8-Iso-Prostaglandin F$_{2\alpha}$ Reduces Trophoblast Invasion and Matrix Metalloproteinase Activity

Anne Cathrine Staff, Trine Ranheim, Tore Henriksen, Bente Halvorsen

Abstract—Preeclampsia is a common pregnancy complication in the latter half of gestation diagnosed by hypertension and proteinuria. A key feature of preeclampsia is an altered placentation with reduced trophoblast invasion. Normal placentation requires controlled invasion of trophoblasts into the maternal uterine wall, with secretion of specific proteolytic enzymes able to degrade basement membranes and extracellular matrix, such as the matrix metalloproteinases (MMPs). 8-Iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) is a marker of oxidative stress in vivo and is biologically active. We have recently reported an elevated content of free 8-iso-PGF$_{2\alpha}$ in preeclamptic gestational tissue at delivery. Assuming an elevated level of 8-iso-PGF$_{2\alpha}$ during the invasion period of the pregnancy, we hypothesized that 8-iso-PGF$_{2\alpha}$ could reduce invasion of JAR cells, a choriocarcinoma cell line. We investigated JAR cell invasion with 2 types of Transwell assays and demonstrated that 8-iso-PGF$_{2\alpha}$ (10 µmol/L) resulted in reduced cell invasion in both the colorimetric and radioactivity Transwell assays (P<0.01). Zymograms revealed reduced MMP-2 and MMP-9 activity in conditioned media from JAR cells incubated with 8-iso-PGF$_{2\alpha}$ (10 µmol/L) (P<0.02). 8-iso-PGF$_{2\alpha}$ (10 µmol/L) also reduced the collagenase type IV activity in the conditioned media of JAR cells (P=0.04). No effects on MMP-2 and MMP-9 mRNA levels were observed after incubation with 8-iso-PGF$_{2\alpha}$ (10 µmol/L), whereas protein levels were significantly decreased (P<0.02), suggesting a posttranscriptional regulation. We hypothesize a potential role for 8-iso-PGF$_{2\alpha}$ in the reduced trophoblast invasion in preeclampsia. (Hypertension. 2000;35:1307-1313.)

Key Words: preeclampsia ■ prostaglandins ■ gelatinases ■ oxidative stress ■ hypertension, pregnancy

Preeclampsia is diagnosed in the latter half of the pregnancy by hypertension and proteinuria. It affects 3% to 7% of all pregnancies and is associated with serious maternal and fetal complications.1 Altered placentation is a key feature of preeclampsia. Normal human placentation requires that cytotrophoblast (CTB) cells from the outer cell mass of the blastocyst acquire an invasive phenotype and invade the inner part of the maternal uterine wall. The vascular endothelium and some of the muscular tunica media of uterine spiral arteries are replaced by the invasive CTBs, resulting in wide, low-resistance, high-flow vessels. In preeclampsia, there is a reduced invasion of trophoblasts and an incomplete transformation of the maternal spiral arteries,2,3 causing inadequate uteroplacental circulation and local ischemia. Ischemia in the uteroplacental unit is proposed to result in release of factor(s) into the maternal circulation.1 These factors are believed to induce the endothelial dysfunction underlying the main characteristics of preeclampsia: hypertension, edema, proteinuria, and activated coagulation.

Isoprostanes are prostaglandin-isomers that are synthesized in vivo from cell membranes by a predominantly nonenzymatic pathway, namely by free radical peroxidation of arachidonic acid. In vivo most arachidonic acids are esterified on phospholipids, and the isoprostanes are produced in situ in the phospholipids. The isoprostanes are later released to free form by the action of phospholipase A$_2$.4 Of the isoprostanes, 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) is of special interest. Besides being a quantitative marker for oxidative stress in vivo, 8-iso-PGF$_{2\alpha}$ is a potent vasoconstrictor; it mediates smooth muscle cell growth, activates platelets,5 and induces derangement of endothelial cell barrier function.6 Elevated urine level of 8-iso-PGF$_{2\alpha}$ has been shown in association with cardiovascular risk factors such as hypercholesterolemia and diabetes.7 The plasma content of free 8-iso-PGF$_{2\alpha}$ is also reported to be elevated in women with preeclampsia.7 We have recently demonstrated an elevated content of lipid peroxides8 and free 8-iso-PGF$_{2\alpha}$9 in decidual tissue (ie, the gestational endometrium) at delivery in preeclamptic pregnancies compared with control pregnancies.

