

# Trimetazidine Improves Recovery During Reperfusion in Isolated Rat Hearts After Prolonged Ischemia

*İzole Sıçan Kalplerinde Uzamış İskemi Sonrasında Trimetazidine Reperfüzyon Strasında Gelişen Düzelmeyi İlerletmektedir*

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## Abstract

**Objective:** Trimetazidine (TMZ) is a cellular anti-ischemic agent, which has been studied in clinical and experimental investigations, and was shown to have protective effects against myocardial ischemia and reperfusion injury. The major objective of this study was to investigate the cardio-protective effects of trimetazidine in prolonged global ischemia subjected Langendorff perfused rat hearts.

**Methods:** Twenty rats (Male Sprague-Dawley) were divided into two study groups. In Group 2 (n=10) TMZ was given by intra-gastric gavage (3 mg/kg BW twice daily for 5 days) prior to operation and was added to the Krebbs-Henseleit perfusate to create a  $10^{-6}$  M solution to perfuse the isolated rat hearts. Group 1 (n=10) reserved as control group and received saline at the same time period. All hearts were paced at 300 beats/min. After a 20-minute of stabilization period, hearts in both groups were arrested for 120 minutes with crystalloid cardioplegia. After ischemic period, the hearts were then reperfused for 30 minutes. Hemodynamic measurements from left ventricular latex balloon, coronary flow, and creatine kinase (CK-MB) and troponin T (cTnT) levels determined from the coronary effluent were analyzed at the end of stabilization and at every 10-min intervals during reperfusion, and results were compared between two groups.

**Results:** No significant differences were observed in all entered hemodynamic and biochemical parameters between two groups at the end of the stabilization. However, peak systolic pressure, end diastolic pressure and  $+dP/dt$  values reflected improved mechanical myocardial recovery in Group 2 hearts after prolonged ischemia. Besides coronary flow measurements were higher in Group 2 compared with Group 1. CK-MB and cTnT levels indicated to less enzymatic damage in trimetazidine treated hearts during reperfusion.

**Conclusion:** In conclusion, both pre-treatment and treatment protocols with TMZ reduce the myocardial damage caused by global ischemia following reperfusion. We could speculate that this beneficial effect of trimetazidine might be useful in open-heart surgery patients, who were subjected to global myocardial ischemia. (*Anadolu Kardiyol Derg 2003; 3: 303-8*)

**Key Words:** Trimetazidine, heart, ischemia, reperfusion.

## Özet

**Amaç:** Bu çalışma bir hücresele anti-iskemik ajan olan trimetazidine'nin (TMZ) izole sıçan kalpleri üzerine olan etkisini incelemek amacı ile gerçekleştirilmiştir.

**Yöntem:** Çalışma amacı ile yirmi sıçan (erkek, Sprague-Dawley) iki gruba ayrılmıştır. Grup 2 (n=10) sıçanlara çalışma öncesi 5 gün boyunca 3 mg/kg TMZ oral yolla verilmiş, ardından çalışma esnasında Krebb's Henseleit perfüzyon sıvısına  $10^{-6}$  M olacak şekilde eklenerek izole kalplere uygulanmıştır. Grup 1 (n=10) sıçanlar ise kontrol grubu olarak çalışılmış ve aynı protokole bağlı kalınarak bu grup sıçanlara TMZ yerine saline uygulanmıştır. Tüm kalpler çalışma esnasında 300 atım/dakika hızında pace edilmiştir. Yirmi dakika stabilizasyon periyodu sonunda her iki grupta da kalpler kristalloid kardiyopleji kullanılarak durduruldu ve 120 dakika global iskemi oluşturuldu. İskemi periyodu sonunda tüm kalpler Krebb's Henseleit solüsyonu ile 30 dakika reperfüze edildi. Deney başlangıcında sol ventriküle yerleştirilen lateks balon yardımı ile elde edilen hemodinamik veriler, koroner akım miktarı ve koroner akım örneklerinden çalışılan creatine kinaz (CK-MB) ve troponin T (cTnT) seviyeleri grupları karşılaştırmak amacı ile analiz edildi.

