Vascular effects of pentoxifylline; towards a novel therapeutic option for preeclampsia

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ABSTRACT

Recently, the non-selective phosphodiesterase inhibitor pentoxifylline (PTX) has gained interest as a possible therapeutic that could target both the generalized endothelial dysfunction and the immune system imbalance in preeclampsia (PE). However not much is known about its effects on the placenta. Therefore, in this study the vascular effects of PTX were evaluated. PTX concentration-response curves after incubation with SQ22536 or L-NAME were constructed in porcine coronary arteries and human chorionic plate arteries of both healthy and PE placentas. The effect of PTX-incubation on vasodilation through the cGMP - and cAMP pathways was studied in segments of the same vessels using the endothelium-independent NO donor sodium nitroprusside and the adenylyl cyclase activator forskolin, respectively. PTX exerted direct vasodilator effects on both porcine coronaries and human chorionic plate arteries, that could be blocked by L-NAME, indicating that this effect is mainly NO/cGMP-mediated. This dilator effect was increased in arteries of PE placentas. PTX enhanced the vasodilator effects of sodium nitroprusside and forskolin in chorionic plate arteries derived from healthy, but not PE placentas. Since PTX induced direct vasodilation in chorionic plate arteries, and even to a greater extent in PE, it could be a beneficial treatment option to improve placental perfusion. As a next step placental transfer of PTX should be studied.

INTRODUCTION

Preeclampsia (PE) is a serious placenta-related pregnancy disorder, affecting approximately 5-8% of all pregnancies.[1] It is characterized by hypertension with an onset after 20 weeks of gestation, accompanied by evidence of maternal organ damage (e.g. proteinuria, elevated liver enzymes, pulmonary - or cerebral edema) and/or fetal growth restriction.[2] Early onset PE, i.e. diagnosis before the 34th week of gestation,[3] is not only associated with an increased risk of maternal and fetal complications during pregnancy, but it can also lead to health problems later in life for both mother and child.[1, 4, 5] Different pathophysiological mechanisms have been proposed for early onset PE, all involving impaired placentation in early pregnancy, leading to increased vascular resistance, generalized endothelial dysfunction and endovascular inflammation. Previous studies showed that PE patients have increased plasma levels of pro-inflammatory cytokines, such as interleukin(IL)-6, interferon(IFN)-γ and tumor necrosis factor(TNF)-α. On the other hand, the anti-inflammatory cytokines IL-5 and IL-10 seem to be suppressed.[6, 7] Furthermore, increased concentrations of pro-inflammatory cytokines have been found in placental tissue of PE patients compared to healthy controls.[8] This imbalance in the immune response attributes to the impaired placentation, endothelial damage and ischemic-reperfusion injury seen in PE.[9-11] It would therefore be very interesting to target both the generalized endothelial dysfunction and the immune system imbalance to treat early onset PE, for example by using the methylxanthine derivative pentoxifylline (PTX). PTX is a non-selective phosphodiesterase (PDE) inhibitor, that increases the intracellular concentration of cAMP.[12] It has been shown to have anti-inflammatory properties, scavenge oxygen radicals, improve endothelial function, induce vasodilation, increase erythrocyte flexibility and inhibit platelet aggregation.[13, 14] Although currently only registered for intermittent claudication, PTX has been suggested as a possible preventive or therapeutic intervention for inflammatory placental diseases such as PE and preterm birth.[15, 16] Previously, Speer *et al*. showed that PTX reduces inflammation in placental explants.[12] Furthermore, it has been indicated that PTX has a beneficial effect on the fetoplacental circulation in patients with imminent preterm labor.[16] However, the mechanisms behind these vascular effects are not well understood. Therefore, the aim of this study was to evaluate the vascular effects of PTX, using both porcine coronary arteries as well as human chorionic plate arteries of healthy and PE placentas.

METHODS

Tissue collection

Placentas of women with uncomplicated singleton pregnancies who underwent an elective cesarean section were collected immediately after delivery at the Erasmus MC

University Medical Center, Rotterdam, the Netherlands. Also, placentas of women with early onset PE (diagnosis before the 34th week of gestation) were collected. The study was exempted from approval by the local institutional Medical Ethics Committee according to the Dutch medical Research with Human Subjects Law (MEC-2016-418 and MEC-2017-418), and all patients gave written consent to use of their placenta and data prior to the experiments. Additionally, porcine hearts were collected from the slaughterhouse.

