

## Original article:

### THE EFFECTS OF TRIMETAZIDINE ON LIPOPOLYSACCHARIDE-INDUCED OXIDATIVE STRESS IN MICE

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#### ABSTRACT

The effects of trimetazidine, a novel anti-ischemic agent, on the development of oxidative stress induced in mice with lipopolysaccharide endotoxin were investigated. The drug was administered orally once daily at doses of 1.8, 3.6 or 7.2 mg/kg for two days prior to intraperitoneal (i.p.) injection of lipopolysaccharide E (200 µg/kg) and at time of endotoxin administration. Mice were euthanized 4 h after administration of the lipopolysaccharide. Lipid peroxidation (malondialdehyde; MDA), reduced glutathione (GSH) and nitric oxide (nitrite/nitrate) concentrations were measured in brain and liver. The administration of lipopolysaccharide increased oxidative stress in both the brain and liver tissue. MDA increased by 33.9 and 107.1 %, GSH decreased by 23.9 and 84.3 % and nitric oxide increased 70.3 and 48.4 % in the brain and liver, respectively. Compared with the lipopolysaccharide control group, brain MDA decreased by 26.2 and 36.7 %, while GSH increased by 18.2 and 25.8 % after the administration of trimetazidine at 3.6 and 7.2 mg/kg, respectively. Brain nitric oxide decreased by 45.3, 50.8 and 57.0 % by trimetazidine at 1.8, 3.6 and 7.2 mg/kg, respectively. In the liver, MDA decreased by 18.7, 30.7 and 49.4 % and GSH increased by 150.3, 204.8 and 335.4 % following trimetazidine administration at 1.8, 3.6 and 7.2 mg/kg. Meanwhile, nitric oxide decreased by 17.3 % by 7.2 mg/kg of trimetazidine. These results indicate that administration of trimetazidine in the presence of mild systemic inflammatory response alleviates oxidative stress in the brain and liver.

**Keywords:** trimetazidine, lipopolysaccharide, oxidative stress, mice

#### INTRODUCTION

Trimetazidine, is an antianginal agent with unique properties. Trimetazidine does not affect systolic blood pressure, coronary blood flow or heart rate (McClellan and Plosker, 1999). The drug is a partial fatty-acid-oxidation inhibitor that selectively inhibits long-chain 3-ketoacyl coenzyme A thiolase (the last enzyme involved in  $\beta$ -oxidation), shifting energy substrate preference from free fatty-acid metabolism towards glucose oxidation, which reduces

oxygen demands and improves myocardial efficiency (Kantor et al., 2000; Fragasso et al., 2009). By optimizing energy metabolism, the drug proved of value in reducing the frequency of anginal symptoms (Cross, 2001) and may improve left ventricular function in patients with heart failure (Fragasso et al., 2006). Trimetazidine preserves cardiac mitochondrial function, thereby reducing the formation of oxidative damage (Baumert et al., 2004). In this sense, the drug represents a novel approach for the management of ischemic heart disease and

terms such as metabolic modulator or cytoprotective has been used to describe its action (Desideri and Celegon, 1998; Belardinelli, 2000; Cross, 2001). Studies have shown that trimetazidine exerts cytoprotective effects under conditions of ischemia in different organs such as the heart (Poloski et al., 2002; Lopaschuk et al., 2003), lung (Inci et al., 2001), kidney (Faure et al., 2004; Jayle et al., 2007), intestine (Tetik et al., 1999; Kuralay et al., 2003), brain (Iqbal et al., 2002) and liver (Settaf et al., 1999). Trimetazidine displays a number of important pharmacological effects. The drug exhibited neuroprotective activity in retina subjected to ischemia by inhibiting extracellular glutamate accumulation (Payet et al., 2004). It showed marked antinociceptive effects in acute pain models, anti-oedematogenic, antidepressive as well as gastric protective properties (Abdel-Salam and El-Batran, 2005). The drug also lessened the development of hepatic damage due to carbon tetrachloride in rats (Abdel-Salam et al., 2005).

