Plasma Thromboxane and Prostacyclin: Comparison During Normal Pregnancy and Pregnancy Complicated by Hypertension

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Abstract

Plasma levels of thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-keto-PGF1α), stable metabolites of two prostanoids with opposing biological effects, TXA2 and prostacyclin, were measured by radioimmunoassay in normal pregnancy (controls) and pregnancy complicated by hypertension (PIH) from 32 to 36 (Period 1; P1) and from 36 to 40 (Period 2; P2) weeks of gestation. The plasma concentration of each compound in the control subjects was 265.6±58.4 (TXB2), 132.4±16.5 (6-keto-PGF1α) for P1 (n=10) and 142.6±11.8 (TXB2), 68.5±5.2 (6-keto-PGF1α) for P2 (n=10) respectively (pg/ml, mean±s.e). In the patients with PIH, TXB2 concentrations increased moderately for P1 (419.2±21.2; n=7) and significantly (P<0.005) for P2 (452.8±31.0; n=7) respectively (pg/ml, mean±s.e), while the plasma levels of 6-keto-PGF1α revealed a slight to moderate decrease both for P1 (84.5±4.0; n=7) and P2 (59.7±8.1; n=7) respectively (pg/ml, mean±s.e). The physiological balance of TXB2 to 6-keto-PGF1α was significantly greater (P<0.005) in the patients with PIH, where the TXB2/6-keto-PGF1α ratio was 5.2±0.7 for P1 and 9.4±2.3 for P2 respectively (mean±s.e) compared with that of the controls, where it was 2.4±0.4 for P1 and 2.0±0.2 for P2 respectively (mean±s.e). Thus, in the patients with PIH, vasoconstricting and proaggregating prostanoid showed a dramatic rise in the maternal circulation, suggesting the possibility that platelet activation would play an important role in eliciting clinical pictures associated with PIH.

Vasoconstrictive substances such as histamine, vasopressin and the extracts from prostatic secretion (prostaglandins or vesiculaglandins), were known as early as the 1930s (von Euler, 1936) to possess contractile effects on smooth muscle of the arteries. However, the contractile action of thromboxane A2 (TXA2), which was discovered in 1975, was reported to be more striking (Hamberg et al., 1975; Svensson et al. 1977). On the other hand, no effective naturally occurring dilators were known until prostacyclin was discovered in 1976 (Moncada et al., 1976). Prostacyclin closely resembles primary prostaglandin, or prostaglandin E1, but it is about four times as effective in relaxing the arteries. In addition to the...
vasoactive actions of these compounds, prostacyclin, mainly generated in the vessel wall, is a powerful inhibitor of platelet aggregation, whereas TXA2, produced mainly by platelets, has counteracting proaggregation effects. A balance between TXA2 and prostacyclin evidently regulates the vascular tonus and platelet aggregation in the systemic circulation (Moncada et al., 1979). It is well established that the impairment of this balance in the arteriolar bed, particularly in the uterus and the kidney, is a characteristic feature of pregnancy induced hypertension (PIH) (Koullapis et al., 1982).

In the present study, we have assessed the plasma levels of these vasoactive and platelet-aggregation related prostanoids to elucidate various clinical pictures associated with PIH.

Materials and Methods

Patients and sampling

All subjects studied in the present work were classified into two periods according to gestational ages, or Period 1 (P1; from 32 to 36 weeks) and Period 2 (P2; from 36 to 40 weeks). The control normotensive subjects included 10 pregnant women for each period and the patients with PIH (blood pressure >150/100 mmHg, proteinuria >1.0 g/day and creatinine clearance <60 ml/min) included 7 pregnant women for each period. The age of all patients ranged from 25 to 30 years (mean ages were 26.4 years for the controls and 28.4 years for the patients with PIH respectively). The patients with PIH preimposed by familial hypertension were excluded from the present study.

Blood samples were collected from the control subjects at out-patient clinic after more than 30 minutes bed rest and from the patients with PIH on admission prior to any treatments under the same conditions as for the controls. Blood was drawn from the antecubital veins without venostasis through an installed teflon catheter with a heparinized syringe and transferred into ice cooled siliconized tubes containing meclofenamate and ethylenediaminetetraacetic acid at concentrations of 10 µg/ml and 2 mg/ml respectively. The plasma was collected after centrifugation at 3,000 rpm for 15 minutes and at 4°C. The extraction of each prostanoid was carried out from the fresh plasma thus obtained within two hours following blood sampling.

