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Predominant basal directional release of thromboxane, but not prostacyclin, by placental trophoblasts from normal and preeclamptic pregnancies

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Abstract

Objective—To investigate apical and basal releases of thromboxane (TX) and prostacyclin (PGI₂) by trophoblasts (TCs) from normal and preeclamptic (PE) placentas.

Methods—TCs isolated from normal and PE placentas were incubated in cell culture inserts for 48hrs. Medium from the upper (apical) and the lower (basal) chambers were then collected separately and measured for TX and PGI₂ by their stable metabolites of TXB₂ and 6-keto PGF_{1α} by ELISA. Apical and basal releases of TX and PGI were also examined with apical exposure of TCs to arachidonic acid (AA)+/-aspirin at different concentrations. Villous tissue expressions for PGI synthase, TX synthase and TX (TP) receptor were examined by immunohistochemistry.

Results—1) TXB₂, but not 6-keto PGF_{1α}, concentrations were significantly higher in the lower than in the upper chambers with both normal and PE TCs (p<0.01); 2) apical exposure of TCs to AA resulted in a significant increase in TX releases towards both the upper and the lower chambers in normal TCs (p<0.01), but only a significant increase in the upper chamber in PE TCs (p < 0.01); 3) aspirin could attenuate AA-induced TX release both in the upper and the lower chambers in normal, but not in PE, TCs (p<0.01), respectively; 4) there were no differences in 6-keto PGF_{1α} productions both in normal and PE TCs treated with AA +/- aspirin; 5) intense staining of TX synthase and TP receptor was seen in syncytiotrophoblast layer, villous core vessels and stromal cells in preeclamptic placental tissue sections.

Conclusion—Predominant basal release of TX together with intense staining of TX synthase and TP receptor in trophoblasts, stromal cells and villous core vessels are found in placentas from PE. We speculate if predominant basal release of TX by TCs occurs *in vivo* as we found in our *in vitro* culture condition, basal released TX may play a significant role in increased placental vasoconstriction such as in PE.

Keywords

Trophoblasts; apical and basal directions; thromboxane; prostacyclin; preeclampsia

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Introduction

Placental trophoblasts are an important bio-interface between the maternal and the fetal compartments during pregnancy. The syncytio-layer of trophoblasts has a unique haemochorial structure: the apical surface of trophoblasts is bathed in the maternal blood in the intervillous space and directly exposed to all components that are present in the maternal circulation, while the basal membrane is opposed to the villous stroma and the fetal vasculature and plays important roles in transferring maternal-derived substances across the placental barrier and controlling trophoblast products released into the fetal compartment.

Emerging evidence has shown that trophoblasts are polarized epithelial cells with distinct functional regulations between the microvillous and the basal membranes (1-4). For example, lactate produced by trophoblasts is a source of energy fuel to the fetus. A study of trophoblast lactate transporter activities/expressions by Settle et al (3) revealed differential localizations of lactate transporter subtypes on microvillous and basal membranes, which corresponded to their roles in the lactate production, lactate release to and lactic acid removal from the fetal circulation (3). Na⁺/H⁺ exchangers also display a differential distribution across the microvillous and basal membranes (4). Differential releases of matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) by syncytiotrophoblasts in culture was reported by Sawicki et al (1). These authors found that more than 90% of MMP-2 and MMP-9 were released towards the basal direction than towards the apical surface by cultured syncytiotrophoblasts (1). MMPs play an important role in tissue remodeling. The finding of dominant basal release of MMPs implicates that the differential directional release of MMPs by placental trophoblasts may have a significant impact on vascular remodeling and angiogenesis during placental development.

