In vitro effects of cyclooxygenase inhibitors in whole blood of horses, dogs, and cats

Christine Brideau, BSc; Carlo Van Staden, BSc; Chi Chung Chan, PhD

Objective—To determine potency and selectivity of nonsteroidal anti-inflammatory drugs (NSAID) and cyclooxygenase- (COX-) specific inhibitors in whole blood from horses, dogs, and cats.

Sample Population—Blood samples from 30 healthy horses, 48 healthy dogs, and 9 healthy cats.

Procedure—Activities of COX-1 and COX-2 were determined by measuring coagulation-induced thromboxane B_2 and lipopolysaccharide-induced prostaglandin E_2 concentrations, respectively, in whole blood with and without the addition of various concentrations of phenylbutazone, flunixin meglumine, ketoprofen, diclofenac, indomethacin, meloxicam, carprofen, 5-bromo-2[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene (DuP 697), 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl) phenyl-2(5H)-furan one (DFU), 3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5H)-furanone (MF-tricyclic), and celecoxib. Potency of each test compound was determined by calculating the concentration that resulted in inhibition of 50% of COX activity (IC_{50}). Selectivity was determined by calculating the ratio of IC_{50} for COX-1 to IC_{50} for COX-2 (COX-1/COX-2 ratio).

Results—The novel compound DFU was the most selective COX-2 inhibitor in equine, canine, and feline blood; COX-1/COX-2 ratios were 77.5, 74, and 69, respectively. Carprofen was the weakest inhibitor of COX-2, compared with the other COX-2 selective inhibitors, and did not inhibit COX-2 activity in equine blood. In contrast, NSAID such as phenylbutazone and flunixin meglumine were more potent inhibitors of COX-1 than COX-2 in canine and equine blood.

Conclusions and Clinical Relevance—The novel COX-2 inhibitor DFU was more potent and selective in canine, equine, and feline blood, compared with phenylbutazone, flunixin meglumine, and carprofen. Compounds that specifically inhibit COX-2 may result in a lower incidence of adverse effects, compared with NSAID, when administered at therapeutic dosages to horses, dogs, and cats. (*Am J Vet Res* 2001;62:1755–1760)

Nonsteroidal anti-inflammatory drugs (NSAID) are inhibitors of **cyclooxygenase (COX)**[1] and have long been used as therapeutic agents in humans because of their anti-inflammatory, analgesic, and antipyretic effects. It is also known that NSAID can cause gastrointestinal tract damage leading to ulcers in some patients.[2] Nonsteroidal antiinflammatory drugs are used in veterinary medicine, especially for their analgesic properties, but they can cause adverse effects such as vomiting, nephrotoxicity, and gastric ulceration.[3]

There are 2 distinct forms of COX that catalyze the bisoxygenation of arachidonic acid to **prostaglandin H_2 (PGH_2)**.[4] **Cyclooxygenase 1 (COX-1)** is considered the constitutive form of the enzyme. It has been implicated in housekeeping functions such as platelet aggregation, regulation of renal blood flow, and gastric cytoprotection. **Cyclooxygenase 2 (COX-2)** is the inducible form of the enzyme and is involved in inflammation. Production of COX-2 can be induced by a variety of natural and artificial stimuli such as phorbol esters, endotoxins, cytokines, stress, and injury.[5] The discovery of COX-2 has led to the identification and development of a novel class of COX-2-specific inhibitors that have little inhibitory effect on COX-1. Thus, COX-2 inhibitors are more therapeutic and less toxic than COX-1 inhibitors.[6]

