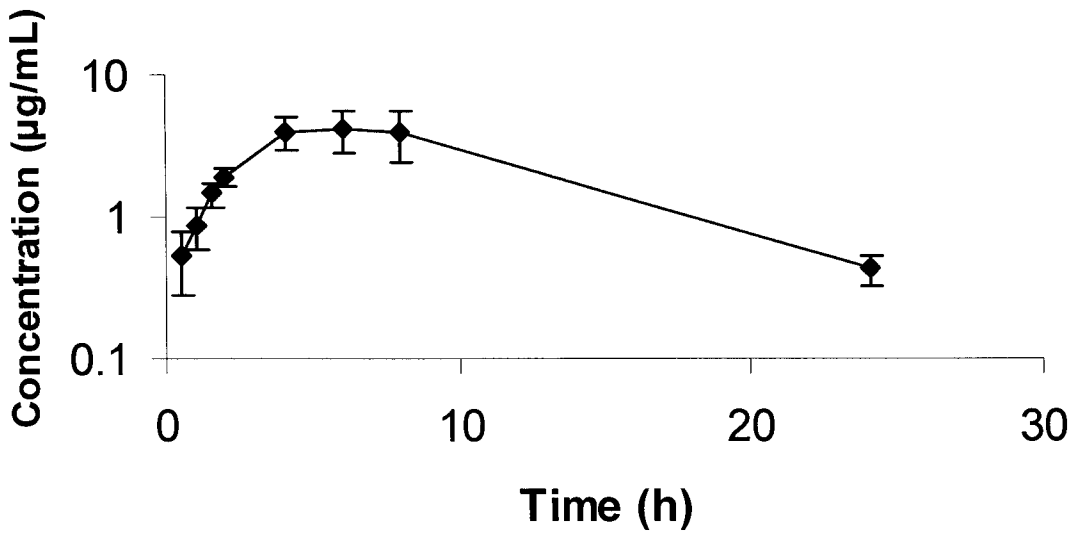

Figure 3-6. Plasma concentration-time profile of celecoxib in the rat following a 40 mg/kg oral dose (n=5).

The values are expressed as mean ± standard deviation.

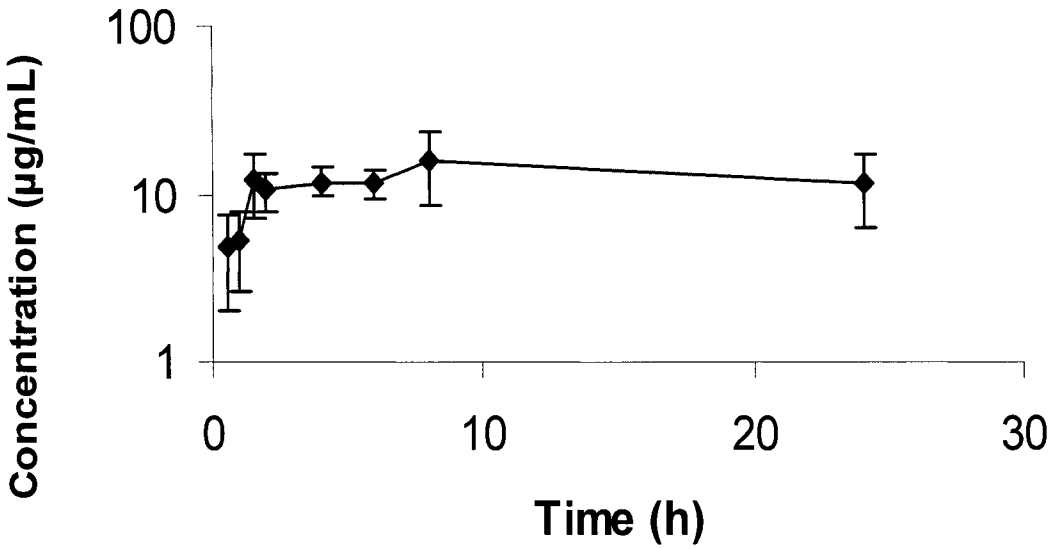

Figure 3-7. Plasma concentration-time profile of meloxicam in the rat following a 3 mg/kg oral dose (n=7).

The values are expressed as mean ± standard deviation.

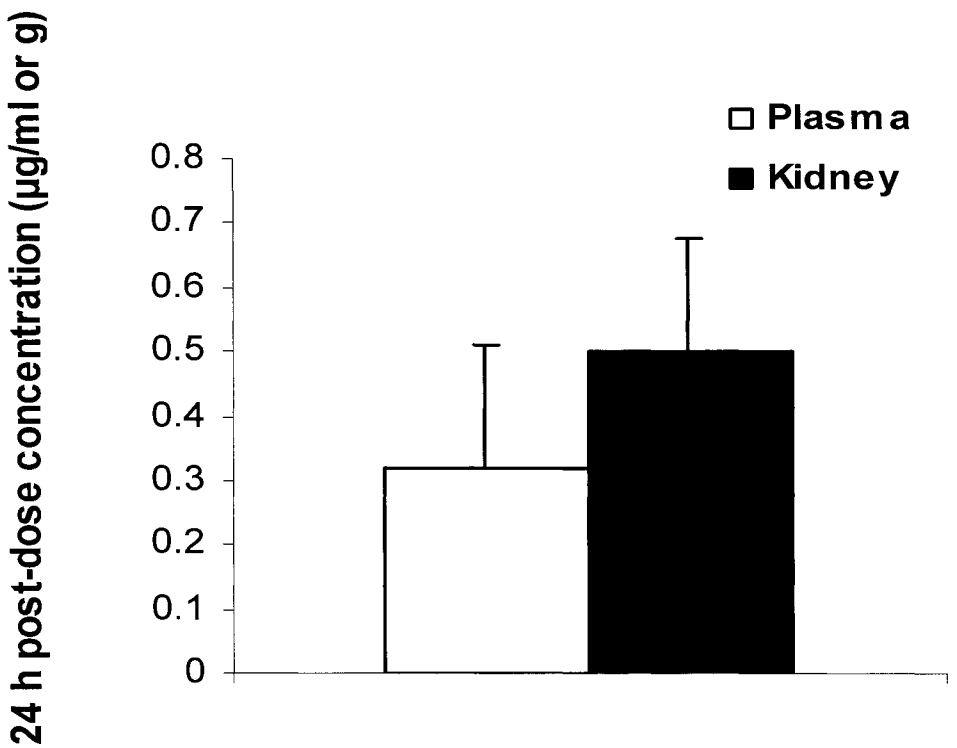

Figure 3-8. 24-h post-dose concentrations of rofecoxib in plasma and kidney of rats following a 10 mg/kg oral dose (n=13).

The values are expressed as mean ± standard deviation.

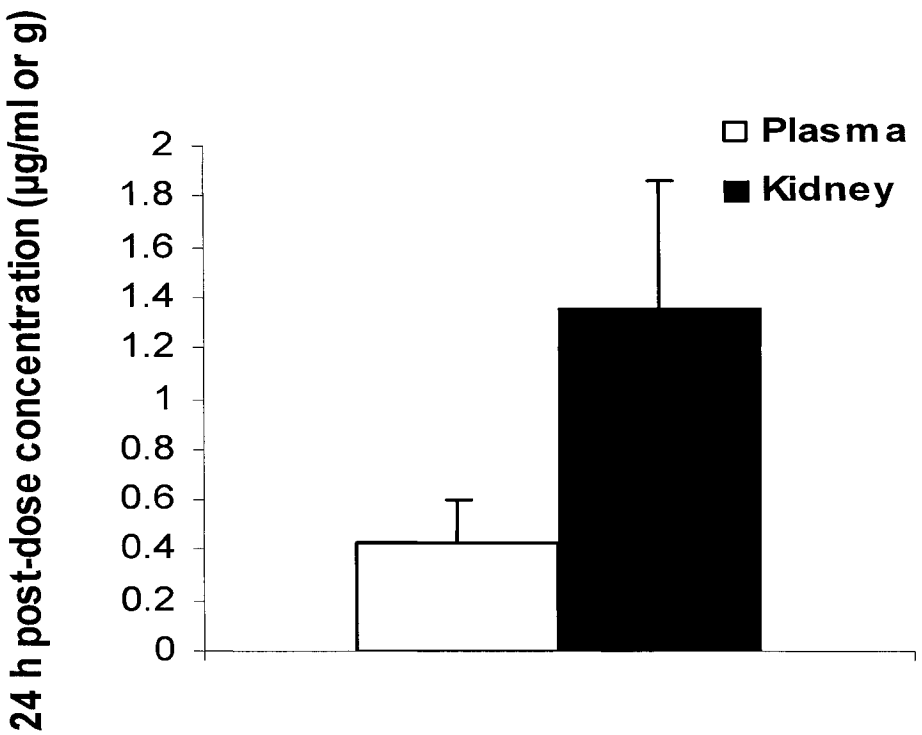

Figure 3-9. 24-h post-dose concentrations of celecoxib in plasma and kidney of rats following a 40 mg/kg oral dose (n=7).

The values are expressed as mean ± standard deviation.

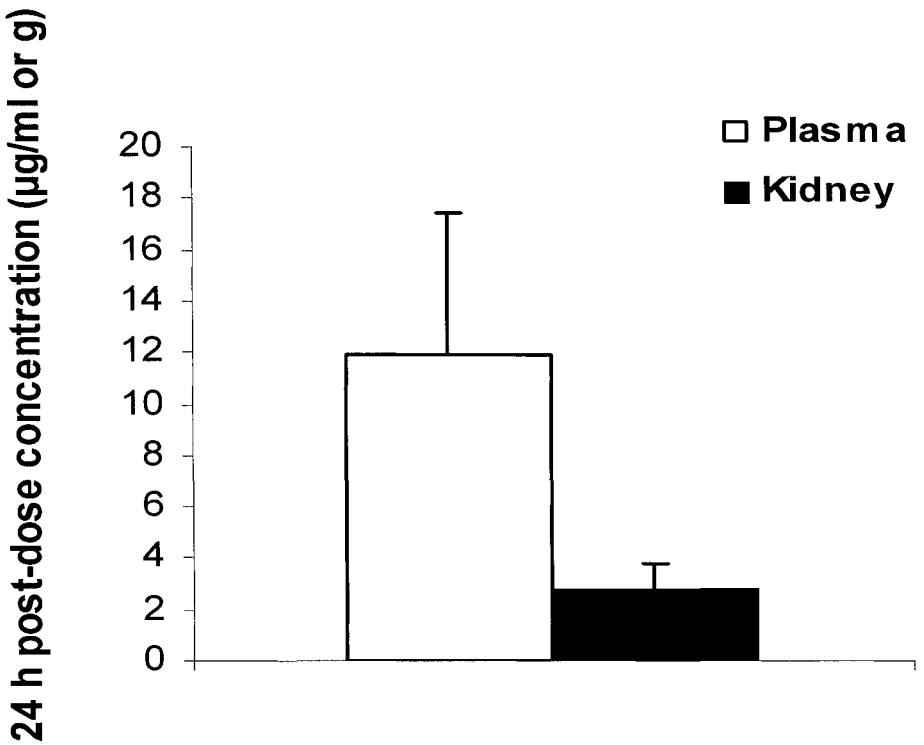

Figure 3-10. 24-h post-dose concentrations of meloxicam in plasma and kidney of rats following a 3 mg/kg oral dose (n=6).

The values are expressed as mean ± standard deviation.