Placental trophoblasts produce a spectrum of vasoconstrictors and vasodilators including thromboxane (TX), angiotensin II (Ang II), endothelin, prostacyclin (PGI₂), and NO (5-7). Maintaining a balance between these vasoconstrictors and vasodilators is critical to achieve a low resistance and high blood flow in the placental vasculature for proper placental growth and fetal development during pregnancy (8). As shown by early studies (5,9), trophoblasts are a major source of TX as compared to its counter partner PGI₂ produced by the placenta. *In vitro* studies have demonstrated trophoblasts from preeclamptic placentas produced more TX than trophoblasts from normal placentas (9,10). The imbalance of increased TX production and reduced PGI₂ production by preeclamptic trophoblasts is believed to contribute to the increased vasoconstriction in preeclampsia. Placental vessels lack autonomic innervations, therefore locally hormonal effects produced by the placenta such as TX and PGI₂ must play an important role in regulation of fetal-placental vascular contractility since the basal membrane of trophoblasts directly contacts to the villous stroma and the fetal vasculature in the placenta. However, little evidence is available regarding the directional release of vasoactivators within the placenta, especially in preeclampsia. In the present study, using an *in vitro* cell insert culture method, we determined apical and basal releases of TX and PGI₂ by trophoblasts isolated from both normal and preeclamptic placentas. We tested our hypotheses: 1) disproportionate releases of TX and PGI₂ towards apical and basal directions by placental trophoblasts; and 2) increased basal release of TX by trophoblasts from preeclamptic placentas. Because our previous study suggests that increased TX production by trophoblast cells from preeclamptic placentas may result from an increase in PLA₂ release that leads to an activation of arachidonic acid (AA) – cyclooxygenase pathway (11), in this study we further examined effects of apical exposure of AA on trophoblast releases of TX and PGI₂ in the presence or absence of aspirin, a cyclooxygenase inhibitor, in our chamber cultures.

Materials and Methods

Placenta collection

Freshly delivered placentas were obtained from the main hospital of Louisiana State University Health Sciences Center – Shreveport (LSUHSC-Sh). A total of 28 placentas (16 normal and 12 preeclampsia) were used in this study. The criteria for normal pregnancy and preeclampsia were followed by ACOG guidelines. This study was approved by the Institutional Review Board (IRB) for Human Research at LSUHSC-Sh. Clinical demographic data are presented in Table 1. None of the study subjects had rupture of membrane or signs of infection. Placentas were either used for isolation of trophoblasts and/or fixed with formalin for immunohistochemical studies.

Trophoblast isolation and culture

Placental trophoblasts were isolated as previously described (12). Briefly, placental villous tissues, excluding chorionic and basal plates, were digested by Dispase (BD Biosciences, Bedford, MA). Isolated trophoblasts were purified by Percoll (Sigma, St. Louis, MO) gradient centrifugation. Contaminated red blood cells were eliminated by incubation of isolated trophoblasts with lysis buffer (155mM NH_4Cl , 10mM KHCO_3 and 0.14M MDTA, pH7.2) on ice for 5 minutes.

Freshly isolated trophoblasts were loaded into cell culture inserts in two seeding steps with a 1 hour interval, which was modified as previously described (1). In the first seeding, trophoblasts were loaded into cell culture inserts at 1×10^6 cells/insert (12 well/plate) and cultured with Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS). The insert was 8 μm pore size purchased from Becton Dickinson Labware (Franklin Lakes, NJ). The second seeding was performed 1 hour after the first seeding, with trophoblasts again at a concentration of 1×10^6 cells per insert. So the total number of trophoblast cells was 2×10^6 cells/per insert with 1.7ml of DMEM in the upper chamber and 1.5ml of DMEM in the lower chamber, which gave approximately a 5mm higher medium level in the upper chamber. After overnight incubation the higher medium level was maintained in the upper chamber, indicating the medium in the upper chamber was unable to freely pass into the lower chamber. The culture was continued for 48 hrs. At the end of incubation, the medium from the upper and the lower chambers were collected separately and measured for TX and PGI₂ by their stable metabolites of TXB₂ and 6-keto PGF₁ α , respectively. The collected medium was stored in a -20C freezer until assay. To investigate if apical exposure of trophoblasts to AA and aspirin could affect TX and PGI production, AA (10 μM or 100 μM) +/- aspirin (10 μM or 100 μM) were added to the upper chamber, respectively, and the medium in the upper and the lower chambers was collected and measured for TXB₂ and 6-keto PGF₁ α . Aspirin was used as a cyclooxygenase inhibitor to attenuate AA induced TX production. All cultures were performed in duplicate and at least 10 trophoblast chambers were used per culture experiment: 2 control chambers and 2 chambers of each that were exposed to AA10 μM , AA100 μM , aspirin 10 μM + AA100 μM and aspirin 100 μM + AA100 μM .