The matrix metalloproteinases (MMPs) are capable of degrading the extracellular matrix.10 Successful implantation and trophoblast invasion are closely linked to expression of MMPs that are able to degrade basement membranes.11,12 The major component of basement membranes is type IV collagen.13 The gelatinases, members of the MMP family, can degrade type IV collagen and consist of MMP-2 (gelatinase...
A, a 72-kDa type IV collagenase) and MMP-9 (gelatinase B, a 92-kDa type IV collagenase); both are believed to be crucial for cellular invasion and tissue remodeling. The MMPs are synthesized as proforms, and their activation is poorly understood. All active MMPs as well as some proforms are inhibited by tissue inhibitors of metalloproteinases (TIMPs), a class of low-molecular-weight proteins.

In this study, we speculated that maternal 8-iso-PGF$_{2\alpha}$ levels could be elevated during the invasion process and investigated a potential role for 8-iso-PGF$_{2\alpha}$, in the invasion of the choriocarcinoma cell line JAR.

Methods

Materials

Reagents, when not otherwise specified, were purchased from Sigma Chemical Co.

Cell Cultures

The choriocarcinoma cell line JAR (ATCC HTB-144) was grown as monolayers in 75-cm$^2$ plastic flasks (Costar) at 37°C in 95% air and 5% CO$_2$ in RPMI-1640 supplemented with 10% heat-inactivated FBS, 1-glutamine (2 mmol/L), penicillin (50 IU/mL), and streptomycin (50 μg/mL) (Bio Whittaker). For subculture, the cells were detached with Accutase (Innovative Cell Technologies).

Transwell Invasion Assay

The effect of 8-iso-PGF$_{2\alpha}$ on JAR cell invasiveness was examined by a Transwell invasion assay with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) from Fluka (Buchs) to stain viable cells. To the upper wells of the invasion system (Transwell plates, 8-μm pore size, from Costar), one of the following test substances was added: 0.009% ethanol (control), 8-iso-PGF$_{2\alpha}$ (1 pmol/L–100 μmol/L) (Cayman Chemical), 1,10-phenanthroline (50 μg/mL), or 8-Bromo-cAMP (8-Br-cAMP) (1.5 mmol/L), a nonhydrolyzable cAMP analogue that induces differentiation of cytotrophoblasts into syncytiotrophoblasts. The invasion was calculated as filter absorbance in percentage of control. The absorbance reflects the number of invaded cells was done as for the colorimetric method, except that gel wash with 2.5% Triton X-100 (wt/vol) and incubation in developing buffer for 40 hours, the gels were fixed, then stained with 0.1% Coomassie blue R-250 (Biorad) and destained in water. The gels were scanned by a laser scanner densitometer (Molecular Dynamics Inc) and quantified with the use of the software Image Quant.

Western Immunoblotting

Western blotting was performed as described elsewhere on JAR cells incubated in growth medium supplemented with either 0.009% ethanol (control), 8-iso-PGF$_{2\alpha}$ (10 μmol/L), or 8-Br-cAMP (3 mmol/L) for 24 hours. The filters were incubated with monoclonal anti-human MMP-2 or MMP-9 (R & D Systems) or monoclonal anti-human TIMP-2 (Calbiochem). Proteins were detected by enhanced chemiluminescence with horseradish peroxidase–labeled anti-mouse IgG (Vector Laboratories).

