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Antianginal effects of FR144420, a novel slow nitric oxide-releasing agent

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Abstract

The aim of this study was to compare the antianginal effects of two compounds that release nitric oxide (NO) spontaneously, i.e. (±)-*N*-[(*E*)-4-ethyl-3-[(*Z*-hydroxyimino]-5-nitro-3-hexenyl]-3-pyridinecarboxamide (FR144420) and (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409), in two different rat models of coronary vasospasm. In the rat methacholine-induced coronary vasospasm model, FR144420 suppressed the elevation of the ST segment dose dependently and significantly at 1.0 mg/kg, i.d. 185 min after its administration. FK409 suppressed the ST elevation only 5 min after its administration at 1.0 mg/kg, i.d. FR144420 and FK409 significantly decreased mean blood pressure at all doses tested only 5 min after their intraduodenal administration, but did not change heart rate at any time. Although the suppression of the ST elevation by FK409 had the same duration as its hypotensive effect, the FR144420-induced suppression of the ST elevation lasted longer than its hypotensive effect. In the rat vasopressin-induced coronary vasospasm model, FR144420 (32 mg/kg) significantly inhibited the depression of the ST segment both 60 min and 120 min after oral administration, whereas FK409 (32 mg/kg) significantly inhibited this ST depression only 60 min after oral administration. These data suggest that FR144420 inhibits coronary vasospasm for longer than FK409 does and particularly shows more prolonged antianginal effects than hypotensive effects in the methacholine-induced coronary vasospasm model. Thus FR144420 is expected to be a useful NO releaser for investigating the in vivo actions of NO.

Keywords: FR144420; FK409; Nitric oxide (NO); Angina

1. Introduction

Recently, we have reported that (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide (FK409) (Fig. 1), which was isolated from the fermentation products of *Streptomyces griseosporeus* (Hino et al., 1989), releases nitric oxide (NO) spontaneously in solution (Kita et al., 1994a). The potent biological actions, such as vasorelaxant and antiplatelet effects, of FK409 are secondary to the release of NO. In in vivo experiments, FK409 shows beneficial effects in anginal models in dogs (Isono et al., 1993) and in rats (Kita et al., 1994b). FK409, however, rapidly decomposes and releases NO in solution (Kita et al., 1994a) and, hence, this compound may have limited clinical use.

We have discovered a derivative ((±)-*N*-[(*E*)-4-ethyl-3-[(*Z*)-hydroxyimino]-5-nitro-3-hexenyl]-3-pyridinecarboxamide; FR144420) (Fig. 1) of FK409. Compared with FK409, FR144420 is more stable and releases NO more slowly in aqueous solution (Kita et al., 1995). In addition, the duration of the hypotensive effect caused by intravenous and oral administration of FR144420 is longer than that of FK409. In the present study, we examined the antianginal effects of FR144420 in two different rat models, i.e. the methacholine- and the vasopressin-induced coronary vasospasm model, in comparison with FK409.

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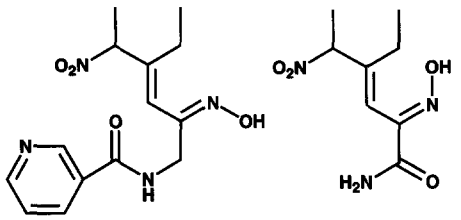

Fig. 1. Chemical structures of FR144420 and FK409.

We discuss whether FR144420 is a useful NO releaser for investigating the in vivo actions of NO.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats and Donryu rats were supplied from Nihon SLC Co. (Shizuoka, Japan) and Charles River Japan Co. (Tokyo, Japan), respectively. In the methacholine- and vasopressin-induced coronary vasospasm models, Sprague-Dawley rats (285–350 g) and Donryu rats (175–260 g), respectively were fasted for 24 h and were given each drug suspended in 0.5% methylcellulose in a volume of 5 ml/kg.

