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FR183998, a Na^+/H^+ exchange inhibitor, suppresses both IL-8 content and myocardial infarct size in a cardiac ischaemia–reperfusion model in rats

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Abstract

The aim of this study was to determine the effect of FR183998 (5-(2,5-dichlorothiophen-3-yl)-3-[(2-dimethylaminoethyl)carbamoyl]benzoylguanidine dihydrochloride), an Na^+/H^+ exchange inhibitor, on myocardial interleukin-8 (IL-8) content and myocardial infarct size in a rat ischaemia and reperfusion model. Rats underwent 30 min of ischaemia followed by 1 to 24 h of reperfusion. IL-8 content rapidly increased in reperfused rat hearts. The maximum increase in IL-8 was obtained after 3 h of reperfusion. Intravenous administration of FR183998 at 1 and 3.2 mg kg^{-1}, 5 min before ischaemia, significantly reduced the IL-8 level after 3 h of reperfusion (122 ± 16 and 149 ± 23 pg mg^{-1} protein, respectively), compared with that of the saline-treated group (258 ± 27 pg mg^{-1} protein). Myeloperoxidase activity after 3 h of reperfusion was also reduced by FR183998 (from 0.83 ± 0.19 unit g^{-1} weight of tissue in the saline-treated group to 0.36 ± 0.09 and 0.33 ± 0.06 unit g^{-1} weight of tissue in FR183998-treated groups at 1.0 and 3.2 mg kg^{-1}, respectively). Myocardial infarction induced by 30 min of ischaemia and 24 h of reperfusion was significantly suppressed by the same doses of FR183998 (14.0 ± 1.5, 13.5 ± 1.9 % at 1.0 and 3.2 mg kg^{-1}), compared with 22.2 ± 2.7 % in the saline-treated group. These results suggest that IL-8 may contribute to the generation of myocardial infarction in an ischaemia and reperfusion model in rats.

Introduction

Na^+/H^+ exchange is known to play a key role in cardiac ischaemia and reperfusion. Myocardial ischaemia activates Na^+/H^+ exchange and leads to a dramatic elevation of Na^+ influx (Frelin et al 1984), subsequently causing intracellular Ca^{2+} overload, which contributes to myocardial injuries (Tani & Neely 1989). In our previous study, inhibition of Na^+/H^+ exchange by 5-(2,5-dichlorothiophen-3-yl)-3-[(2-dimethylaminoethyl)carbamoyl]benzoylguanidine dihydrochloride (FR183998) was shown to reduce the incidence of arrythmias and myocardial infarct size induced by ischaemia and reperfusion (Ohara et al 1999).

Many investigators have shown that inflammation is an additional mediator of myocardial reperfusion injury (Entman et al 1991), including accumulation of leukocytes, which are related to the advancement of myocardial cell necrosis (Chatelain et al 1987). It has been shown that in experimental models of acute ischaemic injury, myocardial infarction could be attenuated by inhibition of leukocyte accumulation such as leukocyte depletion by filter (Engler et al 1986) and using blocking monoclonal antibodies to inhibit leukocyte adhesion (Simpson et al

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1988). In myocardial ischaemia and reperfusion, leukocytes accumulate in myocardial capillaries, leading to capillary plugging, which is known to be a major mechanism in the no-reflow phenomenon (Engler et al 1983) and contributes to the extension of myocardial infarction (Ambrosio et al 1989).

Interleukin-8 (IL-8) is a strong chemoattractant for leukocytes (Yoshimura et al 1987) and has proinflammatory functions. It has been demonstrated that myocardial reperfusion induces IL-8 induction in ischaemic tissue, which triggers subsequent inflammatory responses due to leukocytes (Baggiolini et al 1989). Previous studies have reported that IL-8 content or mRNA levels rose in reperfused hearts in rabbit and canine models (Ivey et al 1995; Kukielka et al 1995). Furthermore, in clinical studies an increase in IL-8 level was observed in patients with acute myocardial infarction undergoing cardiopulmonary bypass surgery (Jorens et al 1993) and percutaneous transluminal coronary angioplasty (Abe et al 1993).

In this study, we examined the effect of FR183998 on IL-8 production in an experimental ischaemia–reperfusion model in rats. As inflammatory processes are implicated in the advancement of myocardial cell necrosis, we also investigated the effect of FR183998 on myocardial infarction after 24 h of reperfusion.