Results of gene disruption studies in mice indicate that COX-1 is necessary for normal platelet function, but, surprisingly, gastric or renal abnormalities did not develop in COX-1-deficient mice.[7] However, COX-2-deficient mice were more sensitive to endotoxin-induced hepatocellular cytotoxicity[8] and developed severe renal abnormalities.[9] It is difficult to extrapolate from results of experiments studying gene-disrupted mice to the in vivo effects of NSAID for several reasons. First, disruption of either the COX-1 or COX-2 gene can result in activation of compensatory mechanisms during pre- or postnatal development that may account for the lack of gastric ulceration in COX-1-deficient mice or the normal inflammatory response observed in the COX-2-deficient strain. Second, NSAID are not selective; they inhibit both isoforms. Treatment with NSAID thus abolishes all prostanoid-mediated functions in tissues. The use of mice deficient in both COX-1 and COX-2 may provide data that are more comparable with the in vivo effects of NSAID; however, such mice die shortly after birth.[10] Data from human clinical studies indicate that administration of a selective COX-2 inhibitor does reduce the incidence of gastric ulcers, compared with administration of NSAID,[11] while still resulting in a reduction in severity of clinical signs in patients with osteoarthritis.[12]

Numerous studies of the differential effects of COX inhibitors on each isoenzyme have been published. However, it is difficult to compare data among studies because of the many variables in assays used to measure COX-1 and COX-2 activities. Cyclooxygenase may be purified from humans or other animals, produced as a recombinant protein, or prepared as microsomal fractions from intact or transfected cells.[6] Other variables that can influence the potency of an inhibitor

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From the Departments of Biochemistry & Molecular Biology (Brideau) and Pharmacology (Van Staden, Chan), Merck Frosst Centre for Therapeutic Research, Kirkland, Quebec, Canada, H9R 4P8. Mr. Van Staden's present address is Amgen Inc, One Amgen Center Dr, Thousand Oaks, CA 91320.

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are the method and time of COX-2 induction and the concentration of substrate (ie, arachidonic acid) used.[13] Assays that incorporate purified enzymes or transfected whole cells can reveal the intrinsic potency of a compound in the absence of other proteins, but such assays do not necessarily reflect in vivo conditions. Typically, in studies that evaluate the effects of gastrointestinal-tract sparing anti-inflammatory agents, the relevant source of enzyme is intact cells from the gastric mucosa (COX-1) or synovium (COX-2). However, these tissues are not readily available, and such purifications cannot be performed routinely. Platelets freshly isolated from blood have been used to study COX-1 activity, whereas isolated blood monocytes have been used to study COX-2 activity. Platelets do not express COX-2 but are a rich source of COX-1.[14] When platelets are activated (eg, by clotting), COX-1 converts arachidonic acid to PGH_2, which is subsequently catalyzed to **thromboxane A_2 (TxA_2)** by thromboxane synthase. Thromboxane A_2 is then quickly converted to its stable metabolite **thromboxane B_2 (TxB_2)**.[15] **Lipopolysaccharide**- (**LPS**-) activated blood monocytes produce COX-2, which also acts to convert arachidonic acid to PGH_2. However, in monocytes, PGH_2 is preferentially converted to **prostaglandin E_2 (PGE_2)** by PGE synthase. Determination of PGE_2 concentration is commonly used as a marker for COX-2 activity.[16,17] In vitro assays designed to measure TxB_2 or PGE_2 concentrations, and hence, COX-1 or COX-2 activity, respectively, are typically performed, using buffers or cell media low in protein content. The use of such buffers may yield an overestimation of NSAID potency,[17] because plasma is rich in albumin and other proteins to which inhibitors bind nonspecifically.

To evaluate the in vitro efficacy of NSAID in clinical studies, a rapid and physiologically sound method that incorporates whole blood is preferred. Because TxB_2 and PGE_2 are lipid mediators, they can easily be detected in tissues from all animal species, using prostanoid-specific antibodies. A whole blood assay is a convenient method to study the in vitro biochemical efficacy and selectivity of NSAID on COX-1 and COX-2 activities in many animal species. This method was developed and established, using whole blood from humans initially, and has been adapted for use with blood from many other animal species.[18] Moreover, the whole blood assay is the ideal system to use for testing potency of novel inhibitors on each COX isoform separately.[19] The purpose of the study reported here was to determine the potency and selectivity of known NSAID, COX-specific inhibitors, and new experimental compounds in whole blood from horses, dogs, and cats.

