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Role of the Mitochondrial Permeability Transition Pore in TNF-α-Induced Recovery of Ventricular Contraction and Reduction of Infarct Size in Isolated Rat Hearts Subjected to Ischemia/Reperfusion

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Abstract—**Pretreatment with tumor necrosis factor-α (TNF-α) is known to trigger cardioprotection. TNF-α can activate multiple downstream signaling cascades. However, it is not known whether the mitochondrial permeability transition pore (MitoPTP) is involved in TNF-α-induced cardioprotection. In the present study, we examined whether TNF-α inhibits MitoPTP opening. In isolated rat hearts subjected to 30 min regional ischemia and 120 min reperfusion, pretreatment with 10 U/ml TNF-α for 7 min followed by 10 min washout improved the recovery of left ventricular developed pressure (LVDP) and rate-pressure product (RPP = LVDP × heart rate) during reperfusion and reduced the infarct size. Administration of 20 μmol/L atractyloside, a MitoPTP opener, for 20 min (last 5 min of ischemia and first 15 min of reperfusion) and pretreatment with 1 μmol/L paxilline, an inhibitor of the Ca^{2+}-activated K^+ channel, for 5 min before ischemia, attenuated the recovery of LVDP and RPP and the reduction of infarct size induced by TNF-α. The findings indicate that, in the isolated heart model, TNF-α protects myocardium against ischemia/reperfusion injury via inhibiting MitoPTP opening as well as by activating the Ca^{2+}-activated K^+ channel.**

Keywords—**Tumor necrosis factor-α, heart, ischemia-reperfusion, mitochondrial permeability transition pore**

I. Introduction

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine that mediates diverse physiological and pathophysiological events, including the ability to evoke protective preconditioning against ischemia and reperfusion injury in isolated, perfused rat and rabbit hearts [1;2]. Hearts from TNF-α-pretreated animals contain lower levels of lactate dehydrogenase than hearts from untreated rats, and TNF-α induces the messenger RNA for manganous superoxide dismutase [3]. In transgenic mice deficient in the TNF-α gene, ischemic preconditioning fails to decrease infarct size, suggesting that endogenous TNF-α is required for cardioprotection [4]. TNF-α activates multiple downstream signaling cascades related to mitochondria. However, the relationship between the cardioprotection evoked by TNF-α and mitochondria has not been elucidated. The mitochondrial permeability transition pore (MitoPTP) is a non-specific pore across the mitochondrial inner and outer membranes. Inhibition of MitoPTP opening is known to be cardioprotective [5]. Therefore, we hypothesized that MitoPTP may play a role in the protective effect of TNF-α in hearts subjected to ischemia and reperfusion. The present study was designed to test whether stimulation or inhibition of MitoPTP opening affects the cardioprotective effect of TNF-α.

II. Methodology

1) *Isolated perfused heart preparation*: Male Sprague-Dawley rats (200-250g) were killed by stunning and cervical dislocation. Hearts were excised rapidly and placed in ice-cold Krebs-Henseleit (K-H) buffer, then mounted on a constant pressure (100 cmH_2O) Langendorff apparatus and perfused at 37°C with K-H buffer. For hearts subjected to regional ischemia, a 5/0 silk suture was passed under the left coronary artery to form a snare. The artery was occluded by pulling the snare to produce ischemia, while reperfusion was achieved by releasing it. A latex, fluid-filled balloon was introduced into the left ventricle through the left atrial appendage and the left ventricular end diastolic pressure (LVEDP) was adjusted to between 4 and 8 mmHg. The cardiac parameters heart rate (HR), left ventricular developed pressure (LVDP) and the rate-pressure product (LVDP × HR) were monitored continuously.

2) *Infarct size measurement*: 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 of 0.5% Evans blue was infused to delineate the non-ischemic zone of the myocardium as a dark blue area. Hearts were then frozen and sliced into 2 mm-thick transverse sections and incubated in 2,3,5-triphenyl-tetrazolium chloride for 10-15 min, then fixed in 10% formalin to visualize the unstained (infarcted) region. Infarct and risk zone areas were determined by planimetry using Image/J software (NIH). Infarct size was expressed as a percentage of the risk zone.

