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SHORT COMMUNICATION

ATP-dependent potassium channels and mitochondrial permeability transition pores play roles in the cardioprotection of theaflavin in young rat

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Abstract Previous studies have confirmed that tea polyphenols possess a broad spectrum of biological functions such as anti-oxidative, anti-bacterial, anti-tumor, anti-inflammatory, anti-viral and cardiovascular protection activities, as well as anti-cerebral ischemia-reperfusion injury properties. But the effect of tea polyphenols on ischemia/reperfusion heart has not been well elucidated. The aim of this study was to investigate the protective effect of theaflavin (TF1) and its underlying mechanism. Young male Sprague-Dawley (SD) rats were randomly divided into five groups: (1) the control group; (2) TF1 group; (3) glibenclamide + TF1 group; (4) 5-hydroxydecanoate (5-HD) + TF1 group; and (5) atractyloside + TF1 group. The Langendorff technique was used to record cardiac function in isolated rat heart before and after 30 min of global ischemia followed by 60 min of reperfusion. The parameters of cardiac function, including left ventricular developing pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), maximal differentials of LVDP ($\pm$LVdP/dt_{max}) and coronary flow (CF), were measured. The results showed: (1) compared with the control group, TF1 (10, 20, 40 μmol/l) displayed a better recovery of cardiac function after ischemia/reperfusion in a concentration-dependent manner. At 60 min of reperfusion, LVDP, $\pm$LVdP/dt_{max} and CF in the TF1 group were much higher than those in the control group, whereas left ventricular end-diastolic pressure (LVEDP) in the TF1 group was lower than that in the control group ($P < 0.01$). (2) Pretreatment with glibenclamide (10 μmol/l), a K_{ATP} antagonist, completely abolished the cardioprotective effects of TF1 (20 μmol/l). Also, most of the effects of TF1 (20 μmol/l) on cardiac function after 60 min of reperfusion were reversed by 5-HD (100 μmol/l), a selective mitochondria K_{ATP} antagonist. (3) Atractyloside (20 μmol/l), a mitochondrial permeability transition pore (mPTP) opener, administered at the beginning of 15 min of reperfusion completely abolished the cardioprotection of TF1 (20 μmol/l). The results indicate that TF1 protects the rat heart against ischemia/reperfusion injury through the opening of K_{ATP} channels, particularly on the mitochondrial membrane, and inhibits mPTP opening.

Keywords Theaflavin · Heart · Ischemia/reperfusion · ATP-dependent potassium channel · Mitochondrial permeability transition pore · Protection

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Introduction

Several brief repeated ischemia/reperfusion (I/R) cycles before long-term ischemia improve cardiac recovery from I/R injury, which is called ischemic preconditioning (IPC) [1]. The heart's tolerance of ischemia can also be enhanced by some other manipulations, including pharmacological preconditioning [2], cardioplegic protection [3] and hypoxic adaptation [4].

Theaflavins are natural polyphenols found in black tea, including theaflavin (TF1), theaflavin 3-gallate (TF2A), theaflavin 3′-gallate (TF2B) and theaflavin 3,3′-gallate (TF3) [5]. These tea polyphenols possess a broad spectrum

of biological functions, such as anti-oxidative, anti-bacterial, anti-tumour, anti-inflammatory, anti-viral and cardiovascular protection activities [6–8]. TF1 has been reported to significantly protect neurons from cerebral I/R injury [9, 10]. The effect of TF1, however, on I/R hearts and the underlying mechanisms are far from clear.

It is well accepted that ATP-dependent potassium (K_{ATP}) channels activated by ischemic or hypoxic preconditioning protect the heart against I/R injury [11]. It has also been reported recently that inhibition of mitochondrial permeability transition pore (mPTP) opening by ischemia preconditioning (IPC) appears to be associated with cardioprotective effects [12]. So it is reasonable to hypothesize that K_{ATP} and mPTP may participate in the protective effects of TF1 against I/R injury.

