BRIEF COMMUNICATION

IN VITRO EFFECT OF MOPIDAMOL ON PLATELET-SUBENDOTHELIUM INTERACTION

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Chemical substitutions in the structure of the pyrimido-pyrimidine nucleus give rise to pyrimido-pyrimidine compounds with platelet antiaggregatory effects, such as RA-8 (dipyridamole), RA-233 (mopidamol) and RA-642. All these compounds inhibit platelet aggregation-induced by ADP through an increase in platelet cAMP levels (1,2). Mopidamol, however, is 3-10 times more potent than dipyridamole and RA-642 both in whole blood and platelet-rich plasma (3) and determines an increase in cAMP levels which is almost 22 times greater than that caused by equimolar concentrations of dipyridamole and RA-642 (2). In contrast to dipyridamole which causes an increase in prostacyclin synthesis, the effect of mopidamol on arachidonic acid metabolism is negligible. On the other hand, platelet thromboxane synthesis is inhibited by mopidamol but unaffected by dipyridamole (4).

Experimental studies of platelet-vessel wall interaction in flowing systems have demonstrated that the formation of platelet adhesions is inhibited by phosphodiesterase inhibitors, such as dipyridamole, whereas the formation of aggregates is particularly reduced by inhibitors of thromboxane synthesis (5-7). Given that mopidamol affects these two mechanisms, a reduction in the formation of both platelet adhesions and aggregates may be expected. To confirm this hypothesis, we have studied comparatively the in vitro effects of mopidamol and dipyridamole on platelet interaction with subendothelium using the Baumgartner perfusion system.

Key words: Mopidamol, Dipyridamole, Mopidamol, Platelets, Subendothelium.
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MATERIALS AND METHODS

Materials.
The annular perfusion chamber was manufactured by Labotron, S.A. (Barcelona, Spain). Dipyridamole was obtained from Boehringer Ingelheim España, S.A. (Barcelona, Spain) and mopidamol from Dr. Karl Thomae Institute (Biberach an der Riss, Germany). The rest of the reagents were obtained from Sigma Chemical Co., (St. Louis, MO, USA).

Design of the study.
Dipyridamole or mopidamol at a final concentration 10 μmol/L were incubated in blood samples from healthy male volunteers (mean age 36.4 ± 2.1 years) who had not received medication known to modify platelet response for 15 days prior to the experiment. The incubation of both pyrimido-pyrimidine derivatives or isotonic saline solution (controls) was maintained at 37°C for 5 min prior to initiation of perfusion studies.

Perfusion studies.
Perfusion studies were carried out in the annular chamber according to the method described by Baumgartner et al. (8,9) with some modifications. Briefly, vessel wall segments were obtained from New Zealand albino male rabbits weighing between 2 and 2.5 kg. Animals were anaesthetized with sodium pentobarbital (100 mg/kg) and their thoracic and abdominal aortas were removed. Aortas were washed with cold phosphate buffer saline (PBS) 0.1 M (pH 7.4), dissected from adjacent fatty tissue, and cut into segments of 1 cm in length which were maintained in PBS. Artery segments were then turned outward, so that endothelial cell layer surfaces were left exposed to the outside. Samples were incubated in a solution of ß-chymotrypsin (0.4 mg/ml in a Tris-calcic buffer 0.1 M) at 37°C for 12 hours while being continuously shaken in order to remove completely the endothelial cell layer. The absence of endothelium was confirmed by scanning electron microscopy in three randomly selected arterial samples from each animal. The remaining arterial segments were kept frozen in PBS at -80°C before perfusion studies which were always carried out within 7 days after removal of the epithelium.

Everted segments from rabbit aorta were mounted in the rod of the annular perfusion chamber and exposed to flowing blood (20 ml) during 10 min at 37°C (wall shear rate 800 s⁻¹). After exposure to blood, the vessel segments were rinsed in PBS, fixed in formaldehyde 15% for 15 min at room temperature, and cut into longitudinal sections which were subsequently fixed in a new solution of formaldehyde for 48-72 hours. Segments were washed in buffer, dehydrated in graded series of alcohol, incubated in xylene, and embedded in paraffin. Blocks from each arterial segment were divided into six parts and 20 series of non-consecutive axial slices of 4 μm thickness were obtained (total 120 sections per arterial segment). Slices were stained with haematoxylin-eosin and examined with a Nikon Labophot-2 microscope (x1,250 magnification) through which section images were projected onto a millimetric graphic scale.

Evaluation of sections was made by one observer who was blind to the origin of the samples. Platelets interacting with subendothelium were assessed at each 10 μm areas according to Baumgartner et al. (8,9) as follows: contact (C), platelets that were attached but not spread onto the subendothelium; adhesion (A), platelets spread on and firmly bound to the subendothelium forming layers of less than 5 μm in height; thrombi (T), were defined as platelet aggregates of 5 μm or more in height. Spread platelets were always observed underneath platelet thrombi. The number of activated platelets (AP) was obtained by adding A + T. Total covered surface was expressed as C + AP. A total of 985 ± 26 interacting points pertaining to each arterial segment
were screened. Before and immediately after blood perfusion, blood cell counts were carried out in duplicate using an automatized counter (Baker 8000, Menarini, Barcelona, Spain).

**Statistical analysis.**
The Student's *t* test for paired data was applied for statistical analysis using the Epistat<sup>®</sup> computer program (T.L. Gustafson, USA, 1985). Statistical significance was set at *p* < 0.05. All values in text, tables and figures are presented as mean ± SEM.

