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Short communication

Measurement of cyclooxygenase inhibition using liquid chromatography–tandem mass spectrometry

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ABSTRACT

Because cyclooxygenases (COX) convert arachidonic acid into pro-inflammatory cyclic endoperoxides, inhibition of these enzymes and especially the inducible COX-2 form is an important therapeutic approach to manage inflammatory diseases and possibly prevent cancer. Due to side effects of existing non-selective and COX-2 selective non-steroidal anti-inflammatory agents, the discovery of new COX inhibitors continues to be an area of active investigation. Since existing assays are slow or lack specificity, a liquid chromatography–tandem mass spectrometry (LC–MS–MS) based COX inhibition assay was developed and validated for the rapid and accurate quantitative analysis of the COX product prostaglandin E_2. The assay was validated using four COX inhibitors, celecoxib, indomethacin, resveratrol, and diclofenac that exhibit different selectivities towards COX-1 and COX-2. The IC_{50} values of celecoxib and resveratrol for ovine and human COX-2 were compared, and the K_m values were determined. Since considerable inter-species variation was observed, human COX-2 should be used for the discovery of COX inhibitors intended for human use. This sensitive and accurate LC–MS–MS based assay is suitable for the rapid screening of ligands for COX-1 and COX-2 inhibition and for IC_{50} determinations.

1. Introduction

Cyclooxygenase (COX)-1 and/or COX-2 are the targets of widely used non-steroidal anti-inflammatory drugs (NSAIDs). Expressed constitutively in all tissues, COX-1 is essential for such physiological processes as maintenance of the gastrointestinal tract, renal function and fever [1]. COX-2 is normally undetectable in most tissues but is induced during inflammatory, degenerative, and neoplastic processes. COX-1 and COX-2 catalyze the conversion of arachidonic acid to the endoperoxide prostaglandin H_2 (PGH_2) (see Fig. 1) which is then metabolized to form prostaglandins, thromboxanes and prostacyclin by non-rate limiting enzymes [2]. In aqueous solution, unstable PGH_2 can rearrange non-enzymatically to form PGD_2 and PGE_2. By inhibiting COX-1 and/or COX-2, NSAIDs prevent the enzymatic conversion of arachidonic acid to pro-inflammatory cyclic endoperoxides. Based on the assumption that selective inhibition of COX-2 might reduce the side effects of NSAIDs, the discovery of selective COX-2 inhibitors has become an important area of pharmaceutical research.

To facilitate the discovery of new COX inhibitors, in vitro screening assays have been developed that utilize cells in culture [3] or purified enzymes. By carrying out enzyme assays using COX-1 and COX-2, the selectivity of inhibitors may be determined based on the ratio of their IC_{50} values (IC_{50} is the concentration of a compound that inhibits enzyme activity by 50%) [4]. Since assays using purified enzymes are faster, more convenient and less expensive than cell-based assays, purified COX-1 and COX-2 were used in this investigation.

Several functional COX assays using purified or recombinant enzymes have been reported and include an oxygen consumption assay [5], a peroxidase co-substrate oxidation assay [5], a radiolabeled chemical inhibition assay [6], and an enzyme-linked immunosorbant assay (ELISA) [5]. The oxygen consumption assay uses a relatively insensitive O_2 sensor and requires more COX than most other assays. The peroxidase co-substrate oxidation assay lacks accuracy since it can respond to various free radical intermediates formed during COX catalysis or might fail to respond when the test inhibitor is an antioxidant [7]. Alternatively, COX inhibition activity can been determined by assessing PGE_2 production using an ELISA or using radiolabeled arachidonic acid and HPLC with radioactivity detection [8]. The ELISA requires almost 2 days per assay and lacks selectivity since it might respond to several prostaglandins, and the radioisotope method