Materials and Methods

Measurement of IL-8 content in rat hearts

All experiments were performed in accordance with the regulations of the Animal Ethical Committee of the Fujisawa Pharmaceutical Co., Ltd. Surgical procedures for the myocardial infarction model in anaesthetized rats have been described previously (Ohara et al 1999). Thoracotomy for coronary artery ligation was performed on the left side of the thorax above the heart of male Sprague-Dawley rats (9–10 weeks of age), anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). The left anterior descending coronary artery was ligated about 2–3 mm from its origin with 6-0 nylon thread penetrating through a polyethylene tube. Reperfusion was performed by cutting the nylon thread on the polyethylene tube. In the measurement of IL-8 content, left coronary arteries were occluded for 30 min followed by differing reperfusion times (1, 3, 6 and 24 h). Saline or FR183998 was administered intravenously 5 min before occlusion. After each reperfusion period hearts were immediately removed and rinsed in saline, and ventricular tissues were frozen in liquid nitrogen and stored at -20°C. Heart tissues were sedimented in ice-cold PBS (–) containing 2 mM phenylmethylsulfonyl fluoride, 1 μg mL^{-1} leupeptin, 1 μg mL^{-1} aprotinin and 0.05% sodium azide (2 mL g^{-1} tissue). The tissues were homogenized and centrifuged at 40000 *g* for 30 min at 4°C and the supernatants used for assay. The IL-8 contents were measured using a rat GRO/CINC-1 assay kit (Immuno-Biological Laboratories Co., Ltd). The protein contents in these supernatants were measured by the Bradford method using serum albumin as the standard.

Determination of myeloperoxidase activity in rat hearts

Myeloperoxidase (MPO) activity was determined according to a previously described method with a little modification (Griswold et al 1988). Surgical procedures in anaesthetized rats were performed as described above. Rat hearts subjected to 30 min of ischaemia and 3 h of reperfusion were immediately removed and rinsed in saline, and ventricular tissues were frozen in liquid nitrogen and stored at -20°C. Saline or FR183998 was administered 5 min before occlusion. Each sample was measured spectrophotometrically at 460 nm and MPO activity was quantified kinetically for 10 min with 1 min of interval. MPO activity was determined by comparison with a standard curve using peroxidase from horseradish. The data are expressed as MPO activity (unit g^{-1} weight of tissue).

Measurement of myocardial infarct size

Surgical procedures in anaesthetized rats were performed in the same way as described above. Saline or FR183998 was given intravenously 5 min before coronary artery ligation and 5 min before reperfusion. After 24 h of reperfusion following 30 min of ischaemia, rat hearts were removed, immediately rinsed with saline and cut into six slices of about 5 mm each, parallel to the atria-ventricular groove. All slices were then stained by incubation in 0.5% triphenyltetrazolium chloride (TTC) in PBS (–) buffer for 10 min at 37°C and immersed in 10% formalin. After 24 h$\pm$15 min they were photographed and the infarct area (TTC negative) and the non-infarct area were determined using a computerized planimetry program (NIH image for Macintosh). The infarct size was calculated as the sum of the necrotic fields of six slices in relation to the entire region (sum of necrotic and non-infarct fields), and expressed as a percentage of the total ventricle area.

Drugs

FR183998 was synthesized by the Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan). All reagents used in the determination of MPO activity were purchased from Sigma Chemicals (St Louis, MO) and Wako Pure Chemical Industries, Ltd (Osaka, Japan).

Statistics

Data are presented as the mean±s.e.m. for the number of experiments indicated. Statistical comparisons for two groups were performed using the Wilcoxon rank sum test. Data were analysed using the Kruskal–Wallis test for multiple comparisons. When this test indicated a significant difference, the data were further analysed with Dunnett's multiple range test.

Results

IL-8 content in rat hearts during reperfusion

Figure 1 shows the IL-8 content in rat hearts during 24 h of reperfusion. The IL-8 content of intact hearts (without any operation) was 15±2 pg mg^{-1} protein (n = 4). In the sham-operated group, a slight rise of IL-8 content was observed, which gradually decreased to the basal level. In contrast, the IL-8 content in hearts subjected to 30 min of ischaemia increased progressively during 24 h of reperfusion, and the peak level was attained at 3 h of reperfusion (499±122 pg mg^{-1} protein). At each time point (1, 3, 6 and 24 h) after reperfusion, the IL-8 level was significantly higher than that of the sham-operated group.

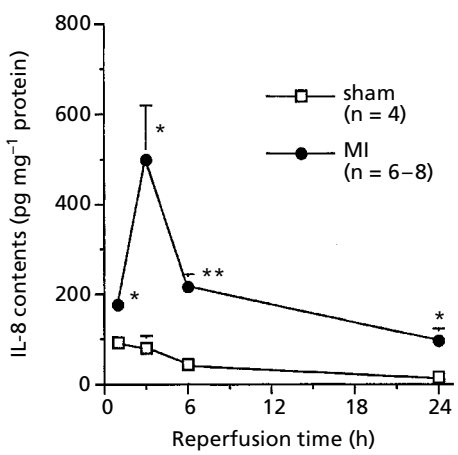

Figure 1 Time-course of IL-8 production in rat hearts subjected to 30 min of ischaemia followed by 1, 3, 6 and 24 h of reperfusion or that of a sham-operated group. Each value represents the mean±s.e.m. of four experiments for the sham-operated group and six to eight experiments for the ischaemia and reperfusion groups. *$P < 0.05$, **$P < 0.01$ compared with the sham-operated group.

