Trimetazidine protects the energy status after ischemia and reduces reperfusion injury in a rat single lung transplant model

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Background: Ischemia-reperfusion injury involves free radical generation and polymorphonuclear neutrophil chemotaxis. Trimetazidine is an anti-ischemic drug that restores the ability of the ischemic cells to produce energy and reduces the generation of oxygen-derived free radicals. We evaluated the effect of trimetazidine against ischemia-reperfusion injury after lung transplantation.

Methods: Rat single lung transplantation was performed in 3 experimental groups (n = 5): (1) the immediate transplantation group was defined as animals undergoing transplantation immediately after harvest without treatment; (2) the ischemic control group was defined as animals undergoing transplantation after 18 hours of cold (4°C) ischemia without treatment; and (3) the trimetazidine-treated group was defined as animals undergoing transplantation after 18 hours of cold (4°C) ischemia and donor and recipient treatment with 5 mg/kg intravenous trimetazidine 10 minutes before harvest and reperfusion, respectively. All donor lungs were flushed with low-potassium dextran-glucose solution. After 2 hours of reperfusion, oxygenation was measured, and lung tissue was frozen and assessed for adenosine triphosphate content, myeloperoxidase activity, and thiobarbituric acid-reactive substances. Peak airway pressure was recorded throughout the reperfusion period.

Results: The trimetazidine group showed significantly higher levels of adenosine triphosphate content (1.73 ± 0.8 pmol vs 0.72 ± 0.2 pmol [ischemic control], $P = .008$), better oxygenation (238.82 ± 113.9 mm Hg vs 89.39 ± 14.7 mm Hg [ischemic control], $P = .008$), and reduced lipid peroxidation (1.28 ± 0.1 nmol/g vs 2.09 ± 0.4 nmol/g [ischemic control], $P = .008$). Adenosine triphosphate levels of the trimetazidine group were comparable with those of the immediate transplantation group (1.73 ± 0.8 pmol vs 1.89 ± 0.5 pmol, respectively; $P = .31$). Peak airway pressure and myeloperoxidase activity were comparable among groups.

Conclusion: Donor and recipient treatment with trimetazidine provided a significant protection of the energy status, better oxygenation, and reduced lipid peroxidation in this experimental model. Our data suggest that trimetazidine may be an important adjunct to prolong ischemic time safely and to decrease lung ischemia-reperfusion injury.

Lung transplantation has become an established therapeutic procedure for end-stage pulmonary disease. Ischemia-reperfusion (I/R) injury after lung transplantation continues to present a potentially life-threatening problem and remains a challenge in lung transplantation. Despite many improved strategies, as many as 10% to 15% of the transplanted pulmonary allografts might

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Figure 1. Arterial blood gas (ABG) analysis of the groups. TMZ-treated group versus IC group, $P = .008$; ImTx group versus IC group, $P = .008$; ImTx group versus TMZ group, $P = .15$.

experience severe graft dysfunction immediately after implantation.¹

Reperfusion of ischemic organs can result in tissue injury that is manifested as microvascular and parenchymal cell dysfunction.² Reactive oxygen metabolites and polymorphonuclear leukocytes have been implicated in the pathophysiology of reperfusion injury. Reactive oxygen metabolites mediate the lipid peroxidation detected in postischemic tissue and promote the formation of inflammatory mediators that recruit and activate polymorphonuclear leukocytes.² Although reperfusion is crucial for oxygen delivery to ischemically injured tissues, tissue reoxygenation is known to be detrimental because it allows the generation of reactive oxygen metabolites, such as superoxide anions, hydroxyl radicals, and hydrogen peroxides.³ Reactive oxygen species stimulate the release and formation of inflammatory mediators with powerful chemotactic potential. These molecules are a distress signal for the immune system. They upregulate adhesion molecules on leukocytes and endothelial cells and recruit leukocytes to the site of injury. Moreover, I/R upregulates the expression of major histocompatibility complex molecules in clinical and experimental studies, rendering postischemic allografts more immunogenic and rejectable.² For this reason, drugs that stabilize or reverse I/R injury should improve the organ to be transplanted.