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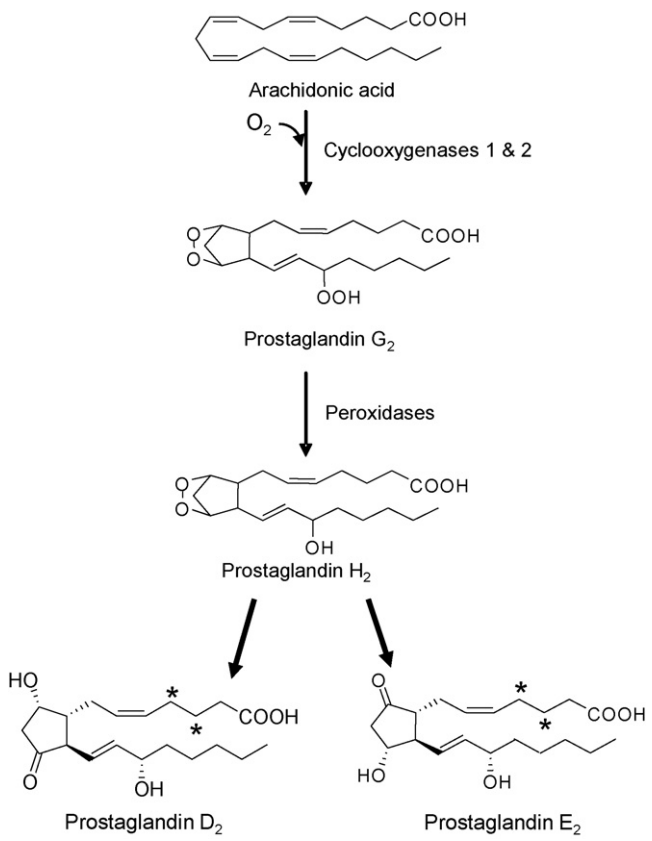

Fig. 1. Arachidonic acid metabolism through the COX pathway. Cyclooxygenases COX-1 and COX-2 convert arachidonic acid to the intermediate prostaglandin PGG_2 and then to PGH_2 which either spontaneously decomposes to form primarily PGE_2 and PGD_2 or is converted by other enzymes to thromboxane-A_2, prostacyclin-GI_2, or prostaglandins such as PGE_2, PGD_2 and PGF_2. (* sites of deuterium labeling in the surrogate standards d_4-PGE_2 and d_4-PGD_2.)

requires specialized licensing, training, handling, and disposal of wastes.

To overcome the limitations of previous assays, we developed an in vitro COX inhibition assay based on the selective and rapid LC–MS–MS quantitative analysis of PGE_2. Although similar to existing ELISA and radioisotope methods and therefore not novel, this assay has technical advantages that include more selective measurement of PGE_2 than either the ELISA or radioisotope assays due to the combination of HPLC separation and tandem mass spectrometric detection, faster analysis than ELISA (less than 1 h total including extraction, reaction and analysis), and no need for radioisotopes. The assay was validated using a range of potent and weak inhibitors of COX-1 and COX-2 inhibitors including celecoxib, indomethacin, resveratrol, and diclofenac.

2. Experimental

2.1. Materials

Ovine COX-1 (oCOX-1), ovine COX-2 (oCOX-2), human recombinant COX-2 (hCOX-2), arachidonic acid, PGE_2, d_4-PGD_2, and d_2-PGE_2 (labeled with deuterium atoms at positions 3 and 4, see Fig. 1) were purchased from Cayman Chemicals (Ann Arbor, MI). The co-factors (−)epinephrine and hematin, and the COX inhibitors indomethacin, resveratrol and diclofenac were purchased from Sigma–Aldrich (St. Louis, MO). Celecoxib was purchased from 3B PharmaChem International (Wuhan, China). All organic solvents were HPLC grade or better and were purchased from Thermo Fisher (Hanover Park, IL). Formic acid was purchased from EMD Chemicals (San Diego, CA). Purified water was prepared by using a Millipore Milli-Q purification system (Millipore, Billerica, MA). All other chemicals and solvents were ACS reagent grade, unless stated otherwise.

2.2. COX inhibition assay

In an Eppendorf tube, 146 μL of 100 mM Tris–HCl (pH 8.0) buffer, 2 μL of 100 μM hematin (co-factor) and 10 μL of 40 mM L-epinephrine (co-factor) were mixed at room temperature. Next, 20 μL of Tris–HCl (pH 8.0) buffer containing 0.2 μg COX-2 or 0.1 μg COX-1 (approximately 1 unit of enzyme; 1 unit COX utilizes 1 nmol O_2/mg/min at 37 °C) was added, and the solution was incubated at room temperature for 2 min. A 2 μL aliquot of the COX inhibitor in DMSO was added to the enzyme solution and preincubated at 37 °C for 10 min. Negative controls were identical except that 2 μL aliquots of DMSO without inhibitor were used instead.

