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Original article

# Trimetazidine inhibits mitochondrial permeability transition pore opening and prevents lethal ischemia–reperfusion injury

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**Abstract**

Trimetazidine (TMZ) affects mitochondrial function during ischemia. Mitochondrial permeability transition is a pivotal event in cardiomyocyte death following acute ischemia. The aim of the present study was to determine whether the anti-ischemic agent TMZ might modulate mitochondrial permeability transition pore (mPTP) opening and limit lethal ischemia–reperfusion injury. Anesthetized NZW rabbits underwent 30 min of coronary artery occlusion followed by 4 hours of reperfusion. Prior to this, they underwent either no intervention (control, C), ischemic preconditioning (PC), or an IV injection of 5 mg $kg^{-1}$ TMZ 10 min before ischemia (TMZ). Additional rabbits (Sham group) underwent no ischemia/reperfusion throughout the experiment. Infarct size was assessed by triphenyltetrazolium staining, and apoptosis via measurement of caspase 3 activity. $Ca^{2+}$-induced mPTP opening was assessed in mitochondria isolated from ischemic myocardium. TMZ and PC significantly reduced infarct size that averaged 34 ± 4% and 21 ± 4% of the risk region respectively, versus 63±6% in controls ($P < 0.005$). Caspase 3 activity was reduced in both TMZ and PC groups: 37 ± 11 and 29 ± 7 respectively, versus 68 ± 9 nmol $min^{-1}$ $mg^{-1}$ mitochondrial protein in controls ($P = 0.01$ versus TMZ and PC). In controls, $Ca^{2+}$ load required for mPTP opening averaged 11 ± 4 μM $mg^{-1}$ mitochondrial protein versus 116±6 in shams ($P < 0.0001$). Pre-treatment by TMZ or PC attenuated this, with $Ca^{2+}$ loads averaging 45 ± 4 and 46 ± 4 μM $mg^{-1}$ mitochondrial proteins, respectively ($P < 0.005$ versus C). These data suggest that TMZ inhibits mPTP opening and protects the rabbit heart from prolonged ischemia–reperfusion injury.


*Keywords:* Trimetazidine; Ischemia; Reperfusion; Myocardial infarction; Preconditioning; Mitochondria

## 1. Introduction

Trimetazidine or 1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride (TMZ) is the first of a promising new class of metabolic agents that act by optimizing energy metabolism in the heart. TMZ is a clinically effective anti-ischemic drug, that is currently used in some European countries for the treatment of stable angina pectoris [1,2]. It has recently been demonstrated that this anti-ischemic effect of TMZ may involve the inhibition of long-chain 3-ketoacyl CoA thiolase activity, with subsequent reduction in fatty acid oxidation and stimulation of glucose oxidation [3]. This mechanism may explain the significant improvement in postischemic functional recovery observed in rat hearts pre-treated with TMZ [4].

Besides this metabolic effect, in vitro evidence suggests that TMZ might also modulate mitochondrial permeability transition [5]. Mitochondrial permeability transition represents a crucial event in both necrotic and apoptotic cardiomyocyte death following a prolonged myocardial ischemia–reperfusion [6,7]. It is due to the opening of a non-specific megachannel (called the mitochondrial permeability transition pore (mPTP)) in the inner mitochondrial membrane. The mPTP, that remains closed throughout ischemia, opens at the time of reperfusion as a consequence of abrupt restoration of pH, $Ca^{2+}$ overload, adenine nucleotide depletion, accumula-

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tion of inorganic phosphate, and production of reactive oxygen species (ROS) [7,8]. Opening of the mPTP results in a collapse of the inner membrane potential ($\Delta\Psi_m$), uncoupling of the respiratory chain, and efflux of small molecules such as cytochrome *c* and other proapoptotic factors [9]. Recent evidence indicates that inhibition of mPTP opening by cyclosporin A (CsA), induce a potent cardioprotection in both in vitro and in vivo experimental models of myocardial infarction [10–12]. Inhibition of mitochondrial permeability transition may explain, at least in part, the cardioprotective effect of ischemic preconditioning (PC) [11,13].

We postulated that TMZ might modulate mPTP opening and limit lethal ischemia–reperfusion injury. Our objective was to determine:

- whether in vivo administration of TMZ might protect the ischemic-reperfused myocardium from necrosis and apoptosis;
- whether any cardioprotective effect of TMZ may be related to an inhibition of mPTP opening.