This study was undertaken to evaluate the cardioprotection of TF1, a major constituent of theaflavins, in I/R heart of rats and to investigate the role of K_{ATP} and mPTP in the cardioprotection of TF1.

Materials and methods

Experiment animal and drugs

Young male Sprague-Dawley (SD) rats weighing 90–120 g were provided by the Experimental Animal Center of Hebei Province, China. All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). TF1 was purchased from Chromadex Inc., and atractyloside, glibenclamide and 5-hydroxydecanoate (5-HD) were purchased from Sigma (St Louis, MO).

Ischemia/reperfusion in isolated heart

Rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and the hearts were quickly excised and mounted on a Langendorff apparatus via the aorta for retrograde perfusion with Krebs-Henseleit (K-H) solution at constant pressure (10 kPa). The K-H solution (in mmol/l) was composed of: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 11.0. The solution was continuously gassed with 95% O_2 and 5% CO_2 (pH 7.4), and maintained at 37°C. A water-filled latex balloon connected to a pressure transducer (Gould P23Db) was introduced into the left ventricle through the atria to record isovolumic left ventricular pressure. The balloon volume was adjusted to achieve a stable left ventricular end-diastolic pressure (LVEDP) of 3–10 mmHg during initial equilibration. Left ventricular developed pressure (LVDP), LVEDP, the maximal differentials of LVDP ($\pm$LVdp/dt_{max}), heart rate (HR) and coronary flow (CF) were monitored with the PowerLab system (ADInstruments Ltd., Australia), which was similar to that previously described by Zhang et al. [13].

Animal group and experimental protocols

Rats were randomly divided into five groups: (1) control group: after stabilization for 20 min with K-H solution, the hearts were subjected to 30 min no-flow global ischemia followed by 60 min of reperfusion; (2) TF1 group: the hearts were treated with 10, 20 or 40 μmol/l TF1 for 10 min before ischemia and reperfusion, respectively; (3) glibenclamide + TF1 group: the hearts were first perfused for 5 min with 10 μmol/l glibenclamide, a K_{ATP} antagonist and then treated with 20 μmol/l TF1 and 10 μmol/l glibenclamide together for 10 min before ischemia and reperfusion; (4) 5-hydroxydecanoate (5-HD) + TF1 group: the hearts were first perfused for 5 min with 100 μmol/l 5-HD, a selective mitochondria K_{ATP} antagonist, and then treated with 20 μmol/l TF1 and 100 μmol/l 5-HD together for 10 min before ischemia and reperfusion; (5) atractyloside + TF1 group: the hearts were treated with 20 μmol/l TF1 for 10 min before ischemia, and atractyloside (20 μmol/l), a mitochondrial permeability transition pore (mPTP) opener, was added at the beginning of 15 min of reperfusion.

Statistical analysis

All data were expressed as mean ± SD. The paired t test was used to compare the data within groups, and ANOVA followed by a Dunnett's post hoc test was used for data between groups. $P < 0.05$ was considered significant.

Results

Protective effects of TF1 on I/R rat hearts

There were no significant differences of functional parameters between the control and TF1 groups under non-ischemic conditions. The values of LVDP, +LVdP/dt_{max}, −LVdP/dt_{max} and CF decreased, while LVEDP increased significantly in both groups during I/R, which indicates damage of left ventricular function ($n = 6$, $P < 0.05$, or $P < 0.01$, Figs. 1, 2). After 60 min reperfusion, LVDP in TF1 at 10, 20 and 40 μmol/l was 21.8 ± 7.5, 29.4 ± 9.1 and 37.1 ± 9.8 mmHg, respectively, and significantly higher than 18.4 ± 6.7 in the control group; LVEDP was 72.4 ± 6.3, 69.8 ± 6.2 and 58.3 ± 5.6 mmHg, respectively, and significantly lower than 81.8 ± 8.9 in the control group; +LVdP/dt_{max} was 916.4 ± 176.8, 1,115.4 ± 218.2