Materials and Methods

Sample population—Blood was collected from 30 healthy male and female horses, 9 male cats, and 48 male Beagles. Maximum volumes obtained from each animal were 80 ml for horses, 30 ml for dogs, and 20 ml for cats. Assays to measure COX-1 and COX-2 activities were typically performed, using blood from the same donor. Some animals were used more than once; in these cases, at least 10 weeks elapsed between sample collections. All procedures were approved by the Animal Care Committees at the Merck Frosst Center for Therapeutic Research according to the guidelines established by the Canadian Council on Animal Care.

Test compounds—Inhibitory effects (selectivity and potency) of several commonly used NSAID and preferential and selective COX-2 inhibitors as well as several novel selective COX-2 inhibitors were evaluated. The NSAID included phenylbutazone,[a] flunixin meglumine,[b] ketoprofen,[c] diclofenac,[c] and indomethacin.[d] Commonly used preferential and selective COX-2 inhibitors included meloxicam[20,e] and carprofen[c] and celecoxib,[21,e] respectively. New selective COX-2 inhibitors included **5-bromo-2[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene (DuP 697)**,[22,e] **5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furan one (DFU)**,[23,e] and **3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5H)-furanone (MF-tricyclic)**.[24,e] All test compounds were dissolved in **dimethyl sulfoxide (DMSO)** before use; however, not all test compounds were evaluated in each species. For instance, only DFU and carprofen were tested in feline blood, whereas all compounds except flunixin meglumine were tested in canine blood.

Determination of COX-1 activity in whole blood—Fresh blood was collected from cats or dogs into sterile anticoagulant-free vacuum tubes. Aliquots (500 µl) were immediately transferred to polypropylene tubes containing 2 µl of DMSO (positive control) or a test compound. Equine blood (2.5 ml) was collected directly into 3-ml sterile anticoagulant-free vacuum tubes containing 10 µl of DMSO or a test compound. Test compounds were evaluated at final concentrations ranging from 0.01 to 400 µ*M*. Tubes were gently inverted several times, then incubated at 37 C for 1 hour to allow blood to clot. After incubation, serum was obtained by centrifugation at 400 × g for 10 minutes. A 100-µl aliquot of serum was mixed with 400 µl of methanol to precipitate protein. The supernatant was collected and diluted 400-fold (canine blood) or 40-fold (feline and equine blood) in **enzyme immunoassay (EIA)** buffer. Concentration of TxB_2 was measured in diluted samples by use of a EIA kit.[f] Amount of TxB_2 in each sample was calculated from the standard curve, which ranged from 4 to 1,000 pg/ml. Recovery of TxB_2 added to equine, canine, and feline serum samples, as measured by use of this EIA, was 100%; **coefficient of variance (CV)** was < 14%. Activity of COX-1 was determined in the positive controls. Background COX-1 activity was taken as the concentration of TxB_2 in plasma obtained from unstimulated blood.

Determination of COX-2 activity in whole blood—Fresh blood was collected from horses, cats, and dogs into sterile vacuum tubes containing heparin. Heparinized whole blood was preincubated for 5 minutes with LPS[g] diluted in PBS solution containing 0.1% (wt/vol) bovine serum albumin.[h] For feline and equine blood, LPS was used at a final concentration of 100 µg/ml, whereas for canine blood, LPS was used at 1 µg/ml. After preincubation, 500-µl aliquots of LPS-treated blood were incubated for 24 hours at 37 C with 2 µl of DMSO (positive control) or 2 µl of a test compound at final concentrations ranging from 1 n*M* to 400 µ*M*. At the end of the 24-hour incubation, blood was centrifuged at 400 × g for 10 minutes to obtain plasma. Plasma from unstimulated (no LPS) blood collected before incubation (at time 0) was used as the negative control. A 100-µl aliquot of plasma was mixed with 400 µl of methanol to precipitate protein. Supernatant and PGE_2 standards were incubated overnight with methyl oxime to convert PGE_2 to its stable methyloximated derivative. The reaction was stopped by addition of PGE_2 **radioimmunoassay (RIA)** buffer to yield a 4-fold (equine and feline samples) or 50-fold (canine samples) dilu-