Northern Blot Analysis

mRNA isolation from JAR cells incubated in growth medium supplemented with either 0.009% ethanol (control), 8-iso-PGF$_{2\alpha}$ (10 μmol/L), or 8-Br-cAMP (3 mmol/L) and Northern blotting was performed as previously described. The TIMP-2, MMP-2, and MMP-9 cDNAs were a gift from Drs Andrew Baker and Gill Murphy. 8-Br-cAMP (1.5 mmol/L, or 1,10-phenanthroline (100 nmol/mL) in the upper wells, quantification of invaded cells was done as for the colorimetric method, except that the radioactivity was counted in a Packard TRICARB 1900 Scintillation Counter.

[3H]Leucine and [3H]Thymidine Incorporation

The JAR cell viability after 8-iso-PGF$_{2\alpha}$ exposure was examined by incorporation of 3-[3,4,5-3H]leucine (Amersham) into cell-associated proteins to measure protein synthesis and by incorporation of [6-3H]thymidine (du Pont-NEN) into DNA to analyze DNA synthesis, as a measure of cell proliferation. At 70% confluence, the cells were preincubated for 22 hours with growth medium supplemented with either 8-iso-PGF$_{2\alpha}$ (1 or 10 μmol/L), 8-Br-cAMP (1.5 mmol/L), or 1,10-phenanthroline (100 ng/mL). The control cells were exposed to 0.009% ethanol. The cells were then incubated for additional 2 hours in fresh growth medium supplied with 5 μCi/mL [3H]leucine or 5 μCi/mL [3H]thymidine. The cells were harvested and analyzed as described previously.

Type IV Collagenase Activity

A modified procedure of Emonard et al was performed to investigate the activity of type IV collagen–degrading enzymes in the conditioned medium (CM) from JAR cells. Briefly, 1×10$^5$ JAR cells were seeded in RPMI-1640 into a 24-well plate precoated with 300 μL of an extracellular matrix gel diluted 1:1 (vol/vol) in DMEM (Bio Whittaker). The cultures received either 0.009% ethanol, 10 μmol/L 8-iso-PGF$_{2\alpha}$, or 100 ng/mL 1,10-phenanthroline. The experiments were performed in quadruplicate in 2 separate experiments. Specific type IV collagenolytic activity was calculated as total type IV enzymatic activity of the CM from 1×10$^5$ cells minus the EDTA-insensitive activity.

Gelatin Zymography of CM

Gelatibase activity was detected in serum-free CM from JAR cells grown on Matrigel-coated Transwell plates (Biomatrix EHS from Boehringer Ingelheim, diluted 1:1 [vol/vol] in RPMI-1640), modified from Sharma et al. Either vehicle (0.009% ethanol), 8-iso-PGF$_{2\alpha}$ (10 μmol/L), 8-Br-cAMP (3 mmol/L), or 1,10-phenanthroline (100 ng/mL) was added to the upper wells; and the cells were incubated for 72 hours. From the CM above the filters, 20 μg protein was incubated at room temperature for 10 minutes with nonreducing loading buffer containing 4% SDS (wt/vol), then subjected to electrophoresis on SDS-polyacrylamide gels containing 0.1% (wt/vol) gelatin (Novex). Both human MMP-2 and MMP-9 standards (Chemicon) were run to ensure identification of MMP activity. After gel wash with 2.5% Triton X-100 (wt/vol) and incubation in developing buffer for 40 hours, the gels were fixed, then stained with 0.1% Coomassie blue R-250 (Biorad) and destained in water. The gels were scanned by a laser scanner densitometer (Molecular Dynamics Inc) and quantified with the use of the software Image Quant.