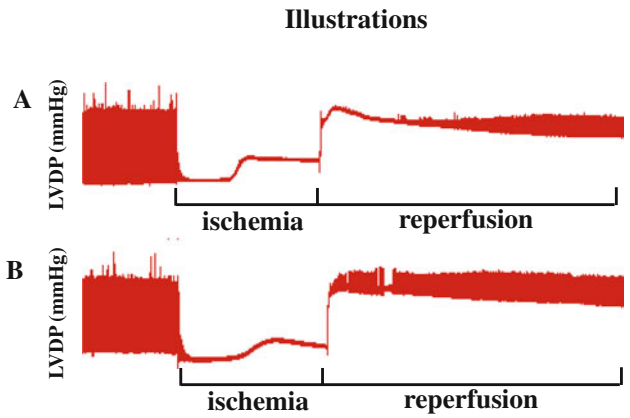

Fig. 1 Original recordings of left ventricular function in isolated rat hearts submitted to 30 min ischemia and 60 min reperfusion. **a** Control group, **b** theaflavin (20 μmol/l) group

and 1,306.2 ± 276.9 mmHg/s, respectively, and significantly higher than 315.5 ± 150.1 mmHg/s in the control group; $-LVdP/dt_{max}$ was −616.5 ± 106.3, −782.0 ± 164.1 and −1,176.4 ± 134.5 mmHg/s, respectively, and significantly higher than −336.7 ± 171.3 mmHg/s in the control group; CF was 3.3 ± 0.8, 4.5 ± 0.9 and 4.9 ± 1.1 ml, respectively, and significantly higher than 2.8 ± 0.4 ml in the control group ($n = 6$, Fig. 3, $P < 0.05$, or $P < 0.01$). All the above results suggest that TF1 increases the tolerance of hearts against I/R injury in a concentration-dependent manner.

Influence of glibenclamide and 5-HD on the protective effects of TF1 against I/R injury in isolated rat hearts

After 60 min reperfusion, the LVDP, LVEDP, $+LVdP/dt_{max}$, $-LVdP/dt_{max}$ and CF in the glibenclamide + TF1 group was 17.3 ± 5.1 mmHg, 83.6 ± 10.6 mmHg, 304.2 ± 76.2 mmHg/s, −316.5 ± 21.0 mmHg/s and 3.0 ± 0.6 ml, respectively, and significantly different from 29.4 ± 9.1, 69.8 ± 3.2, 1,115.4 ± 218.2, −562.464.1 and 4.5 ± 0.9 in the TF1 (20 μmol/l) group ($n = 6$, Fig. 4 $P < 0.05$, or $P < 0.01$), but not different from 18.4 ± 6.7 mmHg, 81.8 ± 8.9 mmHg, 315.5 ± 150.1 mmHg/s, −331.1 ± 21.3 mmHg/s and 2.8 ± 0.4 ml in the control group. However, the LVDP, LVEDP, $+LVdP/dt_{max}$, $-LVdP/dt_{max}$ and CF in the 5-HD + TF1 group was 20.7 ± 4.3 mmHg, 78.7 ± 7.7 mmHg, 785.6 ± 163.6 mmHg/s, −411.7 ± 81.8 mmHg/s and 3.6 ± 0.6 ml, respectively, and significantly different from those in the TF1 (20 μmol/l) group ($n = 6$, Fig. 4, $P < 0.05$, or $P < 0.01$), but not different from those in the control group. These data suggest that the cardioprotective effects of TF1 (20 μmol/l) can be abolished by glibenclamide (10 μmol/l) completely, and by 5-HD (100 μmol/l) mostly.