**Bulgular:** İskemi öncesi elde edilen biyokimyasal ve hemodinamik parametreler incelendiğinde gruplar arasında anlamlı fark saptanamamıştır. Bununla birlikte iskemi sonrası ölçülen tüm parametreler reperfüzyon döneminde grup 2 kalplerde anlamlı olarak daha fazla miyokardiyal iyileşmeyi göstermiştir.

**Sonuç:** Sonuç olarak, TMZ'nin ön tedavi ve hemen iskemi öncesi verilmesi ile oluşturulan tedavi kombinasyonu uzun süreli global iskemi sonrası reperfüzyon döneminde izole sıçan kalpleri üzerinde anlamlı derecede koruyucu etki göstermiştir. (*Anadolu Kardiyol Derg 2003; 3: 303-8*)

**Anahtar Kelimeler:** Trimetazidine, kalp, iskemi, reperfüzyon

## Introduction

Myocardial ischemia is a well-known condition that occurs when the oxygen uptake in the heart is not sufficient to maintain cellular oxidation. Myocar-

dial protection during global ischemia has become an important element in open-heart surgery. Safe and optimal preservation of myocardium provides long cross-clamping time, and less morbidity and mortality in operated patients. Trimetazidine [TMZ; 1-(2,3,4-tri-

methoxibenzyl)-Piperazine dihydrochloride] has been described as a cellular anti-ischemic agent (1), and its clinical application has been focused towards anti-anginal and anti-ischemic effects (2,3). The purpose of this study is to evaluate and further define the cardioprotective effects of TMZ on prolonged global ischemia in isolated rat hearts.

## Material and Methods

This study was approved by the Osmangazi University Institutional local animal care and use committee. Twenty male Sprague-Dawley rats (350-400 g weight) were used for the study. Animals were fed with a standard rat chaw (Oguzlar Yem, Eskisehir, Turkey) and allowed to drink water ad libitum, but they were deprived of food for 12 h before the experiments. They were housed in a single temperature controlled (20–25 °C) cages with a 12-h dark and 12-h light cycles. All procedures were performed in sterilized conditions. All rats were heparinized (300 IU/kg BW) via femoral vein and anaesthetized with intraperitoneal injection of Sodium Pentobarbital (50 mg/kg BW). The hearts were rapidly excised and immersed into ice-cold heparinized saline solution during preparation for aortic cannulation on modified Langendorff perfusion apparatus. Aortic perfusion was initiated at 70 mm Hg constant perfusion pressure with Krebb's-Henseleit (K-H) buffer solution (in millimoles per liter: NaCl 118; KCl 4.7; CaCl<sub>2</sub> 2.0; MgSO<sub>4</sub> 1.2; NaHCO<sub>3</sub> 25; KH<sub>2</sub>PO<sub>4</sub> 1.2; Glucose 11.1). The perfusate was oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> gas mixture and pH was maintained between 7.4 and 7.5. The heart temperature was kept at 37 °C during perfusion and at 24 °C during the ischemic period. Pulmonary arteriotomy was performed to allow free drainage of the coronary effluent. The right atrium was removed and all hearts were paced at rate of 300 beats/min with an external pacemaker (pacer off during ischemia). A water filled latex balloon was inserted to the left ventricle cavity through a small incision in the left atrium, and connected to a pressure transducer (Transpac II, Abbott, USA) by rigid polyethylene tubing. The balloon volume was adjusted to produce 10 mm Hg constant diastolic pressure. Hemodynamic data from the balloon were analyzed using Data Acquisition System (BIOPACK MP 100 system, Santa Barbara, CA) and displayed on PC during experiment.