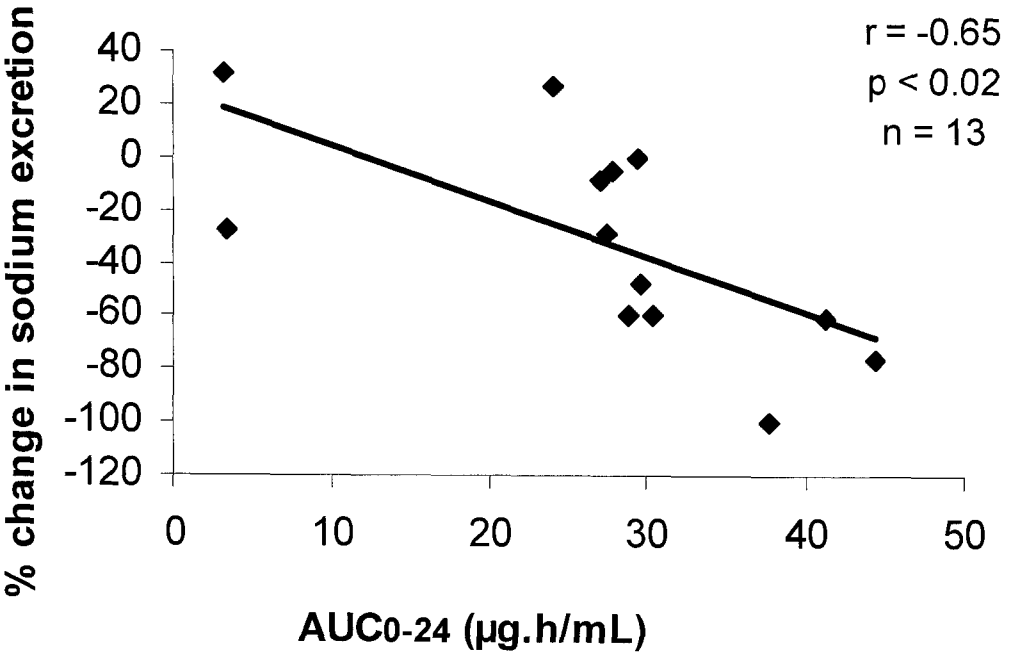

Figure 3-11. Correlation of AUC_{0-24} with sodium excretion change from baseline for rofecoxib.

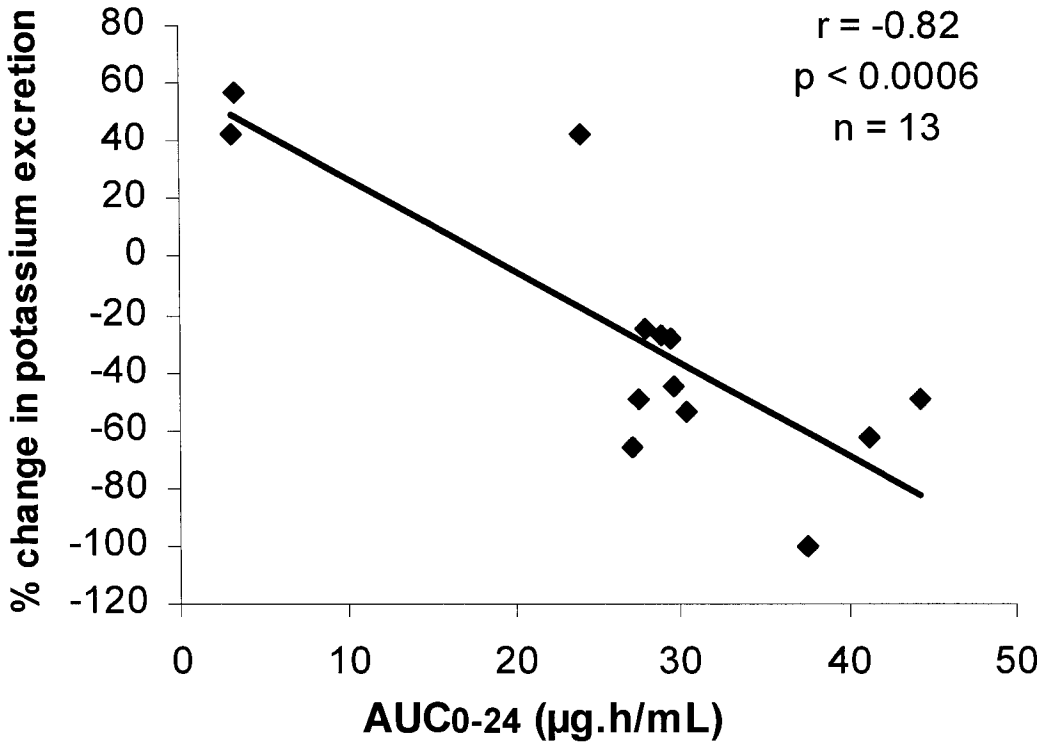

Figure 3-12. Correlation of AUC_{0-24} with potassium excretion change from baseline for rofecoxib.

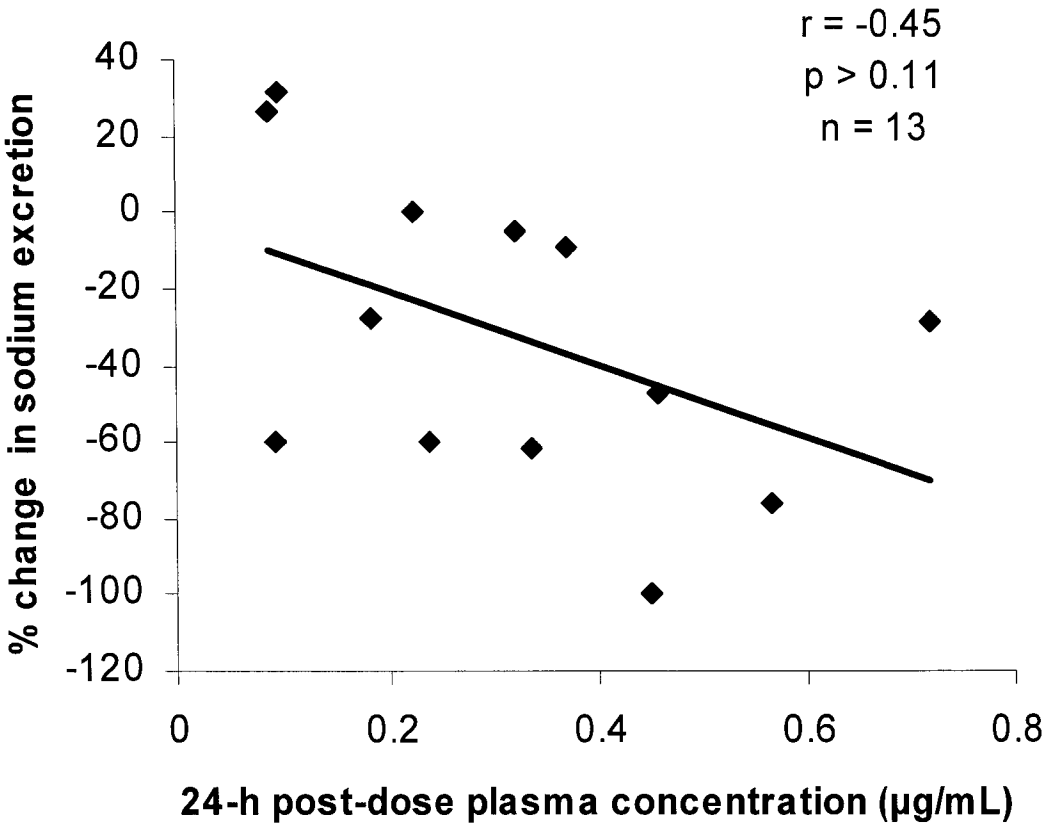

Figure 3-13. Correlation of 24-h post-dose plasma concentration with sodium excretion change from baseline for rofecoxib.

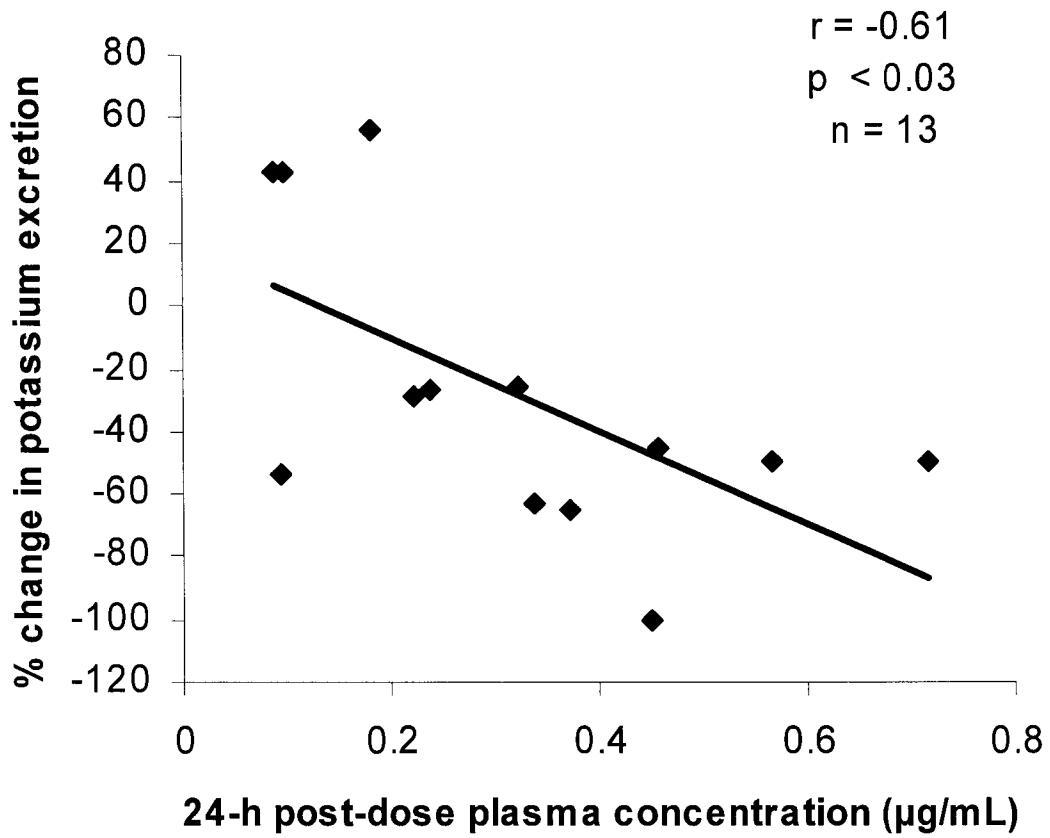

Figure 3-14. Correlation of 24-h post-dose plasma concentration with potassium excretion change from baseline for rofecoxib.

