RESEARCH PAPER

Inhibition of microsomal PGE synthase-1 reduces human vascular tone by increasing PGI$_2$: a safer alternative to COX-2 inhibition

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BACKGROUND AND PURPOSE

The side effects of cyclooxygenase-2 (COX-2) inhibitors on the cardiovascular system could be associated with reduced prostaglandin (PG)I$_2$ synthesis. Microsomal PGE synthase-1 (mPGES-1) catalyses the formation of PGE$_2$ from COX-derived PGH$_2$. This enzyme is induced under inflammatory conditions and constitutes an attractive target for novel anti-inflammatory drugs. However, it is not known whether mPGES-1 inhibitors could be devoid of cardiovascular side effects. The aim of this study was to compare, *in vitro,* the effects of mPGES-1 and COX-2 inhibitors on vascular tone in human blood vessels.

EXPERIMENTAL APPROACH

The vascular tone and prostanoid release from internal mammary artery (IMA) and saphenous vein (SV) incubated for 30 min with inhibitors of mPGES-1 or COX-2 were investigated under normal and inflammatory conditions.

KEY RESULTS

In inflammatory conditions, mPGES-1 and COX-2 proteins were more expressed, and increased levels of PGE$_2$ and PGI$_2$ were released. COX-2 and NOS inhibitors increased noradrenaline induced vascular contractions in IMA under inflammatory conditions while no effect was observed in SV. Interestingly, the mPGES-1 inhibitor significantly reduced (30–40%) noradrenaline-induced contractions in both vessels. This effect was reversed by an IP (PGI$_2$ receptor) antagonist but not modified by NOS inhibition. Moreover, PGI$_2$ release was increased with the mPGES-1 inhibitor and decreased with the COX-2 inhibitor, while both inhibitors reduced PGE$_2$ release.

CONCLUSIONS AND IMPLICATIONS

In contrast to COX-2 inhibition, inhibition of mPGES-1 reduced vasoconstriction by increasing PGI$_2$ synthesis. Targeting mPGES-1 could provide a lower risk of cardiovascular side effects, compared with those of the COX-2 inhibitors.

LINKED ARTICLES

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Abbreviations

C3, compound 3, 1-(1-isopropyl-5,6-dimethyl-1H- benzoimidazol-2-yl)-piperidine-4-carboxylic acid cyclopentylamide; COXIB, selective COX-2 inhibitor; IMA, internal mammary artery; mPGES-1, microsomal PGE synthase-1; NSAIDs, non-steroidal anti-inflammatory drugs; SMC, smooth muscle cell; SV, saphenous vein

Introduction

The effects of selective cyclooxygenase-2 (COX-2) inhibitors (COXIB) or non-steroidal anti-inflammatory drugs (NSAIDs) are mediated through the inhibition of **COX-2** enzyme (Marnett, 2009), which mostly decreases **prostaglandin E2 (PGE$_2$)** production at inflammatory sites (Ibuki *et al.,* 2003; Alvarez-Soria *et al.,* 2008). However, the gastrointestinal side effects of NSAIDs and the cardiovascular side effects associated with both COXIBs and some NSAIDs (such as ibuprofen and diclofenac) limit their use (Bhala *et al.,* 2013; McGettigan and Henry, 2013). Blood pressure and thrombosis are strongly regulated by **prostacyclin (PGI$_2$)**, PGE$_2$ and **thromboxane (Tx)A$_2$** synthesized through the COX pathway (Norel, 2007). PGI$_2$ induces vasodilatation and inhibits platelet aggregation *via* the activation of **IP receptor**s and adenylate cyclase (Reid and Kinsella, 2015). Therefore, the deleterious cardiovascular events induced by COXIBs and NSAIDs have been linked to a decreased level of PGI$_2$. In addition, recent studies have shown that deletion of the COX-2 gene or treatment with COXIBs in mice lead to vascular dysfunction by decreasing **NO** release (Yu *et al.,* 2012) or increasing the endogenous **NOS** inhibitor, **asymmetric dimethylarginine** and production at a renal level (Ahmetaj-Shala *et al.,* 2015). For these reasons, new drugs that only suppress synthesis of pro-inflammatory PGE$_2$ without reducing PGI$_2$ or NO synthesis could be effective in the treatment of inflammatory diseases without increasing cardiovascular risks.

PGE$_2$ controls the vascular tone by inducing contraction (*via* **EP$_3$ receptor**s) in internal mammary arteries (IMA) or relaxation (*via* **EP$_4$ receptor**s) in saphenous veins (SV) (Foudi *et al.,* 2011). Three PGE synthase (PGES) isoforms specifically catalyse the final step of PGE$_2$ biosynthesis from **PGH$_2$**. Among these, microsomal **(m)PGES-1** is the major contributor of PGE$_2$ synthesis in human vascular smooth muscle cells (SMC). This enzyme is constitutively present at a low level or strongly expressed and co-induced with COX-2 in inflammatory conditions (Jakobsson *et al.,* 1999; Camacho *et al.,* 2007; Gomez *et al.,* 2013).

PGE$_2$ has been described as a key mediator of pain and inflammation in many animal studies. Reduced pain hypersensitivity, fever and inflammation have been observed in mice deficient for mPGES-1 (Engblom *et al.,* 2003; Xu *et al.,* 2008) similarly to ablation of COX-2 (Myers *et al.,* 2000). In addition, mPGES-1 knockout mice did not exhibit increased thrombogenesis or increased blood pressure, in contrast with COX-2 knockout mice (Cheng *et al.,* 2006; Wang *et al.,* 2011; Chen *et al.,* 2013). Therefore, mPGES-1 inhibitors could be safer novel anti-inflammatory agents by reducing only PGE$_2$ levels in inflammatory diseases. Consequently, during the past few years, novel mPGES-1 inhibitors [such as MF63, MK886, PF-4693627, YS121, LY3023703 and compound 3 (C3)] have been developed (Leclerc *et al.,* 2013; Jin *et al.,* 2015; Koeberle and Werz, 2015). However, the potential cardiovascular risks of the mPGES-1 inhibitors have not been thoroughly evaluated in *in vitro* models.

For this reason, the aim of our study was to determine and compare the *in vitro* effects of an mPGES-1 inhibitor (C3) and COXIBs on the vascular tone of human vessels such as IMA and SV. The mechanisms underlying these effects associated with PGI$_2$ release were analysed in these vessels with or without inflammation.

Methods

Human vascular preparations

This study was approved by the Institutional Review Board of the Istanbul University Institute of Cardiology and the Ethics Committee of INSERM (the French National Institute for Health and Medical Research). These tissues are considered as surgical waste in accordance with French ethical laws (L.1211-3-L.1211-9). All experiments with human subjects were performed in accordance with the Helsinki Declaration. The study was performed on isolated segments of human IMA and SV, with intact endothelium obtained from patients (IMA: 27 males and 8 females aged 67 ± 2; SV: 35 males and 12 females aged 64 ± 2) who had undergone coronary artery bypass surgery. The vascular preparations have been used either after organ culture (18 h incubation) or in the next hour following surgery without any treatment: 'Normal (0 h)' conditions.

