Interaction between policosanol and prostacyclin on platelet aggregation in rats

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Palabras clave: policosanol, prostaciclina, interacción, agregación plaquetaria.
Key words: policosanol, prostacyclin, interaction, platelet aggregation.

RESUMEN. El policosanol es una mezcla de alcoholes alifáticos de elevado peso molecular, aislada y purificada de la cera de la caña de azúcar (*Saccharum officinarum*, L.), cuyo principal componente es el octacosanol. El tratamiento oral con policosanol reduce las concentraciones séricas de colesterol en diferentes modelos experimentales, voluntarios sanos y pacientes con hiperlipoproteinemia tipo II. Resultados anteriores han demostrado que la administración oral de policosanol inhibe la agregación plaquetaria inducida *in vivo* por colágeno y *ex vivo* por ADP en ratas. También ha sido demostrado una reducción de las concentraciones séricas de tromboxano B_2 en ratas, Mongolian gerbils y ratones, así como un incremento de la prostaciclina en suero de Mongolian gerbils en animales tratados con policosanol. Estos efectos también han sido observados en voluntarios sanos y pacientes con hipercolesterolemia tipo II. La prostaciclina es el más potente inhibidor de la agregación plaquetaria descubierto hasta el momento. El objetivo de este estudio ha sido investigar los efectos de la interacción del policosanol y la prostaciclina sobre la agregación plaquetaria *ex vivo* en ratas. La sensibilidad del plasma rico en plaquetas (PRP) a la prostaciclina exógena fue evaluada midiendo el porcentaje de inhibición de la agregación inducida por ADP (pre-incubado con prostaciclina) y el porcentaje de desagregación después de la adición de prostaciclina cuando la agregación máxima e irreversible al ADP fue alcanzada. Los resultados de este estudio muestran que no solo, como ha sido reportado en trabajos anteriores, la agregación inducida por ADP en PRP de animales pre-tratados con policosanol (200 mg/kg) fue significativamente menor que en animales controles sino, que la sensibilidad a la prostaciclina exógena fue mayor en el PRP de animales tratados con policosanol.

ABSTRACT. Policosanol is a mixture of higher molecular aliphatic primary alcohols, isolated from sugar cane wax, (*Saccharum officinarum*, L.) whose main component is octacosanol. Oral treatment with policosanol reduced serum cholesterol levels in different experimental models, healthy volunteers and patients with type II hiperliproteinemia. Previous results showed that policosanol orally administered inhibits platelet aggregation induced *in vivo* by collagen and induced *ex vivo* by ADP in rats as well as significantly reduces serum thromboxane B_2 levels in rats, Mongolian gerbils and mice and increases prostacyclin serum levels in Mongolian gerbils. It has been shown that policosanol also exhibits antiplatelet properties in human beings. Prostacyclin (PgI_2) is the most potent endogenous inhibitor of platelet aggregation discovered so far. This study was undertaken to investigate the effect of the interaction of policosanol and PgI_2 on *ex vivo* platelet aggregation in rats. The sensitivity of PRP to exogenous PgI_2 was tested measuring the percentage of inhibition on ADP-induced aggregation (pre-incubation with PgI_2) and the percentage of disaggregation after addition of PgI_2 when the maximal and irreversible response of ADP was reached. The results showed that not only, as has been previously reported, ADP induced aggregation in platelet rich plasma (PRP) of policosanol (200 mg/kg) pretreated animals was significantly lower than in control animals but also that the sensitivity to exogenous PgI_2 of PRP in policosanol pretreated animals was also higher than in the controls.

INTRODUCTION

Policosanol is a mixture of higher molecular aliphatic primary alcohols, isolated from sugar cane wax, (*Saccharum officinarum*, L.) whose main component is octacosanol, followed by triacontanol and hexacosanol, while the other alcohols (tetracosanol, heptacosanol, nonacosanol, dotriacontanol and tetratriacontanol) are minor components.