Trimetazidine (TMZ; 1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride) is an anti-ischemic agent known to improve exercise tolerance and cardiac function in patients with ischemic heart disease.⁴ Its anti-ischemic effect has been experimentally assessed in various models, including

cell cultures, isolated and perfused organs, and in vivo models.⁵⁻¹¹ In isolated cardiomyocyte models, TMZ treatment resulted in increased cell resistance to hypoxic stress.⁶ In isolated perfused heart models, it has been found that TMZ protected myocardial energy transformation processes during episodes of ischemia.⁷

The aim of this study was to determine the protective effect of TMZ on post-transplant lung I/R injury, which was assessed by means of blood oxygenation, peak airway pressure, lung tissue adenosine triphosphate (ATP) content, lipid peroxidation, and neutrophil accumulation.

Materials and Methods

Orthotopic single left lung transplantation was performed in male Fischer (F344) rats weighing 280 to 300 g by use of a cuff technique for the anastomoses.¹²

All animals received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The study protocol was approved by the local animal study committee.

Donor Procedure

Animals were anesthetized by means of intraperitoneal injection of 50 mg/kg sodium thiopental (Pentothal; Abbott AG, Baar, Switzerland) and intubated through a tracheostomy with a 16-gauge intravenous catheter. Animals were connected to a volume-controlled ventilator (Harvard Rodent Ventilator, model 683; Harvard Apparatus Co Inc, South Natick, Mass) and ventilated with a fraction of inspired oxygen of 1, a tidal volume of 10 mL/kg at 75 breaths/min, and a positive end-expiratory pressure of 3 cm

Figure 2. Lung ATP content of the groups. TMZ-treated group versus IC group, $P = .008$; ImTx group versus IC group, $P = .008$; ImTx group versus TMZ group, $P = .31$.

H₂O. After this, a median laparosternotomy was performed, and 1000 IU/kg of heparin (Liquemin; Roche Pharma [Schweiz] AG, Reinach, Switzerland) was injected into the inferior vena cava. For the harvest of the heart-lung block, the inferior vena cava was incised, the left atrial appendage was cut, and a 14-gauge cannula was placed into the main pulmonary artery. The lungs were flushed through this cannula with 20 mL of low-potassium dextran-glucose (Perfadex; Xvivo Transplantation Systems AB, Göteborg, Sweden) at 4°C, which also contained 500 µg/L alprostadil (prostaglandin E₁, Prostin VR; Pharmacia & Upjohn, North Peapack, NJ). After the lungs had been flushed, the intratracheal tube was clamped to keep the lungs inflated during storage. Hypothermic condition was maintained during the cuff (16-gauge) placement into the pulmonary artery, pulmonary vein, and main bronchus. The vessels or bronchus was drawn through the center of the cuff, everted circumferentially around it, and secured with a 7-0 silk ligature.

Recipient Procedure

Recipient animals were anesthetized and intubated as described for donor animals. Anesthesia was maintained with 0.5% halothane during the operation and reperfusion period. Ventilation parameters were the same as in donor animals. For measuring the airway pressure during the procedure, a 3-way tap was inserted between the intratracheal tube and the ventilator circuit and connected to a pressure transducer. A left thoracotomy was performed through the fifth intercostal space. The left lung was mobilized by dividing the pulmonary ligament. The hilum of the left lung was dissected, and the pulmonary artery, pulmonary vein, and left main bronchus were isolated. All 3 structures were clamped with microsurgical aneurysm clamps. They were incised on their anterior aspect, and the cuffs of the donor lung were placed into the equivalent recipient structures and fixed with a 6-0 polypropylene suture. The transplanted lung was inflated, and pulmonary vein and arterial clamps

were released. The thoracotomy was closed loosely. The recipient animal was ventilated (with 99.5% oxygen and 0.5% halothane, a tidal volume of 10 mL/kg at 75 breaths/min, and a positive end-expiratory pressure of 3 cm H₂O) for 2 hours.