Each COX reaction was initiated by adding 20 μL of arachidonic acid in Tris–HCl (pH 8.0) buffer to give a final concentration of 5 μM; and the reaction was terminated after 2 min by adding 20 μL of 2.0 M HCl. The surrogate standards d_4-PGE_2 and d_4-PGD_2 (10 μL aliquot of 50 ng/mL solution in methanol) were added to correct for errors or degradation during sample handling and for variation in injection volume or instrument response during LC–MS–MS. After 30 min, PGE_2, PGD_2 and their surrogate standards were extracted from each incubation mixture using 800 μL hexane/ethyl acetate (50:50, v/v). The organic phase was removed, evaporated to dryness, and reconstituted in 100 μL methanol/water (50:50, v/v) for analysis using LC–MS–MS (see details in the next section).

The concentration of PGE_2 in each sample was measured using LC–MS–MS, and the percent of COX inhibition by each test solution was determined by comparing the amount of PGE_2 produced in the experiment with that produced in the negative control incubation. The formation of PGD_2 was measured for quality control purposes, since the levels of PGD_2 should be proportional to those of PGE_2. For IC_{50} value determination, 12 different concentrations of each inhibitor were assayed three times. The IC_{50} value of each inhibitor toward COX-1 or COX-2 was determined by plotting and analyzing the inhibition curve data using Graph Pad Prism 5 software (Mountain View, CA). The selectivity of each inhibitor towards COX-2 was calculated as the ratio of the IC_{50} values (COX-2/COX-1). Using 7 concentrations of arachidonic acid from 0 μM to 32 μM, the initial rates of formation of PGE_2 were determined for ovine COX-1, ovine COX-2 and human COX-2 using LC–MS–MS. From these data, Michaelis–Menten curves were plotted, and the K_m values were determined using SigmaPlot 9 software (Systat Software; San Jose, CA).

2.3. LC–MS–MS

Negative ion electrospray tandem mass spectrometric measurement of PGE_2 was carried out using an Applied Biosystems (Foster City, CA) API 4000 triple quadrupole mass spectrometer equipped with a Shimadzu (Columbia, MD) Prominence HPLC system based on the method of Cao et al. [9]. A Waters (Milford, MA) XTerra MS C_{18} analytical column (2.1 mm × 50 mm, 3.5 μm) was used for HPLC separations with an isocratic mobile phase consisting of acetonitrile/aqueous 0.1% formic acid (35:65; v/v) at a flow rate of 200 μL/min. The deprotonated molecules of m/z 351 and m/z 355 corresponding to PGE_2 and the surrogate standard d_4-PGE_2, respectively, were selected for collision-induced dissociation at a collision energy of −23 eV. The abundant product ions of m/z 271 and m/z 275, corresponding to the $[M-H-2H_2O-CO_2]^-$ product ions of PGE_2 and d_4-PGE_2,

respectively, [9] were measured using selected reaction monitoring.

3. Results and discussion

3.1. Assay optimization

Beginning with the COX incubation conditions cited in the literature [10], multiple parameters were optimized including the enzyme level, the substrate concentration, the reaction time, and the post-reaction extraction time. A preincubation of at least 10 min was found to be necessary before adding the arachidonic acid substrate because potent COX-2 inhibitors such as celecoxib are often time-dependent and require several minutes of interaction with the enzyme to reach full inhibition potency [11]. The optimum amount of COX per incubation was approximately 1 unit which varied from 0.1 μg to 0.2 μg according to the specific activity of the enzyme preparation. A reaction time of 2 min was selected because the production of PGE_2 was linear up to 2 min after which the reaction rate declined. Under the conditions of the COX incubation, the half-life of PGH_2 was determined to be ~5 min. Therefore, samples were extracted after 30 min to allow time for spontaneous, non-enzymatic formation of PGE_2 and PGD_2 from the enzymatic product PGH_2.