Results

Transwell Studies

The MTT colorimetric Transwell assay showed a statistically significant reduction in trophoblast invasion into the filters (Table 1) when the cells were incubated for 24 hours with 8-iso-PGF$_{2\alpha}$ in concentrations from 100 pmol/L (11% reduction of filters relative to control, P=0.04) to 100 μmol/L
TABLE 1. Effects of 24-Hour Incubation With 8-iso-PGF$_{2a}$ on JAR Cell Invasion Into Filters in MTT Transwell Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Invasion of Control</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±2.6</td>
<td>23</td>
<td>1.0</td>
</tr>
<tr>
<td>10 pmol/L 8-iso-PGF$_{2a}$</td>
<td>92.8±3.1</td>
<td>10</td>
<td>0.07</td>
</tr>
<tr>
<td>100 pmol/L 8-iso-PGF$_{2a}$</td>
<td>89.0±4.8</td>
<td>6</td>
<td>0.04</td>
</tr>
<tr>
<td>1 nmol/L 8-iso-PGF$_{2a}$</td>
<td>80.0±7.4</td>
<td>12</td>
<td>0.003</td>
</tr>
<tr>
<td>10 nmol/L 8-iso-PGF$_{2a}$</td>
<td>83.4±2.7</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100 nmol/L 8-iso-PGF$_{2a}$</td>
<td>83.6±5.0</td>
<td>13</td>
<td>0.05</td>
</tr>
<tr>
<td>1 μmol/L 8-iso-PGF$_{2a}$</td>
<td>80.0±7.0</td>
<td>8</td>
<td>0.005</td>
</tr>
<tr>
<td>10 μmol/L 8-iso-PGF$_{2a}$</td>
<td>86.7±3.8</td>
<td>18</td>
<td>0.001</td>
</tr>
<tr>
<td>100 μmol/L 8-iso-PGF$_{2a}$</td>
<td>83.9±4.7</td>
<td>16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Invasion was measured by colorimetric analysis of MTT product formazan, as described in Methods section. Results are given in percentage of control filters and are presented as mean±SEM from 6 to 23 filters from up to 9 separate experiments. (16% reduction, P=0.01). JAR cells incubated with 10 μmol/L 8-iso-PGF$_{2a}$ showed a 20% reduction in filter invasion compared with control cells (P=0.001). No statistically significant effect on the invasion was found at 8-iso-PGF$_{2a}$ concentrations <100 pmol/L (10 pmol/L: 7% reduction, P=0.07)). As expected, 1.5 mmol/L 8-Br-cAMP resulted in a 17% reduction of trophoblast invasion into the filters (n=20 filters; P<0.001). Similarly, 50 ng/mL 1,10-phenanthroline showed a 22% reduction in JAR cell invasion into filters compared with control (n=9; P=0.001). Inhibition of trophoblast and JAR cell invasion by 1,10-phenanthroline has previously been demonstrated. In our study, 1,10-phenanthroline was used as a control for reduced invasion by reducing the MMP activity.

We also demonstrated reduced trophoblast invasion into Transwell filters by using [H]thymidine-radiolabeled JAR cells (Figure 1). Incubation with 10 μmol/L 8-iso-PGF$_{2a}$ reduced the filter invasion by 26.8% (P=0.009, n=20), whereas incubation with 100 nmol/L 8-iso-PGF$_{2a}$ resulted in a reduced invasion that was not statistically significant (7.5% reduction, P=0.5, n=22). There was a 20% reduction of trophoblast invasion when the cells were subjected to 3 mmol/L 8-Br-cAMP (P=0.047, n=17), whereas the 11.6% reduction of invasion after exposure to 100 ng/mL 1,10-phenanthroline did not reach statistical significance (P=0.4, n=12). The cotton swabs, representing JAR cells that had not invaded the filters but were adherent to the filters, showed a statistically significant rise in radioactive activity in wells subject to 100 nmol/L and 10 μmol/L 8-iso-PGF$_{2a}$ (not shown), reflecting that fewer JAR cells had invaded the filters.

