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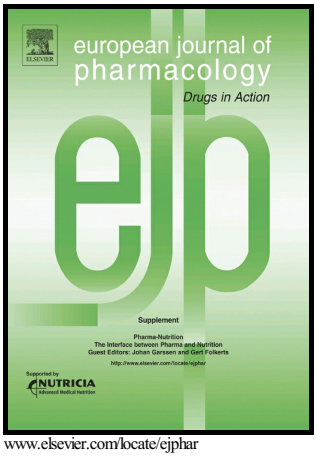

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Prostaglandin D_2 generation from human lung mast cells is catalysed exclusively by cyclooxygenase-1

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Abstract

Mast cells are an exceptionally rich source of prostaglandin D_2 (PGD_2). PGD_2 is pro-inflammatory and can cause bronchoconstriction. The enzyme cyclooxygenase (COX) is central to the generation of prostanoids such as PGD_2. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX. COX exists as two isoforms, COX-1 and COX-2. The principal aim of this study was to establish whether COX-1 and/or COX-2 mediates PGD_2 generation from human lung mast cells. Mast cells were isolated from human lung tissue and purified by flotation over Percoll and immunomagnetic bead separations. The cells were activated with anti-IgE or Stem Cell Factor (SCF). The generation of PGD_2 was determined by ELISA. The effects of NSAIDs (aspirin, ibuprofen, diclofenac, naproxen, indomethacin), COX-1 selective (FR122047), and COX-2 selective (celecoxib) inhibitors on PGD_2 generation were determined. The expression of COX-1 and COX-2 in mast cells was determined by Western blotting. All the NSAIDs tested abrogated stimulated PGD_2 generation from mast cells except aspirin which was only weakly effective. FR122047 was an effective inhibitor of PGD_2 generation (EC_{50} ~25 nM) from mast cells whereas celecoxib was ineffective. Immunoblotting indicated that COX-1 was strongly expressed in all mast cell preparations while COX-2 expression was weak. No induction of COX-2 was observed following activation of mast cells. These findings indicate that COX-1 is the principal isoform involved in generating PGD_2 from human lung mast cells. These studies provide insight into the potential behaviour of NSAIDs in the context of respiratory diseases.

Keywords:

mast cells, cyclooxygenase, NSAIDs, aspirin, prostaglandin D_2

1. Introduction

Mast cells are a very rich source of the prostanoid, PGD_2 (Lewis et al., 1982). Since very few other cells have the capacity to produce PGD_2, the presence of PGD_2 or its metabolites is likely to be indicative of mast cell activation (Bochenek et al., 2004; Dahlén et al., 2004). PGD_2 mediates bronchoconstriction and is largely pro-inflammatory (Beasley et al., 1987; Matsuoaka et al., 2000). Together with other mast cell derived mediators such as histamine and cysteinyl-leukotrienes (cys-LTs), PGD_2 contributes to allergic type reactions (Bingham and Austen, 2000; Bradding et al., 2006). While the recognition that human lung mast cells produce PGD_2 has been acknowledged for some time, the pathway leading to PGD_2 generation in these cells has not been clearly delineated.

The enzyme cyclooxygenase (COX) is central to the generation of prostanoids (Rouzer and Marnett, 2009). COX converts arachidonic acid to a highly labile intermediate, PGH_2. Specific synthases then convert PGH_2 into prostanoids. Whereas COX is found widely, synthases tend to show more restricted distribution (Ueno et al., 2005). Other than PGD_2, important prostanoids include PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 (Woodward et al., 2011). These display varying profiles of activity. For example, PGE_2 is known to inhibit acid secretion from parietal cells in the gut and also appears to be important in mediating inflammation and sensitizing sensory neurones to pain (Tilley et al., 2001; Peskar et al., 2003; Woodward et al., 2011).

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, target COX and, by attenuating prostanoid generation, are useful for the treatment of certain types of inflammation and pain (Cryer and Feldman, 1998). COX is known to exist as two isoforms COX-1 and COX-2. COX-1 is recognised as the isoform that is expressed constitutively whereas COX-2 is thought to be the form that

is induced during inflammation (Mitchell et al., 1994; Cryer and Feldman, 1998; Chan et al., 1999; Warner et al., 1999). However, constitutive expression of COX-2 may also occur in certain tissues indicating that this isoform cannot solely be considered an inducible form of the enzyme (Kirkby et al., 2016). Aspirin and ibuprofen are somewhat non-selective drugs and affect both isoforms of COX (Mitchell et al., 1994; Cryer and Feldman, 1998; Chan et al., 1999; Warner et al., 1999). Any anti-inflammatory benefit of non-selective NSAIDs may therefore be accompanied by non-target effects including inhibition of COX-1 in the gut, leading to a reduction in the production of PGE, increased acid secretion and potential gastropathy (Warner et al., 1999). Since many NSAIDs show limited selectivity for either COX-1 or COX-2, this has led to the development of COX-2 selective drugs such as celecoxib that are less likely to target COX-1 and are gut-sparing (Chan et al., 1999; Warner et al., 1999; Fitzgerald and Patrono, 2001).

Despite the undoubted value of NSAIDs in the treatment of certain types of inflammation and moderate pain, the use of aspirin and related compounds is largely contraindicated in individuals with asthma and respiratory diseases. This is because aspirin can induce exacerbations in a sizeable proportion of asthmatics (Kowalski et al., 2011). The mechanism behind this underlying susceptibility to aspirin and related drugs has not been elucidated but aspirin intolerance has been linked to increased production of both cys-LTs and PGD_2 (O'Sullivan et al., 1996; Cahill et al., 2015). The production of PGD_2 suggests that mast cells are central to the process. While an expectation would be that NSAIDs are likely to inhibit PGD_2 generation from human lung mast cells, there are suggestions that aspirin might activate mast cells by mechanisms that are not clearly understood (Steinke et al., 2014).

To date, no systematic evaluation of the effects of NSAIDs on human lung mast cells has been performed. The present study aims to redress this. Studies in rodent cell systems and in cultured human mast cells suggest that both COX-1 and COX-2 may contribute to PGD_2 production (Murakami et al., 1995; Reddy et al., 1997; Obata et al., 1999). However, it is well recognised that mast cells isolated from different species and different sites from the same species display a great deal of heterogeneity (Pearce, 1983; Lowman et al., 1988). The principal aim of this study, therefore, was to identify which isoform(s) of COX mediates PGD_2 generation from human lung mast cells. The findings of this study may help to explain the actions and behaviour of NSAIDs in the context of respiratory disease.

2. Materials & Methods

2.1 Buffers

Phosphate buffered saline (PBS) was employed in these studies. PBS contained (mM): NaCl 137; $Na_2HPO_4.7H_2O$ 8; KCl 2.7; KH_2PO_4 1.5. +PBS was PBS supplemented with: $CaCl_2.2H_2O$ 1 mM; $MgCl_2.6H_2O$ 1 mM; glucose 5.6 mM; human serum albumin (HSA) 30 µg/ml. The pH of PBS buffers was titrated to 7.3.

