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SHORT COMMUNICATION

Effect of Fantofarone, a New Ca^{2+} Channel Antagonist, on Angioplasty-Induced Vasospasm in an Atherosclerotic Rabbit Model

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ABSTRACT. In order to prevent and treat angioplasty-induced vasospasm, we investigated the effects of a new Ca^{2+} channel antagonist, fantofarone, a nondihydropyridine compound with a novel site of action on the L-type Ca^{2+} channel, in an animal model of angioplasty in rabbits with femoral atherosclerotic lesions. Vasospasm which occurred in saline-treated animals following angioplasty was markedly reduced by fantofarone (50 μg/kg, i.v.) at both the distal and proximal sites. Although it totally inhibited distal vasospasm, isosorbide dinitrate (0.3 mg/kg, i.v.) did not significantly affect proximal diameter decrease. Verapamil (0.2 mg/kg, i.v.) was much less potent than fantofarone in reducing angioplasty-induced vasospasm. Our results confirm the preventive effects of Ca^{2+} blockers on this phenomenon and extend this observation to a potent compound: fantofarone. BIOCHEM PHARMACOL **55**;12:2047–2050, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. vasospasm; angioplasty; calcium channel blocker; fantofarone

Vasospasm occurs after balloon angioplasty in humans in 3–5% of cases [1, 2]. Even if transient, this phenomenon may be associated with acute complications such as thrombosis and artery occlusion. The mechanism of AIV‡ is not completely understood. Several observations showed that vasospasm is related to platelet deposition or release of vasoactive substances, including serotonin, from activated platelets [3]. Local injury also produces vasospasm in the desendothelialized artery segment by depleting relaxing factors such as prostacyclin and NO. In order to prevent and treat AIV, we investigated the effects of a new Ca^{2+} channel antagonist, fantofarone, a nondihydropyridine with a novel site of action on the L-type Ca^{2+} channel [4], in an animal model of AIV previously described by Le Veen *et al.* [5] in rabbits with femoral atherosclerotic lesions.

MATERIALS AND METHODS

Animals and Experimental Design

All animal experiments were approved by the Sanofi Recherche Animal Care and Use Committee. Male New Zealand White rabbits were used in this study (3.0–3.2 kg; Les Dombes, France). All surgical procedures were performed under anaesthesia with a mixture of ketamine and xylazine (Imalgene 0.5 mg/kg and Rompun 0.25 mg/kg, i.m.). At the end of the experiments, the animals were sacrificed by a pentobarbital overdose.

Induction of Focal Atherosclerosis

We used the original air-drying model of Sarembock *et al.* [6]. The proximal femoral arteries were exposed, and the isolated arterial segments were desiccated by air infusion delivered at a rate of 80 mL/min for 8 min. After desiccation was completed, the ligatures were released and flow was restored. This procedure was repeated on the contralateral femoral artery and the wound was sutured. At the day of surgery, a 2% cholesterol/6% peanut oil diet was started for 2 weeks.

Treatment Administration

Before angioplasty, the animals were randomized in 4 groups of 10 animals:

1. Placebo, 1 mL/kg of NaCl 0.9%
2. Isosorbide dinitrate (Risordan®, Rhone-Poulenc Rorer, France), 0.3 mg/kg
3. Verapamil (Sigma), 0.2 mg/kg
4. Fantofarone (Sanofi Recherche, Toulouse, France), 50 μg/kg

The doses of isosorbide dinitrate, verapamil, and fantofarone were defined in a pilot experiment as the highest doses which did not show any hypotensive effect *per se* and were chosen very carefully according to their activity measured in other pharmacological models. These data have been published by Spedding *et al.* [4]. Administration of these