[3H]Leucine and [3H]Thymidine Incorporation

We found no indications of the 8-iso-PGF$_{2a}$-mediated reduction in JAR invasion being due to toxicity because neither [3H]thymidine incorporation into cell-associated DNA nor [3H]leucine incorporation into cell-associated proteins were affected by 1 or 10 μmol/L 8-iso-PGF$_{2a}$ incubation (Table 2). However, 100 μmol/L 8-iso-PGF$_{2a}$ did significantly reduce [3H]thymidine incorporation by 30% (P<0.001), whereas the [3H]leucine incorporation was not reduced. This indicates that this concentration of 8-iso-PGF$_{2a}$ is not toxic to the JAR cells but may impair cell proliferation. This could be the case if there was a differentiation toward syncytiotrophoblast (multinucleated, fused trophoblasts), which would result in an overall reduced cellular invasion. The concentrations ≤10 μmol/L of 8-iso-PGF$_{2a}$ did not result in reduced JAR cell proliferation. 8-Br-cAMP (1.5 mmol/L) reduced the mean cell-associated incorporation of radiolabeled thymidine to 68.4% of control wells (n=27; SEM=3.4, P<0.001), whereas the [3H]leucine incorporation was not affected. 1,10-Phenanthroline (100 ng/mL) similarly reduced the [3H]thymidine incorporation to 83.3% of control (n=19, SEM=1.8, P<0.001), whereas the leucine incorporation was not reduced.

Type IV Collagenase Activity

The specific type IV collagenolytic activity in the CM of JAR cells incubated for 24 hours with 10 μmol/L 8-iso-PGF$_{2a}$ was statistically significantly reduced by 30% (n=8, SEM=11, P=0.04). There was also a 50% reduction of the specific type IV collagenolytic activity in the CM of JAR cells incubated with 100 ng/mL 1,10-phenanthroline as compared with the CM of the control cells (n=8, SEM=4).

Zymography

In general, the MMP activity was higher in the CM from the upper wells compared with the CM from the lower wells of the Transwell assay, and the results presented are from the CM of the upper wells. Several lytic bands, seen as clear areas of lysis in the uniformly stained blue gel, were observed in the zymograms from the CM of the upper wells. The major gelatinolytic bands were activated MMP-2 and MMP-9 (both identified with protein weight markers, mixed MMPs, and a selectively activated MMP-2 marker). The APMA-activated MMP-2 marker comigrated with the MMP-2 band from the mixed MMPs marker, indicating that the SDS that was added to the samples activated the MMPs. MMP-2 was the predominant of these 2 enzymes. Incubation with 10 μmol/L 8-iso-
PGF$_2\alpha$ for 72 hours resulted in a reduction in both MMP-2 and MMP-9 activity (42% and 33% reduction, respectively, $P<0.003$ and $P<0.019$) (Figure 2). As expected, the MMP inhibitor 1,10-phenanthroline (100 ng/mL) reduced MMP activity compared with control in the CM, both for MMP-2 and MMP-9 activity (36% and 32% reduction, respectively). Incubation with 8-Br-cAMP (3 mmol/L) resulted in a 2-fold–augmented MMP-2 activity ($P<0.006$), whereas the MMP-9 gelatinolytic activity was unaltered. Identical gels were incubated in parallel in the presence of EDTA (10 mmol/L), which is a metal chelator. The disappearance of lytic bands in these gels after incubation with EDTA confirmed that the gelatinolytic activity observed was metal dependent, which is characteristic of MMPs.

**Western Blot**

Figure 3 demonstrates a 31% reduction in protein level of MMP-9 and a 42% reduction in protein level of MMP-2 when JAR cells were subjected to 8-iso-PGF$_2\alpha$ (10 µmol/L) compared with controls ($P=0.003$ and $P=0.019$, respectively). Incubation with 3 mmol/L 8-Br-cAMP resulted in a significant 1.8-fold augmentation of MMP-9 and a 1.6-fold augmentation of MMP-2 protein levels compared with controls ($P=0.004$ and $P=0.028$). To assess whether an elevated protein level of TIMP-2 could be involved in a downregulation of MMP activity, we investigated TIMP-2 by Western blots (Figure 4). We found a statistically significant 1.5-fold elevation of TIMP-2 protein level in JAR cells subjected to 10 µmol/L 8-iso-PGF$_2\alpha$ ($P=0.014$). On the other hand, incubation with 3 mmol/L 8-Br-cAMP did not alter TIMP-2 protein levels ($P=0.2$).