**RESULTS**

The percentage of red blood cells and leukocytes found on the subendothelium did not show a statistically significant variation following perfusion of blood samples incubated with physiologic salt solution; however, almost 30% of platelets were found on the subendothelium. In contrast, the percentage of platelets showed a decrease of 31.5% in perfusion studies with blood samples incubated with dipyridamole and a decrease of 63.7% in those incubated with mopidamol (Table I).

With regard to morphological evaluation of platelet-vessel wall interaction, dipyridamole and mopidamol caused a decrease of 9.1% and 38% respectively, in the subendothelium surface covered by platelets (Table II). Both pyrimido-pyrimidine derivatives produced an increase in the surface of subendothelium covered by contacts, whereas the surface of subendothelium covered by platelet adhesions showed a 25% and 20% decrease in perfusion studies with dipyridamole and mopidamol, respectively (Table II).

The mean surface of subendothelium covered by platelet aggregates was significantly reduced by mopidamol (18% inhibition) but remained unchanged in the experiments using dipyridamole (1.2% reduction).

The percentage of subendothelium surface covered by activated platelets (adhesions and aggregates) showed a statistically significant decrease following incubation with mopidamol (32.8% inhibition), whereas the decrease caused by dipyridamole (12.2% inhibition) was not significant (Figure 1).

**DISCUSSION**

The present study demonstrates that there is a clear activation of platelets as a result of platelet-vessel wall interaction following blood perfusion in the annular chamber of Baumgartner with a wall shear rate of 800 s<sup>-1</sup>. Our results of the morphological analysis of control samples are quantitatively lower than those reported by others including Baumgartner et al. (9), although similar than morphometric data of Aznar-Salatti et al. (6) using the perfusion chamber of Sakariassen et al. (10) and subendothelium from human endothelial cell culture. In these studies, however, the more prolonged perfusion time (20-30 min) contributed to induce a greater platelet activation.

Mopidamol and dipyridamole were incubated at a concentration of 10 μmol/L. This concentration is included within the range of plasma levels following *ex vivo* administration of these compounds, and coincides with the range of concentration that produces 50% inhibition of platelet aggregation (IC<sub>50</sub>) in whole blood (3-5, 11-13).
### TABLE I

Percentage of Platelets, Red Blood Cells and Leukocytes on the Subendothelium Following 10-min Perfusion (800 s⁻¹) in Control Samples and Samples Incubated with 10 µmol/L of Dipyridamol and Mopidamol

<table>
<thead>
<tr>
<th>Blood Cells</th>
<th>Percentage of Change versus Pre-Perfusion Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Platelets</td>
<td>-29.8 ± 2.6</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>+2.0 ± 0.3</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>+3.4 ± 0.3</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>+0.6 ± 0.2</td>
</tr>
</tbody>
</table>

Minus sign: decrease; plus sign: increase. ¹p = 0.013 vs. controls; ²p = 0.0003 vs. controls and p = 0.0008 vs. dipyridamole group.

### TABLE II

Percentage of subendothelium surface occupied by platelets following 10-min perfusion (800 s⁻¹) in control samples and samples incubated with 10 µmol/L dipyridamole or mopidamol

<table>
<thead>
<tr>
<th>Covered by</th>
<th>Subendothelium surface (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Platelets</td>
<td>18.7 ± 1.1</td>
</tr>
<tr>
<td>Contacts</td>
<td>23.1 ± 3.2</td>
</tr>
<tr>
<td>Adhesions</td>
<td>38.5 ± 4.7</td>
</tr>
<tr>
<td>Aggregates</td>
<td>38.2 ± 4.5</td>
</tr>
</tbody>
</table>

¹p = 0.004 vs. controls and p = 0.0016 vs. dipyridamole. ²p = 0.04 vs. controls; ³p = 0.05 vs. controls; ⁴p = 0.007 vs. controls and p = 0.001 vs. dipyridamole.

With regard to the effect of the pyrimido-pyrimidine derivatives on platelet interaction with subendothelium, mopidamol exerted a greater inhibitory effect on platelet retention in the vascular sample. This finding is in agreement with the more potent inhibition of either ADP or collagen-induced platelet aggregation in whole blood or platelet-rich plasma caused by mopidamol as compared with dipyridamole and RA-642 (3,4).
In the perfusion system used in our study, those agents that increase intraplatelet cAMP levels cause a decrease in the formation of platelet adhesions and show a mild effect on the formation of aggregates. These coincide with our results, since both dipyridamole and mopidamol are phosphodiesterase inhibitors. With respect to mopidamol, no data has been published in literature with which the results of our study could be compared. Only Lichtner et al. (14) and Gastpar et al. (15) have reported that mopidamol inhibited platelet adhesion to cancer cells and vice versa. Based on this effect, mopidamol has been used as adjuvant medication in antimetastatic chemotherapy.

The fact that generation of thrombi in the subendothelium largely depends upon complete granular release and platelet thromboxane synthesis (9,16) explains the differential effects of mopidamol and dipyridamole on the formation of platelet aggregates. In contrast to dipyridamole which does not inhibit thromboxane synthesis at therapeutic concentration range, mopidamol inhibits thromboxane synthesis without affecting vascular prostacyclin biosynthesis. The dose of 16 μmol/L of mopidamol that produced 50% inhibition of thromboxane synthesis (4) is very similar to that used in this study. Moreover, mopidamol caused a reduction in the overall size of platelet aggregates.

Given the whole effect on activated platelets, the subendothelium surface covered by platelet adhesions and aggregates was smaller in blood samples incubated with mopidamol, since this compound shows a similar effect to that of dipyridamole with respect to the inhibition of adhesions and a higher inhibitory effect on the formation of aggregates.

In summary, characteristic biochemical properties of dipyridamole and mopidamol result in different effects on in vitro platelet-vessel wall interaction in a flowing system.
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REFERENCES