Bacterial lipopolysaccharide is used widely in experimental animals to study the effect of inflammatory stimuli on organ functions. Systemic administration of lipopolysaccharide E has been shown to impair antioxidant mechanisms, induce lipid peroxidation and impairment in mitochondrial redox activity (Noble et al., 2007; Jacewicz et al., 2009) and causes brain inflammation (Jeong et al., 2010) as well as neuronal damage (Qin et al., 2007). The present study therefore aimed to investigate the effect of the metabolic modulator trimetazidine on the oxidative stress in the brain and liver after the administration of bacterial lipopolysaccharide in mice.

## MATERIAL AND METHODS

### *Animals*

Swiss male albino mice 20-22 g of body weight (age: 5-6 weeks) were used. Mice were obtained from animal house colony of the National Research Centre (Cairo, Egypt). Standard laboratory food and water were provided *ad libitum*. Animal proce-

dures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Equal groups of 6 mice each were used in all experiments.

### *Drugs and chemicals*

Lipopolysaccharide derived from *Escherichia coli* (Serotype 055: B5, Sigma, St Louis, MO, USA) was used and dissolved in sterile saline, aliquoted, and frozen at  $-20^{\circ}\text{C}$ . Trimetazidine (Amrya Pharm. Ind., Cairo, Egypt) was used and dissolved in isotonic (0.9 % NaCl) saline solution immediately before use. The doses of trimetazidine in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes (1964) conversion tables.

### *Study design*

Mice were randomly divided into 5 equal groups (6 mice each). Mice were treated with saline (group 1), trimetazidine (groups 2, 3, 4) once daily orally for 2 days before the administration of lipopolysaccharide and at time of endotoxin administration (200 mg/kg, i.p.). In addition, a fifth group (n = 6) received only saline (-ve control). Mice were euthanized 4 h after lipopolysaccharide injection by decapitation under ether anaesthesia, brains and livers were then removed, washed with ice-cold saline solution (0.9 % NaCl), weighed and stored at  $-80^{\circ}\text{C}$  for the biochemical analyses. The tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10 % w/v for the biochemical assays.

### *Determination of lipid peroxidation*

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA). Malondialdehyde forms a 1:2 adduct with thiobarbituric acid which can be measured by spectrophotometry. Malondialdehyde was determined by measuring

thiobarbituric reactive species using the method of Ruiz-Larrea et al. (1994), in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

#### Determination of reduced glutathione

Reduced glutathione (GSH) was determined by Ellman's method (1959). The procedure is based on the reduction of Ellman's reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid. The nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically.

#### Determination of nitric oxide

Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al. (1995) where nitrite, stable end product of nitric oxide radical, is mostly used as indicator for the production of nitric oxide.

#### Statistical analysis

Data are expressed as mean  $\pm$  SEM. The data were analyzed by one way ANOVA followed by Duncan's multiple range test, using SPSS software (SAS Institute Inc., Cary, NC). A probability value of less than 0.05 was considered statistically significant.

## RESULTS

#### Effect of trimetazidine on brain oxidative stress

The administration of lipopolysaccharide significantly increased brain MDA by 33.9 % ( $22.53 \pm 1.30$  vs  $16.82 \pm 0.90$  nmol/g,  $p < 0.05$ ). GSH decreased by 23.9 % ( $3.02 \pm 0.18$  vs  $3.97 \pm 0.22$   $\mu$ mol/g,  $p < 0.05$ ), while nitric oxide increased by 70.3 % ( $47.70 \pm 3.10$  vs  $28.00 \pm 1.70$   $\mu$ mol/g,  $p < 0.05$ ) after lipopolysaccharide injection compared with the saline control group. Brain MDA was not significantly altered by treatment with trimetazidine at 1.8 mg/kg. However, MDA decreased by 26.2 and 36.7 % after trimetazidine at 3.6 and 7.2 mg/kg, respectively

( $16.63 \pm 0.70$  and  $14.27 \pm 0.80$  vs  $22.53 \pm 1.30$  nmol/g) (Figure 1A). The administration of trimetazidine at 3.6 and 7.2 mg/kg resulted in 18.2 and 25.8 % increase in GSH ( $3.57 \pm 0.22$  and  $3.80 \pm 0.26$  vs  $3.02 \pm 0.18$   $\mu$ mol/g,  $p < 0.05$ ) (Figure 1B). Meanwhile, the level of nitric oxide decreased by 45.3, 50.8 and 57.0 % after the administration of 1.8, 3.6 and 7.2 mg/kg trimetazidine, respectively. Values of nitric oxide were:  $26.10 \pm 1.60$ ,  $23.45 \pm 1.80$  and  $20.50 \pm 1.30$  for trimetazidine doses of 1.8, 3.6 and 7.2 mg/kg, respectively vs lipopolysaccharide control value of  $47.70 \pm 3.10$   $\mu$ mol/g,  $p < 0.05$  (Figure 1C).