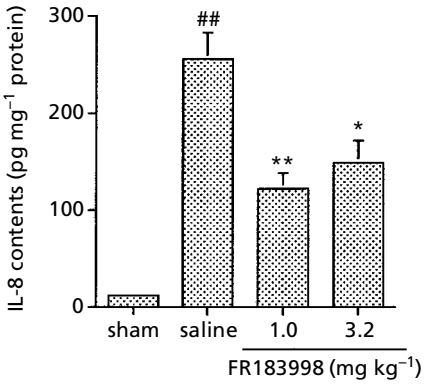

Figure 2 Inhibitory effect of FR183998 (1.0 and 3.2 mg kg^{-1}) on IL-8 production in rat hearts after 30 min of ischaemia followed by 3 h of reperfusion. FR183998 was administered intravenously, 5 min before coronary artery occlusion. Each value represents the mean±s.e.m. of four experiments for the sham-operated group and six or seven experiments for the saline- or FR183998-treated groups. ##$P < 0.01$ compared with the sham-operated group and *$P < 0.05$, **$P < 0.01$ compared with the saline-treated group.

Effect of FR183998 on IL-8 content during reperfusion

The effect of FR183998 on IL-8 production in rat hearts was evaluated at 3 h of reperfusion, as peak IL-8 content was obtained at this point (Figure 1). In the sham-operated group, the IL-8 content was 12±3 pg mg^{-1} protein. As shown in Figure 2, the IL-8 content progressively increased to 258±27 pg mg^{-1} in the control group treated with saline. This elevation of IL-8 production was reduced by 1.0 and 3.2 mg kg^{-1} of FR183998 to 122±16 and 149±23 pg mg^{-1} protein, respectively. These values were statistically significant compared with the control group. Also, IL-8 content in the control group, but not in FR183998-treated groups, reached a statistically significant level compared with the sham-operated group.

MPO activity in reperfused rat hearts

MPO activity was measured as a marker of neutrophil infiltration in rat hearts after 3 h of reperfusion. As shown in Figure 3, MPO activity was reduced in the hearts treated with FR183998 at 1.0 and 3.2 mg kg^{-1} (from 0.83±0.19 in the control group to 0.36±0.09 and 0.33±0.06 unit g^{-1} in FR183998-treated groups at 1.0 and 3.2 mg kg^{-1}, respectively). The reduction in MPO activity in the FR183998-treated groups reached a statistically significant level.

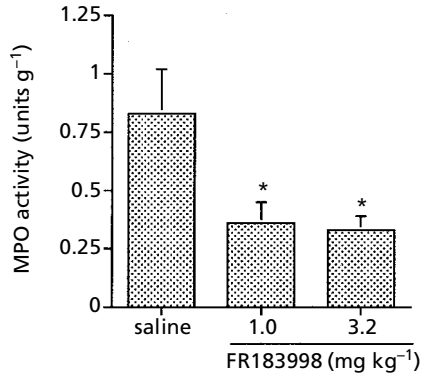

Figure 3 Inhibitory effect of FR183998 (1.0 and 3.2 mg kg^{-1}) on MPO activity in rat hearts after 30 min of ischaemia followed by 3 h of reperfusion. FR183998 was administered intravenously, 5 min before coronary artery occlusion. Each value represents the mean$\pm$s.e.m. of eight experiments for the saline-treated group and six experiments for FR183998-treated groups. *$P < 0.05$ compared with the saline-treated group.

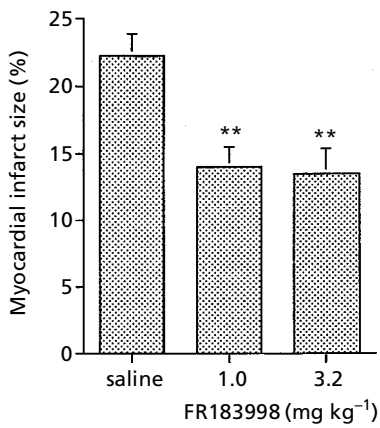

Figure 4 Inhibitory effect of FR183998 on myocardial infarct size in rats after 30 min of ischaemia and 24 h of reperfusion. FR183998 was administered intravenously 5 min before coronary artery occlusion and 5 min before reperfusion. Each value represents the mean$\pm$s.e.m. of 15 experiments for the saline-treated group, and 14 and 16 experiments for the 1.0 and 3.2 mg kg^{-1} FR183998-treated groups, respectively. **$P < 0.01$ compared with the saline-treated group.