Experimental Setting

Animals were randomized into 3 groups ($n = 5$ each): the immediate transplantation (ImTx) group, the ischemic control (IC) group, and the TMZ-treated group. In the IC and TMZ groups, transplantation was carried out after 18 hours of cold ischemia (4°C). There was no treatment in the ImTx and IC groups. In the TMZ group, donors received 5 mg/kg TMZ 10 minutes before harvest by means of injection into the inferior vena cava, and the recipient received 5 mg/kg TMZ 10 minutes before reperfusion by means of injection into the left superior vena cava. In the TMZ group, flush and preservation solution contained 10⁻⁶ mol/L TMZ. Right donor lungs ($n = 5$) were assessed for ATP, myeloperoxidase (MPO), and thiobarbituric acid-reactive substances (TBARS) to obtain baseline values in normal lungs.

MPO Assay

Quantitative MPO activity, as measured for neutrophil migration to the graft, was determined as previously described.¹³ Frozen lung tissue (100 mg) was homogenized in 1 mL of 0.5% hexadecyltrimethylammonium bromide, 5 mmol/L ethylenediamine tetraacetic acid, and 50 mmol/L potassium phosphate buffer (pH 6.2) with a tissue grinder. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was assayed for total soluble protein by the method of Pierce Laboratories,¹⁴ as well as for MPO activity. Enzyme activity was measured spectrophotometrically. Ten milligrams of 5-fold supernatant was combined with 0.6 mL of Hanks bovine serum albumin, 0.5 mL of 100 mmol/L potassium phosphate buffer (pH 6.2), 0.1 mL of 0.05% H₂O₂, and 0.1 mL of 1.25 mg/mL *o*-dianisidine. The reaction was stopped by

Figure 3. TBARS levels of the groups. TMZ-treated group versus IC group, $P = .008$; ImTx group versus IC group, $P = .008$; ImTx group versus TMZ group, $P = .84$.

addition of 1% NaN_3 after 5 and 20 minutes at room temperature, respectively. The optical density (OD) was measured at 460 nm with a spectrophotometer (Kadas 100; Dr Lange, AG, Zurich Switzerland). Color development from 5 to 20 minutes was linear. Enzyme activity is expressed as the change in optical density (ΔOD) unit per milligram of tissue protein per minute.

TBARS

TBARS levels were measured according to the method of Okhawa and coworkers¹⁵ in 10% wet weight per volume homogenate to determine the lipid peroxidation in the graft tissue. Aliquots (0.2 mL) of this homogenate were added to tubes containing 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution adjusted to 3.5 pH with NaOH, and 1.5 mL of 0.8% solution of thiobarbituric acid. The mixture was brought to a volume of 4 mL by addition of distilled water, heated at 95°C for 60 minutes, and then cooled with tap water. One milliliter of distilled water and 5 mL of butanol/pyridine (15:1) was added (all chemicals from Fluka Chemie AG, Buchs, Switzerland). The solution was centrifuged at 4000 rpm for 10 minutes. The absorbance of the upper layer was measured at 532 nm with a spectrophotometer (Kadas 100; Dr Lange, AG Zurich). The TBARS levels were determined by reference to a standard curve of 1,1,3,3-tetramethoxypropane (Sigma Chemicals, Buchs, Switzerland), and the results were expressed as nanomoles of malondialdehyde per gram of wet lung.

Lung ATP Content

Frozen lung tissue (100 mg) was homogenized in 0.9 mL of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid buffer. As an extractant, 200 μL of trichloroacetic acid (1% final concentration, Fluka AG) was used, and 100 μL of releasing agent was added to this homogenate. The homogenate was centrifuged at 3000 rpm for

15 minutes at 4°C. The supernatant was removed and adjusted to pH 7 by addition of 0.1 N NaOH. ATP concentration in the supernatant was determined enzymatically with an ATP assay kit (Calbiochem ATP Assay Kit, Calbiochem-Novobiochem Corporation, San Diego, Calif). The results were expressed as picomoles of ATP.