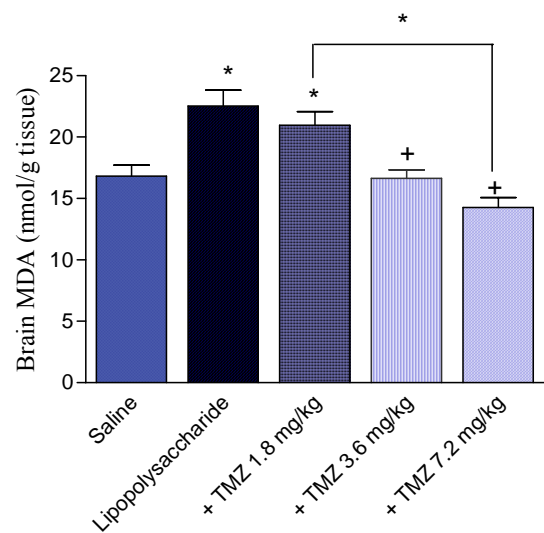


Figure 1A

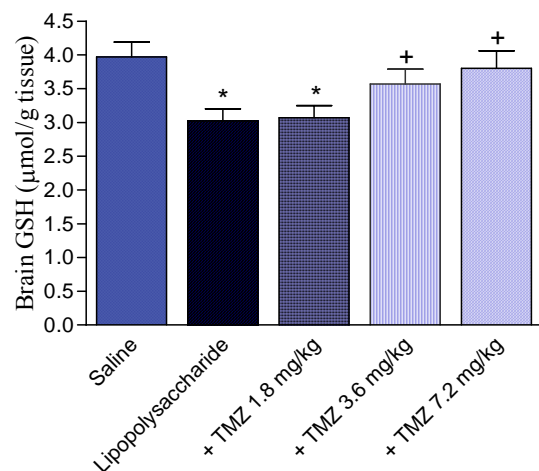


Figure 1B

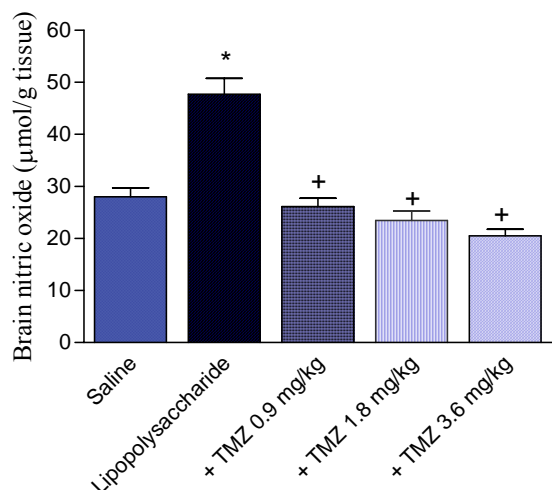


Figure 1C

**Figure 1 A-C:** Effect of trimetazidine (TMZ) on the brain tissue level of (A) malondialdehyde (MDA; nmol/g. tissue); (B) reduced glutathione (GSH; µmol/g. tissue); (C) nitric oxide (nitrite/nitrate; µmol/g. tissue) in lipopolysaccharide-treated mice. Asterisks indicate significant change from saline group and between different groups as shown. The plus sign indicates significant change from the lipopolysaccharide control group. One-way analysis of variance and Duncan multiple range test for *post hoc* comparison of group means.

#### Effect of trimetazidine on liver oxidative stress

Liver MDA was increased significantly by 107.1 % following lipopolysaccharide injection (200 µg/kg, i.p.) ( $46.10 \pm 2.31$  vs  $22.26 \pm 1.92$  nmol/g,  $p < 0.05$ ). The administration of trimetazidine at 1.8, 3.6 and 7.2 mg/kg resulted in a significant and dose decrease in liver MDA by 18.7, 30.7 and 49.4 % compared to the lipopolysaccharide E control group, respectively. Values of MDA were:  $37.47 \pm 1.18$ ,  $31.95 \pm 2.10$  and  $23.30 \pm 1.12$  for trimetazidine doses of 1.8, 3.6 and 7.2 mg/kg, respectively vs lipopolysaccharide control value of  $46.10 \pm 2.31$  nmol/g,  $p < 0.05$  (Figure 2A).