2.2 Preparation of compounds

Aspirin, naproxen, diclofenac, ibuprofen, indomethacin, FR122047, celecoxib and wortmannin were prepared as stock solutions (10 mM) in ethanol and stored at –20 °C in appropriate aliquots. Stem Cell Factor (SCF) was prepared as a stock solution (100 µg/ml) in water and stored frozen in small aliquots. Monoclonal anti-human IgE antibody (2 mg/ml stock) was stored frozen over the long-term but a working stock was kept at 4 °C for up to a month.

2.3 Lung tissue

Lung tissue was obtained following surgery. Seventy-seven lung preparations were used in this study, 42 preparations were derived from males and 35 from females. The age range of participants was 28 to 89 years with a median age of 72. Informed written consent was obtained. This study was approved by the National Research Ethics' Service (REC reference: 15/NW/0657).

2.4 Cell isolation

Mast cells were isolated from human lung tissue using methods described in detail elsewhere (Lewis et al., 2013). Macroscopically normal tissue from resections was disrupted physically and enzymatically (collagenase Ia) to generate a mixed cell suspension of which 3 to 13% of the cells were mast cells. Mast cells were visualized by microscopy using an alcian blue stain. This method generated approximately 6 x 10^5 mast cells per g of tissue. Mast cells of enhanced purity (10 to 69% purity; median 35%) were generated by flotation over discontinuous Percoll gradients (Weston et al., 1997). Mast cells were further purified using a MACS magnetic cell sorting system (Miltenyi Biotec, Surrey, UK) according to the manufacturer's instructions and as described in detail elsewhere (Havard et al., 2011). Mast cell purities using this method ranged from 88 to 98% (median 90%). When longer term incubations (4 or 20 h) were necessary, cells were reconstituted in RPMI-1640 buffer supplemented with penicillin (10 Units/ml), streptomycin (10 μg/ml), gentamicin (50 μg/ml) fungizone (1 μg/ml) and FBS (5%).

2.5 Mediator release

Mediator release experiments were performed essentially as described elsewhere (Lewis et al., 2013). Experiments were performed in +PBS buffer. Mediator release was initiated either with a mouse anti-IgE or SCF. Human lung mast cells express endogenous IgE so the cells are responsive to anti-IgE without the need for passive sensitization with exogenously added IgE (Lewis et al., 2017). SCF was used in this study as a non-IgE-dependent activator that has previously been shown to be an effective driver of PGD_2 generation from human lung mast cells (Lewis et al., 2013).

When COX inhibitors were used, the cells were incubated for 15 min with the inhibitor before challenge with stimulus. Wortmannin, a PI3K inhibitor, was also included in certain experiments acting as a positive inhibitory control. Histamine released into supernatants was measured using an automated fluorometric method (Ennis, 1991). Total histamine content was determined by lysing aliquots of the cells with perchloric acid at a final concentration of 1.6%. Cells incubated in buffer alone served as a measure of spontaneous histamine release which ranged from 2 to 8% of the total histamine content. Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release. PGD_2 and cys-LT content in the supernatants were determined using commercially available kits (Cayman Chemical Company, Ann Arbor, MI, USA). All experiments were performed in duplicate.

2.6 RT-PCR

RNA was extracted from purified mast cells using Tri-Reagent (1 ml). In order to generate cDNA, samples were processed essentially as described (Kay et al., 2013). Amplification of cDNA was performed by PCR using a modification of the conditions and primer pairs for human COX-1 and COX-2 described elsewhere (Picado et al., 1999). The house-keeping gene, β-actin, was also amplified. Primers were synthesised by Sigma (Poole, UK). PCR primers used in this study can be found in Table S1 (Supporting Information repository). PCR products were sequenced in-house to ensure that correct amplification had taken place as described in more detail elsewhere (Kay et al., 2013).

2.7 Immunoblotting

Cell extracts were prepared for use in immunoblotting using a lysis buffer (Tris 50 mM; NaCl 150 mM; PMSF 50 µg/ml; SBTI 50 µg/ml; leupeptin 5 µg/ml; aprotinin 5 µg/ml; Triton X-100 0.5%; pH 8.0) according to methods described elsewhere (Fruman et al., 1992). Cell lysates were prepared for electrophoresis as described (Havard et al., 2011). Samples were subjected to SDS-PAGE (Mini-Sub Cell GT System; Bio-Rad, Hemel Hempstead, UK) using either 10 or 12% gels and then the separated proteins transferred electrophoretically to nitrocellulose membranes. Membranes were probed with a goat polyclonal antibody to COX-2 followed by secondary antibody (donkey anti-goat IgG-HRP). The membrane was stripped (0.2 M NaOH, 15 min) and probed with a rabbit polyclonal antibody to actin followed by secondary antibody (goat anti-rabbit IgG-HRP). The membrane was stripped again and probed with goat polyclonal antibody for COX-1 followed by secondary antibody (donkey anti-goat IgG-HRP). Using a similar approach, the expression of lipocalin-type PGD synthase (L-PGDS) and haematopoeitic-type PGDS (H-PGDS) was also evaluated in mast cells. Protein bands were visualised by the addition of enhanced chemiluminescence (ECL) reagents and signals detected on ECL film. Membranes were visualized using a BioRad ChemiDoc imaging system (Bio-Rad, Watford, UK).

2.8 Materials

The following were purchased from the sources indicated; aspirin, collagenase, DNase, HSA, ibuprofen, indomethacin, Percoll (Sigma, Poole, UK); gentamicin, penicillin/streptomycin, fungizone, RPMI 1640 (Invitrogen, Paisley, UK); diclofenac, FR122047, naproxen (Cayman Chemical Company, Ann Arbor, MI, USA);

wortmannin (Calbiochem, Nottingham, UK); celecoxib (BioVision, Milpitas, CA, USA); anti-human IgE, clone HP6061 (Stratech Scientific Ltd, Newmarket, UK); SCF (Peprotech, Rocky Hill, NJ, USA); ECL reagents, nitrocellulose membranes (GE Healthcare Life Sciences, Little Chalfont, UK). All primary and secondary antibodies were purchased from Santa Cruz Biotechnology (Heidelberg, Germany) with the exception of rabbit polyclonal antibody to actin (Sigma, Poole UK) and goat anti-rabbit IgG-HRP (Dako/Agilent, Santa Clara, CA, USA). All other reagents were from Sigma (Poole, UK).

2.9 Data analysis

Potencies (EC_{50}) were determined by non-linear regression analysis (GraphPad Prism, version 5.0d). Statistical significance was assessed utilising repeated measures ANOVA. When appropriate post hoc analysis was performed using Dunnett's test.

3. Results

3.1 Effects of COX inhibitors on PGD_2 generation

The effects of a range of commonly used NSAIDs on PGD_2 generation from human lung mast cells were investigated. The concentration (1 μM) of NSAIDs used in these preliminary experiments was considered to be maximally effective based on previous studies (Mitchell et al., 1994; Cryer and Feldman, 1998; Chan et al., 1999; Warner et al., 1999). IgE-dependent PGD_2 generation was inhibited effectively by naproxen, ibuprofen and diclofenac (Fig. 1A). These same inhibitors had no effect on either IgE-dependent cys-LT generation (Fig. 1B) or histamine release (Fig. 1C). By contrast, in these same experiments, aspirin was an ineffective inhibitor of PGD_2 generation, and also had no effect on either cys-LT generation or histamine release. Further studies demonstrated that a higher concentration (10 μM) of aspirin could inhibit PGD_2 generation (Table 1) but the inhibition was far from complete (~38% inhibition) unlike the alternative NSAIDs studied which essentially abolished PGD_2 generation (Fig. 1A).

