Comparison of Plasma Prostanoid Levels in the Human Cord Artery in Normal and Fetal Distressed Deliveries

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Abstract

Prostaglandin E2 (PGE2), thromboxane B2 (TXB2; as a stable metabolite of TXA2), prostaglandin F2α (PGF2α) and 6-keto-PGF1α (as a stable end product of prostacyclin) have been measured by using specific radioimmunoassay in the plasma of the cord artery immediately after delivery before the cord was clamped. Plasma prostanoid concentrations in normal deliveries (n=8, as controls) were 24.8±2.6 (PGE2), 246.8±37.0 (TXB2), 122.2±13.3 (PGF2α) and 82.1±7.7 (6-keto-PGF1α) respectively (pg/ml, mean±s.e). On the other hand, in fetal distressed deliveries showing continuous bradycardia (n=6), they increased significantly to 275.4±20.1 (PGE2), 948.6±102.5 (TXB2), 218.0±21.4 (PGF2α) and 1498.6±298.4 (6-keto-PGF1α) respectively (pg/ml, mean±s.e, p<0.005). However, both PGF2α/PGE2 and TXB2/6-keto-PGF1α ratios declined significantly from 4.70±0.33 to 0.68±0.05 and from 3.07±0.37 to 0.68±0.12 respectively (mean±s.e, p<0.005) in the fetal distressed group compared with those of the controls. From these results, it may be concluded that the cord artery, which is known as the patent source for the production of PGE2 and prostacyclin, did exert a sufficiently strong reaction to overcome the undesirable haemodynamic changes to maintain the fetal well-being in utero.

Confirmation of the absence of nerves in the human cord artery (Meyer et al., 1978) has led many investigators to assess humoral mediators regulating the cord circulation. As early as the 1930s, von Euler showed that histamine, vasopressin and the extracts from prostatic secretion, or prostaglandins, contracted the cord artery (von Euler, 1936). However, the contractile action of thromboxane A2 (TXA2) on the human cord artery was proved to be more striking (Svensson et al., 1977). On the other hand, no effective naturally occurring dilators were known until prostacyclin was discovered in 1976. Prostacyclin closely resembles primary prostaglandin, or PGE1, but it is about four times as effective in relaxing the human cord artery (Hamberg et al., 1979). Since very few data exist concerning TXA2 and prostacyclin in normal and pathological pregnancies, or in cord circulation (Remuzzi et al., 1979; Ylikorkala et al., 1980), the factors regulating fetal placental blood flow of the cord artery are not completely understood.

To elucidate the importance of prostaglandins in cord artery regulation, in vivo and in vitro studies on the effects of primary
prostaglandins, TXA2 and prostacyclin under various conditions would be of great value. The establishment of highly specific and reproducible radioimmunoassay system of PGE2, TXB2 (as a stable metabolite of TXA2), PGF2α and 6-keto-PGF1α (as a stable end product of prostacyclin) prompted us to investigate the plasma concentrations of these compounds in the cord artery in uneventful vaginal deliveries and the pathological changes in forceps deliveries due to severe fetal distress with continuous bradycardia.

Materials and Methods

Subjects and cord blood sampling
We used eight full-term uncomplicated pregnancies undergoing uneventful vaginal deliveries as controls and six full-term uncomplicated pregnancies undergoing prompt termination by forceps due to severe fetal distress showing continuous bradycardia on the cardiotocogram. None of the fourteen patients had pathological symptoms, such as pregnancy induced hypertension, diabetes mellitus, or other medical morbidities, during pregnancy. Antenatal screening of fetal well-being was repeatedly carried out, including estimation of the fetal growth and monthly non-stressed test after 25 weeks of pregnancy. From the results obtained, all cases studied were considered to be entirely normal until the onset of labor. In these fourteen patients in whom labor was of spontaneous onset with the neonates presenting the vertex, blood samples were collected from the cord artery with a heparinized syringe and 18 gauge needle immediately after delivery and before the cord was clamped. An aliquot of the blood thus obtained was used for blood gas analysis.

Blood samples were immediately 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 at 4 °C. The extraction of each prostanoid was carried out from the fresh plasma thus obtained within two hours after blood sampling.