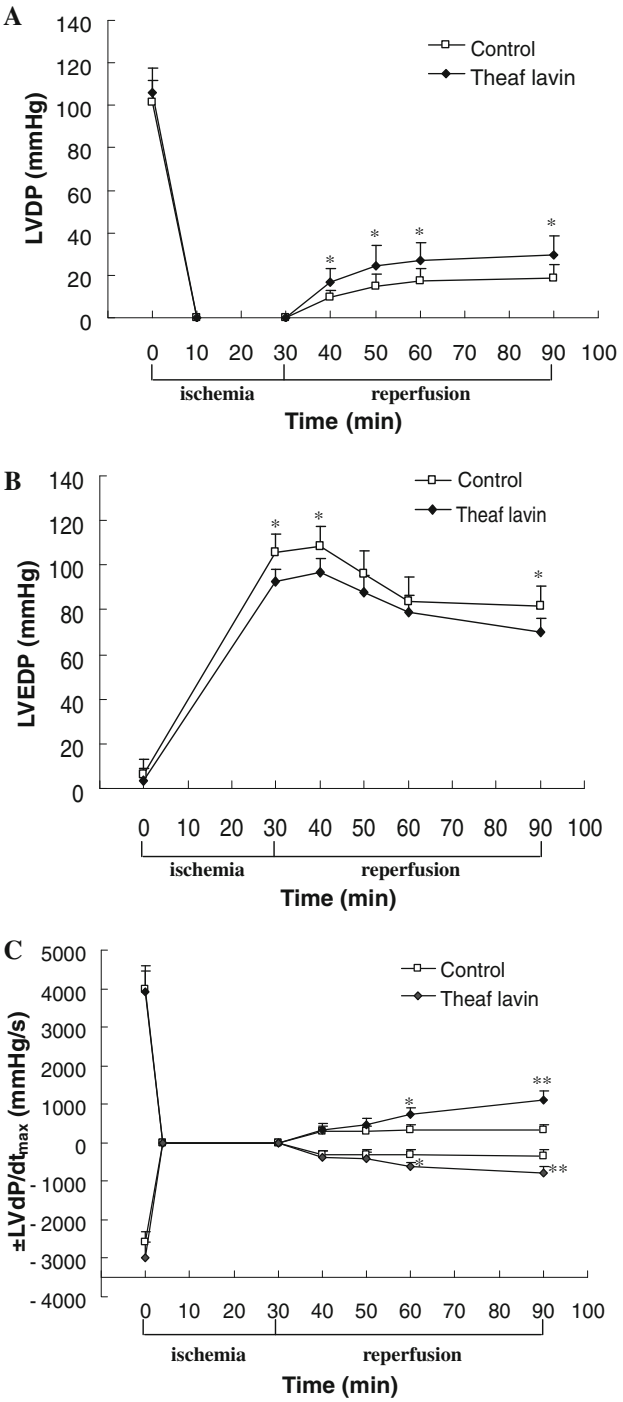

Fig. 2 The effects of theaflavin (20 μmol/l) on cardiac functional parameters in isolated rat hearts subjected to 30 min of ischemia and 60 min of reperfusion ($n = 6$ in each group). **a** LVDP, **b** LVEDP, **c** $\pm LVdP/dt_{max}$. $^*P < 0.05$, $^{**}P < 0.01$ versus control group

Influence of atractyloside on the protective effects of TF1 against I/R injury in isolated rat hearts

After 60 min reperfusion, the LVDP, LVEDP, $+LVdP/dt_{max}$, $-LVdP/dt_{max}$ and CF in the atractyloside + TF1 group were

