Letter to the Editor

Antioxidant UPF1 attenuates myocardial stunning in isolated rat hearts☆

Jaak Kals,a,b,⁎ Joel Starkopfc, Mihkel Zilmera, Tõnis Pruler a, Kristel Pulges a, Monika Hallaste a, Mart Kals d, Andres Pulgese,f, Ursel Soomets a

a Department of Biochemistry, Centre of Molecular and Clinical Medicine, University of Tartu, 19 Ravila Street, Tartu 50411, Estonia
b Endothelial Centre, University of Tartu, 8 Puusepa Street, Tartu 51014, Estonia
c Department of Anaesthesiology and Intensive Care, University of Tartu, 8 Puusepa Street, Tartu 51014, Estonia
d Department of Mathematical Statistics, University of Tartu, 2 Liivi Street, Tartu 50409, Estonia
e Department of Cardiology, University of Tartu, 8 Puusepa Street, Tartu 51014, Estonia
f Centre of Cardiothoracic Surgery, North Estonian Regional Hospital, Siitiste tee 19, Tallinn 13419, Estonia

Received 28 November 2006; accepted 1 January 2007

Abstract

Oxidative stress is a crucial pathophysiological mechanism of myocardial ischaemia–reperfusion injury (IRI). We evaluated the cardioprotective effects of a novel glutathione analogue, UPF1 (4-methoxy-L-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine; MW 483.5), on an isolated rat heart model of thirty-minute global ischaemia followed by 90 min of reperfusion. Treatment with UPF1 (1 mg/ml) prior to ischaemia improved the recovery of post-ischaemic left ventricular end-diastolic pressure (p=0.046), developed pressure (p=0.002) and coronary flow (p=0.01). No protective effect was observed when the hearts were treated with UPF1 after ischaemia. Administration of UPF1 had no influence upon infarct size or enzyme leakage from the heart. The results suggest that glutathione analogue type of biomolecules could possess a therapeutic potential in clinical situations where myocardial IRI is presented as myocardial stunning rather than tissue infarction.

Keywords: Antioxidants; Cardioprotection; Glutathione; Ischaemia–reperfusion injury; Rat heart

Oxidative stress plays an important role in myocardial ischaemia–reperfusion injury (IRI) [1,2]. An increased production of reactive oxygen species (ROS) at reperfusion leads to prolonged but reversible post-ischaemic left ventricular dysfunction (myocardial stunning). The intracellular glutathione system is the key element ensuring the redox capacity of the cells, which is necessary for reducing ROS [3]. Glutathione analogues have been extensively studied as molecules with possible pronounced antioxidant properties [4,5].

We designed and synthesized a new tetrapeptide analogue of glutathione, named UPF1 (assignee Vulpes Ltd., no. 110035500, PCT/SE01/01351), which is more hydrophobic than glutathione and can more strongly interact with plasma membrane and/or with hydrophobic binding sites of different proteins. We have previously shown that UPF1 is non-toxic to primary neuronal cultures and has 60-fold higher antioxidativity compared to glutathione [6]. In a model of global cerebral ischaemia, UPF1 appeared to be an effective agent against brain IRI in rats [6]. In the present study the effects of the glutathione analogue UPF1 on IRI are evaluated in an isolated rat heart.

The glutathione analogue UPF1: 4-methoxy-L-tyrosinyl-γ-L-glutamyl-L-cysteinyl-glycine (4-MeO-Tyr-γ-Glu-Cys-Gly; MW 483.5) (Fig. 1) was synthesized manually using Fmoc-chemistry [7]. Male Wistar rats were anaesthetized with sodium-pentobarbital and a standard technique of retrograde perfusion of the isolated heart, using the Langendorff apparatus (TSE-GmbH, Homburg, Germany), was applied [4]. Global ischaemia was achieved by clamping of aortic

☆ This study was supported by grants Nos. 4913, 5833, 6503 and 6588 of the Estonian Science Foundation and by the target financing PARBK 06906.

⁎ Corresponding author. Department of Biochemistry, Centre of Molecular and Clinical Medicine, University of Tartu, 19 Ravila Street, Tartu 50411, Estonia. Fax: +372 7318 457.

E-mail address: Jaak.Kals@kliinikum.ee (J. Kals).
cannula. After 30 min the aortic clamp was removed and the hearts were reperfused for 90 min. UPF1 was dissolved in the gassed Krebs–Henseleit buffer and administered with an automatic syringe into the stop-cock just above the aortic cannula either for 10 min before ischaemia (preUPF1, \(n = 10\)) or during the first 10 min of reperfusion (postUPF1, \(n = 10\)). Ten control hearts received a buffer for 10 min before ischaemia.

After the end of the reperfusion period the hearts were immediately frozen at \(-20^\circ\)C for 24 h. Thereafter, they were cut manually into 2 mm transverse slices and stained with triphenyl tetrazolium chloride. The sections were visualized using a computer imaging system (WinTV 32, Happauge Computer Works, Happauge, USA). The infarcted area was marked and calculated with the aid of the Adobe Photoshop 6.0 software and expressed as the percentage of the left ventricle.

The cardiac troponin T and the creatine kinase MB isoenzyme were measured using an electrochemiluminescence immunoassay on the Roche Elecsys 2010 analyser (Roche Diagnostics GmbH, D-68298, Mannheim, Germany). Functionality data from the experiments with the isolated hearts were compared by using two-factor analysis of variance with treatment as one factor and time as the other factor (free software R, version 2.0.1 for Windows). Differences between the groups in enzyme level and in infarct size were tested by one-way analysis of variance. A \(p\)-value less than 0.05 was considered significant.

