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Short communication

SSR69071, an elastase inhibitor, reduces myocardial infarct size following ischemia–reperfusion injury

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Abstract

Neutrophil elastase contributes to the severity of cardiac damage following coronary ischemia and reperfusion. We evaluated the effects of 2-(9-(2-piperidinoethoxy)-4-oxo-4*H*-pyridol[1,2-*a*]pyrimidin-2-yloxymethyl)-4-(1-methyethyl)-6-methoxy-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide hemihydrate (SSR69071), a novel, potent and selective inhibitor of neutrophil elastase, on infarct size in anaesthetized rabbits subjected to coronary artery occlusion for 30 min followed by reperfusion for 120 min. SSR69071 (3 mg/kg i.v.) reduced cardiac infarct size when administered before ischemia (− 39%, $P < 0.05$) or just prior to reperfusion (− 37%, $P < 0.05$). Subsequent experiments using the latter administration protocol confirmed the ability of SSR69071 (1 and 3 mg/kg i.v.) to reduce infarct size. This cardioprotective activity was associated with inhibition of cardiac elastase.

Keywords: Neutrophil elastase inhibition; SSR69071; Ischemia–reperfusion injury, cardiac; Infarct size

1. Introduction

Neutrophil activation occurring during inflammatory conditions or ischemia–reperfusion injury results in the liberation of free radicals and diverse proteases including elastase. Elastase is capable of degrading many structural proteins notably elastin, collagens, fibrinogen and is thought to participate in endothelial cell, vascular and cardiac damage encountered in various pathologies. Elastase inhibitors have been reported to demonstrate protective effects in animal models of viral myocarditis (Lee et al., 1998), pulmonary hypertension (Cowan et al., 2000), repetitive cardiac ischemia and infarction (Tiefenbacher et al., 1997) or hyperoxic lung injury (Yamamoto et al., 2000).

In this paper, we report the effects of a novel, potent, selective neutrophil elastase inhibitor, 2-(9-(2-piperidinoethoxy)-4-oxo-4*H*-pyridol[1,2-*a*]pyrimidin-2-yloxymethyl)-4-(1-methyethyl)-6-methoxy-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide hemihydrate (SSR69071) (Kapui et al., in press) on myocardial infarction induced by coronary ischemia–reperfusion in the rabbit. We were particularly interested in determining whether SSR69071 demonstrated cardioprotective properties if administered in a delayed fashion, after the onset of coronary ischemia.

2. Materials and methods

2.1. Coronary ischemia–reperfusion in anaesthetized rabbits

Male New Zealand white rabbits weighing 2–3 kg (ESD, France) were anaesthetized by i.m. injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Anaesthesia was maintained by an i.v. perfusion of the combination of ketamine (30 mg/kg/h) and xylazine (70 mg/kg/h) using a catheter placed in the jugular vein. This study was performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Sanofi-Synthelabo Research. The animals were artificially ventilated and blood pO_2,

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pCO_2 and pH were measured and kept within normal limits. Body temperature was maintained at 38 ± 1 °C. Arterial blood pressure and heart rate were measured from a catheter placed in the carotid artery using a pressure transducer (Gould, Longjumeau, France). A left thoracotomy was performed in the fourth intercostal space after which the animal was allowed to stabilize itself during 30 min. Cardiac ischemia–reperfusion was induced by 30 min occlusion of the marginal branch of the left coronary artery followed by 120 min of reperfusion. At the end of this protocol, the heart was removed, mounted in a Langendorff perfusion circuit and, after having re-tied the coronary artery ligature, ink was injected in order to colour the perfused tissue, thus permitting identification of the non-perfused tissue, the area at risk. The left ventricle was separated and cut into transverse layers (thickness: 1.5 mm) from the position of the coronary ligature to the apex. These ventricular sections were incubated in 1% triphenyltetrazolium (Sigma, St. Quentin Fallaver, France) at 37 °C during 15 min to distinguish the infarcted zone from viable tissue. Tissue sections were conserved during 48 h in 10% formaldehyde in order to accentuate the colour contrast between the two zones and then weighed before measurement in a blinded fashion by computerized image analysis (Visiolab 1000, Biocom). The infarct size was calculated taking into account tissue weight and was expressed as a percentage of the area at risk.

