TRIFLUSAL VS ASPIRIN ON THE INHIBITION OF HUMAN PLATELET AND VASCULAR CYCLOOXYGENASE

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Abstract—1 Triflusal is a salicylic derivative that inhibits platelet aggregation in human whole blood with a minimal inhibition of prostacyclin production.

2 Aspirin inhibits platelet aggregation at concentrations that reduce vascular prostacyclin production.

INTRODUCTION

Triflusal (TR) (2-acetoxy-4-trifluoromethyl benzoc acid) is a platelet aggregation inhibitor structurally related to acetylsalicylic acid (ASA), proven to possess both in vitro and ex vivo platelet antiaggregatory activity (Garcia Rafanell et al., 1979; De La Cruz et al., 1988a,b,c). After administration to humans, TR and ASA are decetylated to their main metabolites, salicylic acid (SA) and 2-hydroxy-4-trifluoromethyl benzoc acid (HTB), respectively (Garcia Rafanell et al., 1979; Rimbau et al., 1981).

TR and ASA are irreversible inhibitors of platelet cyclooxygenase (Pro), whereas HTB and SA are weak reversible Poo inhibitors (De Castellarnau et al., 1988). However, TR and HTB show different pharmacological profiles as compared with ASA and SA: (1) they are more potent as inhibitors of platelet aggregation (PA) induced by ADP (De La Cruz et al., 1988b), (2) red blood cells increase their antiaggregatory effect (De La Cruz et al., 1988c), (3) they increase platelet cyclic AMP content at therapeutic concentration (Garcia Rafanell et al., 1986), and (4) the half life of HTB in humans is about 48 hr (Ramis et al., 1990).

In rats, De Castellarnau et al. (1988) demonstrated that only high doses of TR (40 mg/kg i.v.) inhibited rat aortic prostacyclin production. Our group found that 6-keto-PGF1α plasma levels in diabetic patients were inhibited by 97.8 ± 3.1% by ASA, but only 8.8 ± 0.6% by TR at equivalent doses (De La Cruz et al., 1988a).

All these data strongly suggest that TR and ASA, although they have chemical structural similarities, manifest important differences in pharmacological activity and mechanism of action. The purpose of this study was to evaluate the inhibition of prostacyclin production by TR, ASA, HTB, and SA using human mesenteric artery preparations, and to compare this effect with the inhibition of human PA and of Thromboxane B2 (TxB2) formation by the compounds, using arachidonic acid as the stimulus.

MATERIALS AND METHODS

Materials

TR and HTB were purchased from Unach (Barcelona, Spain). ASA and SA were from Sigma Chemical Co (U.S.A.). Arachidonic acid was from Bio-data Co. (U.S.A.). Thromboxane B2[3H] and 6-keto-PGF1α[3H] RIA kits were purchased from Amersham International plc. All the reagents, except the arachidonic acid, were from Sigma Chemical Co (U.S.A.).

Methods

Platelet aggregation. PA studies were made in whole blood (WB) (n = 30) and in platelet-rich plasma (PRP) (n = 26), in an electronic aggregometer (Chrono Log Corporation, Haverton, Pa), according to the method described by Cardinal and Flower (1980). PRP was obtained by centrifugation of WB at 180 g for 10 min. Male blood donors did not receive any medication for at least 10 days prior to the study.

Blood was anticoagulated with 3.8% trisodium citrate in the proportion 9:1. Final platelet counts (Cell Counter System 8000, Baker Instruments) were 253,000 ± 15,800/μl in WB samples and 242,000 ± 16,500/μl in PRP samples. A volume of 480 μl of WB or PRP was placed in the aggregometer cuvette (37°C, 1000 rpm) which also contained 500 μl of saline and 10 μl of different concentrations of TR, ASA, HTB, or appropriate solvent (control). Samples were incubated for 5 min and then 10 μl of arachidonic acid (AA) was added (0.8 mM final concentration). The PA curve was recorded, and the maximum change in electrical impedance (Ω) was measured 10 min after the addition of AA.

Percentage of inhibition vs control was determined at different drug concentrations and IC50 values were calculated from the dose-response curves.

Platelet production of TxB2. Platelets in PRP samples (n = 22) were aggregated for 10 min using AA as the aggregation inducer (see above). Samples were then centrifuged at 12,000 g for 3 min and the supernatants were kept to −40°C until assay for TxB2, (stable metabolite of TxA2).

TxB2 was determined by radioimmunoassay (RIA) using a [3H]-TxB2 RIA kit, and the IC50 values were calculated from the respective dose–response curves.