Previous clinical studies (O'Sullivan et al., 1996; Cahill et al., 2015) and in vitro studies using peripheral blood derived mast cells (Steinke et al., 2014) suggest that aspirin may directly activate mast cells. However, in this study, we were unable to demonstrate that aspirin or alternative NSAIDs were capable of inducing mediator release from human lung mast cells.

In further studies the effects of FR122047 (COX-1 selective) and celecoxib (COX-2 selective) as well as the NSAID, indomethacin, on PGD_2 generation from human lung mast cells were investigated. The PI3K inhibitor, wortmannin, was included in these experiments as a positive inhibitory control. Both indomethacin and FR122047 were effective inhibitors of PGD_2 generation induced by anti-IgE whereas

celecoxib was ineffective (Fig. 2A). None of the COX inhibitors had much of an effect on the generation of cys-LTs (Fig. 2B) or release of histamine (Fig. 2C).

Previous studies have demonstrated an increase in the mast cell growth factor, SCF, in asthma (Al-Muhsen et al., 2004; Kowalski et al., 2005; Da Siva et al., 2006). A recent study of ours has shown that SCF is also an effective driver of mediator release from human lung mast cells especially PGD_2 generation (Lewis et al., 2013). The effects of FR122047 and celecoxib as well as indomethacin on PGD_2 generation driven by SCF from human lung mast cells were investigated. Both FR122047 and indomethacin were very effective inhibitors of PGD_2 generation whereas celecoxib was ineffective (Fig. 3A). None of the COX inhibitors had any effect on the SCF-induced generation of cys-LTs (Fig. 3B) or release of histamine (Fig. 3C).

Further studies were performed with FR122047 using an extended concentration range (10^{-11} – 10^{-6} M). FR122047 inhibited PGD_2 generation by either anti-IgE or SCF in a concentration-dependent manner (Fig. 4). FR122407 was slightly more potent as an inhibitor of PGD_2 generation induced by SCF than anti-IgE (EC_{50} for FR122047 of 9 and 38 nM, respectively).

3.2 Expression of COX isoforms by mast cells

RT-PCR was performed in order to determine whether mast cells express message for COX-1 and/or COX-2 (Fig. 5). The data demonstrate that mast cells express mRNA for both COX-1 and COX-2. Further studies were performed to determine expression of COX-1 and COX-2 at the protein level. The data show that, overall, mast cells strongly express COX-1 and weakly express COX-2 (Fig. 6).

3.3 Effects of longer-term activation of mast cells on PGD_2 generation

In further studies, we investigated whether longer-term activation of mast cells influenced PGD_2 generation. Mast cells were activated with either anti-IgE or SCF for discrete time intervals (0.5 to 4 h) and PGD_2 generation monitored. The data show that PGD_2 generation showed no time-dependency when the cells were activated with anti-IgE (Fig. 7A) whereas when the cells were activated with SCF (Fig. 7B), significantly ($P < 0.05$) more PGD_2 (more than twice as much) was generated at 4 h compared to 30 min.

Experiments were then performed to determine whether this effect of SCF might be related to the up-regulation of COX isoforms. Treatment with SCF (4 or 20 h) had no effect on the expression of either COX-1 or COX-2 (Fig. 8A, 8B). We also investigated whether LPS was able to affect COX expression in mast cells since our own studies demonstrate that LPS is a strong inducer of COX-2 in lung macrophages (Fig S1, Supporting Information repository). However our data show that LPS has no effect on COX expression in human lung mast cells (Fig. 8B).

We also investigated whether mast cell activation might lead to up-regulation of PGDS expression by mast cells. Mast cells expressed haematopoeitic-type but not lipocalin-type PGDS, the latter synthase more usually associated with the central nervous system (Fig. 8C). Following activation with SCF (4 or 20 h) or LPS (20 h) there was no increase in expression of either of these synthases (Fig. 8A, 8C).

4. Discussion

In the present study, we have assessed the effects of a variety of COX inhibitors and investigated COX expression in human lung mast cells. Taken together, these studies indicate that COX-1 is the principal isoform responsible for PGD_2 generation in these cells.

Studies with COX-selective inhibitors were particularly informative. That FR122047 abolished IgE-dependent PGD_2 generation provided strong evidence that COX-1 is responsible for the production of PGD_2 in human lung mast cells. FR122047 inhibits human recombinant COX-1 with an IC_{50} of ~30 nM and is about 2000 fold more potent against COX-1 than COX-2 (Ochi et al., 2000). The potency of FR122047 (EC_{50} ~25 nM) as an inhibitor of PGD_2 generation from human lung mast cells was very much in keeping with an effect at COX-1. By contrast, that celecoxib was unable to inhibit PGD_2 argues against a role for COX-2. It should be noted that although celecoxib shows some modest selectivity for COX-2 over COX-1, it is possible that at higher concentrations, celecoxib may target COX-1 to some extent (Chan et al., 1999; Warner et al., 1999). Nonetheless, in the present study, celecoxib was ineffective. Collectively, these data suggest that IgE-dependent PGD_2 generation from human lung mast cells is driven by COX-1.

In further studies, we investigated the effects of COX inhibitors on SCF driven PGD_2 generation from mast cells. Elevated concentrations of the mast cell growth factor, SCF, have been observed in asthma (Al-Muhsen et al., 2004; Kowalski et al., 2005; Da Silva et al., 2006) and our recent work has shown that SCF is unexpectedly effective at stimulating PGD_2 generation from mast cells (Lewis et al., 2013). Previous studies have shown that mouse bone marrow derived mast cells express both COX-1 and COX-2 and that whereas IgE-dependent PGD_2 generation mobilises

COX-1, cytokine driven PGD_2 generation activates COX-2 (Murakami et al., 1995). However, in the present study, SCF-induced PGD_2 generation was inhibited effectively by FR122047 whereas celecoxib was ineffective. Overall, these findings suggest that COX-1 is the primary isoform that mediates both IgE- and SCF-dependent PGD_2 generation. These findings strongly support clinical studies showing that COX-1 is likely to be the main driver of PGD_2 generation in asthma (Daham et al., 2011).

The effects of commonly used NSAIDs on PGD_2 production from human lung mast cells were instructive inasmuch as these compounds are largely considered to be non-selective although data from a number of sources indicate some preference for either COX-1 or COX-2 (Mitchell et al., 1994; Cryer and Feldman, 1998; Chan et al., 1999; Warner et al., 1999). Selectivity for a given isoform may be dependent on the enzyme system studied. Taking the literature collectively, indomethacin and aspirin appear to be predominantly COX-1 selective, diclofenac is somewhat COX-2 selective, naproxen and ibuprofen overall display only fractional selectivity for one or other isoform. It is of interest that all the NSAIDs studied inhibited PGD_2 generation very effectively with the notable exception of aspirin which was a surprisingly weak inhibitor. The reasons for this are not really apparent. If an assumption is made that COX-1 is the primary isoform present in mast cells then aspirin is at least as potent at COX-1 as some of the other NSAIDs studied such as ibuprofen yet ibuprofen was effective at blocking PGD_2 generation. Why aspirin was relatively ineffective is difficult to understand unless there are issues with aspirin accessing and/or inactivating mast cell COX.