**Experimental Protocol:** Twenty rats were randomly divided into two groups. In Group 2 (n=10)

rats TMZ (IRIS, Institut de Recherches Internationales, Servier, France) was given by intra-gastric gavage 3 mg/kg BW twice daily for 5 days before experiment. Hearts in each group were stabilized for 20 minutes with K-H solution. Trimetazidine was also added to the K-H solution (10<sup>-6</sup> M) during the stabilization period in Group 2 hearts. Hearts in Group 1 (n=10) were assigned as control, and saline was given according to the same protocol. After 20 minutes of stabilization period, all hearts were arrested with cold (4 °C) crystalloid cardioplegia solution (Plegisol, Abbott, USA). After 120 minutes of global ischemic period at 24 °C, all rats were then reperfused with K-H solution at 37 °C for 30 minutes.

**Hemodynamic Data:** The left ventricular pressure wave from the latex balloon was analyzed and peak systolic pressure (PSP), end diastolic pressure (EDP) and the maximum rate of increase of left ventricular pressure (+dP/dtmax) values were recorded at the end of the stabilization period, and at every 10-minute interval at reperfusion. Coronary effluent was collected in a reservoir for one minute and measured as coronary flow (CF) at the end of the stabilization period and every 10-minute interval of reperfusion.

**Biochemical Assay:** The coronary effluents were collected for 1 minute at the end of the stabilization period, and at the first minute and last minute of reperfusion period. The samples were immediately stored at -70 °C, and ischemic damage was assessed using creatinine kinase activity (CK-MB) and troponin T (cTnT) levels. CK-MB activity was measured with a Boehringer Mannheim kit (BM/Hitachi system 911 automated analyzer) and cTnT levels were assayed with an Electrochemiluminescence immunoassay kit (Elecys-2010 immunoanalyzer).

**Data Analysis:** Results are expressed as means ± standard error of the mean. Statistical analysis was performed using the unpaired Student's t test. A statistical significant difference was considered if p<0.05.

## Results

The hemodynamic and biochemical results are presented in Table 1. The percentage values of results are expressed in figures.

### Hemodynamic Measurements

Peak systolic pressure: Although, Group 2 (TMZ treated group) hearts displayed lower PSP values than in Group 1 during the stabilization period, no

statistical difference was observed between each group ( $p>0.05$ ). Nevertheless, PSP values were significantly higher in Group 2 than in Group 1 at the 10-minute of reperfusion ( $p<0.05$ ). But there was no statistical difference between two groups at the 20 and 30-minutes of reperfusion ( $p>0.05$ ) (Fig 1).

**End Diastolic Pressure:** EDP values were significantly lower in Group 2 than in control group at the 10th and 20th minute of reperfusion ( $p<0.05$ ). During 30th minute of reperfusion the EDP values showed no statistical difference between groups ( $p>0.05$ ) (Fig 2).

**+dP/dtmax:** At the 10th minute of reperfusion, +dP/dtmax measurements were significantly higher in Group 2 than in control group ( $p<0.05$ ). No statistical differences were observed in +dP/dtmax values

at the 20th and 30th minute of reperfusion between two groups ( $p>0.05$ ) (Fig 3).

**Coronary Flow**

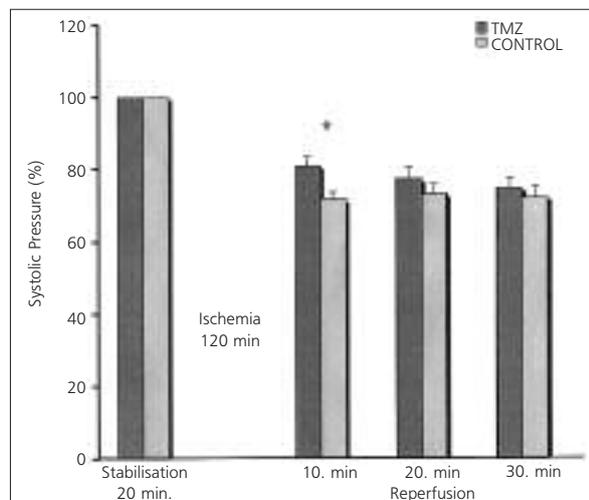
Although statistics could not show any significance, CF measurements were slightly higher in TMZ treated group than in control one during stabilization. However, at the 10th minute of reperfusion period CF values were clearly higher in Group 2 than in control group ( $p<0.01$ ). In addition, at the 20th minute of reperfusion, difference in CF measurements between two groups was statistically significant ( $p<0.05$ ). We could not show any significant difference in CF values at the 30th minute of reperfusion period ( $p>0.05$ ) (Fig 4).