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‡ *Abbreviation:* AIV, angioplasty-induced vasospasm.
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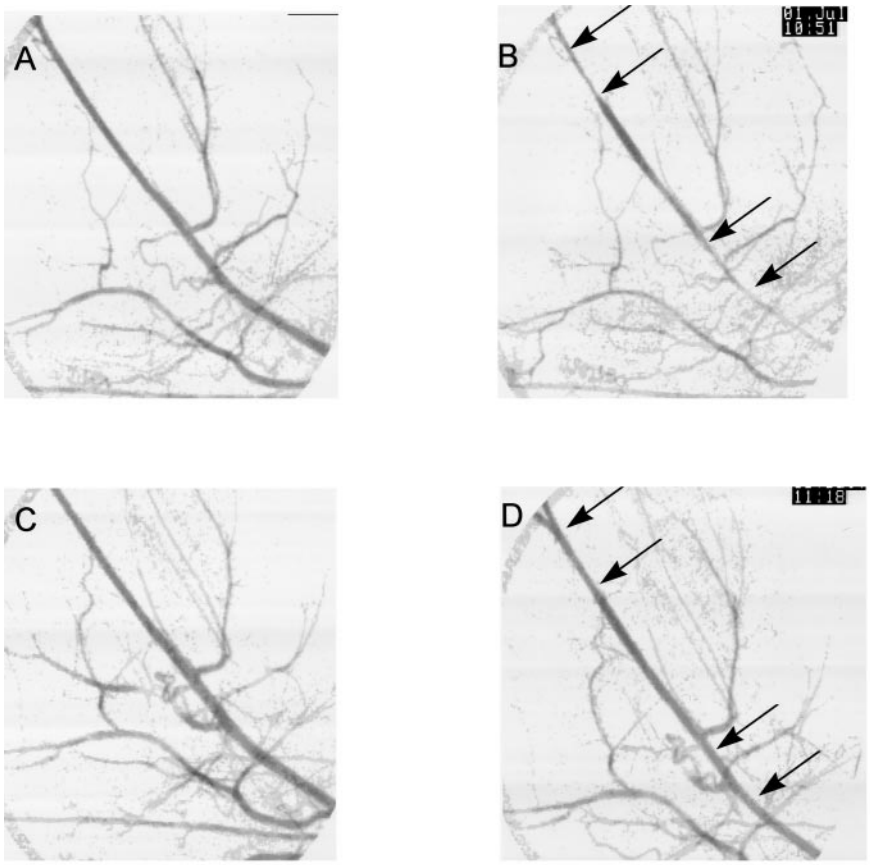
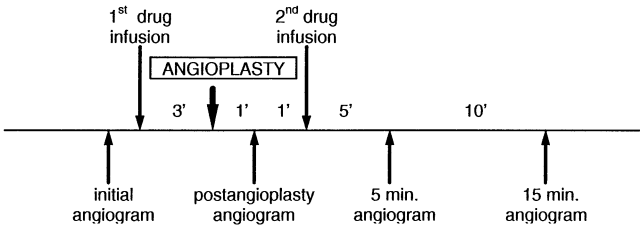


FIG. 1. Effect of fantofarone on AIV or rabbit femoral arteries following angioplasty. Angiographies of femoral arteries of vehicle (A, B) or fantofarone-treated (C, D) (50 μg/kg, i.v.) rabbits before (A, C) and after (B, D) angioplasty. Results are from a representative experiment. Arrows shown delimit the measured segments (proximal and distal).

drugs was performed by a slow intra-arterial injection through the angiographic catheter for at least 1 min. To evaluate the haemodynamic effects of these drugs, intra-arterial blood pressure was continuously monitored with a "Narco-Bio-System, Physiograph" recorder.

