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Vasoconstrictor effects of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ on human umbilical vein

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Abstract

The present study was undertaken to determine whether 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ posses contractile action on human umbilical vein and to evaluate the possible involvement of prostanoid TP receptors in this effect. Human umbilical vein rings were mounted in organ baths and concentration–response curves to 8-iso-prostaglandin E_2 or 8-iso-prostaglandin $F_{2\alpha}$ were constructed. Both isoprostanes evoked concentration-dependent contraction. 8-iso-prostaglandin E_2 (pEC_{50}=6.90±0.03) was significantly more potent than 8-iso-prostaglandin $F_{2\alpha}$ (pEC_{50}=6.10±0.04). However, both isoprostanes were equieffective. The prostanoid TP receptor antagonists, ICI-192,605 (4-(*Z*)-6-(2-*o*-Chlorophenyl-4-*o*-hydroxyphenyl-1,3-dioxan-*cis*-5-yl)hexenoic acid) and SQ-29548 (7-[3-[[2-[(phenylamino)-carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-[1*S*(1α,2α(*Z*),3α,4α)]-5-Heptenoic acid) produced a competitive rightward shift of 8-iso-prostaglandin E_2 concentration–response curves with pK_B values of 8.91±0.04 and 8.07±0.07, respectively. When ICI-192,605 (1 nM) and SQ-29548 (10 nM) were evaluated against 8-iso-prostaglandin $F_{2\alpha}$ they produced a parallel rightward displacement of 8-iso-prostaglandin $F_{2\alpha}$ concentration–response curves without affecting the maximum responses giving pA_2 values of 9.02±0.12 and 8.26±0.13, respectively. In conclusion, the present study describes for the first time the vasoconstrictor action of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ in human umbilical vein. Furthermore, the affinity values obtained with ICI-192,605 and SQ-29548 provide strong pharmacological evidence of prostanoid TP receptors involvement in this effect.

Keywords: Human umbilical vein; Isoprostane; Prostanoid TP receptor; Vasoconstriction

1. Introduction

The isoprostanes are a group of prostaglandin-like compounds produced in vivo by free radical-catalyzed peroxidation of arachidonic acid independent of cyclo-oxigenase activity (Morrow et al., 1990). Isoprostanes differ structurally from prostaglandins by the *cis*-orientation at the cyclopentane ring junction compared to the *trans*-orientation in the classical prostanoids (Morrow and Roberts, 1996).

These compounds can be quantified as reliable markers of oxidative stress in vivo (Cracowski et al., 2002). On the other hand, isoprostanes themselves are biologically active compounds and there are increasing reports of them eliciting a wide variety of responses in tissues including airway (Janssen et al., 2000), intestinal (Elmhurst et al., 1997), uterine (Crankshaw, 1995) and vascular smooth muscle (Kromer and Tippins, 1996; Zhang et al., 1996). Most of the studies of isoprostanes effects have been done in animal tissues and relatively few have been performed with human preparations (Janssen, 2001). There is strong evidence for species related differences in the responses to isoprostanes; therefore, in humans the biological effects of these compounds are already poorly understood.

The specific receptors involved in mediating the effects of isoprostanes have not been clearly defined. Many observations suggest that isoprostanes exert their effects through prostanoid receptors (Crankshaw, 1995; Elmhurst et al., 1997; Oliveira et al., 2000; Sametz et al., 2000). However, other studies proposed an activity on a unique

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isoprostane receptor (Fukunaga et al., 1993, 1997; Longmire et al., 1994).

The human umbilical vein transports the oxygenated blood from the placenta to the foetus; therefore, a normal blood flow is crucial for foetal life. Umbilical and placental vessels lack autonomic innervation (Reilly and Russell, 1977; Fox and Khong, 1990) and regulation of its vascular tone depend on the release of vasoactive substances which are locally produced or conveyed through the blood stream. Walsh et al. (2000), demonstrated that the human placenta contains, produces and secretes isoprostanes into both, maternal and foetal effluents; and that the rate of secretion is increased during preeclampsia. Therefore, isoprostanes may be relevant in the modulation of human umbilical vein tone under physiological or pathophysiological conditions.

