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Moderate inhibition of myocardial matrix metalloproteinase-2 by ilomastat is cardioprotective

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ABSTRACT

Pharmacological inhibition of matrix metalloproteinase-2 (MMP-2) is a promising target for acute cardioprotection against ischemia/reperfusion injury. Therefore, here we investigated if the MMP inhibitor ilomastat administered either before ischemia or before reperfusion is able to reduce infarct size via inhibition of MMP-2, the most abundant MMP in the rat heart.

Infarct-size limiting effect of ilomastat (0.3–6.0 μmol/kg) was tested in an in vivo rat model of myocardial infarction induced by 30 min coronary occlusion/120 min reperfusion. Ilomastat at 0.75 and 1.5 μmol/kg decreased infarct size significantly as compared to the vehicle-treated (dimethyl sulfoxide) group (from 66.1 ± 4.6% to 45.3 ± 7.0% and 46.7 ± 5.5% of area at risk, $p < 0.0.5$, respectively), when administered 5 min before the onset of ischemia. Ilomastat at 6.0 μmol/kg significantly reduced infarct size from its control value of 65.4 ± 2.5% to 52.8 ± 3.7% of area at risk ($p < 0.05$), when administered 5 min before the onset of reperfusion. Area at risk was not significantly affected by ilomastat treatments. To further assess the cytoprotective effect of ilomastat, primary cardiomyocytes isolated from neonatal rats were subjected to 240 min simulated ischemia followed by 120 min simulated reperfusion in the presence of ilomastat (5 nM–5 μM). Ilomastat at 500 nM and 5 μM significantly increased cell viability when compared to vehicle treated group. To assess the in situ MMP-2 inhibitory effect of ilomastat, in separate experiments in situ zymography was performed in cardiomyocytes. The cytoprotective concentration of ilomastat (500 nM) showed a moderate (approximately 25%) inhibition of intracellular MMP-2 in ischemic/reperfused cardiomyocytes. In these cells, MMP-2 immunostaining showed a 90% colocalization with the in situ gelatinolytic activity.

We conclude that the MMP inhibitor ilomastat reduces infarct size when administered either before the onset of ischemia or before the onset of reperfusion in vivo. Furthermore, this is the first demonstration that a moderate inhibition of intracellular MMP-2 is sufficient to confer cardiocytoprotection.

1. Introduction

Acute myocardial infarction and its complications are the leading cause of death in the industrialized countries. The treatment of acute ischemic heart disease has entered a new era through early reperfusion therapy, however, irreversible cell injury leading to apoptosis and necrosis may be precipitated by reperfusion, which may contribute to the development of infarction [1,2]. Therefore, to protect the heart from acute ischemic and reperfusion injury, i.e. to reduce infarct size is of great clinical relevance.

The pathomechanism of myocardial ischemia and reperfusion injury is not completely revealed. Since the original observation by the research group of Richard Schulz, the involvement of matrix metalloproteinases (MMP) in acute myocardial ischemia/reperfusion injury has been well-established [3–9]. MMPs are zinc dependent, neutral endopeptidases involved in several physiological processes, such as embryogenesis, angiogenesis and re-building of extracellular matrix (ECM). Gelatinase types of MMPs, MMP-2 and -9, are implicated in numerous cardiovascular diseases including ischemia/reperfusion injury [10]. Recently, the presence of MMP-2 has been shown in the cytosol of intact

Abbreviations: DMSO, dimethyl sulfoxide; ILO, ilomastat; LAD, left anterior descending coronary artery; MMP, matrix metalloproteinase; PBS, phosphate buffer solution; PI, propidium iodine.

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cardiomyocytes [11]. Moreover, several cardiac contractile proteins, such as titin and troponins, were shown to be potential targets of acute intracellular MMP-2 activation during ischemia/reperfusion [12]. Therefore, MMP-2 became a major target for drug development in acute cardiovascular pathologies including myocardial infarction [13,14].

We have previously reported that MMP-2 activity was moderately decreased during ischemic preconditioning [6] and that exogenous inhibition of MMPs by ilomastat, a non-selective MMP inhibitor, diminished ischemia-induced MMP-2 activity in isolated rat hearts [5]. Furthermore, we have described that the activities of MMP-2 and MMP-9 were decreased significantly in an in vivo rat model of ischemic late preconditioning [3]. Moreover, we and others have shown that ilomastat reduces infarct size in rats and mice ([3,15], for review see Refs. [10,16]). Nevertheless, the dose–response relationships of ilomastat administered before the onset of ischemia as well as before the onset of reperfusion are still unknown. Moreover, there is no proof if ilomastat-induced cardioprotection is due to MMP-2 inhibition. Furthermore, it is also not known, what extent of intracellular MMP-2 inhibition is needed for effective cardioprotection.

Therefore, in the present study, we aimed to investigate the dose–response relationships of ilomastat administered before the onset of ischemia as well as before the onset of reperfusion in an in vivo rat model of myocardial infarction. Furthermore, to test if ilomastat-induced cardioprotection is due to (and what extent of) MMP-2 inhibition, we performed gelatin zymography and in situ zymography followed by immunostaining of MMP-2 in cardiomyocytes subjected to simulated ischemia/reperfusion.

2. Materials and methods

2.1. Animals

Animal handling and the investigation was in conjunction with Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health publication 85-23, revised 1996), and it was approved by a local animal ethics committee.

