Cardiotrophin-1 (CT-1) can protect the adult heart from injury when added both prior to ischaemia and at reperfusion

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Abstract

Objectives: To determine whether the cytokine cardiotrophin-1 (CT-1) can protect the adult heart against ischaemia/reperfusion when added either prior to ischaemia or at reperfusion. Background: CT-1 has previously been shown to protect cultured embryonic or neonatal cardiocytes from cell death. To assess the therapeutic potential of CT-1, it is necessary to determine whether this effect can be observed in adult cardiac cells both in culture and most importantly in the intact heart. Methods: We examined the protective effect of CT-1 both in cultured adult rat cardiocytes and in the rat intact heart. In both cases, the cardiac cells were exposed to hypoxia/ischaemia followed by reoxygenation/reperfusion and CT-1 was administered either prior to hypoxia/ischaemia or at reoxygenation/reperfusion. Results: CT-1 has a protective effect in reducing ischaemic damage in the intact heart ex vivo as assayed by infarct size to area at risk ratio (20% compared to 35%). Similar protective effects against cell death were noted in adult cells in vitro. Both in vitro and ex vivo CT-1 can exert a protective effect when added at the time of reoxygenation/reperfusion as well as prior to the hypoxic/ischaemic stimulus (cell death reduced from 50 to 20% in TUNEL assay, infarct size to zone at risk ratio reduced from 35 to 20%). These protective effects are blocked by an inhibitor of the p42/p44 MAPK pathway. Conclusion: CT-1 can protect adult cardiac cells both in vitro and in vivo when added both prior to or after the hypoxic/ischaemic stimulus. The potential therapeutic benefit of CT-1 when added at the time of reperfusion following ischaemic damage is discussed.

Condensed abstract

The cytokine cardiotrophin-1 (CT-1) has previously been shown to have protective effects in embryonic or neonatal cardiac cells in culture. Here we show for the first time that CT-1 can protect adult cardiac cells both in culture and in the intact heart exposed to ischaemia/reperfusion. Moreover, this protective effect can be observed when CT-1 is added at reperfusion after ischaemia as well as prior to ischaemia. The ability of CT-1 to protect the intact adult heart when given at reperfusion suggests it may have therapeutic potential in the clinical situation. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Apoptosis; Cytokines; Hypoxia/anoxia; Reperfusion

1. Introduction

Cardiotrophin-1 (CT-1) was initially isolated as a factor, which was capable of inducing cardiac myocyte hypertrophy in vitro [1] and was subsequently shown to have a similar effect when injected into adult mice [2]. Subsequent studies established that CT-1 is a member of a family of cytokines including interleukin-6 (IL-6), leukaemia inhibitory factor (LIF), CNTF, oncostatin M...
and IL-11 [3,4]. The receptor for each of these factors contains a common component known as gp130 (for review see Refs. [5,6]). Although the IL-6, CNTF and OSM receptors contain in addition a specific subunit which is unique to each of these receptors [5,6], LIF and CT-1 and in certain cells, OSM share a common second receptor component, which is known as LIF receptor sub-unit β [3,7]. Following binding to the receptor, members of the IL-6 family activate both the MAP kinase pathway and the Jak/STAT pathway resulting, respectively, in the phosphorylation of the transcription factors NF-IL6 and STAT-3 allowing them to stimulate transcription [5,8–10].

As well as its potentially damaging hypertrophic effects, CT-1 also has significant protective effects. The apoptotic death of unstimulated embryonic or neonatal cardiac myocytes in defined serum-free medium can be reduced by treatment with CT-1 [11,12] and this effect was inhibited by inactivation of p42/p44 MAP kinase, which had no effect on the hypertrophic response to CT-1 [12]. This effect, together with the expression of CT-1 in the early mouse embryonic heart tube, suggests that CT-1 may have significant effects on cardiac survival which are important during heart development [11]. Moreover, CT-1 may also play a key role in protecting cardiac cells against stressful stimuli. Thus, treatment of cultured neonatal cardiomyocytes with CT-1 protects them against subsequent exposure to thermal or hypoxic stress both in terms of the total amount of cell death observed and the extent of apoptosis [13].