2.2. Rat methacholine-induced coronary vasospasm model

The methacholine-induced coronary vasospasm model was prepared as described previously (Sakai et al., 1981). Briefly, Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg s.c., in saline). The trachea, duodenum and left femoral artery were cannulated with polyethylene tubing. For bolus injection of methacholine into the ostia of the coronary artery, a double-walled cannula was introduced through the exposed right carotid artery to a point near the aortic valve. Mean blood pressure and heart rate were measured by means of cannula in the femoral artery with a pressure transducer (TP-400T, Nihon Kohden Co., Tokyo, Japan) connected to an amplifier (AP-601G, Nihon Kohden Co.) and a heart rate counter (AT-600G, Nihon Kohden Co.) respectively and they were recorded on a polygraph (PEN OSCILLOGRAPH, NEC San-ei Instruments, Tokyo, Japan). Single doses of methacholine (8 μg) in a volume of 0.01 ml were injected into the aorta over 1 s, using microsyringes, before and 5, 50, 95, 140 and 185 min after intraduodenal administration of vehicle or each drug. Drugs were given intraduodenally while the rats were under anesthesia to observe the duration of the stable effects in the same rat. The standard limb lead II of the electrocardiogram was recorded with an electrocardiograph (LABO SYSTEM ZS-501, Fukuda ME Kogyo Co., Tokyo, Japan). The difference between the amplitude of the ST segment after and just before the administration of methacholine was presented as the elevation of ST segment. The effects of each drug on ST elevation, mean blood pressure and heart rate are expressed as percentages of pre-administration values.

2.3. Rat vasopressin-induced coronary vasospasm model

Donryu rats were anesthetized with sodium pentobarbital (60 mg/kg i.p., in saline) 15 min before intravenous administration of vasopressin. A polyethylene catheter filled with saline was inserted into the left femoral vein in the anesthetized rat. According to the method of Hiramatsu et al. (1970), coronary vasospasm was induced. Vasopressin (0.2 I.U./kg, in saline) was injected intravenously via the cannula placed in the femoral vein. The standard limb lead II of the electrocardiogram was recorded with an electrocardiograph. As previously described (Hatano et al., 1980), the difference between the amplitude of the ST segment after and just before the administration of vasopressin was presented as the depression of ST segment (ΔST). The amplitude of the ST segment was measured at intervals of 0.5 min for 5 min after administration of vasopressin. When the amplitude of the ST segment was measured, the rats were anesthetized. The mean ΔST from 1.0 to 5.0 min was expressed as $|\Delta ST|$. Each drug was administered to conscious rats orally 60 and 120 min prior to the administration of vasopressin.

2.4. Drugs

FR144420 and FK409 were synthesized by Fujisawa Pharmaceutical Co. (Osaka, Japan). Methacholine chloride and arginine vasopressin were purchased from Wako Pure Chemical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO, USA), respectively.

2.5. Statistical analysis

Data are presented as the means $\pm$ S.E.M. of the number of experiments performed. For multiple comparisons, data were analyzed using a one-way analysis of variance followed by Dunnett's test.

3. Results

3.1. Effects in the rat methacholine-induced coronary vasospasm model

Fig. 2 shows the effects of FR144420 and FK409 on methacholine-induced coronary vasospasm. The ST segment elevation before intraduodenal administration of vehicle, FR144420 (0.32, 1.0 and 3.2 mg/kg) and FK409 (1.0 mg/kg) was 0.446 ± 0.110, 0.367 ± 0.082, 0.554 ± 0.122, 0.469 ± 0.185 and 0.321 ± 0.108 mV, respectively. These values were not significantly different between any

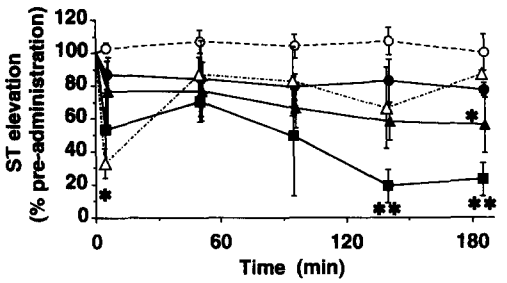

Fig. 2. The effects of vehicle (○), FR144420 (●, 0.32; ▲, 1.0; and ■, 3.2 mg/kg) and FK409 (△, 1.0 mg/kg) on methacholine-induced ST elevation. Effects of each drug are expressed as percentages of pre-administration values. Each value represents the mean ± S.E.M. for 4–5 experiments. * $P < 0.05$, ** $P < 0.01$ compared with the vehicle-treated group.

of the groups. FK409 significantly inhibited the ST elevation caused by methacholine only 5 min after its administration at 1.0 mg/kg, i.d. This result was in agreement with our recent report (Kita et al., 1994b). FR144420 inhibited the ST elevation caused by methacholine dose dependently and significantly at 1.0 mg/kg. In addition, FR144420 showed more prolonged inhibitory effects than did FK409. At 1.0 mg/kg, FR144420 significantly inhibited the ST elevation 185 min after its administration.

