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Journal of Molecular and Cellular Cardiology 39 (2005) 893–899
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

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

Laurent Argaud [a,b,c], Ludovic Gomez [a,b], Odile Gateau-Roesch [a,b], Elisabeth Couture-Lepetit [a,b], Joseph Loufouat [a,b], Dominique Robert [c], Michel Ovize [a,b,*]

[a] *Inserm, E0226, 69003 Lyon, France*
[b] *Laboratoire de physiologie Lyon-Nord, université Claude-Bernard Lyon-I, 69008 Lyon, France*
[c] *Département d'urgence et de réanimation médicale, hospices civils de Lyon, hôpital Édouard-Herriot, 69003 Lyon, France*

Received 25 April 2005; received in revised form 8 September 2005; accepted 9 September 2005

Available online 21 October 2005

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-

* Corresponding author. Laboratoire de physiologie Lyon-Nord, Inserm E0226, 8, avenue Rockefeller, 69373 Lyon, France. Tel.: +33 4 78 77 70 74; fax: +33 4 78 77 71 75.
E-mail address: ovize@sante.univ-lyon1.fr (M. Ovize).

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doi:10.1016/j.yjmcc.2005.09.012

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.

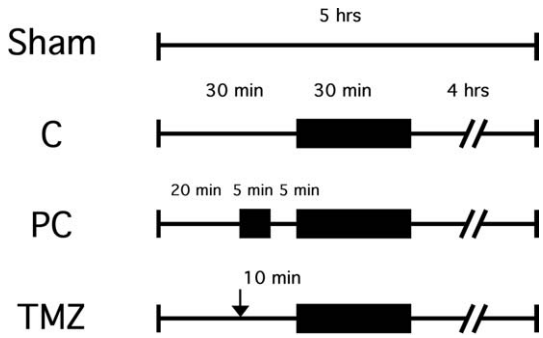

Fig. 1. Experimental design.
Animals underwent 30 min of ischemia followed by 4 hours of reperfusion. PC consisted of one episode of 5 min of ischemia and 5 min of reperfusion. TMZ was administered as an IV bolus (arrows), 10 min before ischemia. C: control group, PC: preconditioned group, TMZ: trimetazidine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

(brick red) myocardial area [16]. The slices were then rephotographed. Enlarged projections of these slices were traced for determination of the boundaries of the AR and area of necrosis (AN). Extent of the AR and AN was quantified by computerized planimetry and corrected for the weight of the tissue slices. Total weights of the AR and the AN were then calculated and expressed in grams and as percentage of total left ventricle (LV), and of the AR weight, respectively ($N = 10$ per group). We decided prospectively that hearts with a risk region less than 10% of the LV weight would be excluded from the study.

2.4. Preparation of mitochondrial and cytosolic fractions of myocardial tissue

At the end of the 4 hours reperfusion period, hearts were harvested while still beating, and mitochondria isolated from the myocardium at risk for further assessment of Ca^{2+}-induced MPT pore opening. Mitochondrial and cytosolic and fractions were separated by differential centrifugation as previously described in [11,17,18]. All operations were carried out in the cold. Heart pieces (0.5–1.0 g) were placed in isolation buffer A containing 70 mM sucrose, 210 mM mannitol, 1 mM EDTA in 50 mM Tris–HCl pH 7.4. The tissue was finely minced with scissors and then homogenized in the same buffer (10 ml buffer g^{-1} tissue), using successively a Kontes tissue grinder and a Potter Elvejem. The homogenate was centrifuged at 1300 g for 3 min. The supernatant was poured through cheesecloth and centrifuged at 10,000 × *g* for 10 min. The mitochondrial pellet was suspended in isolation buffer B containing 70 mM sucrose, 210 mM mannitol, 0.1 mM EDTA in 50 mM Tris–HCl pH 7.4. Protein content was routinely assayed according to Gornall's procedure using bovine serum albumin as a standard [19]. Mitochondria, by aliquots of 5 mg proteins, were washed in isolation buffer B, centrifuged at 6800 × *g* for 10 min and stored as pellets. Pellets were kept over ice prior to mPTP opening experiments. Post-mitochondrial supernatant was centrifuged for 45 min at 16,000 × *g*, and the resulting supernatant was used as cytosolic fraction for determination of caspase 3 activity.

