ANTIPLATELET EFFECT OF PENTOXIFYLLINE IN HUMAN WHOLE BLOOD

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Abstract—1. Pentoxifylline inhibits platelet aggregation in whole blood more than in platelet-rich plasma.
2. An inhibition of the erythrocyte uptake of adenosine contributes to the antiaggregatory effect of pentoxifylline.

INTRODUCTION

Pentoxifylline [1-(5'-oxohexyl)-3,7-dimethylxanthine] is a hemorrheologic drug prescribed for the treatment of peripheral vascular disease (Müller, 1981). The mechanism of action appears to be related to improve membrane fluidity in red blood cells (Aviado and Dettelbach, 1984); its hemorrheological effect reduces blood viscosity and increases erythrocyte deformability (Angelkort et al., 1979). Other contributing effects to pentoxifylline action include inhibited platelet aggregation (Ambrus et al., 1979), increased white cell deformability and decreased leukocyte adhesiveness (Schmalzer et al., 1984). Pentoxifylline is known to increase cyclic AMP (cAMP) levels in polymorphonuclear cells (Bessler et al., 1986) and in endothelial cells (Stefanovich, 1973), which reduces superoxide anion generation (Ciuffetti et al., 1991); this inhibition seems to be the mechanism underlying the vasoactive effect mediated by endothelium-derived relaxing factor (EDRF) (Berkenboom et al., 1991).

An increase in platelet cAMP (via inhibition of membrane-bound phosphodiesterase) (Stefanovich, 1974) and an increase in prostacyclin synthesis by the endothelium (Matzky et al., 1982), seem to be the mechanisms of its antiaggregatory effect. Most of the investigations about this effect have been carried out in platelet-rich plasma (PRP) aggregometry; other drugs that inhibit cAMP-dependent phosphodiesterase, such as dipyridamole, show a higher antiplatelet effect when erythrocytes are present in the sample. The aim of the present study was to assess whether or not pentoxifylline exerts a different antiplatelet behavior in whole blood vs PRP samples in human blood.

MATERIAL AND METHODS

The study was divided into two phases: (a) "In vitro" experiments, involving determination of the antiaggregatory dose–response relationships of pentoxifylline in whole blood (WB) vs PRP, and determination of the possible effect on erythrocyte uptake of 8-14C-adenosine and on arachidonate-induced platelet thromboxane B2 production; (b) "Ex vivo" experiments: 10 male healthy volunteers (26.8 ± 1.5 yr old) participated in the study after giving informed consent. None of them had ingested medication for at least 15 days prior to the experience. Under basal conditions, blood was drawn from the antecubital vein and 30 min later, pentoxifylline (Elorgan®, Hoechst Ibérica S.A., Barcelona, Spain) was administered in a dose of 1200 mg/day (400 x 3 mg/day) for 15 days. One hour after the last dose, a blood sample was drawn again. In each blood sampling, platelet aggregometry in WB and PRP, erythrocyte uptake of 8-14C-adenosine and arachidonate-induced platelet thromboxane B2 production, were assessed.

Platelet aggregometry

Platelet impedance aggregations in WB and PRP were measured with a Chrono-Log dual channel aggregometer (Chrono-Log Corp., Haverton P.A.), according to the method described by Cardinal and Flower (1980). Blood was anticoagulated with 3.8% trisodium citrate in the proportion 1:10. PRP was prepared by centrifugation of blood at 180g for 10 rain, and platelet-poor plasma (PPP) was obtained by centrifugation of PRP at 1800g for 15 min. In the studies with WB, the samples were diluted with isotonic saline until 32-35% hematocrit was achieved (Cardinal and Flower, 1980); PRP samples were also diluted with PPP until the same platelet count in the homologous diluted WB was achieved.

Platelet aggregation was induced by ADP, adrenaline, collagen or arachidonic acid, in different final concentrations (Diagnostica Stago, Asnières-sur-Seine, France; Bio-Data Corp., Hatboro, PA, U.S.A.). All tests were made at 37°C, with a sample agitation speed of 1000 rpm. Platelet aggregation was measured as the maximum increase in impedance (ohms), 10 min after the addition of the aggregating agent. In the in vitro experiments, the IC50 value (concentration of pentoxifylline producing 50% inhibition of the maximum aggregation) was calculated graphically from each experience.