Graft Assessment

Peak airway pressure was recorded after intubation; after the chest was entered; before reperfusion; at 1, 5, 10, and 15 minutes after reperfusion; and then every 15 minutes thereafter. At the end of 2 hours of reperfusion, oxygenation of the graft was evaluated by sampling the blood directly from the pulmonary vein of the transplanted lung by means of aspiration with a heparinized needle (29-gauge) inserted distal to the anastomotic cuff.¹⁶ The transplanted lung was excised, divided into 3 pieces, and put into the liquid nitrogen and stored at -80°C for further evaluation of ATP, TBARS, and MPO.

Statistical Analysis

Data analysis was performed with SPSS for Windows 8.0 (SPSS Inc, Chicago, Ill). All data are expressed as mean values $\pm$ SD. Because it was not possible to check for the normality of the variables, with only 5 observations per group, we used nonparametric procedures. We performed the Kruskal-Wallis test to compare among the 3 groups. When a significant difference was found, we then performed the Mann-Whitney test for comparison between 2 groups. Analysis of variance for repeated measures was used to evaluate the statistical difference between the groups regarding the peak airway pressure over the 2-hour reperfusion period (which consists of 14 measurements). The normal values were given for the purpose of comparison but were not used in any testing procedures.

Figure 4. MPO activity of the groups. Kruskal-Wallis test result among the groups was not significant ($P = .827$).

Results

Blood Gas Analysis

Oxygenation 2 hours after graft reperfusion was higher in the ImTx group (405.83 ± 142.6 mm Hg) than in the TMZ and IC groups (238.82 ± 113.9 mm Hg and 89.39 ± 14.7 mm Hg, respectively). The Kruskal-Wallis test showed significance ($P = .005$) among the 3 groups, and the Mann-Whitney test revealed a significant difference between the IC group versus the TMZ group ($P = .008$) and the IC group versus the ImTx group ($P = .008$). No statistical difference was found between the ImTx and TMZ groups ($P = .15$, Figure 1).

Peak Airway Pressures

The analysis of variance for repeated measures with all measurements made during the reperfusion period did not show any significant differences among the groups ($P = .14$). At the end of 2 hours of reperfusion, peak airway pressure was 12.2 ± 1.3 mm Hg in the TMZ group, 13.2 ± 1.09 mm Hg in the IC group, and 10.8 ± 2.2 mm Hg in the ImTx group. The Kruskal-Wallis test performed at this time point did not show significance among the groups ($P = .134$).

Lung ATP Content

Lung ATP content in the normal lung was 8.5 ± 2.08 pmol of ATP. The TMZ group showed higher levels of ATP content (1.73 ± 0.87 pmol of ATP) than in the IC group (0.72 ± 0.2 pmol ATP) and comparable levels with those of the ImTx group (1.89 ± 0.51 pmol of ATP). The Kruskal-Wallis test revealed a significant difference among the groups ($P = .007$). The Mann-Whitney test showed a significant difference between the IC group

versus the TMZ group ($P = .008$) and the IC group versus the ImTx group ($P = .008$). No statistical difference was found between the ImTx and TMZ groups ($P = .31$, Figure 2).

TBARS

The normal lungs had a mean TBARS level of 0.14 ± 0.03 nmol of malondialdehyde per gram of wet lung. The amount of lipid peroxidation was comparable between the TMZ and ImTx groups (1.28 ± 0.14 nmol/g and 1.31 ± 0.15 nmol/g, respectively), whereas the IC group had higher levels (2.09 ± 0.42 nmol/g). The Kruskal-Wallis test showed a significant difference among the groups ($P = .009$). The Mann-Whitney test showed a significant difference between the IC group versus the TMZ group ($P = .008$) and the IC group versus the ImTx group ($P = .008$). The difference between the ImTx and TMZ groups was not significant ($P = .84$, Figure 3).