Organ cultures

The IMA and SV were dissected free from connective tissue, cut into rings of 2–4 mm width and placed immediately into 12-well plates containing RPMI supplemented with PSA (penicillin, 1000 IU·mL^{-1}; streptomycin, 100 μg·mL^{-1}; amphotericin, 0.25 μg·mL^{-1}). In addition, two conditions were tested: in the presence or absence of both **IL-1β** (100 ng·mL^{-1}) and **LPS** (100 μg·mL^{-1}), named 'Inflammation (18 h)' or 'Normal (18 h)' respectively. The volume of the culture medium was adjusted to 1 mL for 70 mg of tissue. All tissue incubations were done at 37°C in a humidified atmosphere of 5% CO$_2$ in air using a culture incubator. After 18 h incubation, different protocols were performed for each sample. One part of the samples was set up in organ bath system for vascular reactivity studies. The second part of samples from the same patient was frozen −80°C for Western blot analysis. Organ culture and organ bath solutions were also kept at −80°C for prostanoid measurements.

Vascular reactivity studies

After the incubation period previously described, inflammatory conditions, IMA and SV preparations (cut as rings) were set up in 10 mL organ baths containing Tyrode's solution (concentration mM): NaCl 139.2, KCl 2.7, CaCl$_2$ 1.8, MgCl$_2$ 0.49, NaHCO$_3$ 11.9, NaH$_2$PO$_4$ 0.4, glucose 5.5, gassed with

5% CO_2 and 95% O_2 at 37°C and pH 7.4. Each ring was initially stretched to an optimal load (IMA: 1.0–1.5 g; SV: 1.5–2.0 g). Changes in force were recorded by isometric force displacement transducer (Narco F-60). Rings were equilibrated for 90 min with bath fluid changes taking place every 10 min.

After the equilibration period, the viability (contractility) of the vessel specimens was checked with KCl (40 mM) stimulation and the preparations were washed until the initial resting tone was reestablished. Thereafter, the vessels were contracted with increasing concentrations of noradrenaline (0.01–100 μM) in a cumulative manner to establish the concentration–response relationship. When a maximal effect (E_{max}) was obtained, the preparations were washed with Tyrode's solution until they returned to the resting basal tone. Subsequently, these preparations were incubated for 30 min with the following pharmacological treatments: the COX-2-selective inhibitors (DuP-697, 1 μM or DFU, 1 μM), the PGI_2 receptor (IP) antagonist [CAY10441 (**RO1138452**), 1 μM], the PGE_2 receptor (EP4) antagonist (**GW627368X**, 1 μM), the mPGES-1 inhibitor (C3, 10 μM) (Leclerc *et al.,* 2013) and the NOS inhibitor (**L-NOARG, nitroarginine**; 100 μM). Some preparations were incubated without any compounds for 30 min and served as a time control. After this 30 min incubation period, a second noradrenaline concentration–response curve was obtained, after which the organ bath solution was stored at −80°C for prostanoid measurements. Each preparation was used for one protocol, comprising two concentration–response curves of noradrenaline, separated with an incubation period. In order to compare the effect of different compounds between cultured under inflammatory (18 h) conditions and fresh vessels, these protocols have been performed with IMA and SV samples 'Normal (0 h)'.

Western blot analysis

Following incubation 'Normal (18 h)' or 'Inflammatory (18 h) conditions', IMA and SV samples were homogenized under liquid nitrogen, using a porcelain mortar. The homogenates were resuspended in RIPA solution [Tris–HCl buffer (in mM): Tris: 50, pH: 8; NaCl: 150; EDTA: 5; Triton X-100: 1%; sodium desoxycholate 1%; SDS 0.1%] at 4°C (1 mL·100 mg^{-1} of tissue) with a protease inhibitor cocktail. The homogenates were centrifuged at 4000 x g for 20 min, at 4°C. The supernatants were assayed for protein concentration using a bicinchoninic acid (BCA) protein assay kit. Samples containing 50 μg of protein were loaded on SDS-PAGE. Proteins were transferred to nitrocellulose membranes. The membranes were subsequently blocked for 1 h in TBS, 0.1% Tween 20 and 5% non-fat dry milk and incubated overnight at 4°C with an anti-COX-2 or anti-mPGES-1 monoclonal antibody diluted to 1:250 and 1:1000, respectively, in TBS/0.1% Tween-20. Subsequently, the membranes were incubated with alkaline phosphatase-conjugated goat anti-mouse or rabbit secondary antibody. Bands were visualized using enhanced chemiluminescent (ECL) plus kit. For quantification, the film was scanned and the integrated optical density of the bands was estimated with Scion image (Scion Corporation, NIH, Frederick, MD, USA) and normalized to β-actin.

Prostanoid measurements

The supernatants of organ culture in the presence or absence of inflammatory conditions were collected after 18 h. In addition, the organ bath solutions after 30 min incubation with the COXIB (DuP-697, 1 μM) and the mPGES-1 inhibitor (C3, 10 μM) with or without noradrenaline (100 μM) stimulation were also harvested. The concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) were measured in both supernatants using an enzyme immunoassay (EIA) kit according to the manufacturer's instructions. The prostanoid concentrations in supernatants were expressed as pg or ng·(mg tissue wet weight)$^{-1}$. Technical replicates were used to ensure the reliability of single values.

Data and statistical analysis

The data and statistical analysis in this study comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.,* 2015). All results obtained from different patients (n) were expressed as means ± SEM. The first concentration–response curve induced by noradrenaline was expressed in absolute terms, as mN, but the second concentration–response curve induced by noradrenaline was expressed as % of the E_{max} of the first curve. Where possible, using SigmaPlot version 12.0 (Systat Software, Point Richmond, CA, USA), a four-parameter logistic equation of the form

$$E = \frac{E_{max}[A]^{nH}}{EC_{50}^{nH} + [A]^{nH}}$$

was fitted to data (E) obtained from each organ bath protocol to provide estimates of the maximal contraction (E_{max}) induced by noradrenaline [A], the half-maximum effective concentration values (EC_{50}), and Hill slope (nH) parameters. The pEC_{50} values were calculated. Statistical analysis was performed by Student's *t*-test (paired data derived from the same patient or unpaired) or repeated-measures (RM) two-way ANOVA and Bonferroni's correction for multiple comparisons *post hoc* tests. If there was more than one preparation (same condition and protocol) for one patient, the results were averaged before statistical analysis. Values of $P<0.05$ indicated significant differences between means. Statistical analyses were performed using SigmaStat version 3.5 (Systat Software, Point Richmond, CA, USA).

Materials

The protease inhibitor cocktail, IL-1β, LPS, noradrenaline, KCl, antibiotics and antimycotics were purchased from Sigma–Aldrich (St. Louis, MO, USA). CAY10441, DuP-697, DFU, GW627368X, **U46619**, **iloprost**, PGE_2 and 6-keto-$PGF_{1\alpha}$ EIA kits were obtained from Cayman Chemical (Ann Arbor, MI, USA). RPMI was obtained from Gibco Invitrogen (Paisley, UK). BCA protein assay kit was from Thermo (Rockford, USA). Nitrocellulose membranes and ECL Plus system were obtained from Amersham Biosciences (Buckinghamshire, UK). C3 [1-(1-isopropyl-5,6-dimethyl-1H-benzoimidazol-2-yl)-piperidine-4-carboxylic acid cyclopentylamide] was a generous gift from NovaSAID AB and Dr Per-Johan Jakobsson (Karolinska Institutet, Stockholm, Sweden). Antibodies against mPGES-1 and COX-2 were from Oxford Bio Therapeutics (Oxford, UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA) respectively.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.,* 2015a,b,c).