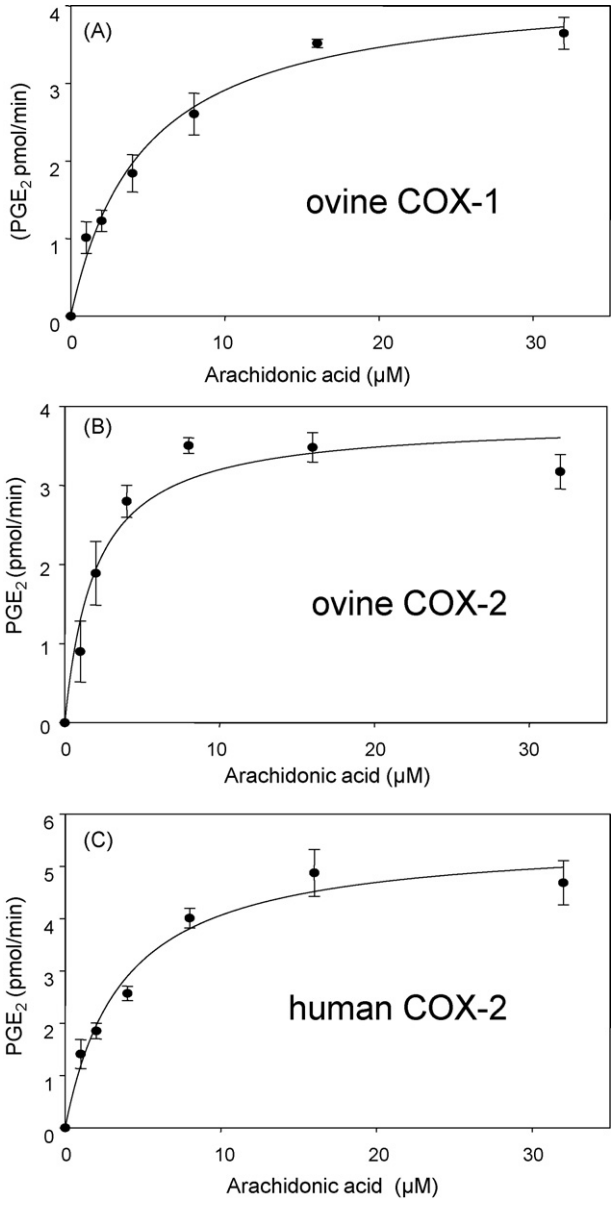

Fig. 2. Michaelis–Menten curves for (A) ovine COX-1; (B) ovine COX-2; and (C) human COX-2. Using these data, K_m values were determined to be 4.67 ± 0.56 μM, 1.94 ± 0.39 μM and 3.66 ± 0.51 μM, respectively.

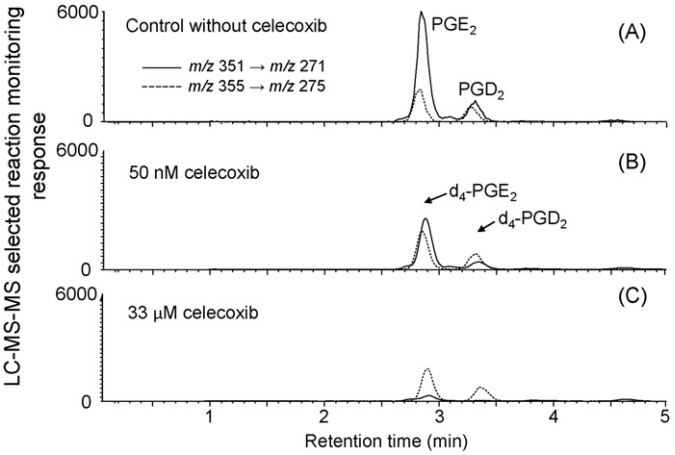

Fig. 3. Examples of LC–MS–MS selected reaction monitoring (SRM) data showing the detection of PGE_2 (solid line, SRM m/z 351 → m/z 271, retention time 3.0 min) in reaction mixtures after incubation of COX with arachidonic acid and (A) no inhibitor (control); (B) 50 nM celecoxib producing 50% inhibition of PGE_2 formation; and (C) 33 μM celecoxib producing >90% inhibition of PGE_2 formation. The surrogate standards [d_4]-PGE_2 and [d_4]-PGD_2 (dashed line, SRM m/z 355 → m/z 275) were added at a constant level to each sample.

Since Marnett and Kalgutkar [12] reported that substrate concentration can affect the selectivity of COX inhibition, this variable was investigated using the COX-2 selective inhibitor celecoxib. We confirmed that as the concentration of arachidonic acid was increased, the COX-2 selectivity of celecoxib also increased. For example, using celecoxib at 10 μM, and arachidonic acid concentrations of 1 μM, 5 μM or 100 μM, the selectivity of celecoxib for ovine COX-2 increased from 0.99 to 2.52 and then to >1000, respectively. This observation confirmed the report of Marnett and Kalgutkar [12].

The K_m values of ovine COX-1, ovine COX-2 and human COX-2 were determined to be 4.67 ± 0.56 μM, 1.94 ± 0.39 μM and 3.66 ± 0.51 μM, respectively. The Michaelis–Menten curves for these determinations are shown in Fig. 2. These values are similar to those determined previously using radiolabeled arachidonic acid for ovine and human COX-1 of 3.7 ± 0.5 μM [13] and 2.9 μM [14], respectively. Based on this information, an arachidonic acid concentration of 5 μM was selected for use in all subsequent COX reactions.