MPO Activity

MPO activity in the normal lungs was $0.5 \pm 0.17 \Delta OD \cdot mg^{-1} \cdot min^{-1}$. MPO activity in the ImTx, IC, and TMZ groups was $1.68 \pm 0.96 \Delta OD \cdot mg^{-1} \cdot min^{-1}$, $2.81 \pm 1.8 \Delta OD \cdot mg^{-1} \cdot min^{-1}$, and $2.49 \pm 1.3 \Delta OD \cdot mg^{-1} \cdot min^{-1}$, respectively. The difference among the groups was not significant, as determined with the Kruskal-Wallis test ($P = .827$, Figure 4).

Discussion

In this study with a rat single lung transplant model, we showed that donor and recipient treatment with TMZ result-

ed in significantly improved graft function. Energy status was protected, and lipid peroxidation was reduced after 18 hours of cold storage and 2 hours of reperfusion. According to our and others' experience, 18 hours of cold ischemia is a satisfactory time period to obtain severely injured lungs.¹⁷ The severity of I/R injury was examined 2 hours after the start of reperfusion. This time point was chosen in the rat model because it corresponds well with the early appearance of reperfusion injury in the clinical setting.¹⁷

We used low-potassium dextran-glucose solution for preservation and flush, which corresponds to our clinical practice. The concentration of TMZ used in flush and preservation solution was 10^{-6} mol/L. This concentration has been shown to be effective in restoring ATP synthesis of isolated mitochondria previously decreased by Ca^{2+} overload.⁵ Also, a previous study has demonstrated that higher concentrations of TMZ exerted no protective effect.¹⁸

In anaerobic metabolism, instead of 38 mol of ATP, only 2 mol is produced, representing a 94% reduction of energy production for essential cellular processes.¹⁹ Failure of ion pumping with rapid loss of electrochemical gradients results in translocation of ions, notably Ca^{2+} . Free Ca^{2+} rapidly accumulates and triggers many adverse effects, including phospholipase activation.¹⁹ Another consequence of failed ion pumping is that intracellular Na^+ accumulates and K^+ is lost to the extracellular fluid. Cell swelling and interstitial fluid accumulation produce edema and increase diffusion distances, further compromising oxygen and substrate delivery.¹⁹ The production of oxygen-derived free radicals is considerably increased during tissue ischemia caused by dissociation of oxidative phosphorylation, which results in univalent reduction of oxygen, catabolism of ATP into hypoxanthine and uric acid, and infiltration of damaged tissues by polymorphonuclear leukocytes and phagocytosis.⁵

Various experimental studies have shown that TMZ has preserved the intracellular concentrations of ATP and inhibited the extracellular leakage of potassium during cellular ischemia. Additionally, it prevents excessive release of free radicals, which are particularly toxic to phospholipid membranes and are responsible for both the fall in the intracellular ATP concentration and the extracellular leakage of potassium. It also reduces intracellular accumulation of sodium and calcium.²⁰ Its mechanism of action is not fully understood, but data indicate that it affects both metabolic functions and ion permeabilities in mitochondria.²¹ It has been shown that TMZ restored the ATP synthesis in isolated mitochondria previously exposed to Ca^{2+} overload.²² The formation of a giant pore, called the mitochondrial transition pore, might be involved, allowing the exchange of small solutes (<1500 d) across the inner membrane.²³ It has been stated that TMZ acts on mitochondrial function in at least 2 different ways, as a mitochondrial Ca^{2+} releaser when the giant pore is closed, and by inducing its closure when the pore is open.²¹

TMZ is a cytoprotective drug that counteracts the metabolic disorders occurring at the level of ischemic cells.²⁴ The effect of TMZ on kidneys after 24 hours of cold storage and subsequent reperfusion goes along with protection of cells from acidosis during cold storage.²⁴ With the ^{32}P nuclear magnetic resonance technique, it has been described that TMZ counteracted the fall in ATP levels induced by ischemia and favored the restoration of ATP levels during reperfusion.²⁵

In a rat liver model of I/R injury, TMZ lowered the increase in liver enzymes and maintained higher concentrations of hepatic ATP.²⁶ In isolated rat liver mitochondria, TMZ sustained the normal functions of mitochondria by inhibiting mitochondrial swelling, the decrease in reduced nicotinamide adenine dinucleotide phosphate level, and the decrease in ATP synthesis.²⁷

In our study a marked decrease in lung ATP level was observed in the IC group, confirming that this index of function is rapidly and seriously affected by I/R. Lung grafts of animals treated with TMZ and stored for 18 hours showed ATP contents similar to those of lung grafts without storage. We assume that the higher ATP content is not only the result of better protected tissue by lesser I/R injury but also a direct effect of ATP use itself. These results are in agreement with those of the various other investigators.