**Biochemical Assay**

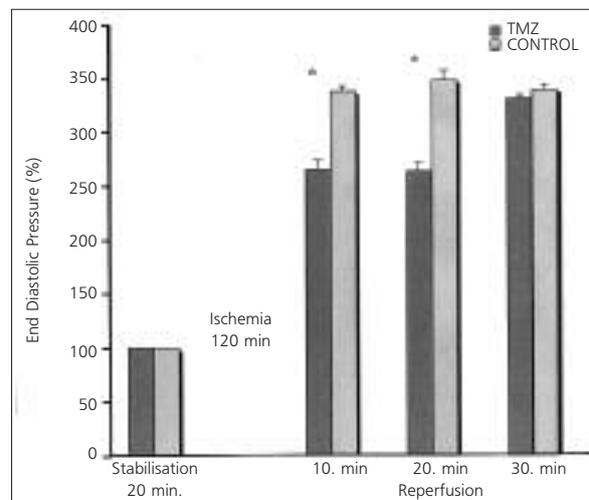
**Creatine Kinase Washout:** No significant difference was found in CK-MB levels at stabilization pe-

**Table 1: Changes in hemodynamic and biochemical parameters during reperfusion in Sprague-Dawley rats hearts**

	Group	Stabilisation Period	Reperfusion 1. min	Reperfusion 10. min	Reperfusion 20. min	Reperfusion 30. min
Systolic Pressure (mmHg)	1	106.4±4.16	-	86.1±2.21	82.5±2.60	79.6±2.55
	2	110.5±6.67	-	79.3±1.44	80.8±2.40	79.7±2.74
End Diastolic Pressure (mmHg)	1	7.91±0.26	-	20.9±1.9	20.8±1.7	26.1±1.04
	2	7.98±0.35	-	26.9±1.4	27.8±2.3	27.0±1.36
+dP/dtmax	1	4419.9±234	-	2939.5±171	2503.9±134	2246.4±127
	2	4323.9±142	-	2366.5±147	2440.9±132	2484.9±141
Coronary Flow (ml/min)	1	20±1.3	-	21.7±1.6	21.2±1.2	22.8±1.3
	2	18±1.0	-	15.3±1.2	17.6±0.9	20.0±1.2
CK-MB (I.U./L)	1	9±1.4	89.4±16.6	-	-	84.5±12.9
	2	11.9±1.5	143.7±15.3	-	-	119.9±8.5
Troponin-T (mg/L)	1	0.59±0.11	1.45±0.31	-	-	1.52±0.29
	2	0.53±0.11	2.68±0.43	-	-	2.36±0.23



**Figure 1: Changes in systolic pressure during reperfusion in Sprague-Dawley rats heart.**



**Figure 2: Changes in left ventricular diastolic pressure during reperfusion in Sprague-Dawley rats hearts.**

riod between two groups. However, CK-MB levels were significantly higher in control group than in TMZ treated group at the 1st and 30th minutes of reperfusion ( $p < 0.05$ ) (Fig 5).

**Troponin T (cTnT):** Although no significant differences were observed between groups at the stabilization, the cTnT levels were significantly lower in TMZ treated hearts than in control hearts at the 1st and 30th minutes of reperfusion ( $p < 0.05$ ) (Fig 6).