2.2. Measurement of cardiac elastase activity

Cardiac elastase activity was measured in separate groups of animals using the following method. Following the ischemia–reperfusion protocol described above, sections of the left ventricle taken from the ischemic zone were immersed in potassium phosphate buffer (50 mM, pH 6, containing 0.9% sodium chloride) and then homogenized using a Potter S Homogenizer (B. Braun Melsungen). After centrifugation at $2800 \times g$ for 30 min at 4 °C, the supernatant was discarded, and 2 ml of potassium phosphate buffer (50 mM, pH 6, containing 0.9% sodium chloride and 0.5% cetyltrimethylammonium bromide) was added to the tissue pellet and homogenized again. The homogenate was frozen and thawed three times. After the last thawing, the homogenate was centrifuged at $2800 \times g$ for 30 min at 4 °C and this supernatant was used as enzyme source. The protein concentration of the supernatant was determined by BIO-RAD Protein Assay kit (Bio-Rad Laboratories, Munchen). Elastase activity was measured using the specific chromogenic substrate Meo-Suc-Ala-Ala-Pro-Val-pNa. The assay mixture contained 100 μl substrate (final concentration: 400 μM), 150 μl buffer (50 mM HEPES/NaOH, pH 7.8 containing 0.5 M NaCl and 0.1 mg/ml bovine serum albumin) and 50 μl of supernatant. Final volume of the assay was 300 μl. The assay was performed in microtiter plates placed in a kinetic plate reader (Vmax kinetic plate reader, Molecular Devices). The change in absorbance at 405 nm was continuously monitored at 25 °C for 0.5 h and the initial velocity of product formation was determined. The elastase activity was expressed as pmol standard elastase/mg protein, using the absorbance of standard elastase (human sputum elastase, Elastase Product, Owensville, MO, USA).

2.3. Treatment protocols

In order to evaluate effects on infarct size, SSR69071 or vehicle were administered i.v. to anaesthetized rabbits prepared as described above in two separate studies. In the first protocol, SSR69071 (3 mg/kg) or vehicle were each administered to two groups of animals either 15 min before coronary ligation or 25 min after coronary ligation (5 min before reperfusion). In the second study, different doses of SSR69071 (1, 3 and 10 mg/kg) or vehicle were administered 25 min after coronary ligation (5 min before reperfusion).

Measurement of cardiac elastase activity was performed in a separate study involving three groups of rabbits. A group treated with SSR69071 (3 mg/kg i.v.) administered 25 min after coronary ligation (5 min before reperfusion), a vehicle group and a sham group (thoracotomy but no coronary ligation).

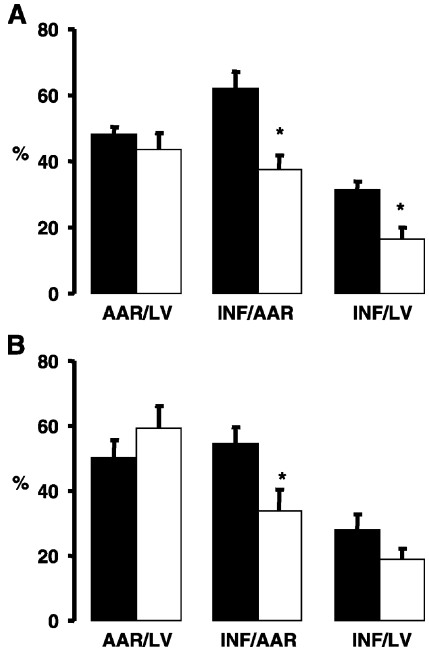

Fig. 1. The effects of SSR69071 (3 mg/kg i.v.) on infarct size in anaesthetized rabbits submitted to 30-min coronary ischemia followed by 120-min reperfusion. Panel A shows the effects of pretreatment with SSR69071 (administered 15 min before coronary ligation). Panel B shows the effects of treatment post-ischemia where SSR69071 was administered 25 min after the start of ischemia 5 min before reperfusion. Black columns represent vehicle group; open columns represent SSR69071-treated animals. Values are means ± S.E.M., $n = 5-7$, showing area at risk as a percentage of the left ventricle (AAR/LV), infarct size as a percentage of the area at risk (INF/AAR) and infarct size as a percentage of the left ventricle (INF/LV). *$P < 0.05$.