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Table 1—Characteristics of assays designed to assess activities of cyclooxygenase (COX)-1 and -2 in whole blood

Species	COX-1 assay*				COX-2 assay†			
	Bkg TxB_2 (ng/ml)	Induced TxB_2 (ng/ml)	Intra-assay CV (%)	Intra-animal CV (%)	Bkg PGE_2 (ng/ml)	Induced PGE_2 (ng/ml)	Intra-assay CV (%)	Intra-animal CV (%)
Equine‡	2.0 ± 2.0	26 ± 12	22%	34%	0.2 ± 0.2	1.7 ± 1.0	18%	57%
Canine§	3.0 ± 1.0	465 ± 33	16%	44%	1.2 ± 0.2	329 ± 30	16%	63%
Feline||	1.5 ± 0.4	33 ± 8	19%	40%	0.7 ± 0.03	8.3 ± 1.8	21%	35%

Concentration data are reported as mean ± SEM.
*The COX-1 assay measured concentration of TxB_2 in serum after whole blood was allowed to clot (induced TxB_2). Background concentration was the concentration in plasma (negative controls). †The COX-2 assay measured concentration of PGE_2 in plasma after heparinized whole blood was stimulated with lipopolysaccharide. Background concentration was the concentration in plasma from unstimulated whole blood. ‡Blood samples from 29 and 30 horses were used in the COX-1 and COX-2 assays, respectively; intra-animal CV was determined by repeating assays with at least a 10-week interval in 8 horses. §Blood samples from 42 and 48 dogs were used in the COX-1 and COX-2 assays, respectively; intra-animal CV was determined by repeating assays with at least a 10-week interval in 5 dogs. ||Blood samples from 8 and 9 cats were used in the COX-1 and COX-2 assays, respectively; intra-animal CV was determined by repeating assays with at least a 10-week interval in 2 cats.
Bkg = Backround. TxB_2 = Thromboxane B_2. CV = Coefficient of variance. PGE_2 = Prostaglandin E_2.

tion of extracted samples. Concentration of PGE_2 was determined by use of a commercial RIA kit,[i] and amount of PGE_2 in each sample was calculated from the standard curve, which ranged from 0.4 to 3,200 pg/ml. Recovery of PGE_2 added to equine and feline plasma, as measured by use of this RIA, was 100%, whereas recovery from canine plasma was only 52%. The low recovery of PGE_2 from canine plasma may have been attributable to the use of multiple dilution steps. Canine plasma was diluted 1,000-fold for the RIA, and no correction was made for extraction efficiency. As with recovery of TxB_2, CV was < 14% for recovery of PGE_2 from all spiked samples. Activity of COX-2 was determined in LPS-stimulated positive controls. Background activity was taken as the concentration of PGE_2 in negative control samples.

Determination of potency and selectivity of test compounds—Potency of each test compound was determined by calculating the **concentration that resulted in inhibition of 50% of COX activity (IC_{50})**. Selectivity of each test compound was determined by calculating the **ratio of IC_{50} for COX-1 to IC_{50} for COX-2 (COX-1/COX-2 ratio)**.

Statistical analyses—All statistical analyses were performed using the Student *t*-test. Significance was set at $P \leq 0.05$.

Results

Concentration of TxB_2 and PGE_2 in whole blood—In positive control samples from all 3 species, concentrations of TxB_2 and PGE_2 increased a minimum of 13- and 8-fold, respectively, over background concentrations, indicating that COX-1 and COX-2 activity was present and measurable (**Table 1**). The concentration of TxB_2 in positive control canine serum was 14- to 17-fold greater than that in feline or equine serum, whereas concentration of PGE_2 varied among species. To obtain a substantial and consistent increase in PGE_2 production in equine and feline blood, LPS was used at a final concentration of 100 µg/ml. In contrast, 100-fold less LPS (1 µg/ml) was required to stimulate COX-2 activity in canine blood. Despite that, concentration of LPS-induced PGE_2 in canine plasma was 193-fold greater than that in equine plasma and 40-fold greater than that in feline plasma. Concentration of PGE_2 in feline plasma was 5-fold greater than in equine plasma.