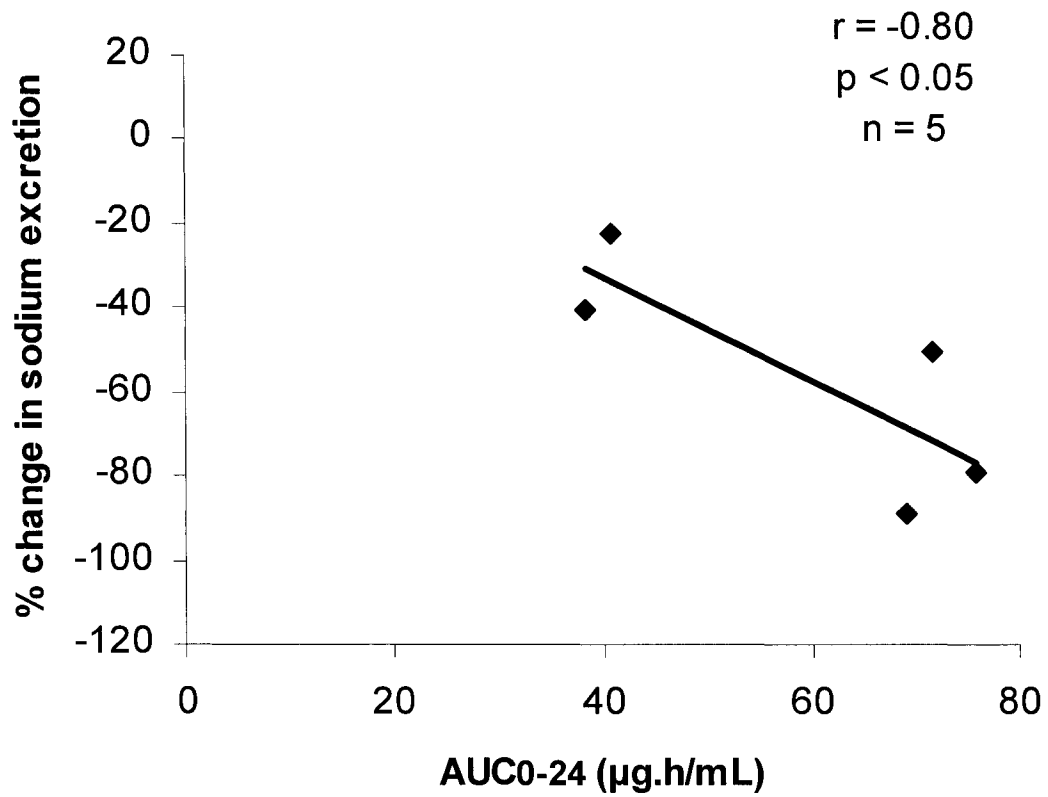

Figure 3-15. Correlation of $AUC_{0\text{-}24}$[†] with sodium excretion change from baseline for celecoxib.

[†] Calculated for five rats

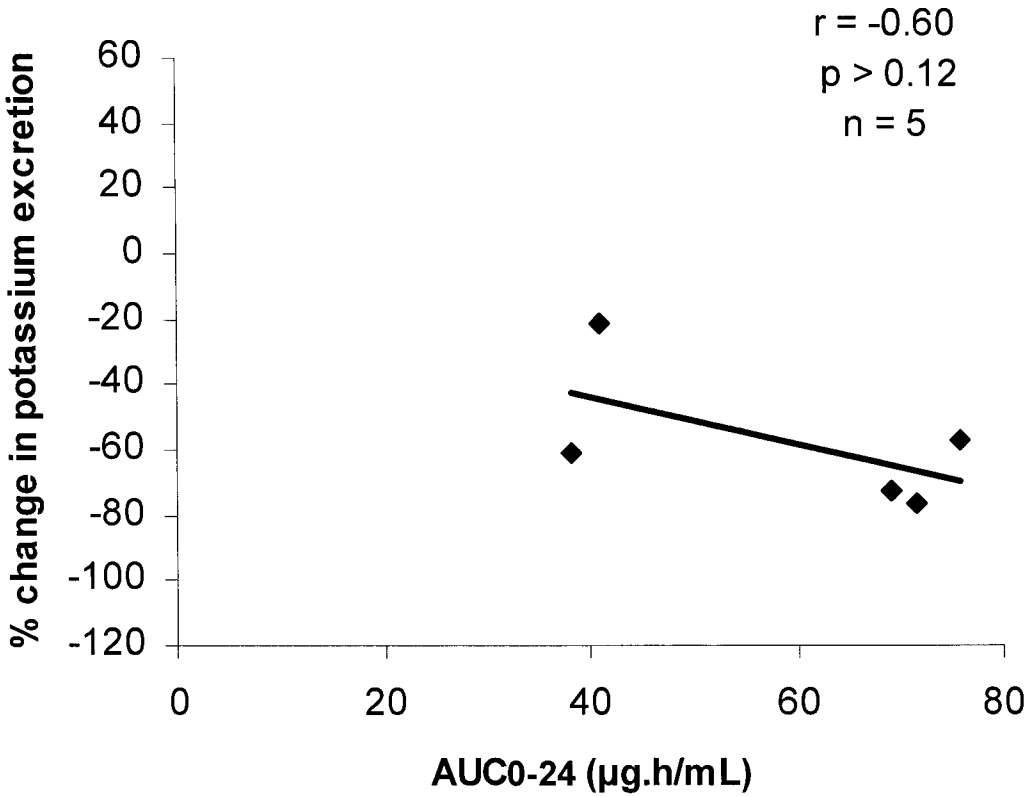

Figure 3-16. Correlation of $AUC_{0\text{-}24}$[†] with potassium excretion change from baseline for celecoxib.

[†] Calculated for five rats

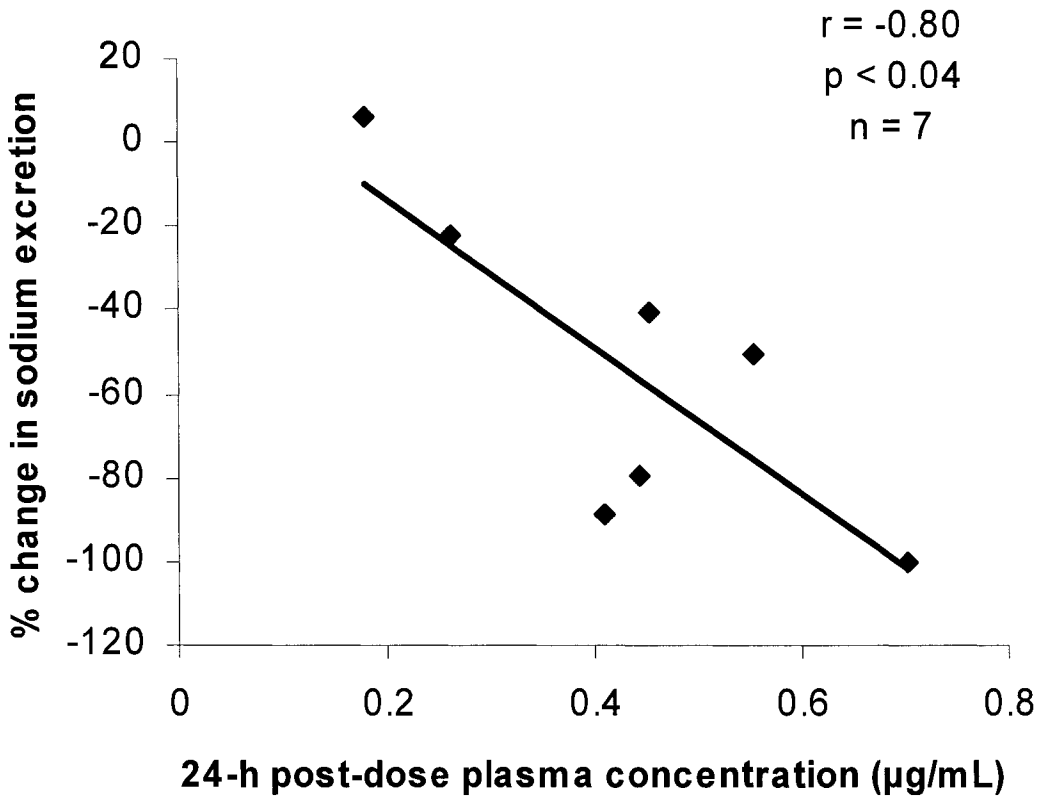

Figure 3-17. Correlation of 24-h post-dose plasma concentration with sodium excretion change from baseline for celecoxib.

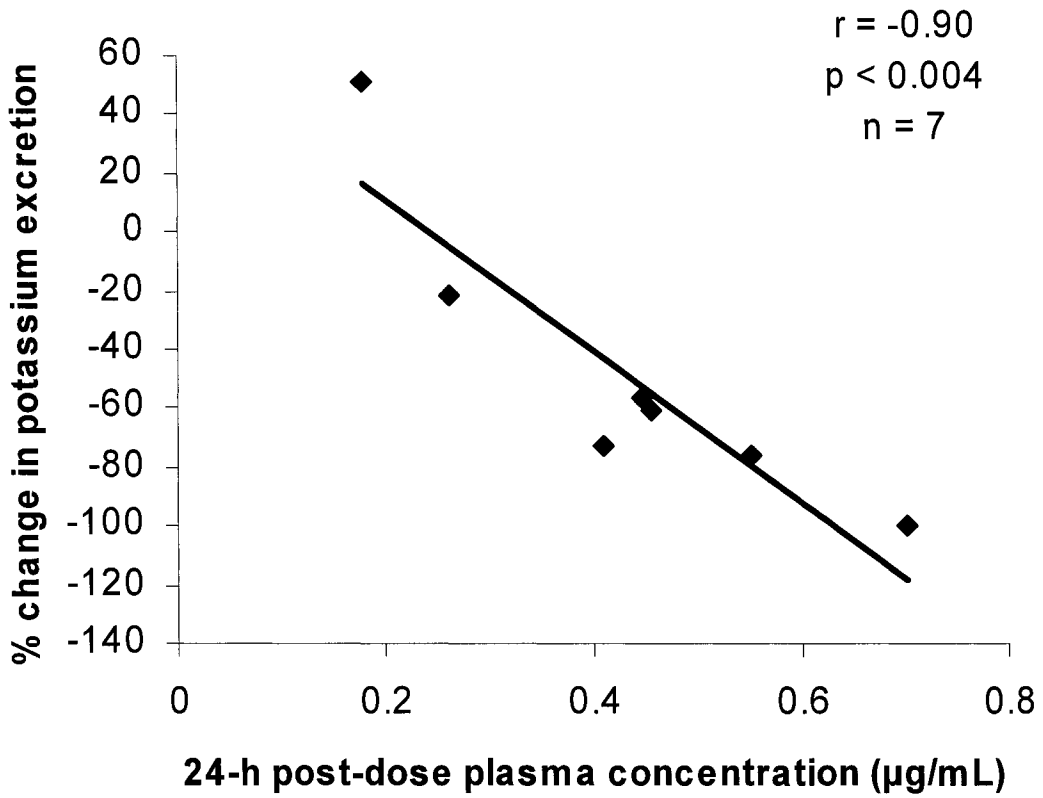

Figure 3-18. Correlation of 24-h post-dose plasma concentration with potassium excretion change from baseline for celecoxib.