The aim of the present study was to determine if the most widely studied isoprostanes, 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$, have contractile effects on human umbilical vein and to evaluate the possible involvement of prostanoid TP receptors in this action, taking into account that we have recently demonstrated that human umbilical vein functionally expresses these prostanoid receptors (Daray et al., 2003).

2. Materials and methods

2.1. Human umbilical vein preparations

Approximately 15–35 cm segments were excised from human umbilical cords (n=25) midway between the placenta and newborn. These cords were collected from healthy and normotensive patients after full-term vaginal or caesarean deliveries. Written informed consent was obtained from each parturient. Cords were immediately placed at 4 °C in modified Krebs solution of the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0, EDTA 0.004 and D-glucose 11. Indomethacin (30 μM) was added to the bath solution 30 min before starting the concentration–response curves in order to avoid any effect induced by the release of endogenous prostanoids. Human umbilical vein samples were placed onto dissecting dishes containing Krebs solution. The vein (internal diameter approximately 5 mm) was carefully dissected free from Warthon's jelly using microdissecting instruments. Vascular preparations with intact endothelium, confirmed by histology, were cut as rings of approximately 3 mm width. The preparations were suspended in 10 ml organ baths and stretched with a resting tension of 3–5 g defined by previous studies (Bertrand et al., 1993; Errasti et al., 1999). Passive tension was maintained and adjusted periodically throughout the equilibration period. The time from delivery until the tissue was set up in the organ baths was approximately 3 h. Changes in tension were measured with Grass isometric transducers (FT 03C, Grass Instrument, Quincy, MA, USA) and displayed on Grass polygraphs (Model 7D). During the equilibration period, Krebs solution was maintained at 37 °C and at pH 7.4 by constant bubbling with 95% O_2/5% CO_2. The bath solution was replaced every 15 min with fresh warmed Krebs. After 70 min of equilibration period, each preparation was contracted with 40 mM KCl in order to test the functional state of the tissue.

2.2. Experimental protocols

After an equilibration period of 120 min, only one concentration–response curve to the agonist was obtained per each ring. Control versus treated experiments were performed in parallel in rings from the same segment of vein. Concentration–response curves to 8-iso-prostaglandin E_2 (0.01 to 3 μM) and 8-iso-prostaglandin $F_{2\alpha}$ (0.01 to 3 μM) were obtained by cumulative addition of agonists to the organ bath in 0.25 log increments. Thromboxane A_2 mimetic, U-46619 1 μM (full prostanoid TP receptor agonist) was applied on precontracted vessels at the end of each experiment in order to determine the maximum vasoconstrictor response induced by the prostanoid TP receptors in HUV (Daray et al., 2003). To evaluate the possible involvement of prostanoid TP receptors in the effect of isoprostanes, concentration–response curves to 8-iso-prostaglandin E_2 were obtained alone (control) and in the presence of prostanoid TP receptor selective antagonists ICI-192,605 (1, 3 and 10 nM) or SQ-29548 (10, 30, 100 nM). In other series of experiments concentration–response curves 8-iso-prostaglandin $F_{2\alpha}$ were obtained in the absence (control) and in the presence of ICI-192,605 (1 nM) or SQ-29548 (10 nM). The prostanoid TP receptor antagonists were applied 30 min before the cumulative addition of agonists. The lowest concentrations used of each prostanoid TP receptor antagonist were in the range of pK_B previously described in human umbilical vein (Daray et al., 2003).

2.3. Chemicals

ICI-192,605 (4-(*Z*)-6-(2-*o*-Chlorophenyl-4-*o*-hydroxyphenyl-1,3-dioxan-*cis*-5-yl)hexenoic acid) was purchased from Tocris (Ballwin, MO, USA). U-46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$), indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] and SQ-29548 (7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-[1*S*(1α, 2α(*Z*),3α,4α)]-5-Heptenoic acid) were purchased from Biomol Research Laboratories (Plymouth Meeting, PA, USA). 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ were purchased from Cayman Chemical (Ann Arbour, MI, USA).

U-46619, indomethacin and SQ-29548 stock solutions were made up with ethanol and subsequent dilutions were prepared in bidistilled water. ICI-192,605, 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ were dissolved with dimethyl sulphoxide (DMSO) to give stock solutions which

were further diluted with bidistilled water. All stock solutions were stored frozen in aliquots, thawed and diluted daily. All concentrations of drugs are expressed as a final concentration in the organ bath. Control experiments in the presence of corresponding concentrations of ethanol and DMSO were performed in order to rule out any possible non-specific action of these solvents on tonus or contractility of the preparation.