Intra-assay and intra-animal (or bleed-to-bleed) CV for the TxB_2 and PGE_2 assays were calculated from concentrations in positive control samples, as these samples yielded the maximal TxB_2 or PGE_2 response. Although DMSO (0.4%) was present in all samples,

Table 2—In vitro potency and selectivity of COX inhibitors

Inhibitor	IC_{50} (µ*M*) for COX-1	IC_{50} (µ*M*) for COX-2	COX-1/ COX-2*
Equine blood			
DFU	29.44 ± 2.06 (3)	0.38 ± 0.06 (3)	77.5†
MF-tricyclic	16.61 ± 2.55 (14)	0.44 ± 0.07 (16)	38†
Carprofen	9.20 ± 0.26‡ (3)	5.87 ± 0.14‡ (3)	1.6
Phenylbutazone	6.15 ± 1.83‡ (4)	3.79 ± 0.72 (6)	1.6
Flunixin meglumine	0.06 ± 0.02‡ (3)	0.18 ± 0.03 (4)	0.3
Indomethacin	0.036 ± 0.004‡ (2)	0.43 ± 0.01 (3)	0.08
Canine blood			
DFU	63.50 ± 8.14 (16)	0.86 ± 0.31 (15)	74†
MF-tricyclic	54.46 ± 5.05 (15)	1.13 ± 0.32 (17)	48†
Meloxicam	4.33 ± 0.77‡ (9)	0.45 ± 0.07 (12)	10†
Celecoxib	14.72 ± 1.50‡ (9)	1.58 ± 0.46 (9)	9†
DuP 697	6.77 ± 2.53‡ (3)	0.93 ± 0.58 (5)	7
Carprofen	65.19 ± 7.55 (10)	10.00 ± 1.89‡ (12)	6.5
Indomethacin	0.74 ± 0.19‡ (21)	0.16 ± 0.02 (19)	4†
Diclofenac	0.41 ± 0.16‡ (8)	0.34 ± 0.08 (6)	1
Ketoprofen	0.13 ± 0.02‡ (6)	0.23 ± 0.02 (3)	0.6†
Phenylbutazone	17.77 ± 4.78‡ (5)	27.57 ± 5.08‡ (8)	0.6
Feline blood			
DFU	33.08 ± 10.58 (5)	0.48 ± 0.07 (5)	69§
Carprofen	8.93 ± 1.60 (3)	1.64 ± 0.19‡(3)	5.5§

IC_{50} data are reported as mean ± SEM (n).
*Ratio of IC_{50} for COX-1 to IC_{50} for COX-2. †Significantly ($P < 0.01$) different from IC_{50} for COX-1 determined for the same compound. ‡Significantly ($P < 0.01$) different from IC_{50} determined for DFU. §Significantly ($P < 0.05$) different from IC_{50} for COX-1 determined for the same compound.
IC_{50} = Concentration of inhibitor that resulted in inhibition of 50% of COX activity.

DMSO (up to 5%) does not affect COX-1 or COX-2 activity.[j] In addition, DMSO is a common vehicle used in COX-2 induction assays and assays that measure purified enzyme activity.[23]

Inhibitory effects of test compounds in equine blood—Of the COX-2 preferential inhibitors, the novel drugs DFU and MF-tricyclic were the most potent inhibitors of COX-2 in equine blood. In contrast, carprofen was the weakest COX-2 inhibitor. Activity of carprofen was significantly different from DFU (**Table 2**). In the NSAID class, flunixin meglumine and indomethacin were more potent than phenylbutazone. Of all compounds tested, DFU and MF-tricyclic were significantly more selective for COX-2 than COX-1. In comparison, phenylbutazone- or carprofen-induced inhibition of COX-2 was only 1.6-fold greater than that of COX-1. Flunixin and indomethacin appeared to be selective COX-1