Results

Vasoconstriction induced by noradrenaline in a model of inflammation

The contraction of samples of human IMA and SV induced by noradrenaline was significantly decreased under
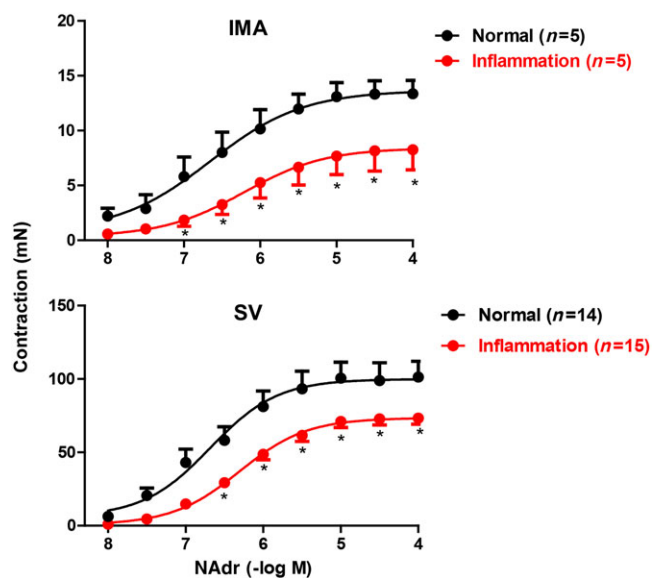

Figure 1

Vascular reactivity induced by noradrenaline in IMA and SV following incubation under 'Normal (0 h)' or 'Inflammatory (18 h)' conditions. Contractions obtained from first noradrenaline (NAdr) concentration–response curve are expressed in mN. * $P < 0.05$, significantly different; two-way ANOVA. Values are means ± SEM derived from (*n*) different patients (see Table 1 for pEC_{50}, E_{max} values and statistics).

'Inflammatory (18 h) conditions' compared with 'Normal (0 h)' or 'Normal (18 h)' conditions. The values of pEC_{50} were lower after incubation (18 h) either under normal or inflammatory conditions, compared with 'Normal (0 h)' conditions (Figure 1; Table 1). On the other hand, there was no difference in the KCl-induced contractions between normal and inflammatory conditions in both vessels (Supporting Information Figure S1).

Expression of COX-2 and mPGES-1 and prostanoid release in a model of inflammation

In inflammatory conditions, expression of COX-2 and mPGES-1 protein was significantly increased in IMA, compared with 'Normal (18 h)' conditions (Figure 2A–C). In SV, only COX-2 expression was increased in inflammatory conditions as the increase in mPGES-1 expression in inflammatory conditions did not reach statistical significance. In addition, concentrations of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) and PGE_2 in culture supernatants were significantly higher under inflammatory conditions, compared with normal conditions, in both IMA and SV (Figure 2D, E). The expression of mPGES-1 and COX-2 and the production of PGE_2 were greater in SV than in the IMA, in both conditions (Figure 2).

Effects of the mPGES-1 inhibitor on vasoconstriction induced by noradrenaline

The mPGES-1 inhibitor (C3, 10 μM) decreased the vascular contractile response and sensitivity (pEC_{50}) to noradrenaline, under normal or inflammatory conditions, in both IMA and SV (Figure 3; Table 2). Inhibition of noradrenaline-induced contractions following C3 incubation was significantly greater in SV, compared with IMA under inflammatory conditions (Supporting Information Figure S2). The decreased vascular tone and sensitivity to noradrenaline induced by C3 were reversed by co-incubation with the IP receptor antagonist (CAY10441, 1 μM) in SV and IMA (Figure 3; Table 2). Interestingly, under inflammatory conditions, the whole of the concentration-response curves induced by noradrenaline after co-incubation with C3 and CAY10441 were significantly higher in IMA and lower in SV as compared with their respective controls (Figure 3C, D). On the other hand, vascular response after co-incubation with C3 and the **TP receptor** antagonist (**BAY u3405**, 1 μM) was comparable
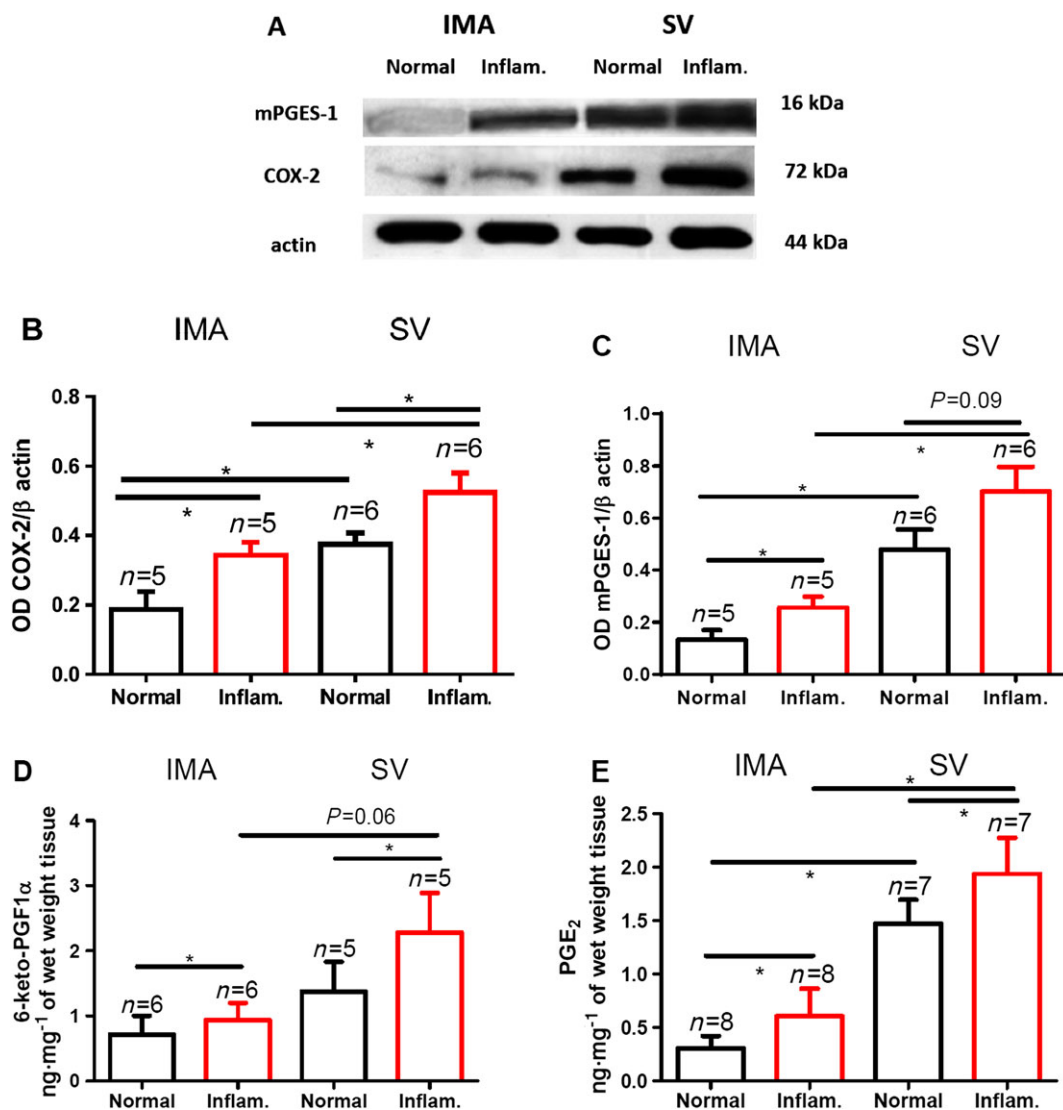
Table 1