Assay for PGE2, TXB2, PGF2α and 6-keto-PGF1α
Plasma levels of these compounds were determined by the method previously reported with slight modifications (Okahara et al., 1981; Okahara et al., 1983). Tritium labeled PGE2, TXB2, PGF2α 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 five volumes of petroleum ether to remove neutral lipids, followed by extraction with ethyl acetate after being acidified with three volumes of 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. The dried materials were kept frozen at −80 °C until separation by silic acid column chromatography (0.6 g, 100 mesh, Mallinkrodt, Missouri, Fig. 1). The separation of each prostanoid was started within four weeks after the extraction procedure, and two groups were obtained. A mixture of benzene, ethyl acetate and methanol at various concentrations

Blood in siliconized tube containing meclofenamate (10 μg/ml) & EDTA (2mg/ml)

\[ \downarrow \]

Removal of neutral lipids from the plasma by adding 5vol of petroleum ether

\[ \rightarrow \text{Organic phase discarded} \]

Plasma

\[ \downarrow \]

Rapid acidification following addition of ethyl acetate

Isopropanol: 0.1N HCl, 3:3:1 by vol (3vol)

\[ \rightarrow \text{Extraction with ethyl acetate (2 vol)} \]

\[ \rightarrow \text{Addition of distilled water (3 vol)} \]

\[ \rightarrow \text{Organic phase} \]

\[ \downarrow \]

Drying under N2 gas at 55°C

\[ \rightarrow \text{Kept frozen at-80°C until separation by silic acid column chromatography} \]

Fig. 1. The extraction procedure is illustrated. The details are described in the text
was used for the elution. The separation profile of the major groups is shown in Fig. 2. Dried materials after N2 gas evaporation were used for radioimmunoassay.

The antisera to PGE2 and PGF2α were purchased from the Pasteur Institute (Paris, France) and the related cross reactivities have previously been reported (Dray et al., 1975). The antisera to 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 to 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%). The antiserum to TXB2 did not cross react significantly with other prostaglandins (less than 0.1%). Fifty per cent displacement of the radioligands of PGE2, TXB2, PGF2α and 6-keto-PGF1α represents approximately 36, 60, 150 and 110 pg respectively. The recovery of the initially added [3H] PGE2, [3H] TXB2, [3H] PGF2α and [3H] 6-keto-PGF1α was 65.2±0.7%, 65.0±0.9%, 71.3±0.8% and 54.8±0.6% respectively (mean±S.D, n=80).

Results

Plasma concentrations of each compound in the cord artery were 24.8±2.6 (PGE2), 246.8±37.0 (TXB2), 122.2±13.3 (PGF2α) and 82.1±7.7 (6-keto-PGF1α) respectively (pg/ml, mean±s.e) in the uneventful vaginal deliveries. On the other hand, in the fetal distressed group, in which the pH of the blood was 7.098±0.034 (mean±s.e), each prostanoid showed a significant rise to 275.4±20.1 (PGE2), 948.6±102.5 (TXB2), 218.0±21.4 (PGF2α) and 1498.6±298.4 (6-keto-PGF1α) respectively (pg/ml, mean±s.e,
Table I. Plasma prostanoid levels in the cord artery are summarized by the normal control group and fetal distressed group.

<table>
<thead>
<tr>
<th>Prostanoid</th>
<th>Normal Control Group (N=8)</th>
<th>Fetal Distressed Group (N=6)</th>
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<tbody>
<tr>
<td>PGE2</td>
<td>24.8 ± 2.6a</td>
<td>275.4 ± 20.1a</td>
</tr>
<tr>
<td>TXB2</td>
<td>246.8 ± 37.0b</td>
<td>948.6 ± 102.5b</td>
</tr>
<tr>
<td>PGF2α</td>
<td>122.2 ± 13.3c</td>
<td>218.0 ± 21.4c</td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>82.1 ± 7.7d</td>
<td>1499.6 ± 298.4d</td>
</tr>
</tbody>
</table>

a,b,c,d P<0.005 (mean ± s.e : pg/ml)