Vascular production of 6-keto-PGF1α. The production of the main stable metabolite of prostacyclin, 6-keto-PGF1α, was determined in vascular rings of the human inferior mesenteric artery (HIMA) (n = 28) obtained after surgery from patients with colon tumors. Each sample of HIMA was analyzed histologically and it was confirmed that it was not affected by tumor infiltrations. The mean time which elapsed between the surgical extraction and the chemical study was 3.4 ± 0.2 hr.

The HIMA was dissected from adipose tissue and placed in a cold buffer containing (in g/l). 0.29 of KCl, 6.2 of NaCl,
1 6 of NaHCO₃, 0.28 of Na₂SO₄, 5 5 of sodium citrate, 0 5 of glucose, and 0 6 of TRIS (pH 8.2) The HIMA was divided into 4 mm ring sections (196 ± 15 3 mg) and each one was placed in separate tubes containing 98 0 μl of the same buffer, and 10 μl of different concentrations of TR, ASA, HTB or SA The samples were incubated at 37°C for 5 min, after which 10 μl of AA (final concentration 0 8 mM) was added After 10 min, supernatants were stored at −40°C until assayed for 6-keto-PGF₁₀, determined by RIA using a [³H]-6-keto PGF₁₀ RIA kit IC₅₀ values were calculated graphically from the corresponding dose-response curves.

Statistical analysis Data are mean ± standard error of the mean. RIA data were processed with the aid of the Aria program (Elsever Bosoft, 1987) and statistical analysis was made by the EpiStat program (T L Gustafson, 1985), using the unpaired Student's t-test

RESULTS
There were no statistically significant inter-group differences in the baseline values of the different parameters. The mean basal values were as follows: 13.2 ± 1.4 Ω (PA-WB), 10 9 ± 1.3 Ω (PA-PRP), 68.8 ± 8.2 ng/10⁶ platelets (TxB₂ production), and 64.2 ± 10.5 pg/mg tissue (6-keto-PGF₁₀ production).

TR, ASA and HTB produced a dose-dependent inhibition of PA, and of TxB₂ and 6-keto-PGF₁₀ (Fig 2) production. SA was the least active of the compounds in all the parameters, producing a less than 50% inhibitory effect at 5000 μM. Table 1 shows the IC₅₀ values for these compounds.

Figure 3 shows percent inhibition values of PA-PRP, TxB₂, and 6-keto-PGF₁₀, at the concentration of each compound that produces 50% inhibition of PA-WB, which was calculated from the dose–response curves. Except for SA which was unable to produce 50% inhibition of PA-WB at the maximum concentration tested which was 5000 μM.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>PA-WB</th>
<th>PA-PRP</th>
<th>TxB₂</th>
<th>6-Keto-PGF₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>0.9 ± 0.09</td>
<td>10.9 ± 1.1</td>
<td>0.9 ± 0.01</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>(10)</td>
<td>(7)</td>
<td>(9)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Triflusal</td>
<td>48.3 ± 3.3</td>
<td>693 ± 58 3</td>
<td>468 ± 33 7</td>
<td>339 ± 54 7</td>
</tr>
<tr>
<td>(7)</td>
<td>(6)</td>
<td>(8)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>HTB</td>
<td>202 ± 15 3</td>
<td>2328 ± 339</td>
<td>1323 ± 122</td>
<td>4725 ± 523</td>
</tr>
<tr>
<td>(7)</td>
<td>(5)</td>
<td>(8)</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
</tr>
<tr>
<td>(6)</td>
<td>(6)</td>
<td>(7)</td>
<td>(5)</td>
<td></td>
</tr>
</tbody>
</table>

Number of experiments are indicated in brackets
Intra- and inter-group differences were statistically significant (P < 0.01), except when compared IC₅₀ (TxB₂) vs IC₅₀ (6-keto-PGF₁₀) of triflusal (P = 0.065)
Our results demonstrate that TR inhibits human vascular cyclooxygenase in vitro giving an IC₅₀ value about 1000 times higher than that of ASA and lower than that of HTB SA, at concentrations of 100–5000 μM, only produces a weak inhibition of 6-keto-PGF₁α formation. These data, using human vascular tissue, confirm those obtained by De Castellarnau et al. (1988) in rats after intravenous administration of high doses of TR and ASA. The inhibitory effect of ASA on human vascular prostacyclin production has also been demonstrated by other authors (Burch et al., 1978, Baenziger et al., 1979).