Assay for TXB2 and 6-keto-PGF1α

Plasma levels of TXB2 (a stable metabolite of TXA2) and 6-keto-PGF1α (a stable end product of prostacyclin) were determined by the method previously reported (Okahara et al., 1981, 1982 and 1983). Tritium labeled TXB2 and 6-keto-PGF1α (8,000–10,000 cpm, New England Nuclear, New Jersey) were alternatively added to 3.0 ml of plasma to determine the recovery rate. The mixture was initially extracted with 5 volumes of petroleum ether to remove neutral lipids, followed by extraction with ethyl acetate after being acidified by 3 volumes of the solvent consisting of ethyl acetate: isopropanol: 0.1N HCl, 3:3:1 by volume. The organic phase thus obtained was dried under N2 gas at 55°C. Dried materials were kept frozen at −80°C until separation by silic acid column chromatography (0.6 g, 100 mesh, Mallinckrodt, Missouri). The separation of each prostanoid was started within four weeks after the extraction procedure, then two groups were achieved. A mixture of benzene, ethyl acetate and methanol was used for the elution. The separation profile for each group is shown in Fig. 1. Dried materials after N2 gas evaporation were applied for radioimmunoassay.

The antiserum for TXB2 and 6-keto-PGF1α were kindly donated by Dr. T. Inagawa at Ono Pharmaceutical Company and Dr. K. Nishikawa at Takeda Chemical Company respectively. The antiserum of TXB2 did not cross-react significantly with other prostaglandins (less than 0.1%), with only exception for 2, 3-dinor-TXB2 (58.7%). The antiserum of 6-keto-PGF1α cross-reacted with PGF1α (6.0%), 6, 15-diketo-PGF1α (0.4%) and 6, 15-diketo-13, 14-dihydro-PGF1α (0.3%). Fifty per cent displacement of the radioligands of TXB2 and 6-keto-PGF1α represents 60 and 110 pg respectively. The recovery of the initially added [3H] TXB2 and [3H] 6-keto-PGF1α was 65.0±0.9% and 54.8±0.6% respectively (mean±S.D.; n=80).

Results

Plasma prostanoid levels in the control subjects

Plasma levels of TXB2 and 6-keto-PGF1α in the control subjects were 265.6±58.4,
Fig. 1. A typical separation profile of thromboxane B₂ and 6-keto-PGF₁α, using silic acid column chromatography. [³H] thromboxane B₂ (circles) and 6-keto-PGF₁α (triangles) were added to 3.0 ml of plasma. Extraction and separation were carried out as described in the text. The ordinate is the percentage of radioactivity and the abscissa is the elution volume of the solvent system, benzene (B), ethyl acetate (E) and methanol (M) by volume.

132.4±16.5 for P₁ (n=10) and 142.6±11.8, 68.5±16.5 for P₂ (n=10) respectively (pg/ml, mean±s.e). The TXB₂/6-keto-PGF₁α ratios were found to be 2.4±0.4 (P₁) and 2.0±0.2 (P₂) respectively (mean±s.e ; n=10).

**Plasma prostanoid levels in the patients with PIH**

Plasma levels of TXB₂ and 6-keto-PGF₁α in the patients with PIH were 419.2±21.2, 84.5±4.0 for P₁ (n=7) and 452.8±31.0, 59.7±8.1 for P₂ (n=7) respectively (pg/ml, mean±s.e). The TXB₂/6-keto-PGF₁α ratios were found to be 5.2±0.7 (P₁) and 9.4±2.3 (P₂) respectively (mean±s.e).

**Statistical comparison during normal pregnancy and pregnancy complicated by hypertension**

(Table 1)

For P₁ period, there was no significant difference between the control and hypertension group TXB₂ concentrations, though plasma levels of TXB₂ showed a moderate increase in the patients with PIH compared with those of the controls. However, during the P₂ period, the TXB₂ concentration in plasma showed a significant rise (p<0.005) in the patients with PIH compared with those of the controls.

Regarding plasma levels of 6-keto-PGF₁α, they were lower in the patients with PIH to a slight to moderate degree compared with those of the controls, though no statistical differences were noted.

In contrast to the absolute values for each prostanoid in the circulation, it is
Table 1. Comparison between normal pregnancy (control) and pregnancy induced hypertension (PIH).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>PIH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TXB2</td>
<td>265.6±58.4</td>
<td>419.2±21.2</td>
</tr>
<tr>
<td>6-keto-PGF 1α</td>
<td>132.4±16.5</td>
<td>84.5±4.0</td>
</tr>
<tr>
<td>TXB2/6-keto-PGF 1α ratio</td>
<td>2.4±0.4*</td>
<td>5.2±0.7†</td>
</tr>
<tr>
<td>TXB2</td>
<td>142.6±11.8*</td>
<td>452.8±31.0*</td>
</tr>
<tr>
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<td>TXB2/6-keto-PGF 1α ratio</td>
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<td>9.4±2.3*</td>
</tr>
</tbody>
</table>

TXB2, 6-keto-PGF 1α; pg/ml, mean±s.e
TXB2/6-keto-PGF 1α ratio; mean±s.e
1, 2, 3; p<0.005 Others are not significantly different

notable that the physiological balance between TXB2 and 6-keto-PGF 1α significantly increased (p<0.005) in the patients with PIH compared with that of the controls.