2.4. Drugs

SSR69071 (Kapui et al., in press) was synthetised by Sanofi-Synthélabo Research (Chinoin Pharmaceutics, Budapest, Hungary) and was dissolved in methane sulphonic acid before being diluted in 0.9% saline. Other drugs used were as follows: ketamine (Merial, Lyon, France) and xylazine (Bayer, Puteaux, France).

2.5. Statistical analysis

All values shown are means ± S.E.M. Statistical analysis was performed using a Student's *t*-test with the level of significance set at $P < 0.05$.

3. Results

Cardiac infarct size (measured as a percentage of the area at risk) was similar in the three vehicle groups used in these studies at 62 ± 5%, 55 ± 5% and 58 ± 4%, respectively.

In the first study, SSR69071 (3 mg/kg i.v.) significantly reduced infarct size (− 39%, $P < 0.05$) when administered as a pre-treatment (before coronary ligation). The same dose administered at the end of the ischemia period just before coronary reperfusion produced an equivalent reduction in infarct size (− 37%, $P < 0.05$). These data are shown in Fig. 1.

In the second study, investigating different doses of SSR69071 administered at the end of the ischemia period just prior to reperfusion, statistically significant reductions in infarct size were observed for 1 mg/kg i.v. (− 31%, $P < 0.05$, $n = 10$) and 3 mg/kg i.v. (− 35%, $P < 0.05$, $n = 10$) but not for 10 mg/kg i.v. (− 21%, ns, $n = 9$). The haemodynamic parameters measured during this study are shown in Table 1. Coronary ischemia resulted in a sustained reduction in blood pressure and a minor transient increase in heart rate in all experimental groups. No significant difference in blood pressure occurred between vehicle and SSR69071-treated groups. Significant but modest differences in basal heart rate were observed between vehicle and certain drug-treated groups during the pre-treatment period.

Cardiac elastase activity (measured as pmol/mg protein) was low in the sham group of animals (0.53 ± 0.10), but increased substantially in animals submitted to cardiac ischemia–reperfusion (4.39 ± 0.41, vehicle group). Treatment with SSR69071 (3 mg/kg i.v.) just prior to reperfusion significantly reduced cardiac elastase activity (2.92 ± 0.35, $P < 0.05$ versus vehicle group).

Table 1
Haemodynamic effects of SSR69071 in an anaesthetized rabbit model of myocardial infarction following coronary ischaemia–reperfusion

	Control (pre-ischaemia)	Ischaemia (20 min)	5-min post-drug ischaemia (30 min)	Reperfusion (2 h)
Mean arterial pressure (mm Hg)				
Vehicle	91 ± 6	71 ± 5	71 ± 8	67 ± 6
SSR69071— 1 mg/kg i.v.	91 ± 2	71 ± 2	79 ± 3	70 ± 3
SSR69071— 3 mg/kg i.v.	85 ± 4	71 ± 5	70 ± 5	69 ± 6
SSR69071— 10 mg/kg i.v.	89 ± 2	72 ± 2	65 ± 4	75 ± 4
Heart rate (bpm)				
Vehicle	185 ± 6	195 ± 6	183 ± 6	163 ± 5
SSR69071— 1 mg/kg i.v.	164 ± 5[a]	174 ± 4[a]	171 ± 5	160 ± 7
SSR69071— 3 mg/kg i.v.	174 ± 5	189 ± 6	171 ± 7	163 ± 5
SSR69071— 10 mg/kg i.v	163 ± 8[a]	169 ± 8[a]	167 ± 10	144 ± 12

SSR69071 or vehicle was administered 25 min after coronary artery occlusion.
Values shown are means ± S.E.M. $n = 9–11$.
[a] $P < 0.05$ versus vehicle group.

