Antiplatelet Effects of Fenflumizole, a New Anti-Inflammatory Drug, in Dogs

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Abstract—Fenflumizole (2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole) was given to dogs in a single oral dose of 3 or 10 mg/kg. The plasma concentrations of fenflumizole and the two metabolites (mono- and di-demethyl forms) attained to the peak level 1–2 hr after dosing of fenflumizole, returning to near the predose levels 8 hr after the dosing. Fenflumizole (10 mg/kg) given orally significantly inhibited collagen- and ADP-induced platelet aggregations *ex vivo* over 4 hr after the dosing. Fenflumizole effectively inhibited *in vitro* collagen-induced platelet aggregation, but failed to prevent ADP-induced aggregation. The mono-demethyl form of fenflumizole inhibited *in vitro* ADP- and collagen-induced aggregations, but the di-demethyl form was ineffective in inhibiting them.

Fenflumizole, 2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole, is one member of a novel series of imidazole derivatives with anti-inflammatory, analgesic and anti-pyretic properties (1). Corell et al. (2) reported that fenflumizole also possesses a potent cyclooxygenase inhibitory activity. Non-steroidal anti-inflammatory agents are generally limited in their clinical application because of the high incidence of gastrointestinal ulcerations associated with the use of these drugs (3). However, fenflumizole seems to have retained therapeutic activities but reduced side effects, compared to other non-steroidal anti-inflammatory drugs, particularly on the gastro-intestinal tract (1, 4). Recently, its anti-platelet effects against various aggregating agents have been noted in animals (2, 4, 5) and humans (2, 6, 7). The present study was undertaken to evaluate further the anti-platelet effect of fenflumizole against collagen and ADP, together with its pharmacokinetics, in dogs.

Male beagle dogs weighing 9.5 to 12.0 kg were used.

In vitro studies: Three animals were anesthetized *i.v.* with 30 mg/kg of Na-thiobarbitone. The blood was withdrawn from the cannulated left carotid artery into glass syringes containing 3.8% sodium citrate solution (citrate : blood = 1:9). Platelet-rich plasma (PRP) was prepared by centrifuging the blood for 8 min at 1100 rpm (190×g). After PRP was separated, the remaining aliquot was centrifuged for 15 min at 3000 rpm (1300×g), and platelet-poor plasma (PPP) was obtained. A platelet counter (Model ZB1, Coulter Electronics) was used to adjust the number of platelets in PRP to a constant value (3×10^5 platelets/ μ l) in each experiment with homologous PPP. Each sample was prewarmed for 5 min at 37°C and stirred at 1100 rpm before addition of drugs. Then, 20 μ l of drug solution or vehicle was added to each sample, and 2 min later, 20 μ l of aggregating agents were also added. The final concentrations of aggregating agents were 30 μ g/ml collagen and 7.5 μ M ADP, concentrations which induced about 75% of the maximal aggregation. The aggregation curve was monitored by means of a blood platelet aggregometer (NKK platelet

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aggregation tracer, model PAT-4M, SSR Engineering Co.). Inhibition of platelet aggregation was assessed by comparing maximal optical transmission changes of the drug-treated PRP sample with the vehicle-treated PRP sample and expressed as percentage changes.

Ex vivo studies: Five animals were given orally fenflumizole (3 or 10 mg/kg as tablets containing 5 or 50 mg of fenflumizole) or placebo (lactate) at random once a week, approximately 1 hr after the intake of food (9 a.m.). Venous blood samples (5.0 ml) were withdrawn from the cephalic vein before and 1, 2, 3, 4, 6 and 8 hr after the oral dosing. Platelet aggregation tests were performed according to the same procedure as described in the *in vitro* studies. Inhibition of platelet aggregation was assessed by comparing maximal optical transmission changes of the pre-dosing PRP sample with the post-dosing PRP sample and expressed as percentage changes.

In the same preparation, the pharmacokinetic study was made. To avoid photodecomposition of fenflumizole, blood samples were carefully protected from light. Blood was centrifuged for 10 min at 3000 rpm with a Hitachi Refrigerated Centrifuge (05PR-22). Plasma was separated and frozen at -80°C until assayed (within one week). The plasma concentrations of fenflumizole and the two metabolites (Fig. 1) were determined according to a small modification of the method of Midskov (8).

The drugs used were: 2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole, fenflumizole, and mono- and di-demethyl fenflumizole (all from Dumex, Denmark; see Fig. 1); collagen (Hormonchemie, München GMBH); and ADP (adenosine 5'-diphosphate from equine muscle, Sigma). Fenflumizole and its metabolites were dissolved in 2% dimethyl sulfoxide solution and diluted with 0.9% saline solution. Collagen and ADP were dissolved in and diluted with 0.9% saline solution.

The data in the text are expressed as values of the mean $\pm$ S.E. Significant differences between mean values were estimated by using Student's *t*-test. A *P* value smaller than 0.05 was considered statistically sig-

Fig. 1. The mean plasma concentration profile of fenflumizole (FEN) and the two metabolites in dogs given orally 3 and 10 mg/kg fenflumizole. The vertical bars represent S.E. Each point shows the mean of 5 observations on 5 preparations.

nificant.