2.4. Data analysis and statistics

All data are presented as means±S.E.M. The number (n) of rings and veins is denoted: number rings/number veins, where each vein is obtained from a different umbilical cord and typically four or eight rings of each vein were employed. The responses are expressed as g of developed isometric contraction.

The concentration–response curves were fitted to a four-parameter logistic model, where estimates of EC_{50} value, the agonist concentration that produces 50% of the maximum, and the slope factor (n_H) were obtained using ALLFIT (DeLean et al., 1978). The EC_{50} values were transformed into pEC_{50} ($-\log EC_{50}$). Agonist log concentration ratio (r) was determined by subtracting the pEC_{50} value of the agonist in the presence of the antagonist from the pEC_{50} in control preparation. When the criteria for competitive antagonism were satisfied, that is the antagonist produced a parallel rightward shift of the agonist curve without attenuation in the maximum response over a wide range of concentrations (1–2 log units), antagonist pA_2 values and slope of Schild regressions were calculated as described by Arunlakshana and Schild (1959). In those cases, where the slope of Schild's plot was no significantly different from the unity, the regression was recalculated with Schild's slope constrained to unity and the affinity value obtained was then referred as pK_B (Jenkinson et al., 1995). In the cases of ICI-192,605 (1 nM) or SQ-29548 (10 nM) versus 8-iso-prostaglandin $F_{2\alpha}$, antagonist affinities were obtained by "single concentration" analysis (assuming a Schild's regression slope of 1) according to the equation: $pA_2 = -\log[\text{Antagonist}] + \log(r-1)$ (Lachnit et al., 1997; Rogines-Velo et al., 2002). Statistical analysis was performed by means of unpaired Student's t-test. P-values lower than 0.05 were taken to indicate significant differences between means. Terms and equations are as recom-

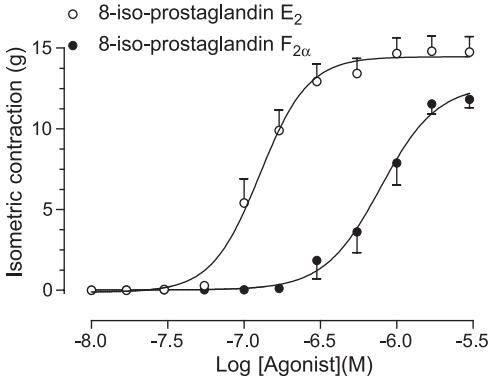

Fig. 1. Vasoconstrictor effects of isoprostanes on human umbilical vein. Concentration–response curves to 8-iso-prostaglandin E_2 (n=15/15) and 8-iso-prostaglandin $F_{2\alpha}$ (n=13/13). Each symbol represents the mean and vertical lines represent S.E.M.

Table 1
Summary of pEC_{50} values of 8-iso-prostaglandin E_2 or 8-iso-prostaglandin $F_{2\alpha}$ in different human vessels

Human vessel	8-iso-prostaglandin E_2	8-iso-prostaglandin $F_{2\alpha}$
Bronchial artery[a]	6.8±0.2	6.5±0.1
Internal mammary artery[b]	7.03±0.1	6.12±0.2
Pulmonary artery[c]	6.3±0.2	5.9±0.5
Umbilical artery[d]	6.5±0.1	5.8±0.2
Pulmonary vein[c]	6.3±0.3	5.4±0.4
Saphenous vein[e]		6.31±0.12
Umbilical vein[f]	6.90±0.03	6.10±0.04*

[a] Tazzeo et al., 2003.
[b] Cracowski et al., 2001.
[c] Janssen et al., 2001.
[d] Oliveira et al., 2000.
[e] Gardan et al., 2000.
[f] Data obtained from the present study.
* Significantly different from the corresponding value of 8-iso-prostaglandin E_2 (P<0.05).