These findings clearly indicate a protective effect for CT-1 in cultured embryonic or neonatal cardiomyocytes. However, in contrast to the hypertrophic effects of CT-1, such protective effects have not yet been demonstrated in the intact heart, although a protective effect of CT-1 has been demonstrated using atrial appendages derived from adult human cardiac patients [14]. Indeed, CT-1 has never been tested for its protective effect in cultured adult as opposed to embryonic or neonatal cardiac cells. This is of particular importance since CT-1 has been shown to induce the synthesis of protective heat shock proteins such as hsp70 or hsp90 and other protective stimuli which induce these proteins have been shown to be less effective in cells from aged compared to younger animals both in terms of hsp induction and protective effect [15,16]. Moreover, a wide variety of differences exist between cultured neonatal and adult cardiac cells [17,18]. Thus, neonatal cardiac cells are not fully differentiated and retain the ability to undergo one cell division following plating. In contrast, cultured adult cardiac cells are non-dividing and are terminally differentiated. In addition, since mitochondrial structure/arrangement in the cell and energy metabolism differ in adult compared to neonatal myocytes, the responses of adult myocytes to ischaemia differ from those of neonatal cells and therefore need to be studied directly. Similarly, cardiac cells from animals of different ages show differences in contractility and its response to calcium channel antagonists [19].

Here we demonstrate that CT-1 has a protective effect in both cultured adult cardiac cells and in the intact adult heart ex vivo subjected to ischaemia/reperfusion. Moreover, we demonstrate that both in vitro and ex vivo, CT-1 can have a protective effect when added at the time of reperfusion/reoxygenation as well as when added prior to the ischaemic/hypoxic period and that these protective effects are blocked by an inhibitor of the p42/p44 MAPK pathway.

2. Methods

2.1. Culture of adult cardiac cells

Adult cardiac myocytes were isolated using a combination of enzymatic and mechanical dispersion after Langendorff perfusion of the whole heart [20–22]. The hearts from adult female Sprague–Dawley rats, ranging in age from 2 to 7 months, were excised and then rapidly mounted on to the aortic cannula of a Langendorff perfusion system. The perfusion flow rate was kept constant at 9 ml/min per g of heart tissue. All buffers were heated to 37 °C and oxygenated with medically supplied oxygen (BOC gases). Initially, the heart was perfused for 2 min with modified Krebs–Henselit (KH) buffer containing low concentration of physiological Ca$^{2+}$. This contained 130 mM NaCl, 5.4 mM KCl, 5 mM HEPES, 0.4 mM NaH$_2$PO$_4$, 3.5 mM MgCl$_2$, 750 μM CaCl$_2$·2H$_2$O, 10 mM glucose, pH 7.4, O$_2$–CO$_2$ (95:5). The hearts were then perfused with the above buffer containing 100 μM EGTA for 4 min and finally with a Collagenase type 2 enzyme solution consisting of KH buffer supplemented with 0.8 mg/ml Collagenase type 2 (Worthington Biochem) and 200 μM Ca$^{2+}$ for 15 min.

The left ventricle was removed from the heart and cut into small pieces to increase the surface area for enzymatic digestion in oxygenated “shake solution” [KH buffer supplemented with 0.8 mg/ml collagenase type 2, 10% bovine serum albumin (BSA) (Sigma), 200 μM Ca$^{2+}$] for 5 min in a 37 °C shaking water bath. The digested heart tissue was passed through nylon gauze for filtration with restore solution consisting of KH buffer supplemented with 10% BSA, 200 μM CaCl$_2$. The cells were sedimented under gravity for 5–10 min and isolated cells were washed with restore solution twice. The cells were then washed with 50% (v/v) restore solution and 50% (v/v) DMEM (Gibco) supplemented with 80 μM EGTA, 1% (v/v) penicillin/streptomycin (PS) (Gibco) (wash solution) twice and finally with the wash solution once. The cells were then plated at a concentration of 2·10$^5$ cells/ml in DMEM containing 80 μM EGTA, 1% (v/v) PS and 1% (v/v) fetal calf serum (FCS) and plated on 3 cm dishes that were coated with Laminin (Sigma) at a concentration of 15 μg Laminin/ml in phosphate-buffered saline (PBS) for 2 h.
room temperature in a laminar flow hood, prior to plating. For the annexin V labelling and TUNEL studies, cardiac myocytes were plated onto tissue culture slides (NUNC). The cells were pre-plated for 2 h and media was replaced with fresh media for 24 h prior to experimentation. Cells were maintained in a 37 °C Heraeus incubator in a humidified atmosphere under 5% CO₂ in air.