The effect of inflammation on vascular contraction induced by noradrenaline

Condition	pEC$_{50}$	IMA E$_{max}$ (mN)	*n*	SV pEC$_{50}$	E$_{max}$ (mN)	*n*
Normal (0 h)	6.80 ± 0.16	13.36 ± 1.21	5	6.71 ± 0.11	101.30 ± 10.83	14
Normal (18 h)	6.27 ± 0.24[#]	17.36 ± 4.09	6	6.29 ± 0.10[#]	108.13 ± 8.61	15
Inflammation (18 h)	6.26 ± 0.15[#]	8.25 ± 1.83*[#]	5	6.27 ± 0.06[#]	73.31 ± 4.15*[#]	15

pEC_{50} and E_{max} (maximal contraction expressed as mN) values derived from first concentration–response curve induced by noradrenaline in IMA and SV under 'Normal (0 h)', 'Normal (18 h)' or 'Inflammation (18 h)' conditions.
*$P < 0.05$, significantly different from 'Normal (18 h)'; Student's *t*-test.
[#]$P < 0.05$, significantly different from 'Normal (0 h)'; Student's *t*-test. Values are means ± SEM derived from (*n*) different patients. The E_{max} values indicated in this table correspond to 100% values presented in Figures 3, 4 and 6 and Table 2 of our study.

Figure 2

The expression of COX-2 and mPGES-1 and release of 6-keto-PGF$_{1\alpha}$ (stable metabolite of PGI$_2$) and PGE$_2$ in IMA or SV following 18 h incubation under 'Normal' or 'Inflam' (inflammatory) conditions. A representative image of Western blot is presented (A). Histograms represent Western blot quantification of COX-2 (B) and mPGES-1 (C) corresponding bands. Optical density (OD, arbitrary units) was quantified by Scion Image and normalized by actin. The release of 6-keto-PGF$_{1\alpha}$ (D) and PGE$_2$ (E) in organ culture supernatant after 18 h incubation were expressed as ng·(mg tissue wet weight)$^{-1}$. * $P < 0.05$ significantly different as indicated; Student's t-test. Values are means ±SEM derived from (n) different patients.

with the response obtained following incubation with C3 alone (Supporting Information Figure S3). C3 significantly decreased the potent vasoconstriction response induced by **U46619** (TxA$_2$ analogue) in IMA under inflammatory conditions (Supporting Information Figure S4).

Effects of the IP-receptor antagonist and COX-2 inhibitors on vasoconstriction induced by noradrenaline

Under 'Normal (0 h)' conditions, the IP receptor antagonist (CAY10441, 1 µM) did not modify the contractions induced by noradrenaline in IMA and SV (Figure 3A, B; Table 2). Under 'Inflammatory (18 h) conditions', incubation with

CAY10441 or the COX-2 inhibitor DuP-697 (1 µM) increased the contractions induced by noradrenaline in IMA while pEC$_{50}$ values were not changed (Figures 3C and 4A; Table 2). There was no statistical difference between maximal contraction obtained after these treatments under inflammatory condtions (DuP-697: 15 ± 8 mN, CAY10441: 14 ± 5 mN) and those obtained under normal conditions in IMA (17 ± 4 mN, Table 1). In contrast, these effects of CAY10441 and DuP-697 were not observed in SV (Figures 3D and 4B; Table 2). For this reason, another COXIB (DFU, 1 µM) and an EP$_4$ receptor antagonist (GW627368X, 1 µM) were tested and found to have no effect on the vascular response to noradrenaline in SV under inflammatory conditions (Figure 4B; Table 2; Supporting Information Table S1).
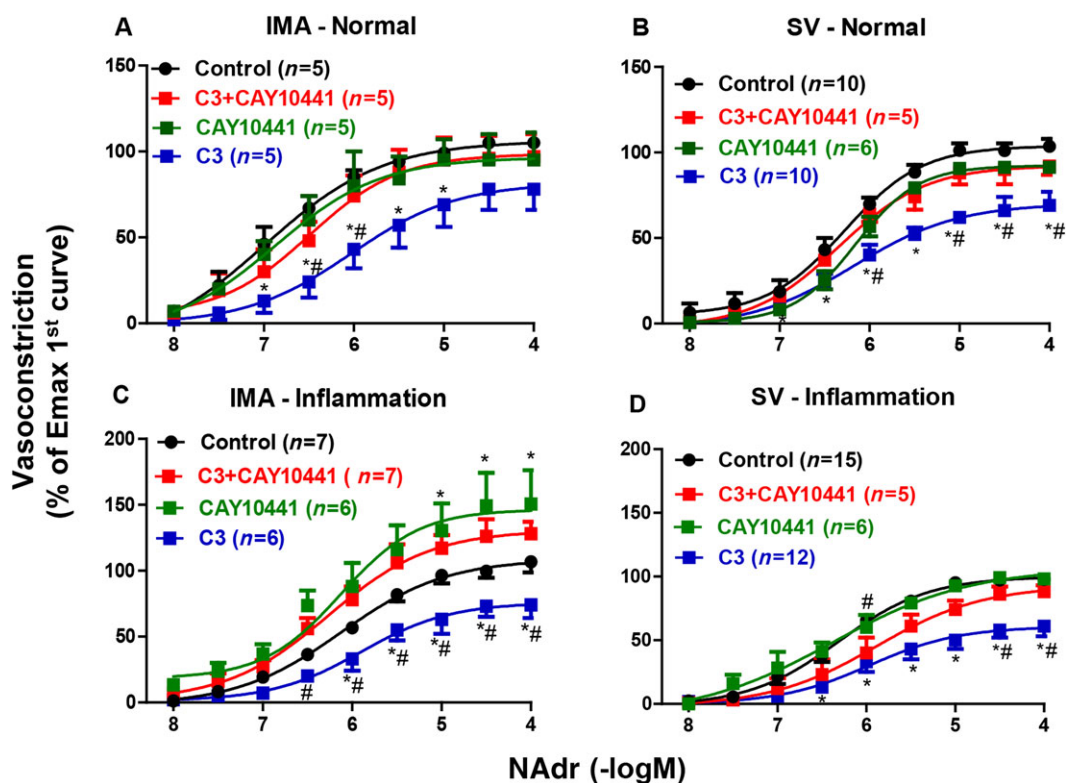
Figure 3

The effects of mPGES-1 inhibitor (C3, 10 μM, 30 min), IP receptor antagonist (CAY10441, 1 μM, 30 min) and co-incubation of these treatments on vasoconstriction induced by noradrenaline (NAdr) in IMA and SV. Vessels were used 'Normal (0 h)' (A, B) or after incubation under 'Inflammatory (18 h)' (C, D) conditions. The contraction was expressed as % of the maximal noradrenaline contraction obtained in the first concentration–response curve. * $P < 0.05$, significantly different from Control (without any treatment in the organ bath): #$P < 0.05$, significantly different from C3 + CAY10441; repeated measures two-way ANOVA. Values are means ± SEM derived from (n) different patients (see Table 2 for pEC_{50}, E_{max} values and statistics).

