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SPECIAL REPORT

Close correlation of the cardioprotective effect of FK409, a spontaneous NO releaser, with an increase in plasma cyclic GMP level

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FK409 ((±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide), which has been reported by us to be a new spontaneous nitric oxide (NO) releaser, prevented myocardial infarction following occlusion and reperfusion in rat coronary artery and increased plasma cyclic GMP level in rats, dose-dependently and significantly at 32 mg kg^{-1}. Isosorbide dinitrate (ISDN), which is the most popular orally active NO donor used in the treatment of ischaemic cardiovascular diseases, did not show significant effects at 32 mg kg^{-1} in either experiment. Therefore, it is suggested that FK409 can attenuate myocardial injury during ischaemia and reperfusion in contrast to ISDN and a change in plasma cyclic GMP level may serve as an indicator of the cardioprotective effect of NO-releasing drugs.

Keywords: FK409; ISDN; nitric oxide; cyclic GMP; myocardial infarction

Introduction FK409 ((±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide) is a spontaneous nitric oxide (NO) releaser, which shows potent vasorelaxant and antiplatelet effects (Kita *et al.*, 1994). In our former paper, we have reported that FK409 is the first NO donor which can increase the plasma guanosine 3':5'-cyclic monophosphate (cyclic GMP) level (Kita *et al.*, 1994). Recently, in experiments with isosorbide dinitrate (ISDN) as a NO donor, it has been reported that a change in plasma cyclic GMP level cannot serve as a surrogate measurement of haemodynamic changes because ISDN does not increase the plasma cyclic GMP level at the dose, at which ISDN shows haemodynamic effects (Shotan *et al.*, 1993). However, the correlation between cardioprotective effect and a change in plasma cyclic GMP level by NO donors remains unclear.

In this paper, we evaluated the cardioprotective effect of FK409 during ischaemia and reperfusion in rat coronary artery, applying myocardial infarct areas as the endpoint. Furthermore, we examined whether a change in plasma cyclic GMP level could serve as an indicator of the biological action of NO-releasing drugs.

Methods *Myocardial infarction model* Male SD rats, purchased from Nihon SLC Co. (Sizuoka, Japan) weighing 285–320 g, were fasted for 24 h before the experiment. They were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) and were respired artificially with air through a tracheal cannula. Subsequently the chest was opened. After the heart was exposed, the left coronary artery was ligated for 60 min with a nylon thread and then reperfused for 60 min. Vehicle (0.5% methyl cellulose), FK409 or ISDN (each drug suspended in vehicle) was given orally in a volume of 5 ml kg^{-1}, 30 min before the ligation. Standard second lead of the electrocardiogram (Labo System ZS-501, Fukuda ME Kogyo Co., Tokyo, Japan) was used to evaluate ST segment elevation. Peak ST segment elevation was measured during coronary occlusion. Sixty min after reperfusion, the heart was excised and was divided into six slices perpendicular to the apex-base axis. These were then placed in 0.5% triphenyltetrazolium chloride in phosphate buffered saline without calcium chloride at 37°C for 10 min to dye the normal region. The infarct and normal area were analysed on photographed tissue slices with a digitizer (KD-4300, Graphtec Co., Tokyo, Japan). The myocardial infarct area was expressed as a percentage of the total ventricle area.

Determination of plasma cyclic GMP level Blood from male SD rats weighing 240–325 g, which were anaesthetized with diethyl ether before and 5, 30, 60, 120 and 240 min after the administration of vehicle, FK409 or ISDN, was collected from the abdominal aorta into plastic vessels containing 2% EDTA (20^{-1} volume) on ice. Cyclic GMP levels were determined according to Kita *et al.* (1994). The plasma cyclic GMP level was expressed as a percentage of the pre-administration level.

Materials FK409 and ISDN were synthesized by Fujisawa Pharmaceutical Co. (Osaka, Japan).

Statistical analysis Data are presented as mean ± s.e. mean of the number of experiments as indicated. For multiple comparisons, data were analysed by one-way analysis of variance followed by Dunnett's test.

Results Figure 1a shows the effects of oral FK409 and ISDN on myocardial infarction. FK409 reduced the infarct area dose-dependently and showed significant suppression (45.5% suppression vs. vehicle-treated group) at 32 mg kg^{-1} while ISDN did not reduce the infarct area significantly compared with the vehicle-treated group at 32 mg kg^{-1}.

Figure 1b shows the effects of FK409 and ISDN on plasma cyclic GMP level 5 min after oral administration. Plasma cyclic GMP levels before the administration of vehicle, FK409 (10 and 32 mg kg^{-1}) and ISDN (32 mg kg^{-1}) were 11.7 ± 0.7, 10.1 ± 1.2, 10.3 ± 1.7 and 8.8 ± 1.0 pmol ml^{-1}, respectively. These values were not significantly different. FK409 increased the plasma cyclic GMP level dose-dependently, a significant increase being induced after the administration of 32 mg kg^{-1} FK409. No significant increase in plasma cyclic GMP level was observed after the administration of ISDN, 32 mg kg^{-1}. FK409 increased plasma cyclic GMP level only 5 min after administration, whilst ISDN had no effect at any time (data not shown).