Transwell electrical resistance (TER) was also measured in some trophoblast culture experiments. An EndohmTM EVOM ohmmeter (World Precision Instruments, Sarasota, FL) was used. The mean reading from triplicate measures of each insert was used for data calculation. The value obtained from a blank insert was subtracted to give the net resistance, which was multiplied by the membrane area to give the resistance in area corrected units (0.90cm²/insert). Our results showed that the mean TER value was not significantly different between inserts after overnight culture, and between the control cells and cells exposed to AA after 48 hours.

Measurement of lactate dehydrogenase activity

Trophoblast cell viability was assessed by measuring lactate dehydrogenase (LDH) activity in the culture medium modified as previously described (13). Briefly, an aliquot of 100 μ l of culture medium was mixed with 40 μ l of pyruvate (7.6mM) and 60 μ l of NADH (1220 μ M)/per well in a 96 well ELISA plate. The plate was incubated in the dark for 5 minutes and read at OD340nm by a microplate reader VERSA_{MAX} (Molecular Devices, Sunnyvale, CA). LDH, purchased from Cayman Chemical (Ann Arbor, MI), was used to create a standard curve. All samples were measured in duplicate. The enzymatic activity for LDH was μ U/ml/min/ 2×10^6 cells. There was no difference for LDH activity between the control cells and cells treated with 10 or 100 μ M AA after 48 hours of culture, which were measured in 4 independent experiments.

Measurements of thromboxane and prostacyclin

Medium concentrations of TX and PGI₂ were measured by their stable metabolites of TXB₂ and 6-keto PGF₁ α by an enzyme immunoassay (EIA). The EIA kits for both TXB₂ and 6-keto PGF₁ α were purchased from the Cayman Chemical Company, MI. The range of the standard curve for TXB₂ was 7.8–1,000 pg/ml. The range of the standard curve for 6-keto PGF₁ α was 3.9–500pg/ml. Samples for measuring TXB₂ were diluted with a ratio of 1:50 with dilution buffer provided by the kit. Medium samples were directly measured for 6-keto PGF₁ α . All assays were performed in duplicate. Within and between assay variations were < 5% and < 8% for both assays, respectively. Data are presented as ng/ml/ 2×10^6 cells.

Immunohistochemistry

To examine the cellular localization of PGI synthase (PGIS), TX synthase (TXS) and TX receptor (TP receptor) in the placental villous tissue, tissue sections (4 μ m thickness) were immunostained with antibodies against PGIS, TXS and TP receptor. ABC staining kit (Santa Cruz Biotechnology, Inc., San Diego, CA) was used. Rabbit polyclonal antibodies for TXS and PGIS were purchased from Cayman Chemical (Ann Arbor, MI). Rabbit polyclonal TP receptor was from Novus Biochemicals, Littleton, CO. Standard immunostaining procedures were used: deparaffinization, dehydration, quenching of endogenous peroxidase activity with H₂O₂ followed by blocking nonspecific binding with rabbit serum. The concentration of primary antibodies used was 4 μ g/ml for TXS, 2 μ g/ml for PGIS, and 20 μ g/ml for TP receptor. After overnight incubation with primary antibodies, sections were incubated with a goat anti-rabbit biotinylated IgG secondary antibody at the concentration of 1 μ g/ml for 2 hours and with biotinylated horseradish peroxidase and avidin mixture for 30 min. Slides stained with a secondary antibody only was used as negative control. The nuclei were counterstained by hematoxylin. Stained slides were examined by an Olympus microscope (Olympus IX71). Images were captured by a digital camera with PictureFrame computer software (Uptronics, Inc., Sunnyvale, CA) and recorded into a microscope-linked PC computer.

Statistical analysis

Data are expressed as mean $\pm$ SE and analyzed by paired t-test or ANOVA with Fisher's PLSD test as a post hoc test for statistical analyses. A computer software program, StatView (Cary, NC), was used. A probability level of $p < 0.05$ was considered statistically significant.