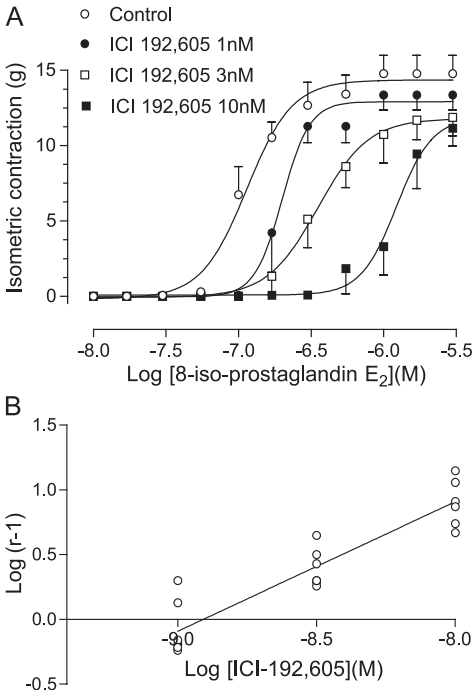

Fig. 2. Antagonism of 8-iso-prostaglandin E_2 by ICI-192,605 in human umbilical vein. (A) Concentration–response curves to 8-iso-prostaglandin E_2 on control rings (n=10/10) and on tissues previously exposed to ICI-192,605 (1 nM, n=6/6; 3 nM, n=7/7; 10 nM, n=6/6). Each symbol represents the mean and vertical lines represent S.E.M. (B) Schild's plot for ICI-192,605 versus 8-iso-prostaglandin E_2 was constructed with concentration-ratios from individual experiments. The slope parameter was found to be not significantly different from unity and it was subsequently constrained to unity to estimate a pK_B of 8.91±0.04 (n=19/10).

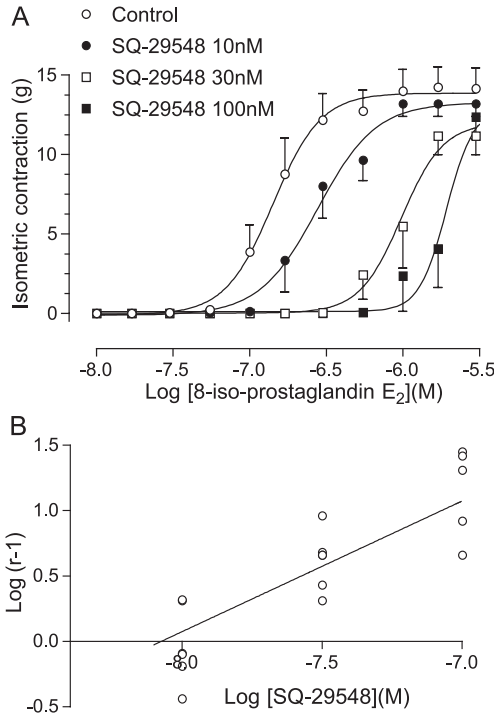

Fig. 3. Antagonism of 8-iso-prostaglandin E_2 by SQ-29548 in human umbilical vein. (A) Concentration–response curves to 8-iso-prostaglandin E_2 on control rings (n=8/8) and on tissues previously exposed to SQ-29548 (10 nM, n=6/6; 30 nM, n=6/6; 100 nM, n=5/5). Each symbol represents the mean and vertical lines represent S.E.M. (B) Schild's plot for SQ-29548 versus 8-iso-prostaglandin E_2 was constructed with concentration-ratios from individual experiments. The slope parameter was found to be not significantly different from unity and it was subsequently constrained to unity to estimate a pK_B of 8.07±0.07 (n=17/8).

mended by the IUPHAR Committee on Receptor Nomenclature and Drug Classification (Jenkinson et al., 1995).

3. Results

3.1. Contractile responses to isoprostanes in human umbilical vein

The isoprostanes 8-iso-prostaglandin E_2 (n=15/15) and 8-iso-prostaglandin $F_{2\alpha}$ (n=13/13) evoked concentration-dependent contraction in human umbilical vein (Fig. 1). 8-iso-prostaglandin E_2 was significantly more potent than 8-iso-prostaglandin $F_{2\alpha}$ in this tissue (Fig. 1, Table 1). Both isoprostanes were, however, equieffective (8-iso-prostaglandin E_2, 14.76±0.95 g and 8-iso-prostaglandin $F_{2\alpha}$, 12.12±0.53 g). Moreover, the contractile responses elicited by the isoprostanes represented the 90% and 81% of the maximal response to U-46619 (1 μM) applied at the end of the concentration–response curves (16.36±0.89 and 14.97±0.74 g, respectively). The slopes of the concentration response curves obtained for 8-iso-prostaglandin E_2 (n_H=2.74±0.44) and 8-iso-prostaglandin $F_{2\alpha}$ (n_H=2.35±0.42) were nearly identical.