Marnett and Kalgutkar [12] predicted that if the COX reaction mixture is static and is not stirred or shaken, higher COX-2 selectivity might be observed for a COX inhibitor than when the reaction mixture is agitated. This prediction was based on the hypothesis that a selective COX-2 inhibitor might dissociate rapidly from COX-1 but remain tightly bound to COX-2, resulting in a longer duration of action against COX-2 than COX-1. Using celecoxib and ovine COX, we found that gently shaking the COX reaction mixture during incubation produced more reproducible results that those obtained using static reaction conditions. However, the IC_{50} values and ovine COX-2 selectivity of celecoxib were identical for both groups regardless of agitation. Therefore, all subsequent COX reactions were carried out using gentle shaking to mix the samples and thereby enhance the reproducibility of the measurements.

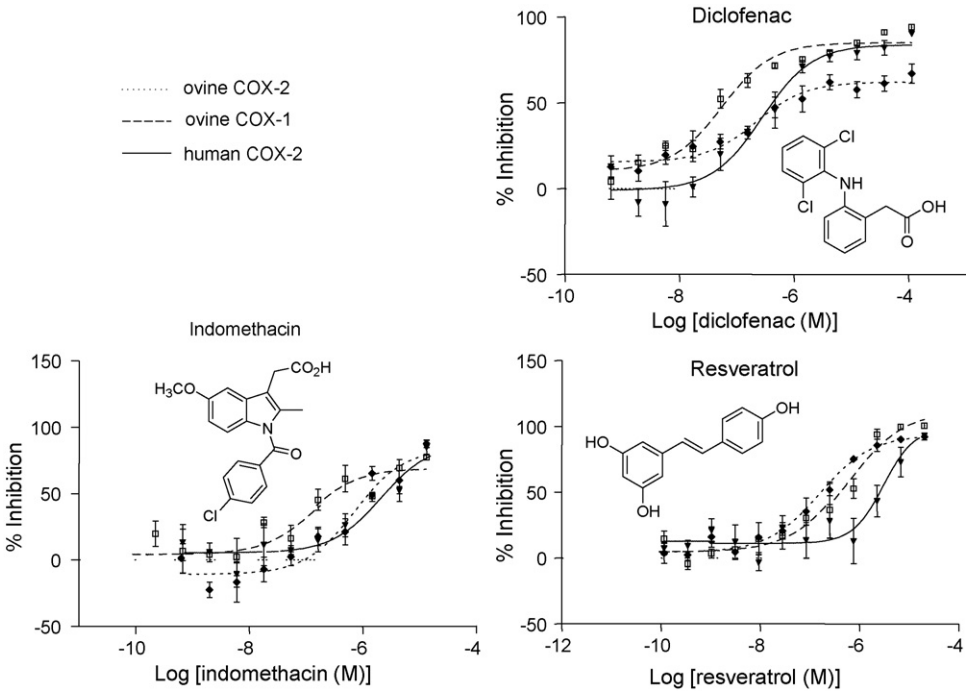

Fig. 4. Determination of IC_{50} values for the inhibition of COX-1 and COX-2 by indomethacin, resveratrol and diclofenac. These data were obtained using the new LC–MS–MS based assay. The IC_{50} values of these compounds for human COX-2, ovine COX-2 and ovine COX-1 that were determined from these data are shown in Table 1.

3.2. COX inhibition assay validation

During LC–MS–MS, the chromatographic peaks corresponding to PGE_2 and PGD_2 were separated completely with retention times of 3.0 min and 3.5 min, respectively (see LC–MS–MS chromatograms in Fig. 3). Since an isocratic mobile phase was used, samples could be injected onto the LC–MS–MS system at least every 5 min. The surrogate standards d_4-PGE_2 and d_4-PGD_2 co-eluted with the corresponding unlabeled prostaglandins providing correction factors for errors during sample preparation or fluctuations in mass spectrometer response. Since the concentration of PGE_2 always exceeded that of PGD_2 (Fig. 3), PGE_2 levels were used instead of PGD_2 levels for the determination of COX inhibition and IC_{50} values. The ratio of PGE_2 to PGD_2 remained a constant value of 4.6 for these reaction conditions. Any deviation in this ratio would be an indication of assay problems such as contamination of the samples

