Comparative study of policosanol and grape seed extract on platelet aggregation in rats

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RESUMEN. La enfermedad arterial coronaria constituye una de las causas principales de morbilidad y mortalidad en el mundo. Las plaquetas están involucradas en el desarrollo de la enfermedad aterosclerótica, por lo que la reducción de la actividad plaquetaria mediante el uso de medicamentos reduce la incidencia y severidad de esta enfermedad. El policosanol, mezcla de alcoholes alifáticos primarios de alto peso molecular obtenida de la cera de caña y el extracto de semilla de uva, un producto natural que contiene derivados polifenólicos, poseen propiedades antiplaquetarias demostradas en animales de experimentación y en humanos. Este trabajo comparó los efectos del policosanol y el extracto de semilla de uva sobre la agregación plaquetaria inducida *ex vivo* por ADP y colágeno en plasma rico en plaquetas de ratas. Las ratas se distribuyeron en siete grupos: un control tratado con el vehículo y seis grupos que recibieron dosis orales únicas de policosanol (25,50 y 200 mg/kg) y extracto de semilla de uva (25,50 y 200 mg/kg). La administración oral de dosis únicas de policosanol y extracto de semilla de Uva a ratas produjo una reducción significativa de la agregación plaquetaria inducida *ex vivo* por ADP y colágeno cuando se comparó con el grupo control. No se encontraron diferencias significativas al comparar similares dosis de policosanol y extracto de semilla de uva, lo cual indica que la potencia y eficacia antiplaquetaria fue similar. Ambas sustancias fueron más efectivas para reducir la agregación al colágeno que al ADP.

ABSTRACT. Coronary artery disease constitutes a major cause of morbidity and mortality worldwide. Since platelets are involved in the development of atherosclerosis, the use of anti-platelet agents reduces the incidence and severity of this disease. Both policosanol, a mixture of primary high molecular weight aliphatic alcohols purified from sugar cane wax, and grape seed extract (GSE), which mainly contain polyphenolic compounds, have been shown antiplatelet activity in experimental and clinical studies. This work compared the effects of policosanol and GSE on platelet aggregation induced *ex vivo* by ADP and collagen in plasma rich in platelets of rats. Rats were randomized into seven groups: a vehicle control, three groups treated with policosanol (25.50 and 200 mg/kg, respectively) and three groups with similar doses of GSE. Single oral doses of policosanol and GSE administered to rats significantly inhibited ADP-and collagen–induced platelet aggregation compared with the control group. ADP-induced aggregation was inhibited up to about 23 % by the highest dose of policosanol (22.8 %) and GSE (22.6 %). In turn, collagen induced aggregation was reduced up to 78.6 % (policosanol) and 83.7 % (GSE). The effects of Policosanol, not of GSE, on collagen-induced platelet aggregation were dose dependent, meanwhile the effects of both substances on ADP-induced platelet aggregation were devoid at dose dependence. Comparisons between treated groups did not show significant differences. In conclusion, both substances reduced similarly platelet aggregation to ADP and collagen being more effective in collagen induced platelet aggregation.

INTRODUCTION

Coronary artery disease (CAD) constitutes a major cause of morbidity and mortality worldwide. Since platelets are involved in the development of atherosclerosis and superimposed thrombosis,[1] the use of anti-platelet agents reduces the incidence and severity of CAD.[2,3]

Policosanol is a mixture of high molecular weight of primary aliphatic alcohols purified from sugar cane wax, with antiplatelet activity demonstrated in experimental[4,5] and clinical studies,[6-8] which has been associated with a decreased serum levels of thromboxane A2 (TxA_2) and increased levels of prostacyclin (PgI_2).[9,10]

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Grapes (*Vitis vinifera*) and grape products contain polyphenolic compounds, including flavonoids, which have antioxidant and anti-thrombotic properties.[11,12] Grape seed extract (GSE) is a nutritional supplement with *in vitro* antiplatelet effects associated to its antioxidant activity.[13] There are few reports about the *ex vivo* antiplatelet effects of GSE. So, Shanmuganayagam and cols. (2002) reported an *ex vivo* antiplatelet effect of GSE in combination with grape skin extract (GSK) in dogs feeding during eight days, but the extracts individually did not affect platelet aggregation.[14] Interestingly, a clinical study showed that *ex vivo* platelet aggregation in male smokers was inhibited after single feeding with a flavanol-rich GSE.[15] Procyanidin, however, a polyphenol contained in GSE, has been shown both *in vitro* and *in vivo* anti-thrombotic effects in mice.[16]

In light of these facts, this work was undertaken to compare the effects of Policosanol and GSE on *ex vivo* induced platelet aggregation in rats.