2.5. Measurement of caspase 3 activity

Caspase 3 activity was measured using the fluorogenic substrate peptide DEVD-AMC (BACHEM® Biochimie, France) as previously described in [20]. Aliquots containing 80 µg of cytosolic proteins in 50 µl of buffer C (5 mM $MgCl_2$, 1 mM EGTA, 1 mM PMSF, 10 µg ml^{-1} peptidase A, 10 µg ml^{-1} leupeptine, in 25 mM Hepes pH 7.5) were dissolved with 225 µl of freshly prepared buffer D [0.1% (w/v) 3-[(cholamidopropyl) dimethylammonio]-1-propane sulfonate, 10 mM DTT, 1 mM PMSF, 10 µg ml^{-1} aprotinin, in 25 mM HEPES pH 7.5] containing 167 µM substrate, and incubated for 60 min at 37 °C. Fluorescence was measured using a Perkin–Elmer fluorimeter (excitation at 342 nm, emission at 441 nm). The amount of released 7-amino-4-methyl coumarin (AMC) was calculated by transposing the fluorescence measurement of each point onto a scale with purified AMC ($N = 7–8$ per group).

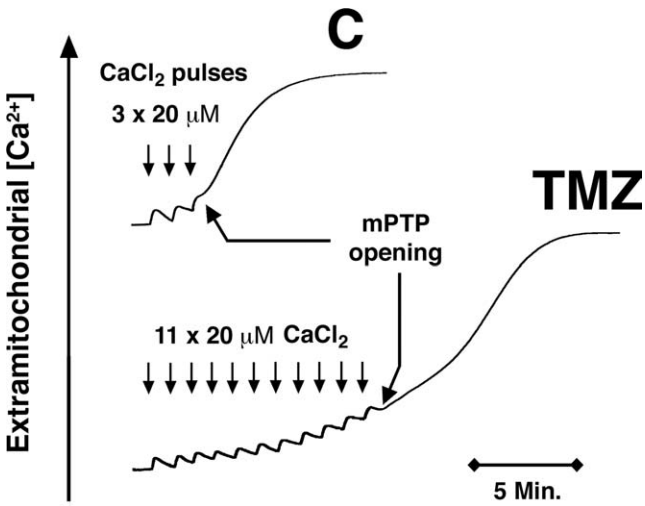

Fig. 2. Ca^{2+}-induced MPT pore opening.
Typical example of a Ca^{2+}-induced MPT pore opening recording in mitochondria isolated from control (C), and animals treated by TMZ before ischemia (TMZ). mPTP opening was defined as the massive release of Ca^{2+} by isolated mitochondria following progressive Ca^{2+} overload of the suspension medium. In the control heart, a Ca^{2+} overload of 60 µM (three pulses of 20 µM) was required to induce MPT pore opening versus 220 µM Ca^{2+} in hearts treated by TMZ.

2.6. Calcium-induced mPTP opening in isolated mitochondria

Opening of the mPTP pore opening was assessed following in vitro Ca^{2+} overload, as previously described in [11,12]. Isolated mitochondria (5 mg proteins) were suspended in 100 µl buffer B, and added in 900 µl of buffer E (150 mM sucrose, 50 mM KCl, 2 mM KH_2PO_4, 5 mM succinic acid in 20 mM Tris–HCl pH 7.4) within a Teflon chamber equipped with a Ca^{2+}-selective microelectrode, in conjunction with a reference electrode [11,21,22]. Modifications of the medium (i.e. extra-mitochondrial) Ca^{2+} concentration were continuously recorded using a custom made Synchronie® software. Mitochondria were gently stirred for 1.5 min. At the end of the pre-incubation period, 20 µM $CaCl_2$ pulses were performed every 60 s. As depicted in Fig. 2, each 20 µM $CaCl_2$ pulse was recorded as a peak of extramitochondrial Ca^{2+} concentration. Ca^{2+} is then very rapidly taken up by the mitochondria resulting in a return of extramitochondrial Ca^{2+} concentration to near baseline level. Following sufficient Ca^{2+} loading, extra-mitochondrial Ca^{2+} concentration abruptly increases indicating a massive release of Ca^{2+} by mitochondria due to MPT pore opening (Fig. 2). The amount of Ca^{2+} necessary to trigger this massive Ca^{2+} release was used here in the four groups as an indicator of the susceptibility of MPT pore to Ca^{2+} overload, and expressed as µM mg^{-1} mitochondrial proteins ($N = 6–8$ per group).