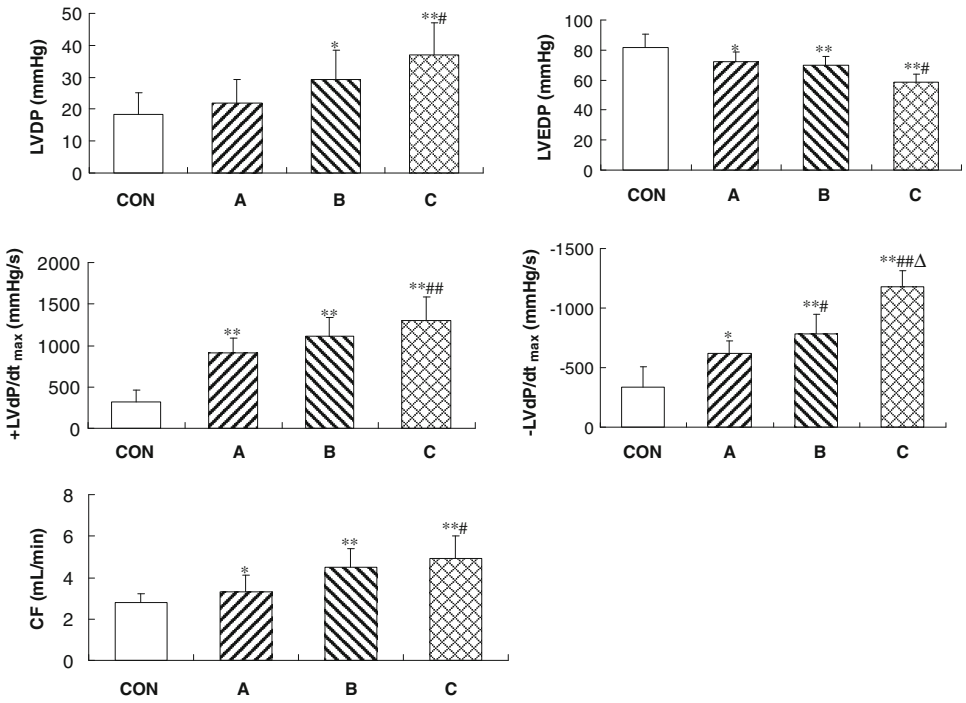

Fig. 3 Effects of theaflavin on cardiac function after I/R ($n = 6$ in each group), *CON* control group, **a** theaflavin (10 μmol/l) group, **b** theaflavin (20 μmol/l) group, **c** theaflavin (40 μmol/l) group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus theaflavin (10 μmol/l) group; $^{\Delta}P < 0.05$ versus theaflavin (20 μmol/l) group