MATERIALS AND METHODS

Animals

Adult male Sprague Dawley rats, weighing 200-250 g, were purchased from the National Centre for Laboratory Animals Production (CENPALAB, Havana, Cuba). The animals were adapted to laboratory conditions (temperature (25 ± 3) °C, relative humidity (60 ± 5) %, light/dark cycles of 12 h) for seven days. Food (rodent chow obtained from CENPALAB) and water were provided *ad libitum*.

Animal handling was conduced in accordance with the Cuban Regulations and ethical principles for animal management. Experiments were carried out following Good laboratory Practices. An independent ethical board approved the study protocol and the use of the animals.

Administration and dosage

Policosanol was supplied by the Plant of Natural Products purchased of the National Center for Scientific Research (Havana City, Cuba), after corroborate that they met the quality criteria for batch release. The composition of policosanol batch was tetracosanol 0.07 %, hexacosanol 4.9 %, heptacosanol 0.8 %, octacosanol 63.8 %, nonacosanol 0.5 %, triacontanol 12.8 %, dotriacontanol 6.8 %, tetratriacontanol 2.4 %.

GSE (85 % in proanthocyanidine) came from Blackmores (Sydney, Australia).

Policosanol and GSE powders were suspended in acacia gum/H_2O (10 mg/mL) and administered as single oral doses by gastric gavages two hours before blood extraction.

Collagen and ADP were purchased from Sigma, and used as aggregating reagents.

Experiment design

Rats were randomized into seven groups (10 rats /group): a vehicle control, three groups treated with policosanol (25, 50 and 200 mg/kg, respectively) and three with similar doses of GSE.

The treatments were administered two hours before platelet aggregation determination. All rats were anaesthetized with thiopental (40 mg/kg) and blood samples were drawn from vena cava and mixed with 3.8 % (w/v) sodium citrate (9 volumes of blood per of anticoagulant). Blood was centrifuged at 1 000 r/min for 10 min to obtain platelet-rich plasma (PRP). Once PRP was isolated, the remainder was centrifuged at 2 500 r/min for 10 min to obtain platelet-poor plasma (PPP). Platelet aggregation was quantified by Born turbidimetric method.[17] In brief, PRP 250 μL aliquots were pre-incubated for 2 min at 37 °C on a Biodata Corporation (USA) aggregometer, at 1 000 r /min and stimulated by the addition of ADP ($1{,}3 \cdot 10^{-5}$ mol/L) and collagen (16 μg/mL). Platelet aggregation was determined by calibrating the equipment at 0 % light transmission for PRP and at 100 % for PPP. Aggregation curves were recorded for 5 min and were expressed in aggregation percentages.

Statistical analysis

Comparisons between groups were performed with the Kruskal Wallis test and paired comparisons versus the control group with the Mann Whitney U test. The level of statistical significance was set at $\alpha = 0.05$. All analyses were performed using Statistics software for Windows (Release 6.0).

RESULTS

The effects of single oral doses of policosanol and GSE on platelet aggregation induced *ex vivo* by ADP in PRP of rats are shown in Table 1. Policosanol (50 and 200 mg/kg) significantly, but modestly reduced ADP induced-platelet aggregation (24.4 and 22.2 %, respectively), and similar effects were obtained with GSE (50 and 200 mg/kg) (24.4 and 22.6 %, respectively).

The effects on collagen induced platelet aggregation are shown in Table 2. Policosanol (25.50 and 200 mg/kg) significantly, markedly and dose dependently inhibited collagen-induced platelet aggregation (56.2; 63.7 and 78.6 %, respectively). Similar effects, but not dose dependent, were obtained with GSE (62.5; 65.7 and 83.7 %, respectively).

Table 1. Effect of policosanol and GSE on platelet aggregation induced *ex vivo* by ADP in rats.