Erythrocyte uptake of 8-14C-adenosine

Uptake of 8-14C-adenosine (Amersham International plc, Amersham, U.K., specific activity 53 µCi/mmol) by erythro-

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Table 1. Inhibitory concentrations 50% (IC50) of pentoxifylline on platelet aggregation

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Whole blood</th>
<th>Platelet-rich plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (1.2 μmol/l)</td>
<td>168 ± 23</td>
<td>733 ± 62*</td>
</tr>
<tr>
<td>Adrenaline (12.5 μmol/l)</td>
<td>348 ± 40</td>
<td>895 ± 78**</td>
</tr>
<tr>
<td>Collagen (1 μg/ml)</td>
<td>221 ± 27</td>
<td>664 ± 32*</td>
</tr>
<tr>
<td>Arachidonic acid (0.8 mmol/l)</td>
<td>594 ± 25</td>
<td>615 ± 23</td>
</tr>
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</table>

* P < 0.0001; ** P < 0.005 vs whole blood values. Each value is mean of 10 independent experiments.

cytes has been determined by a method described by Roos and Pfleger (1972). Briefly, blood samples were centrifuged at 1800 g for 15 min, and 3 ml of the cell sediment was diluted 1:1 with Tris(5 mmol/l)-NaCl(153.8 μmol/l)-glucose(5 mmol/l) (pH 7.4); the cell suspension was centrifuged at 1000 g for 20 min, and the supernatant was discarded. The process was repeated twice. Erythrocyte sample was diluted with the same buffer to 200 red cells x 10⁹/l.

Ten microliters of 1 nmol/l of 8-14C-adenosine were added to the appropriate aliquots of red cells, and the samples were incubated at 37°C for 30 min. Reaction was stopped by cooling at 0°C for 1 min. Four control samples with 1 mmol/l of cold adenosine (Sigma Chemical Co, St Louis, MO, U.S.A.) were processed simultaneously to determine the non-specific uptake of 8-14C-adenosine. The red cell samples were centrifuged at 10,000 g for 3 min; the supernatant and pellet were each mixed with 5 ml of scintillation liquid and counted in a Betamatic counter (Kontron, Switzerland). Uptake of 8-14C-adenosine was calculated as the percentage of radioactivity in the pellet.

Platelet production of thromboxane B₂

The blood samples used in the arachidonate-induced platelet aggregation were centrifuged at 10,000 g for 3 min, after adding 100 μmol/l of indomethacin (Sigma Chemical Co, St Louis, MO, U.S.A.). Supernatants were frozen at −80°C until testing. Levels of thromboxane B₂ were determined by radioimmunoassay using a commercial kit (1H-Thromboxane B₂, Amersham International plc, Amersham, U.K.) (Granstrom et al., 1976).

RESULTS

In vitro experiments

Pentoxifylline significantly reduced platelet aggregation, both in WB and in PRP, induced by all the aggregating agents (Table 1). IC₅₀ values were statistically higher in PRP than in WB, except when arachidonic acid was used as inducer.

Incubation of erythrocytes with platelets raised the antiaggregatory effect of pentoxifylline to that obtained in ADP-induced platelet aggregation in WB (Fig. 1). In erythrocytes, pentoxifylline inhibits the 8-14C-adenosine in a concentration-dependent manner (Table 2).

Table 2. Inhibition of erythrocyte uptake of 8-14C-adenosine by pentoxifylline

<table>
<thead>
<tr>
<th>Pentoxifylline concentration (mmol/l)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>8.5 ± 2.8</td>
</tr>
<tr>
<td>0.25</td>
<td>10.5 ± 3.3</td>
</tr>
<tr>
<td>0.50</td>
<td>22.3 ± 4.6</td>
</tr>
<tr>
<td>1</td>
<td>40.0 ± 4.5</td>
</tr>
<tr>
<td>1.50</td>
<td>52.9 ± 4.8</td>
</tr>
<tr>
<td>2</td>
<td>72.1 ± 3.5</td>
</tr>
</tbody>
</table>

IC₅₀ (mmol/l) 1.4 ± 0.2

Basal uptake: 2.7 ± 0.09 nmol/10⁹ erythrocytes. Each value is mean of 12 independent experiments.