In diabetic patients, we have demonstrated that 600 mg/day of TR did not produce a significant change in the 6-keto-PGF₁α plasma levels, whereas 400 mg/day of ASA (equimolecular dose) inhibited them by 97.8 ± 3.1% (De La Cruz et al., 1988a). These results are in agreement with the present findings using human vascular rings.

If we compare the inhibition of human vascular cyclooxygenase with the decrease of platelet TxB₂ production, it can be observed that TR, ASA and HTB show a similar behaviour in both AA pathways. Thus, ASA inhibits TxB₂ biosynthesis with an IC₅₀ value 5-fold lower than IC₅₀ against 6-keto-PGF₁α formation. In comparison with our findings, Burch et al. (1978) and Baenziger et al. (1979) observed that ASA was 30–40 times more potent as an inhibitor of cyclooxygenase in platelets than in endothelial cells, this apparent discordance could be explained by the use by those authors of culture cells from umbilical veins, and our use of another type of vascular tissue (rings of human inferior mesenteric artery). On the other hand, TR inhibits both TxB₂ and 6-keto-PGF₁α formation to a similar extent, and HTB is, like ASA, about 3.5 times more potent as an inhibitor of TxB₂ formation, but has a much lower IC₅₀ value of 1323 μM. In any case, human plasma concentrations of HTB of about 1000 μM have been found after repeated dose administration of triflusal at 900 mg/day, orally (Rams et al., 1990; Albors et al., 1987).

In previous studies we have demonstrated an anti-aggregatory activity of TR, HTB and ASA in WB higher than that in PRP, using ADP and collagen as aggregation inducers (De La Cruz et al., 1986, 1987, 1988a,c). The present study shows similar differences in activity using AA as the aggregation inducer.

It is known that leukocytes and, to a lesser extent, red blood cells, potentiate the antiplatelet effect of ASA in WB, whereas red blood cells are the only cause of this potentiation for HTB (De La Cruz et al., 1987, 1988c). Both leukocytes and erythrocytes contribute similarly to the increase of antiplatelet activity of TR (De La Cruz et al., 1988c). The contribution of blood cells to the antiplatelet activity of drugs shows the importance of using WB instead of PRP in in vitro platelet aggregation studies, because this permits a better extrapolation of the results to in vivo situations (Cardinal and Flower, 1980; Gresele et al., 1983, Ingerman-Wojenski and Silver, 1984).

**DISCUSSION**

**Fig 3** Percent of inhibition of platelet aggregation in platelet-rich plasma (PA-PRP), thromboxane B₂ (TxB₂) and 6-keto-PGF₁α production, at concentrations that produce 50% inhibition of the platelet aggregation in whole blood (PA-WB).
contribute to the antiplatelet activity of TR and of its main metabolite HTB. Furthermore, it is important to notice that both compounds inhibit platelet aggregation in human whole blood (Table 1) at concentrations that have been found in human plasma after repeated oral doses of triflusal at 600 mg/day in healthy volunteers (Albors et al., 1987; Ramis et al., 1990).

In conclusion, the results of this study show that (a) TR and its main metabolite HTB inhibit AA-induced platelet aggregation in human whole blood at concentrations that do not produce significant inhibition of TxB2 and 6-keto-PGF1α formation. With ASA, it was not possible to dissociate the antiplatelet activity in human WB and the inhibition of production from the inhibition of 6-keto-PGF1α formation. (b) Besides cyclooxygenase inhibition, TR and HTB seem to inhibit platelet aggregation in human whole blood through other mechanisms where cyclooxygenase inhibition does not play the principal role.

SUMMARY

The aims of the present study were to compare the inhibitory effects of triflusal (TR), acetylsalicylic acid (ASA) and of their main metabolites, 2-hydroxy-4-trifluoromethyl acid (HTB) and salicylic acid (SA), on human vascular prostacyclin production, in vitro, and to compare the inhibition of vascular prostacyclin production with the inhibition of human platelet thromboxane B2 (TXB2) formation, and of platelet aggregation induced by arachidonic acid in whole blood (PA-WB). TR inhibited prostacyclin production by 60% (IC₅₀) at 339 ± 55 μM whereas the value of HTB was 4725 ± 523 μM. ASA was about 1000 times more potent than triflusal (0.33 ± 0.05 μM) and SA had no significant inhibitory effect at 5 mM. On the other hand, ASA inhibited PA-WB at concentrations higher than those needed to inhibit TXB2 production, while the opposite occurred with TR and HTB. These results lead us to conclude that TR can inhibit PA-WB in humans without causing a significant inhibition of vascular prostacyclin formation, and that other mechanisms besides platelet cyclooxygenase inhibition may contribute to the antiplatelet effect of triflusal and HTB.

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