Reduced glutathione was markedly and significantly reduced by 84.3 % by administration of lipopolysaccharide ( $1.47 \pm 0.18$  vs  $9.36 \pm 0.22$  µmol/g,  $p < 0.05$ ). Treatment with trimetazidine resulted in significant increase in GSH by 150.3 %, 204.8 and 335.4, respectively ( $3.68 \pm 0.26$ ,  $4.48 \pm$

$0.22$  and  $6.40 \pm 0.28$  for trimetazidine doses of 1.8, 3.6 and 7.2 mg/kg, respectively vs lipopolysaccharide control value of  $1.47 \pm 0.18$  µmol/g,  $p < 0.05$ ) (Figure 2B).

Nitric oxide (the level of nitrite/nitrate) increased by 48.4 % following lipopolysaccharide injection ( $46.90 \pm 3.10$  vs  $31.60 \pm 2.00$  µmol/g,  $p < 0.05$ ). The administration of trimetazidine at doses of 1.8 or 3.6 mg/kg did not change liver nitric oxide level. However, the level of nitric oxide was significantly decreased by 17.3 % ( $p < 0.05$ ) by trimetazidine administration at 7.2 mg/kg vs lipopolysaccharide control value ( $38.80 \pm 2.30$  vs  $46.90 \pm 3.10$  µmol/g tissue,  $p < 0.05$ ) (Figure 2C).