4. Discussion

The principal finding of this study is that SSR69071, a novel small-molecule neutrophil elastase inhibitor (Kapui et al., in press), is capable of reducing infarct size when administered at the end of the period of cardiac ischemia, just prior to reperfusion. We have demonstrated, in a single dose comparison, that the reduction in infarct size (measured as a percentage of the area at risk) is equivalent (− 39% vs. − 37%) whether SSR69071 is administered before ischemia or just prior to reperfusion. Since coronary collateral blood flow is negligible in the rabbit (Miura et al., 1989), this latter protocol allows SSR69071 to reach the ischemic area only once blood flow has been restored. This suggests that the drug does not possess significant anti-ischemic properties and that its cardioprotective effect is due to inhibition of reperfusion-induced cardiac injury. This is consistent with reports concerning the role of elastase in this pathology. In our study, cardiac elastase activity was elevated following ischemia–reperfusion compared with sham animals and this elevation was significantly inhibited by SSR69071 (3 mg/kg i.v.). In man, increased neutrophil elastase release has been demonstrated in unstable angina and acute myocardial infarction (Dinerman et al., 1990). The role of activated neutrophils in reperfusion injury is well documented (Go et al., 1988) and elastase inhibition decreases cardiac leucocyte infiltration and improves myocardial histology following thrombolysis of coronary artery thrombosis in the dog (Nicolini et al., 1991). SSR69071 is a potent and selective elastase inhibitor (Kapui et al., in press). Nevertheless, our study does not allow us to establish the mechanism of the cardioprotective activity observed with SSR69071. Although we believe that this is due to elastase inhibition, we cannot rule out the possibility that other mechanisms could be involved.

Although pharmacological agents acting by several different pharmacological mechanisms demonstrate cardioprotective properties (i.e., reduce infarct size) when administered before the ischemic period, not all are capable of protecting the heart when administered during the ischemic period just prior to reperfusion. For example, using rabbit models of coronary ischemia–reperfusion similar to our own, other groups have demonstrated that nicorandil, a K_{ATP} channel opener (Yamada et al., 2000) and *N*-[(1*S*, *trans*)-2-hydroxycyclopentyl]adenosine (GR79236), an adenosine A_1 receptor agonist (Baxter et al., 2000) no longer reduce infarct size when administered at the reperfusion. The sodium/proton exchange inhibitors, one member of which (cariporide) has been studied clinically in cardiac ischemia–reperfusion injury, have been reported to be either inactive (Miura et al., 1997) or similarly active (Yamada et al., 2000) when given at the reperfusion in the rabbit model. This mode of administration has clinical relevance in the context of the treatment of acute myocardial infarction. Priority is given to interventions such as balloon angioplasty, thrombolysis or coronary bypass graft which rapidly restore perfusion to the ischemic myocardium with the hope of salvaging jeopardized cardiac tissue. Paradoxically, such interventions risk triggering reperfusion injury (Farb et al., 1993), so, in theory, the simultaneous administration of a cardioprotective agent at the time of reperfusion could prove beneficial in this respect.

We confirmed in the dose-ranging study that SSR69071 (1 and 3 mg/kg i.v.) significantly reduced infarct size when administered just prior to reperfusion; however, the inhibitory effect of the highest dose tested (10 mg/kg) did not reach statistical significance. Throughout this dose-range, SSR69071 produced no significant haemodynamic effects versus the vehicle group. We noted, however, a tendency (ns) for a lower heart rate and higher mean arterial pressure at the end of the study (after 120-min reperfusion) in the high dose group, which may explain the smaller reduction in infarct size.

In conclusion, we have demonstrated that SSR69071, a novel neutrophil elastase inhibitor, reduces infarct size in an acute model of coronary ischemia–reperfusion injury when administered just prior to reperfusion. Further studies will be necessary to establish the long-term consequences of this effect for cardiac function.

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