Effects of mPGES-1 and COX-2 inhibitors on prostanoid release in inflammatory conditions

After 18 h culture under inflammatory conditions, vascular preparations were set up in an organ bath. The measurements of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) and PGE_2 in organ bath solutions, were performed after 30 min incubation with the respective treatments. The levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the organ bath solutions (without any pharmacological treatment) were increased after noradrenaline (100 μM) stimulation (Figure 5). Interestingly, incubation with the mPGES-1 inhibitor (C3, 10 μM) increased the basal release of 6-keto-$PGF_{1\alpha}$ while the COX-2 inhibitor (DuP-697, 1 μM) caused a significant decrease of 6-keto-$PGF_{1\alpha}$ concentrations, in IMA and SV (Figure 5A, B). PGE_2 release with or without noradrenaline stimulation was significantly decreased in IMA and SV after either C3 or DuP-697 incubation (Figure 5C, D).

Effect of NOS inhibitor on vasoconstriction induced by noradrenaline

Under normal and inflammatory conditions, contractions induced by noradrenaline in IMA (but not in SV) were significantly increased in the presence of the inhibitor of NO synthesis (L-NOARG, 100 μM; Figure 6A–D; Table 2). These vascular contractions after co-incubation with C3 and the NOS inhibitor (L-NOARG, 100 μM) were comparable with

those obtained after incubation with C3 alone in IMA under inflammatory conditions (Figure 6C).

Discussion

COX-2 inhibitors are effective in the treatment of inflammatory disease by decreasing PGE_2 levels. However, their cardiovascular side effects, associated with the reduction of PGI_2 production, have limited their use (Grosser et al., 2010). In recent years, the inhibition of mPGES-1 has gained importance as an alternative to inhibition of COX-2. Our study focused on the effects of mPGES-1 inhibitor on human vascular reactivity in inflammatory conditions. The present report shows that in contrast to the COX-2 inhibitor, the mPGES-1 inhibitor (C3) reduced the contractions induced by noradrenaline (Figures 3 and 7) by increasing (twofold) PGI_2 production (Figures 5 and 7). Furthermore, treatment with this mPGES-1 inhibitor on human vessels was associated with reduced levels (threefold to fivefold) of PGE_2 (Figure 5). These results suggest that C3 could have a potent anti-inflammatory effect, without the increased cardiovascular risk associated with COX-2 inhibitors. In addition, our study provides a comparison of the regulation of vascular tone in IMA and SV, induced by selective inhibitors of PGI_2, PGE_2 and NO pathways (Figure 7).

Table 2

The effects of different treatments on vascular contractions induced by noradrenaline

Condition	Treatments	IMA			SV		
		pEC_{50}	E_{max} (%)	n	pEC_{50}	E_{max} (%)	n
Normal (0 h)	Control	6.57 ± 0.31	105 ± 2	5	6.43 ± 0.08	103 ± 4	10
Normal (0 h)	C3	$5.75 \pm 0.40^{*\#}$	78 ± 12	5	$6.07 \pm 0.13^{*\#}$	$69 \pm 8^{*\#}$	10
Normal (0 h)	C3 + CAY10441	6.71 ± 0.18	97 ± 13	5	6.58 ± 0.11	92 ± 5	5
Normal (0 h)	CAY10441	6.04 ± 0.10	95 ± 16	5	6.38 ± 0.12	91 ± 2	5
Inflam. (18 h)	Control	6.15 ± 0.18	106 ± 8	7	$6.36 \pm 0.09^{\#}$	98 ± 3	15
Inflam. (18 h)	C3	5.91 ± 0.08	$74 \pm 10^{*\#}$	6	$5.59 \pm 0.18^{*}$	$61 \pm 8^{*\#}$	12
Inflam. (18 h)	C3 + CAY10441	6.22 ± 0.25	128 ± 9	7	5.82 ± 0.31	88 ± 5	5
Inflam. (18 h)	CAY10441	6.38 ± 0.27	$150 \pm 25^{*}$	6	6.41 ± 0.19	98 ± 2	5
Inflam. (18 h)	Control	6.20 ± 0.10	103 ± 12	5	6.35 ± 0.09	88 ± 2	6
Inflam. (18 h)	DuP-697	6.42 ± 0.18	$165 \pm 22^{*}$	5	6.41 ± 0.11	90 ± 2	6
Inflam. (18 h)	DFU	nt	nt	–	6.27 ± 0.09	85 ± 3	6
Normal (0 h)	Control	6.68 ± 0.15	105 ± 4	5	6.32 ± 0.12	103 ± 4	5
Normal (0 h)	L-NOARG	6.14 ± 0.22	$160 \pm 20^{*}$	5	6.28 ± 0.15	92 ± 5	5
Inflam. (18 h)	Control	6.18 ± 0.04	102 ± 2	5	6.52 ± 0.15	89 ± 5	5
Inflam. (18 h)	L-NOARG	5.82 ± 0.07	$142 \pm 13^{*}$	6	6.37 ± 0.13	95 ± 1	5

pEC_{50} and E_{max} (maximal contraction) values derived from the second concentration–response curve induced by NE in IMA or SV under 'Normal (0 h)' and 'Inflammatory (18 h)' (Inflam.) conditions. E_{max} are expressed as % of the maximal noradrenaline contraction obtained in the first concentration–response curve. The treatments used are as follows: mPGES-1 inhibitor (C3, 10 μM), COX-2 inhibitor (DuP-697 or DFU, 1 μM), NOS inhibitor (L-NOARG, 100 μM) and IP receptor antagonist (CAY10441, 1 μM). Values are means ± SEM derived from (n) different patients.
$^{*}P < 0.05$, significantly different from their respective paired Control (without any treatment in organ bath);
$^{\#}P < 0.05$, significantly different from C3 + CAY10441; Student's t-test. nt indicates protocol not tested.
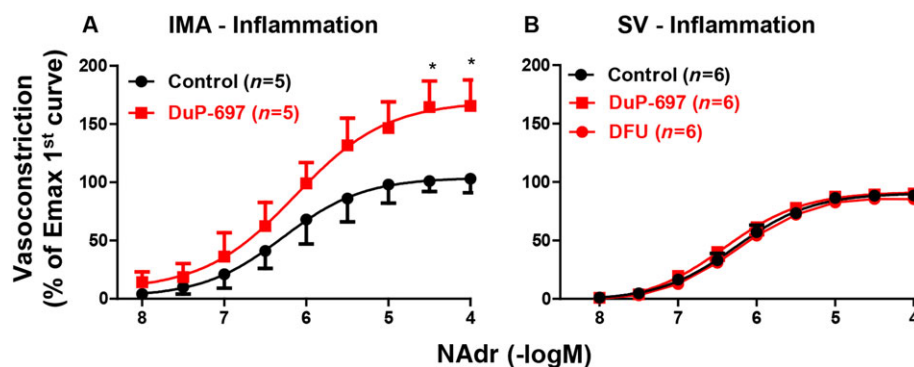
Figure 4

The effects of COX-2 inhibitors (DuP-697, DFU 1 μM, 30 min) on vasoconstriction induced by noradrenaline. IMA and SV were used after incubation under 'Inflammatory (18 h)' conditions. The contraction was expressed as % of the maximal noradrenaline (NAdr) contraction obtained in the first concentration–response curve. $^{*}P < 0.05$, significantly different from Control (without any treatment in the organ bath); repeated measures two-way ANOVA. Values are means ± SEM derived from (n) different patients (see Table 2 for pEC_{50}, E_{max} values and statistics).