## 2. Materials and methods

The investigation confirms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

### 2.1. Surgical preparation

Male New Zealand White rabbits, weighing 2.2–2.5 kg were anesthetized by intramuscular injections of xylazine (5 mg $kg^{-1}$) and ketamine (50 mg $kg^{-1}$), as previously described in [14]. An intravenous infusion of a mixture of xylazine (20–50 μg $kg^{-1}$ $min^{-1}$) and ketamine (40–100 μg $kg^{-1}$ $min^{-1}$) was then maintained throughout the experiment. After a midline cervical incision, a tracheotomy was performed and animals were ventilated with room air. A cannula was inserted into the right internal jugular vein for administration of drugs and fluids and into the left carotid artery for measurement of blood pressure. A left thoracotomy was performed in the fourth left intercostal space. The pericardium was opened and the heart exposed. A 3.0 silk suture attached to a small curved needle was passed around a marginal branch of the left circumflex coronary artery. Both ends of the thread were passed through a small vinyl tube to form a snare that could be tightened to occlude and loosened to reperfuse the artery. Body temperature was monitored via an intraperitoneal thermometer and kept constant by means of a heating pad. Heart rate (HR) and mean blood pressure (MBP) were monitored continuously throughout the experiment on a Gould® recorder (Gould Inc., Cleveland, OH). After the surgical procedure, a 20 min stabilization period was observed.

### 2.2. Experimental design

All animals underwent a test ischemic insult consisting of a coronary artery occlusion followed by reperfusion, as previously described in [11,14]. Prior to this, control rabbits underwent no intervention (control group, C), while preconditioned received 5 min of ischemia followed by 5 min of reperfusion (preconditioned group, PC). Treated rabbits received an intravenous bolus of 5 mg $kg^{-1}$ TMZ, 10 min before coronary occlusion (TMZ group). An additional group of rabbits (Sham) underwent no ischemia/reperfusion throughout the experiment. At the end of this experimental procedure, hearts were harvested for further analysis.

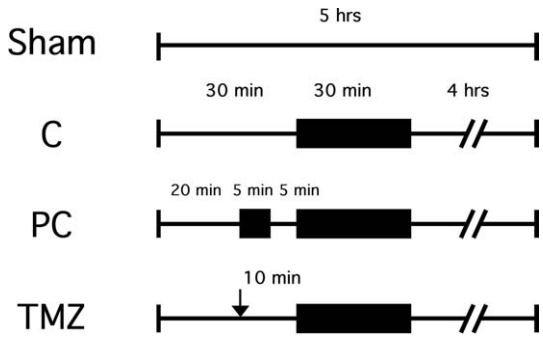


Fig. 1. Experimental design.
Animals underwent 30 min of ischemia followed by 4 hours of reperfusion. PC consisted of one episode of 5 min of ischemia and 5 min of reperfusion. TMZ was administered as an IV bolus (arrows), 10 min before ischemia. C: control group, PC: preconditioned group, TMZ: trimetazidine group.

We performed two independent and parallel protocols in which all animals received 30 min of ischemia followed by 4 hours of reperfusion (Fig. 1). The first one was designed to assess infarct size ($N = 10$ per group). In the second protocol, performed in different animals, myocardium was used to address $Ca^{2+}$-induced mPTP opening ($N = 6–8$ per group) and caspase 3 activity ($N = 7–8$ per group).

In an additive protocol, all rabbits underwent 10 min of ischemia followed by 5 min of reperfusion. Prior to this, they received ($N = 6–8$ per group), either no intervention (control), PC, or TMZ injection as described above. At the end of these experiments, myocardium from the area at risk (AR) was excised for assessment of $Ca^{2+}$-induced mPTP opening.

### 2.3. AR and infarct size determination

At the end of the 4 hours reperfusion, the coronary artery was briefly reoccluded and 0.5 mg $kg^{-1}$ Uniperse blue pigment (Ciba–Geigy®, Hawthorne, NY) was injected intravenously to delineate the in vivo AR, as previously described in [15]. With this technique, the previously non-ischemic myocardium appears blue, whereas the previously ischemic myocardium (AR,) remains unstained. Anesthetized rabbits were then euthanized by an intravenous injection of 4 ml KCl 10%. The heart was excised and cut into five to six 2 mm thick transverse slices, parallel to the atrioventricular groove. After removing right ventricular tissue, each heart slices were weighed. The basal surface of each slice was photographed for later measurement of the AR. Each slice was then incubated for 15 min in a 1% solution of triphenyltetrazolium chloride at 37 °C to differentiate infarcted (pale) from viable