p<0.005; Table I). The PGF2α/PGE2 ratio was 4.70 ± 0.33 and the TXB2/6-keto-PGF1α ratio was 3.07 ± 0.37 (mean ± s.e) in the control group. However, in the fetal distressed group, both the PGF2α/PGE2 and TXB2/6-keto-PGF1α ratios declined to a significant degree to 0.68 ± 0.05 and 0.68 ± 0.12 respectively (mean ± s.e, p<0.005) regardless of a significant increase in each prostanoid against the control values. It is worth noting that the most dramatic changes were observed in PGE2 and 6-keto-PGF1α, where the former showed more than a ten fold increase and the latter increased to approximately twenty times the values for the control group. This evidence agrees closely with in vitro results obtained with the cord endothelial monolayers, in which striking production of PGE2 and prostacyclin was achieved. The mean maximal production of PGE2 and 6-keto-PGF1α in the in vitro study was 604.1 and 1930.7 respectively (pg/ml/x10⁶ cells; n=5, unpublished data).

Discussion

There are a couple of key points in the determination of plasma prostanoid levels in cord circulation. The great importance of the timing for cord blood sampling has been well documented by Mitchell and his co-workers (Mitchell et al., 1978), who assessed the difference in prostaglandin concentrations in the cord vessels. They reported that the significant rise in plasma prostaglandin levels was associated with expulsion of the placenta, but no report shows that whether samples were collected before or after cord clamping made any difference statistically. The timing of cord clamping may be employed in measuring primary prostaglandins, though such labile prostanoids as TXA2 and prostacyclin should be determined in blood plasma obtained in free flowing blood. Thus, in the present study, we have applied strict criteria in blood sampling.

Determination of the plasma levels of PGE2 and PGF2α has been reported as less reliable than measurement of their metabolites (Satoh et al., 1979). However, our data emphasize the necessity for determining the concentrations of PGE2 and PGF2α in the cord circulation (which is a closed compartment, reflecting in situ fetal circulation) in that the relative balance of these two biologically opposed compounds attracts fresh concern on the neonatal morbidities (Demers et al., 1976). Moreover, plasma PGE2 in fetal circulation has been shown to play an important role in the etiology of a patent ductus arteriosus (Coceani et al., 1983; Clyman et al., 1983).

Regarding the absolute concentrations of 6-keto-PGF1α in the cord arterial plasma, these vary greatly from several hundred picograms (Mitchell et al., 1980) to a single nanogram (Ylikorkala et al., 1981) per milliliter. The remarkable variation in assay values among laboratories would depend on the differences in the assay procedures, including 1) whether fresh plasma or frozen plasma was used, 2) whether the extraction procedure is started early or late, 3) how to keep extracted materials, and 4) how soon separation and assay are done. Therefore, in the present work, meticulous care has been taken in each procedure and our data were obtained according to these strict criteria.

Evaluation of the pathological changes in the plasma prostanoid levels in the cord artery should be reviewed considering the
results obtained by *in vitro* studies. The endothelial monolayers of the human cord have been clearly shown to produce prostanoids, among which PGE2 and prostacyclin were considered to be the major active prostanoids formed (Hamberg *et al.*, 1979; Eldor *et al.*, 1983). Our unpublished data also confirmed the possibility that the cord artery possesses the capacity to form a moderate amount of PGF2α and much less TXA2. Similar reports have been found in a few papers, suggesting such a possibility as that the endothelial cells of the cord vessels can produce TXA2 (Tuveno *et al.*, 1976; Ingerman-Wojenski *et al.*, 1981). Anyway it may be considered that the cord artery is potentially endowed with the capacity to generate PGE2 and prostacyclin predominantly in response to various stimulations.

In view of these results, it is very likely that a significant increase in PGE2 and prostacyclin is of endothelial origin in response to severe hypoxegenation (Tuveno, 1980), while a striking increase in PGF2α and TXA2 might originate predominantly in the fetal compartment. Furthermore, the cord artery did react sufficiently to surpass the undesirable haemodynamic changes occurring in the cord circulation in order to lower the vessel tonus and to prevent thromboembolic changes in the fetal placental circulation. In view of this for the time being, we shall have to take great care when using prostaglandin synthetase inhibitors during pregnancy.

**References**


and thromboxane release in the dog kidney. Prostaglandins. 25, 373–383.