2.2. Hypoxia

To produce hypoxia in vitro, cultured cardiocytes were incubated in 1 ml of hypoxic buffer (137 mM NaCl, 12 mM KCl, 0.49 mM MgCl₂, 0.9 mM CaCl₂, 2H₂O, 4 mM HEPES, 20 mM sodium lactate, pH 6.2) for 6 h in an atmosphere of CO₂–argon (5:95) at 37 °C producing a PO₂ level of 4 mmHg [23]. CT-1 (Genentech) at a concentration of 20 ng/ml was added to the cardiac myocytes for 24 h and removed prior to a lethal hypoxic insult (Fig. 1). For assays of the protective effect of CT-1 added prior to hypoxia the cells were analysed immediately after the hypoxic period. The MEK1 inhibitor (PD98059) (New England Biolabs, Beverly, MA, USA) [24,25] was stored at −20 °C at a concentration of 100 mM and was added at a concentration of 50 μM, 10 min prior to the addition of CT-1.

2.3. Reoxygenation

For analysis of the protective effect of CT-1 added at the time of reoxygenation, cardiac cell cultures were exposed to 1 h of lethal hypoxia as described above and then incubated in a normoxic environment for 2 h (reoxygenation) (Fig. 1). At the point of reoxygenation the hypoxic buffer was removed and replaced with 1 ml minimal media with and without the addition of 20 ng/ml of CT-1 for 2 h. To investigate the downstream signalling pathway which mediates CT-1 cardioprotection in reperfusion injury the cardiac myocytes were incubated for 10 min in 1 ml of minimal medium containing signal transduction inhibitors, prior to the 2-h incubation with 20 ng/ml of CT-1.

2.4. TUNEL labelling

DNA degradation was assessed by the end labelling of DNA 3’ ends with dUTP-FITC, using a modification of the TUNEL method [26] as previously described [27–29].

2.5. Annexin V staining

Surface staining with annexin V to test for the translocation of phosphatidyl serine to the outer surface of the plasma membrane which occurs early in apoptosis [30] was carried out as previously described [27–29]. Only cells staining positive for annexin V and negative for propidium iodide were scored as apoptotic to eliminate membrane permeable necrotic cells which take up both stains.

2.6. Animals

Male Sprague–Dawley rats (280–400 g body weight) were used. All animals were obtained from the same supplier, fed a standard pelleted diet with free access to water, housed under the same conditions and received humane care in accordance with the Guidelines on the Operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty’s Stationery Office (London, UK).

2.7. Heart perfusion and treatment protocol

The animals were anaesthetised by i.p. administration of sodium pentobarbital (55 mg/kg) and were given heparin (300 IU). The hearts were excised, placed in chilled buffer solution and within 1 min mounted on a constant pressure (80 mmHg) Langendorff perfusion apparatus. They were perfused retrogradely through the ascending aorta with a modified KH hydrogen carbonate buffer containing (in mM): NaCl 118.5, NaHCO₃ 25, KCl 4.8, MgSO₄ 1.2 KH₂PO₄ 1.2, CaCl₂ 1.7, glucose 12. All solutions were filtered through a Whatman microfiber filter (2.0 μm pore), gassed with O₂–CO₂ (95:5) and maintained at 37 °C with the help of water heated double jacket chambers. The temperature was permanently monitored by a thermocouple inserted in the right ventricle. The pH of the perfusate was ascertained with a blood gas analyser (AVL type 993; AVL Instruments, Stone, UK) and adjusted as necessary to maintain pH close to 7.4 (±0.05) by modifying the gas output. A latex isovolumic balloon was introduced into the left ventricle through an insertion in the left atrial appendage and inflated to give a preload of 2–15 mmHg. The balloon catheter was attached by a pressure transducer to a chart recorder (Lectromed, Welwyn Garden City, UK).
Left ventricular developed pressure, heart rate and coronary flow were registered at regular intervals.