3.2. Sensitivity of isoprostane vasoconstrictor responses to prostanoid TP receptor antagonists in human umbilical vein

To determine whether prostanoid TP receptors are involved in the vasoconstrictor effects of both isoprostanes on human umbilical vein, contractile responses were obtained in the presense of two well known prostanoid TP selective and competitive antagonists, ICI-192,605 and SQ-29548.

ICI-192,605 produced a parallel rightward shift of 8-iso-prostaglandin E_2 concentration–response curve without affecting the maximum response (Control, 14.79±1.21 g; 1 nM, 13.37±1.01 g; 3 nM, 11.87±1.28 g; 10 nM, 11.17±1.19 g) indicative of competitive antagonism (Fig. 2A). Analysis of the data by Schild regression gave a slope (0.96±0.10) which was no significantly different from unity and a pA_2 value of 8.93 yielding a pK_B value from constrained Schild plots of 8.91±0.04 (n=19/10, Fig. 2B). Furthermore, increasing concentrations of SQ-29548 produced a rightward shift of 8-iso-prostaglandin E_2 concentration–response curves without modifying the maximum response (Control, 14.14±1.31 g; 10 nM, 13.18±0.78 g; 30 nM, 11.17±1.18 g; 100 nM, 12.35±1.37 g) indicative of competitive antagonism (Fig. 3A). Data analysed by Schild regression gave a slope (1.19±0.17) which was no significantly different from unity and a pA_2 value of 7.99

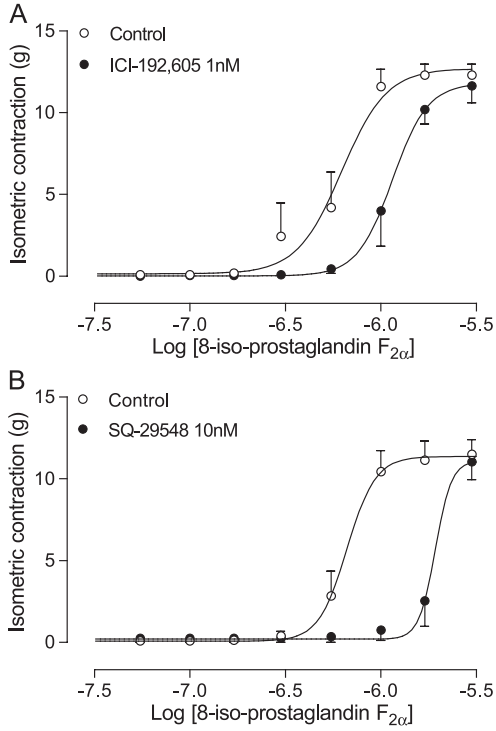

Fig. 4. Antagonism of 8-iso-prostaglandin $F_{2\alpha}$ by ICI-192,605 or SQ-29548 in human umbilical vein. (A) Concentration–response curves to 8-iso-prostaglandin $F_{2\alpha}$ in the presence of ICI-192,605 (1 nM, n=5/5). (B) Concentration–response curves to 8-iso-prostaglandin $F_{2\alpha}$ in the presence of SQ-29548 (10 nM, n=5/5). Each symbol represents the mean and vertical lines represent S.E.M.

yielding a pK_B value from constrained Schild plots of 8.07±0.07 (n=17/8, Fig. 3B).

In other series of experiments the effect of both prostanoid TP receptor selective antagonists against 8-iso-prostaglandin $F_{2\alpha}$ was tested. ICI-192,605 (1 nM) produced a parallel rightward displacement of 8-iso-prostaglandin $F_{2\alpha}$ concentration–response curves without affecting the maximum responses (Control, 12.30±1.18 g; treated, 11.65±1.05 g) giving a calculated pA_2 of 9.02±0.12 (n=5/5, Fig. 4A). When 8-iso-prostaglandin $F_{2\alpha}$ concentration–response curves were treated with SQ-29548 (10 nM) a competitive rightward shift without attenuation of the maximum response was observed (Control, 11.50±0.89 g; treated, 11.05±1.10 g) and the calculated pA_2 value was 8.26±0.13 (n=5/5, Fig. 4B).