Figure 1 shows the mean plasma concentration-time curves of fenflumizole and its metabolites (demethylation products) after oral dosing of 3 or 10 mg/kg fenflumizole. The plasma levels of fenflumizole and the two metabolites attained to the peak level 1–2 hr after oral dosing of fenflumizole, reaching near the predose levels 8 hr after the dosing. The maximum plasma concentrations ($C_{\max}$) of fenflumizole and the metabolites were as follows: fenflumizole, 144.1 ± 47.1 and 700.5 ± 140.3 ng/ml; the mono-demethyl form, 14.8 ± 3.9 and 67.6 ± 12.2 ng/ml; and the di-demethyl form, 9.1 ± 1.8 and 39.2 ± 13.7 ng/ml for 3 and 10 mg/kg dosing of fenflumizole, respectively (each $n=5$).

The effects of fenflumizole were examined on collagen- and ADP-induced platelet aggregations *ex vivo*, at different time points, before and 1, 2, 3, 4, 6 and 8 hr after fenflumizole administration. Fenflumizole given orally in a dose of 10 mg/kg significantly

Fig. 2. Time course of the effects of fenflumizole given orally on collagen- and ADP-induced platelet aggregations ex vivo in dogs. The vertical bars represent S.E. Each point shows the mean of 5 observations on 5 preparations. * $P < 0.05$, ** $P < 0.01$ compared to the corresponding values obtained from the placebo group.

inhibited both of collagen- and ADP-induced aggregations, although the effect on ADP-induced aggregation was less potent, compared with that on the collagen-induced one (Fig. 2). The inhibitory effects on the aggregations lasted over 4 hr. At a dose of 3 mg/kg, fenflumizole had a significant inhibitory effect, at 1 hr after the dosing, only on collagen-induced aggregation.

In in vitro studies, fenflumizole produced a concentration-dependent inhibition of collagen-induced platelet aggregation in the range of 3×10^{-8} to 3×10^{-7} M. A metabolite of fenflumizole, the mono-demethyl form, in concentrations of 3×10^{-7} to 3×10^{-6} M, also effectively inhibited the aggregation. The 50% effective concentrations (EC₅₀) of fenflumizole and the mono-demethyl form for inhibiting the aggregation were 7.1×10^{-8} and 9.2×10^{-7} M, respectively. The potency of fenflumizole in inhibiting the aggregation

was approximately 13 times higher than that of the mono-demethyl form. Even in a considerably high concentration (10^{-6} M), fenflumizole hardly inhibited ADP-induced aggregation, but the mono-demethyl form, unlike fenflumizole, was effective in inhibiting the aggregation, although its potency was relatively weak (10^{-6} M, $24.8 \pm 4.2\%$, $n=5$). The di-demethyl form of fenflumizole, even in high concentrations of 10^{-6} to 3×10^{-6} M, scarcely had inhibitory effects on either collagen- or ADP-induced aggregation.

The present results demonstrated that fenflumizole given orally to the dog effectively inhibited collagen- and ADP-induced platelet aggregations. Grande et al. (6) claimed that the threshold concentrations for arachidonate- and collagen-induced aggregations ex vivo were significantly increased during treatment of healthy human volunteers with oral fenflumizole, first with 50 mg b.i.d. for 4 or 5 days and then with 200 mg/day for 5 days, but no significant changes were observed in ADP-induced aggregation. However, recent investigations by Grauholt et al. (personal communication by A. Grauholt, P. Grande, J. Wadt and K. Koch, University of Copenhagen and Dumex Ltd., 1985) revealed that in patients with unstable angina pectoris, fenflumizole given orally effectively inhibited ex vivo the platelet aggregations induced by ADP as well as by arachidonate and collagen. Thus, some difference was observed in the inhibitory effect of fenflumizole on ADP-induced platelet aggregation between healthy human volunteers and patients with ischemic heart disease.

According to our previous (4, 5) and present studies, fenflumizole was effective in inhibiting arachidonate- and collagen-induced aggregations in vitro, while it did not prevent ADP-induced platelet aggregation. Thus, fenflumizole seems to have no inhibitory effect on ADP-induced platelet aggregation. It should be noted, however, that fenflumizole given orally was converted mainly to the two demethylation products, the mono- and di-demethyl forms of fenflumizole, commonly in rats (4), dogs and humans (9), and that the mono-demethyl form was effective in inhibiting ADP-induced aggregation in vitro, even

though its inhibitory effect appeared relatively weak.

It has been generally known that platelet hyperresponsiveness occurs in patients with ischemic heart disease, probably contributing to the cause of vasospasm, anginal pain and eventually coronary vascular thrombosis (10). Actually, Frishman et al. (11, 12) evidenced that patients with angina pectoris demonstrate increased platelet sensitivity to aggregating concentrations of ADP and epinephrine when compared to the normal subjects exposed to the same concentrations of these agents. Fenflumizole may elicit more easily the antiplatelet effects against various aggregating agents in patients with ischemic heart disease than in healthy human volunteers.

In conclusion, the present result indicates that fenflumizole may be a candidate for anti-platelet therapy; and in addition, some active metabolites involving the monodemethyl form formed in the process of a biotransformation of fenflumizole seems to in part contribute to the antiaggregatory effect of fenflumizole. Further studies are needed to evaluate the anti-platelet activity, particularly in patients with ischemic heart disease and in those receiving chronic doses of fenflumizole.

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