Groups	Doses (mg/kg)	Aggregation (%) ADP ($1{,}3 \cdot 10^{-5}$ mol/L)	Inhibition (%)
Control	0	42.9 ± 2.9	—
Policosanol	25	37.9 ± 2.4	11.6
Policosanol	50	32.4 ± 2.3 **	24.4
Policosanol	200	33.1 ± 1.9 **	22.8
GSE	25	34.8 ± 3.3	18.8
GSE	50	32.4 ± 2.9 *	24.4
GSE	200	33.2 ± 1.6 **	22.6

GSE Grape seed extract * $p < 0.05$; ** $p < 0.01$ Comparison with control group.

Table 2. Effect of policosanol and GSE on platelet aggregation induced *ex vivo* by collagen in rats.

Groups	Doses (mg/kg)	Aggregation (%) (collagen 16 μg/mL)	Inhibition (%)
Control	0	35.0 ± 6.4	—
Policosanol	25	15.3 ± 6.6*	56.2
Policosanol	50	12.7 ± 4.2*	63.7
Policosanol	200	7.5 ± 4.6**	78.6
GSE	25	13.1 ± 7.5 *	62.5
GSE	50	12.0 ± 3.4*	65.7
GSE	200	5.7 ± 3.8**	83.7

GSE Grape seed extract * $p < 0.05$; ** $p < 0.01$ Comparison with control group.

DISCUSSION

This study demonstrates that single oral doses of both policosanol and GSE (25-200 mg/kg) significantly inhibited platelet aggregation induced *ex vivo* by ADP and collagen in rats. Both substances were more effective in collagen than in ADP-induced aggregation as indicates the effect achieved with the highest dose used (78.6 and 83.7 %, respectively). Despite these inhibitions were marked we can't affirm that they correspond to the maximal effects since no *plateau* effect was obtained and no higher doses were assayed, a fact that remarks the high efficacy of policosanol and GSE for lowering collagen-induced platelet aggregation. Thus, policosanol and GSE were much more effective for inhibiting collagen than ADP induced platelet aggregation.

Dose dependence only was observed with policosanol for inhibiting collagen-induced platelet aggregation, while the other dose-schemes fall to produce a response-dose relation.

The platelet physiology is different among the diverse animal species. So, the reactivity of platelet of rat is not the same against different agonists.[18] In PRP of rats, ADP produces a unique aggregation curve without formation of TxA_2 or serotonine, and without ATP secretion.[17] Then, the ADP receptor antagonists like ticlopidine or clopidrogel are very effective for inhibiting ADP-induced platelet aggregation.[20, 21]

On the other hand, collagen only produces platelet aggregation at concentrations higher than those required for inducing aggregation in human platelets.[19] Indeed, collagen induces changes of form, aggregation and secretion of platelets that can be suppressed by inhibitors of prostaglandins generation, like indomethacin or aspirin,[22] and wherein endogen TxA_2 plays an essential role.[23]

The mechanism whereby policosanol and GSE inhibit significantly ADP-induced aggregation is unclear, but the fact that this effect is modest suggests that it does not involve the antagonism of ADP receptor, being rationale to suspect that it could be related with prostaglandin synthesis inhibition.

The marked reductions of collagen-induced aggregation by Policosanol, however, agree with previous reports. Thus, policosanol has been shown to decrease TxA_2 and to increase PgI_2 serum levels,[9,10] and to reduce lipid peroxidation in PRP after aggregation process.[5] On the other hand, the antiplatelet effects of GSE have been related with the antioxidant capacity of its flavonoids, which decrease the oxygen reactive species formed during platelet aggregation.[24] Although no specific mechanistic proof support specific effect on aggregation to collagen, since an effect of GSE on TxA_2 serum levels has not been reported. (Entrez Pubmed review from 2000-2010).

In light of these facts, we think that the dose-dependence relationship observed with the policosanol for inhibiting platelet aggregation against collagen could be associated to TxA_2 reducing effects previously reported, due to the important role of TxA_2 in collagen-induced platelet aggregation but not by ADP in PRP of rats. An explanation about the absence of dose-effect relation of GSE on platelet aggregation here observed could be linked to non-effect on TxA_2 synthesis; however this point must be clarified for further studies.

In conclusion, both substances reduced similarly platelet aggregation to ADP and collagen being more effective in collagen-induced than in ADP-induced platelet aggregation.

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