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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 morbidad 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 antiplaquetarias fue similar. Ambas sustancias fueron más efectivas para reducir la agregación al colágeno que al ADP.

Palabras clave: policosanol, extracto de semilla de uva, agregación plaquetaria, ratas.

Key words: policosanol, grape seed extract, platelet aggregation, rats.

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 experimental4,5 and clinical studies,6-8 which has been associated with a decreased serum levels of thromboxane A2 (TxA2) and increased levels of prostacyclin (PgI2).9,10

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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
Grapes (*Vitis vinifera*) and grape products contain polyphenolic compounds, including flavonoids, which have antioxidant and anti-thrombotic properties. 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. Interestingly, a clinical study showed that ex vivo platelet aggregation in male smokers was inhibited after single feeding with a flavonol-rich GSE. Procyanidin, however, a polyphenol 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 acaic gum/H 2O (10 mg/mL) and administered as single oral doses by gavage two hours before blood extraction. Collagen and ADP were purchased from Sigma, used as aggregating reagents.

**Experiment design**

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

The treatments were administered two hours before the platelet aggregation determination. All rats were anesthetized 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. 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 with the addition of ADP(1.3·10−5 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 as 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 α = 0.05. All analyses were performed using Statistics software (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, and dose dependently inhibited collagen-induced platelet aggregation (62.2; 63.7 and 78.3 %, respectively). Similar effects, but not dose dependent, were obtained with GSE (62.5; 63.7 and 83.7 %, respectively).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>Aggregation (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>42.9 ± 2.9</td>
<td>—</td>
</tr>
<tr>
<td>Policosanol</td>
<td>25</td>
<td>37.9 ± 2.4</td>
<td>11.6</td>
</tr>
<tr>
<td>Policosanol</td>
<td>50</td>
<td>32.4 ± 2.3 **</td>
<td>24.4</td>
</tr>
<tr>
<td>Policosanol</td>
<td>200</td>
<td>33.1 ± 1.9 **</td>
<td>22.8</td>
</tr>
<tr>
<td>GSE</td>
<td>25</td>
<td>34.8 ± 3.3</td>
<td>18.8</td>
</tr>
<tr>
<td>GSE</td>
<td>50</td>
<td>32.4 ± 2.9 *</td>
<td>24.4</td>
</tr>
</tbody>
</table>
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 indicated 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. In PRP of rats, ADP produces a unique aggregation curve without formation of TxA2 or serotonin, and without ATP secretion. Then, the ADP receptor antagonists like ticlopidine or clopidogrel are very effective for inhibiting ADP-induced platelet aggregation.

On the other hand, collagen only produces platelet aggregation at concentrations higher than those required for inducing aggregation in human platelets. Indeed, collagen induces changes of form, aggregation and secretion of platelets that can be suppressed by inhibitors of prostaglandins generation, like indomethacin or aspirin, and wherein endogenous TxA2 plays an essential role. 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 rational 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 TxA2 and to increase PGI2 levels, and to reduce lipid peroxidation in PRP after aggregation process. On the other hand, the antplatelet effects of GSE have been mechanistic proof support specific effect on aggregation to collagen, since an effect of GSE on TxA2 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 TxA2 reducing effects previously reported due to the important role of TxA2 in collagen-induced platelet aggregation but not by ADP in PRP of rats. An explanation about the absence of dose-effect relationship of GSE on platelet aggregation here observed could be linked to non-effect on TxA2 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.

BIBLIOGRAPHIC REFERENCES


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

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

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