A 4/0 silk suture on a round bodied surgical needle (Mersilk type W546, Ethicon, Edinburgh, UK) was passed under the left main coronary artery and the ends of the suture were passed through a small plastic tube to form a snare. Regional ischaemia was induced by tightening the snare and clamping in place with haemostat forceps. Reperfusion was instituted by releasing the ends of the suture.

Six groups are included in the study (Fig. 6). Group (i): control, (n=8) 20 min perfusion without intervention followed by 35 min coronary occlusion and 120 min reperfusion; Group (ii): CT-1 pre-ischaemia, (n=8) 30 min perfusion of 10 μg CT-1/1 of the buffer was followed by a 35 min period of ischaemia and 120 min reperfusion. Group (iii): (n=8) following 35 min ischaemia CT-1 (10 μg/l) was perfused for 30 min at the beginning of 120 min reperfusion. Group (iv): (n=8) following 35 min ischaemia PD (5 μM) and CT-1 (10 μg/l) was perfused together for 30 min at the beginning of 120 min reperfusion. Group (v): (n=6) following ischaemia PD (5 μM) alone was perfused for 30 min at the beginning of 120 min reperfusion. Group (vi): (n=8) CT-1 (10 μg/l) was perfused during the 35 min ischaemia. Infarct size was measured as previously described [27].

2.8. Statistical analysis

Data for in vitro experiments are expressed as means±S.D. Single-factor analysis of variance (ANOVA) was performed for each group of treatments and significance was assumed when the P-value was less than 0.05. Differences among means were compared within the treatment groups using the Student’s t-test. The experiments were repeated at least three times for each experiment, each n number corresponds to the mean of three random fields per well of cells with a minimum of 250 cells scored per view. Infarct size data were tested for group differences by one-way ANOVA combined with Tukey’s posthoc test. ANOVA, P values and the Student’s t-test values that were less than *<0.05, were considered significant.

3. Results

Previous animal work showing protective effects of CT-1 has been conducted in embryonic or neonatal cardiac cultures which are of limited relevance to any potential therapeutic situation [11–13]. We therefore wished to evaluate the protective effect of CT-1 in cultures from adult rats and compare it to that observed in neonatal cultures. Accordingly, CT-1 was added at different doses to cardiocyte cultures derived from rats of different ages and 24 h later the cells were exposed to hypoxia (see Fig. 1 for details of the experimental protocol). Cell survival was assayed by the trypan blue exclusion assay which measures total cell death, both necrotic and apoptotic on the basis of the ability of live cells to exclude trypan blue.

As illustrated in Fig. 2, a dose of 20 ng/ml was able to produce a similar statistically significant protection against hypoxia in cells from neonatal rats aged 2 days or adult rats aged 2, 4 and 7 months. Interestingly, however, whilst a significant protective effect could be observed in the neonatal cultures at a dose of CT-1 of 2 ng/ml in accordance with our earlier results [13], this was not seen in the adult cultures. In other experiments, doses of 5 ng/ml or 10 ng/ml of CT-1 were also ineffective in adult cultures whereas 30 ng/ml CT-1 had the same effect as 20 ng/ml (data not shown). Hence, adult cultures can be protected against subsequent exposure to hypoxia by CT-1 but a higher dose is required compared to that needed in cultures from younger neonatal animals. 20 ng/ml CT-1 was used in all subsequent experiments.

As well as investigating the effect of CT-1 using the trypan blue exclusion assay we also utilised the TUNEL labelling and annexin V assays to measure the effect on cell death using two additional methods in adult cultures. In these experiments (Fig. 3), CT-1 treatment produced a clear protective effect in cultures of adult cells as assayed by the two different methods confirming the protective effect of CT-1 in cultured adult cardiac cells exposed to hypoxia.