Fig. 3a,b shows the effects of FR144420 and FK409 on mean arterial blood pressure and heart rate, respectively. The mean blood pressure and heart rate before intraduodenal administration of vehicle, FR144420 (0.32, 1.0 and 3.2 mg/kg) and FK409 (1.0 mg/kg) were 95 ± 8 and 377 ± 12, 84 ± 5 and 358 ± 5, 88 ± 6 and 346 ± 5, 90 ± 5 and

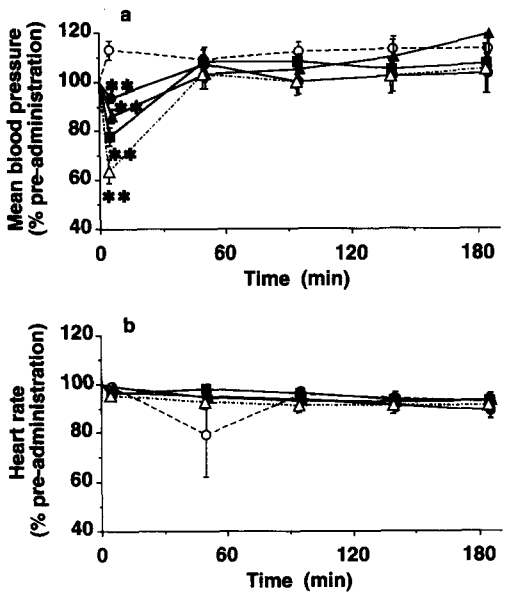

Fig. 3. The effects of vehicle (○), FR144420 (●, 0.32; ▲, 1.0; and ■, 3.2 mg/kg) and FK409 (△, 1.0 mg/kg) on (a) mean blood pressure and (b) heart rate in rats. Effects of each drug are expressed as percentages of pre-administration values. Each value represents the mean ± S.E.M. for 4–5 experiments. * $P < 0.05$, ** $P < 0.01$ compared with the vehicle-treated group.

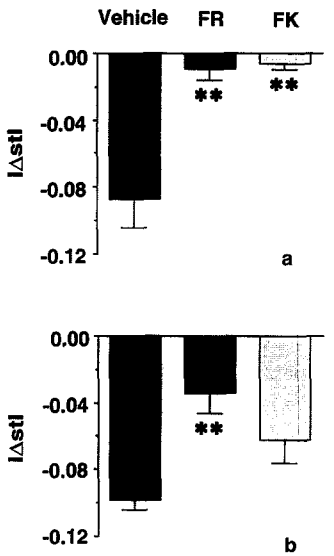

Fig. 4. The effects of FR144420 (FR) and FK409 (FK) on vasopressin-induced ST depression in rats. Each drug was administered orally at 32 mg/kg (a) 60 and (b) 120 min before intraveneous administration of vasopressin. The |ΔST| expresses the mean depression of the ST segment from 1.0 to 5.0 min. Each value represents the mean ± S.E.M. for 6 experiments. ** $P < 0.01$ compared with the vehicle-treated group.

388 ± 12, and 98 ± 11 mm Hg and 368 ± 18 beats/min, respectively. These values were not significantly different between any of the groups. At all doses tested, FR144420 and FK409 decreased mean blood pressure significantly only 5 min after intraduodenal administration. However, the compounds did not change heart rate at any time.

3.2. Effects in the rat vasopressin-induced coronary vasospasm model

In the vehicle-treated group, depression of the ST segment was observed from 0.5 min after the administration of vasopressin. One minute later, the ST depression (ΔST) reached its maximum level (−0.175 ± 0.016 mV; $n = 12$), and 5 min later, ΔST returned to −0.019 ± 0.006 mV ($n = 12$). As shown in Fig. 4, FR144420 (32 mg/kg) significantly inhibited the ST depression caused by vasopressin 60 and 120 min after oral administration by 90 and 65%, respectively. FK409 (32 mg/kg) caused significant inhibition of the ST depression by 93% only 60 min after oral administration.

4. Discussion

FR144420, a derivative of FK409, is more stable and releases NO more slowly in vitro than FK409 (Kita et al., 1995). Furthermore, in in vivo experiments, FR144420 has a longer duration of hypotensive activity after intravenous and oral administration in conscious rats. In the present

study, we compared the antianginal effects of FR144420 in two different rat angina models, i.e. the methacholine- and the vasopressin-induced coronary vasospasm model, with those of FK409. In the methacholine-induced coronary vasospasm model, methacholine administered intraarterially evoked an elevation of the ST segment on the electrocardiogram. As methacholine was injected selectively into the coronary vascular bed, methacholine induces coronary vasoconstriction and coronary vasospasm selectively (Sakai et al., 1981). In the vasopressin-induced coronary vasospasm model, when vasopressin was administered intravenously, it produced a depression of the ST segment. Vasopressin administered peripherally not only causes coronary vasospasm but also induces generalized vasoconstriction and produces hypertension. Therefore, the changes in the ST segment in this model can be influenced by hypotension more than those in the methacholine-induced coronary vasospasm model. Generally, the ST elevation represents transmural ischemia caused by transient total occlusion of a large epicardial artery. The ST depression represents subendocardial ischemic occlusion of a major artery with collaterals from an uninvolved artery (Rothman and Khan, 1991).