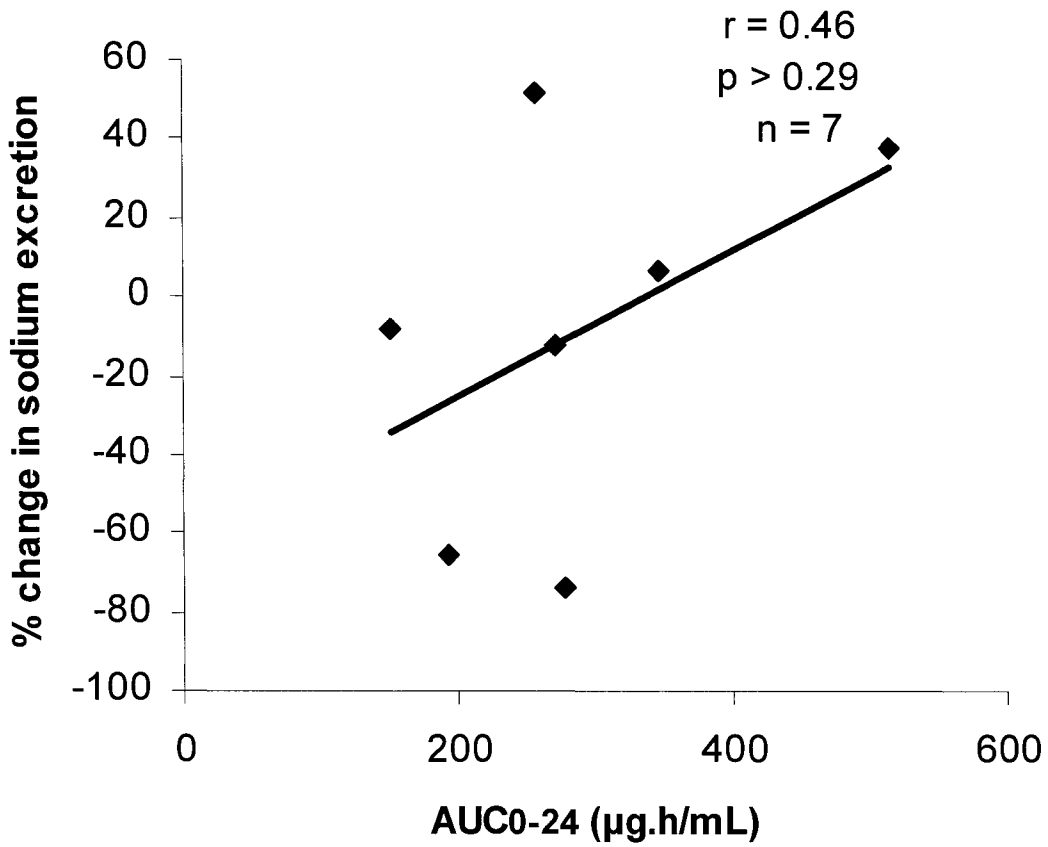

Figure 3-19. Correlation of AUC_{0-24} with sodium excretion change from baseline for meloxicam

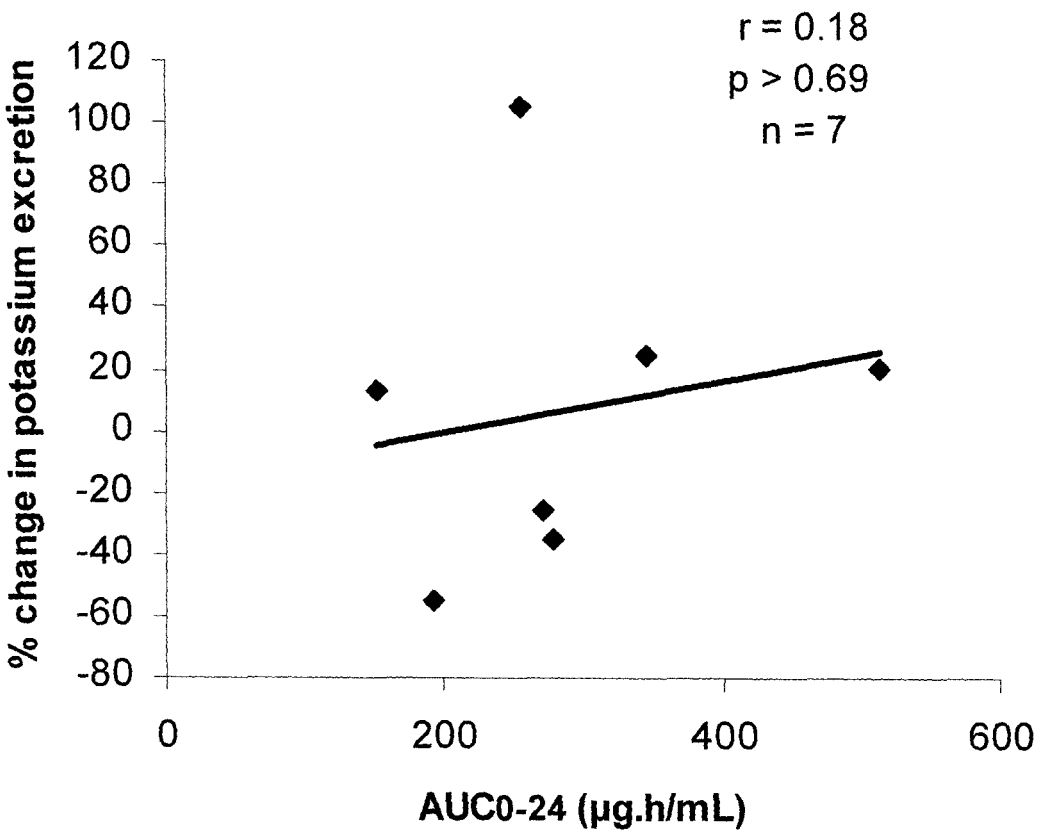

Figure 3-20. Correlation of AUC_{0-24} with potassium excretion change from baseline for meloxicam.

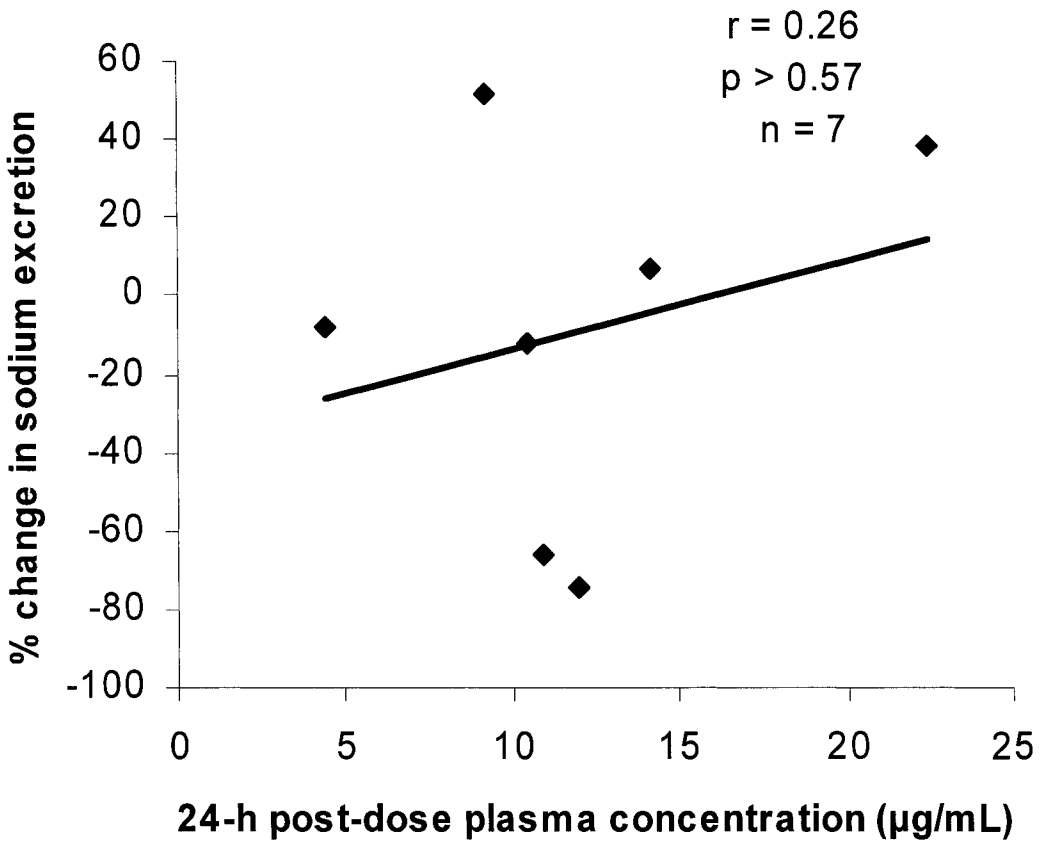

Figure 3-21. Correlation of 24-h post-dose plasma concentration with sodium excretion change from baseline for meloxicam.

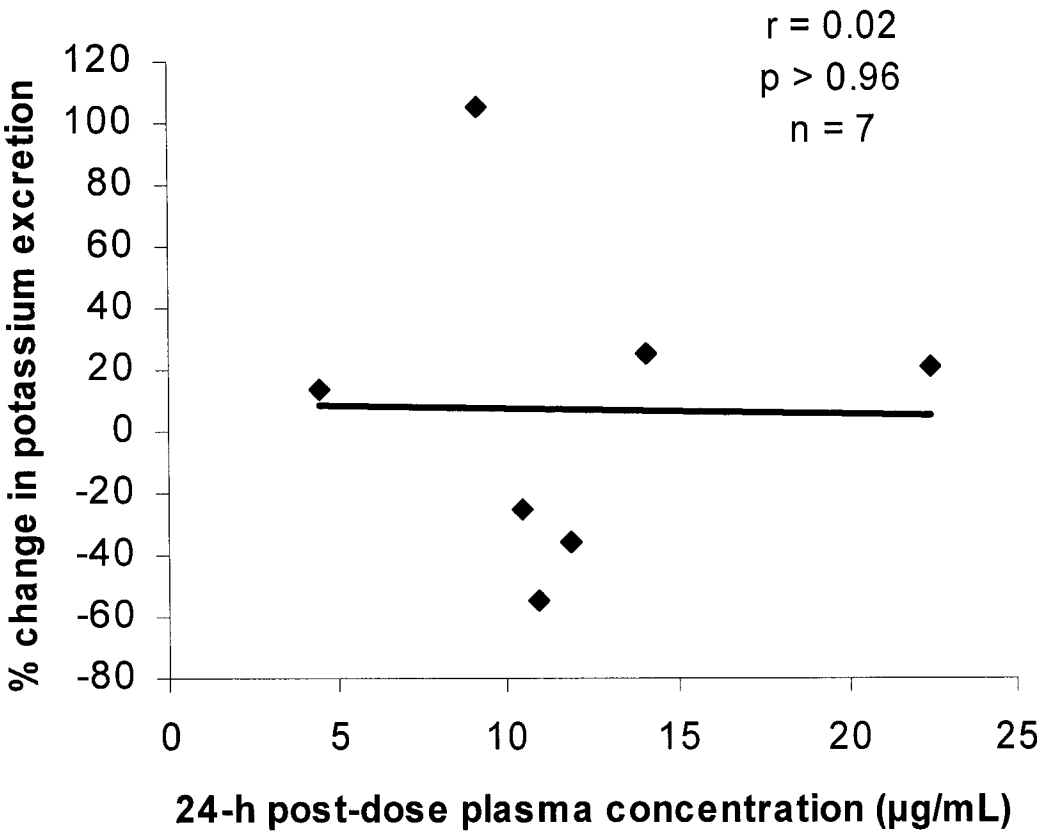

Figure 3-22. Correlation of 24-h post-dose plasma concentration with potassium excretion change from baseline for meloxicam

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Chapter 4[4]

Effect of Inflammation on Kidney Function and Pharmacokinetics of Nonsteroidal Anti-inflammatory Drugs, with Different COX-2 Selectivity

[4] **This chapter is dealt with three hypotheses that inflammation changes the kidney function, inflammation influences the pharmacokinetics of rofecoxib, and the distribution of drug into the kidney is altered by inflammation.**

4.1 INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease which targets joints and results in pain, inflammation, and disability (1). It is estimated that RA occurs in 1% of population (2).

