SHORT COMMUNICATIONS

Proc. Estonian Acad. Sci. Biol. Ecol., 2001, **50**, 1, 33–36

https://doi.org/10.3176/biol.ecol.2001.1.04

SENSITIVITY OF PLATELETS TO PROSTAGLANDINS IN PATIENTS WITH CORONARY ARTERY DISEASE

Vilja MARDLA[a], Gennadi KOBZAR[a], Indrek RÄTSEP[b], Madis LÕHMUS[a], Ivar JÄRVING[a], and Nigulas SAMEL[a]

[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

Received 31 August 2000, in revised form 3 November 2000

Abstract. The platelet sensitivity to the antiaggregatory prostaglandins (PGE_1, 13,14-dihydro-PGE_1, and 5,6-dihydro-PGE_3) 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; PGE_1 = prostaglandin E_1; 13,14-dihydro-PGE_1 = prostaglandin 13,14-dihydro-E_1; 5,6-dihydro-PGE_3 = prostaglandin 5,6-dihydro-E_3; PGI_2 = prostaglandin I_2, prostacyclin; ADP = adenosine diphosphate; CAD = coronary artery disease; PRP = platelet-rich plasma.

INTRODUCTION

Prostaglandin E_1 (PGE_1) and its natural analogues prostaglandin 13,14-dihydro-E_1 (13,14-dihydro-PGE_1) and prostaglandin 5,6-dihydro-E_3 (5,6-dihydro-PGE_3) interact with the same platelet receptor as prostacyclin (PGI_2). 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 PGE_1 and 13,14-dihydro-PGE_1 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_1 and 5,6-dihydro-PGE_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 ≥ 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 × *g* or 4000 × *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 °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_{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 ± 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 °C, and diluted with saline before the experiment. PGE_1 was obtained from Kemasol Ltd. (Tallinn, Estonia). 5,6-Dihydro-PGE_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_1 was synthesized from PGE_1 by the reduction of the 13,14 double bound with H_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

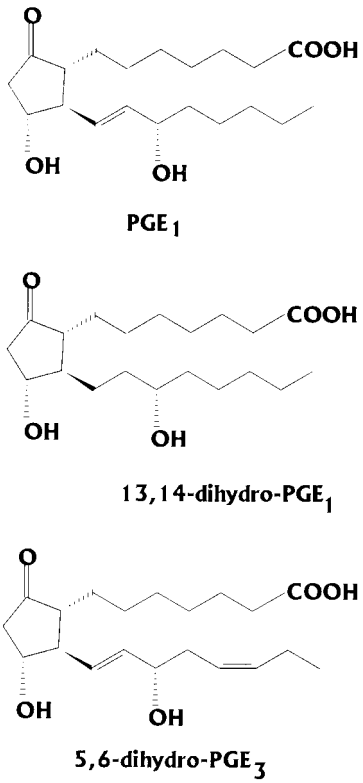

Fig. 1. Chemical structure of the prostaglandins under study.

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

Compound	Healthy subjects		CAD patients		*P*-value
	IC_{50}, nM	*n*	IC_{50}, nM	*n*	
PGE_1	56 ± 5	11	120 ± 20	11	< 0.01
13,14-Dihydro-PGE_1	35 ± 6	11	86 ± 12	11	< 0.01
5,6-Dihydro-PGE_3	58 ± 6	10	110 ± 9	10	< 0.01

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 PGI_2 (Mehta et al., 1980) and to PGE_1 (Sinzinger et al., 1981). This effect may be explained by the fact that PGI_2 synthesis is increased in blood vessels of CAD patients (Fisch et al., 1997) and long-term exposure of platelets to PGI_2 may result in desensitization of PGI_2

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

The research was supported by the Estonian Science Foundation (grant No. 3736).

REFERENCES

Born, G. V. R. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*, **194**, 927–929.

Fisch, A., Tobusch, K., Veit, K., Meyer, J. & Darius, H. 1997. Prostacyclin receptor desensitization is a reversible phenomenon in human platelets. *Circulation*, **96**, 756–760.

Kobzar, G., Mardla, V., Järving, I., Lõhmus, M., Vahemets, A., Samel, N. & Lille, Ü. 1993. Comparison of the inhibitory effect of E-prostaglandins in human and rabbit platelet-rich plasma and washed platelets. *Comp. Biochem. Physiol.*, **106C**, 489–494.

Lõhmus, M., Vahemets, A., Järving, I., Samel, N., Lille, Ü. & Pehk, T. 1990. Preparative separation of natural prostaglandin E. *Prep. Chromat.*, **1**, 279–300.

Mehta, J., Mehta, P. & Conti, C. R. 1980. Platelet function studies in coronary heart disease. IX. Increased platelet prostaglandin generation and abnormal platelet sensitivity to prostacyclin and endoperoxide analog in angina pectoris. *Am. J. Cardiol.*, **46**, 943–947.

Sinzinger, H., Schernthaner, G. & Kaliman, J. 1981. Sensitivity of platelets to prostaglandins in coronary heart disease and angina pectoris. *Prostaglandins*, **22**, 773–781.

Sinzinger, H., Neumann, I., O'Grady, J., Rogatti, W. & Peskar, B. A. 1998. Effects of prostaglandin E_1 metabolites on the induction of arterial thromboresistance. *Prostaglandins Other Lipid Mediators*, **55,** 265–275.

KORONAARTÕVEGA PATSIENTIDE TROMBOTSÜÜTIDE TUNDLIKKUS PROSTAGLANDIINIDE 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 adenosiindifosfaadi toimel indutseeritud trombotsüütide agregatsiooni. Koronaarhaigusega patsientidel oli nende prostaglandiinide antiagregatiivne aktiivsus tunduvalt madalam võrreldes tervete kontrollgrupiga.