4. Discussion

Isoprostanes are a complex family of prostaglandin-like compounds produced in vivo from arachidonic acid via a free-radical-catalyzed non-enzymatic peroxidation (Morrow et al., 1990). They have been used clinically and experimentally as markers for many disease states in which oxidative stress is a prominent feature, including atherosclerosis (Pratico et al., 1997), hypercholesterolemia (Oguogho et al., 1999), myocardial and renal ischemia–reperfusion injury (Takahashi et al., 1992; Mobert and Becker, 1998) and preeclampsia (McKinney et al., 2000). Furthermore, some isoprostanes posses biological activity mediating nociception, broncoconstriction and vasoconstriction (Cracowski et al., 2002). The vasoconstrictor effect of several isoprostanes has been described in some human vessels, being 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ the most potent and efficacious compounds studied (Oliveira et al., 2000; Cracowski et al., 2001; Janssen et al., 2001; Tazzeo et al., 2003). Therefore, we decided to focus on both isoprostanes the study of the possible vasoconstrictor effect in human umbilical vein.

In the present study, both isoprostanes caused concentration-dependent contraction of human umbilical vein. The pEC_{50} values estimated for 8-iso-prostaglandin E_2 (6.90±0.03) and for 8-iso-prostaglandin $F_{2\alpha}$ (6.10±0.04) were consistent with previous reports indicating greater potency of 8-iso-prostaglandin E_2 compared with 8-iso-prostaglandin $F_{2\alpha}$ in human vascular tissues (Table 1). On the other hand, unresponsiveness to isoprostanes has been reported in some animal vessels like ovine coronary (Kromer and Tippins, 1996) and canine pulmonary arteries (Janssen et al., 2001). Moreover, in human umbilical vein both isoprostanes are equieffective in accordance with previous results in other human vascular preparations such as umbilical artery (Oliveira et al., 2000); internal mammary artery (Cracowski et al., 2001); pulmonary vein (Janssen et al., 2001) and bronchial artery (Tazzeo et al., 2003). However, in rat pial arterioles the effect induced by 8-iso-prostaglandin $F_{2\alpha}$ was higher than 8-iso-prostaglandin E_2 (Hoffman et al., 1997). Furthermore, isoprostanes maximal contractile effects in human umbilical vein represent the 90% (8-iso-prostaglandin E_2) and 81% (8-iso-prostaglandin $F_{2\alpha}$) of U-46619 (prostanoid TP receptor full agonist) maximal response. Therefore, in this tissue they act practically as full agonists in contrast with their activity as partial agonists in guinea pig aorta (Zhang et al., 1996) and porcine and bovine coronary arteries (Kromer and Tippins, 1996). Altogether these data support the view that isoprostanes vascular effects depend both on the vessel and species studied (Cracowski et al., 2000; 2001).

McKinney et al. (2000) have previously reported plasma free 8-iso-prostaglandin $F_{2\alpha}$ concentration values of 150±11 pg/ml (4.23×10^{-10} M) in maternal circulation during normotensive pregnancies and a significative increase in this values to 342±50 pg/ml (9.65×10^{-10} M) in women with severe preeclampsia. Preeclampsia is associated with increased activation and generation of oxygen-free radicals by circulating leukocytes (McKinney et al., 2000). Moreover, Walsh et al. (2000) have demonstrated that human placenta contains, produces and releases 8-iso-prostaglandin $F_{2\alpha}$ and that the production rate is increased in preeclampsia suggesting that the raise in circulating levels of this isoprostane could be originated from the placenta. The plasma concentration of 8-iso-prostaglandin $F_{2\alpha}$ in normal pregnancies and in women with severe preeclampsia are markedly lower to the potency estimated in the present study ($CE_{50}=7.9\times10^{-7}$) but the concentration at the site of free radical injury might be sufficiently high to induce regional vasoconstriction (Cracowski et al., 2002). To our knowledge, no data have been already published concerning the plasma levels of 8-iso-prostaglandin E_2 during pregnancy, but taking into account that in the present study it is almost 1 logarithmic unit more potent that 8-iso-prostaglandin $F_{2\alpha}$ this isoprostane could be more relevant as a possible endogenous vasoconstrictor agent. Therefore, further studies should be focused in quantifying the plasma levels of 8-iso-prostaglandin E_2 in systemic circulation and in blood from the umbilical cord under physiological or pathophysiological conditions as preeclampsia.