Male Wistar rats (Charles-River, Germany) weighing 280–370 g were used in the experiments housed in individually ventilated cages. Animals were fed with standard murine chow and unlimited access to water was ensured prior to the surgical intervention. For the cell culture experiments, neonatal Wistar rats were purchased from the local live-stock of the University of Szeged.

2.2. In vivo studies

2.2.1. Surgical procedure of coronary occlusion

Rats were anesthetized by intraperitoneal injection of 60 mg/kg sodium pentobarbital (Euthasol, Produlab Pharma b.v., Raamsdonksveer, The Netherlands). Animals were mechanically ventilated (Model 683, Harvard Apparatus, Holliston, MA) with room air in a volume of 6.2 ml/kg and a frequency of 55 ± 5 breath/min according to body weight. Rats were placed in supine position on a heating pad to maintain body core temperature in physiological range (37.0 ± 1.0 °C). Right carotid artery was cannulated to measure mean arterial blood pressure by a pressure transducer (Experimetria Inc., Budapest, Hungary). Mean arterial blood pressure and body surface electrocardiogram (ECG) was monitored throughout the experiments (Haemosys, Experimetria Inc., Budapest, Hungary). Right jugular vein was also cannulated for fluid substitution and drug administration. Left anterior descending coronary artery (LAD) occlusion was induced by left thoracotomy. A 5-0 Prolene suture (Ethicon, Johnson & Johnson, Budapest, Hungary) was placed around LAD artery and a small plastic knob, which was threaded through the ligature and placed in contact with the heart, was used for making occlusion for 30 min. Appearance of ischemia was confirmed by ST segment elevation and arrhythmias. After 30-min ischemia, hearts were reperfused for 120 min by releasing the ligature. Restoration of blood flow was confirmed by arrhythmias observed in the first minutes of reperfusion.

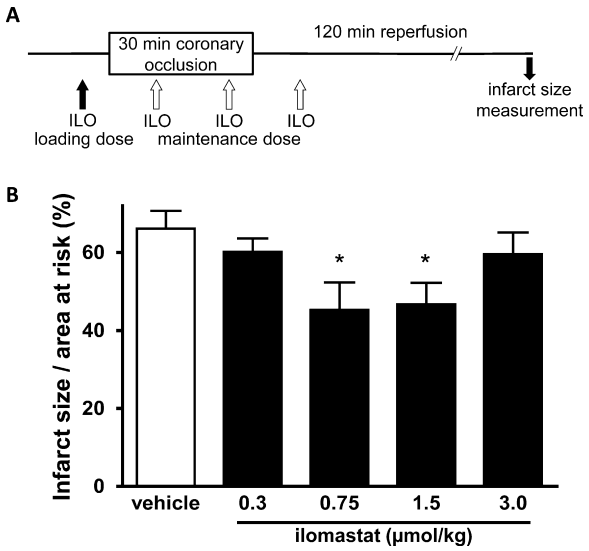

Fig. 1. *Panel A*: In vivo experimental protocol: rats were subjected to 30 min ischemia/120 min reperfusion to measure infarct size. Ilomastat at 0.3, 0.75, 1.5 and 3.0 μmol/kg or vehicle (DMSO) was administered intravenously (upward closed arrow) at 5 min before the onset of ischemia. To maintain serum level of ilomastat, repeated boluses with half dose of the first bolus were administered in every 15 min, three times: at the 10th and 25th min of ischemia and at the 10th min of reperfusion (upward open arrows). *Panel B*: Effect of ilomastat treatment on infarct size when administered before ischemia. $^{*}p < 0.05$ compared to vehicle-treated group, $n = 7–8$, data are shown as mean ± S.E.M.

2.2.2. Experimental groups

In first series of in vivo experiments, ilomastat was administered before the onset and during the 30-min ischemia. Animals were divided into five groups. Dimethyl sulfoxide (DMSO; 11.6 w/v% solution diluted with physiological saline) as vehicle or 0.3, 0.75, 1.5, and 3.0 μmol/kg ilomastat were administered intravenously in slow bolus 5 min before ischemia (Fig. 1A). To maintain serum level of ilomastat, additional 3 boluses of vehicle (5.8 w/v% DMSO solution) or ilomastat with half dose (0.15, 0.375, 0.75; and 1.5 μmol/kg, respectively), were given at the 10th, 25th min of ischemia and at the 10th min of reperfusion. We estimated the maintaining doses of ilomastat according to its half-life based on pharmacokinetic data described previously in rodents after a single intravenous bolus injection [17].

In the second series of in vivo experiments DMSO (11.6 w/v% solution) or ilomastat (0.75, 1.5, 3.0, and 6.0 μmol/kg) bolus was injected at the 25th min of ischemia. Maintaining boluses (5.8 w/v% DMSO or 0.375, 0.75, 1.5, and 3.0 μmol/kg, respectively) were administered at the 10th, 25th and 40th min of reperfusion (Fig. 2A) to maintain constant ilomastat concentration in blood during the early phase of reperfusion.

2.2.3. Determination of infarct size

After 120 min of reperfusion hearts were isolated for infarct size measurements. Hearts were perfused in Langendorff perfusion system with 37 °C Krebs–Henseleit buffer (composition given in Ref. [18]) to remove blood from the coronary vessels. After 5 min of perfusion, risk area was re-occluded, and hearts were perfused with 4 ml of 0.1% Evans blue dye through the ascending aorta.