Both COX-2 and mPGES-1 enzymes are co-induced by inflammatory stimuli in many cells and are the main enzymes responsible for PGE_2 synthesis (Jakobsson *et al.*, 1999). In an earlier study, the induction of COX-2 enzyme, after 24 h incubation of IMA with IL-1β and LPS, was accompanied by increased levels of PGE_2 and PGI_2 (Foudi *et al.*, 2009). These results were confirmed in the present report after 18 h incubation with the same inflammatory stimuli (Figure 2A, B, D, E). In addition, we demonstrated here that the mPGES-1 enzyme

was also induced in inflammatory conditions in IMA (Figure 2A, C). This acute inflammation (either 18 or 24 h incubations with inflammatory stimuli) reduced the contraction induced by noradrenaline (Figure 1, Foudi *et al.*, 2009). This decreased reactivity can be explained, at least in part, by the inflammation-induced synthesis of PGI_2 and its vasodilatory effect. This hypothesis was confirmed by the fact that the decreased contraction under inflammatory conditions was increased after incubation with the IP receptor
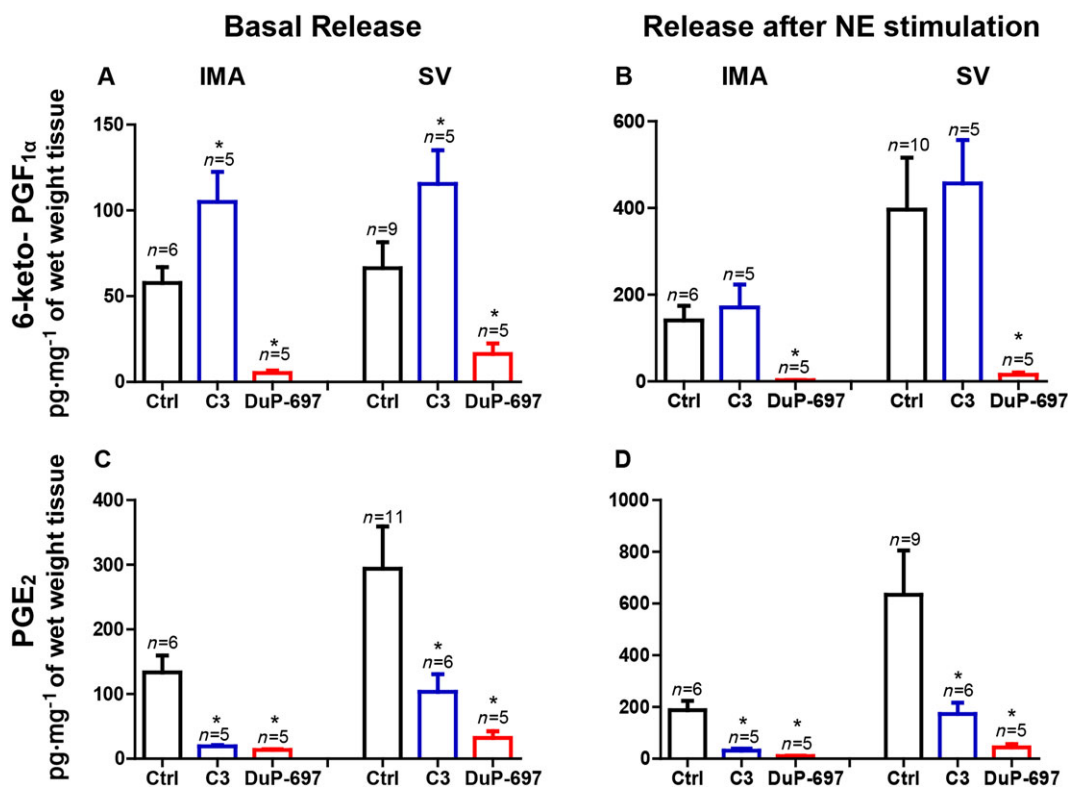
Figure 5

The effects of mPGES-1 inhibitor (C3, 10 µM, 30 min) and COX-2 inhibitor (DuP-697, 1 µM, 30 min) on the release of 6-keto-PGF$_{1\alpha}$ (stable metabolite of PGI$_2$; A, B) and PGE$_2$ (C, D) in basal and after noradrenaline (maximal stimulation (100 µM). The production of prostanoids was measured in organ bath solutions containing IMA and SV after incubation under 'Inflammatory (18 h)' conditions. The release of prostanoids were expressed as pg·(mg tissue wet weight)$^{-1}$. * $P < 0.05$, significantly different from control (Ctrl) of respective vessel; Student's t-test. Values are means ± SEM derived from (n) different patients.

antagonist (CAY10441) or a COX-2 inhibitor (DuP-697, DFU, etoricoxib) (Figures 3C and 4A; Foudi *et al.*, 2009). Both treatments were able to restore the contractions to a level, similar to that observed under normal conditions (Table 1). This increase in vascular tone due to inhibition of PGI$_2$ synthesis induced by COX-2 inhibitors could be associated with their cardiovascular side effects such as arterial hypertension. As several studies have shown that PGE$_2$ is a key mediator of inflammation, drugs that specifically inhibit PGE$_2$ while sparing other prostanoids would be more specific and possibly safer anti-inflammatory therapy. From this aspect, mPGES-1 inhibition represents a promising target for drug development (Wang and FitzGerald, 2010).