Although TMZ is not known to have a direct effect on neutrophils, the number of infiltrating neutrophils was significantly lower in TMZ-treated rabbits in a myocardial I/R model.¹⁰ Additionally, in a rat intestinal I/R injury model, decreased MPO activity after TMZ therapy has been reported.⁸ In contrast, in our study we were not able to show a difference in MPO activity among our study groups.

Free radical oxygen species can be measured indirectly by using malondialdehyde as a marker. The colorimetric reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, has been widely adopted as a sensitive assay method for lipid peroxidation in animal tissues.¹⁵ The high levels of malondialdehyde observed in this and previous studies support the notion that lipid peroxidation occurs during I/R injury. We have shown that lipid peroxidation of the TMZ-treated group was significantly less than that of the nontreated IC group. This reduction in malondialdehyde levels is consistent with the powerful antioxidant effect of TMZ. Significant reduction of lipid peroxidation has also been reported by other investigators.^{3,8,28,29}

In conclusion, in the rat single lung transplant model, we found that donor and recipient treatment with TMZ decreased the severity of I/R injury as shown by a significant protection of the energy status, better oxygenation, and reduced lipid peroxidation. Our data suggest that TMZ may be an important adjunct to prolonging ischemic time safely in lung transplantation.

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References

- Hosepund JD, Novic RJ, Bennet LE, et al. The registry of the International Society for Heart and Lung Transplantation: thirteenth official report-1996. *J Heart Lung Transplant*. 1996;15:655-74.
- Zimmerman BJ, Granger DN. Mechanisms of reperfusion injury. *Am J Med Sci*. 1994;307:284-92.
- Hauet T, Bauza G, Goujon JM, et al. Effects of trimetazidine on lipid peroxidation and phosphorus metabolites during cold storage and reperfusion of isolated perfused rat kidneys. *J Pharmacol Exp Ther*. 1998;285:1061-7.
- Brottier L, Barat JL, Combe C, et al. Therapeutic value of a cardioprotective agent in patients with severe ischaemic cardiomyopathy. *Eur Heart J*. 1990;11:207-12.
- Harpey C, Clauser P, Labrid C, Freyria JL, Poirier JP. Trimetazidine, a cellular anti-ischemic agent. *Cardiovasc Drug Rev*. 1989;6:292-312.
- Fantini E, Demaison L, Sentex E, Grynberg A, Athias P. Some biochemical aspects of the protective effect of trimetazidine on rat cardiomyocytes during hypoxia and reoxygenation. *J Mol Cell Cardiol*. 1994;26:949-58.
- Rossi A, Lavanchy N, Martin J. Antiischemic effect of trimetazidine: ^{31}P -NMR spectroscopy study in the isolated rat heart. *Cardiovasc Drug Ther*. 1990;4:812-3.
- Tetik C, Ozden A, Calli N, et al. Cytoprotective effect of trimetazidine on 60 minutes of intestinal ischemia-reperfusion injury in rats. *Transpl Int*. 1999;12:108-12.
- Tsimoyiannis EC, Moutesidou KJ, Moschos CM, et al. Trimetazidine for prevention of hepatic injury induced by ischemia and reperfusion in rats. *Eur J Surg*. 1993;159:89-93.
- Williams FM, Tanda K, Kus M, Williams TJ. Trimetazidine inhibits neutrophil accumulation after myocardial ischemia and reperfusion in rabbits. *J Cardiovasc Pharmacol*. 22;1993:828-33.