Wire-myography experiments with porcine coronary arteries

Coronary arteries were dissected from the hearts and stored overnight in cold, oxygenated Krebs-Henseleit buffer (in mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4). Vessel segments of 4 mm were suspended on stainless steel hooks in 15-ml organ baths, filled with Krebs-Henseleit buffer at 37°C and aerated with 95% O_2 – 5% CO_2. After a period of equilibration, vessel segments, stretched to a stable force of about 15 mN, were exposed to 30 mmol/l KCl twice. Subsequently, maximum contractile responses were determined using 100 mmol/l KCl. After washout of the KCl, segments were pre-incubated for 30 min in the absence or presence of one of the following inhibitors: the adenylate cyclase inhibitor SQ22536 (100 µmol/l), the NOS-inhibitor L-NAME (100 µmol/l) or PTX (100 µmol/l). All vessel segments were then pre-constricted using the thromboxane A2 agonist U46619 (1 µmol/l). Segments that were pre-incubated with SQ22536 or L-NAME were used to construct concentration-response curves to PTX (1 nmol/l - 300 µmol/l), and segments that were pre-incubated with PTX were exposed to sodium nitroprusside (SNP, 1 nmol/l - 100 µmol/l) or forskolin (1 nmol/l - 30 µmol/l). Vessel segments without any additives were used as a control for all concentration-response curves. Changes in tissue contractile force were recorded with a Harvard isometric transducer (South Natick, MA, USA).

Wire-myography experiments with human chorionic plate arteries

Segments of second order branches of chorionic plate arteries (2 mm in length) were mounted in 6-ml organ baths (Danish Myograph Technology, Aarhus, Denmark), filled with Krebs-Henseleit buffer at 37°C and aerated with 95% O_2 – 5% CO_2. Tension was normalized to 90% of the estimated diameter at 38 mmHg effective transmural pressure to mimic the physiological circumstances of placental vessels. Maximum contractile responses were determined using 100 mmol/l KCl. Experimental protocols were similar to those used for the porcine coronary arteries. After washout of KCl, all vessel segments were pre-constricted with U46619 (10 nmol/l). Concentration-response curves for PTX (1 nmol/l - 300 µmol/l) were performed in a subset of segments, that had been pre-incubated for 30 min with either SQ22536 (100 µmol/l), L-NAME (100 µmol/l) or both. Again vessel segments without any additives were used as a control. Concentration-response curves for SNP (1 nmol/l - 100 µmol/l) and forskolin (1 nmol/l - 30 µmol/l) were constructed in the presence or absence of PTX (100 µmol/l).

Chemicals

PTX (Trental®) was acquired from the hospital pharmacy of Erasmus MC, Rotterdam, the Netherlands. All other compounds came from Sigma-Aldrich, Schnelldorf, Germany.

Data analysis

Data are presented as mean±SEM. Vascular responses are expressed as a percentage of the contractile response to 100 mmol/l KCl or U46619. Log10-transformed values at which the half-maximal response (pEC_{50}) occurred were individually estimated using sigmoid curve fitting software (GraphPad Prism 5, La Jolla, CA, USA). In case of normally distributed data, groups were analyzed using 1-way ANOVA with Bonferroni post-hoc evaluation. When 1-way ANOVA was significant, individual comparisons were made with Student's *t* test. A p-value of <0.05 was considered statistically significant.

RESULTS

Porcine coronaries

Figure 1A shows the concentration-response curves to PTX in porcine coronary arteries. The maximum relaxation (E_{max}) induced by PTX was 68±5% of U46619 pre-constriction, with a pEC_{50} of -4.2±0.1. Incubation with L-NAME or SQ22536 reduced E_{max} to 30±9% and 55±7% respectively, however this was only significant for L-NAME (p=0.003, Table 1). Incubation with either antagonist shifted the PTX curve to the left, evidenced by a decrease in pEC_{50} (p=0.05, Table 1). Incubation with PTX tended to shift the SNP (p=0.09) and forskolin (p=0.06) curves to the left, without altering E_{max} (Figures 1B and 1C and Table 1).

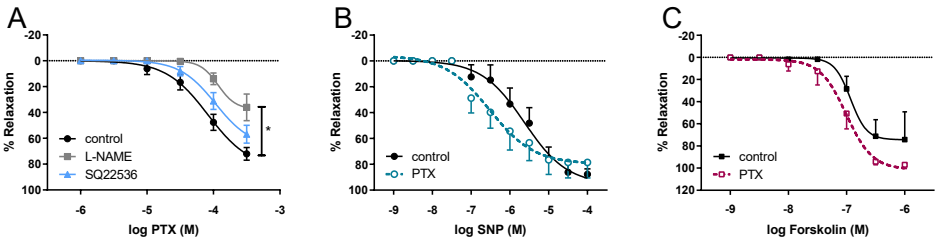

Figure 1. Results of the wire-myography experiments using porcine coronary arteries. Panel A shows the concentration-response curves to pentoxifylline (PTX) in control segments (circles) or after incubation with SQ22536 (triangles) or L-NAME (squares). Panel B shows the concentration-response curves to sodium nitroprusside (SNP) in control segments (closed circles) or after incubation with of 100 µmol/l PTX (open circles). Panel C shows the concentration-response curves to forskolin in control segments (closed squares) or after incubation with 100 µmol/l PTX (open squares). Data are mean±SEM (n=5-9). *p<0.01, 1-way ANOVA for repeated measures with Bonferroni post-hoc evaluation.