In this study, we have chosen C3 as an mPGES-1 inhibitor because this compound is a selective mPGES-1 inhibitor in humans and no concentration-dependent inhibition has been detected against COX-1, COX-2, PGIS or haematopoietic PGD synthase, at concentrations up to 50 µM C3 (Leclerc *et al.*, 2013). The novel finding of our study was that C3 attenuated the noradrenaline-induced vasoconstrictions of human vessels (IMA and SV) by increasing PGI$_2$ production within the vascular wall. This shift towards increased PGI$_2$ synthesis after mPGES-1 inhibition was confirmed by both measuring the PGI$_2$ metabolite, 6-keto-PGF$_{1\alpha}$, and using a specific IP receptor

antagonist in organ bath studies (Figures 3 and 5A). However, the effect of C3 on PGI$_2$ levels after noradrenaline stimulation (100 µM) was not significant (Figure 5B) while a decreased contraction was still measured (Figure 3). The high concentration of PGI$_2$ (threefold to sixfold) produced after noradrenaline stimulation, the metabolism of 6-keto-PGF$_{1\alpha}$ and the accumulation of the metabolites in organ bath solutions could account for this discrepancy. The redirection of PGH$_2$ metabolism towards PGI$_2$ has been reported with various mPGES-1 inhibitors in preparations derived from animals (PF-9184, MF63, LY3023703, C3) (Mbalaviele *et al.*, 2010; Leclerc *et al.*, 2013; Jin *et al.*, 2015; Chandrasekhar *et al.*, 2016). In addition, our data obtained with C3 are in accordance with higher concentrations of PGI$_2$ observed in mPGES-1 knockout mice (Cheng *et al.*, 2006; Wang *et al.*, 2006; Raouf *et al.*, 2016a). Moreover, in these mice, blood pressure was not elevated (Cheng *et al.*, 2006) while increased blood pressure was observed in COX-2 knockout mice (Qi *et al.*, 2002). These *in vivo* murine data are in agreement with our *in vitro* human data, which show an increased contraction in the presence of the COX-2 inhibitor (Figure 4A) and a decreased contraction by the mPGES-1 inhibitor (Figure 3). The shift in favour of PGI$_2$ production induced by mPGES-1 inhibitors might be a great improvement in the cardiovascular safety of anti-inflammatory drugs in comparison to COX-2 inhibitors.
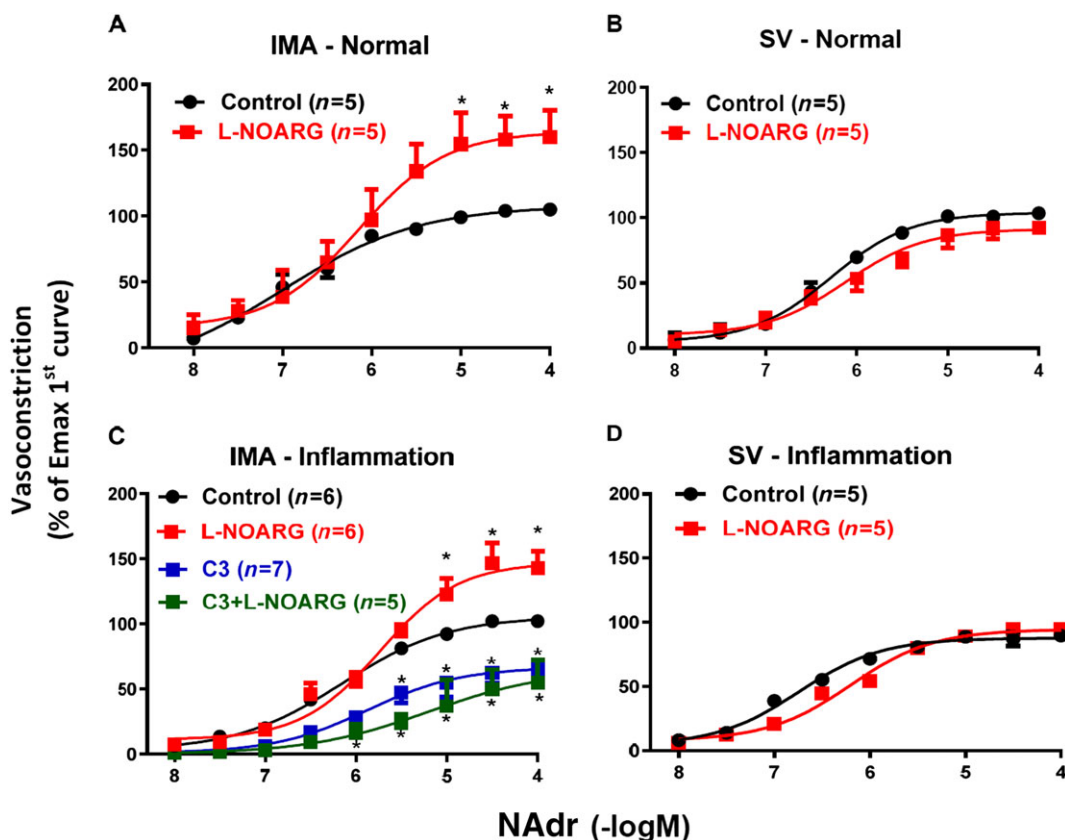
Figure 6

The effect of the NOS inhibitor (L-NOARG, 100 μM, 30 min) on vasoconstriction induced by noradrenaline (NAdr) in IMA and SV in 'Normal (0 h)' or 'Inflammatory (Inflam. 18 h)' conditions. The effect of co-incubation with mPGES-1 inhibitor (C3, 10 μM, 30 min) and NOS inhibitor (L-NOARG, 100 μM, 30 min) was tested in IMA under inflammatory conditions. * $P < 0.05$, significantly different from Control (without any treatment in the organ bath); repeated measures two-way ANOVA. Values are means ± SEM derived from (n) different patients (see Table 2 for pEC_{50}, E_{max} values and statistics).

A recent study has demonstrated that isolated aorta preparations derived from mPGES-1 knockout mice exhibit greater ACh-induced relaxation than those from the wild-type mice (Raouf *et al.*, 2016a). As the relaxation of ACh is mostly dependent on NO and PGI_2 releases in murine aorta (Shen *et al.*, 2013), the increased ACh relaxation in mPGES-1 knockout mice (Raouf *et al.*, 2016a) could be due to either increased PGI_2 or NO levels. In our study, we tested the possibility for an augmentation of NO levels after mPGES-1 inhibition. Our results demonstrated that co-incubation with NOS inhibitor and C3 exhibited similar profile to treatment with C3 alone on vascular tone (Figure 6C). This result suggests that C3 did not affect NO levels.

In addition to the beneficial effects of mPGES-1 inhibitors described above, the present study could provide new aspects for improving the patency of graft materials by using a mPGES-1 inhibitor. In this study, we used IMA and SV, which are frequently used as a graft material to bypass stenosed coronary arteries (Goldman *et al.*, 2004). During bypass surgery or the post-operative period, vascular cells are likely to be exposed to inflammatory conditions,

resulting in an increase of prostanoid production. In addition, the PGE_2 produced by the vascular wall exacerbates atherothrombosis (Gross *et al.*, 2007) while PGI_2 has a reverse effect. All patients are prescribed antiplatelet therapy including aspirin after bypass surgery. Another recent study showed that the inhibition of mPGES-1 also prevents platelet activation (Raouf *et al.*, 2016b). For all these reasons, we suggest that using an mPGES-1 inhibitor such as C3 in the post-operative period could prevent graft spasm, thrombosis or occlusion, not only by increasing PGI_2 release but also by decreasing PGE_2 levels.

