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# Interleukin-2 Confers Cardioprotection by Inhibiting Mitochondrial Permeability Transition Pore

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*Abstract*—**In the present study, we determined whether interleukin-2 (IL-2) confers cardioprotection by inhibiting mitochondria permeability transition pore (MPTP) opening. In isolated rat hearts subject to 30 min ischemia and 120 min reperfusion (IR), IL-2 (50 U/ml) decreased the infarct size and LDH release, effects blocked by a selective kappa-opioid receptor antagonist, Nor-BNI (5 microM) or an opener of MPTP, atractyloside (Atr, 20 microM). In isolated ventricular myocytes subjected to anoxia and reoxygenation (AR), which reduced both the amplitude of the electrically induced $[Ca^{2+}]i$ transient and diastolic $[Ca^{2+}]i$, IL-2 attenuated the AR-induced alterations and their effects were abolished by Atr. In addition, IL-2 attenuated the reduction in calcein fluorescence in myocytes subject to AR and reduced calcium-induced swelling in mitochondria of rat hearts subjected to IR, which were similar to effect of inhibitor of MPTP. The observations indicated that IL-2 confers cardioprotection by inhibiting the MPTP opening.**

*Keywords*—**Interleukin-2, heart, ischemia/reperfusion, mitochondria permeability transition pore**

## I. Introduction

Ischemic preconditioning (IPC) is a phenomenon in which transient nonlethal period of ischemia increase the resistance to a subsequent prolonged ischemic period [1]. It has been demonstrated that Gi protein coupled receptors (GPCR) mediate cardioprotection of IPC [2]. Kappa opioid receptor (κ-OR) is one of these G-protein coupled receptors [3]. Recently it has been shown that interleukin-2 (IL-2), a member of cytokine family, reduced cardiac injury of the rat heart subject to myocardial ischemia and reperfusion and its effect was attenuated by blockade of κ-OR with Nor-BNI, a κ-OR antagonist, indicating that IL-2 confers cardioprotection via κ-OR [4].

The mitochondria permeability transition pore (MPTP) is a multiprotein complex formed at the contact sites between the inner and outer mitochondrial membranes [5]. Opening of MPTP causes apoptosis/necrosis, which may eventually lead to cell death [6]. The MPTP has been shown to stay closed during ischemia and only open in the first few minutes of reperfusion [7]; the latter results in myocardial injury [8]. So inhibition of MPTP opening may be beneficial and in fact it was demonstrated that blockade of MPTP opening conferred cardioprotection of IPC [9]. It is therefore hypothesized that IL-2 may confer cardioprotection by inhibiting MPTP.

Therefore, the aim of this study was to determine the role of the MPTP in cardioprotection of IL-2.

## II. Methodology

1) *Isolated perfused heart preparation*: Hearts from male Sprague-Dawley rats of 250-300 g body weight were excised rapidly and placed in ice-cold Krebs–Henseleit (K-H) perfusion buffer before being mounted on a Langendorff apparatus for perfusion at 37°C with K-H buffer at a constant pressure (100 cm $H_2O$). The buffer, that had the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.25, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0, was equilibrated with 95% $O_2$/5% $CO_2$. For hearts subjected to regional ischemia, a silk suture was placed around the left coronary artery to form a snare. The coronary artery was occluded by pulling the snare to produce ischemia. Reperfusion was achieved by releasing the snare.

2) *Measurement of the area of risk*: For determination of infarct size in hearts subjected to regional ischemia, the coronary artery was re-occluded at the end of the reperfusion period and a solution with 2.5% Evans blue was perfused to delineate the area of risk. Hearts were then frozen and cut into slices, which were then incubated in a sodium phosphate buffer containing 1% w/v 2, 3, 5-triphenyl-tetrazolium chloride (TTC) for 15 min to visualize the unstained infracted region. Infarct and risk zone areas were determined with planimetry by using a software Image/J from NIH and infarct was expressed as a percentage of the risk zone.

