FORMATION OF 13,14-DIHYDRO-PROSTAGLANDIN E₁ DURING INTRAVENOUS INFUSIONS OF PROSTAGLANDIN E₁ IN PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE

B.A. Peskar¹, W.H. Hesse¹, W. Rogatti², C. Diehm³, G. Rudofsky⁴, H. Schweer⁵ and H.W. Seyberth⁶

¹Department of Pharmacology and Toxicology, Ruhr University, D-4630 Bochum, FRG, ²Schwarz Pharma AG, D-4019 Monheim, FRG, ³Department of Internal Medicine, University of Heidelberg, D-6900 Heidelberg, FRG, ⁴Department of Internal Medicine, University of Essen, D-4300 Essen, FRG, and ⁵Children's Hospital, University of Marburg, D-3550 Marburg, FRG

ABSTRACT

Formation of 13,14-dihydro-prostaglandin (PG) E₁ during intravenous infusions of PGE₁ in patients with peripheral arterial occlusive disease was investigated. Using both high performance liquid chromatography (h.p.l.c.) combined with radioimmunoassay and gas chromatography/triple stage quadrupole mass spectrometry (GC/MS/MS) basal levels of 13,14-dihydro-PGE₁ were found to be close to or below the detection limits of the assay methods. Levels of the PGE₁ metabolite increased significantly during the infusion periods and decreased after their end. Since 13,14-dihydro-PGE₁, in contrast to its precursors 15-keto-PGE₁ and 15-keto-13,14-dihydro-PGE₁, is biologically active, its formation could contribute to the beneficial effects of PGE₁ administered intravenously in patients with peripheral arterial occlusive disease.

INTRODUCTION

For more than a decade intravenous PGE₁ infusions have been used in the treatment of peripheral arterial occlusive disease (1,2). The beneficial effect of PGE₁ administered intravenously is remarkable, since a major portion of circulating PGE₁ is rapidly metabolized during passage through the human lung (3). Initial metabolism of PGE₁ occurs via the 15-hydroxy-PG-dehydrogenase/Δ₁³-reductase pathway (4) resulting in formation of 15-keto-13,14-dihydro-PGE₁ as the major circulating metabolite. This compound lacks almost completely biological activity (5). It was, therefore, of interest to investigate the possible formation of 13,14-dihydro-PGE₁, a biologically active metabolite (5,6), during intravenous infusions of PGE₁.

METHODS

PGE₁ (Prostavasin®, Schwarz Pharma AG, Monheim, FRG) was infused intravenously either at 80 µg/60 min/patient (n=6, age 21-79, 4 male, 2 female) or at 60 µg/30 min/patient (n=4, age 60-70, 2 male, 2 female). In the first group of patients blood was taken before and 40 min after the start as well as at the end (60 min) and 30 min after the end of the infusion period. In the second group of patients blood was taken before and at the end of the infusion period (30 min) as well as 60 min later. Blood (20 ml) was collected into syringes con-
PROSTAGLANDINS

taining sodium EDTA as anticoagulant and indomethacin as cyclooxygenase inhibitor (final concentrations 5.4 and 0.1 mM, respectively). Plasma was separated by immediate centrifugation (1500 x g, 4°C, 15 min) and analyzed for 13,14-dihydro-PGE₁ using h.p.l.c. (7) combined with radioimmunoassay of eluted fractions for the first group of 6 patients. The anti-PGE₁ antiserum (8) used for the radioimmunoassay exhibits 100% relative cross-reaction with 13,14-dihydro-PGE₁. Blood from the second group of 4 patients was analyzed by GC/MS/MS (to be published) using a purification procedure described previously (9). Standard amounts of 13,14-dihydro-PGE₁ (100 pg - 1 ng) added to human whole blood were shown to be stable for at least 2 - 3 hours.

The results were calculated as means ± S.E.M. Statistical analysis was performed by use of Student's t-test for paired values.

RESULTS

The results are shown in Table I. While preinfusion plasma levels of 13,14-dihydro-PGE₁ were close to or below the detection limits of

Table I

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>h.p.l.c./radioimmunoassay</th>
<th>GC/MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5 ± 2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>n.d.</td>
<td>13 ± 1 ***</td>
</tr>
<tr>
<td>40</td>
<td>19 ± 6 *</td>
<td>n.d.</td>
</tr>
<tr>
<td>60</td>
<td>22 ± 5 **</td>
<td>n.d.</td>
</tr>
<tr>
<td>90</td>
<td>13 ± 5 +</td>
<td>4 ± 0.2 +++</td>
</tr>
</tbody>
</table>

n.d.: not determined; * p < 0.05, ** p < 0.01, *** p < 0.001 as compared to 0 min; + p < 0.05, +++ p < 0.001 as compared to end of infusion period (60 or 30 min, respectively).

the assay methods, concentrations at the end of the infusion periods (60 and 30 min, respectively) had significantly increased to 22 ± 5 pg/ml (n=6, p < 0.01) and to 13 ± 1 pg/ml (n=4, p < 0.001), respectively. Thirty min after the end of the infusion period levels of 13,14-dihydro-PGE₁ had significantly (p < 0.05) decreased to 13 ± 5 pg/ml in those 6 patients that had received 80 µg PGE₁ for 60 min. Similarly a significant (p < 0.001) decrease in the metabolite levels
to 4 ± 0.2 pg/ml was observed 1 hour after the end of the infusion period in those 4 patients that had received 60 μg PGE1 for 30 min (Table 1).

**DISCUSSION**

The results clearly demonstrate for the first time formation of 13,14-dihydro-PGE1 during intravenous administration of PGE1 to man. On the other hand, formation of this metabolite in vitro (5,10,11) as well as in the rat in vivo (12) has long been known to occur. In man, the analogous metabolite 13,14-dihydro-PGF2α has been found to occur in plasma after intravenous administration of PGF2α (13,14).

Similar to PGE1, 13,14-dihydro-PGE1 has been shown to lower blood pressure (5) and to inhibit platelet aggregation (6). Although the mechanism of beneficial action of PGE1 in peripheral arterial occlusive disease remains unknown and the concentrations of 13,14-dihydro-PGE1 formed during the infusions of PGE1 are rather low, the biologically active metabolite could contribute to the therapeutic effects of intravenous infusions of PGE1 observed in such patients (1,2).

**ACKNOWLEDGEMENTS**

We thank Mrs. K. Rezny for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Pe 256/4-1 and Se 263/9).

**REFERENCES**


performance liquid chromatography. J. Chromatogr., in press


Editor: P.W. Ramwell Received: 10-17-90 Accepted: 12-7-90