Expression of COX isoforms was determined both by RT-PCR and by Western blotting. Strong mRNA expression was observed for both COX-1 and COX-

2. However, at the protein level the picture was somewhat different and it was clearly evident that human lung mast cells strongly express COX-1 and only weakly express COX-2 in the majority of mast cell preparations. These findings are at odds with studies in murine systems as well as cultured human mast cells wherein both COX-1 and COX-2 are thought to co-exist (Murakami et al., 1995; Reddy et al., 1997; Obata et al., 1999).

Based on previous studies in a mouse mast cell line (Reddy et al., 1997), the possibility was considered that discrete COX isoforms might be mobilised in a time-dependent fashion. Mast cells were activated for time intervals of 30 min to 4 h with either anti-IgE or SCF. It was of interest that the extent of PGD_2 generation induced by anti-IgE was the same after 30 min or 4 h whereas when SCF was used as a stimulus, over twice as much PGD_2 was generated after 4 h compared to 30 min. These data suggested that SCF can drive PGD_2 generation continuously over at least 4 h.

We considered whether this continuous release of PGD_2 by SCF might be due to the induction of COX-2. However, our data showed that COX-2 levels did not change following longer-term activation of mast cells with SCF. In point of fact a well-recognised inducer of COX-2, LPS, was also investigated to determine whether this would induce COX-2 (Hempel et al., 1994). However, LPS failed to induce up-regulation of COX-2 in mast cells but readily did so in isolated human lung macrophages. The possibility that SCF might induce PGDS was also considered but, similarly, there was no indication that SCF induced PGDS. Presumably, the nature of the SCF/c-kit interaction transduces signals that permit continuous ongoing activation of the pathway leading to PGD_2 generation.

In the clinical context, these studies may have some bearing on mechanistic aspects related to aspirin-intolerant asthma. Aspirin-intolerant asthma has been shown to be accompanied by increases in cys-LTs and PGD_2 (O'Sullivan et al., 1996; Cahill et al., 2015). This association with PGD_2 has led to suggestions that aspirin directly activates mast cells by an unidentified mechanism (Steinke et al., 2014). While the possibility exists that mast cells in asthmatic lung or discrete subsets of mast cells found in particular areas of the lung may behave differently in response to aspirin, we were unable to demonstrate that aspirin can activate human lung mast cells directly.

Another mechanism that has been forwarded to explain some of the adverse effects of NSAIDs in respiratory conditions is that blocking COX can lead to increased availability of arachidonic acid for metabolism by the lipoxygenase pathway and thereby enhanced generation of cys-LTs (Hamad et al., 2004). Overall, data in the present study suggested that such a mechanism would be unlikely to occur (see Fig. 2B and Fig 3B). One reason why a clear enhancement of cys-LT generation was not readily observed may have been related to the fact that, in these experiments, maximally effective concentrations of stimuli were used. It is possible therefore that if optimal levels of cys-LTs were already being generated by a stimulus then there may have been limited opportunity to see any enhancement with a COX inhibitor. For this reason, additional experiments were performed utilising a submaximal concentration of anti-IgE (0.1 µg/ml), 20-fold lower than maximal, to determine whether selected COX inhibitors might enhance cys-LT generation from activated mast cells. Although there was some variability among experiments, overall there was no evidence for enhancement of cys-LTs by COX inhibitors under these conditions (see Fig S2, Supporting Information repository). These data are not altogether unexpected since, in other systems, inhibition of COX does not promote increased conversion of

arachidonic acid by the lipoxygenase pathway (Sud'ina et al., 2008). Although not explored in the present study, the possibility that arachidonic acid might also be converted to other active metabolites by alternative pathways cannot be excluded (Feltenmark et al., 2008; Wendell et al., 2014).

An alternative possibility that has been put forward to explain aspirin-intolerant asthma is that COX inhibition by aspirin may reduce PGE_2. Whereas in general PGE_2 is known to have pro-inflammatory effects, paradoxically in the lung, PGE_2 is a bronchodilator and inhibits human lung mast cells (Gauvreau et al., 1999; Hartert et al., 2000; Buckley et al., 2011; Kay et al., 2013). The loss of an endogenous stabiliser of mast cells in PGE_2 could permit an increase in the release of mast cell derived mediators. If, as we have shown in the present study, aspirin was also ineffective at inhibiting mast cell COX, this might then be reflected by even greater levels of PGD_2 generation. Thus an increased elaboration of PGD_2 by aspirin may reflect an inability to contain PGD_2 generation rather than direct activation of the mast cell.

In summary, we have shown that PGD_2 generation by mast cells is driven by COX-1. We have been unable to demonstrate any supporting role for COX-2. While the possibility cannot be excluded that mast cells in different disease states may behave differently, our findings strongly support the notion that COX-1 is the principal isoform driving PGD_2 from human lung mast cells.

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Conflict of interest statement

None

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Table 1 Effects of aspirin on mediator release from human lung mast cells

Aspirin (*μM*)	*PGD_2* (*ng/10^6 mast cells*)	*cys-LT* (*ng/10^6 mast cells*)	*histamine release* (*%*)
-	82 ± 25	85 ± 26	26 ± 8
1	72 ± 20	79 ± 28	25 ± 7
10	51 ± 23*	93 ± 29	24 ± 7

Mast cells were incubated (15 min) without (-) or with aspirin before challenge for a further 25 min with anti-IgE for mediator release. Values are means ± S.E.M., for 5 different mast cell preparations. Statistically significant reduction in PGD_2 generation compared to control (-) is indicated, *$P < 0.05$

Figure Legends

Fig. 1 Effects of NSAIDs on mediator release. Mast cells were incubated (15 min) without (-) or with either aspirin (ASP), naproxen (NAP), ibuprofen (IBU) or diclofenac (DIC) before challenge for a further 25 min with anti-IgE for mediator release. Effects of NSAIDs on (A) PGD_2, (B) cys-LT and (C) histamine generation were evaluated. Values are means ± S.E.M., for 4 different mast cell preparations. Statistically significant reductions in PGD_2 generation compared to control (-) are indicated, $*P < 0.05$.

Fig. 2 Effects of COX-selective inhibitors on IgE-dependent mediator release. Mast cells were incubated (15 min) without (-) or with either indomethacin (IND), FR122047 (FR), celecoxib (CEL) or wortmannin (W) before challenge for a further 25 min with anti-IgE (2 μg/ml) for mediator release. Effects of NSAIDs on (A) PGD_2, (B) cys-LT and (C) histamine generation were evaluated. Values are means ± S.E.M., for 4 different mast cell preparations. Statistically significant alterations in mediator release generation compared to control are indicated, $*P < 0.05$.

