Platelet PGI₂ receptor affinity is reduced in pre-eclampsia

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Prostacyclin (PGI₂) receptors were studied in platelet membrane preparations from women with normal pregnancy, pregnancy-induced hypertension (PIH) or pre-eclampsia. Patient groups showed no differences in gestational week at delivery. A markedly lower birth weight, however, was found in pre-eclampsia. No differences between groups could be detected in platelet PGI₂ receptor number. In contrast, the binding affinity to the PGI₂ mimetic iloprost was considerably reduced in pre-eclampsia, whereas receptor affinity between PIH and normal pregnancy did not differ significantly.

Keywords prostacyclin receptor platelets pregnancy-induced hypertension pre-eclampsia

Introduction

Reduced formation of PGI₂ is a typical feature of PIH and pre-eclampsia [1, 2]. It is, however, still unsettled whether possible consequences of PGI₂ deficiency such as platelet activation, reduced blood flow and intrauterine growth retardation may be counterbalanced by increased number and avidity of corresponding receptors. So far nobody has investigated PGI₂ receptors in pregnancies complicated by hypertension. The present study was designed to determine platelet PGI₂ receptor characteristics in normal pregnancy, PIH and pre-eclampsia and to assess any relation to the clinical outcome.

Methods

Patients were randomly selected from the local department of obstetrics and comprised 15 healthy pregnant women, nine patients with PIH and 10 with pre-eclampsia. Informed consent was obtained from all individuals. Hypertension was defined as a sitting diastolic blood pressure ≥ 90 mmHg measured several times with a mercury sphygmomanometer. Proteinuria was defined as ≥ 300 mg day⁻¹ or persistently $\geq 2+$ on dipstick testing. Patients with chronic diseases were excluded as well as those receiving any medication. Particularly, no antihypertensive drugs or cyclo-oxygenase inhibitors were taken at least until blood sampling was performed.

Platelet membranes were prepared as described previously [3]. In short, 40 ml venous blood was drawn

from an antecubital vein into a syringe containing 10 ml acid citrate/dextrose (ACD, Biostabil®, Biotest, Frankfurt, Germany). Platelet-rich plasma was prepared by centrifugation (250 g, 10 min, 20° C). The pH was adjusted to 6.5 with ACD. After a final centrifugation (900 g, 10 min, 20° C) the supernatant was removed and the pellet stored at -80° C. Platelet membranes were prepared by repeated freezing and thawing and isolated by differential centrifugation [3].

For ligand binding studies [4], the pellets were resuspended in washing buffer (mM): NaCl (140), KCl (5), Tris (10), sodium citrate (2), glucose (5), citric acid (5), pH 7.4 and centrifuged (105000 g, 15 min, 4° C). Thereafter, the pellets were resuspended in assay buffer (mM): NaCl (140), KCl (5), Tris (10), glucose (5), CaCl₂ (5), pH 6.0. Membrane suspensions (100 µl) were incubated with 50 µl [³H]-iloprost (5 nM, Amersham, Braunschweig, Germany) and 50 µl of unlabelled iloprost (Schering, Berlin, Germany) at concentrations of 1–10000 nM (final) for 90 min at 15° C. Bound ligand was separated from free by rapid filtration (Brandel cell harvester, Gaithersburg, USA). Radioactivity was measured by liquid scintillation counting. Binding affinity (K_d^{-1}) and capacity (B_{max}) were calculated from the displacement data by computerized curve-fitting analysis (Enzfitter, Biosoft, Cambridge, UK). Membrane protein was measured using a commercial assay kit (Biorad, Richmond, USA).

For statistical comparison of binding affinities between groups, log K_d values were analysed by ANOVA and subsequent Student-Newman-Keuls test. Since the B_{max} values and the clinical data were not normally distributed, comparisons between groups were carried out by using Kruskal-Wallis one-way analysis of variance and

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subsequent Mann–Whitney U-test with Bonferroni correction. P levels <0.05 were considered significant. Calculations were accomplished using PROC GLM, NPAR1WAY and UNIVARIATE of SAS software, release 6.08.