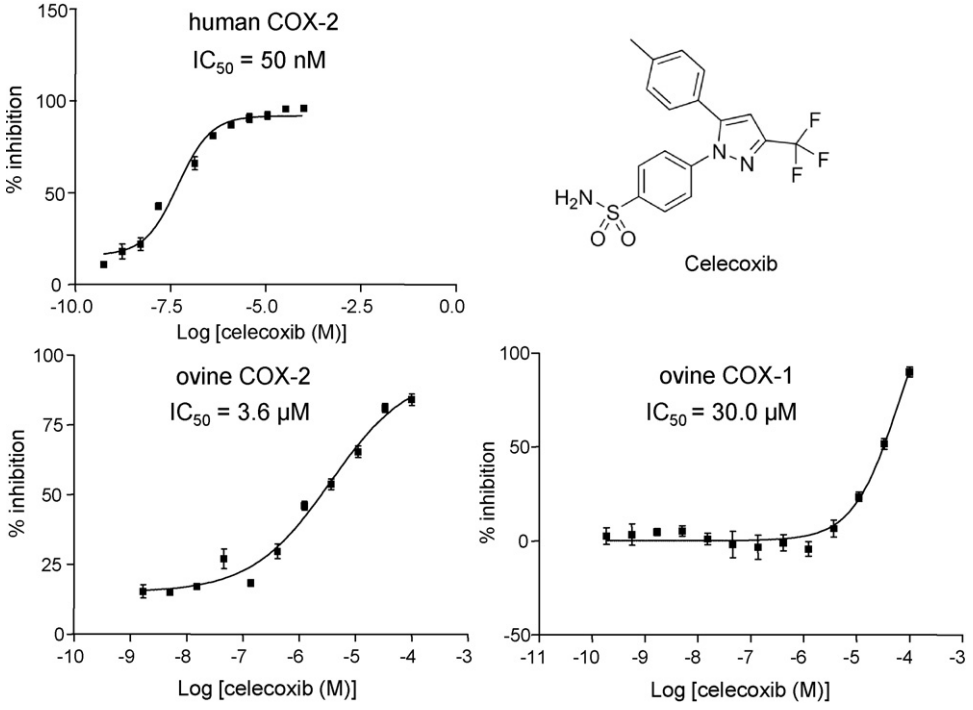

Fig. 5. Determination of the IC_{50} values of celecoxib, a COX-2 selective inhibitor, for the inhibition of purified ovine COX-1, purified ovine COX-2 and human recombinant COX-2. These data were obtained using the LC–MS–MS based assay. Note that human COX-2 was more sensitive to inhibition by celecoxib than was ovine COX-2.

Table 1
IC_{50} values and selectivity of standard COX inhibitors determined using LC–MS–MS quantitative analysis of PGE_2.

COX inhibitor	Measured IC_{50} (μM) ($N = 3$; ±std. dev.)			Selectivity	
	Ovine COX-1	Ovine COX-2	Human COX-2	Ovine COX1/ovine COX2	Ovine COX1/human COX2
Resveratrol	0.86 ± 0.10	0.15 ± 0.06	3.06 ± 2.06	5.57	0.28
Celecoxib	30.0 ± 15.3	3.16 ± 2.16	0.05 ± 0.03	9.49	600
Indomethacin	0.42 ± 0.21	1.32 ± 0.49	2.75 ± 2.59	0.32	0.16
Diclofenac	0.06 ± 0.02	0.79 ± 0.21	0.40 ± 0.13	0.08	0.16

with PGE_2 or PGD_2, contamination of COX with prostaglandin synthases, or co-eluting substances interfering with the LC–MS–MS analysis.

The calibration curve for the measurement of PGE_2 was linear ($r^2 > 0.999$) over the range 0.10–500 ng/mL. The limit of detection and limit of quantitation were 20 pg/mL and 100 pg/mL, respectively. The intraday and interday accuracies of PGE_2 measurement were from 99.4% to 100.8% and the precision was within 3.2% for concentrations of 500 pg/mL to 10 ng/mL. For additional details concerning the measurement of PGE_2 using LC–MS–MS, see Cao et al. [9].