3) *Determination of myocardial injury by lactate dehydrogenase efflux*: The effluent from the isolated perfused heart was collected at 5 min of reperfusion and lactate dehydrogenase (LDH) was spectrophotometrically assayed using a kit purchased from Sigma Chemical Co. LDH activity was expressed as units per liter.

4) *Preparation of isolated ventricular myocytes*: Single ventricular myocytes were prepared from the heart of male Sprague-Dawley rats by enzymatic dissociation. Only rod-shaped cells with clear cross striations were used for experiments.

5) *Intracellular calcium recording*: Intracellular $Ca^{2+}$ and its transient were determined by a spectrofluorometric

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method using the sensitive dye fura-2 as $Ca^{2+}$ indicator. Fluorescence was measured on an Olympus inverted microscope equipped with a fluorometer system (T.I.L.L., Germany). The $Ca^{2+}$-dependent signal of fura-2 was obtained by illuminating at 340 and 380 nm and recording the emitted light at 510 nm. The $[Ca^{2+}]i$ transient was induced by supra-threshold stimuli at 0.2 Hz delivered by a stimulator through two platinum field-stimulation electrodes in the bathing fluid.

6) *Measurement of MPTP opening with calcein*: To determine the opening of MPTP, calcein release from myocytes was measured. Cells were loaded with 1 micro} . calcein-AM for 20 min at room temperature, and the quenching of cytosolic and nuclear calcein was achieved by addition of 5 mM cobalt chloride (CoCl2) to the solution. Calcein fluorescence (excitation at 488 nm and emission at 505 to 550 nm) were recorded with the fluorometer system.

7) *Statistical analysis*: Values presented here are means±standard error of means. Statistical comparisons were performed by One-way analysis of variance and Newman-Keuls test. Differences of $p<0.05$ are regarded as significant.

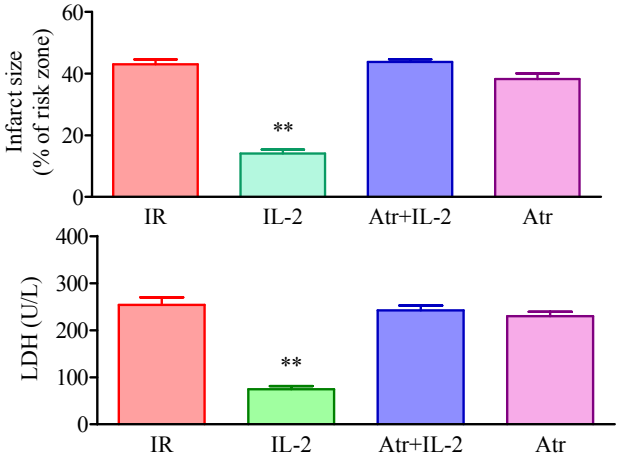
## III. Results

### *A. Effect of IL-2 on myocardial infarct induced by myocardial ischemia and reperfusion in the presence of a MPTP opener*

An infarct size of 43.1±7.7% was induced by 30 min of ischemia and 120 min of reperfusion. The infarct size was significantly reduced when IL-2 at 50 U/ml was administered for two cycles of 5 min each interspersed with a 5 min of drug-free perfusion before ischemia (Fig. 1). Pretreatment of the heart with Atr (20 microM) blocked the effect of IL-2 on infarction. Pretreatment of the heart with 50 U/ml IL-2 significantly reduced the release of LDH (Fig. 1), which was blocked by Atr.