The protective effect of CT-1 against cell death in
neonatal cardiocytes grown in serum-free medium or exposed to hypoxia has previously been shown to be inhibited by PD98059, a specific inhibitor of the p42/p44 MAP kinase pathway which acts by inhibiting the activity of the upstream activator of these kinases MEK1 [12,29]. The protective effect of CT-1 against hypoxia was therefore investigated in our adult cultures using the trypan blue exclusion assay in the presence or absence of this inhibitor. In these experiments (Fig. 4) the PD98059 p42/p44 MAPK inhibitor completely blocked the protective effect of CT-1 in cultures from 6-month-old animals. This suggests that, as with the protective effect in neonatal cultures, the protective effect of CT-1 involves the MEK1 kinase, which specifically activates the p42/p44 MAP kinase pathway. No significant effect of PD98059 on cell survival was observed in the absence of CT-1 confirming that the effect of PD98059 specifically involved blocking the protective effect of CT-1. Similar inhibition of the protective effect of CT-1 by PD98059 was also observed in TUNEL and annexin V assays (data not shown).

Having established the pattern of protection by CT-1 when added before hypoxia we wished to establish whether CT-1 could have a protective effect when added solely during the period of reoxygenation. In these experiments, CT-1 was added at the end of the hypoxic period and cells analysed after two h reoxygenation (see Fig. 1 for experimental protocol). In these experiments we were able to observe a protective effect using trypan blue, TUNEL labelling or staining with labelled annexin V (Fig. 5) when CT-1 was added at the time of reoxygenation.

The protective effect of CT-1 when added prior to hypoxia and more particularly its protective effect when added immediately prior to reoxygenation in cultures of cardiac cells from adult rats suggested a potential therapeu-
Fig. 6. Protocol used to test the protective effect of CT-1 in the isolated heart on a Langendorff perfusion apparatus and to investigate the effect of PD98059 on this protection.

However, CT-1 had a clear protective effect when added prior to the period of ischaemia, significantly reducing the degree of damage observed in the heart as measured by the ratio of infarct size to zone at risk (Fig. 7 and Table 3).

Most importantly however, a significant protective effect was also observed when CT-1 was added immediately prior to reperfusion and subsequent to the period of ischaemia. Hence, CT-1 can protect against injury when added after the ischaemic period and unlike the great majority of proposed protective agents (such as β-blockers, calcium antagonists and nitrates) can have an effect when added after, as well as before, the period of ischaemia. Moreover, as observed in our in vitro experiments, the protective effect of CT-1 added at reperfusion was

### Table 1
Baseline haemodynamics of all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDP</th>
<th>RPP</th>
<th>CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.38±0.42 (n=8)</td>
<td>30 903±1929 (n=8)</td>
<td>16.25±0.84 (n=8)</td>
</tr>
<tr>
<td>CT-1 PreI</td>
<td>7.88±1.04 (n=8)</td>
<td>33 986±4070 (n=8)</td>
<td>17.05±0.91 (n=8)</td>
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<tr>
<td>CT-1 Rep</td>
<td>7.38±0.87 (n=8)</td>
<td>33 779±2882 (n=8)</td>
<td>16.6±0.84 (n=8)</td>
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<tr>
<td>PD&amp;CT-1</td>
<td>5.75±1.01 (n=8)</td>
<td>26 610±1597 (n=8)</td>
<td>14.31±0.79 (n=8)</td>
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<td>PD Rep</td>
<td>7.67±1.50 (n=6)</td>
<td>33 103±2374 (n=6)</td>
<td>15.13±0.48 (n=6)</td>
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<tr>
<td>CT-1 Isch</td>
<td>6±0.73 (n=9)</td>
<td>25 372±1885 (n=9)</td>
<td>15.58±0.95 (n=9)</td>
</tr>
</tbody>
</table>

All data expressed as mean±S.E. There are no significant differences between groups.