Fig. 3 Effects of COX-selective inhibitors on SCF-induced mediator release. Mast cells were incubated (15 min) without (-) or with either indomethacin (IND), FR122047 (FR), celecoxib (CEL) or wortmannin (W) before challenge for a further 25 min with SCF (100 ng/ml) for mediator release. Effects of NSAIDs on (A) PGD_2, (B) cys-LT and (C) histamine generation were evaluated. Values are means ± S.E.M., for 5 different mast cell preparations. Statistically significant reductions in mediator release generation compared to control are indicated, $*P < 0.05$.

Fig. 4 Effect of FR122047 on PGD_2 generation. Mast cells were incubated without or with FR122047 for 15 min before challenge for a further 25 min with either anti-IgE (2 μg/ml) or SCF (100 ng/ml) for PGD_2 generation. Results are expressed as the % inhibition by FR122047 of the control unblocked PGD_2 generation which was 191±30 ng of PGD_2 per 10^6 mast cells for anti-IgE and 84±24 ng of PGD_2 per 10^6 mast cells for SCF. Values are means ± S.E.M., for 6 different mast cell preparations.

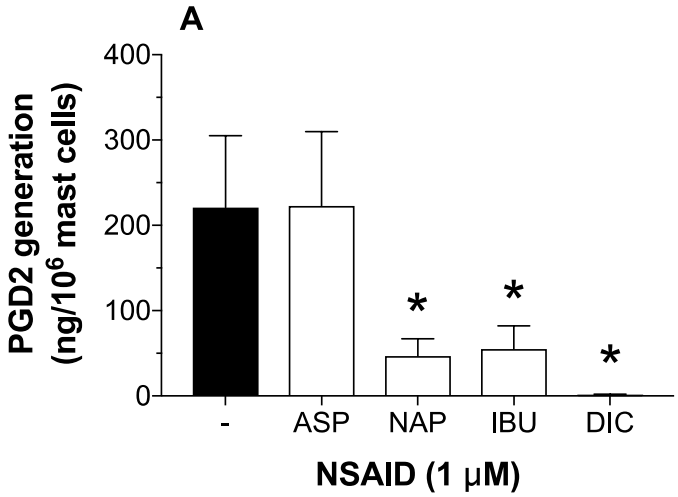
Fig. 5 COX isoform expression in human lung mast cells. Isolated RNA was converted to cDNA by reverse trancriptase (+) and this step was also carried out in the absence of reverse transcriptase for control purposes (-). Amplification of cDNA was performed using primers for COX-1 and COX-2 and for β-actin. Expression profiles for 3 mast cell preparations (HLMC-1, HLMC-2 and HLMC-3) are shown. Data are representative of a total of 6 different mast cell preparations in excess of 95% purity. Lanes at either end of each gel represent molecular weight standards (mw) comprising a 100 bp ladder.

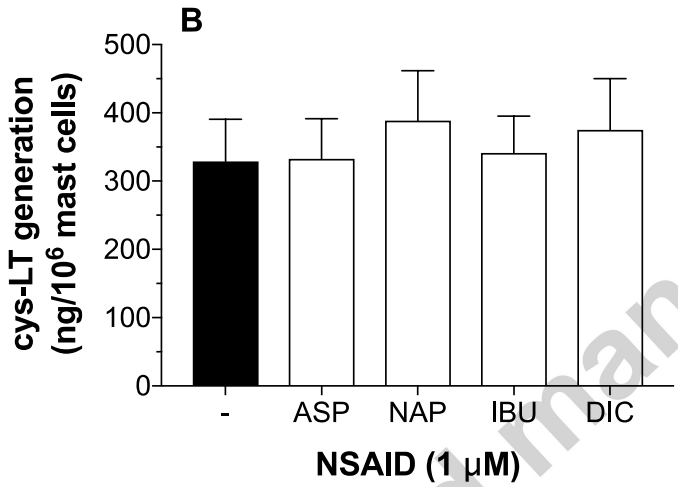
Fig. 6 Immunoblot for COX isoform expression in human lung mast cells. (A) Three mast cell preparations (HLMC-1, HLMC-2 and HLMC-3) were solubilized and subjected to SDS-PAGE along with a mixed lung cell (MLC) preparation. Following electrophoretic transfer to a nitrocellulose membrane, the membrane was probed with an antibody to COX-2, the membrane stripped and probed with antibody to actin and the membrane stripped again and probed with antibody to COX-1. mw stands for molecular weight standards. (B) COX-1 and COX-2 content in mast cells was determined by densitometry. Content is expressed relative to the same MLC preparation that was used as a control in all blots and normalised relative to cell actin.

Open symbols represent the COX content in individual mast cell preparations, filled symbols the mean value. Data are for 13 different mast cell preparations.

Fig. 7 Time-dependence of PGD_2 generation. Mast cells were incubated for between 30 min to 4 h with either (A) anti-IgE (2 μg/ml) or (B) SCF (100 ng/ml) after which PGD_2 generation was assessed. Values are means ± S.E.M., for 4 different mast cell preparations. There was significantly greater generation of PGD_2 after 4 h with SCF compared to a 30 min challenge, $*P < 0.05$.

Fig. 8 Effects of LPS and SCF on COX and PGDS expression. (A) Two mast cell preparations (HLMC-1 and HLMC-2) were incubated (4 h) without (-) or with SCF (100 ng/ml) and expression of COX-1, COX-2, H-PGDS and β-actin evaluated. A mixed lung cell (MLC) preparation was also included. (B) A single mast cell preparation was incubated (20 h) in buffer (-), LPS (100 ng/ml) or SCF (100 ng/ml) and expression of COX-1 and COX-2 evaluated. An MLC preparation was also included. (C) A single mast cell preparation was incubated (20 h) in buffer (-), LPS (100 ng/ml) or SCF (100 ng/ml) and expression of H-PGDS and L-PGDS evaluated. A solubilized mouse brain preparation was also included as a control for L-PGDS. Experiments were performed 3 or more times for (A), (B) and (C). mw stands for molecular weight standards.