Results

Bidirectional releases of TX and PGI₂ by trophoblasts

Figure 1 shows the 6-keto PGF₁ α and TXB₂ concentrations in the upper and the lower chambers cultured with TCs from 8 normal and 8 preeclamptic placentas for 48 hours. No difference was found for 6-keto PGF₁ α concentrations between the upper and the lower chambers cultured with normal-TCs: 0.40 ± 0.18 vs. 0.32 ± 0.08 ng/ml/ 2×10^6 cells and cultured

with PE-TCs: 0.35 ± 0.06 vs. 0.34 ± 0.05 ng/ml/ 2×10^6 cells, respectively. In contrast, TXB2 concentrations were significantly higher in the lower chamber than in the upper chamber cultured with both normal-TCs and PE-TCs, normal-TCs: 2.83 ± 0.23 vs. 1.55 ± 0.27 ng/ml/ 2×10^6 cells, $p < 0.01$; PE-TCs: 6.65 ± 0.93 vs. 4.61 ± 0.68 ng/ml/ 2×10^6 cells, $p < 0.01$, respectively. Compared to normal TCs, PE-TCs produced significantly more TX towards both apical and basal directions than that by normal-TCs, $p < 0.01$, respectively. These results indicate: 1) trophoblasts could release PGI2 and TX into both the apical and the basal directions; 2) trophoblasts from both normal and preeclamptic placentas released a greater amount of TX than PGI2 into the basal direction; and 3) trophoblasts from preeclamptic placentas released significantly more TX than those from normal placentas into both the apical and the basal directions.

Expressions of PGIS, TXS and TP receptor

Immunostaining of PGI synthase, TX synthase and TP receptor expressions was examined in 4 normal and 6 preeclamptic placental villous sections. Intense staining of PGI synthase, TX synthase and TP receptor was noticed in all preeclamptic tissue sections relative to normal tissue sections. Representative staining recordings from a normal placenta and a preeclamptic placenta are shown in Figure 2. For PGI synthase, the staining is diffused all throughout the cytoplasm of syncytiotrophoblasts for both normal (Fig. 2A) and preeclamptic (Fig. 2B) tissue sections. Positive staining of PGI synthase in villous core vessel endothelium is also observed in both normal (Fig. 2A) and preeclamptic (Fig. 2B) tissue sections. For TX synthase, the immunostaining signal is mainly localized at the apical surface of syncytiotrophoblasts and tapers to the basal membrane direction in the normal tissues (Fig. 2C). In contrast, in preeclamptic placentas strong TX synthase immunostaining is observed not only in the entire syncytiotrophoblast layer but also in villous core vessels and villous stromal cells (Fig. 2D). A higher magnification for TX synthase staining focusing on syncytiotrophoblast layer between normal and preeclamptic placentas is shown in Figure 3. For TP receptor, the expression pattern in the preeclamptic placenta is similar to that of TX synthase: intense staining in the syncytiotrophoblast layer, villous core vessels and stromal cells (Fig. 2F). Normal syncytiotrophoblasts exhibit a weak staining for TP receptor (Fig. 2E). Negative staining was observed with slides stained with secondary antibody only.

AA promotes TX, but not PGI2, production/release by trophoblast cells into both the apical and basal directions

We then determined trophoblast releases of TX and PGI2 stimulated with AA. Arachidonic acid is the substrate of cyclooxygenase, which converts AA to TX, PGI2, and prostaglandins via their synthase. AA at the concentrations of $10 \mu\text{M}$ or $100 \mu\text{M}$ was added to the upper chamber (apical exposure). After 48 hours of culture, the medium in the upper and the lower chambers were collected separately and measured for 6-keto PGF1 α and TXB2. Our data showed no changes for 6-keto PGF1 α concentrations in both the upper and the lower chambers with or without apical exposure of AA in normal and preeclamptic trophoblasts, Figure 3 (A and B). Apical exposure of trophoblasts to AA resulted in significant increases in the release of TX to the upper and lower chambers by normal trophoblasts, but only to the upper chamber by preeclamptic trophoblasts (Figure 3C and D).