## Discussion

Ischemia is defined as an inadequate oxygen supply to cells and subsequently results in a decrease in oxidative metabolism, and if left untreated, can

progress to cell necrosis (1). Trimetazidine is an anti-ischemic agent, which affects the intracellular concentration of adenine nucleotides particularly ATP and intracellular pH (4), and has beneficial effects in preventing high myocardial calcium content with long-term therapeutic procedures (5). Guarnieri et al. (6) have shown that TMZ preserves both mitochondrial activity and cardiac ATP levels by a non-specific calcium antagonist effect. This effect is related to the protection of mitochondrial function and reduction of calcium induced ATP hydrolysis (6). However, in acidotic conditions TMZ reduces the accumulation of  $\text{Na}^+$  and  $\text{Ca}^{++}$  ions in cardiac cells as well as prevents intracellular acidosis. Lavanchy et al. (7) have shown that TMZ reduces cellular acidosis and preserves

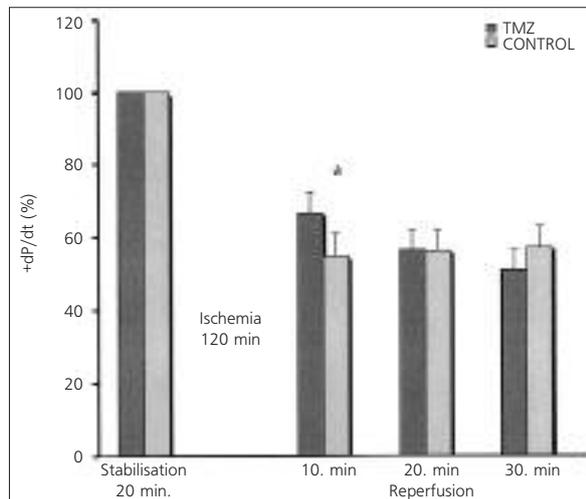


Figure 3: Changes in left ventricular dP/dt during reperfusion in Sprague-Dawley rats hearts.

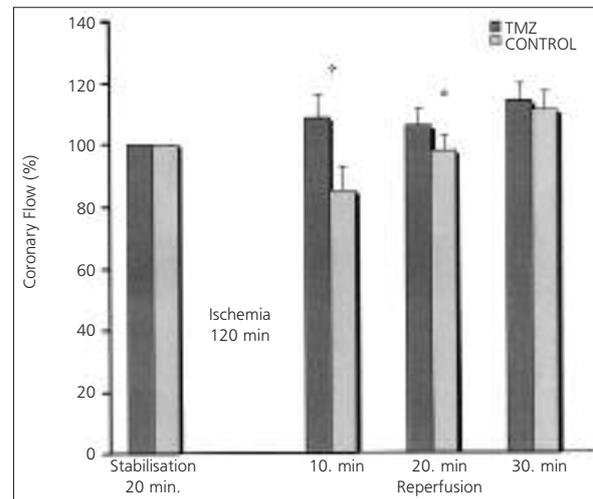


Figure 4: Changes in coronary flow during reperfusion in Sprague-Dawley rats hearts.

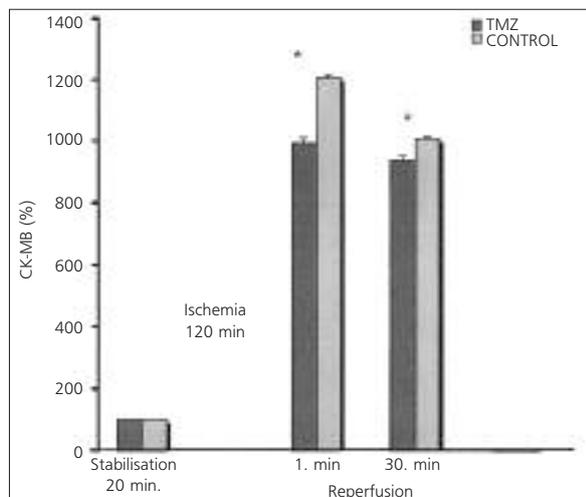


Figure 5: Changes in CK-MB during reperfusion in Sprague-Dawley rats hearts.