Balloon Angioplasty Procedure and Induction of Vasospasm

The different phases of the protocol are summarized in Scheme 1:



Each rabbit underwent balloon angioplasty 1 month after the induction of focal atherosclerotic lesions. The right common carotid artery was isolated, and a 5F introducer was placed and advanced to the junction of the aortic arch. Sodium heparin (1 mg/kg, Diosynth, Oss, Netherlands) was injected intra-arterially. An initial femoral angiogram (Siemens Siremobil angiograph) was performed via a 4F angiographic catheter that was positioned above the aortic bifurcation. Serial angiograms were obtained after the injection of 6 mL of Omnipaque (Nycomed, Stockholm, Sweden) (1/1 NaCl 0.9%). Dilatation was performed on the most severe of the 2 femoral stenoses. Under fluoroscopic guidance, a 2.0-mm balloon angioplasty catheter (Advanced Cardiovascular Systems, Paris, France) was introduced, advanced over a 0.036 cm guide wire, and positioned across the stenosis. Angioplasty was started 3 min after treatment infusion, and the balloon was inflated 3 times at 10 atm for 1 min with a handheld inflator, with 1-min intervals between inflations. After balloon dilatation, the angioplasty catheter was withdrawn. A post-procedure angiogram was performed 1 min after the last inflation. One min later, the drug was again administered and 2 other angiograms were performed 5 and 15 min after this second administration. The animal was then euthanized.

Vasospasm Quantification

All angiograms were analyzed by a computer-assisted method of image analysis (Imagenia 5000 Biocom, Lyon, France). Artery diameter quantification was performed by an observer blinded to the treatment. Zones of interest (distal external iliac artery 1 cm proximal to the dilatation site and femoral artery 1 cm distal to the dilatation site)

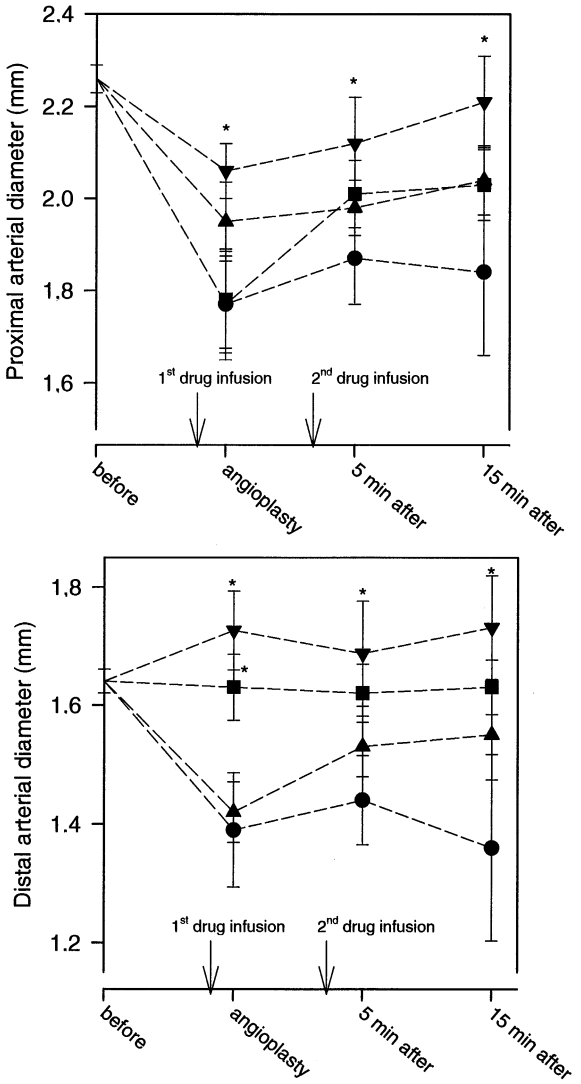


FIG. 2. Effect of different compounds on AIV or rabbit femoral arteries following angioplasty. Proximal (upper panel) and distal (lower panel) arterial diameters of femoral arteries of vehicle- (●), isosorbide dinitrate- (0.3 mg/kg, i.v.) (■), verapamil- (0.2 mg/kg, i.v.) (▲) or fantofarone-treated (50 μg/kg, i.v.) (▼) rabbits were measured at the indicated times before and after angioplasty. Results are means of 10 animals per group. Statistical analysis: *P < 0.05.

were located with collaterals. The arterial diameter value of these segments was the mean of 20 measurements.