LVEDP=Left ventricular end diastolic pressure; RPP=rate pressure product (heart rate x developed pressure); CFR=coronary flow rate; CT-1 PreI=perfusion with CT-1 preischaemia for 30 min; CT-1 Rep=perfusion with CT-1 at the beginning of reperfusion for 30 min; PD&CT-1=perfusion with PD and CT-1 at the beginning of reperfusion for 30 min; PD Rep=perfusion with PD at the beginning of reperfusion for 30 min; CT-1 Isch=perfusion with CT-1 during the 35 min of ischaemia.
abolished by addition of PD98059 indicating it could be prevented by an inhibitor of the p42/p44 MAPK pathway.
Interestingly CT-1 also produced a protective effect when added during ischaemia. This effect is likely to be partly dependent on the small amount (approximately 6%) of collateral flow, which occurs in the rat [31]. However, it is also likely to be due to this CT-1 entering the heart at reperfusion on release of the occlusion and producing a protective effect in this manner.

4. Discussion

The development of methods for reducing the damage suffered by individuals undergoing episodes of cardiac ischaemia is evidently of central importance in cardiology. Many studies in this area have been carried out with cardiac cultures from neonatal animals since these are easier to culture than those from adult animals. However, these neonatal cells are different from adult cells in their ability to divide in culture and are not fully differentiated in terms of morphology, biochemistry, etc. Most impor-

Table 2
Percentage decrease in haemodynamics from baseline values in all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Ischaem-35’</th>
<th>Reperf-30’</th>
<th>Reperf-60’</th>
<th>Reperf-90</th>
<th>Reperf-120’</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>RPP</td>
<td>56±2.6</td>
<td>40±4.8</td>
<td>52±5.5</td>
<td>66±5.8</td>
<td>69±4.9</td>
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<td></td>
<td>CFR</td>
<td>50±4.0</td>
<td>44±5.5</td>
<td>56±4.9</td>
<td>71±5.4</td>
<td>70±2.9</td>
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<td>CT-1 PreI</td>
<td>RPP</td>
<td>60±3.9</td>
<td>42±13.7</td>
<td>47±13.7</td>
<td>48±13.9</td>
<td>61±10.9</td>
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<tr>
<td></td>
<td>CFR</td>
<td>50±7.1</td>
<td>46±9.2</td>
<td>48±9.2</td>
<td>56±11.1</td>
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<tr>
<td>CT-1 Rep</td>
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<td>58±5.5</td>
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<td>CFR</td>
<td>45±5.4</td>
<td>54±4.5</td>
<td>56±3.9</td>
<td>59±2.3</td>
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<td>PD&amp;CT-1</td>
<td>RPP</td>
<td>49±7.4</td>
<td>50±4.8</td>
<td>55±7.6</td>
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<td>74±4.7</td>
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<td>CFR</td>
<td>48±6.3</td>
<td>55±4.5</td>
<td>59±3.9</td>
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<td>PD Rep</td>
<td>RPP</td>
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<tr>
<td>CT-1 Isch</td>
<td>RPP</td>
<td>54±5.4</td>
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<td>55±8.9</td>
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<tr>
<td></td>
<td>CFR</td>
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<td>42±6.3</td>
<td>53±6.0</td>
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All data expressed as mean±S.E. There are no significant differences between groups.
Ischaem-35’=At 35 min of ischaemia; Reperf-30’=at 30 min of reperfusion; Reperf-60’=at 60 min of reperfusion; Reperf-90’=at 90 min of reperfusion; Reperf-120’=at 120 min of reperfusion; RPP=rate pressure product (heart rate x developed pressure); CFR=coronary flow rate; CT-1 PreI=perfusion with CT-1 preischaemia for 30 min; CT-1 Rep=perfusion with CT-1 at the beginning of reperfusion for 30 min; PD&CT-1=perfusion with PD and CT-1 at the beginning of reperfusion for 30 min; PD Rep=perfusion with PD at the beginning of reperfusion for 30 min; CT-1 Isch=perfusion with CT-1 during the 35 min of ischaemia.