Discussion The data presented in this study clearly showed a beneficial effect of FK409, a new spontaneous NO releaser, on myocardial infarction produced during occlusion and reperfusion in rat coronary artery. The peak ST segment elevations in the electrocardiogram during myocardial

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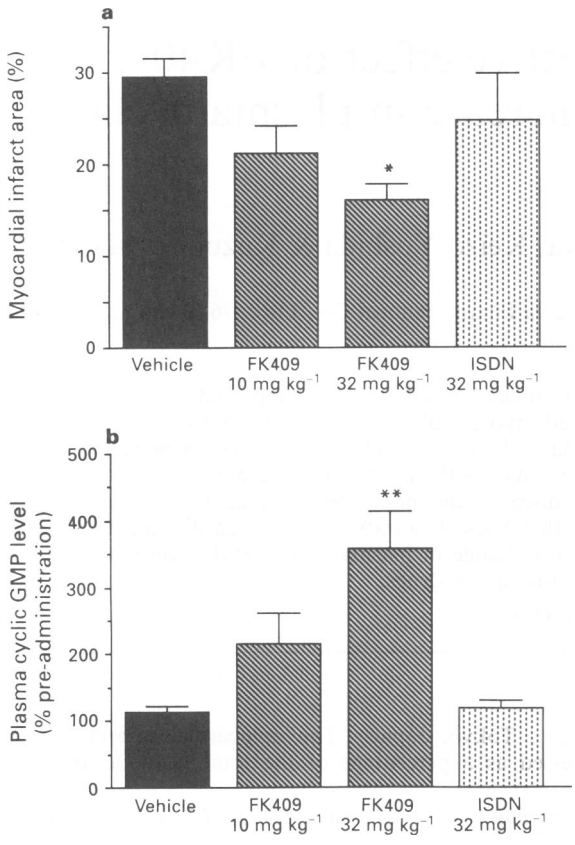

Figure 1 (a) The effects of FK409 and ISDN on myocardial infarction produced during occlusion and reperfusion in rat coronary artery. Each value represents the mean ± s.e.mean of eight and six experiments for vehicle-treated group and drug-treated groups, respectively. $*P<0.05$ compared with vehicle-treated group. (b) The effects of FK409 and ISDN on plasma cyclic GMP level in rats. Each value represents the mean ± s.e.mean of four experiments. $**P<0.01$ compared with vehicle-treated group.

ischaemia were equivalent in both the vehicle-treated and drug-treated groups, indicating a comparable degree of ischaemic insult (data not shown). Nevertheless, FK409 was able to reduce myocardial infarct area dose-dependently and significantly at 32 mg kg^{-1}, while ISDN, which is the most popular orally active NO donor used in the treatment of ischaemic cardiovascular diseases, was not cardioprotective at 32 mg kg^{-1}.

The mechanism of myocardial injury during ischaemia and reperfusion is not fully understood, but thrombus formation (Laws *et al.*, 1983; Bender *et al.*, 1985) and the generation of cardioactive and vasoactive mediators (Stahl *et al.*, 1988; Smith *et al.*, 1989) by activated platelets may be important aspects of this pathogenic mechanism. The reduction of circulating platelets by the treatment with rabbit antiserum against rat platelets has reduced myocardial infarct area in rats (Kanayama *et al.*, 1992). We have reported that FK409 shows much more potent antiplatelet effects than ISDN (Kita *et al.*, 1994). Therefore, the antiplatelet action of FK409 via NO is probably an important factor for the cardioprotective effect of the compound.

FK409 increased the plasma cyclic GMP level dose-dependently and significantly at 32 mg kg^{-1}. FK409 is the first NO donor to be reported to increase plasma cyclic GMP levels *in vivo*. This increase may be attributed to the copious production of intracellular cyclic GMP mediated by NO, which is released spontaneously from FK409. The dose-dependency of FK409-induced cardioprotection coincided with the increase in plasma cyclic GMP level, while ISDN, which can release NO dependently on sulphydryl group (Feelish & Noack, 1987a,b), did not show any increase in plasma cyclic GMP level or a cardioprotective effect at 32 mg kg^{-1}. Thus a change in plasma cyclic GMP level may serve as an indicator of the cardioprotective effect of NO-releasing drugs.

In conclusion, we suggest that FK409, a new spontaneous NO releaser, suppresses myocardial infarction produced during ischaemia and reperfusion. The cardioprotective effect of NO closely correlates with a rapid increase in the plasma cyclic GMP level.

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