Statistical Analysis

The results are shown as means ± SEM. Data were statistically analyzed using the Kruskal-Wallis nonparametric analysis of variance with Holm-Bonferroni alpha adjustment taking $P < 0.05$ to indicate a significant difference.

RESULTS

Figure 1B shows an example of AIV which occurred in saline-treated animals. In these animals, as shown in Fig. 2, both mean distal and proximal artery diameters measured on the femoral artery, 1 cm below and above the angioplasty site (see arrows in Fig. 1B), strongly decreased following angioplasty and remained at the same level throughout the experiment. In 50% of the controls ($N = 25$), vasospasm was diffuse (proximal and distal) whereas in the other 50%, vasospasm was largely predominant on proximal segments. In some cases (10%), we observed very severe vasospasms progressively resulting in complete arterial occlusion.

TABLE 1. Effect of different compounds on MABP during angioplasty

	Before angioplasty	Angioplasty	5 Min after angioplasty
Vehicle	90.2 + 1.3	91.4 + 0.3	90.4 + 0.5
Isosorbide dinitrate (0.3 mg/kg, i.v.)	91.0 + 1.1	88.9 + 1.1	90.8 + 1.1
Verapamil (0.2 mg/kg, i.v.)	89.0 + 0.7	89.2 + 0.8	88.9 + 1.7
Fantofarone (50 μg/kg, i.v.)	91.2 + 1.0	91.0 + 0.3	89.4 + 1.8

Values are means + SD (mm Hg).

Following treatment with isosorbide dinitrate (0.3 mg/kg, i.v.) or fantofarone (50 μg/kg, i.v.), we observed a reduction in the occurrence and severity of vasospasm, whereas verapamil (0.2 mg/kg, i.v.) was much less effective (Fig. 2). Although it totally inhibited distal AIV, isosorbide dinitrate did not significantly affect proximal diameter decrease. The most potent compound with regard to both the distal and proximal vasospasms was fantofarone, which significantly reduced AIV throughout the experiment. A typical example of the effect of this compound is shown in Fig. 1D. Verapamil did not reduce AIV significantly.

Variations of mean arterial blood pressure showed that, compared to the placebo group, all treatments induced a slight but nonsignificant reduction in arterial blood pressure (Table 1).

DISCUSSION

Abnormal constriction of arteries has been described after renal and coronary angioplasty [7, 8]. An increased vasoconstrictor responsiveness has also been shown to contribute to the pathogenesis of myocardial ischemia in spastic angina [9]. In order to study this phenomenon after experimental angioplasty of femoral, iliac, aorta or carotid arteries, several animal models have been described [5, 10, 11]. In that respect, the mechanisms involved in angioplasty-induced vasospasm are not well understood. This vasoconstriction generally occurs in muscular arteries and can be explained by a myogenic response of vascular smooth muscle following depletion of desendothelialized segments in relaxing factors such as prostacyclin and NO. Thrombosis and platelet release of vasoconstrictor factors such as serotonin, platelet-activating factor, adenosine 5′

diphosphate, thromboxane A2 and thrombin [3] may also play an important role, and the effects of different treatments on the prevention of AIV could elucidate the influence of these different pathways.

One explanation for AIV is the impairment of atherosclerotic and desendothelialized arteries in NO production. Our study confirms the efficiency of a NO donor (isosorbide dinitrate) in improving dilated artery diameter. This compound, which acts as a NO donor, induces smooth muscle cell relaxation by stimulating the soluble guanylate cyclase. This ability to restore normal artery diameter is well known and routinely used during coronary angioplasty when vasospasm occurs [5, 10–12].

Our results confirm the preventive effects of Ca^{2+} blockers on AIV as previously suggested by Le Veen *et al.* with verapamil [5] but extend this observation to a potent compound: fantofarone. This preventive effect has now to be proven in current clinical practice for AIV and spastic angina prevention and treatment.

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