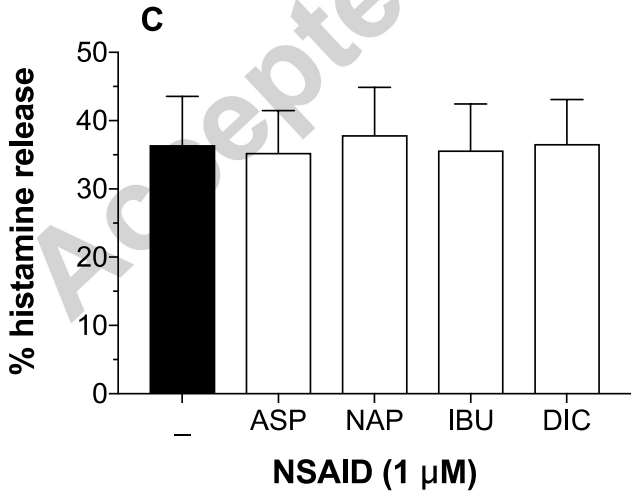


Fig. 1

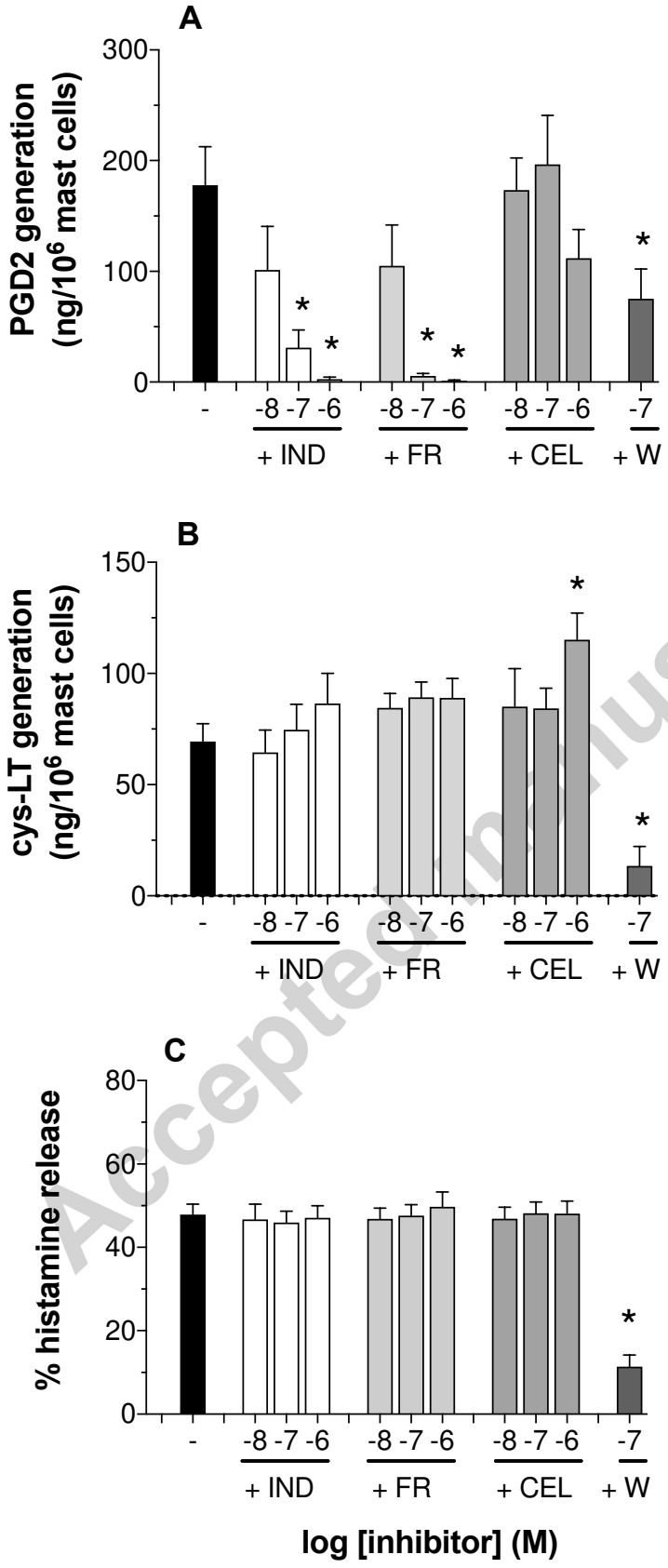


Fig. 2

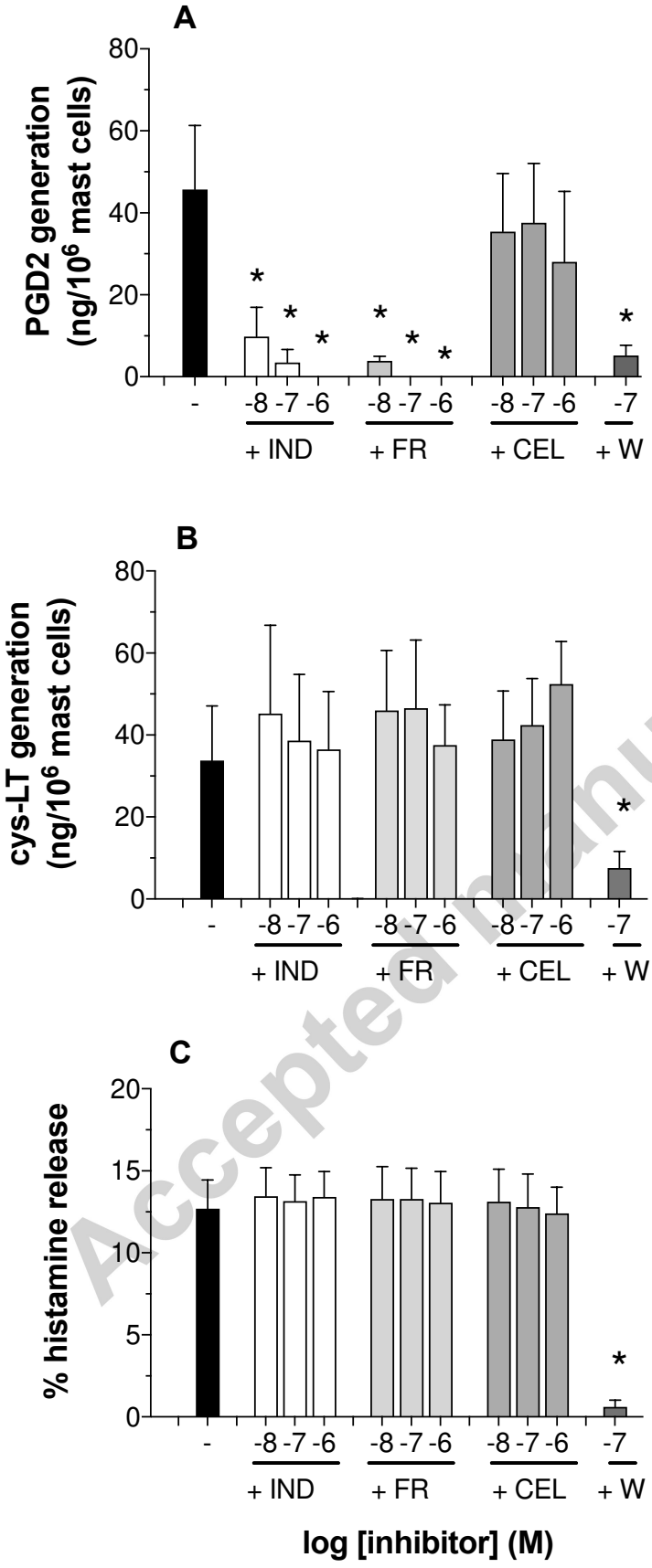