**Expression of mRNA**

Figure 5 shows unaltered mRNA signal density of MMP-2 in JAR cells subjected to 10 µmol/L 8-iso-PGF$_2\alpha$ (incubation time up to 24 hours). On the other hand, the signal density of JAR cells exposed to 3 mmol/L 8-Br-cAMP shows a time-dependent transcriptional induction of MMP-2, being 1.5-
fold induced at 2 hours and 6-fold induced at 24 hours, compared with control cells (both $P < 0.014$). Similar results were obtained for MMP-9, showing unaltered mRNA level after incubation of JAR cells with 10 $\mu$mol/L 8-iso-PGF$_2\alpha$ up to 24 hours. Incubation with 3 mmol/L 8-Br-cAMP gave a time-dependent induction of MMP-9 mRNA, being 1.5-fold induced compared with control at 2 hours and 8-fold induced after 24-hour incubation (both $P < 0.014$). We also investigated whether there was an elevated transcription of the TIMP-2 gene after JAR cell incubation with 8-iso-PGF$_2\alpha$ (10 $\mu$mol/L). Analogous to the study by Graham et al.,$^{18}$ our study shows that JAR trophoblasts exhibit the 1.0-kb as well as the 3.5-kb TIMP-2 transcript. There were no differences between the signal densities of the 1.0- and 3.5-kb mRNAs relative to $\beta$-actin. Therefore, only the results from the 3.5-kb transcript is presented in Figure 6. The TIMP-2 mRNA level was unaltered after incubation of JAR cells with either
10 μmol/L 8-iso-PGF$_{2α}$ or 3 mmol/L 8-Br-cAMP up to 24 hours.

Statistical Analysis
When statistical analyses were performed comparing more than 2 groups, we used the Bonferroni method to control the overall type I error at a level ≤5% of statistical significance. Where Mann-Whitney U tests were <0.05, the Bonferroni method still resulted in a corrected probability value <0.05 (not shown). An exception to this is the reduced invasion of 20% (Figure 1) in JAR cells incubated with 3 mmol/L 8-Br-cAMP compared with control cells incubated with [$^{3}$H]thymidine-labeled JAR cells. This difference does not reach statistical significance with the use of the Bonferroni method (corrected P=0.188). In addition, the effect of 3 mmol/L 8-Br-cAMP on augmenting the MMP-2 protein levels in Western blots with 60% (Figure 3) compared with control cells is of borderline statistical significance when corrected for multiple comparisons (corrected P=0.056). These corrections do not affect the results regarding the effects of 8-iso-PGF$_{2α}$ on invasion of JAR cells and possible MMP mechanisms.

Discussion
Our present work demonstrates that 8-iso-PGF$_{2α}$ reduces the invasiveness of a choriocarcinoma cell line, JAR. We also show that JAR cells incubated with 10 μmol/L 8-iso-PGF$_{2α}$ exhibit reduced MMP-2 and MMP-9 expression, both at enzyme activity and protein levels, together with diminished type IV collagenolytic activity.

The relative roles of MMP-2 and MMP-9 in invasion of first-trimester CTBs is controversial. One study showed that CTB production and activation of both MMP-2 and MMP-9 in vitro was most pronounced during the first trimester of the pregnancy, in which period the CTB invasion is maximal.

An in vitro study of first trimester trophoblasts showed predominantly zymographic expression of MMP-9, whereas in another study MMP-2 was highly expressed.

A specific MMP-9 antibody completely inhibited first-trimester CTB invasion in vitro, suggesting that MMP-9 is critical for CTB invasion.

It has been demonstrated that CTB from preeclamptic placentas failed to upregulate MMP-9 expression both at the protein and mRNA levels in contrast to control CTBs, and the invasiveness of these CTBs was also greatly reduced.

The diverse opinions on the relative involvement of MMP-2 and MMP-9 in placentation may be explained by contamination of other cell types and/or diverse proportions of early stages of differentiated CTBs.

