SENSITIVITY OF PLATELETS TO PROSTAGLANDINS IN PATIENTS WITH CORONARY ARTERY DISEASE

Vilja MARDLAa, Gennadi KOBJARa, Indrek RÄTSEPb, Madis LÖHMUSa, Ivar JÄRVINGa, and Nigulas SAMELa

a Institute of Chemistry, Tallinn Technical University, Akadeemia tee 15, 12618 Tallinn, Estonia; vilja@chemnet.ee
b Mustamäe Hospital, Sütiste tee 19, 13419 Tallinn, Estonia

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Abstract. The platelet sensitivity to the antiaggregatory prostaglandins (PGE1, 13,14-dihydro-PGE1, and 5,6-dihydro-PGE3) was studied in patients with coronary artery disease. Platelets of healthy subjects were used as control. The sensitivity was tested in vitro by inhibiting the adenosine diphosphate-induced platelet aggregation with these prostaglandins of various concentrations. In the patients the antiaggregatory potency of the prostaglandins was significantly lower than in healthy subjects.

Key words: platelets, prostaglandins, coronary artery disease.

Abbreviations: PG = prostaglandin; PGE1 = prostaglandin E1; 13,14-dihydro-PGE1 = prostaglandin 13,14-dihydro-E1; 5,6-dihydro-PGE3 = prostaglandin 5,6-dihydro-E3; PGI2 = prostaglandin I2, prostacyclin; ADP = adenosine diphosphate; CAD = coronary artery disease; PRP = platelet-rich plasma.

INTRODUCTION

Prostaglandin E1 (PGE1) and its natural analogues prostaglandin 13,14-dihydro-E1 (13,14-dihydro-PGE1) and prostaglandin 5,6-dihydro-E3 (5,6-dihydro-PGE3) interact with the same platelet receptor as prostacyclin (PGI2). These prostaglandins (PGs) are synthesized in the organism in very low quantities, but are very potent inhibitors of platelet aggregation (Kobzar et al., 1993). Sinzinger et al. (1998) showed that PGE1 and 13,14-dihydro-PGE1 inhibit platelet adhesion to the vessel wall as well as platelet thrombus formation and
induce thromboresistance in experimental animals and in man. There are no data in the literature on the effect of 13,14-dihydro-PGE\textsubscript{1} and 5,6-dihydro-PGE\textsubscript{3} on platelets of patients suffering from coronary artery disease (CAD). Therefore in this work we compared the antiaggregatory potency of these PGs in patients suffering from advanced CAD with that of age and sex matched healthy subjects.

**MATERIALS AND METHODS**

The studied patients suffered from CAD and underwent coronary artery bypass grafting. Preliminary coronary angiography showed $\geq 75\%$ lumen narrowing in at least one coronary artery. All patients gave informed consent and the local ethics committee approved the study. Donors of the local blood bank served as control. Blood samples were taken from the radial artery through an indwelling catheter before cardiopulmonary bypass in the patients and from the antecubital vein in the healthy subjects.

Blood was collected into 10-mL plastic centrifuge tubes containing 1 mL of 3.8\% trisodium citrate. Platelet-rich plasma (PRP) and platelet-poor plasma were prepared by centrifugation of the citrated blood at $160 \times g$ or $4000 \times g$ for 10 min at room temperature, respectively.

Aggregation was measured photometrically in a Chrono-Log aggregometer according to the method of Born (1962) by detecting the transmission of the light through PRP. An aggregometer cuvette containing 0.5 mL of an aliquot of PRP was maintained at 37 $^\circ$C and stirred with a Teflon coated stirring bar at 1200 rpm. Inhibition of platelet aggregation was determined by incubation of PGs with PRP. The IC\textsubscript{50} was calculated from dose–effect curves as a concentration necessary to reduce the ADP-induced aggregation by 50\% of its control amplitude. Data were expressed as arithmetic mean $\pm$ standard error (SEM) of separate experiments with PRP of $n$ subjects. Statistical significance of healthy donors versus CAD patients was determined using the Student’s $t$-test for an unpaired experiment.