Additional experiments using mitochondria isolated from sham hearts were performed to appreciate in vitro the effect

Table 1
Hemodynamic measurements in different experimental groups

Groups	Baseline		Pre-CO		30 min CO		1 h Reperf		4 h Reperf	
	HR beats per min	MBP mmHg	HR beats per min	MBP mmHg	HR beats per min	MBP mmHg	HR beats per min	MBP mmHg	HR beats per min	MBP mmHg
C	193 ± 9	71 ± 4	193 ± 10	67 ± 5	208 ± 11	51 ± 3 *	201 ± 12	52 ± 4 *	204 ± 13	46 ± 4 *
PC	176 ± 7	70 ± 5	178 ± 8	63 ± 4	199 ± 13	58 ± 2	174 ± 15	66 ±5	166 ± 26	51 ± 7 *
TMZ	178 ± 7	71 ± 7	170 ± 8	75 ± 6	192 ± 6	52 ± 7 *	184 ± 6	58 ± 7	185 ± 9	49 ± 9 *

Values are means ± S.E.M. $N = 10$ per group. CO: coronary occlusion; Reperf: reperfusion; HR, heart rate; MBP, mean blood pressure. *$P < 0.05$ versus baseline.

of CsA (1 μM) and TMZ concentrations ranging from 1 μM to 1 mM ($N = 4$–9 per concentration value) on Ca^{2+}-induced mPTP opening.

2.7. Chemicals

TMZ used in this present study was a generous gift of Servier® (Neuilly-sur-Seine, France). TMZ was used either in vivo (5 mg kg^{-1}, IV), or in vitro (1, 10, 100 μM, and 1 mM), and was dissolved in saline. Other chemicals were purchased from Sigma Chemical® (St. Louis, MO).

2.8. Statistical analysis

Differences in the relationship between infarct size and AR were evaluated by analysis of covariance (ANCOVA) with infarct size as the dependent variable and AR as the covariate. For Ca^{2+} overload and caspase 3 data, comparisons were performed using one-way analysis of variance (ANOVA). Statistical analysis of hemodynamics was performed by using two-way ANOVA with repeated measures on one factor. Means were compared by the Fisher's test when a significant F value was obtained. Statistical calculations were performed using Statview® 5.0 Power PC version (SAS Institute Inc., Cary, NC). All values are expressed as mean ± standard error of mean (S.E.M.). Statistical significance was defined as a value of $P < 0.05$.

3. Results

3.1. Hemodynamics

HR and MBP were comparable at baseline among all three groups (Table 1). In all experimental groups, sustained ischemia–reperfusion resulted in a comparable decrease in MBP. TMZ had no significant hemodynamic effect (Table 1).

3.2. Cardioprotective effect of TMZ

3.2.1. Infarct size

AR was comparable among the three groups of rabbits, with mean values averaging 1.14 ± 0.12 g, 1.04 ± 0.10 g, 1.36 ± 0.14 g, in C, PC, and TMZ groups respectively (P = ns among groups). As expected, ischemic PC significantly reduced infarct size that averaged 21 ± 4% of the AR versus 63 ± 6% in the control group ($P < 0.0001$) (Fig. 3). AN of TMZ group averaged 34 ± 4% of the risk region ($P = 0.0004$ vs. control group; P = ns versus PC group). This cardioprotective effect of both PC and TMZ was confirmed by ANCOVA indicating that for any size of the risk region, TMZ or preconditioned hearts developed significantly smaller infarcts than controls.

3.2.2. Myocardial apoptosis

Caspase 3 enzymatic activity averaged 24 ± 5 pmol AMC min^{-1} mg^{-1} of cytosolic proteins in sham animals. Caspase 3 activity significantly increased after ischemia–reperfusion in the C group (68 ± 12 pmol mg^{-1}, $P = 0.0008$ versus sham group). Both PC and TMZ prevented this phenomenon, with caspase 3 activity averaging 29 ± 6, and 37 ± 11 pmol min^{-1} mg^{-1}, respectively ($P < 0.01$ versus C, P = ns among sham, PC and TMZ groups) (Fig. 4).