- Baumert H, Goujon JM, Richer JP, et al. Renoprotective effects of trimetazidine against ischemia-reperfusion injury and cold storage preservation: a preliminary study. *Transplantation*. 1999;68:300-3.
- Mizuta T, Kawaguchi, Nakahara K, Kawashima Y. Simplified rat lung transplantation using a cuff technique. *J Thorac Cardiovasc Surg*. 1989;97:578-81.
- Goldblum SE, Wu KM, Jay M. Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. *J Appl Physiol*. 1985;59:1978-85.
- Smith Pk, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985;150:768-85.
- Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95:351-8.
- Fischer S, Hopkinson D, Liu M, Keshavjee S. Raffinose improves the function of rat pulmonary grafts stored for twenty-four hours in low-potassium dextran solution. *J Thorac Cardiovasc Surg*. 2000;119:488-92.
- Fischer S, MacLean AA, Liu M, Kalirai B, Keshavjee S. Inhibition of angiotensin-converting enzyme by captopril: a novel approach to reduce ischemia-reperfusion injury after lung transplantation. *J Thorac Cardiovasc Surg*. 2000;120:573-80.
- Boucher FR, Hearse DJ, Opie LH. Effects of trimetazidine on ischemic contracture in isolated perfused rat hearts. *J Cardiovasc Pharmacol*. 1994;24:45-9.
- Zelenock GB, D'Alecy LG. Tissue ischemia. In: Greenfield LJ, Mulholland MW, Oldham KT, Zelenock GB, Lillemoie KD, editors. *Surgery: scientific principles and practice*. 2nd ed. Philadelphia: Lippincott-Raven; 1997. p. 1643-55.
- Labrid C. Cellular disorders induced by ischemia: the action of trimetazidine. *Presse Med*. 1986;15:1754-7.
- Morin D, Elimadi A, Sepana R, et al. Evidence for the existence of $[\text{H}^3]$ -trimetazidine binding sites involved in the regulation of the mitochondrial permeability transition pore. *Br J Pharmacol*. 1998;123:1385-94.
- Salducci MD, Chauvet-Monges AM, Tillement JP, et al. Trimetazidine reverses calcium accumulation and impairment of phosphorylation induced by cyclosporin A in isolated rat liver mitochondria. *J Pharmacol Exp Ther*. 1996;277:417-22.
- Haworth RA, Hunter DR. The Ca^{+2} -induced membrane transition in mitochondria. II. Nature of the Ca^{+2} trigger site. *Arch Biochem Biophys*. 1979;195:460-7.
- Hauet T, Bauza G, Mothes D, et al. Beneficial effect on rat kidney preservation of the antiischemic agent trimetazidine during cold storage and reperfusion: assessment by ^{31}P nuclear magnetic resonance spectroscopy. *Transplant Proc*. 1997;29:2343-4.
- Lavancy N, Martin JR, Rossi A. Antiischemic effects of trimetazidine: ^{31}P -NMR spectroscopy in the isolated rat heart. *Arch Int Pharmacodynamics Ther*. 1987;286:97-110.
- Settaf A, Morin D, Lamchouri F, Elimadi A, Cherrah Y, Tillement JP. Trimetazidine ameliorates the hepatic injury associated with ischemia-reperfusion in rats. *Pharmacol Res*. 1999;39:211-6.
- Elimadi A, Settaf A, Morin D, et al. Trimetazidine counteracts the hepatic injury associated with ischemia-reperfusion by preserving mitochondrial function. *J Pharmacol Exp Ther*. 1998;286:23-8.
- Richer JP, Gibelin H, Tallineau C, et al. Limitation of lipid peroxidation and renal medullary cell injury of the kidney after 48- and 72-hour cold storage in University of Wisconsin solution: effect of trimetazidine. *Transplant Proc*. 2000;32:46.
- Hauet T, Mothes D, Goujon JM, et al. Trimetazidine reverses deleterious effects of ischemia-reperfusion in the isolated perfused pig kidney. *Nephron*. 1998;80:296-304.