Boers et al. (3) who studied the effect of inflammation on kidney function of 35 arthritic patient, reported a decrease in glomerular filtration rate (GFR), elevated urinary tubular enzyme levels, and proteinuria in some patients. Although the patients who were taking known nephrotoxic drugs other than drug used for the treatment of RA were excluded from the study, the authors did not rule out the possibility of the influence of nonsteroidal anti-inflammatory drugs (NSAIDs) or disease-modifying drugs on kidney function. Since it is difficult to find patients diagnosed with RA who are not treated with the latter drug, it is reasonable to use animal models of arthritis to study the effect of inflammation on the kidney function. Moreover, it is known that NSAIDs alter sodium and potassium excretion (4). Accordingly, we examined the effect of inflammation on urinary electrolyte excretion in rats. In addition, we looked at the effect of inflammation on total protein, plasma creatinine, and blood urea nitrogen (BUN).

The literature contains many reports describing the effect of inflammation on the pharmacokinetics of drugs (5-9). Many arthritic patients take nonsteroidal anti-inflammatory drugs (NSAIDs). Since the change in pharmacokinetics of drugs may have therapeutic consequences (5), we investigated the effect of inflammation on the pharmacokinetics of rofecoxib and meloxicam. In order to induce inflammation in rats, we used the pre-adjuvant arthritis model established by Ling and Jamali (8). We chose

this model because the animals were exposed to less pain and anxiety compared with the other chronic models of inflammation.

The other aim of this study was to investigate whether inflammation changes the extent of distribution of selective cyclooxygenase-2 (COX-2) inhibitors into the kidney. Recently, we have shown that renal effects of selective COX-2 inhibitors, rofecoxib and celecoxib, are explained by their pattern of distribution into the kidney. Our unpublished data shows that altered sodium excretion, caused by rofecoxib and celecoxib treatment, is associated with the accumulation of drug in the kidney of rats. We have shown that renal effects of NSAIDs are correlated with 24-h plasma concentrations of those drugs.

Didier et al. (10) reported that tumor necrosis factor-α (TNF-α) and endothelin-1 (ET-1) induced the breakdown of human brain microvascular endothelial cells that were co-cultured with astrocytes, suggestive of an increase in permeability into the brain. Although the structures of kidney and brain membranes are different, we examined the possibility that inflammation may change the distribution of NSAIDs into the kidney.

4.2 METHODS

4.2.1 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, Michigan, USA). The immunoassay kit for measurement of TNF-α was purchased from BioSource International (Camarillo, California, USA).

4.2.2 Dosage Forms 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.

4.2.3 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 to 8 hours on day 0. In order to measure the baseline nitrite, TNF-α, total protein, plasma creatinine, and BUN, blood samples were collected via tail vein. After urine and blood collection, rats were randomly divided into 6 groups (n=6 or 7). Group PL/CONT, MEL/CONT, and ROF/CONT received 0.2 mL of sterile normal saline into the ischiadic lymph node at the tail base. Group PL/INF, MEL/INF, and ROF/INF received 0.2 mL of a 50 mg/mL of heat killed *Mycobacterium butyricum*, suspended in squalene, into the ischiadic lymph node at the tail base. The animals were then transferred to standard rat cages, 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. 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, groups PL/CONT and PL/INF received methyl cellulose, MEL/CONT and MEL/INF received meloxicam, 3 mg/kg; and ROF/CONT and ROF/INF received rofecoxib, 10 mg/kg. Rats were then transferred to metabolic cages. Blood samples (0, 0.25, 1, 2, 4, 6, 8, and 24 hour in group MEL/CONT, MEL/INF, ROF/CONT and ROF/INF; 24 hours in group PL/CONT and PL/INF) were collected. Urine was collected up to 8 hours. On day 14, rats were sacrificed and kidneys were excised and stored at -70° C until analyzed.

The dose of rofecoxib (10 mg/kg) and meloxicam (3 mg/kg) was chosen based on the result our previous study (11).

4.2.4 Total Protein, BUN and Plasma Creatinine Analysis

A Vet Test 8008 analyzer (Idexx Laboratories Inc, Westbrook, MA) determined total protein, BUN and plasma creatinine. The basis of analysis is based on a 'dry' chemistry analyzer system. Briefly, the biochemical reactions are occurred on films, supplied by the manufacturer. Then, an optical device is used to measure the intensity of reaction.

4.2.5 Nitrite Assay

Nitrite was measured in plasma of all rats using a method reported by Grisham et al. (12). Briefly, 100 μL of plasma was incubated with *Asperigillus* nitrate reductase and treated with flavine adenine dinucleotide, 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 x 340 plate reader (Bio-Tek Instruments, Fisher Scientific). Calibration was performed using standard solutions of sodium nitrate and sodium nitrite.

4.2.6 TNF-α Analysis

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.

4.2.7 Rofecoxib and Meloxicam Assays

Rofecoxib and meloxicam concentrations were determined in plasma and kidney using the validated reverse-phase HPLC methods described in chapter 3 (pages 73-74 and 75-76).

4.2.8 Electrolytes Analysis

Urine samples were analyzed for sodium and potassium using instrumental neutron activation analysis as previously described in chapter 3 (pages 76-77).

4.2.9 Data Treatment and Statistical Analysis

Urinary electrolyte excretion (μmol/min) was calculated from: $C_X * V_X / T$, where C_X is the concentration of urine electrolyte (mmol/L), V_X is the urine volume (mL), and T is the urine collection time (min). Urine flow rate (mL/h) 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, California, USA) was used to estimate pharmacokinetic parameters. Pharmacokinetic parameters and kidney/plasma concentration ratios calculated for the inflamed groups treated with rofecoxib or meloxicam were compared with those of respective controls using the two-tailed Student t-test. Renal data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Statistical significance was set at $p < 0.05$. All data are presented as mean ± standard deviation.

4.3 RESULTS

4.3.1 The Presence of Inflammation

The presence of inflammation was confirmed with a significant increase in plasma concentrations of nitrite in groups PL/INF, MEL/INF, and ROF/INF on day 9 compared with the baseline values (53.7 ± 20.5 vs. 28.2 ± 12.2, 88.4 ± 7.74 vs. 33.3 ± 17.6, and 54.2 ± 18.4 vs. 28.2 ± 12.3 μmol/L, respectively) (Figure 4-1). As shown in Figure 4-1, nitrite concentrations in groups PL/INF, MEL/INF, and ROF/INF on day 13 were also significantly higher than baseline values (51.3 ± 11.0 vs. 28.2 ± 12.2, 65.8 ± 14.8 vs. 33.3 ± 17.6, and 45.4 ± 16.8 vs. 28.2 ± 12.3 μmol/L, respectively) (Figure 4-1). TNF-α concentration increased significantly in group MEL/INF (12.7 ± 3.70 vs. 4.12 ± 1.17 pg/mL) on day 9 and in groups MEL/INF, and ROF/INF on day 13 (14.55 ± 3.73 vs. 4.12 ± 1.17 and 15.2 ± 10.42 vs. 6.87 ± 3.92 pg/mL, respectively) compared with those of baseline values (Figure 4-2).

4.3.2 Effects on Body Weight

On day 9, average weight of rats in inflamed groups was significantly less than control group, while no meaningful change was seen between control group and two groups did not receive adjuvant. On the thirteenth day of experiment, there was a significant difference between the average weight of control group and inflamed groups (Table 4-1).

4.3.3 The Effect of Inflammation on Kidney Function

The results of BUN, plasma creatinine, and total protein for all groups are presented in Table 4-2. Compared with baseline values, mean BUN was significantly increased in groups PL/INF and ROF/INF on day 9 ($p < 0.0001$ and $p < 0.0007$, respectively) and in groups MEL/INF ($p < 0.0007$) on day 13. For plasma creatinine, the difference from baseline was significant in all inflamed groups on days 9 and 13. Total protein was not affected by inflammation. The values ranged from 51.8 ± 1.64 to 54.3 ± 2.14, 50.8 ± 1.60 to 55.0 ± 1.67, and 51.8 ± 4.75 to 56.8 ± 2.32 g/L on days 0, 9, and 13, respectively.

4.3.4 Effects on Urinary Electrolyte Excretion and Urine Flow Rate

Table 4-3 summarizes the results of urine flow rate and sodium and potassium excretion. Inflammation elevated urine output in inflamed rats that did not receive any drug. Treatment with rofecoxib significantly decreased sodium excretion rate in normal ($p < 0.011$) and inflamed ($p < 0.005$) rats. Also, potassium excretion was significantly decreased in normal rats received rofecoxib ($p < 0.023$). There was no change in urinary sodium and potassium excretion and urine flow rate in rats receiving meloxicam.

4.3.5 The Effect of Inflammation on Pharmacokinetics Parameters

Examining the plasma concentration-time profile of rofecoxib (Figure 4-3), a delay in the time to reach peak plasma level (t_{max}) was observed in the inflamed rats. Table 4-4 summarizes the pharmacokinetic parameters of rofecoxib in the absence and presence of inflammation. Inflammation caused a significant decrease (30%) in oral clearance of rofecoxib ($p < 0.006$). As a result, the area under the plasma concentration-time curve from time of dosing to 24 h post-dose (AUC_{0-24}) of drug was significantly increased in inflamed rats ($p < 0.004$). However, terminal elimination half-life ($t_{1/2}$) and apparent volume of distribution (V_d) were not significantly altered by inflammation.

Figure 4-4 shows the plasma concentration-time curve of meloxicam. Inflammation did not affect the pharmacokinetic parameters of meloxicam (Table 4-5). Terminal elimination $t_{1/2}$, oral clearance (CL), and apparent V_d of meloxicam were not estimated due to insufficient data in time of elimination.

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

Although the concentrations of rofecoxib in kidney and plasma were higher in inflamed rats compared with those of normal rats, the ratios of kidney/plasma concentration were not significantly different ($p > 0.79$) between the two groups (Figure 4-5). There was no significant difference ($p > 0.14$) in the ratio of kidney/plasma concentration of meloxicam in normal and inflamed rats (Figure 4-6).

4.4 DISCUSSION

It has been shown that the levels of nitric oxide (13) and TNF-α (14) increase in RA patients. Mayo et al. (6) have reported a significant correlation between the severity of disease and concentrations of nitrite in serum. We also found a significant increase in concentrations of nitrite on days 9 or 13 post treatment with adjuvant that confirms the presence of inflammation in those groups (Figure 4-1). In this study, we have shown that TNF-α concentrations significantly increased in groups MEL/INF, on days 9 and 13, and ROF/INF, on day 13 (Figure 4-2) compared with baseline values. Although TNF-α concentration elevated on days 9 and 13 post-injection in groups PL/INF, the values did not reach significance. In addition, the weight of inflamed rats was significantly less than control group on day 9 and 13.

This study provides the evidence that kidney dysfunction occurs in rats inflamed with adjuvant. In order to assess GFR in normal and inflamed rats, we measured the levels of creatinine and BUN concentrations in the rat plasma. Both plasma creatinine (15) and BUN (16) reflect the GFR. In agreement with the result of earlier study (17), we observed an increase in plasma creatinine on day 9 and 13 (Table 4-2). In addition, the levels of BUN increased in inflamed rats after induction of inflammation on day 9 or 13 (Table 4-2). Accordingly, we provided evidence indicating inflammation causes altered renal function.