It has been demonstrated that the excitatory effects of 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ are sensitive to a wide variety of agents that are structurally distinct but exhibit prostanoid TP receptor blocking activity, including ICI-192,605 (Crankshaw, 1995; Janssen et al., 2000; 2001; Sametz et al., 2000; Tazzeo et al., 2003) and SQ-29548 (Crankshaw, 1995; Kromer and Tippins, 1996; Zhang et al., 1996; Hoffman et al., 1997). It has been debated whether this indicates activity at the classic prostanoid TP receptors or on a unique isoprostane receptor that bears some similarity to the prostanoid TP receptor (Janssen, 2001). In human umbilical vein, SQ-29548 and ICI-192,605 produced a dose-dependent rightward displacement of 8-iso-prostaglandin E_2 concentra-

Table 2
Antagonist potency of SQ-29548 and ICI-192,605 versus U-46619, 8-iso-prostaglandin E_2 or 8-iso-prostaglandin $F_{2\alpha}$ in human umbilical vein

Agonist	Antagonist	
	ICI-192,605	SQ-29548
U-46619[a]	9.07±0.07[c]	7.96±0.09[c]
8-iso-prostaglandin E_2[b]	8.91±0.04[c]	8.07±0.07[c]
8-iso-prostaglandin $F_{2\alpha}$[b]	9.02±0.12[d]	8.26±0.13[d]

[a] Daray et al., 2003.
[b] data obtained from the present study.
[c] pK_B values.
[d] pA_2 values.

tion–response curves fulfilling the criteria of competitive antagonism. Moreover, the pK_B values estimated for the selective prostanoid TP receptor antagonists ICI-192,605 (8.91±0.04) and SQ-29548 (8.07±0.07) versus 8-iso-prostaglandin E_2 are almost identical to those previously estimated against U-46619 under the same experimental conditions in human umbilical vein (Table 2). Although our group have recently described that human umbilical vein functionally expresses not only prostanoid TP receptors but also prostanoid FP receptors (Daray et al., 2003), the fact that the Schild plot slope for both prostanoid TP receptor antagonists was not different from unity suggest that 8-iso-prostaglandin E_2 is acting through an homogeneous receptor population (Kenakin, 1982). When both prostanoid TP receptor antagonists against 8-iso-prostaglandin $F_{2\alpha}$ were used, due to the lower potency of the agonist, just one concentration of the antagonist was tested and therefore a pA_2 calculated was obtained (see Material and methods). Again, the pA_2 values for ICI-192,605 (9.02±0.12) and SQ-29548 (8.26±0.13) were in accordance with the affinity values estimated against U-46619 in human umbilical vein (Table 2). Moreover, the pK_B and pA_2 values estimated for both antagonists versus 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ are in the same range as those determined in other human smooth muscle preparations which expresses functional prostanoid TP receptors: 8.1–9.2 for ICI-192,605 (umbilical artery, Boersma et al., 1999, and nonpregnant myometrium, Senchyna and Crankshaw, 1996, respectively) and 7.6–9.1 for SQ-29548 (umbilical artery, Boersma et al., 1999, and penile resistance artery, Angulo et al., 2002, respectively). In conclusion, the affinity values estimated with the prostanoid TP receptor antagonists versus 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ provide strong pharmacological evidence that both isoprostanes mediate vasoconstriction through prostanoid TP receptors in human umbilical vein. If a novel class of isoprostane receptors exists we are not able to discriminate them with these pharmacological tools.

In summary, the present study describes for the first time the biological activity of two endogenous isoprostanes, 8-iso-prostaglandin E_2 and 8-iso-prostaglandin $F_{2\alpha}$ in human umbilical vein, inducing vasoconstriction. Furthermore, the findings obtained with the TP receptor selective antagonists provide clear evidence of the involvement of prostanoid TP receptors mediating the vasoconstriction induced by isoprostanes in human umbilical vein.

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