Fig. 3

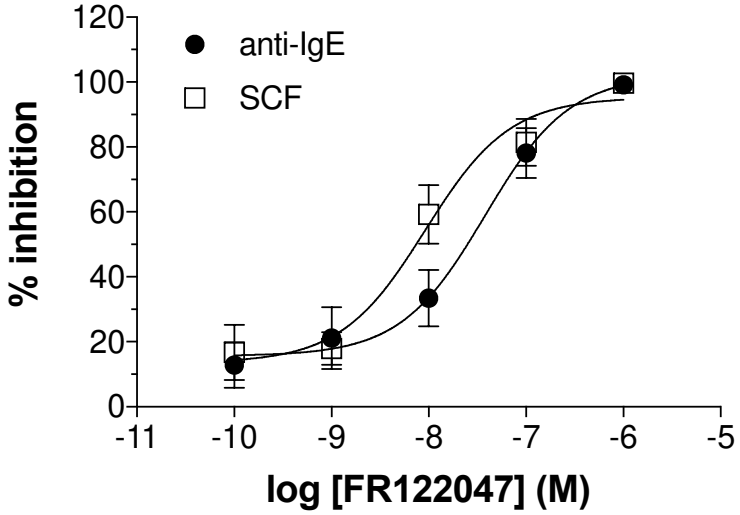


Fig. 4

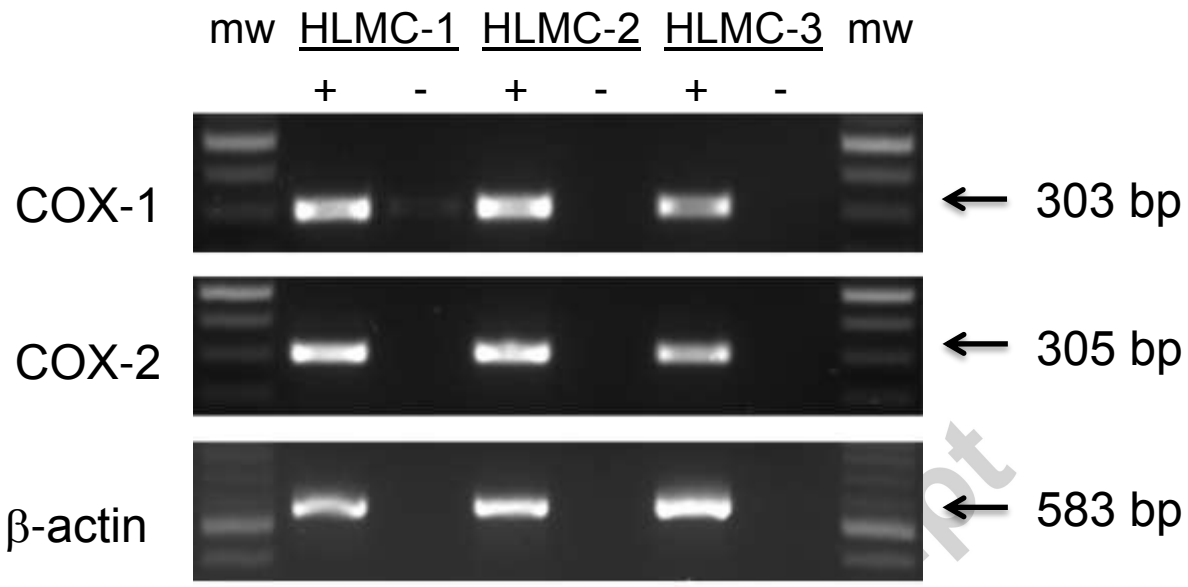


Fig. 5

