The ex vivo effect of triflusal and acetylsalicylic acid (ASA) on platelet interaction with the subendothelium using the Baumgartner perfusion system (wall shear rate 350 s⁻¹) was assessed in blood from 10 healthy volunteers who given a 15-day course of triflusal 600 mg per day and ASA 400 mg per day in a crossover trial.

The percentage of platelets on the subendothelium showed a decrease of 62% in samples from subjects on ASA and a decrease of 93% in those from subjects on triflusal (P < 0.005). The percentage of the subendothelial surface covered by platelets was reduced by 23.3% after treatment with ASA, mainly due to inhibition of aggregates (75.2%), and by 29.9% after treatment with triflusal, mainly due to inhibition of aggregates (89.6%) and of adhesion (25%). The subendothelial surface covered by activated platelets (adhesions and thrombi) showed 32.5% inhibition after treatment with triflusal and 11.6% after treatment with ASA (P < 0.043 vs. triflusal). In the in vitro experiments, 10 μmol·l⁻¹ triflusal did not modify the percentage of the subendothelium covered by platelets. HTB 1 mmol·l⁻¹ inhibited adhesion (26%) and aggregates (18%).

We conclude that HTB participates in the ex vivo effects of triflusal on the platelet-subendothelium interaction.

Key words Triflusal, Acetylsalicylic acid; platelet activation, platelet-vessel wall interaction, subendothelium

Triflusal, 2-(acetyloxy)-4-(trifluoromethyl)benzoic acid, is an antithrombotic agent with a chemical structure similar to that of acetylsalicylic acid (ASA), but with a different pharmacokinetic and pharmacodynamic profile [1-3]. A deacetylated metabolite of triflusal, 2-hydroxy-4-trifluoromethyl-benzoic acid (HTB), shows a longer half-life (approximately 40 hours) than its deacetylated congener, salicylic acid (SA). Triflusal and HTB are inhibitors of platelet cyclooxygenase [1, 4], but in contrast to SA which prevents binding of ASA to platelet cyclooxygenase [5], HTB potentiates the effect of triflusal [6]. Triflusal and in particular HTB increase intraplatelet cAMP levels. The effect of ASA or SA on cAMP levels in platelets is negligible [7, 8]. With regard to the platelet antiaggregatory effects of these compounds, triflusal inhibits platelet aggregation induced by ADP, with a stronger inhibitory effect in the presence of red blood cells, whereas the latter do not potentiate the antiaggregatory activity of ASA and SA [9].

In clinical practice, triflusal has been shown to be an effective inhibitor of platelet aggregation in diabetic patients [10], to reduce the evolution of background diabetic retinopathy [11], and to prevent aortocoronary vein-graft occlusion [12].

In this study we have determined ex vivo the effect of triflusal on platelet interaction with the subendothelium using the Baumgartner perfusion system. In order to investigate the possible influence of its main metabolite HTB on this effect, we also performed a similar in vitro experiment using triflusal, ASA, HTB and SA.

Materials and methods

Materials

The annular perfusion chamber was manufactured by Labotron, S.A. (Barcelona, Spain). Triflusal (Disgren®) was obtained from Laboratorios J. Uriach & Cia., (Barcelona, Spain), ASA (Adiro®) from Química Farmacéutica Bayer, S.A. (Barcelona, Spain), malondialdehyde-bis-diethyl-acetal from Aldrich Chemical Co
Design of the study

Ex vivo study

Ten healthy male volunteers with a mean (SD) age of 25.6 (3.1) years took part in the study. No subject had received medication known to modify the platelet response for 15 days prior to the experiment. They were distributed at random into two groups according to the antiaggregatory treatment administered: five subjects received ASA 400 mg per day (200 x 2 mg per day) ASA and five received triflusal 600 mg per day (300 x 2 mg per day) for 15 days. After a washout phase of 15 days, those subjects treated with ASA received triflusal, and those treated with triflusal received ASA for a period of 15 days. The doses of triflusal were chosen according to the usual clinical protocol in patients with thrombotic risk factors; the dose of ASA was equimolar to that of triflusal. Venous blood samples were collected under basal conditions prior to the administration of either antiplatelet drug, 1 h after the last dose of the first drug treatment phase, at the end of the washout period, and 1 h after the last dose of the second drug treatment phase. Blood was anticoagulated with 3.8% trisodium citrate in the proportion 1:10.

The results given are the means of at least 8-10 independent samples.

Perfusion studies

Perfusion studies were carried out in the annular chamber according to a modification of the method described by Baumgartner et al. [14, 15]. Briefly, vessel wall segments were obtained from New Zealand white male rabbits weighing 2 to 2.5 kg. Animals were anaesthetised with sodium pentobarbital (100 mg·kg⁻¹) and samples were anticoagulated with 3.8% trisodium citrate in the proportion l:10.

Platelet aggregometry

Platelet aggregation was measured in whole blood samples collected before and immediately after blood perfusion by the electric impedance method described by Cardinal and Flower [16], as the maximum change in impedance (Ohm) 10 min after addition of the aggregating agent. Samples were diluted in physiological saline (pH 7.4, 1:1 v/v) and aggregometry was performed at 37°C with continuous stirring at 1,000 r.p.m. Different concentrations of ADP or collagen were used to induce aggregation.

Lipid peroxidation

The formation of malondialdehyde (MDA) by platelets was taken as an indicator of enzymatic lipid peroxidation, which was induced with arachidonic acid [17]. Briefly, platelet-rich plasma (PRP) was obtained from whole blood by centrifugation at 180 g for 10 min, and platelet-poor plasma (PPP) by centrifugation at 1,800 g for 15 min. PRP and PPP samples were incubated at 37°C for 5 min in 0.4 mmol·l⁻¹ arachidonic acid. Trichloacetic acid 20% was then added and the product was centrifuged at 10,000 x g for 3 min. Subsequently, 250 μl thiobarbituric acid 0.5% were added and samples were incubated at 100°C for 15 min. The amount of MDA produced was measured as the spectrophotometric absorbance of the supernatant at 532 nm. The absorbances obtained were compared with that of a standard curve using malondialdehyde-bis-diehtyl-acetol with the value obtained in PPP as the blank. Results are expressed in nmol MDA/10⁶ platelets.

Statistical analysis

The Student’s t test for paired data was used for statistical analysis, using the Epistat® 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 with SEM.