Effects of Ifenprodil Glucuronide Derivative on Platelet Aggregation and Vasocontraction

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Abstract—It has been supposed that ifenprodil glucuronide derivative, detectable in large amount in rabbit plasma, is related to the pharmacological actions by ifenprodil tartrate. However, a synthesized ifenprodil glucuronide derivative was found to have no effect on platelet aggregation and vasocontraction in vitro. These results indicate that ifenprodil itself rather than its glucuronide derivative manifested the pharmacological actions.

Ifenprodil (dl-erythro-4-benzyl-a-(4-hydroxyphenyl)-(3-methyl-1-piperidin-ethanol) is a vasodilator and an inhibitor of platelet aggregation (1, 2). The inhibitory effect on platelet aggregation ex vivo by oral administration of ifenprodil in rabbits was manifested only after the plasma ifenprodil level had reached its peak. The ex vivo effect of ifenprodil was found to be stronger than its effect under in vitro condition (1). Furthermore, significant increase in rat vertebral blood flow was not manifested until 10 min after intravenous administration of this drug (3). These three findings suggest the possibility that metabolites of ifenprodil inhibit platelet aggregation ex vivo and relax cerebral blood vessels in vivo. We found that ifenprodil was readily metabolized into an ifenprodil glucuronide derivative which was readily detectable in significantly large amounts in rabbit plasma. Therefore, to study whether the ifenprodil glucuronide derivative manifests pharmacological actions in vivo and ex vivo, we synthesized the ifenprodil glucuronide derivative and investigated its effects on platelet aggregation and contraction of basilar artery in vitro.

Ifenprodil tartrate was orally administered at 10 mg/kg to male rabbits 3–3.5 kg; the citrated blood was collected at 0.5, 1, 2 and 4 hr after administration. Free-form ifenprodil in plasma was extracted with ether under the alkaline condition (pH 10). Ifenprodil glucuronide derivative was transferred to the free-form ifenprodil by hydrolysis with β-glucuronidase (3000 Fishman units/ml) and extracted in the same way. Ifenprodil was determined by gas chromatography-mass spectrometry. Analytical conditions were as follows: column, 1% OV-17 on Gas-chrom Q (80–100 mesh) and 0.5 mm x 0.3 mm i.d.; column temp., 225°C; flow rate of helium, 40 ml/min; electron energy, 70 eV; internal standard, 1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-[2-(4-methylphenyl)ethyl]-2,6-methano-3-benzazocin-8-ol; selected ions (m/z), 202 (ifenprodil) and 302 (internal standard).

Since ifenprodil glucuronide derivative is presumed have a structure in which a glucuronic acid is bound to the phenolic hydroxy group of ifenprodil (4), this derivative was synthesized by the method of Berrang et al. (5). Platelet aggregation in vitro was measured by using platelet-rich plasma from citrated rabbit blood by the previously reported method (6). The measurement of K+-induced basilar artery contraction was carried out as previously described (2). Ifenprodil and the ifenprodil derivative used were solubilized in saline.

Only a very small amount of free-form ifenprodil and a trace amount of a free-form metabolite of ifenprodil, probably dl-erythro-2-[4-(4-hydroxybenzyl)-piperidino]-1-(4-hydroxyphenyl)-1-propanol, were de-
detected in rabbit plasma after oral administration of ifenprodil tartrate, but a significantly large amount of ifenprodil glucuronide derivative was detected (Fig. 1). The areas

![Graph showing concentrations of ifenprodil and ifenprodil glucuronide over time](image)

**Fig. 1.** Free-form ifenprodil (○) and ifenprodil glucuronide (●) concentrations in rabbit plasma after oral administration of 10 mg/kg ifenprodil tartrate. Rabbit blood (9 vol.) was collected from the femoral artery by using 3.8% trisodium citrate (1 vol.), an anti-coagulant. The plasma was separated by centrifugation, and ifenprodil and ifenprodil glucuronide were determined as described in the text. The circles and the bars represent the mean±S.E. of four animals.

![Graphs showing effect of ifenprodil and ifenprodil glucuronide derivative on platelet aggregation and vasomotor responses](image)

**Fig. 2.** Effect of ifenprodil and ifenprodil glucuronide derivative on platelet aggregation in vitro induced by 2 μM ADP (a) and 10 μg/ml of collagen (b) and on K+-induced contraction of canine basilar artery (c). Platelet-rich plasma was separated from citrated rabbit blood, and platelet aggregation was measured as described in the previous report (6). Basilar artery from mongrel dog was prepared as described previously (2). A: ADP. C: collagen, K+: 50 mM KCl.
under the curve (0→4 hr) of unchanged ifenprodil and ifenprodil glucuronide derivative concentrations in the plasma were 16.08±4.91 ng/hr·ml⁻¹ and 16.73±1.16 µg/hr·ml⁻¹, respectively. From these results, comparison of the effects of free-form ifenprodil and synthesized glucuronide derivative on ADP- and collagen-induced platelet aggregation and on K⁺-induced contraction of basilar artery strip were carried out. Free-form ifenprodil from 1 µM to 100 µM inhibited platelet aggregation induced by ADP or collagen (Fig. 2 a, b) and also relaxed the contraction of basilar artery by K⁺ (Fig. 2c), but the ifenprodil glucuronide derivative had no effect on platelet aggregation and arterial contraction. These results indicate that the increased potency of ifenprodil under ex vivo and in vivo conditions and the delay in its manifestation of pharmacological actions are not due to its metabolized glucuronide derivative. In the previous report (1), we indicated that it is possible that ifenprodil at a concentration too low to have an effect on platelet aggregation in vitro may manifest an ex vivo effect by interacting with endogenous PG₁₂. Therefore, the present results support that ifenprodil itself manifested the pharmacological actions in vivo and ex vivo.

References

1. Irino, O., Saitoh, K., Hayashi, T. and Ohkubo, K.: Inhibitory mechanism of ifenprodil tartrate on rabbit platelet aggregation. Folia Pharmacol. Japon. 85, 379–385 (1985) (Abs. in English)
2. Honda, H. and Sakai, S.: The mode of action of ifenprodil tartrate in isolated canine cerebral and femoral arteries. Arch. Int. Pharmacodyn. 285, 211–225 (1987)
3. Shibuya, T., Watanabe, Y., Shimura, H., Honda, H. and Matsuda, H.: A study on the simultaneous measurement of blood flow in the various blood vessels. In III World Conference of Clinical Pharmacology and Therapeutics, Abstract, p. 78 (1986)
4. Nakagawa, H., Matsubara, Y., Yamano, S., Hiraoka, K. and Suga, T.: Metabolic fate of dl-erythro-2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)-1-propanol (Ifenprodil). II. Separation and identification of metabolites in urine and bile of rats. Pharmacometrics 10, 841–848 (1975)
5. Berrang, B., Twine, C.E., Hennessey, G.L. and Carroll, F.I.: Synthesis of morphine-3-glucuronide. Synthetic Communications 5, 231–236 (1975)
6. Fukawa, K., Saitoh, K., Irino, O., Ohkubo, K. and Hashimoto, S.: Inhibitory mechanism of dipyridamole on platelet aggregation ex vivo. Thromb. Res. 27, 333–340 (1982)