Fig. 1. Percent inhibition caused by 200 μmol/l of pentoxifylline of ADP (1.2 μmol/l)-induced platelet aggregation in the presence or absence of erythrocytes. * P < 0.0001 vs whole blood and PRP + RBC; ** P > 0.2 vs whole blood.
Arachidonate-induced platelet thromboxane B₂ production was not inhibited significantly by pentoxifylline (89.6 ± 8.4 ng/ml) after 2 μmol/l of pentoxifylline, from 83.5 ± 6.2 ng/ml, neither when 0.8 mmol/l of arachidonate was used nor when 0.4, 0.2 or 0.1 mmol/l.

ADP (1.2 μmol/l)-induced platelet aggregation in PRP was inhibited 10.4 ± 0.9% by 100 μmol/l of caffeine (Sigma Chemical Co, St Louis, MO, U.S.A.), and 15.8 ± 1.2% by 125 μmol/l of pentoxifylline; when both compounds were incubated in association, 40.1 ± 2.6% inhibition were obtained (P < 0.001 vs caffeine or pentoxifylline values).

Ex vivo experiments

The administration of 1200 mg/day of pentoxifylline reduces significantly platelet aggregation induced by ADP, adrenaline (3.1 μmol/l) and collagen (0.75 μg/ml), in WB (Table 3). In PRP aggregometry no significant inhibition of platelet aggregation was found after treatment. Percent inhibition of aggregation in WB were higher than those obtained in PRP when ADP and 0.75 μg/ml of collagen were used as inducers.

After pentoxifylline treatment, the 8-14C-adenosine uptake by erythrocytes was inhibited 50.1 ± 6.5% (P < 0.0001 vs pretreatment values). Arachidonate-induced platelet TxB₂ production was not modified (0.2 ± 0.1% of maximal inhibition, P > 0.2).

DISCUSSION

The results obtained in our study demonstrate that pentoxifylline presents a higher antiplatelet effect in whole blood than in platelet-rich plasma. The ratios IC₅₀ PRP/IC₅₀ WB in the in vitro experiments are as follows: 4.36 for ADP, 2.57 for adrenaline, 3.02 for collagen, and 1.23 for arachidonic acid induced platelet aggregation. For the ex vivo experiments we observed these differences on the ADP and collagen induced platelet aggregation. Concerning the results obtained in whole blood aggregometry, we have not found any data with which we can compare our results. In platelet-rich plasma, our results are in agreement with those of several authors: Deguchi et al. (1979) demonstrated that ADP-induced aggregation was inhibited after incubation with 500 μmol/l of pentoxifylline, while the oral administration of the drug does not inhibit this test; likewise, Itoh and Satoh (1979) demonstrated that 10–20% inhibition in the ADP-induced aggregation, and no inhibition of adrenaline-induced aggregation, were observed after pentoxifylline administration to patients suffering cerebrovascular attacks.

Therefore, our study demonstrates that pentoxifylline inhibits platelet aggregation in whole blood to a greater extent than in platelet-rich plasma.

Concering the mechanism involved in the antiaggregatory effect of pentoxifylline in whole blood, red blood cells and/or leukocytes may be responsible for increasing this effect. Drugs that inhibit platelet thromboxane synthesis, such as aspirin or triflusal, show a higher antiaggregating effect mainly in the presence of leukocytes (De La Cruz et al., 1987, 1988), while inhibitors of cAMP-dependent phosphodiesterase, such as dipyridamole or RA-642, inhibit platelet aggregation more in PRP plus red blood cells, than in platelet-rich plasma (Gresele et al., 1983; De La Cruz et al., 1991). Pentoxifylline is a drug that acts on red blood cells and increases platelet cAMP (Angelkort et al., 1979; Stefanovich, 1974), without inhibition of platelet thromboxane B₂ synthesis (see Results); for that reason, we think that red blood cells can influence the antiplatelet effect of pentoxifylline. In fact, incubation with platelets of the same red blood cell count as that obtained in whole blood, increases the antiaggregatory effect of pentoxifylline to the values obtained in whole blood (Fig. 1). Pentoxifylline could thus exert a greater effect in whole blood through a possible effect on the red blood cells.