Oral treatment with policosanol reduced serum cholesterol levels in different experimental models,[1,2] healthy volunteers[3] and patients with type II hiperliproteinemia.[4-9]

Previous results showed that policosanol orally administered inhibits platelet aggregation induced in vivo by collagen and induced ex vivo by ADP in rats[10] as well as significantly reduces serum thromboxane B_2 levels in rats, Mongolian gerbils and mice[10-12] and increases prostacyclin serum levels in Mongolian gerbils.[11]

It has been shown that policosanol also exhibits antiplatelet properties in human beings.[13]

Prostacyclin (PgI_2) is the most potent endogenous inhibitor of platelet aggregation discovered so far. It also disaggregates platelets *in vitro*[14-15] and can prevent or reverse *in vivo* or *ex vivo* platelet aggregation.[16] An increase in cAMP levels of platelets has been proposed be the mechanism of this antiplatelet effect of PgI_2.[17]

There are alternative approaches for enhancing the therapeutic action of PgI_2.One of them is the concomitant use of a phosphodiesterase in-

hibitor such as theophylline or dipyridamole which may potentiate the effect of PgI_2 by preventing the breakdown of intracellular cAMP in platelets.[15-18]

Taking into account these facts this study was undertaken to investigate the effect of the interaction of policosanol and PgI_2 on *ex vivo* platelet aggregation in rats.

MATERIAL AND METHODS

Animals

Male Sprague Dawley rats weighting 250-300 g obtained from Centro Nacional para la Producción de Animales de Laboratorio were used and adapted to experimental conditions for 7 d with food and water *ad libitum*. Rats were randomly distributed in two groups.

Reagents

ADP equine muscle (Sigma); acacia gum (BDH); prostacyclin (Sigma).

Administration and dosage

Policosanol was prepared as a suspension in acacia gum (10 mg/kg) and administered by gavage at 1 mL/200 g body weight. Control animals received the same volume of acacia gum vehicle. All animals were fasted but had free access to water for 16 h prior to the experiment.

PgI_2 was kept in a stock solution (1 mmol/L ethanol) and diluted in Tris HCl buffer pH 7.4 immediately before use.

Platelet aggregation induction

Two hours after treatments all animals were anaesthetized with 40 mg/kg sodium pentobarbital and blood samples were drawn from cava vein and mixed with 3,8 % sodium citrate (9 volumes of blood per 1 of anticoagulant). Blood was centrifuged at 250 g for 10 min to obtain platelet-rich-plasma (PRP). Once PRP was isolated, the remainder was centrifuged at 1 300 g for 15 min to obtain platelet-poor-plasma (PPP).

Platelet aggregation was quantified by Born turbidimetric method.[19] PRP (250 µL) aliquots were preincubated for 2 min at 37 °C on a Elvi 840 aggregometer, stirred at 1 000 r/min and stimulated by the addition of 5 or 12 µmol/L ADP (concentrations obtained in the incubation medium). Platelet aggregation levels were measured after calibrating the equipment at 0 % light transmission for PRP and at 100 % for PPP. Aggregation curves were recorded for 5 min .

The antiaggregatory activity of PgI_2 was assessed by determining the percentage of inhibition on ADP (5 µmol/L)-induced PRP aggregation in the presence of PgI_2 ($1 \cdot 10^{-8}$ mol/L) or Tris HCl buffer added to PRP 1 min before ADP in PRP of controls and policosanol pretreated animals.

The disaggregatory activity of PgI_2 was assessed by determining the percentage of disaggregation on ADP (12µmol/L)-induced PRP irreversible aggregation after the addition of PgI_2 ($2 \cdot 10^{-8}$ mol/L).

Statistical analysis

Comparisons between groups were done using the Mann-Whitney U test.

RESULTS

The results showed that not only, as has been previously reported, ADP induced aggregation in PRP of policosanol (200 mg/kg) pretreated animals was significantly lower than in control animals but also that the sensitivity to exogenous PgI_2 of PRP in policosanol pretreated animals was also higher than in the control's (Table 1).

The sensitivity of PRP to exogenous PgI_2 was tested measuring the percentage of inhibition on ADP-induced aggregation (pre-incubation with PgI_2) and the percentage of disaggregation after addition of PgI_2 when the maximal and irreversible response of ADP was reached (Table 2).

DISCUSSION

A number of disease states have now been related to an imbalance in the vascular PgI_2 and thromboxane A_2 (TxA_2) system.

Patients with arterial thrombosis, deep vein thrombosis and recurrent venous thrombosis produce more of the preoaggregatory compounds: PG endoperoxides and TxA_2 in their platelets. Atheroscelrotic rabbits and patients who have survived myocardial infarction produce more TxA_2 than controls and they are also very sensitive to other aggregating agents. [20-21]

Not only diabetic rats release more TxA_2 than normal rats but also their blood vessels show reduced generation of PgI_2,[22] blood vessels from diabetic patients show reduced production of PgI_2[23, 24] and circulating levels of 6 oxo PgF1a, the metabolite of PgI_2 are reduced in diabetic patients with proliferative retinopathy.[25]

Smooth muscle cells obtained from atherosclerotic lesions and cultured *in vitro* also produced less PgI_2 than normal vascular smooth muscle.[26]

Increased levels of low density proteins (LDL) has been suggested to be a risk factor associated with ischaemic heart disease,[27,28] meanwhile an inverse relation is shown regarding high density lipoprotein (HDL) levels. LDL reduces the generation of prostacyclin-like substance from human endothelial cells whereas HDL stimulated PgI_2 synthesis. It is likely that the lipid per-

Table 1. Sensitive to exogenous PgI_2 in PRP of control and policosanol treated animals.