### *B. Effect of IL-2 on the changes of intracellular calcium of isolated ventricular myocytes induced by anoxia and re-oxygenation in the presence of a MPTP opener*

As shown in Fig. 2 the amplitude of the electrical stimulated $[Ca^{2+}]i$ transient was markedly reduced during anoxia and recovered slightly during re-oxygenation. On the other hand, the fluorescence of end-diastolic calcium level was increased during anoxia and increased further during re-oxygenation. Pretreatment of the myocytes with IL-2 (50 U/ml) attenuated the effects on $[Ca^{2+}]i$ transient and end-diastolic $[Ca^{2+}]i$ induced by anoxia and re-oxygenation. The effect of both IL-2 were blocked by the κ-OR antagonist, 5 microM Nor-BNI (Fig 2). When Atr (20 microM) was administered for 25 min before anoxia, effect of IL-2 on electrical stimulated $[Ca^{2+}]i$ transient and end-diastolic $[Ca^{2+}]i$ were abolished (Fig. 2). Inhibition of MPTP opening with CsA (0.2 microM) attenuated the alterations in $[Ca^{2+}]i$ induced by anoxia and re-oxygenation (Fig. 2).




Fig, 1. Effect of interleukin 2 (IL-2, 50 U/ml) on myocardial infarct (upper) or LDH release (bottom) in the isolated perfused rat heart subject to ischemia of 30 min and reperfusion of 2 h and in the presence of atractyloside (Atr, 20 microM). Data were expressed as mean±SE. (n=10 in each group)
** $p<0.01$ compared with IR group

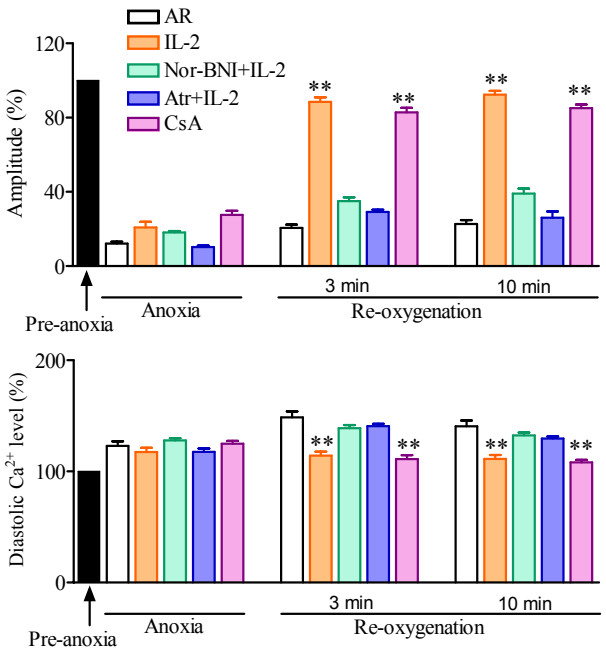



Fig. 2 Effect of pretreatment of interleukin-2 (IL-2, 50 U/ml) on the amplitude of the electrically induced $[Ca^{2+}]i$ transient (upper) and end-diastolic $[Ca^{2+}]i$ level (bottom) in isolated ventricular myocytes subject to 5 min anoxia and 10 min re-oxygenation and in the presence or absence of Nor-BNI (5 microM). Data were expressed as mean±SE. (n=16 in each group)
** $p<0.01$ compared with AR group

*C. Effect IL-2 on the fluorescence of calcein in the isolated ventricular myocyte subject to anoxia and re-oxygenation*

Perfusion with K-H solution for 10 min decreased the calcein signal by 15% while anoxia and reperfusion decreased the calcein signal by 80%, indicating opening of MPTP. In the presence of CsA (0.2 microM), the reduction in calcein signal was only 27%, indicating a marked attenuation compared with the group subject to anoxia and re-oxygenation.

Pretreatment of the myocyte with IL-2 at 50 U/ml also attenuated the decrease of the calcein signal induced by AR (Fig. 3). After incubation the myocytes with Nor-BNI (5 microM) for 10 min, the effect of IL-2 was attenuated.