3.3. Inhibition of Ca^{2+}-induced mPTP opening by TMZ

In the sham group, the amount of Ca^{2+} required to open the mPTP averaged 116 ± 6 μM mg^{-1} of mitochondrial proteins (Fig. 5A). In hearts that received 30 min of ischemia followed by 4 hours of reperfusion, Ca^{2+} overload required to trigger mPTP opening was significantly reduced in the control group C, averaging 11 ± 4 μM mg^{-1} proteins ($P < 0.0001$ versus sham) indicating an increased susceptibility of the mPTP to opening following Ca^{2+}overload. PC

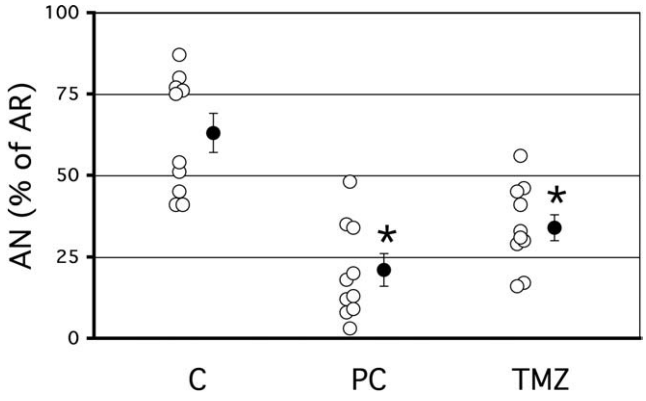

Fig. 3. Infarct size.
AN expressed as the percentage of the risk region weight (AR). Empty circles represent individual data while full circles indicate the mean values for each group. Infarct size was significantly reduced in PC and TMZ groups. C, PC and TMZ indicate controls, preconditioned, and TMZ-treated animals, respectively. *$P < 0.0005$ versus C.

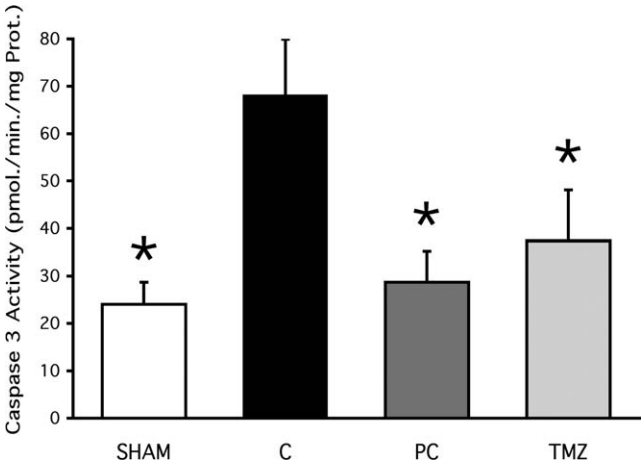

Fig. 4. Caspase 3 activity.
Bars indicate enzymatic activity in sham hearts, and after ischemia–reperfusion in controls (C), preconditioned (PC) and TMZ-treated hearts. There was a significant increase in caspase 3 activity after sustained ischemia–reperfusion: it was prevented by PC or TMZ. $N = 7–8$ per group. *$P<0.01$ versus C.

significantly attenuated this phenomenon, with a Ca^{2+} overload required to mPTP opening averaging 46 ± 4 μM mg^{-1} proteins ($P = 0.0004$ versus C). TMZ had a comparable effect to PC, with a Ca^{2+} overload required to open the mPTP averaging 45 ± 4 μM mg^{-1} proteins ($P = 0.0005$ versus control, P = ns versus PC) (Figs. 2 and 5A).

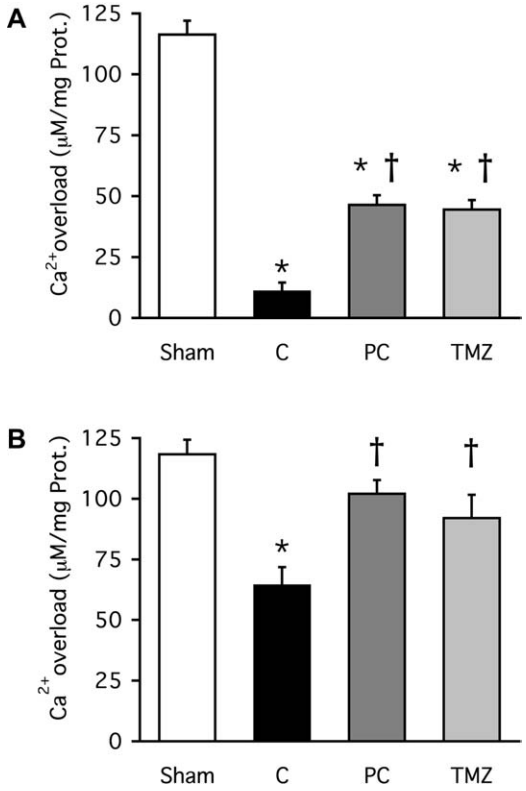

Fig. 5. Ca^{2+} overload required for mPTP pore opening.
Panel A: Prolonged ischemia. Panel B: Reversible ischemia. In the control group (C), Ca^{2+} overload required for MPT pore opening was significantly reduced versus sham animals. PC and TMZ inhibited mPTP opening. $N = 6–8$ per group. *$P < 0.01$ versus sham; † $P \leq 0.05$ versus C.