3) *Experimental protocol*: All hearts were allowed to equilibrate for at least 20 min and were subsequently subjected to a standard 30 min of regional ischemia followed by 120 min of reperfusion. The hearts were randomly assigned to one of the following 7 groups: (1) hearts subjected to regional ischemia and reperfusion only (IR); (2) hearts perfused with TNF-α (10 U/ml for 7 min) followed by 10 min drug-free perfusion before the standard ischemia; (3) 0.2 μmol/L cyclosporin A, an inhibitor of

MitoPTP opening, for 20 min (last 5 min of ischemia and first 15 min of reperfusion); (4) NS1619 (10 μmol/L), an activator of the Ca^{2+}-activated K^+ (K_{Ca}) channel for 10 min before the standard ischemia; (5) hearts treated with TNF-α and 20 μmol/L atractyloside, an opener of MitoPTP, for 20 min (last 5 min of ischemia and first 15 min of reperfusion); (6) treatment with both NS1619 and atractyloside; and (7) hearts treated with TNF-α and 1 μmol/L paxilline, a blocker of the K_{Ca} channel, for 5 min before the standard ischemia.

III. Results

A. *Effect of TNF-α, atractyloside and paxilline on ventricular contraction during ischemia and reperfusion*

In hearts subjected to ischemia and reperfusion only, LVDP and LVDP×HR decreased during ischemia and continued to fall during the following 120 min of reperfusion, while LVEDP was elevated. Treatment with 10 U/ml TNF-α or 0.2 μmol/L CsA attenuated these changes. Administration of atractyloside (20 μmol/L) and paxilline (1 μmol/L) reduced the effect of TNF-α (Figs. 1, 2, 3).

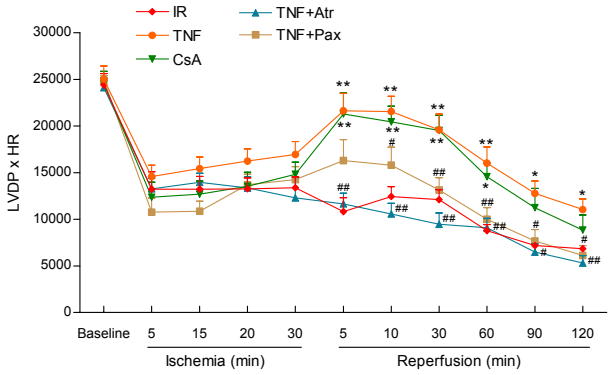

Fig. 1. Effect of TNF-α (10 U/ml), cyclosporin A (CsA, 0.2 μmol/L), atractyloside (Atr, 20 μmol/L) and paxilline (Pax, 1 μmol/L) on LVDP x HR in isolated rat heart subjected to ischemia and reperfusion (IR). Data expressed as mean ± SEM, n = 7-8.
*p<0.05, **p<0.01 vs IR group; #p<0.05, ## p<0.01 vs TNF group.

B. *Effect of TNF-α, atractyloside, paxilline and NS1619 on infarct size*

The infarct size of hearts after 30 min ischemia followed by 120 min reperfusion was 40.72 ± 6.47% of the risk zone. Treatment with TNF-α, CsA and NS1619 significantly reduced infarct size. However, atractyloside attenuated the reduction of infarct size induced by TNF-α and NS1619. Pretreatment with paxilline diminished the reduction of infarct size induced by TNF-α (Fig. 4).

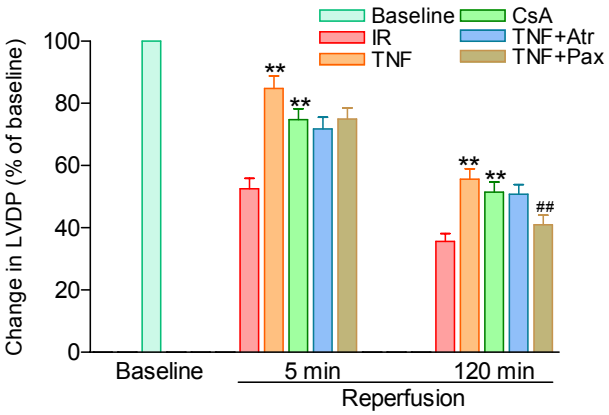

Fig. 2. Effect of TNF-α (10 U/ml), cyclosporin A (CsA, 0.2 μmol/L), atractyloside (Atr, 20 μmol/L) and paxilline (Pax, 1 μmol/L) on LVDP at 5 min and 120 min of reperfusion in isolated rat heart subjected to ischemia and reperfusion (IR). Data expressed as mean ± SEM, n = 7-8.
**p<0.01 vs IR group; ##p<0.01 vs TNF group.