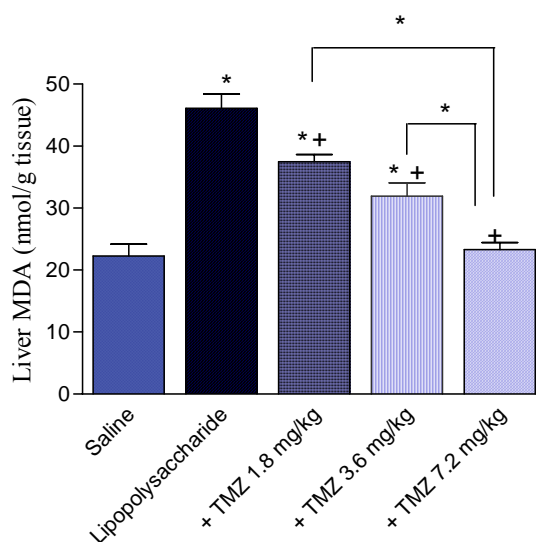


Figure 2A

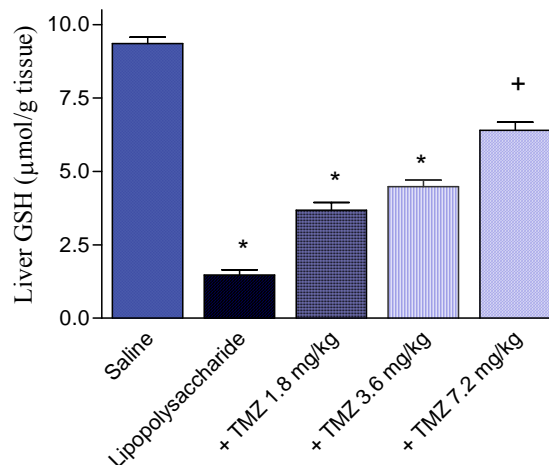


Figure 2B

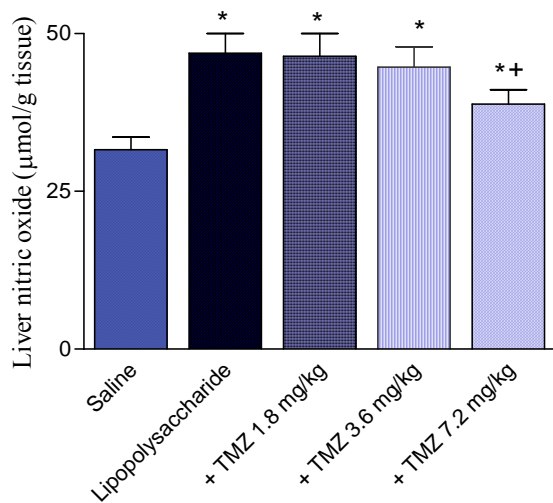


Figure 2C

**Figure 2 A-C:** Effect of trimetazidine (TMZ) on the liver tissue level of (A) malondialdehyde (MDA; nmol/g. tissue); (B) reduced glutathione (GSH; µmol/g. tissue); (C) nitric oxide (nitrite/nitrate; µmol/g. tissue) in lipopolysaccharide-treated mice. Asterisks indicate significant change from saline group and between different groups as shown. The plus sign indicates significant change from the lipopolysaccharide control group. One-way analysis of variance and Duncan multiple range test for *post hoc* comparison of group means.

## DISCUSSION

The present study provided evidence that the metabolic modulator and anti-ischemic agent trimetazidine alleviates oxidative stress caused by lipopolysaccharide endotoxin in mice. In both the brain and liver, the presence of mild systemic inflammatory illness evoked by systemic administration of lipopolysaccharide led to increased malondialdehyde (MDA) an index of lipid peroxidation which indicates increased free radical production and consequent damage to macromolecules such as lipids (Gutteridge, 1995). MDA in the brain and liver showed significant decrease by trimetazidine treatment, thereby suggesting decreased free radicals. Reduced glutathione, an important intracellular antioxidant is also markedly decreased after bacterial endotoxin administration. This thiol is common in all tissues and has an important role in maintaining the cellular redox bal-

ance and in protection against oxidative injury due to reactive oxygen species (Wang and Ballatori, 1998). These findings are in line with other studies reporting decreased brain GSH, glutathione reductase activity and increased lipid peroxidation after single intraperitoneal administration of lipopolysaccharide in rat and mice (Noble et al., 2007; Jacewicz et al., 2009). The significance of this observation derives from the accumulating evidence that links decreased GSH to the development of a number of neurodegenerative diseases, possibly due to consumption by free radicals (Schulz et al., 2000). In the liver, also, studies indicated that reduced glutathione is important in protecting the liver against toxic injury (Cnubben et al., 2001). The thiol is lower in red cells from patients with chronic liver disease compared with the controls (Czuczejko et al., 2003). In both the brain and liver of mice given lipopolysaccharide, the administration of trimetazidine led to marked increase in the level of GSH, thereby, suggesting a beneficial effect for the drug in conditions of excessive oxidative stress.

In the present study, nitric oxide (the concentrations of nitrite/nitrate) is also increased in the brain and liver tissue following lipopolysaccharide injection. The administration of trimetazidine led to marked reduction of the elevated nitric oxide in the brain and to lesser extent in the liver. Nitric oxide is an important signaling molecule in biological systems involved in neurotransmission and in control of vascular tone (Moncada et al., 1991). Studies indicated increased hepatic lipid peroxidation, serum liver enzymes and bilirubin in CCl<sub>4</sub>-treated rats (Muriel, 1998) and impaired liver regeneration (Rai et al., 1998) after inhibition of nitric oxide synthase, while increasing nitric oxide availability with L-arginine improved hepatic arterial and portal blood flow and sinusoidal oxygenation in experimental hepatic steatosis (Ijaz et al. 2005) and enhanced regeneration of reduced-size livers (Cantré et al., 2008). Increased production of nitric oxide can occur in response to pro-inflammatory cytokines due

to the action of inducible form of nitric acid synthase (Moncada et al., 1991). Studies indicated the involvement of both inducible as well as neuronal nitric oxide in the induction of lipopolysaccharide-induced fever (Kozak and Kozak, 2003). The increase in nitric oxide levels can be deleterious to tissue functions. In this context, synthesis of nitric oxide by both the inducible and constitutive nitric oxide synthase isoforms has been shown to contribute to the activation of apoptotic pathways in the brain during systemic inflammation induced by i.p. lipopolysaccharide injection (Czapski et al., 2007). Nitric oxide is also increased in patients with chronic liver disease and cirrhosis and correlated with disease stage (Arkenau et al., 2002; Pârvu et al., 2005). Nitric oxide itself is a free radical and can react with many other free radicals e.g., superoxide radical generating peroxy nitrite radical capable of causing oxidative changes to macromolecules e.g., proteins, lipids and DNA (Estévez and Jordán, 2002). Nitric oxide also binds to cytochrome c oxidase, and is able to inhibit cell respiration in a process that is reversible and in competition with oxygen. This action can also lead to the release of superoxide anion from the mitochondrial respiratory chain (Moncada and Bolanos, 2006). Thus increased nitric oxide and consequent vasodilatation can benefit tissue function against toxic insults, but another consequence of the elevated levels of nitric oxide is its cellular toxicity and lipid peroxidation.