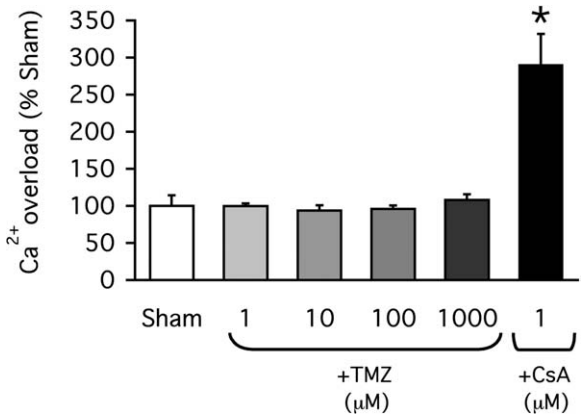

Fig. 6. In vitro effect of TMZ on mitochondrial permeability transition.
Unlike CsA (1 μM), exposure to TMZ using concentrations ranging from 1 μM to 1 mM, did not alter Ca^{2+}-induced mPTP opening in mitochondria isolated from sham myocardium. $N = 4–9$ per group.

In rabbit hearts that underwent the 10 min reversible ischemic insult followed by 5 min of reperfusion, the Ca^{2+} overload required to open the mPTP averaged 64 ± 7 μM mg^{-1} proteins in controls ($P < 0.01$ versus 118 ± 6 μM mg^{-1} in the sham group). Both PC and TMZ significantly increased the Ca^{2+} load that opened the mPTP to 102 ± 6 and 92 ± 10 μM mg^{-1} proteins, respectively ($P < 0.05$ versus C) (Fig. 5B).

We evaluated in vitro the direct effect of increased concentrations of TMZ (ranging from 1 μM to 1 mM) on Ca^{2+}-induced mPTP opening in mitochondria isolated from sham hearts. Whatever the dose, exposure to TMZ for 1 min before the first 20 μM Ca^{2+} pulse had no effect on Ca^{2+}-induced mPTP opening (Fig. 6). As a comparison, 1 μM CsA, the well known mPTP inhibitor, increased Ca^{2+} load required to open the transition pore up to 290 ± 42% of sham (Fig. 6).

4. Discussion

In the present study, we demonstrated, that in vivo TMZ attenuates myocardial apoptosis and necrosis to a similar extent than ischemic PC, and inhibits mPTP opening in the rabbit heart model of myocardial infarction.

The present findings are in agreement with previous studies, performed in the anesthetized rat or rabbit heart models, demonstrated that TMZ reduces infarct size following a prolonged ischemic insult [23–25]. In the present study, we further showed that infarct size reduction by TMZ is comparable to that seen in preconditioned hearts, indicative of a powerful protective effect. In apparent contradiction with the above-mentioned studies, Minners et al. reported that TMZ could limit the ability of ischemic PC to reduce infarct size in the isolated rat heart model, yet they did not assess the effect of TMZ per se (i.e. in the absence of PC) in their model [26]. We demonstrated here that TMZ could also limit apoptotic cardiomyocyte death. This demonstration of an anti-apoptotic effect of TMZ in cardiomyocyte is supported by recent findings by Yin et al. [27], as well as Gabryel et al. [28] who

showed similar antiapoptotic effect in cultured astrocytes submitted to hypoxia-reoxygenation.