The IC_{50} values for the inhibition of COX by the NSAIDs celecoxib, indomethacin, diclofenac, and the natural product resveratrol are shown in Table 1, and the inhibition curves used to calculate the IC_{50} values of these compounds are shown in Figs. 4 and 5. The inhibition of ovine COX-1, ovine COX-2 and human COX-2 were evaluated, but no human COX-1 was available from commercial sources for this study. Diclofenac is a potent, non-selective inhibitor of COX that exhibits a time-dependent two-step mechanism of inhibition [15]. The IC_{50} values for diclofenac inhibition of ovine COX-1, ovine COX-2 and human COX-2 were 0.06 μM, 0.79 μM and 0.40 μM, respectively, and the IC_{50} ratio (ovine COX-1/human COX-2) was 0.15. Diclofenac did not show any species differences when comparing the data for ovine COX-2 and human COX-2. Dannhardt and Ulbrich [16] reported similar results with IC_{50} values for COX-1 and COX-2 that were 0.01 μM and 0.03 μM, respectively, and an IC_{50} ratio (COX-1/COX-2) of 0.33. Please note that Dannhardt et al., used bovine aortic coronary endothelial cells for the evaluation of COX-1 and COX-2 inhibitors instead of purified enzymes.

Indomethacin is a time-dependent COX inhibitor with slightly higher affinity for COX-1 than for COX-2 [17]. Previously, IC_{50} values of 0.08 μM and 1 μM were reported for ovine and human COX-1 and COX-2, respectively [17]. In this investigation, the IC_{50} value of indomethacin for ovine COX-1 was 0.42 μM, and the IC_{50} value for human COX-2 was 2.75 μM, which are similar to the literature values. Indomethacin did not show any significant species differences between inhibition of ovine COX-2 and human COX-2 (Table 1).

The only natural product evaluated in this study, resveratrol, has been reported to non-selectively inhibit COX-1 and COX-2 with IC_{50} values of 0.83 ± 0.44 μM and 0.99 ± 0.40 μM, respectively [18]. In that paper, the authors did not mention whether ovine, human or other form of COX was used. As shown in Table 1, we found similar IC_{50} values of 0.86 ± 0.70 μM for ovine COX-1 and 3.06 ± 2 μM for human COX-2. Resveratrol showed species selectivity of ~20-fold for inhibition of ovine COX-2 vs. human COX-2 (Table 1).

Celecoxib is a time-dependent, high affinity COX-2 selective inhibitor [17], that inhibited ovine COX-1 and human COX-2 with IC_{50} values of 30 μM and 50 nM, respectively (Table 1 and Fig. 5). As expected, celecoxib showed high COX-2 selectivity (600:1) when comparing the ratio of the IC_{50} values for human COX-2 to ovine COX-1 (Table 1). However, celecoxib inhibited ovine COX-2 with an IC_{50} value of only 3.6 μM. Our data are consistent with the results of Penning et al. who reported celecoxib IC_{50} values of 40 nM toward COX-2 and 15 μM to COX-1 in *in vitro* COX-1 and COX-2 assays [19]. Also, the 600-fold selectivity of celecoxib measured in our functional assay is similar to the previously reported 300-fold selectivity of celecoxib for COX-2 compared to COX-1 [12,17].

Despite examining COX inhibition data for celecoxib and other compounds in the literature, we could find no other examples of large species differences in selectivity between ovine COX-2 and human COX-2. The other NSAIDs that we investigated did not show IC_{50} species differences for COX-2 inhibition as large as those observed for celecoxib (Table 1). Since celecoxib showed the highest affinity for human COX-2 among the inhibitors evaluated in this study, its high species selectivity might be related to a unique fit in the human COX-2 active site that cannot occur in the structurally different ovine COX-2 active site. Because of the possibility of strong species selectivity of COX-2 ligands, we recommend using human COX-2 instead of ovine COX-2 for the discovery and optimization of new COX-2 inhibitors.

Although this LC–MS–MS COX assay is based on the formation of PGE_2, the COX-catalyzed formation of prostaglandins from arachidonic acid involves first, a cyclooxygenase reaction, and second, a peroxidase reaction (Fig. 1). Therefore, drugs discovered using this assay might inhibit the cyclooxygenase and/or the peroxidase reactions. For newly discovered COX inhibitors, the specific mechanism of inhibition would need to be investigated using additional experiments.

4. Conclusions

A COX inhibition assay based on the selective measurement of PGE_2 using LC–MS–MS has been developed, optimized and validated by analyzing known COX inhibitors. This sensitive assay uses small amounts of enzyme that are comparable to ELISA-based assays. The assay is also fast and requires less than 5 min per sample for LC–MS–MS analysis. When comparing the IC_{50} values of well characterized COX inhibitors for ovine COX-2 vs. human COX-2, significant species differences were observed for some inhibitors such as resveratrol and celecoxib. In view of this species selectivity for some COX inhibitors, the use of human COX-2 is recommended whenever possible.