Aspirin attenuates AA-induced TX production by trophoblasts

To further determine if apical exposure of cyclooxygenase inhibitor aspirin could attenuate TX release affected AA, trophoblast cells were pretreated with aspirin at concentrations of $10 \mu\text{M}$ and $100 \mu\text{M}$ for 30 minutes and then AA at a concentration of $100 \mu\text{M}$ were added to the upper chamber (apical exposure). Again, after 48 hours of culture, the medium from the upper and the lower chambers were collected separately and measured for 6-keto PGF1 α and TXB2. Our

results showed that aspirin had no effects on PGI₂ production by trophoblasts from normal and preeclamptic placentas, Figure 4 (A and B). However, aspirin at concentrations of 100 μ M, but not 10 μ M, significantly attenuated TX release in the presence of AA towards both the apical and the basal directions by normal, but not by preeclamptic, trophoblasts ($p < 0.01$), Figure 4 (C and D).

Discussion

In the present study, we investigated the directional releases of PGI₂ and TX by placental trophoblast cells using a chamber cell culture method. By measuring 6-keto PGF_{1 α} and TXB₂, the stable metabolites of PGI₂ and TX, concentrations separately in the upper and the lower chamber medium, we found that TXB₂ concentrations were significantly higher in the lower chambers than in the upper chambers cultured with trophoblasts from both normal and preeclamptic placentas. In contrast, there was no difference for 6-keto PGF_{1 α} concentrations between the two chambers in both normal and preeclamptic trophoblast cultures. The distinct patterns of 6-keto PGF_{1 α} and TXB₂ concentrations in the upper and the lower chambers suggest that trophoblast cells could differentially release these agents into the apical and the basal directions. We further noticed that TXB₂ concentration in the lower chamber with preeclamptic trophoblasts was significantly higher than that with normal trophoblasts, indicating preeclamptic trophoblasts released even more TX than normal trophoblasts towards the basal membrane direction. These findings are important regarding the possibility of trophoblast-derived TX in regulation of fetal-placental vascular activity. Apically released TX could enter the maternal circulation, although it has a very short half-life (30 seconds), while basally released TX could bind to its receptor in trophoblasts, or diffuse/shuttle to the adjacent villous stroma/fetal-placental vessels. *In vitro* study showed that placental trophoblast cells produce more TX (9,10). The imbalance of increased TX and decreased PGI₂ productions by trophoblast cells is believed to contribute to the increased placental vasoconstriction in preeclampsia. Therefore, our findings of predominant basal release of TX by trophoblast cells together with intensive staining of TX synthase and TP receptor in the stromal cells and villous core vessels in preeclamptic placentas further support the concept that increased trophoblast-derived TX production may have a great impact on fetal-placental vasculature in preeclampsia.

Tight junction formation is critical for polarized epithelial cells carrying out specific vectorial transport functions of absorption and secretion (14), and maintaining the protein and lipid differences between apical and basolateral membrane domains (15). Although we did not study tight junction formation in the present study, previous electron microscopic and freeze-fracture studies of full term human placentas have shown the presence of the tight junction (zonulae occludente and maculae occludente) structure within apical invaginations of the syncytiotrophoblastic layer (16,17), which support the concept that placental syncytiotrophoblasts are polarized epithelial cells. A study by Hemmings et al also demonstrated that tight junction structure could be formed in *in vitro* cultured trophoblasts (2). The findings of dominant basolateral releases of MMPs by Sawicki et al (1) and TX by our present study further support this concept.