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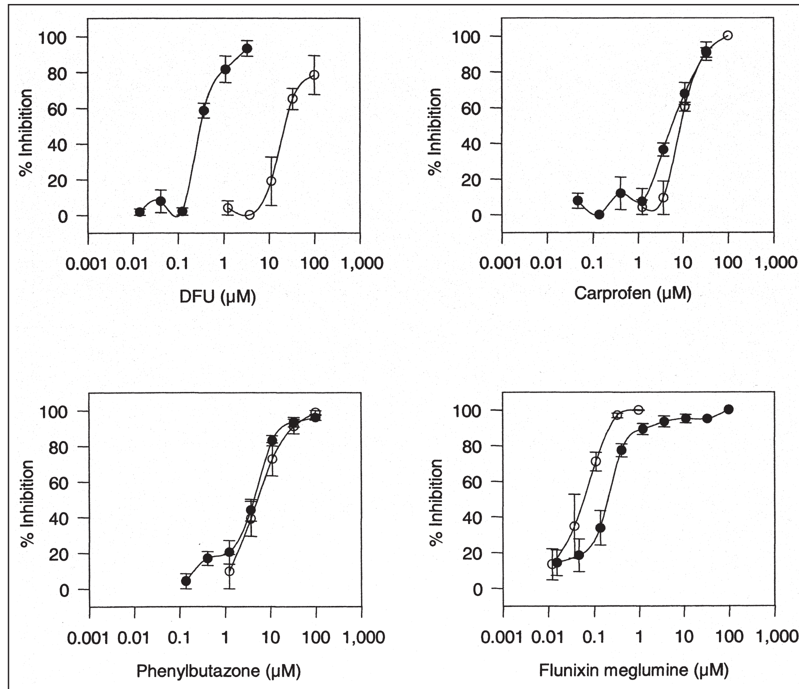

Figure 1—Mean ± SEM percentage of inhibition of cyclooxygenase-1 (COX-1; open circles) and cyclooxygenase-2 (COX-2; closed circles) in equine whole blood versus concentration of DFU (n = 3), carprofen (3), phenylbutazone (6), and flunixin meglumine (4).

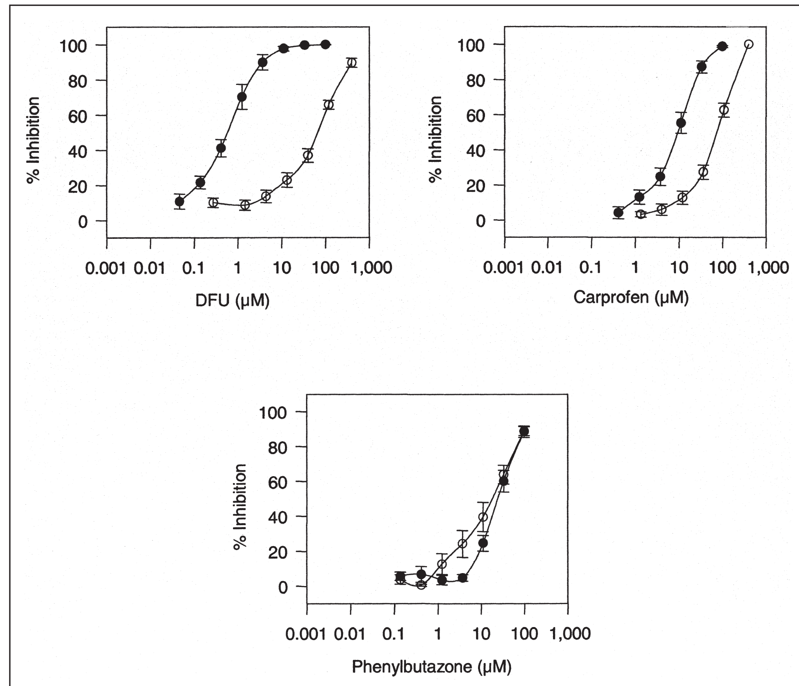

Figure 2—Mean ± SEM percentage of inhibition of COX-1 (open circles) and COX-2 (closed circles) in canine whole blood versus concentration of DFU (n = 16), carprofen (12), and phenylbutazone (8).