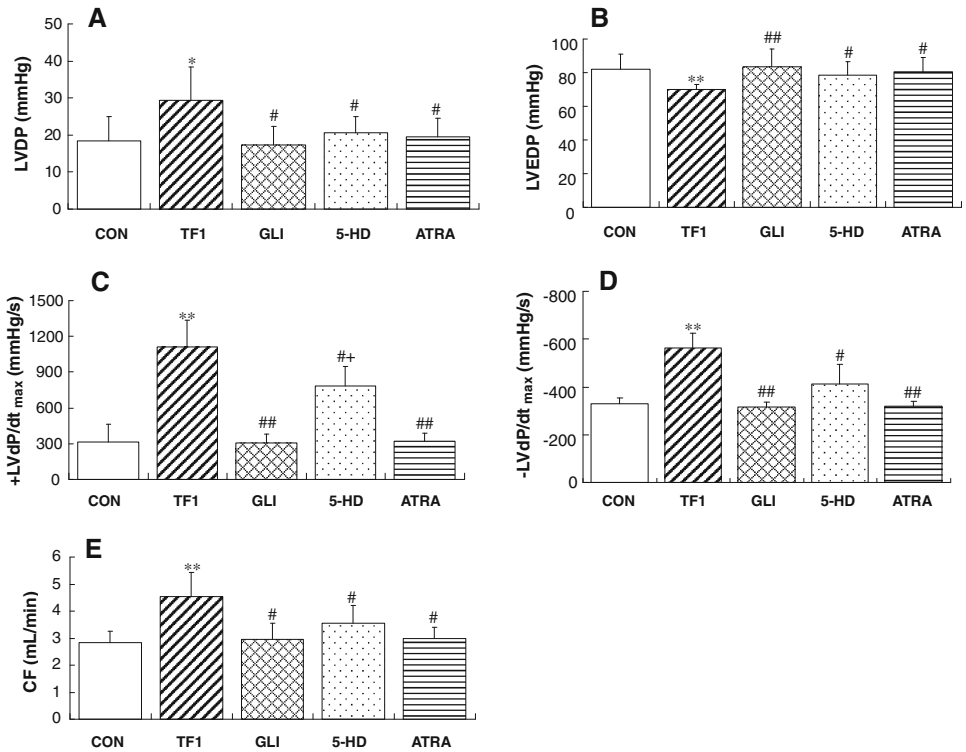

Fig. 4 Influence of K_{ATP} antagonist and mPTP opener on the protective effects of theaflavin against myocardial ischemia/reperfusion injury (measured after 60 min of reperfusion, $n = 6$ in each group). *CON* control group, *TF1* theaflavin group (20 μmol/l), *GLI* glibenclamide group, *5-HD* 5-hydroxydecanoate group, *ATRA* atractyloside group. **a** LVDP, **b** LVEDP, **c** $+LVdp/dt_{max}$, **d** $-LVdp/dt_{max}$, **e** CF. $^{*}P < 0.05$, $^{**}P < 0.01$ versus CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus TF1 group; $^{+}P<0.05$ versus GLI group

19.5 ± 5.2 mmHg, 80.4 ± 8.7 mmHg, 318.1673 ± 73.3 mmHg/s, −318.2 ± 22.1 mmHg/s and 3.0 ± 0.4 ml, respectively, and significantly different from those in the TF1 (20 μmol/l) group ($n = 6$, Fig. 4, $P < 0.05$ or $P < 0.01$), but not different from those in the control group. These data suggest that the cardioprotective effects of TF1 (20 μmol/l) can be abolished by atractyloside (20 μmol/l).

Discussion

In this study, the Langendorff technique was employed, and TF1 in three common concentrations of 10, 20 and 40 μmol/l [14] was used to investigate the effect of TF1 on isolated I/R heart for the first time. The results show that short-term administration of TF1 before ischemia has a

clear protective effect against I/R injury on the heart in young rats, manifested as an improved recovery of post-ischemic ventricular function. The protective effects of TF1 could be abolished by glibenclamide, a K_{ATP} antagonist, 5-HD, a selective mitochondria K_{ATP} antagonist, and atractyloside, an mPTP opener, which suggests that K_{ATP} and mPTP are involved in the cardiac protection afforded by TF1.

ATP-sensitive potassium (K_{ATP}) channels exist in high density in the sarcolemmal membrane as well as the mitochondrial membrane of cardiomyocytes. The K_{ATP} channel is a weakly inward-rectifying K^+ channel that is inhibited by intracellular ATP and activated by intracellular nucleoside diphosphates. Under physiological conditions, the K_{ATP} channel exists mainly in a closed, inactive form. The probability of the channel opening, however, is increased during myocardial ischemia, as the intracellular ATP concentration falls and ischemic metabolites (ADP, lactate, H^+) accumulate. This results in an enhanced outward repolarizing flow of K^+ and cell membrane hyperpolarization. Consequently, the myocardial action potential duration (APD) is shortened, and the voltage-dependent calcium current and myocardial contractility are decreased in which ATP is preserved during ischemia. Generally, it is thought that K_{ATP} channels have a protective property in myocardial ischemic diseases [15]. In this study, the cardioprotection of TF1 was abolished by glibenclamide, a non-selective K_{ATP} inhibitor, suggesting K_{ATP} channels are involved in the protective effect of TF1. In recent years, it was found that an ATP-sensitive K^+ channel in the mitochondrial inner membrane was involved in the signaling cascade of myocardial ischemic preconditioning and that it played an important role in cardiac protection against myocardial ischemic injuries [16]. A number of studies have proved the role of mitochondrial K_{ATP} channels in ischemic and pharmacological preconditioning based on the ability of 5-HD to block cardioprotection [17, 18]. In our study, the addition of 5-HD, the specific mitochondrial K_{ATP} channel blocker, abolished mostly the cardioprotection of TF1 on reperfusion-induced injury, which suggests that the K_{ATP} channel, especially the mitochondrial K_{ATP} channel, may be involved in the cardioprotective effect of TF1. A recent study on theaflavins has demonstrated that a PKCε-dependent regulation is involved in myocardial contraction [19]. It was reported that PKC activation resulted in opening of the mitochondrial K_{ATP} channel and consequently induced the postconditioning of human myocardium [20]. Thus, we guess that the opening of the mitochondrial K_{ATP} channel in TF1 cardioprotection resulted from the activation of PKC.