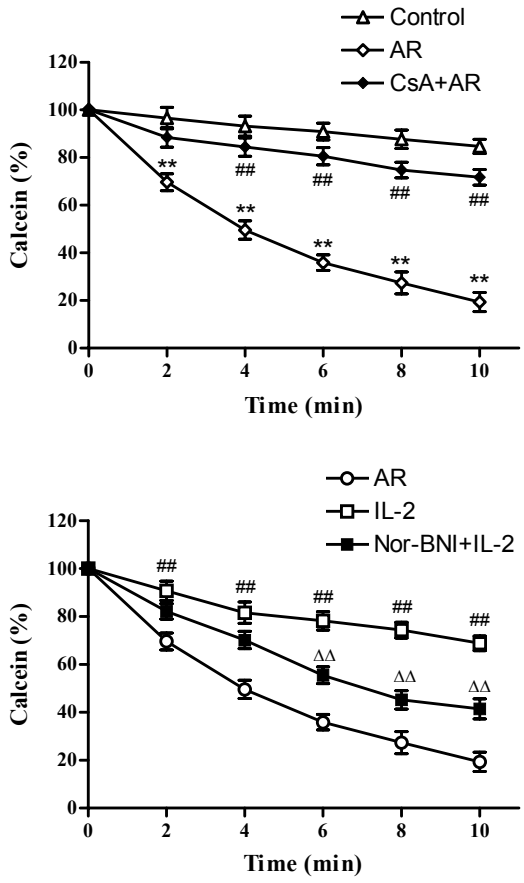



Fig. 3. Time effect of anoxia/re-oxygenation (AR), cyclosporine A (CsA, 0.2 microM), interleukin-2 (IL-2, 50 U/ml) on fluorescence of calcein in ventricular myocytes. For AR myocytes were subject to 5 min anoxia followed by 10 min re-oxygenation. CsA (0.2 microM), IL-2 (50 U/ml), or Nor-BNI (5 mM) were administered. Calcein signals were measured for 10 min during re-oxygenation. Data were expressed as mean±SE. (n=15 in each group)
** p<0.01 compared with control; ## P<0.01 compared with AR group; Δ P<0.05 compared with IL-2 group.

## IV. Discussion

The main observation of the present study was that κ-OR stimulation with IL-2 conferred cardioprotection, inhibited opening of the MPTP and attenuated alterations of $[Ca^{2+}]i$, effects also produced by an inhibitor of the opening of MPTP, cyclosporin A. Opening of the MPTP with a pore opener reversed the effects. These are the first evidence that cardioprotection of κ-OR stimulation by IL-2 involves the inhibition of opening of MPTP. Since κ-OR has been shown to mediate cardioprotection of preconditioning with ischemic insults, the finding supports an important role of inhibition of MPTP in cardioprotection of ischemic preconditioning as shown previously [10].

In the present study we observed that inhibition of opening of MPTP by IL-2 or by the inhibitor of MPTP, that conferred cardioprotection, also attenuated the $[Ca^{2+}]i$ overload particularly during reperfusion, a period when severe cardiac infarction and arrhythmia occur [11]. On the other hand, opening of MPTP that abolished cardioprotection of IL-2, also abolished the attenuating effect of IL-2 on $[Ca^{2+}]i$ overload. The observation is in agreement with the notion that $[Ca^{2+}]i$ overload leads to mitochondrial $Ca^{2+}$ accumulation, which leads to opening of MPTP and cardiac injury/death [12].

In addition to cardiac injury, we also observed that inhibition of opening of MPTP by IL-2 or CsA caused restoration of amplitude of the electrically induced $[Ca^{2+}]i$ transient reduced by ischemic insult and reperfusion while opening of MPTP abolished the beneficial effect of IL-2. Since the electrically induced $[Ca^{2+}]i$ transient represents influx of $Ca^{2+}$ across the L-type $Ca^{2+}$ channel and release of $Ca^{2+}$ from the sarcoplasmic reticulum during excitation-contraction coupling and is directly correlated to contraction, the observations indicate that inhibition of opening of MPTP restores the cardiac contractility by restoring the $Ca^{2+}$ homeostasis, impaired by ischemia/reperfusion, which is in agreement with our present understanding.

## V. Conclusion

In conclusion, the present study has provided the first evidence that IL-2 confers cardioprotection by inhibiting the opening of MPTP.

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