We demonstrated that mitochondria, isolated from hearts that underwent a prolonged ischemia–reperfusion and that had been either pre-treated by TMZ or preconditioned, display a delayed Ca^{2+}-induced mPTP opening. Griffiths et al. first demonstrated that the mPTP, that remains closed during prolonged myocardial ischemia, opens in the early minutes of reperfusion [8]. Upon reflow, the abrupt correction of pH, combined to ATP depletion, increased phosphate inorganic, enhanced ROS production, and Ca^{2+} overload, favors opening of the transition pore in the inner mitochondrial membrane. Opening of the mPTP represents a crucial event in both necrotic and apoptotic cardiomyocyte death following a prolonged myocardial ischemia–reperfusion. Increasing evidence suggests that interventions that reduce lethal reperfusion injury are associated with a decrease in mPTP opening [6,7]. Several groups including ours recently demonstrated that direct pharmacological inhibition of the mPTP by CsA or its derivatives dramatically attenuates both necrotic and apoptotic cardiomyocyte death following a prolonged ischemia–reperfusion [11–13,26,29]. Recently, we reported that ischemic PC inhibits mPTP opening [11,30]. Although we did not demonstrate a causal relation between inhibition of mPTP opening and limitation of cell death in TMZ-treated hearts, the present study strongly suggests a significant link between the two phenomena.

We performed additional experiments to determine whether TMZ and PC improve the resistance of the mPTP to Ca^{2+}loading after a 10 min ischemia. In both treated group, a higher Ca^{2+} load was required to open the mPTP when compared to control. Since myocardial injury is fully reversible after 10 min of coronary artery occlusion, these data demonstrate that the observed beneficial effect of TMZ or PC is not due to a putative confounding effect of the isolation procedure but actually reflects the protection afforded by both treatments.

How TMZ may alter mitochondrial permeability transition is largely unknown. The anti-ischemic effect of TMZ is usually attributed to its effect on the balance between fatty acid and glucose oxidation. During ischemia, the imbalance between oxygen demand and supply compromises energy supply to the heart. Mitochondrial oxidative metabolism decreases, with fatty acid oxidation dominating over glucose oxidation. Consecutive increased production of lactate and protons decreases cardiac efficiency at a time when the heart is already starved of energy. Via the inhibition of the mitochondrial long-chain 3-ketoacyl CoA thiolase, TMZ favors a shift from fatty acid oxidation to glucose oxidation, and thereby attenuate the detrimental effects of ischemic-reperfusion [3].

However, recent evidence (including the present study) suggests that TMZ may be cardioprotective through additional mechanisms. Previous in vitro investigation indicates that TMZ may bind to the inner mitochondrial membrane, and dose-dependently prevent mitochondrial permeability transition in rat liver mitochondria [5,31]. Although we demonstrated that mitochondria isolated from hearts that had been pre-treated in vivo by TMZ are less prone to undergo permeability transition upon Ca^{2+} overload, unlike CsA, TMZ did not inhibit mPTP opening when directly applied to untreated isolated cardiac mitochondria in vitro. The fact that CsA protects mitochondria in vitro is an additional factor suggesting that TMZ acts indirectly. Our data rather suggest that the cardioprotective effect of TMZ likely results from an indirect effect on mitochondrial permeability transition.

How TMZ might indirectly modify mPTP opening in vivo remains to be determined. Recent studies indirectly support our findings and give some insight into the effect of TMZ on mitochondrial permeability transition. Monteiro et al. [32] recently reported in the isolated rat heart that TMZ prevents ischemia-induced decrease in respiratory chain complex I activity. This preservation of complex I activity during ischemia might decrease the production of ROS, with subsequent attenuation of mitochondrial membrane damage and limitation of mPTP opening [32]. Sentex et al. [33] showed that TMZ increases the synthesis of cardiolipin in ventricular myocytes. Cardiolipin is a phospholipid localized almost exclusively in the inner mitochondrial membrane, where it is used, at least partly, to anchor cytochrome c. Cardiolipin is particularly rich in unsaturated fatty acid (90% represented by linoleic acid), being thus a possible and early target of ROS attack. Paradies et al. [34,35] and others reported that the myocardial content of cardiolipin decreases dramatically following ischemia–reperfusion. TMZ, by directly increasing cardiolipin synthesis, and by inhibiting its degradation via the reduction of ROS production, might prevent release of cytochrome c and improve tolerance to ischemia–reperfusion. These hypotheses remain however to be confirmed.

Besides the known effect of TMZ on fatty acid/glucose oxidation, the present study suggests that complementary mechanisms are involved to indirectly limit mitochondrial permeability transition and attenuate cardiomyocyte death following a prolonged ischemic insult. It would be useful to address the possible link between the effects of TMZ on the "metabolic shift" from lipid oxidation to glucose oxidation on the one hand, with mitochondrial permeability transition, on the other hand.

Acknowledgments

We thank Servier® (Neuilly-sur-Seine France) for providing TMZ.

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