Myocardium is a typically aerobic tissue, and its metabolism totally depends on oxygen availability in mitochondria. It was confirmed that I/R could damage the mitochondrial functions, including depression of energy production, disruption of ionic homeostasis and generation of free radicals [21]. The mPTP is a non-specific large pore in the inner mitochondrial membrane and usually opens in response to oxidative stress during reperfusion of the ischemic myocardium. The mPTP opening allows water and solutes to enter the mitochondria, leading to matrix swelling, inner membrane potential collapse, uncoupling of the respiratory chain, efflux of Ca^{2+} and release of small proteins such as cytochrome *c* [22]. Recent studies have found that suppression of mPTP opening during the first few minutes of reperfusion may be important for IPC [21, 23]. The inhibitors of mPTP opening, such as cyclosporin A (CsA) and sanglifehrin A, have already been shown to protect the heart against I/R injury [24–26]. In this study, atractyloside, an mPTP opener, completely abolished the protective effects of TF1, suggesting that reduction of mPTP opening during reperfusion plays an important role in the cardiac protection of TF1.

Ca^{2+} overloading induces mPTP opening, which appears to be a critical event in the transition from reversible to irreversible myocardial injury following an ischemic insult [21, 27]. This permeability transition leads to the collapse of the mitochondria membrane potential, massive mitochondrial swelling and loss of low-molecular weight components (such as cytochrome c) from the intermembrane place, which contributes to cell death [28, 29]. Opening of mitochondrial K_{ATP} channels dissipates mitochondrial membrane potential and releases Ca^{2+} from mitochondria into the cytoplasm, leading to a decrease in the driving force for Ca^{2+} uptake into the mitochondria and prevents mitochondrial Ca^{2+} overloading. A previous study showed that mitochondrial K_{ATP} channel activation might inhibit mPTP opening at reperfusion [21], but the mechanism is not clear, and the link between mitochondrial K_{ATP} channels and mPTP needs further investigation.

In summary, the present study demonstrated firstly that TF1 protects the rat heart against I/R injury through the opening of K_{ATP} channels, particularly on the mitochondrial membrane, and secondly inhibits mPTP opening.

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References

1. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136
2. Bradamante S, Piccinini F, Barenghi L, Bertelli AA, De Jonge R, Beemster P, De Jong JW (2000) Does resveratrol induce pharmacological preconditioning? Int J Tissue React 22:1–4