Although Dijoseph et al. (17) have reported an increase in urine output as a result of inflammation, the result of our study showed that urine output was increased in only one group (group PL/INF) (Table 4-3). The discrepancy between the result of that study and ours is most likely due to the difference in the models of inflammation used by two

studies. It is probable that the severity of inflammation associated with the adjuvant-induced arthritic used by Dijoseph et al. (17) is different from our model, pre-adjuvant arthritis. The other plausible explanation might be a difference in the length of experiments. They have noticed the significance on day 16 of experiment, while we continued the experiment only for 13 days.

In this study, we investigated the effect of inflammation on the total protein concentrations, as well. We did not detect any change in total protein on day 9 or 13 in normal or inflamed rats (Table 4-2). Besides, treatment with rofecoxib or meloxicam did not affect those concentrations. The unchanged concentrations of total protein could provide evidence that the pre-adjuvant arthritis, similar to the adjuvant-induced arthritis, is not a form of nephritic syndrome which is characterized by massive proteinuria (17). In our model of study, the degree of injury was not sufficient to produce protein leakage.

As it is shown in Table 4-3, while sodium and potassium excretion rates were not altered by inflammation, treatment with rofecoxib significantly decreased sodium excretion rate in normal and inflamed rats. Potassium excretion rate was decreased in normal rats treated with rofecoxib. The mechanisms underlying renal effects of NSAIDs are well documented in the literature. A decrease in GFR has been linked to the inhibition of COX-1 after administration of indomethacin to healthy elderly subjects (18). Whereas, the expression of COX-2 in thick ascending limb cells containing Na-K-ATPase proposes a role for the enzyme in regulation of sodium in rat kidney (19-21). NSAIDs may change potassium balance by suppression of the renin–aldosterone axis and/or a reduction in GFR (22). In spite of GFR alteration, confirmed by BUN and plasma creatinine elevation (Table 4-2), our results did not reveal any change in sodium and potassium excretion rate

after induction of inflammation. One explanation could be that the extent of glomerular damage and/or the period of experiment were not adequate to see the change. Also, we did not perform any test to evaluate the tubular damage to the kidney. Since sodium and potassium are exchanged in renal tubules, further studies are needed to investigate the possible tubular damage to the kidney.

Electrolyte excretion was altered by rofecoxib, but not meloxicam, on day 13 (Table 4-3). This study confirms the result of the previous study that we compared the effect of various NSAIDs on the urinary excretion of sodium and potassium in the rat (11) (Chapter 2). We found that rofecoxib, celecoxib, diclofenac, and flurbiprofen but not meloxicam significantly reduced the electrolytes excretion.

We have also shown that inflammation altered the pharmacokinetics of rofecoxib (Table 4-4), but not meloxicam (Table 4-5). It has been reported that inflammatory conditions alter the clearance of drugs which are mainly metabolized in the liver (23). In order to calculate hepatic extraction ratio of rofecoxib in the rat, we used pharmacokinetic data, after intravenous administration, reported by Halpin et al. (24). Based on their data, rofecoxib can be considered as an intermediate extraction ratio drug ($E \approx 0.50\text{-}0.55$). For a drug that undergoes first pass metabolism, shows linear pharmacokinetic profile, and has complete absorption, systemic availability is calculated from $F = Q / (Q + CL_I)$, where Q is the hepatic blood flow and CL_I is the intrinsic clearance of drug (25). Since CL_I can be replaced by $CL_I'*fu$, where CL_I' is the intrinsic clearance of free drug and fu is unbound fraction of drug. For drugs with intermediate hepatic extraction ratios, liver blood flow, unbound fraction of drug in the blood, and intrinsic clearance of unbound fraction are important parameters that influence the

systemic availability of drug. Since inflammation does not have any effect on Q (26), an increase in systemic exposure of rofecoxib (Table 4-4) by inflammation can be explained by a change in protein binding and/or intrinsic clearance. In a study performed to compare the plasma protein binding of propranolol and chlorpromazine between healthy and people diagnosed with inflammatory diseases, the higher plasma protein binding among patients with inflammatory disease was attributable to the elevated α_1-acid glycoprotein concentrations (7). Ling and Jamali (8) have shown that pre-adjuvant arthritis decreases the content of cytochrome P450 (CYP) enzymes. 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 reported by Monshouwer et al. (9).

For a low extraction ratio drug, since Q is much higher than CL_I, the above equation can be theoretically reduced to F=Q/Q. Therefore, a change in blood flow, protein binding, or intrinsic clearance cannot alter the systemic availability of drug. Hepatic extraction ratio was calculated for meloxicam to be less than 0.1, using the intravenous data reported by Aghazadeh-Habashi and Jamali (27). Not surprisingly, inflammation did not affect the systemic exposure of meloxicam (Table 4-5).

Although, based on radioactivity studies, the mean oral bioavailability of rofecoxib in human subjects is reported to be 93%, due to low solubility and consequently unavailability of intravenous dosage form, absolute bioavailability of drug in human is not known (28). Therefore, more studies are needed to compare the systemic exposure of drug in healthy and arthritic subjects. On the other hand, literature contains intravenous data for meloxicam suggesting the absolute bioavailability of drug is more

than 99% (29). Therefore, based on our data in rat, one may extrapolate that inflammation does not alter systemic availability of meloxicam in human.

The other objective of this study was to understand whether inflammation could change the distribution of NSAIDs into the kidney. Although it has been shown that expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 may change the permeability of the blood-brain barrier (30), the results of our study showed that 24-h post dose kidney/plasma concentration ratios of rofecoxib (Figure 4-5) and meloxicam (Figure 4-6) groups were not different between normal and inflamed rats indicating that inflammation did not change the permeability across the kidney membrane.

In summary, inflammation produces a kidney dysfunction in rat. The altered renal function is verified by an increase in concentrations of BUN and plasma creatinine. However, urinary sodium and potassium excretion are not altered by inflammation. Oral clearance of rofecoxib decreases by inflammation. A decline in unbound fraction or oral first pass metabolism of drug may be responsible for the change in pharmacokinetics of rofecoxib. The degree of distribution of rofecoxib and meloxicam to kidneys are not influenced by inflammation.

Table 4-1. The effect of inflammation on the weight of normal rats treated with placebo (PL/CONT, n=6), inflamed rats treated with placebo (PL/INF, n=6), normal rats treated with a 3 mg/kg oral dose of meloxicam (3 mg/kg), (MEL/CONT, n=7), inflamed rats treated with a 3 mg/kg oral dose of meloxicam (MEL/INF, n=7), normal rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/CONT, n=7), and inflamed rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/INF, n=6) on day 13 post-inflammation.

	Group					
Time	**PL/CONT**	**PL/INF**	**MEL/CONT**	**MEL/INF**	**ROF/CONT**	**ROF/INF**
Baseline	268 ± 2.58	268 ± 2.58	273 ± 9.83	262 ± 3.36	265 ± 5.00	268 ± 2.58
Day 9	337 ± 8.16	298 ± 21.8†	331 ± 8.61	284 ± 14.6†	339 ± 6.27	298 ± 21.8†
Day 13	362 ± 18.9	324 ± 19.9†	346 ± 13.6	302 ± 22.0†	354 ± 13.0	323 ± 19.9†

Data are mean ± standard deviation
†Significantly different from control

Table 4-2. Changes in BUN, plasma creatinine, and total protein in normal rats treated with placebo (PL/CONT, n=6), inflamed rats treated with placebo (PL/INF, n=6), normal rats treated with a 3 mg/kg oral dose of meloxicam (3 mg/kg), (MEL/CONT, n=7), inflamed rats treated with a 3 mg/kg oral dose of meloxicam (MEL/INF, n=7), normal rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/CONT, n=7), and inflamed rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/INF, n=6) on day 13 post-inflammation.

		Group					
Parameter	Time	PL/CONT	PL/INF	MEL/CONT	MEL/INF	ROF/CONT	ROF/INF
BUN (mmol/L)	Baseline	3.84 ± 0.93	4.48 ± 0.83	4.24 ± 0.91	4.22 ± 1.06	4.78 ± 0.81	4.48 ± 0.83
	Day 9	4.57 ± 1.05	6.22 ± 0.63***	4.79 ± 0.66	4.61 ± 0.88	4.42 ± 0.66	6.22 ± 0.63***
	Day 13	5.58 ± 0.69	8.04 ± 0.78	5.33 ± 1.78	10.6 ± 9.3***	7.69 ± 0.82	7.33 ± 2.79
Plasma creatinine (µmol/L)	Baseline	15.2 ± 3.43	14.33 ± 3.78	26.5 ± 2.07	20.6 ± 2.88	26.4 ± 2.88	14.3 ± 3.78
	Day 9	18.8 ± 3.42	28.8 ± 3.43***	29.2 ± 2.0	24.3 ± 2.43*	26.9 ± 3.98	28.8 ± 3.43***
	Day 13	21.67 ± 3.67	32.2 ± 4.96*	28.0 ± 5.4	39.4 ± 34.2*	34.2 ± 5.85	48.2 ± 32.7***
Total protein (g/L)	Baseline	51.8 ± 1.64	53.5 ± 4.18	52.5 ± 1.64	52.3 ± 1.11	54.3 ± 2.14	53.5 ± 4.18
	Day 9	55.00 ± 1.67	50.8 ± 1.60	54.7 ± 1.75	52.9 ± 1.95	53.0 ± 2.16	50.8 ± 1.60
	Day 13	51.8 ± 4.75	56.8 ± 2.32	53.8 ± 2.93	55.4 ± 0.89	55.2 ± 3.43	52.7 ± 2.58

Data are mean ± standard deviation

***Significantly different from baseline, p<0.05**

****Significantly different from baseline. P<0.01**

*****Significantly different from baseline, p<0.001**

Table 4-3. Changes in urine flow rate, sodium excretion, and potassium excretion in normal rats treated with placebo (PL/CONT, n=6), inflamed rats treated with placebo (PL/INF, n=6), normal rats treated with a 3 mg/kg oral dose of meloxicam (3 mg/kg), (MEL/CONT, n=7), inflamed rats treated with a 3 mg/kg oral dose of meloxicam (MEL/INF, n=7), normal rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/CONT, n=7), and inflamed rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/INF, n=6) on day 13 post-inflammation.

		Group					
Parameter	**Time**	**PL/CONT**	**PL/INF**	**MEL/CONT**	**MEL/INF**	**ROF/CONT**	**ROF/INF**
Urine flow rate (mL/h)	**Baseline**	0.75 ± 0.11	0.44 ± 0.21	0.54 ± 0.17	0.59 ± 0.30	0.61 ± 0.13	0.44 ± 0.21
	Post treatment	0.92 ± 0.4	0.71 ± 0.20*	0.63 ± 0.16	0.65 ± 0.26	0.55 ± 0.23	0.31 ± 0.13
Sodium excretion rate (µmol/min)	**Baseline**	1.83 ± 0.29	1.38 ± 0.41	1.19 ± 0.53	0.89 ± 0.37	1.62 ± 0.95	1.38 ± 0.41
	Post treatment	1.37 ± 0.46	1.57 ± 0.61	0.82 ± 0.50	0.76 ± 0.37	0.98 ± 0.56*	0.62 ± 0.41*
Potassium excretion rate (µmol/min)	**Baseline**	2.81 ± 0.87	1.58 ± 0.61	2.08 ± 0.55	1.81 ± 0.64	2.55 ± 0.95	1.58 ± 0.61
	Post treatment	2.10 ± 0.72	2.18 ± 0.80	2.27 ± 0.78	1.41 ± 0.46	1.63 ± 0.82*	1.08 ± 0.53

Data are mean ± standard deviation
***Significantly different from baseline**

Table 4-4. The pharmacokinetic parameters of rofecoxib (10mg/kg) in the absence or presence of inflammation.