Another important finding of the present study is that the apical exposure of trophoblast cells to AA resulted in an increase in TX, but not PGI₂, release towards both the apical surface and the basal membrane directions, and the increased TX release was concentration-dependent. The mechanism for the unaltered secretion of PGI₂ in response to AA is not known. However, our results suggest that the different regulatory mechanisms exist between TX synthase and PGI₂ synthase in placental trophoblast cells. Two concentrations of arachidonic acid, 10 and 100 μ M, were used in our study. This was based on a previously published work by McCoy et al (18). These authors measured AA concentrations in the maternal plasma from 17 pregnant women complicated with chronic hypertension or preeclampsia by high-performance liquid

chromatography. They found the mean plasma AA concentration is approximately 100 μ M (18). We believed that the AA concentrations used in our study were relatively compatible to the maternal levels in women with preeclampsia. Our data also showed that AA at 100 μ M concentration had no difference for LDH release compared to the control cells. Cellular and soluble phospholipase A2 activity could specifically catalyze the hydrolysis of membrane phospholipids sn-2 ester bond to liberate AA, which is the substrate of cyclooxygenase that converts AA to TX, PGI₂, and PGs *via* their synthase. In fact, elevated maternal levels of soluble phospholipase A2 and AA have been reported in women with preeclampsia (19,20). Recently, we and others found that trophoblast cells from preeclamptic placentas released more soluble phospholipase A2 than cells from normal placentas (11,21) and increased trophoblast phospholipase A2 activity is positively correlated to the higher levels of TX produced by trophoblasts from preeclamptic placentas. Under an oxidative stress condition, both phospholipase A2 activity and TX production are increased in trophoblasts from preeclampsia (11). It is believed that the activation of phospholipase A2 - AA pathway favors the TX synthesis in placental trophoblasts. Therefore, under an *in vivo* condition such as in preeclampsia, increased phospholipase A2 activity and AA levels in the maternal circulation, as well as enhanced phospholipase A2 activity in trophoblast cells, would promote TX synthesis by trophoblasts.

Aspirin is a cyclooxygenase inhibitor. Previous *in vitro* studies showed that aspirin could differentially inhibit TX and PGI productions by placental explants or villous core tissue, but not by trophoblasts (22,23). In the present study, we further examined if apical exposure of aspirin could attenuate AA-induced apical/basal releases of TX by normal and/or preeclamptic trophoblasts. The concentrations of aspirin used in this study (10 μ M and 100 μ M) were relatively compatible to the concentrations of aspirin measured in the umbilical circulation after maternal ingestion of aspirin of 100 mg or 500 mg (23,24) and similar to the previously published *in vitro* works (22,23). Our results showed that neither apical nor basal release of PGI₂ was affected in cultured trophoblasts with apical exposure of aspirin, although previous reports showed that the same concentration of aspirin 100 μ M exhibited an inhibitory effect on PGI₂ production (22,23). The different results of aspirin on PGI₂ and TX synthesis obtained in the present study from the previous published works could be explained by: 1) isolated trophoblasts vs. whole villous explant or villous core tissues (22,23); 2) different culture condition, i.e. trophoblasts cultured on cell culture filters which could provide matrix supports on cell basal membrane vs. cells directly grown on plastic surface of culture plates (22); 3) culture medium containing serum vs. serum free medium (22); and 4) cell with vs. without AA stimulation. The relative lesser degree of the inhibitory effect on TX release by preeclamptic trophoblasts than normal trophoblasts might be due to enhanced oxidative stress and increased cyclooxygenase activity in preeclamptic trophoblasts. Therefore, the aspirin concentration may not be high enough to inhibit cyclooxygenase activity in preeclamptic trophoblasts. Another explanation is that aspirin may inhibit cyclooxygenase, but not TX synthase, activity in trophoblasts. As a result, aspirin might not be able to attenuate increased TX synthesis/production due to enhanced TX synthase in preeclamptic trophoblasts. This possibility is supported by enhanced TX synthase expression observed in preeclamptic tissues. Since the terminal villi trophoblast basement membrane directly contacts capillary basement membrane as shown in Figure 3B, it is likely that basal released TX could diffuse towards basement membrane of intravillous capillaries and affect/mediate vascular responses.

In the present study, we found predominant basal directional release of TX by the placental trophoblasts, especially by trophoblasts from preeclamptic placentas. We also found that apical exposure of trophoblasts to AA could induce a dose-dependent increase in TX production towards both the apical and the basal directions without effecting PGI₂ production. Our results further showed that aspirin could attenuate AA-induced TX production by normal, but not by preeclamptic, trophoblasts. The findings of predominant basal release of TX together with

intense staining of TX synthase and TP receptor in trophoblasts, stromal cells and villous core vessels in preeclamptic placentas led us to speculate that if predominant basal release of TX by trophoblasts occurs *in vivo* as we found in our *in vitro* study, basal released TX combined with TX produced by stromal cells and villous core vessels may contribute significantly to the increased placental vasoconstriction in preeclampsia.