3. Guyton RA, Gott JP, Brown WM, Craver JM (1996) Cold and warm myocardial protection techniques. Adv Card Surg 7:1–29
4. Dong JW, Zhu HF, Zhu WZ, Ding HL, Ma TM, Zhou ZN (2003) Intermittent hypoxia attenuates ischemia/reperfusion induced apoptosis in cardiac myocytes via regulating Bcl-2/Bax expression. Cell Res 13:385–391
5. Gupta S, Saha B, Giri AK (2002) Comparative antimutagenic and anticlastogenic effects of green tea and black tea: a review. Mutat Res 512:37–65
6. Mukhtar H, Ahmad N (2000) Tea polyphenols: prevention of cancer and optimizing health. Am J Clin Nutr 71:1698S–1702S (discussion 1703S–1704S)
7. Higdon JV, Frei B (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 43:89–143
8. Dreger H, Lorenz M, Kehrer A, Baumann G, Stangl K, Stangl V (2008) Characteristics of catechin- and theaflavin-mediated cardioprotection. Exp Biol Med (Maywood) 233:427–433
9. Cai F, Li C, Wu J, Min Q, Ouyang C, Zheng M, Ma S, Yu W, Lin F (2007) Modulation of the oxidative stress and nuclear factor kappaB activation by theaflavin 3,3′-gallate in the rats exposed to cerebral ischemia–reperfusion. Folia Biol (Praha) 53:164–172
10. Cai F, Li CR, Wu JL, Chen JG, Liu C, Min Q, Yu W, Ouyang CH, Chen JH (2006) Theaflavin ameliorates cerebral ischemia–reperfusion injury in rats through its anti-inflammatory effect and modulation of STAT-1. Mediators Inflamm 2006:30490
11. O'Rourke B (2000) Myocardial K(ATP) channels in preconditioning. Circ Res 87:845–855
12. Javadov SA, Clarke S, Das M, Griffiths EJ, Lim KH, Halestrap AP (2003) Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. J Physiol 549:513–524
13. Zhang LP, Yang CY, Wang YP, Cui F, Zhang Y (2008) Protective effect of polydatin against ischemia/reperfusion injury in rat heart. Sheng Li Xue Bao 60:161–168
14. Leung LK, Su Y, Chen R, Zhang Z, Huang Y, Chen ZY (2001) Theaflavins in black tea and catechins in green tea are equally effective antioxidants. J Nutr 131:2248–2251
15. Fujita A, Kurachi Y (2000) Molecular aspects of ATP-sensitive K+ channels in the cardiovascular system and K^+ channel openers. Pharmacol Ther 85:39–53
16. Papp Z, Csapo K, Pollesello P, Haikala H, Edes I (2005) Pharmacological mechanisms contributing to the clinical efficacy of levosimendan. Cardiovasc Drug Rev 23:71–98
17. Schultz JE, Qian YZ, Gross GJ, Kukreja RC (1997) The ischemia-selective KATP channel antagonist, 5-hydroxydecanoate, blocks ischemic preconditioning in the rat heart. J Mol Cell Cardiol 29:1055–1060
18. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC (1999) Opening of mitochondrial K_{ATP} channel induces early and delayed cardioprotective effect: role of nitric oxide. Am J Physiol 277:H2425–H2434
19. Li D, Yang C, Chen Y, Tian J, Liu L, Dai Q, Wan X, Xie Z (2008) Identification of a PKCepsilon-dependent regulation of myocardial contraction by epicatechin-3-gallate. Am J Physiol Heart Circ Physiol 294:H345–H353
20. Lemoine S, Puddu PE, Durand C, Lepage O, Babatasi G, Ivascau C, Massetti M, Gérard JL, Hanouz JL (2010) Signaling pathways involved in postconditioning-induced cardioprotection of human myocardium, in vitro. Exp Biol Med (Maywood) 235:768–776
21. Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM (2002) Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? Cardiovasc Res 55:534–543
22. Borutaite V, Brown GC (2003) Mitochondria in apoptosis of ischemic heart. FEBS Lett 541:1–5
23. Hausenloy DJ, Yellon DM, Mani-Babu S, Duchen MR (2004) Preconditioning protects by inhibiting the mitochondrial permeability transition. Am J Physiol Heart Circ Physiol 287:H841–H849
24. Clarke SJ, McStay GP, Halestrap AP (2002) Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. J Biol Chem 277:34793–34799
25. Halestrap AP, Connern CP, Griffiths EJ, Kerr PM (1997) Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury. Mol Cell Biochem 174:167–172
26. Hausenloy DJ, Duchen MR, Yellon DM (2003) Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. Cardiovasc Res 60:617–625
27. Halestrap AP, Kerr PM, Javadov S, Woodfield KY (1998) Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. Biochim Biophys Acta 1366:79–94
28. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida H, Kong YY, Mak TW, Zuniga-Pflucker JC, Kroemer G, Penninger JM (2001) Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 410:549–554
29. Di Lisa F, Bernardi P (1998) Mitochondrial function as a determinant of recovery or death in cell response to injury. Mol Cell Biochem 184:379–391