	Condition	
Parameter	**Normal (n=7)**	**Inflamed (n=7)**
AUC_{0-24} (μg.h/mL)	21.57 ± 3.41	30.51 ± 4.03†
C_{max} (μg/mL)	2.63 ± 0.52	2.44 ± 0.37
t_{max} (h)	2.7 ± 1.2	5.6 ± 1.7
CL/F (mL/h/kg)	470 ± 75	331± 42†
Vd/F (mL/kg)	3895 ± 765	3888 ± 756
$t_{1/2}$ (h)	5.2 ± 0.9	6.5 ± 2.6

Note: The values are expressed as mean ± standard deviation
†Significantly different from normal rats

Table 4-5. The pharmacokinetic parameters of meloxicam (3mg/kg) in the absence or presence of inflammation.

	Condition	
Parameter	**Normal (n=7)**	**Inflamed (n=7)**
AUC_{0-24} (μg.h/mL)	366.57 ± 44.11	324.58 ± 81.70
C_{max} (μg/mL)	19.70 ± 3.75	16.85 ± 3.96
t_{max} (h)	6.7 ± 1.6	6.8 ± 1.8
CL/F (mL/h/kg)	‡	‡
Vd/F (mL/kg)	‡	‡
$t_{1/2}$ (h)	‡	‡

Note: The values are expressed as mean ± standard deviation
‡Not estimated

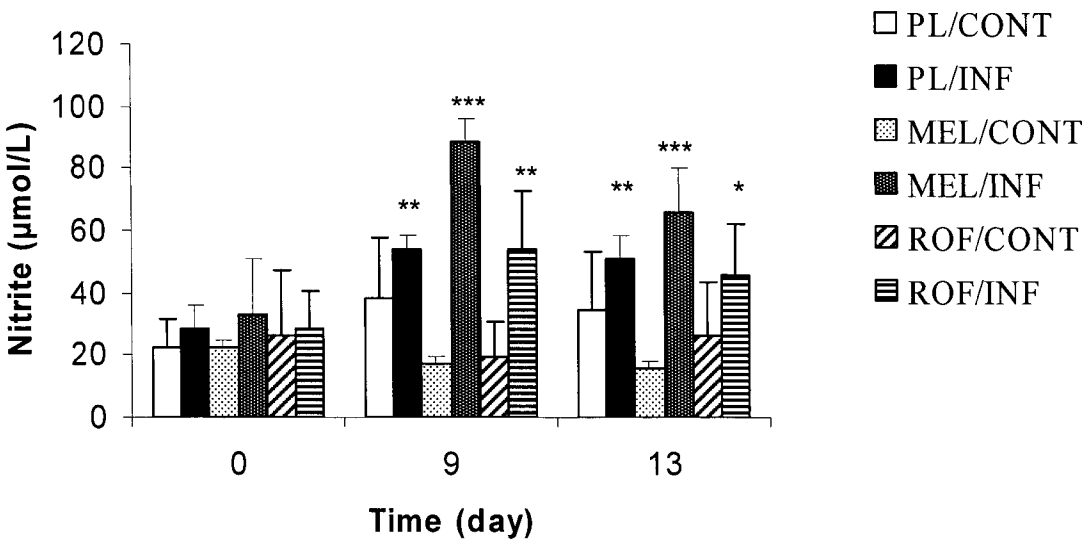

Figure 4-1. The changes in nitrite in normal rats treated with placebo (PL/CONT, n=6), inflamed rats treated with placebo (PL/INF, n=6), normal rats treated with a 3 mg/kg oral dose of meloxicam (3 mg/kg), (MEL/CONT, n=7), inflamed rats treated with a 3 mg/kg oral dose of meloxicam (MEL/INF, n=7), normal rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/CONT, n=7), and inflamed rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/INF, n=6) on day 13 post-inflammation.

*Significantly different from baseline, $p < 0.05$

**Significantly different from baseline, $p < 0.01$

***Significantly different from baseline, $p < 0.001$

The values are expressed as mean ± standard deviation.

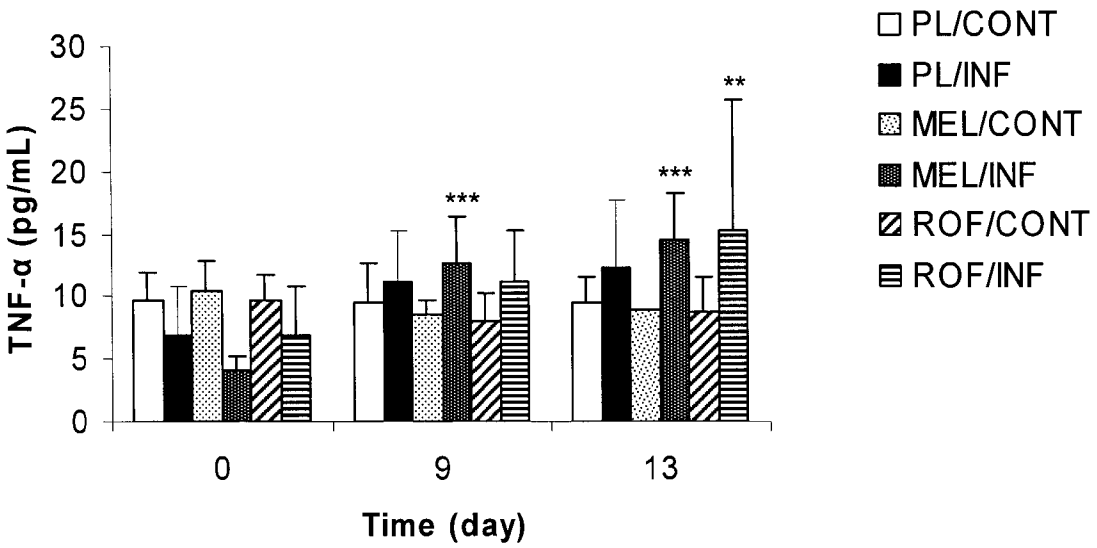

Figure 4-2. The changes in TNF-α normal rats treated with placebo ((PL/CONT, n=6), inflamed rats treated with placebo (PL/INF, n=6), normal rats treated with a 3 mg/kg oral dose of meloxicam (3 mg/kg), (MEL/CONT, n=7), inflamed rats treated with a 3 mg/kg oral dose of meloxicam (MEL/INF, n=7), normal rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/CONT, n=7), and inflamed rats treated with a 10 mg/kg oral dose of rofecoxib (ROF/INF, n=6) on day 13 post-inflammation.

**Significantly different from baseline, $p < 0.01$

***Significantly different from baseline, $p < 0.001$

The values are expressed as mean ± standard deviation.

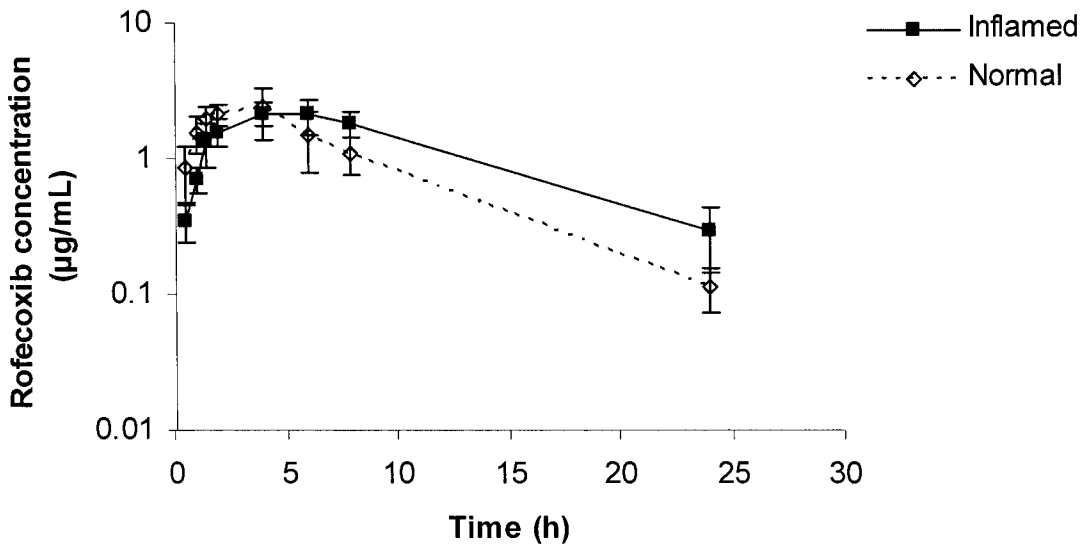

Figure 4-3. Plasma concentration-time profile of rofecoxib in absence (n=7) or presence (n=6) of inflammation following a 10 mg/kg oral dose.

The values are expressed as mean ± standard deviation.

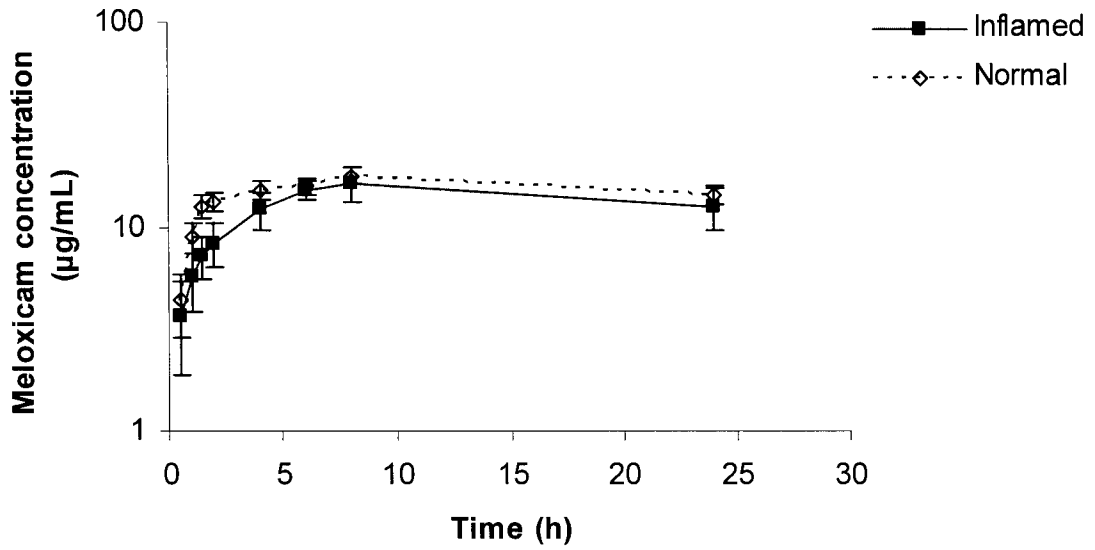

Figure 4-4. Plasma concentration-time profile of meloxicam in absence (n=7) or presence (n=7) of inflammation following a 3 mg/kg oral dose.

The values are expressed as mean ± standard deviation.