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References

[1] C. Yokoyama, T. Tanabe, Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme, Biochem. Biophys. Res. Commun. 165 (1989) 888.
[2] N.V. Chandrasekharan, D.L. Simmons, The cyclooxygenases, Genome Biol. 5 (2004) 241.
[3] W.A. Cromlish, B.P. Kennedy, Selective inhibition of cyclooxygenase-1 and -2 using intact insect cell assays, Biochem. Pharmacol. 52 (1996) 1777.
[4] B.R. Battistini, R. Botting, Y.S. Bakhle, COX-1 and COX-2: towards the development of more selective NSAIDs, Drug News Perspect. 7 (1994) 501.

[5] R.J. Kulmacz, Prostaglandin G2 levels during reaction of prostaglandin H synthase with arachidonic acid, Prostaglandins 34 (1987) 225.
[6] H.L. White, A.T. Glassman, A simple radiochemical assay for prostaglandin synthetase, Prostaglandins 7 (1974) 123.
[7] M. Cuendet, A.D. Mesecar, D.L. DeWitt, J.M. Pezzuto, An ELISA method to measure inhibition of the COX enzymes, Nat. Protoc. 1 (2006) 1915.
[8] D.R. Huntjens, M. Danhof, O.E. Della Pasqua, Pharmacokinetic–pharmacodynamic correlations and biomarkers in the development of COX-2 inhibitors, Rheumatology (Oxford) 44 (2005) 846.
[9] H. Cao, L. Xiao, G. Park, X. Wang, A.C. Azim, J.W. Christman, R.B. van Breemen, An improved LC–MS/MS method for the quantification of prostaglandins E(2) and D(2) production in biological fluids, Anal. Biochem. 372 (2008) 41.
[10] Y. Noreen, T. Ringbom, P. Perera, H. Danielson, L. Bohlin, Development of a radiochemical cyclooxygenase-1 and -2 in vitro assay for identification of natural products as inhibitors of prostaglandin biosynthesis, J. Nat. Prod. 61 (1998) 2.
[11] R.A. Copeland, J.M. Williams, J. Giannaras, S. Nurnberg, M. Covington, D. Pinto, S. Pick, J.M. Trzaskos, Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 11202.
[12] L.J. Marnett, A.S. Kalgutkar, Cyclooxygenase 2 inhibitors: discovery, selectivity and the future, Trends Pharmacol. Sci. 20 (1999) 465.
[13] W. Chen, T.R. Pawelek, R.J. Kulmacz, Hydroperoxide dependence and cooperative cyclooxygenase kinetics in prostaglandin H synthase-1 and -2, J. Biol. Chem. 274 (1999) 20301.
[14] D.C. Swinney, A.M. Mak, J. Barnett, C.S. Ramesha, Differential allosteric regulation of prostaglandin H synthase 1 and 2 by arachidonic acid, J. Biol. Chem. 272 (1997) 12393.
[15] A.L. Blobaum, L.J. Marnett, Molecular determinants for the selective inhibition of cyclooxygenase-2 by lumiracoxib, J. Biol. Chem. 282 (2007) 16379.
[16] G. Dannhardt, H. Ulbrich, In-vitro test system for the evaluation of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitors based on a single HPLC run with UV detection using bovine aortic coronary endothelial cells (BAECs), Inflamm. Res. 50 (2001) 262.
[17] J.K. Gierse, C.M. Koboldt, M.C. Walker, K. Seibert, P.C. Isakson, Kinetic basis for selective inhibition of cyclo-oxygenases, Biochem. J. 339 (1999) 607.
[18] S.S. Kang, M. Cuendet, D.C. Endringer, V.L. Croy, J.M. Pezzuto, M.A. Lipton, Synthesis and biological evaluation of a library of resveratrol analogues as inhibitors of COX-1, COX-2 and NF-kappaB, Bioorg. Med. Chem. 17 (2009) 1044.
[19] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, E.G. AndersonGd, J.N. Burton, S.A. Cogburn, C.M. Gregory, W.E. Koboldt, K. Perkins, A.W. Seibert, Y.Y. Veenhuizen, P.C. Zhang, Isakson, Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib), J. Med. Chem. 40 (1997) 1347.