Discussion

It is well documented that severe toxemic patients have several clinical entities of disseminated intravascular coagulation (Pritchard et al., 1976), whereby the platelet count is reduced remarkably in association with activation of the fibrinolytic system. In fact, the patients with PIH studied showed a moderate elevation of fibrin degraded products (>15 µg/dl). Furthermore, they showed a moderate fall in renal function. As previously reported, vasoconstricting and platelet aggregating prostanoid, or TXA2, exerts its physiological effects predominantly at the levels of uterus and the kidney (Koullapis et al., 1982) in the patients with PIH. In this aspect, a moderate to significant elevation of TXA2 in the maternal circulation may well reflect locally occurring vasoconstriction and platelet aggregation, leading to additional renal symptoms. It is worth noting that platelet aggregation elicited by adenosine diphosphate in vitro is remarkably reduced regardless of platelet activation, which reflects an increased generation of TXA2 (Sakamoto, 1983). This evident discrepancy needs further explanation. One possible explanation is that platelet activation may deleted number of platelets, which in turn facilitates platelet turnover resulting in macrothrombocytosis (Garg et al., 1972; Paulus, 1975). Therefore, decreased aggregation by adenosine diphosphate in vitro might result from exhaustion (Sakamoto, 1983) and/or shortened life-span of viable platelets regardless of the facilitated platelet turnover.

Regarding the absolute concentrations of TXB2 and 6-keto-PGF 1α in human plasma, of pregnant or non-pregnant subjects, they vary greatly among the institutions. The plasma levels of TXB2 showed a great difference from a single picogram (Granstrom et al., 1984) to hundreds of picograms (Ylikorkala et al., 1983) per milliliter. Moreover, that of 6-keto-PGF 1α in pregnant women has been reported to be in the magnitude ranging from double picogram (Mitchell et al., 1978) to hundreds of picograms (Ylikorkala et al., 1983) per milliliter. The remarkable variation in the absolute assay values among laboratories would depend on the differences, including 1) whether using fresh plasma or frozen plasma, 2) whether the extraction procedure is started soon or late, 3) how the extracted materials are kept, 4) what kinds of separation methods are used, silic acid column chromatography or HPLC, and 5) what kinds of assay methods are applied, GC-MS quan-
titration or radioimmunoassay. Therefore, in the present study, meticulous care has been involved in each procedure, and our data were obtained under these strict conditions, as previously described.

Furthermore, regarding the plasma concentration of 6-keto-PGF1α in the patients with PIH, there is profound confusion whether production of this compound is significantly increased (Koullapis et al., 1982), slightly reduced (Goodman et al., 1982; Sakamoto, 1983), or does not differ from normal pregnancy (Ylikorkala et al., 1981 and 1983). The results obtained in our present work have clearly demonstrated that the balance between TXA2 and prostacyclin shifts to a dominance of TXA2. There are two possible explanations to be considered for reduced production of prostacyclin. One possible explanation, reported by Hope and his co-workers (Hope et al., 1979), is that the release of β-thromboglobulin contained in α-granules of the platelets is facilitated in response to platelet activation to inhibit prostacyclin biosynthesis by the endothelial cells. Another possible explanation, is compatible with the exhausted platelet theory for PIH. Prostaglandin endoperoxides, which are released by platelets and are precursors both for TXA2 and prostacyclin biosynthesis (Moncada et al., 1979), are relatively deleted by platelet activation since elevation of TXA2 precedes reduced production of prostacyclin. In this respect, further intensive studies of platelet-to-vessel wall interaction are necessary.

It is generally considered as more effective to evaluate the physiological balance between TXA2 and prostacyclin than the absolute values of these compounds for the perspectives of vasoconstriction and platelet aggregation (Koullapis et al., 1982). However, it is still most important to endeavour to determine the plasma levels of these prostanoids during pregnancy and pregnancy complicated by hypertension in order to escape from the present confusion.

Finally, it should be emphasized that the clinical usefulness of measuring the metabolites of TXA2 and prostacyclin in plasma depends, for the time being, on the evaluation of the physiological balance between TXA2 and prostacyclin, or TXB2/6-keto-PGF1α ratio.

References