The unaltered JAR expression of mRNA levels for MMP-2 and MMP-9 in our study (Figure 5) shows that 8-iso-PGF$_{2α}$ did not exert its effect on the MMP-2 and MMP-9 enzymes at gene level. In our study, 8-iso-PGF$_{2α}$ decreased JAR cell protein levels of MMP-2 and MMP-9 (Figure 3) and reduced the gelatinolytic activity of MMP-2 and MMP-9 in the CM from JAR cells (Figure 2). This indicates a posttranscriptional effect of 8-iso-PGF$_{2α}$ on MMP-2 and MMP-9 in JAR cells, possibly affecting the stability of mRNA. The regulation of MMPs is believed to occur mainly at the transcriptional level, besides activation of latent proenzymes and inhibition of proteolytic activity. In addition, other studies have reported posttranscriptional regulation of MMP expression, both increasing the mRNA half-life and decreasing mRNA MMP stability. A destabilization of mRNA is a possible posttranscriptional regulatory mechanism by which 8-iso-PGF$_{2α}$ reduces MMP-2 and MMP-9 activity. However, the exact mechanism(s) for a posttranscriptional MMP-2 and MMP-9 regulation by 8-iso-PGF$_{2α}$ still remains to be elucidated.

The activation and activity of MMPs are regulated by a family of endogenous inhibitors referred to as TIMP. TIMP-2 is proposed to be involved in autoregulation of trophoblast invasion.

In our study, we could not demonstrate any effect of 8-iso-PGF$_{2α}$ on TIMP-2 mRNA level (Figure 6), whereas Western blots demonstrated a 1.5-fold increase at the protein level (Figure 4). We cannot exclude the possibility that augmented TIMP-2 protein level is involved in the 8-iso-PGF$_{2α}$-mediated reduction of gelatinolytic activity of MMP-2 in JAR cells. Another member of the TIMP family, TIMP-1, inhibits all MMPs in the activated form and MMP-9 in both latent and active forms.

We found that JAR cells expressed only barely detectable mRNA levels of TIMP-1, which is in accordance with a previous study. We have recently demonstrated an elevated content of free 8-iso-PGF$_{2α}$ in decidual tissue (ie, the gestational endometrium) at delivery in preeclamptic pregnancies compared with control pregnancies, whereas the rationale for this in vitro study is based on an assumption of elevated 8-iso-PGF$_{2α}$ during the invasion process, which is normally completed by week 20 of the pregnancy. To the best of our knowledge, no information is available on the level of 8-iso-PGF$_{2α}$ in plasma nor decidual tissue during the invasion process. On the other hand, dyslipidemia is a feature of pregnancy in general and of preeclampsia in particular and has been demonstrated already in the first half of pregnancies (week 17 to 19) later complicated by preeclampsia.

Other dyslipidemic conditions, such as diabetes mellitus, are also characterized by elevated levels of 8-iso-PGF$_{2α}$. It is therefore possible that 8-iso-PGF$_{2α}$ is elevated at this time of trophoblast invasion and remodeling of the maternal tissues. The concentrations of 8-iso-PGF$_{2α}$ chosen in this study are in the range observed in vivo and similar to previous studies examining molecular effects of 8-iso-PGF$_{2α}$.

It has previously been demonstrated that the choriocarcinoma cell line JAR has the ability to invade in vivo. We chose this cell line because it provides a large number of uniform cells. JAR choriocarcinoma cells share many of the characteristics of early placental trophoblasts, such as placentone hormone production, and the ability to differentiate into syncytiotrophoblast-like cells in vitro and some of the same molecular mechanisms of invasion. Other studies report differences in vitro between choriocarcinoma cell lines and human first-trimester trophoblasts in the regulation of invasiveness. Therefore, these in vitro findings performed with a malignant cell line do not necessarily represent the in vivo situation of invasion, where many cell types in addition to trophoblasts are involved in the invasion process.

In conclusion, we have demonstrated that 8-iso-PGF$_{2α}$ reduces the invasion of JAR cells. Reduced protein levels of MMP-2 and MMP-9, as well as reduced enzymatic activity,
were also observed. We hypothesize that 8-iso-PGF<sub>2α</sub> could reduce trophoblast invasion in vitro by reducing the MMP activity and that 8-iso-PGF<sub>2α</sub> could contribute to reduced trophoblast invasion in vivo in preeclampsia.

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