Treatment	n	Aggregation		Inhibition
		(Buffer)	(PgI_2)	
		(%)		
Control	6	59.5 ± 18.6	38.6 ± 4.0	31.3 ± 6.4
Policosanol 20 mg/kg	6	49.1 ± 20.3	23.5 ± 11.0	46.9 ± 10.0*
Policosanol 200 mg/kg	6	31.2 ± 14.6*	10.6 ± 9.0**	71.0 ± 10.6**

*p < 0.05; **p < 0.01. Mann Whitney U test. Comparisons with control group.

Table 2. Disaggregation induced by PgI_2 in control and policosanol treated animals.

Treatment	n	Disaggregation
		(%)
Control	6	15.4 ± 11.9
Policosanol 20 mg/kg	6	58.8 ± 3.6*
Policosanol 200 mg/kg	6	61.6 ± 15.5*

*p < 0.05 Mann Whitney U test.

oxides found in LDL (and not in HDL) may be responsible for the inhibition of PgI_2 synthesis.[29]

Blood vessels of the brain of all animals species, including man, generate PgI_2.[30,31] Several investigators have suggested that PgI_2 may be involved in the regulation of blood flow in the brain.[32]

These results suggest that prostanoids (TxB_2 and PgI_2) have a role in platelet function and are involved in cardiovascular as well as cerebrovascular disorders.

This concept has provided the basis for the development of compounds with therapeutic potential in reducing intravascular thrombus formation as: cyclooxigenase inhibitors, thromboxane synthetase inhibitors, thromboxane antagonists and PgI_2 analogues.

Previous results have shown that single doses of policosanol not only are able to reduce TxA_2 and malondyaldehide,[10] but also increases PgI_2 serum levels in several animal models,[11] suggesting an thromboxane synthetase inhibitor like effect.

On the other hand, platelets of hyperlipoproteinemic (HLP) patients exhibit a significantly reduced sensitivity to prostacyclin,[33] iloprost and PgE1.[34] Thus, considerably higher concentrations of these prostaglandins are required to inhibit platelet function in HLP than in healthy controls.

Oliva *et al.*[34] have shown that the number of prostacyclin binding sites in platelet membranes, prepared from platelet –rich plasma, was significantly reduced in type IIa HLP whereas the K_D, indicating the affinity of the binding sites, remained unchanged.

Jaschonek *et al.*[35] using cholesterol-hemisuccinate-loaded platelets, have shown that incorporation of cholesterol results in a decrease of membrane fluidity and an apparent loss of iloprost binding sites while administration of local anesthetics, leading to increased membrane lipid fluidity, exerted the opposite effect.

It has been argued that if the changes in platelet reactivity, sensitivity to PgI_2, and eicosanoids biosynthesis reported in hypercholesterolemia are a direct consequence of elevated plasma lipid levels, then one would expect that cholesterol lowering by dietary means, plasmapheresis, or lipid-lowering drugs would result in normalization of platelet function.[36] In fact, LDL apheresis, fibric acid derivatives, ion exchange resins, probucol, and HMG-CoA reductase inhibitors have all been evaluated for their effects on platelet function. In these studies, except those carried out with simvastatin, despite LDL-C reductions on the order of 10 to 30 % , no change in platelet aggregation[37] and platelet sensitivity to iloprost[38] could be demonstrated *ex vivo*.

The results of the present work show that policosanol at the same doses reduces thromboxane and increases PgI_2 serum levels, enhances exogenous PgI_2 antiaggregatory effect in rats.

The fact that policosanol increases not only PgI_2 serum levels but also the sensitivity of exogenous PgI_2 suggests that it could act preventing the breakdown of PgI_2 to its inactive metabolite (6 oxo PgF1a).

Although the exact mechanism of antiplatelet action of policosanol is unknown, these results can explain at least partially the protective effect of policosanol on atherosclerotic lesions in experimental models[39] and human beings.[40]

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