Table 3
Body weight and risk to left ventricular volume ratio and Infarct to risk volume ratio of all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>R/LV (%)</th>
<th>I/R (%)</th>
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<tr>
<td>Control</td>
<td>338±7</td>
<td>52±1.9</td>
<td>33.9±2.4</td>
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<td>(n=8)</td>
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<tr>
<td>CT-1 PreI</td>
<td>327±3</td>
<td>53±2.4</td>
<td>20.3±2.0</td>
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<tr>
<td>(n=8)*</td>
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<tr>
<td>CT-1 Rep</td>
<td>359±13</td>
<td>51±3.1</td>
<td>20.5±1.7</td>
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<td>(n=8)*</td>
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<td>(n=8)</td>
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<tr>
<td>PD Rep</td>
<td>323±5</td>
<td>48±2.5</td>
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<td>(n=6)</td>
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<tr>
<td>CT-1 Isch</td>
<td>327±11</td>
<td>51±3.2</td>
<td>25.7±3.4</td>
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<tr>
<td>(n=9)*</td>
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All data expressed as mean±S.E. *P<0.05 vs. control.
R/LV= Risk to left ventricular volume ratio. I/R=Infarct to risk volume ratio. CT-1 PreI=Perfusion with CT-1 preischaemia for 30 min. CT-1 Rep=Perfusion with CT-1 at the beginning of reperfusion for 30 min. PD&CT-1=Perfusion with PD and CT-1 at the beginning of reperfusion for 30 min. PD Rep=Perfusion with PD at the beginning of reperfusion for 30 min. CT-1 Isch=Perfusion with CT-1 during the 35 min of ischaemia.

Fig. 7. Infarct size compared to the zone at risk in hearts subjected to ischaemia/reperfusion on a Langendorff perfusion apparatus. Results are shown for control hearts, or hearts given CT-1 either pre-ischaemia, during ischaemia or at reperfusion and for hearts given CT-1 with PD98059 or PD98059 alone at reperfusion. The results for each group are shown together with the mean value and the number of animals (n). The bars show the standard error of the mean. Both groups of CT-1 treated hearts showed significant protection compared to controls (P<0.05 in each case).
Cytokines of the IL-6 family such as CT-1 have previously been shown to activate both the p42/p44 MAP kinase pathway and the Jak/STAT pathway [8–10, 37]. In neonatal cardiocytes the protective effect of CT-1 against apoptosis is dependent upon its ability to activate p42/p44 MAP kinase and appears to be independent of its activation of the Jak/STAT pathway [12, 29]. In contrast, the hypertrophic effect of CT-1 was not inhibited by inhibition of the p42/p44 MAPK pathway [12]. In this work presented here, we have similarly demonstrated that the protective effect of CT-1 in adult cardiac cells both in vitro and in the intact heart ex vivo can be abolished by treatment with PD98059, a specific inhibitor of the p42/p44 MAP kinase pathway. Hence these novel protective effects are also likely to be dependent on this pathway.

IL-6 family cytokines are able to activate the NF-IL6 transcription factor by MAP kinase-dependent phosphorylation [8, 37]. Hence, newly activated NF-IL6 could activate the transcription of genes encoding protective proteins resulting in the protective effect of CT-1. Indeed, we have previously demonstrated that in neonatal cardiocytes CT-1 can enhance the expression of the protective heat shock proteins hsp70 and hsp90 whose transcription is known to be stimulated by activated NF-IL6 [38]. We also observed such induction in adult cells in our study (data not shown), although it is still unclear whether the induction of these proteins is essential for the protective effect of CT-1.

Further studies will be required in order to demonstrate the protective effect of CT-1 in the intact adult animal as well as in the ex vivo heart preparation. Similarly, it will be important to identify the mechanisms by which CT-1 activation of the p42/p44 MAP kinase pathway results in enhanced protection in adult cardiac cells. This is of particular importance since it may well lead to a method of activating this protective pathway either with CT-1 or related agents without activating the potentially damaging hypertrophy pathway which is also activated by CT-1, hence allowing therapeutic advantage to be taken of the ability of CT-1 to have a protective effect in the adult heart when given at the time of reperfusion following an ischaemic episode.

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