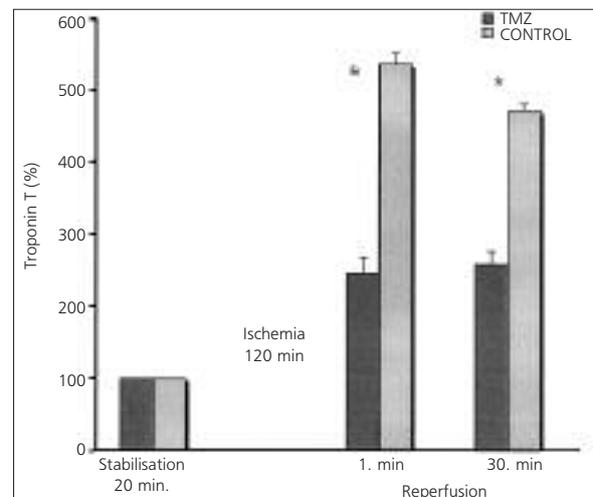


Figure 6: Changes in troponin T during reperfusion in Sprague-Dawley rats hearts.

high-energy phosphates during ischemia in isolated rat hearts.

Although the anti-ischemic effects of TMZ on cellular changes associated with ischemia, the drug was ineffective in normoxic conditions (8). Trimetazidine has been evaluated in animal models (9,10) and in human studies (11,12), and has been shown to have no effect in the absence of ischemia.

Noble et al. (13) concluded that pre-treatment with TMZ in the blood perfused rabbit heart is effective in reducing myocardial infarct size. In addition, several experimental results have also indicated that the addition of TMZ to the perfusate in isolated hearts improves myocardial recovery after short global ischemia periods (7,14). Opie and colleagues (15) have pre-treated the rats with 3 mg/kg body weight orally per day for 5 days, and perfused with  $10^{-6}$  M TMZ in their study, and showed low ischemic contact level in combination of pre-treatment and perfusion by TMZ. It has also been shown that the protective effects of TMZ at  $10^{-6}$  M concentration on post ischemic recovery were completely lost when TMZ was used at a higher concentration ( $10^{-4}$  M) (16). Being in agreement with the mentioned studies, we applied both pre-treatment and treatment protocols in Group 2 with TMZ as described in the methods section.

The hemodynamic results have shown improved myocardial mechanical recovery in TMZ treated group in our study. This beneficial effect of TMZ may be related to protecting the ATP pool and mitochondrial function in myocyte during reperfusion. Lavanchy et al. (7) demonstrated that restoration of ATP levels in TMZ treated hearts was 38 % higher than in controls after ischemia, and confirmed that TMZ accelerates the reconstitution of energy pools during reperfusion.

Some investigators (7,14) have not been able to show the beneficial effect of TMZ on coronary flow. In contrast, the coronary flow measurements in our study were higher in TMZ treated hearts during reperfusion. Takenata et al. (17) administered TMZ in anaesthetized open-chest dogs and also observed a dose-dependent increase in coronary blood flow.

Enzymatic leakage is a well-described index of ischemia and induced structural damage. Creatinine levels were decreased by 57 % in the presence of TMZ  $10^{-6}$  M (4). Troponin T a regulatory protein of muscle tissue, binds tropomyosin and thus transfers calcium-induced conformational changes to the thin filament of muscle (18). Yamahara et al. (19) showed

that cardiac cTnT is a useful indicator of myocardial cell damage and can be used to evaluate the degree of myocardial cell damage. In recent clinical studies (20-23) it has been also demonstrated that cTnT is a good indicator in diagnosis of ischemic myocardial cell damage. Our study consistently showed CK-MB and cTnT levels in the coronary effluents were significantly higher in the non-treated group during reperfusion. This improvement in myocardial preservation is probably the result of reduction in ATP breakdown and lactate accumulation that causes cellular acidosis (1).

Trimetazidine as an anti-ischemic agent also has beneficial effects on myocardial protection against long global ischemic periods. This experiment indicates that TMZ pre-treatment as well as its addition to the perfusate significantly improves myocardial recovery after 120 minutes of global ischemia. Since the protective effect of TMZ mentioned above, the drug can take part in medical treatment options for open-heart surgery patient; especially who had long cross-clamping time.

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