inhibitors. The COX-1 and COX-2 inhibition curves for carprofen and phenylbutazone overlapped, indicating a lack of selectivity for these 2 drugs, whereas the COX-1 inhibition curve for flunixin was shifted to the left of the COX-2 curve, indicating that this drug preferentially inhibited COX-1. In contrast, the COX-1 inhibition curve for DFU was shifted greatly toward the right of the COX-2 curve, indicating that DFU was selective for COX-2 even when used at the highest concentration (3 μ*M*; **Fig 1**).

Inhibitory effects of test compounds in canine

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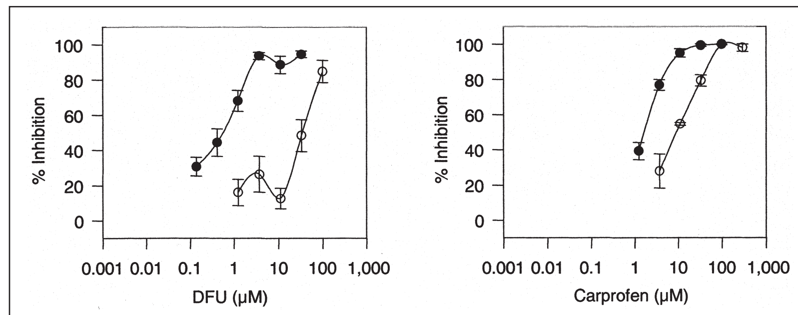

Figure 3—Mean ± SEM percentage of inhibition of COX-1 (open circles) and COX-2 (closed circles) in feline whole blood versus concentration of DFU (n = 3) and carprofen (3).

blood—In canine blood, ketoprofen and diclofenac were potent COX-1 and COX-2 inhibitors in terms of absolute IC_{50} values, but neither was a selective inhibitor. Phenylbutazone was the weakest inhibitor of COX-2; indeed, this drug appeared to selectively inhibit COX-1. Of the selective COX-2 inhibitors, carprofen was the least effective and selective. In contrast, DFU was the most potent and selective COX-2 inhibitor (Table 2). Inhibition curves for DFU, carprofen, and phenylbutazone were derived; COX-1 and COX-2 inhibition curves for phenylbutazone overlapped, indicating a lack of selectivity. In contrast COX-2 inhibition curves for carprofen and DFU were shifted to the left of the COX-1 curves, indicating that these drugs selectively inhibited COX-2 (**Fig 2**).

Inhibitory effects of test compounds in feline blood—Only 2 test compounds were evaluated in feline blood. Both DFU and carprofen selectively inhibited COX-2 (**Fig 3**). However, DFU was > 12-fold more selective for COX-2 than was carprofen (Table 2).

Discussion

In this study, we described a simple in vitro method for determining selectivity of COX inhibitors in whole blood from 3 animal species. The COX-1 inhibitory assay incorporates a natural clotting process in which blood platelets are stimulated to aggregate and activate COX-1. Results of this assay indicated that clotting-induced COX-1-mediated TxB_2 production in canine blood was at least 10-fold greater than that in equine or feline blood. Activity of COX-2, as measured by PGE_2 production, also varied greatly among species. We determined from a preliminary LPS-dose response experiment that 100 µg of LPS/ml was required for sustained and consistent PGE_2 production in feline and equine blood, whereas only 1 µg/ml was required in canine blood. In addition, we detected variability in results of these assays when blood collected at 2 different times from the same animal was assayed; however, IC_{50} of the test compounds remained constant from bleed to bleed.