Results

There were no differences between healthy pregnant women, patients with PIH and those with pre-eclampsia in maternal age (31 [17–40], 33 [20–40], 26 [22–34] years; medians and ranges), gestational week at blood collection (36 [29–42], 38 [31–41], 38.5 [31–42]) and gestational week at delivery (40 [34–42], 40 [33–42], 39 [31–42]). Although without statistical significance, foetal heart rate decelerations requiring caesarean section, umbilical pH <7.20 and an Apgar score <7 at 5 min were more frequent in pre-eclampsia (in three of 10 subjects) than in the other groups (once in each case). Foetal birth weight was moderately lower in PIH and markedly ($P<0.05$) lower in pre-eclampsia compared with normal pregnancy (Figure 1).

Specific binding of [^3H]-iloprost to platelet membranes showed one single binding site in all patients. Non-specific binding was generally less than 30% of the total binding. Platelet binding capacity for iloprost did not differ between groups, as shown by similar B_{max} values (medians and 25th–75th percentiles) of 989 (454–1810) fmol mg $^{-1}$ protein in normal pregnancy, 600 (386–1325) in PIH and 739 (462–989) in pre-eclampsia. In contrast, the K_d values were considerably increased in pre-eclampsia ($P<0.05$ vs control, $P<0.05$ vs PIH), whereas in PIH only a small increase was observed. This indicates a substantial reduction in platelet PGI $_2$ receptor affinity in pre-eclamptic patients (Figure 2).

Figure 1 Birth weight in normal pregnancy (CON, $n=15$), pregnancy-induced hypertension (PIH, $n=9$) and pre-eclampsia (PE, $n=10$). Horizontal bars represent the medians.

Figure 2 Dissociation constant for iloprost binding to platelet membranes of normal pregnancy (CON, $n=15$), pregnancy-induced hypertension (PIH, $n=9$) and pre-eclampsia (PE, $n=10$).

Discussion

Reduced formation of PGI $_2$, the most potent endogenous inhibitor of platelet reactivity, may play a role in PIH and pre-eclampsia since an enhanced platelet activation is considered to be an important pathogenetic factor [5, 6]. In addition, PGI $_2$ deficiency precedes the manifestation of the disease [1, 2]. Any PGI $_2$ -mediated effects, however, not only depend on PGI $_2$ formation but may also be modulated by alterations at the receptor level. The absence of any differences in platelet PGI $_2$ receptor number between patient groups reported here, however, argues against a possible compensatory receptor regulation. In contrast, we found a tendency to a reduced PGI $_2$ receptor affinity in PIH and a significant reduction in pre-eclampsia. Thus, PGI $_2$ -mediated actions may be reduced in pre-eclampsia, while this is uncertain in PIH. The clinical data, although based on a small number of observations, seem to be consistent with a decreased PGI $_2$ activity in pre-eclampsia. In these patients, signs of reduced uteroplacental blood flow were noticed, since the rate of foetal distress was somewhat higher and fetal growth was significantly reduced. In agreement with the nonsignificant reduction of PGI $_2$ receptor affinity in PIH, there was only a moderate impairment of fetal development.

Clearly, an important role of reduced PGI $_2$ receptor affinity in pre-eclampsia is not proven by our results. Horn *et al.* [7] found a trend for an impaired PGI $_2$ receptor function in hypertensive pregnancies (PIH and pre-eclampsia), as indicated by a non-significant reduction of platelet cyclic AMP accumulation in response to iloprost. Unfortunately, the patients with pre-eclampsia have not been evaluated by subgroup analysis. More clear evidence in favour of PGI $_2$ receptor dysfunction in pre-eclampsia comes from earlier studies, showing reduced platelet sensitivity to PGI $_2$ analogues in this disorder [8, 9], which agrees with the reduced

platelet PGI₂ receptor affinity in pre-eclampsia reported here. Similar receptor characteristics could also apply to vascular PGI₂ receptors, because so far only one gene encoding for the PGI₂ receptor has been identified [10]. The present data should stimulate further studies directed to clarify the functional consequences of pre-eclampsia-associated reduction of PGI₂ receptor affinity.

In conclusion, there is no basis for the assumption that compensatory alterations at the receptor level counterbalance PGI₂ deficiency in PIH and pre-eclampsia. In contrast, reduced PGI₂ receptor affinity may result in decreased PGI₂ activity and subsequent platelet hyperreactivity in pre-eclampsia.

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