Table 1. Organ bath experiments with porcine coronaries.

			Incubation	
CRC	**Control**	**SQ22536**	**L-NAME**	**PTX**
pEC_{50}				
PTX	-4.2±0.1	-4.0±0.1	-3.9±0.1*	
SNP	-5.7±0.3			-6.4±0.3
Forskolin	-6.6±0.2			-7.1±0.2
E_{max}				
PTX	68±5	55±8	30±9**	
SNP	91±4			79±12
Forskolin	99±3			98±2

pEC_{50}: log10-transformed concentrations at which the half-maximum response occurred. E_{max}: maximum effect expressed as % relaxation of U46619 constriction. *p<0.05 and **p<0.01 compared to control, 1-way ANOVA for repeated measures with Bonferroni post-hoc evaluation.

Human chorionic plate arteries

The concentration-response curves to PTX, SNP and forskolin in human chorionic plate arteries are shown in Figure 2. In chorionic plate arteries of healthy placentas, PTX induced an E_{max} of 74±8% of U46619 pre-constriction, with a pEC_{50} of -4.4±0.1 (Table 2). Incubation with L-NAME, SQ22536, or both did not affect E_{max} (Table 2, Figure 2A), but L-NAME with or without SQ22536 did shift the PTX curve to the left (p=0.02 and p=0.04, respectively). Incubation with PTX potentiated both SNP and forskolin, evidenced by a decreased pEC_{50} (p=0.002 and p=0.008, respectively, Figure 2B and 2C, Table 2). In chorionic plate arteries of PE placentas, the E_{max} induced by PTX tended to be larger than in healthy placentas (p=0.07, Table 3). None of the antagonists altered the pEC_{50} of

Table 2. Organ bath experiments with human chorionic plate arteries of healthy placentas.

			Incubation		
CRC	**Control**	**SQ22536**	**L-NAME**	**SQ22536 + L-NAME**	**PTX**
pEC_{50}					
PTX	-4.4±0.1	4.3±0.1	-4.1±0.1*	4.1±0.1*	
SNP	-7.9±0.1				-8.7±0.2**
Forskolin	-5.8±0.2				-6.6±0.1**
E_{max}					
PTX	74±8	64±10	59±9	53±10	
SNP	90±6				90±4
Forskolin	88±8				113±10

pEC_{50}: log10-transformed concentrations at which the half-maximum response occurred. E_{max}: maximum effect expressed as % relaxation of U46619 constriction. *p<0.05 and **p<0.01 compared to control, Student's *t* test.

PTX in PE vessels (Figure 2D, Table 3). Furthermore, PTX did not alter the response to SNP and forskolin in PE arteries (Figure 2E and 2F, respectively).