Drugs that show a similar effect to pentoxifylline, such as dipyridamole, inhibit the erythrocyte uptake of adenosine (Roos and Pfleger, 1972), thereby increasing its inhibitory effect on the cAMP-dependent phosphodiesterase (Gresele et al., 1986). Our results demonstrate that pentoxifylline inhibits the erythrocyte uptake of 14C-adenosine in a concentration-dependent manner (Table 2); however the IC₅₀ values are higher than those necessary to inhibit ADP-induced platelet aggregation in whole blood.

Our experiments with caffeine (see Results) and the IC₅₀ values described for inhibition of platelet cAMP-dependent phosphodiesterase (620–660 μmol/l) (Stefanovich, 1974), could explain why the inhibition of phosphodiesterase is important to the antiaggregatory effect of pentoxifylline; however the inhibition of

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Table 3. Percent inhibition of platelet aggregation after 15 days of treatment with 1200 mg/day of pentoxifylline

<table>
<thead>
<tr>
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<tr>
<td>ADP (μmol/l)</td>
<td>1.2</td>
<td>30.3 ± 4.5*</td>
</tr>
<tr>
<td>Adrenaline (μmol/l)</td>
<td>0.6</td>
<td>21.7 ± 3.0***</td>
</tr>
<tr>
<td>Collagen (μg/ml)</td>
<td>0.75</td>
<td>34.5 ± 7.8***</td>
</tr>
<tr>
<td>Arachidonic acid (mmol/l)</td>
<td>0.8</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

*P < 0.001; **P < 0.01; ***P < 0.05 vs pretreatment values. †P < 0.005; ††P < 0.01 vs whole blood.
erythrocyte uptake of adenosine could possibly contribute to the above mentioned mechanism, because adenosine increases adenylcyclase activity, raising the platelet cAMP levels, which decreases its metabolization to AMP due to pentoxifylline inhibition of phosphodiesterase.

In the ex vivo experiences pentoxifylline shows a lower antiaggregatory effect than expected after the in vitro experiments; plasmatic levels of pentoxifylline from 193 to 898 μmol/l have been described after oral administration (Ambrus et al., 1990; Rames et al., 1990). These data are in agreement with the inhibition of erythrocyte uptake of adenosine observed in our experiences, which emphasize the importance of this mechanism on the ex vivo effect of pentoxifylline. Hinze et al. (1972) described 7 organic metabolites of pentoxifylline; of these, metabolites 1 and 5 are more active hemorrheologic agents than the parent compound (Ambrus et al., 1990). The possible effects of these 7 metabolites on platelet behaviour, and their possible influence on the in vivo antiplatelet pentoxifylline action, are unknown.

In conclusion, our results demonstrated a higher antiaggregatory effect of pentoxifylline in whole blood than in platelet-rich plasma, and an inhibitory effect on the erythrocyte uptake of adenosine, which may be complementary to cAMP-dependent phosphodiesterase inhibition. These results may be an explanation for the beneficial use of pentoxifylline in ischemic vascular diseases, such as intermittent claudication.

**SUMMARY**

Pentoxifylline is a xanthine derivative that increases erythrocyte deformability and cAMP-dependent phosphodiesterase. The antiplatelet effect of this drug in whole blood has been evaluated in both in vitro and ex vivo experiences. Pentoxifylline inhibits the in vitro platelet aggregation in whole blood (WB) to a greater extent than in platelet-rich plasma (PRP); the IC₅₀/PRP/IC₅₀ WB ratios were as follows: 4.36 for ADP, 2.57 for adrenaline, 3.02 for collagen and 1.23 for arachidonic acid. Likewise, pentoxifylline inhibits in a concentration-dependent manner the erythrocyte uptake of adenosine, with an IC₅₀ of 1.4 ± 0.2 mmol/l. Incubation of red blood cells with platelets increases the antiaggregatory effect of pentoxifylline from 20.1 ± 2.5% in PRP to 47.1 ± 3.4% in PRP plus red blood cells. In the ex vivo experiments, pentoxifylline inhibits platelet aggregation in whole blood induced by ADP or collagen, and the erythrocyte uptake of adenosine (50.1 ± 6.5%). We conclude that pentoxifylline inhibits platelet aggregation in whole blood, thus implicating the inhibition of adenosine uptake by red blood cells.

**REFERENCES**