Effect of FR183998 on myocardial infarct size induced by 30 min of ischaemia followed by 24 h of reperfusion

As several inflammatory factors have been implicated in the progress of myocardial cell necrosis, we also examined the effect of FR183998 on myocardial infarct size in rats after 24 h of reperfusion. The results are presented in Figure 4. Myocardial infarct size was 22.2$\pm$1.7%, expressed as percentage of the total heart area, in the control group treated with saline. Intravenous administration with FR183998 at 1.0 or 3.2 mg kg^{-1} significantly reduced myocardial infarct size to 14.0$\pm$1.5 and 13.5$\pm$1.9%, respectively. The percentage inhibition of these effects was 37 and 40%, respectively, when compared with the saline-treated group.

Discussion

We have demonstrated that IL-8 content in the myocardium increases rapidly and consistently during reperfusion in an in-vivo rat model, and this increase is suppressed by FR183998, an Na^+/H^+ exchange inhibitor. Also, MPO activity in reperfused hearts was reduced significantly in FR183998-treated groups, suggesting that neutrophil infiltration was attenuated by FR183998. IL-8 is a potent chemoattractant released from endothelial cells, neutrophils and lymphocytes in response to ischaemia and cytokine stimulation. Local generation of IL-8 is an important step in inducing inflammatory responses after reperfusion, and the contribution of IL-8 to reperfusion injury has been shown in a canine ischaemia–reperfusion model (Kukielka et al 1995). To our knowledge this is the first study to examine the relationship between Na^+/H^+ exchange activity and IL-8 content in cardiac ischaemia and reperfusion in rat hearts. Our results agree with previous studies reporting that IL-8 increases in reperfused hearts, and also suggest that Na^+/H^+ exchange may play a key role in IL-8 generation in myocardial ischaemia and reperfusion.

There have been several studies indicating the relationship between Na^+/H^+ exchange and neutrophil activation. It has been reported that intracellular pH regulation due to Na^+/H^+ exchange is essential for neutrophil activities such as migration (Simchowitz & Cragoe 1986) and the production of superoxide (Wright et al 1988). Faes et al (1995) showed that inhibition of Na^+/H^+ exchange with 5-methyl-*N*-isobutyl amiloride could protect hearts from neutrophil-induced reperfusion injury in isolated rat hearts. Also, Gumina et al (2000) showed that inhibition of Na^+/H^+ exchange directly attenuated neutrophil activity in an in-vitro study. These findings and our study suggest the possibility that Na^+/H^+ exchange inhibition could attenuate the neutrophil function directly through pH regulation, as well as indirectly through reduction of IL-8 production.

In this study the myocardial infarct size induced by 30 min of ischaemia followed by 24 h of reperfusion was significantly reduced by the administration of FR183998. We have already shown that FR183998

strongly reduces myocardial infarct size after 1 h of reperfusion in the same model (Ohara et al 1999), which is in agreement with other studies showing the myocardial infarct size-limiting effects of Na^+/H^+ exchange inhibition in the early phase of reperfusion (Bugge & Ytrehus 1995; Rohman et al 1995). The mechanism of this effect is thought to be due to the suppression of Ca^{2+} overload in myocytes during ischaemia and early reperfusion by Na^+/H^+ exchange inhibition. Our present study suggests that FR183998 reduces myocardial infarction not only by suppression of Ca^{2+} overload during ischaemia and in the early phase of reperfusion, but also by attenuation of neutrophil infiltration due to IL-8 reduction in the subsequent phases of reperfusion.

In conclusion, the results of this study indicate that inhibition of Na^+/H^+ exchange with FR183998 can reduce the increase of IL-8 levels and MPO activity in rat hearts during reperfusion. Furthermore, FR183998 also limited subsequent myocardial infarction after 24 h of reperfusion in rats. Although further studies will be needed to clarify the relationship between Na^+/H^+ exchange and inflammation related to IL-8 induction during reperfusion, this study suggests the possibility that inhibition of Na^+/H^+ exchange might attenuate extension of reperfusion injury due to IL-8 accumulation, following derangement of ionic homeostasis.

Conclusion

This study demonstrates the contribution of IL-8 and Na^+/H^+ exchange to cardiac ischaemia and reperfusion injury in an anaesthetized rat model as follows:

- the IL-8 content increased in reperfused rat hearts after 30 min of ischaemia and the maximum increase was obtained after 3 h of reperfusion
- Na^+/H^+ exchange inhibition with FR183998 attenuated the increase of IL-8 content as well as neutrophil accumulation after 3 h of reperfusion
- myocardial infarct size after 24 h of reperfusion was also reduced by Na^+/H^+ exchange inhibition.

These results suggest the possibility that Na^+/H^+ exchange inhibition may possess a more cardioprotective effect due, at least partly, to suppression of the increase of IL-8 and neutrophil accumulation in reperfused rat hearts.

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