Trimetazidine (2,3,4 trimethoxybenzylpiperazine dihydrochloride) is a novel anti-ischemic compound and a clinically effective antianginal agent (Stanley and Marzilli, 2003). It also showed benefit in patients with heart failure (Fragasso et al., 2006) and in idiopathic dilated cardiomyopathy with heart failure (Tuunanen et al., 2008) increasing cardiac function and ejection fraction. The drug has no vasodilator properties and has been described as a cytoprotective or a cellular anti-ischemic agent (Desideri and Celegon, 1998; Belardinelli, 2000) because it increases cell tolerance to

ischemia by maintaining cellular homeostasis (McClellan and Plosker, 1999). The most widely accepted mechanism of action is the inhibition of long-chain 3-ketoacyl coenzyme A thiolase (the last enzyme involved in  $\beta$ -oxidation) by trimetazidine. This shifts energy substrate preference from free fatty-acid metabolism towards glucose oxidation, thereby, optimizing energy metabolism (Kantor et al., 2000; Cross, 2001; Fragasso et al., 2009). In case of the heart, the improvement in cardiac energy metabolism should theoretically translate into enhancement in mechanical efficiency (Belardinelli, 2000). The drug also improved whole-body insulin sensitivity and glucose control in insulin-resistant idiopathic dilated cardiomyopathy patients, thus hypothetically countering the myocardial damage of insulin resistance (Tuunanen et al., 2008). Trimetazidine attenuated myocardial reperfusion injury (Castedo et al., 2005; Khan et al., 2010). In other organs such as the kidney (Faure et al., 2004; Jayle et al., 2007), trimetazidine displayed cytoprotective effects as well. Other mechanisms might be also operable in the cytoprotective action of trimetazidine during tissue ischemia such as (1) limitation of the intracellular accumulation of protons that is responsible for cell acidosis during ischemia and also reduced accumulation of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  in the cell (Renaud, 1988); (2) preservation of the adenosine triphosphate pool during reflow (Allibardi et al., 1998); (3) inhibition of neutrophil accumulation after ischemia and reperfusion (Williams et al., 1993); (4) inhibition of thrombin-induced aggregation and calcium entry into platelets (Astarie-Dequeker et al., 1994); and (5) antioxidant or free radical scavenging effect, thereby diminishing the bioavailability of free radicals and consequently minimizing their toxic effects on cellular macromolecules such as membrane lipids, proteins and DNA (Guarnieri and Muscari, 1993; Bayram et al., 2005). Trimetazidine reduced the level of tissue malondialdehyde in heart mitochondria subjected to oxidative stress (Guarnieri and

Muscari, 1993). In ischemia-reperfusion injury after lung transplantation, trimetazidine increased adenosine triphosphate content, resulted in better oxygenation, and reduced lipid peroxidation (Inci et al., 2001). In rat models of colitis, the elevated colonic nitric oxide levels and myeloperoxidase activity were reduced by trimetazidine administration (Kuralay et al., 2003), whereas total glutathione in tissue is increased by trimetazidine (Girgin et al., 2000). Trimetazidine given before reflow prevented reperfusion-mediated cardiac injury and dysfunction. This protection appears to be mediated by activation of p38 mitogen-activated protein kinase and Akt signaling (Khan et al., 2010). In a model of global myocardial ischemia, trimetazidine had a preferential action on the oxidative system (mainly on complex I), increasing its enzyme activity and decreasing O<sub>2</sub> consumption after phosphorylation; this could decrease oxygen free radical production and increase mitochondrial integrity, thus allowing the maintenance of the electrical potential (Monteiro et al., 2004).

### CONCLUSION

In summary, the present study indicates that administration of trimetazidine alleviates oxidative stress in the brain and liver caused by lipopolysaccharide administration in mice. It is suggested, therefore, that trimetazidine might prove of benefit in therapy of systemic inflammation.

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