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Figure 1.

Bidirectional releases of PGI₂ and TX by placental TCs from normal and PE pregnancies. PGI₂ and TX were measured by their stable metabolites, TXB₂ and 6-keto PGF1 α , separately in the TC medium from the upper and the lower chambers, respectively. There was no difference for 6-keto PGF1 α concentrations between the upper and the lower chambers in both normal TCs and PE TCs (A). However, TXB₂ concentrations were significantly higher in the lower chamber than in the upper chamber in both normal TCs and PE TCs (B). ** $p < 0.01$: PE vs. normal; ## $p < 0.01$: lower vs. upper, respectively.

Figure 2.

Representative villous tissue expressions for PGI synthase, TX synthase and TX receptor (TP receptor). A and B: PGI synthase; C and D: TX synthase; E and F: TP receptor. A, C and E from a 35week normal placenta. B, D, and F from a 34⁺⁵ week preeclamptic placenta. Positive staining of PGI synthase is observed in syncytiotrophoblasts and villous core vessel endothelium in tissue sections from both normal and preeclamptic placentas. Intensive staining of TX synthase and TP receptor are seen in syncytiotrophoblasts, villous core vessels and stromal cells in villous sections from preeclamptic placentas. V: villous core fetal vessel; IVS: intervillous space; ST: villous core stromal. Bar = 10 micron.

Figure 3.

Higher magnification for TX synthase staining focusing primarily on the syncytiotrophoblasts. A: normal tissue section; B: preeclampsia tissue section; and C: negative control – stained with secondary anti-body only. V: villous core fetal vessel; IVS: intervillous space; arrow: syncytiotrophoblast layer. Bar = 5 micron for A and B; Bar = 10 micron for C.

Figure 4.

Effects of apical exposure of TCs to AA on PGI₂ and TX productions. TXB₂ and 6-keto PGF_{1α} were measured separately in the medium from the upper and the lower chambers. There was no difference for 6-keto PGF_{1α} concentrations between the upper and the lower chambers in cells with apical exposure of AA at 10 and 100 μM concentrations compared to the control cells in both normal and PE TC cultures (A and B). In contrast, TXB₂ concentrations were significantly increased in the upper chambers in cells with apical exposure of AA in both normal and PE TCs (C), $p < 0.01$, respectively. Apical exposure of AA resulted in an increase in basal release of TX by normal TCs, but not by PE TCs (D), ** $p < 0.01$, respectively.

Figure 5.

Inhibitory effects of aspirin on AA induced TX productions. TXB2 and 6-keto PGF1α were measured separately in the medium from the upper and the lower chambers. TXB2 concentrations (C and D), but not 6-keto PGF1α concentrations (A and B), were remarkably lower in both the upper and the lower chambers in normal TCs with apical exposure of aspirin + AA compared to the cells exposed to AA alone, ** $p < 0.01$, respectively. TXB2 concentrations in the upper chamber were slightly reduced, but not reach a significant level, in PE TCs with apical exposure of aspirin + AA, Asp 100μM + AA 100μM vs. AA 100μM: $p = 0.094$ (C); $p = 0.353$ (D). Apical exposure of aspirin had no effects on basal release of TX by PE TCs.

Table 1

Demographic characteristics for normal and preeclamptic pregnancies from which placentas were used in the study

Variables	Normal (n=16)	Preeclampsia (n=12)	p value
Maternal age (years)	22±1	26±1	0.137
Racial status			
White	3	1	
Black	13	11	
Gestational age (weeks)	38±4	34±3	<0.01
Blood pressure (mmHg)			
Systolic	125±3	165±5	<0.01
Diastolic	74±3	103±4	<0.01
Mode of delivery			
Vaginal	8	1	
Cesarean Section	8	11	
Placental weight (g)	630±31	380±60	<0.01