In general, test compounds were less potent inhibitors of canine COX, compared with equine and feline COX. Concentrations of DFU and carprofen at which 50% of the COX response was inhibited (ie, IC_{50}) were higher in canine blood, compared with equine or feline blood; however, selectivity was similar among species. The lower potency of these inhibitors may have been attributable to the high concentrations of TxB_2 and PGE_2 in canine blood. Other possible explanations for this difference in potency include differences in the sequence of either COX-1 or COX-2 or differences in substrate concentrations among species.

Inhibition curves for DFU in canine blood indicated that 10 µ*M* DFU inhibited > 90% of COX-2 activity and only 20% of COX-1 activity. To achieve the same degree of COX-2 inhibition with carprofen, concentration was increased to 100 µ*M*. This concentration also resulted in inhibition of > 60% of COX-1 activity. Phenylbutazone at concentrations > 30 µ*M* inhibited both enzymes equally. Thus, in dogs, DFU and carprofen were 74- and 6.5-fold more selective, respectively, for COX-2, whereas phenylbutazone was slightly more selective for COX-1. Our results differed from those of a previous study[25] in which carprofen and phenylbutazone were reported to be 129- and 2.6-fold more selective, respectively, for COX-2. This difference may be attributable to differences in the assays used to measure COX activity. In the previous study, COX-1 activity was measured by challenging isolated platelets with the calcium ionophore A23187 in the presence of 0.2% bovine serum albumin. Activity of COX-2 was assessed by stimulating a transformed canine macrophage-like cell line with LPS overnight and then adding the COX substrate arachidonic acid in the presence of 1% fetal calf serum (5-fold more protein than used in the COX-1 assay). The use of different sources of cells, stimulations, and protein concentrations to determine COX-1 and COX-2 activity make it difficult to determine selectivity of test compounds. The whole blood assays described in the present study used endogenous substrate; protein concentrations also remained constant between assays. In addition, because blood from the same donor was used in both assays, appropriate comparisons could be made for the same test compound.

In equine blood, 3 µ*M* DFU inhibited > 90% of COX-2 activity and 0% of COX-1 activity. This was in contrast to results for carprofen, phenylbutazone, and flunixin; concentrations of these 3 inhibitors (30 µ*M* for carprofen and phenylbutazone and 1 µ*M* for flunixin) that yielded > 90% inhibition of COX-2 activity also yielded > 90% inhibition of COX-1 activity. Similar results for DFU and carprofen were obtained with feline blood. Inhibition of > 93% of COX-2 activity was achieved with 3.7 µ*M* DFU. This concentration

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resulted in inhibition of only 26% of COX-1 activity. On the other hand, > 10 μ*M* of carprofen was necessary to achieve 90% inhibition of COX-2 in feline blood, and this concentration also resulted in > 50% inhibition of COX-1 activity.

We have described a simple in vitro method to study the potency and selectivity of conventional NSAID and new selective COX-2 inhibitors, using whole blood from various animal species. We have shown that COX-1 and COX-2 activity and inhibitor selectivity are species dependent. We have also demonstrated that the novel selective COX-2 inhibitor DFU is a more potent and selective inhibitor of COX-2 in whole blood from dogs, horses, and cats, compared with commonly used COX inhibitors.

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[a]Cayman, Ann Arbor, Mich.
[b]CDMV (Centre de Distribution des Médicaments Vétérinaires), Montreal, QC, Canada.
[c]Sigma Chemical, St Louis, Mo.
[d]Merck & Co, Rahway, NJ.
[e]Synthesized at the Department of Medicinal Chemistry, Merck Frosst Center for Therapeutic Research, Merck Frosst Canada & Co, Kirkland, QC, Canada.
[f]EIA kit #519031, Cayman, Ann Arbor, Mich.
[g]Lipopolysaccharide, *E coli*, serotype 0111:B4, Sigma Chemical Co, St Louis, Mo.
[h]Albumin, bovine fraction V, Sigma Chemical Co, St Louis, Mo.
[i]RIA kit RPA530, Amersham, Oakville, ON, Canada.
[j]Ouellet M, Department of Biochemistry and Molecular Biology, Merck Frosst Canada & Co, Kirkland, QC, Canada: Personal communication, 1999.

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