The functional parameters of the Langendorff-reperfused rat hearts with induced global ischaemia and UPF1 treatment are shown in Table 1 and in Fig. 2. Administration of UPF1 prior to ischaemia, but not at reperfusion, significantly improved heart function. The infarct size, in contrast, was not significantly different between the groups — 46.2 % of left ventricle was infarcted in the control group, versus 48.4 % and 49 % in preUPF1 and postUPF1 group, respectively. Also, the release of cardiac markers, was not influenced by UPF1 (data not shown).

### Table 1

Functional variables of isolated hearts subjected to 30 min of global ischaemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Stabilization 20 min</th>
<th>Ischaemia 25 min</th>
<th>Reperfusion 5 min</th>
<th>Reperfusion 30 min</th>
<th>Reperfusion 60 min</th>
<th>Reperfusion 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left ventricular systolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>103.2 ±7.5</td>
<td>42.0±4.6</td>
<td>107.4±6.2</td>
<td>78.6±6.5</td>
<td>67.4±5.9</td>
<td>58.6±4.4</td>
</tr>
<tr>
<td>PreUPF1</td>
<td>118.8 ±6.9</td>
<td>31.3±6.3</td>
<td>100.5±8.6</td>
<td>92.8±6.9</td>
<td>82.3±6.9</td>
<td>78.1±5.8</td>
</tr>
<tr>
<td>PostUPF1</td>
<td>116.1±7.9</td>
<td>41.3±4.7</td>
<td>103.9±11.6</td>
<td>98.1±6.4</td>
<td>76.8±7.2</td>
<td>70.7±6.2</td>
</tr>
<tr>
<td><strong>Left ventricular end diastolic pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.5±0.3</td>
<td>41.2±4.6</td>
<td>72.3±4.6</td>
<td>48.7±3.5</td>
<td>46.2±3.1</td>
<td>44.1±3.5</td>
</tr>
<tr>
<td>PreUPF1</td>
<td>3.8±0.6</td>
<td>30.3±6.4</td>
<td>62.5±7.6</td>
<td>34.5±4.4*</td>
<td>32.4±3.7*</td>
<td>32.8±3.3*</td>
</tr>
<tr>
<td>PostUPF1</td>
<td>3.9±0.3</td>
<td>40.4±4.7</td>
<td>81.4±5.6</td>
<td>51.6±3.4</td>
<td>46.7±2.9</td>
<td>43.7±2.7</td>
</tr>
<tr>
<td><strong>Coronary flow (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.9±1.3</td>
<td>0.0±0.0</td>
<td>0.6±0.1</td>
<td>2.7±0.4</td>
<td>1.6±0.3</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>PreUPF1</td>
<td>9.3±1.3</td>
<td>0.0±0.0</td>
<td>4.7±1.5*</td>
<td>5.4±1.3</td>
<td>5.3±1.5*</td>
<td>5.7±1.5*</td>
</tr>
<tr>
<td>PostUPF1</td>
<td>9.2±1.0</td>
<td>0.0±0.0</td>
<td>1.6±0.7</td>
<td>3.0±0.7</td>
<td>2.6±0.6</td>
<td>2.2±0.6</td>
</tr>
</tbody>
</table>

The data are given as mean±standard error of the mean, with \(*p<0.05\) for the differences in comparison with the control hearts. Control — control group, PreUPF1 — UPF1 administration before ischaemia, PostUPF1 — UPF1 administration after ischaemia.

---

Please cite this article as: Kals J et al. Antioxidant UPF1 attenuates myocardial stunning in isolated rat hearts. Int J Cardiol (2007), doi:10.1016/j.ijcard.2007.01.032
The present study demonstrates that the novel antioxidant tetrapeptide UPF1 improves recovery of heart function after IRI but fails to diminish infarct size in the isolated rat heart. The cardioprotective effect was evident only when UPF1 infusion was commenced before ischaemia, while UPF1 administration immediately after ischaemia had no protective effect.

Glutathione is the most abundant non-protein intracellular thiol compound with well verified antioxidant properties [3,8]. Administration of glutathione in its pure form is unlikely to be effective due to its rapid extracellular degradation and poor penetration through cell membranes. Glutathione analogues, in contrast, have shown promising clinical perspectives for maintaining/modulating intracellular glutathione levels [9,10]. The present study suggests that the tetrapeptide analogue of glutathione, UPF1, could be one of the most interesting compounds in this line of research into antioxidants.

The fact that UPF1 improved post-ischaemic function but did not diminish myocardial necrosis in the present study is not very surprising. The data support the idea that ROS contribute to myocardial stunning rather than act as primary mediators of myocardial cell death after reperfusion [11]. However, it is somewhat unexpected to see that UPF1 was effective when given before ischaemia, but not at reperfusion. One possible explanation for this is that the need for UPF1 presence is critical at the moment of reperfusion, which was not achieved in the postUPF1 group. Or, more importantly, certain time might be needed to “upload” cells with the glutathione analogue to make them resistant against subsequent oxidative burden.

The exact mechanisms of the protective action of UPF1 remained unclear in the present experiments. In our previous studies we have shown that UPF1 modulates the activity of the G-proteins in the brain tissue [12] and can act as a scavenger [6] or a signal molecule increasing the level of glutathione itself or the glutathione redox ratio (our unpublished data).

In conclusion, the current study shows that treatment with the novel glutathione analogue UPF1 improves post-ischaemic functional recovery of the isolated rat heart but does not reduce the infarct size. Further studies are needed to investigate the cellular mechanisms of afforded protection.

Acknowledgement

The authors are indebted to Ms. E. Jaigma for the linguistic revision of the manuscript.

References