In IMA and SV under inflammatory conditions, mPGES-1 and COX-2 expressions were induced and similar reduction of noradrenaline-induced vasoconstriction was measured (Figures 1 and 2A–C). The mPGES-1 inhibitor reduced vascular tone to the same extent in both IMA and SV under normal conditions (Figures 3A, B). On the other hand, we have found many discrepancies between these vessels. Under inflammatory conditions, the inhibition of noradrenaline-induced vasoconstriction by C3 was less pronounced in IMA (Supporting Information Figures S2) and restauration with CAY10441 was significantly greater (twofold) in IMA than
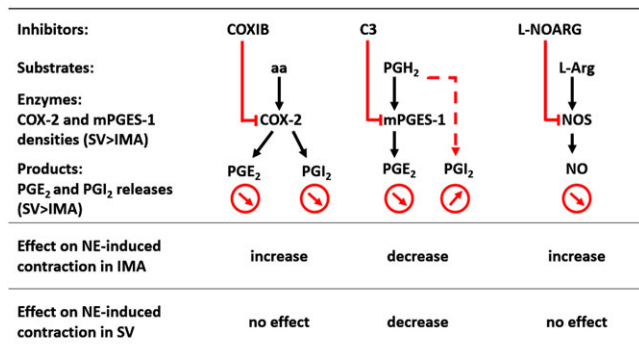
Figure 7

The effects of different inhibitors on vascular tone and their mechanisms of action in human vessels. aa: arachidonic acid, L-arg: L-arginine.

SV (Figures 3C, D; Table 2). Moreover, the inhibition of the vasodilatory effects of PGI_2, either by COX-2 inhibition or by antagonism of IP receptors antagonist, induced an increased contraction in IMA but not in SV under inflammatory conditions (Figures 3C, D and 4A, B; Table 2). In addition, we showed that SV is 100-fold less sensitive than IMA to the relaxation induced by the PGI_2 analogue (**iloprost**, Supporting Information Figure S5 and Table S2) under inflammatory conditions. This could explain the lack of effect of IP receptor antagonism or COX-2 inhibition on vascular tone in SV. In contrast, these treatments augmented the contractile response to noradrenaline (Figures 3C and 4A) in IMA, which was much more sensitive to the PGI_2 analogue (Supporting Information Figure S5 and Table S2). As PGE_2 induces vasodilatation *via* EP_4 receptors in SV but not in IMA (Foudi *et al.,* 2011), the effect of the EP_4 receptor antagonist on vascular tone was tested only in SV and no effect was found (Supporting Information Table S1).

Like COX-2 inhibition and antagonism of IP receptors, the inhibition of NOS also regulated the vascular tone differently between IMA and SV. After incubation with the NOS inhibitor, a greater response to noradrenaline under normal and inflammatory conditions was detected in IMA, while the SV responses were not modified (Figure 6A–D; Table 2). The same variation between IMA and SV was observed in endothelium-denuded vessels after 22 h incubation with LPS (Thorin-Trescases *et al.,* 1995). This different regulation of vascular tone by NO could be due to the higher eNOS activity described in IMA (Hamilton *et al.,* 1997; Shapira *et al.,* 1999), and consequently greater effect of NOS inhibitor on vascular tone was measured in IMA. Globally, these results suggest that the contractions induced by noradrenaline in IMA were under control of both PGI_2 and NO. In contrast, in SV, only the increased level of PGI_2 after C3 incubation controlled the vascular tone (Figure 3B, D) and no role for NOS activity was found (Figure 6B, D).

Another difference observed between IMA and SV was that the expression of mPGES-1, COX-2 and PGE_2 release were greater in SV versus IMA (Figures 2 and 5C, D). A similar result has been described using SMC cultures derived from IMA and SV (Bishop-Bailey *et al.,* 1998). In addition, this study showed that COX-2 expression in arterial SMC, but not in venous SMC, was strongly inhibited by exogenously applied PGE_2 (Bishop-Bailey *et al.,* 1998). This negative feedback could explain the lower levels of COX-2 expression and consequently lower prostanoid productions in IMA presented in Figures 2 and 5. In our results, in spite of these greater enzymic activities in SV, inhibition or antagonism of the PGI_2 pathway (even with high doses) was without effect on SV vascular tone. Only the greater effect of mPGES-1 inhibitor on vascular tone (Supporting Information Figure S2) was associated with higher mPGES-1 expression in SV versus IMA (Figure 2C).

In conclusion; while mPGES-1 and COX-2 inhibitors have similar anti-inflammatory effect in reducing inflammation-induced PGE_2 levels, they affect PGI_2 synthesis and regulation of vascular tone in opposing ways. From our *in vitro* study, the decreased vasoreactivity to noradrenaline due to increased PGI_2 release is the predominant effect induced by mPGES-1 inhibition either in human artery or vein. This effect is predictive of the absence of cardiovascular side effects of mPGES-1 inhibition, such as the increased blood pressure observed with COX-2 inhibitors.

Acknowledgements

Author contributions

G.O. and X.N. designed the study; G.O., X.N., A.D. and L.B. performed experiments; G.O., X.N. and I.G. analysed the data; C.D. and O.T. obtained human vessel samples and recruitment of patients; P.J.J. provisioned of C3 study protocols; G.O and X.N. wrote the manuscript; I.G., A.D., P.J.J., D.L., S.U.D. and G.T. revised manuscript.

Conflict of interest

Per-Johan Jakobsson is a member of the board of NovaSAID AB. The other authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

https://doi.org/10.1111/bph.13939

Figure S1 Contractions induced by potassium chloride (KCl, 40 mM) in internal mammary artery (IMA) and saphenous vein (SV) preparations after incubation with 'Normal (18 h) or Inflammatory (18 h)' conditions.

Figure S2 The effect of mPGES-1 (microsomal prostaglandin E synthase-1) inhibitor (C3, 10 μM, 30 min) on the vasoconstriction induced by noradrenaline (NE) in internal mammary artery (IMA) and saphenous vein (SV) in 'Inflammatory (18 h)' conditions. The values are expressed as % of differences between first concentration–response curve and second concentration–response curve after C3 incubation. * $P < 0.05$, significantly different; two-way ANOVA. Values are means ± s.e.mean derived from (n) different patients.

Figure S3 The effect of mPGES-1 (microsomal prostaglandin E synthase-1) inhibitor (C3, 10 μM, 30 min) with or without thromboxane receptor (TP) antagonist (BAY u3405, 1 μM, 30 min) on vasoconstriction induced by norepinephrine (NE) in saphenous vein (SV) in 'Inflammatory (18 h)' conditions. The contraction was expressed as % of the maximal noradrenaline contraction obtained in the first concentration–response curve. * $P < 0.05$, significantly different from control; two-way ANOVA. Values are means ± SEM derived from (n) different patients.

Figure S4 The effect of mPGES-1 (microsomal prostaglandin E synthase-1) inhibitor (C3, 10 μM, 30 min) on vasoconstriction induced by U46619 (TP receptor agonist, 100 nM) in internal mammary artery under 'Inflammatory (18 h)' conditions. The contraction was expressed as % of KCl (40 mM) induced contraction. * $P < 0.05$, significantly different; Student's *t*-test. Values are means ± SEM derived from ($n = 5$) different patients.

Figure S5 The relaxation response induced by iloprost (PGI$_2$ analogue) in internal mammary artery (IMA) and saphenous vein (SV) precontracted with U46619 (TP receptor agonist, 0.1 μM) after incubation under 'Inflammatory (18 h)' conditions. Values are means ± s.e.mean derived from (n) different patients (see Table S2 for pEC$_{50}$, Emax values and statistics).

Table S1 The effect of an EP$_4$ receptor antagonist (GW627368X) on vascular contractions induced by noradrenaline in saphenous vein.

Table S2 Pharmacological values derived from relaxation-curves induced by iloprost.