In the rat methacholine-induced coronary vasospasm model, FR144420 and FK409 showed significant antianginal effects at 1.0 mg/kg i.d. At this dose, FK409 inhibited the coronary vasospasm caused by methacholine significantly only 5 min after its administration, while FR144420 showed an effect of slow onset and inhibited the vasospasm significantly 185 min after its administration. Thus, the antianginal effect of FR144420 was more prolonged than that of FK409. FR144420 decreased the mean blood pressure only 5 min after its administration in contrast to the longer duration of its antianginal effect. FR144420 showed a more prolonged antianginal effect than hypotensive effect. We consider that FR144420 suppresses the ST elevation caused by methacholine via a direct coronary action induced by NO released from the compound rather than by vasodilation of a peripheral artery, which influences hypotension, because nitroglycerin, which is biotransformed to NO (Chung and Fung, 1992), has been reported to act preferentially on large coronary arteries (Winbury et al., 1969). Previously it has been also reported that FK409 has a greater vasorelaxing effect on the large coronary artery than on the small coronary artery (Isono et al., 1993). Recently we have reported that FK409 has a more potent antianginal effect than hypotensive effect in the methacholine-induced coronary vasospasm model (Kita et al., 1994b). Therefore, FR144420, like FK409 and nitroglycerin, could also act selectively on the large coronary artery. In addition, it is also reported that nitroglycerin and sodium nitroprusside, a nitrovasodilator drug, cause reflex activation of the renin-angiotensin system and sympathetic nervous system (Nielsen et al., 1993; Parker et al., 1992). Thus FR144420, like nitroglycerin and sodium nitroprusside, could cause reflex activation of the renin-angiotensin system and sympathetic nervous system. These responses could diminish the hemodynamic effect of FR144420 even though the compound would remain active. If so, the effect of the compound on exogenous methacholine could remain after the reversal of the hypotensive effect. Additionally, FR144420 is more stable than FK409 and has a longer duration of effect than FK409 in in vivo experiments (Kita et al., 1995). Therefore, it may be relevant that FR144420 shows a longer duration of its antianginal effect than FK409 and a more prolonged antianginal effect than hypotensive effect in the methacholine-induced coronary vasospasm model.

In the rat vasopressin-induced coronary vasospasm model, FR144420 showed significant inhibition of coronary vasospasm both 60 and 120 min after oral administration at 32 mg/kg, whereas FK409 significantly inhibited coronary vasospasm only 60 min after oral administration at the same dose. We have reported that the duration of hypotension caused by the oral administration of FR144420 is longer than that of FK409. At 120 min after administration, FR144420 showed a greater hypotensive effect than FK409 (Kita et al., 1995). Thus, the effect of the two compounds in the vasopressin-induced coronary vasospasm model seems to parallel the hypotensive effects of the compounds. As described above, the level of blood pressure seems to influence the ST segment depression in this model. Taking these facts into account, the effects of the compounds in the vasopressin-induced coronary vasospasm model could be influenced by the hypotensive effects of the compounds more than in the methacholine-induced coronary vasospasm model.

Recently, we have reported that the pharmacokinetic profiles of FK409 and isosorbide dinitrate, which is the most popular orally active nitrate drug for the treatment of ischemic cardiovascular diseases, are very similar and FK409 is about 30 times more potent than isosorbide dinitrate in the rat methacholine-induced coronary vasospasm model (Kita et al., 1994b). Taking these data into account, FR144420 would be expected to be an NO releaser with a more prolonged duration of action in vivo than isosorbide dinitrate.

In conclusion, FR144420 inhibits coronary vasospasms with a longer duration of action than FK409 does in two models of rat angina. In addition, FR144420 shows more prolonged antianginal effects than hypotensive effects in the rat methacholine-induced vasospasm model. These data suggest that FR144420 should be very useful as an NO releaser for investigating the role of NO in in vivo animal models.

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