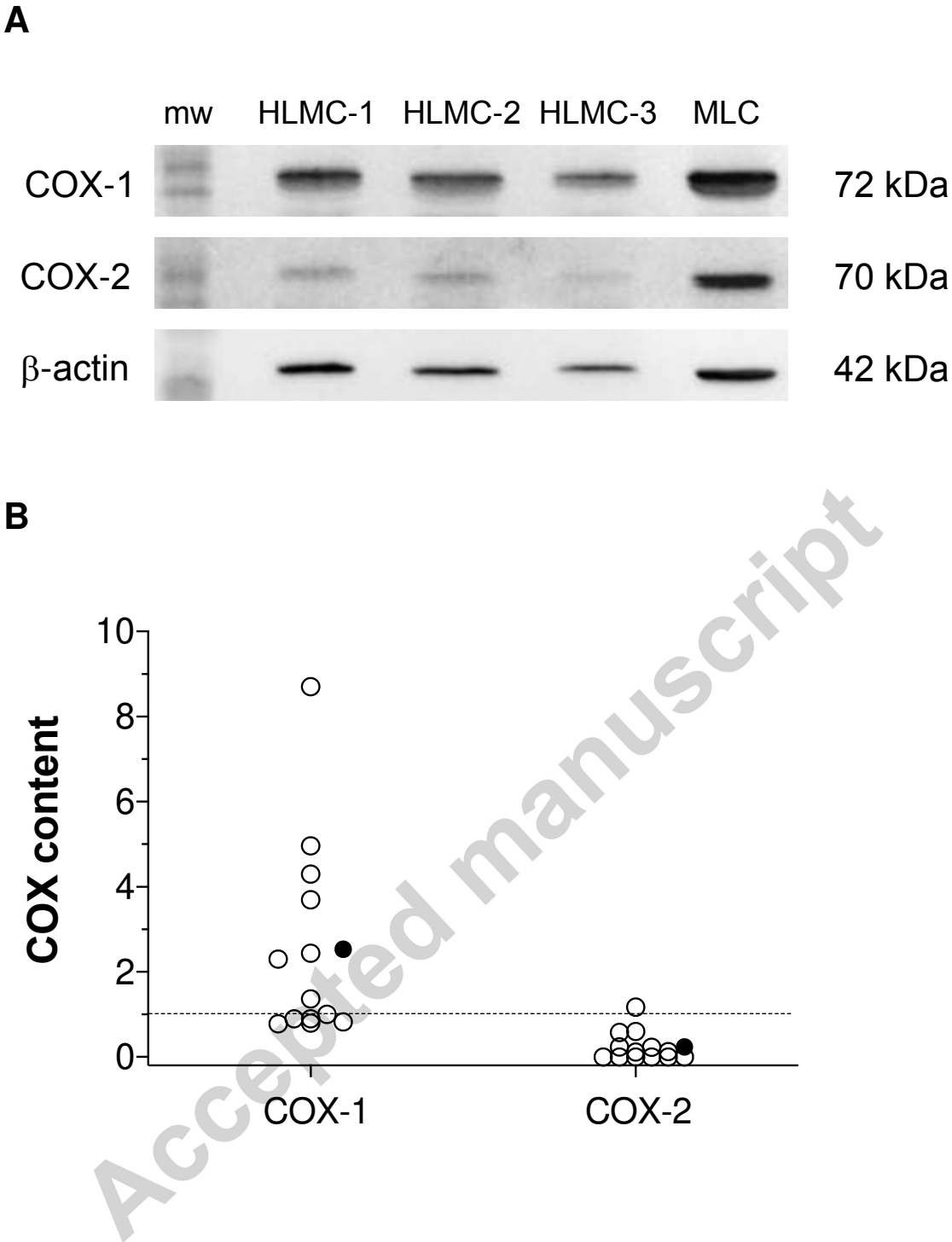


Fig. 6

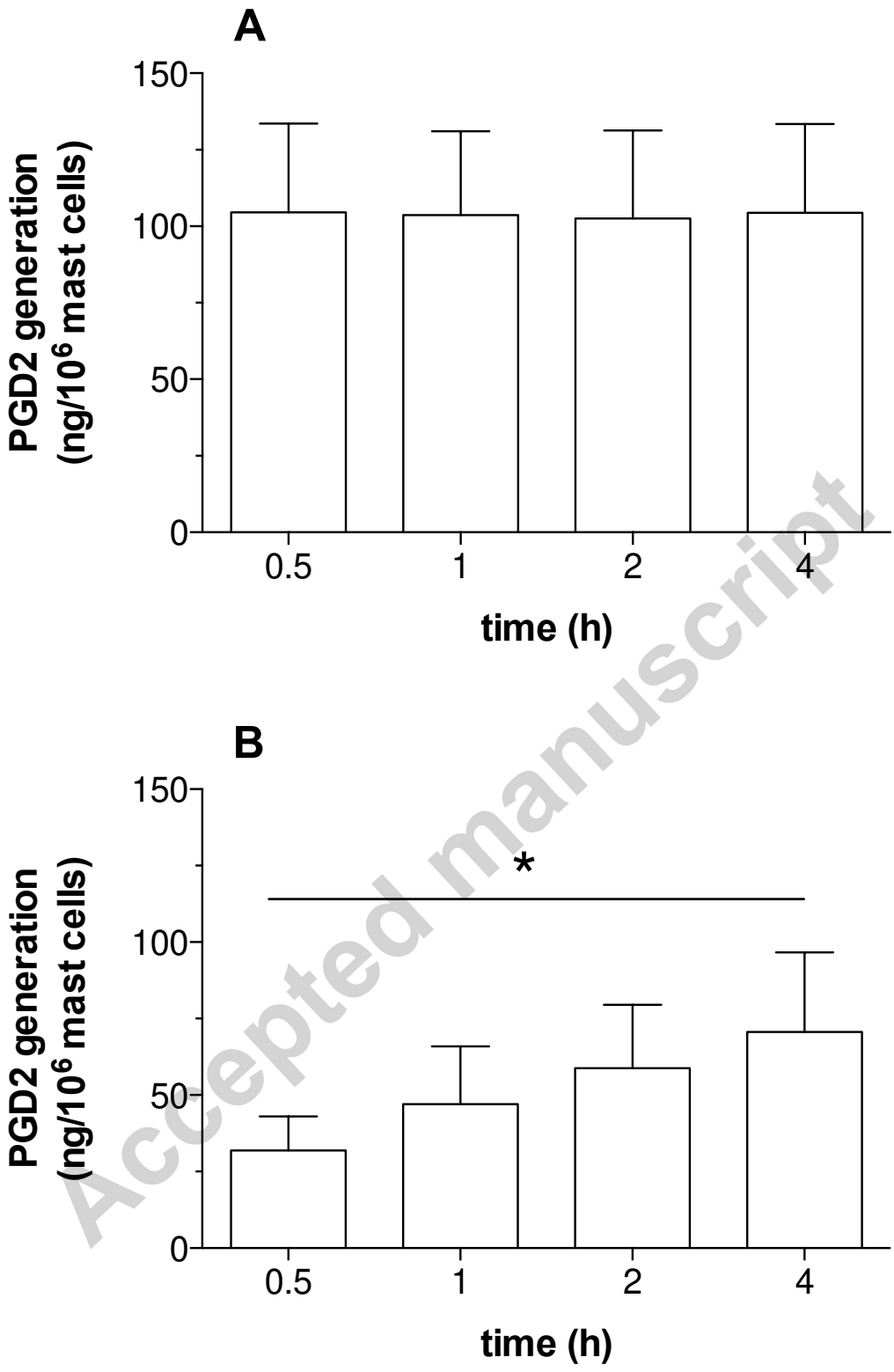


Fig. 7

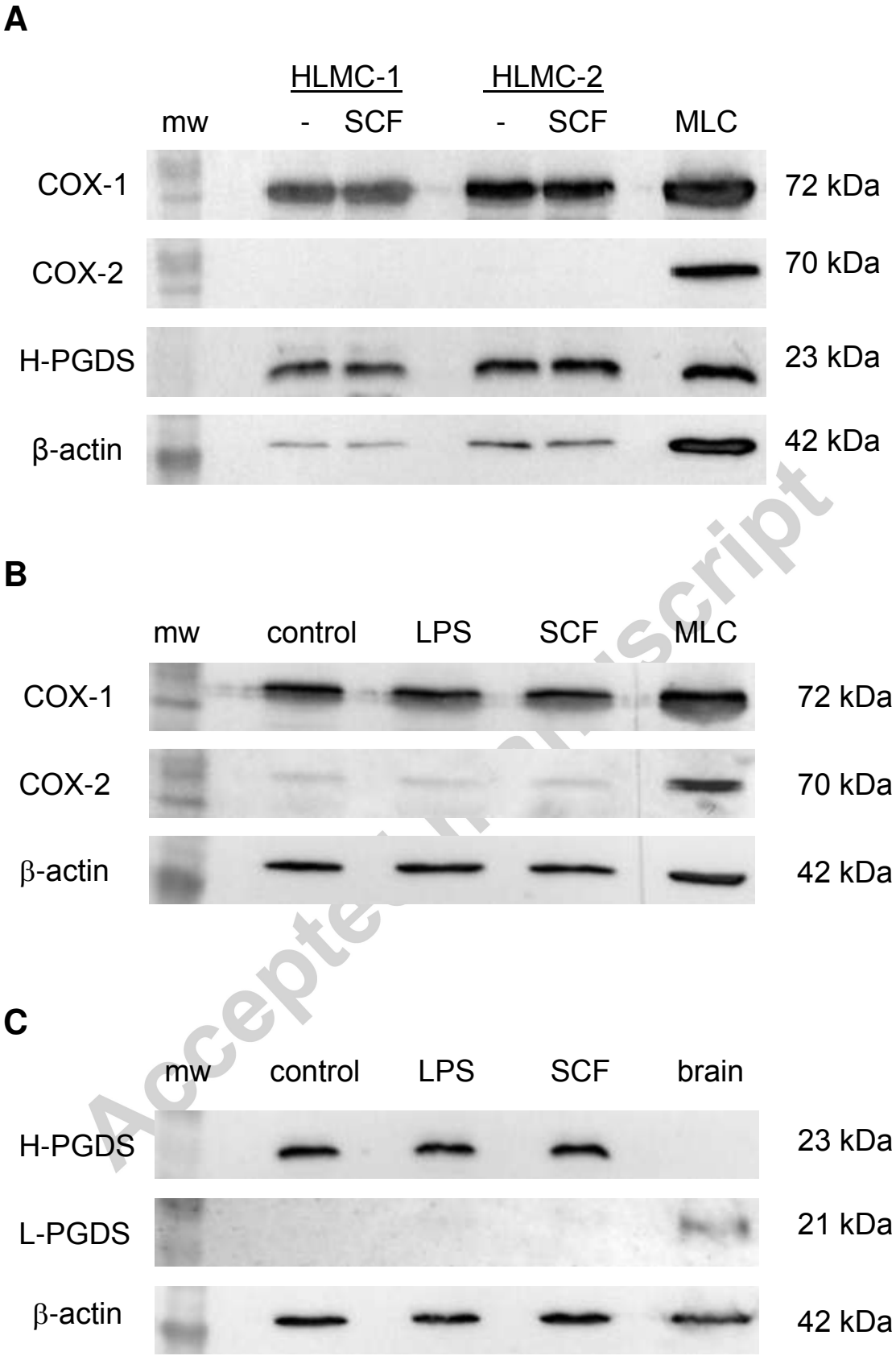


Fig. 8