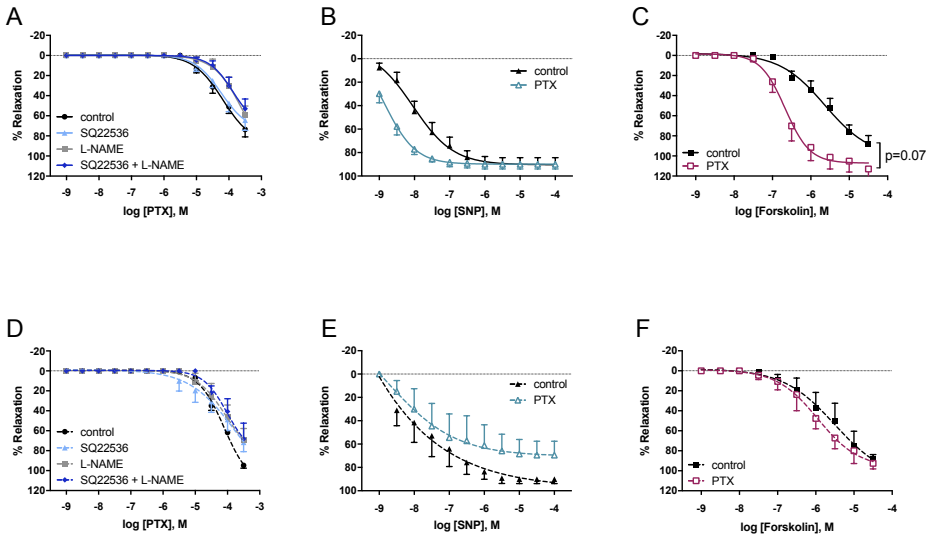

Figure 2. Results of the wire-myography experiments using human chorionic plate arteries. The upper part of the figure (Panels A, B and C) represent the results of chorionic plate arteries of healthy placentas (solid lines, n=6-10) and the lower part (Panels D, E and F) the results of chorionic plate arteries of preeclamptic placentas (dashed lines, n=3-5). Panels A and D show the concentration-response curves to pentoxifylline (PTX) in control segments (circles) or after incubation with SQ22536 (triangles), L-NAME (squares) or both (diamonds). Panels B and E show the concentration-response curves to sodium nitroprusside (SNP) in control segments (closed triangles) or after incubation with of 100 µmol/l PTX (open triangles). Panels C and F show the concentration-response curves to forskolin in control segments (closed squares) or after incubation with 100 µmol/l PTX (open squares). Data are mean±SEM.

Table 3. Organ bath experiments with human chorionic plate arteries of preeclamptic placentas.

		Incubation			
CRC	**Control**	**SQ22536**	**L-NAME**	**SQ22536 + L-NAME**	**PTX**
pEC_{50}					
PTX	-4.1±0.2	-4.0±0.2	-4.1±0.3	-4.0±0.1	
SNP	-7.9±0.5				-7.5±0.6
Forskolin	-5.8±0.3				-5.6±0.6
E_{max}					
PTX	95±3	72±9	69±11	68±16	
SNP	90±4				70±12
Forskolin	89±5				93±6

pEC_{50}: log10-transformed concentrations at which the half-maximum response occurred. E_{max}: maximum effect expressed as % relaxation of U46619 constriction. *p<0.05 and **p<0.01 compared to control, Student's *t* test.

DISCUSSION

This study shows that PTX has direct vasodilator effects on both porcine coronaries and human chorionic plate arteries, and that these effects can be blocked by L-NAME. Besides directly inducing vasodilation, PTX also enhanced the vasodilator effects of the NO donor SNP and the adenylate cyclase activator forskolin in chorionic plate arteries derived from healthy, but not PE placentas.

In line with previously reported effects of PTX on for example rat mesenteric arteries and rabbit aorta,[18, 19] PTX evoked concentration-dependent relaxation in the current study. Similar to the results of Hansen *et al.*[19], who showed inhibition of the PTX response by L-NAME in rat mesenteric vessels, we saw a blocking effect on PTX response by L-NAME in porcine coronary arteries and human chorionic plate arteries. The fact that incubation with the adenylate cyclase inhibitor SQ22536 did not result in similar blocking effects indicates that, at least in these vessels, the acute dilator PTX response is mainly cGMP-mediated. PTX did enhance the vasodilator effects of both SNP and forskolin in healthy chorionic plate arteries, confirming that it affects both cGMP and cAMP degradation. In contrast, no such potentiation was seen in the rabbit aorta,[18] emphasizing the difficulties of translating results from animal studies to humans.

PTX tended to induce stronger relaxant effects in PE arteries, yet was unable to potentiate forskolin or SNP in these arteries. The latter is reminiscent of what was observed for the PDE inhibitors sildenafil and vinpocetine in PE arteries,[17] and suggest that the larger relaxant effects of PTX are unrelated to PDE inhibition. Hence, PTX might still be a beneficial treatment option to improve placental perfusion, acting via a non-PDE-dependent mechanism.

Besides its vasodilator properties, PTX has been shown to reduce inflammation. In placental explants with LPS-induced inflammation, PTX reduced expression and production of the pro-inflammatory cytokines TNF-α, IFN-γ and IL-1β.[12] It could therefore be speculated that PTX is a good candidate to treat placental inflammatory conditions *in vivo*. PTX is already studied as anti-inflammatory treatment in preterm born infants with suspected late-onset sepsis or necrotizing enterocolitis, with promising results.[20, 21]

Before starting a clinical trial in pregnancy, it is essential to gain knowledge on the placental transfer of PTX. No teratogenic effects of PTX have been described in animal studies, and clinical trials with PTX as treatment to prevent preterm labor did not report negative effects, although no long-term follow-up has been performed.[16] Furthermore, in preterm infants PTX treatment was well tolerated without significant adverse effects.[20]

CONCLUSION

Since it has vasodilator and anti-inflammatory properties, PTX is a very interesting drug that could target both the generalized endothelial dysfunction and the immune system imbalance seen in PE. As a next step we suggest to study placental transfer of PTX in both healthy and (preterm) preeclamptic placentas. Furthermore, we should expand our knowledge on the anti-inflammatory effects of PTX in the PE placenta, using placental explants and/or trophoblast cell culture. With this, possible differences between early – and late onset PE, with or without fetal growth restriction should be kept in mind. Furthermore, a pharmacokinetic model of PTX in pregnant women should be made before starting a clinical trial.

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