PGs were dissolved in ethanol, kept at $-20^\circ$C, and diluted with saline before the experiment. PGE\textsubscript{1} was obtained from Kemasol Ltd. (Tallinn, Estonia). 5,6-Dihydro-PGE\textsubscript{3} was separated by repeated HPLC-purification from the mixture of natural E-PGs extracted from ram seminal vesicles (Lõhmus et al., 1990). 13,14-Dihydro-PGE\textsubscript{1} was synthesized from PGE\textsubscript{1} by the reduction of the 13,14 double bound with H\textsubscript{2} on the Pd(C) catalyst. The purity of the PGs used for the platelet antiaggregatory study was more than 99.9\% by HPLC.

**RESULTS**

The chemical structure of the PGs studied is shown in Fig. 1. All three compounds were about two times less effective in CAD patients than in control (Table 1). These data are in accordance with the earlier studies, which showed
Fig. 1. Chemical structure of the prostaglandins under study.

Table 1. Inhibition of ADP-induced platelet aggregation in healthy subjects and patients with CAD

<table>
<thead>
<tr>
<th>Compound</th>
<th>Healthy subjects</th>
<th>CAD patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$, nM</td>
<td>n</td>
<td>IC$_{50}$, nM</td>
</tr>
<tr>
<td>PGE$_1$</td>
<td>56 ± 5</td>
<td>11</td>
<td>120 ± 20</td>
</tr>
<tr>
<td>13,14-Dihydro-PGE$_1$</td>
<td>35 ± 6</td>
<td>11</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>5,6-Dihydro-PGE$_3$</td>
<td>58 ± 6</td>
<td>10</td>
<td>110 ± 9</td>
</tr>
</tbody>
</table>

Data are expressed as arithmetic mean ± SEM of separate experiments with PRP of n subjects. IC$_{50}$ is the concentration causing 50% inhibition of ADP-induced platelet aggregation. The statistical significance of healthy donors versus CAD patients was determined with unpaired Student’s t-test.

A lower sensitivity of platelets of CAD patients to PG	extsubscript{I2} (Mehta et al., 1980) and to PGE$_1$ (Sinzinger et al., 1981). This effect may be explained by the fact that PG	extsubscript{I2} synthesis is increased in blood vessels of CAD patients (Fisch et al., 1997) and long-term exposure of platelets to PG	extsubscript{I2} may result in desensitization of PG	extsubscript{I2}. 

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receptors. The lower sensitivity of platelets to 13,14-dihydro-PGE\(_1\) and 5,6-dihydro-PGE\(_3\), as well as to PGE\(_1\) and PGI\(_2\), may be used as an indicator of CAD.

**ACKNOWLEDGEMENT**

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**REFERENCES**


**KORONAARTÕVEGA PATSIENTIDTE TROMBOTSÜÜTIDE TUNDLIKKUS PROSTAGLANDINIIDE SUHTES**

Vilja MARDLA, Gennadi KOBZAR, Indrek RÄTSEP, Madis LÕHMUS, Ivar JÄRVING ja Nigulas SAMEL

Eksperimentaalselt on uuritud koronaartõvega patsientide trombotsüütide tundlikkust antiagregatiivsete prostaglandiinide (PGE\(_1\), 13,14-dihüdro-PGE\(_1\) ja 5,6-dihüdro-PGE\(_3\)) suhtes. Tervete doonorite trombotsüüte on kasutatud kontrolliks. Trombotsüütide tundlikkust on testitud *in vitro* inhibeerides prostaglandiinidega adenosindifosfaadi toimel indukteeritud trombotsüütide aggregatsiooni. Koronaarhaigusega patsientidel oli nende prostaglandiinide antiagregatiivne aktiivsus tunduvalt madalam võrreldes tervete kontrollgrupiga.