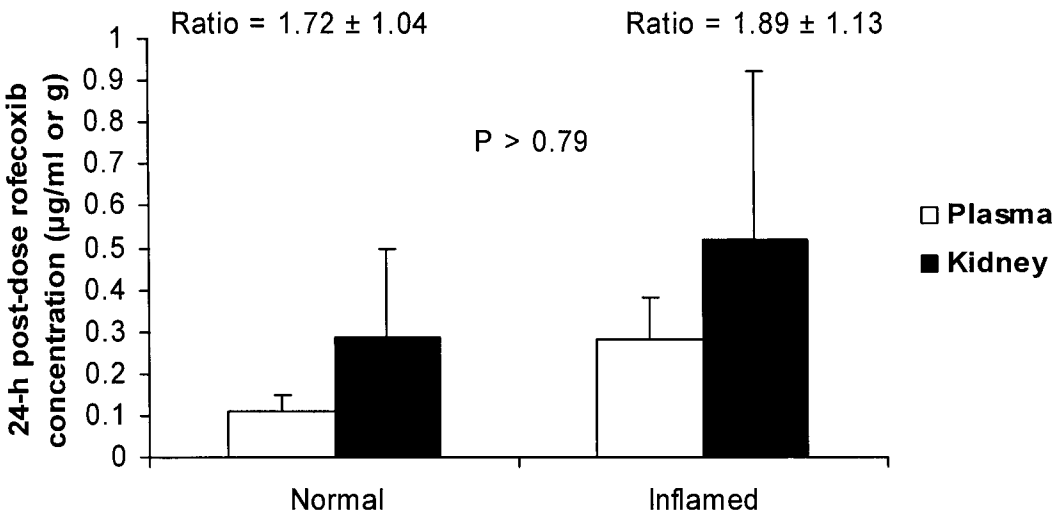

Figure 4-5. 24-h post dose concentrations of rofecoxib in plasma and kidney of normal (n=5) or inflamed (n=6) rats following a 10 mg/kg oral dose.

The values are expressed as mean ± standard deviation.

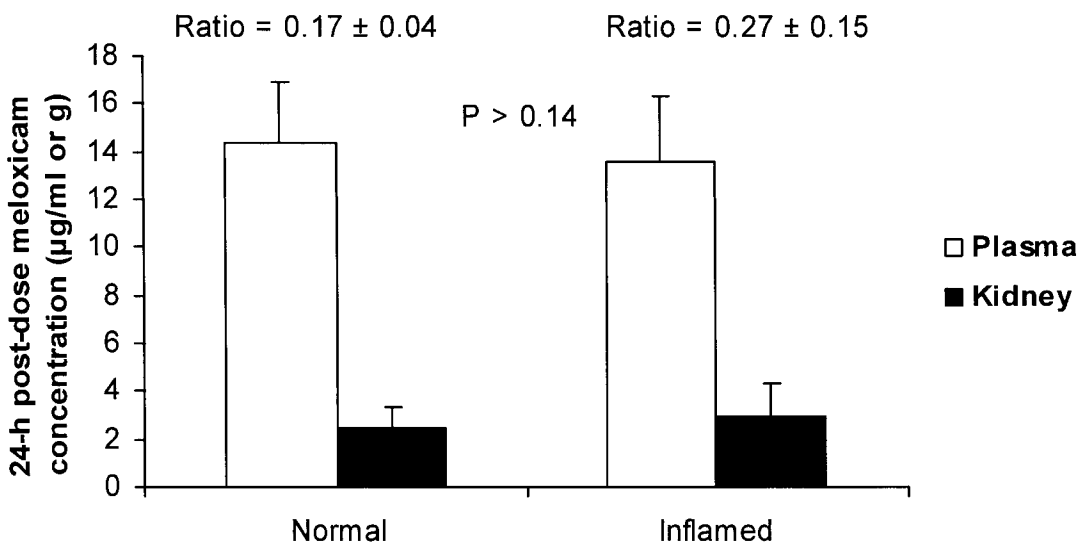

Figure 4-6. 24-h post dose concentrations of meloxicam in plasma and kidney of normal (n=6) or inflamed (n=7) rats following a 3 mg/kg oral dose.

The values are expressed as mean ± standard deviation.

4.5 References

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Chapter 5

General Discussions and Conclusions

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to reduce pain and inflammation associated with arthritis. However, several side effects such as gastrointestinal (GI) symptoms and renal effects limit the use of these compounds in some groups of patients (1). Since it was known that cyclooxygenase-1 (COX-1) is constitutively produced in many cells and tissues through the body and COX-2 is only generated in response to inflammation, it was believed that the development of NSAIDs with more selectivity for COX-2 might lead to fewer side effects (2). However, while this is true with respect to GI side effects (3), the discovery of COX-2 expression in the kidney (4), in the absence of inflammation, raised the question whether selective COX-2 inhibitors may possess other side effects such as cardiovascular and renal effects. Accordingly, we considered the hypothesis that selective COX-2 inhibitors have renal side effects similar to the traditional NSAIDs.

In order to examine our hypothesis, we selected a group of NSAIDs with diverse selectivity for COX-1 and COX-2. For instance, we chose flurbiprofen and diclofenac with high selectivity for COX-1 (5). On the other hand, rofecoxib was picked for its high selectivity for COX-2. Also, celecoxib and meloxicam were chosen for their relatively selectivity on COX-2. The most significant complications related to the use of NSAIDs are reported to be reduction in sodium and potassium excretion and renal perfusion (6). It has been shown that decreased sodium excretion can lead to important clinical events such as edema, weight gain, decreased response to anti-hypertensive drugs, and rarely congestive heart failure and hyperkalemia may cause arrhythmia (6). Therefore, the focus of our study was on the effects of NSAIDs on sodium and potassium exertion. The results of our study demonstrated that administration of rofecoxib, celecoxib, diclofenac, and

flurbiprofen significantly decreased sodium excretion in the rats on normal diet. Additionally, we observed a significant reduction in potassium excretion due to the effect of those NSAIDs. It is worth of noting that meloxicam did not show any effect on sodium and potassium excretion. Meloxicam has been around for many years. Some authors have categorized this drug as a preferential COX-2 inhibitor; however, others have considered it as a selective inhibitor of COX-2. Nevertheless, the unique characteristic of meloxicam concerning renal effects was our motivation to extend our investigation. We were interested to know whether the renal effects of examined NSAIDs were dictated by the degree of selectivity for COX-2 and COX-1. No correlation was found when the COX-2/COX-1 selectivity or potency of drugs versus renal effects was examined. While meloxicam and celecoxib hold the same levels of COX-2/COX-1 selectivity ratio, they showed different effect on renal function. Furthermore, rofecoxib, a highly selective COX-2 inhibitor, altered both sodium and potassium excretion, but meloxicam, with less selectivity for COX-2, did not. Thus, we rejected the hypothesis suggesting a correlation between selectivity for COX enzyme and renal effects.

In this study, we found a significant correlation between the pharmacokinetic parameters of rofecoxib or celecoxib with the renal effect. We provided evidence correlating the drug concentrations and toxic effects of coxibs. The linking pharmacokinetics and pharmacodynamics predicts the concentration- effect relationship of a drug (7). Knowing these relationships assists the prescriber to administer the doses which reaches the therapeutics concentrations and avoids toxic concentrations. In the field of NSAIDs, there are studies that correlate therapeutic effects (8) or toxic effects (9) with drug plasma concentrations. In case of salicylates, plasma concentrations required

for observing therapeutic effects in treatment of arthritis and toxic concentrations for developing side effects such as tinnitus or headaches have been reported (10). These observations show that measurement of steady plasma concentrations of NSAIDs might have clinical benefits. Our findings indicate a basis for therapeutic drug monitoring of selective COX-2 inhibitors in order to avoid the renal effects. Higher steady plasma concentrations of rofecoxib and celecoxib may indicate the undesirable effects. This is important especially for the patients who are at risk to develop renal effects. These are elderly, who have less renal function, congestive heart failure patients, patents who take other medication that have renal effects, and people diagnosed with kidney dysfunction. Our observations provide a need for clinical studies to establish the correlations between plasma concentrations and renal effects in human.

Although we could show the concentration- side effect relationships for rofecoxib and celecoxib, the main question pursuing the lack of renal effect of meloxicam was not answered yet. For many years, it has been known that the use of aminoglycosides or cyclosporine is associated with renal side effects (11;12). The degree of effect is governed by the extent of accumulation of those drugs in the kidney (13;14). Hence, we speculated that the renal effects of NSAIDs might be correlated with the amount of drug in the kidney as well. We measured the 24-h post-dose plasma and kidney concentrations of three COX-2-selective NSAIDs, rofecoxib, celecoxib, and meloxicam. For rofecoxib and celecoxib, the concentrations of drugs were much higher in kidney compared to those of plasma, while the ratio for meloxicam was in opposite direction. This suggests that the concentration of meloxicam in the kidney does not reach the levels needed to exert the renal effects. This may be explained with the difference in solubility of examined

selective COX-2 inhibitors in aqueous solutions. The work of Seedher and Bhatia (15) has been shown that solubility of rofecoxib and celecoxib is very small in response to pH changes. In contrast, meloxicam solubility substantially changed when the pH of solution was increased. Accordingly, the solubility of meloxicam is likely high at physiological pH. Therefore, meloxicam exists in ionized form and unable to pass cell membrane. On the other hand, rofecoxib and celecoxib are likely present in non-ionized form in the blood circulation. Therefore, the lipophilicity of drugs is adequate to pass the tissue membranes and show renal effects.

A review of literature provides evidence that arthritis causes kidney dysfunction (16). These findings indicate that arthritic patients are at risk of developing sub-clinical renal dysfunction. Since it was shown that rofecoxib and celecoxib decreased sodium excretion, it was worth of knowing whether the use of NSAIDs in arthritic patients may make sodium retention worse. In order to study those effects in animal models, we induced inflammation in rats with injection of adjuvant using the model described by Ling and Jamali (17). The presence of inflammation was confirmed with increase in the concentrations of nitric oxide and tumor necrosis factor-α.

We could detect an increase in the concentrations of creatinine and blood urea nitrogen on days 9 or 19 post-adjuvant injection. This indicates a change in glomerular filtration rate attributable to glomerular dysfunction. In contrast, inflammation did not change sodium or potassium excretion in rats. This suggests that inflammation causes glomerular, but not the tubular damage. This may be explained by the notion that sodium is actively transported out of the most portions of the tubule (18). Also, the reabsorption and secretion of potassium take place in proximal tubules and distal tubular cells,

respectively. Accordingly, one may speculate that inflammation does not change the action of renal tubule.

The other objective of this project was to understand the effect of inflammation on pharmacokinetics of selective COX-2 inhibitors. It is well known that inflammation affects the pharmacokinetics of some drugs. As it was expected for a low extraction drug, inflammation did not alter bioavailability of meloxicam. We did not observe any change in the area under the plasma concentration-time curve from time of dosing to 24 h post-dose (AUC_{0-24}) of drug in inflamed rats. In contrast, AUC_{0-24} of rofecoxib was higher in inflamed rats compared to that of control. This suggests that the content and/or activity of enzymes metabolizing rofecoxib were reduced due to inflammation.

These observations in rat may have clinical implications in human. We provided evidence that meloxicam did not alter electrolyte excretion (Chapter 2). Moreover, inflammation did not change the pharmacokinetics of meloxicam due to the low extraction ratio of drug (Chapter 4). Therefore, it can be suggested that meloxicam to be the first choice among selective COX-2 inhibitors in treatment of signs and symptoms of arthritic patients.

In summary, this work provides the evidences that selective COX-2 inhibitors have renal effects similar to traditional NSAIDs. The extent of the effect is correlated with some pharmacokinetic parameters such as AUC and 24-h plasma drug concentration. In addition, inflammation alters the kidney function and pharmacokinetics of rofecoxib. However, Inflammation does not change the permeability of kidney.

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