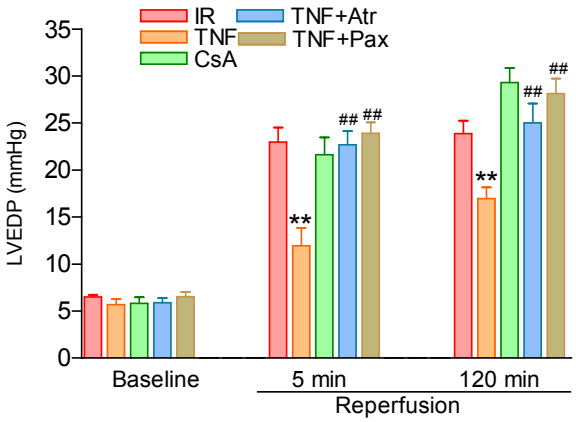

Fig. 3. Effect of TNF-α (10 U/ml), cyclosporin A (CsA, 0.2 μmol/L), atractyloside (Atr, 20 μmol/L) and paxilline (Pax, 1 μmol/L) on LVEDP at 5 min and 120 min of reperfusion in isolated rat heart subjected to ischemia and reperfusion (IR). Data expressed as mean ± SEM, n = 7-8.
**p<0.01 vs IR group; ## p<0.01 vs TNF group.

IV. Discussion

In the present study, we found that pretreatment with TNF-α attenuated the decrease of LVDP, LVDP × HR and the increase of LVEDP in the isolated heart during 120 min reperfusion after 30 min regional ischemia, as well as decreasing the infarct size. This protective effect was attenuated by the MitoPTP opener atractyloside and the K_{Ca} channel blocker paxilline, indicating that cardioprotection induced by TNF-α is associated with inhibiting MitoPTP opening and activating the K_{Ca} channel.

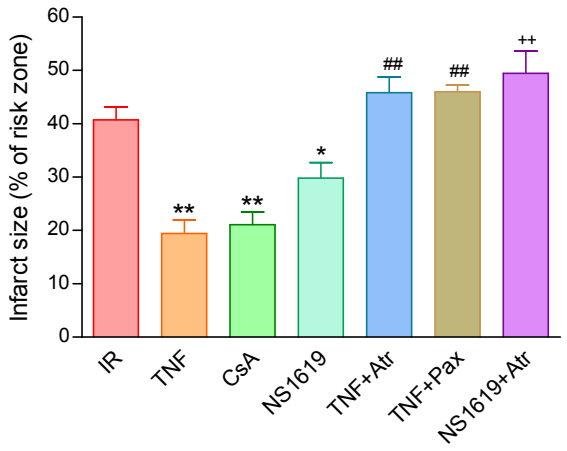

Fig. 4. Effect of TNF-α (10 U/ml), cyclosporin A (CsA, 0.2 μmol/L), atractyloside (Atr, 20 μmol/L), paxilline (Pax, 1 μmol/L) and NS1619 (10 μmol/L) on infarct size in isolated rat hearts. Data expressed as mean ± SEM, n = 7-8.
*p<0.05, **p<0.01 vs IR group; ##p<0.01 vs TNF group; ++p<0.01 vs NS1619 group.

Our study confirmed the cardioprotective effect of TNF-α against ischemia and reperfusion-induced myocardial injury reported by others [2;6]. Although some intracellular signal transduction pathways such as sphingolipid, NF-κB, AKT, and JUN are known to participate in the cardioprotection induced by TNF-α [6;7], the detailed mechanism remains to be elucidated.

Mitochondria are considered to lie at the "heart" of the cardioprotection provided by various triggers [8;9] and MitoPTP is the common target of the intracellular signaling transduction pathway [10].Therefore, since it was not clear how TNF-α exerted its cardioprotective effect we hypothesized that MitoPTP may serve as a target. In the present study, the MitoPTP opener atractyloside abolished the cardioprotective effect of TNF-α, which thus appears to act via MitoPTP inhibition. Furthermore, to verify the role of the K_{Ca} channel, we determined the effect of the K_{Ca} channel blocker paxilline on infarct size and ventricular hemodynamics in the presence of TNF-α. Pretreatment with paxilline attenuated the cardiac effect of TNF-α, which thus also appears to act via the K_{Ca} channel. It has been reported that K_{Ca} channel participates in the pharmacological protection against ischemic myocardial injury by using angiotensin-converting enzyme inhibitor cilazaprilat [11] and the K_{Ca} channel is activated and contributes to the regulation of coronary vascular tone during coronary hypoperfusion [12]. In the present study, the attenuating effect of NS1619, a K_{Ca} channel activator, was reduced by the MitoPTP opener atractyloside. This observation suggests that this channel, probably a mitochondrial K_{Ca} channel, is associated with MitoPTP and cardioprotection, and the K_{Ca} channel is upstream of the MitoPTP.

V. Conclusion

In conclusion, this study shows that cardioprotection against ischemia-reperfusion induced injury by TNF-α is mediated by inhibiting MitoPTP opening and activating the K_{Ca} channel.

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