Ischemia-Reperfusion Injury During Experimental Heart Transplantation. Evaluation of Trimetazidine's Cytoprotective Effect

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Introduction and objectives. The objectives of this study were to analyze the ischemia-reperfusion injury due to free radicals that occurs during heart transplantation and to determine the potential cytoprotective effect of trimetazidine.

Material and method. A total of 21 orthotopic heart transplantations were performed in pigs. We divided the experimental animals into 2 groups: in group A (n=11), standard myocardial protection was used; in group B (n=10), trimetazidine was added to the cardioplegic solution used to protect the donor heart and to the solution administered to the recipient prior to release of the aortic clamp (trimetazidine, 10^{-5} mol/L), and recipients were pretreated with trimetazidine, 2.5 mg/kg. Blood samples were taken from the recipient's coronary sinus at three times: at baseline, during ischemia, and during reperfusion. We measured the levels of malondialdehyde, a marker of lipid peroxidation, and of several antioxidants: glutathione peroxidase, glutathione reductase, superoxide dismutase, α-tocopherol, and retinol. The total antioxidant status was also determined.

Results. Malondialdehyde production and enzymatic antioxidant activity rose during ischemia and reperfusion, while the retinol level decreased. The increases in malondialdehyde level and glutathione peroxidase activity that occurred between baseline and reperfusion were significantly higher in group A.

Conclusions. The degree of lipid peroxidation and the level of activity of intracellular antioxidant mechanisms increased progressively throughout transplantation. Trimetazidine had a cytoprotective effect. It ameliorated free radical-induced reperfusion injury and modified the response pattern of several defense mechanisms.

Daño por isquemia-reperfusión durante el trasplante cardíaco experimental. Evaluación del papel citoprotector de la trimetazidina

Introducción y objetivos. El objetivo de este trabajo fue analizar el daño por isquemia-reperfusión mediado por radicales libres que se produce durante el trasplante cardíaco y evaluar el posible efecto citoprotector de la trimetazidina (TMZ).

Material y método. Se realizaron 21 trasplantes cardíacos ortotópicos en cerdos. Dividimos los experimentos en 2 grupos: A (n = 11), en el que se realizó una protección miocárdica estándar, y B (n = 10), en el que se administró TMZ en la cardioplejía empleada para parar el corazón donante (TMZ, 10^{-5} mol/l), como pretratamiento intravenoso del receptor (TMZ, 2,5 mg/kg) y como parte de la cardioplejía infundida en el receptor antes de despinzar la aorta (TMZ, 10^{-5} mol/l). Se tomaron muestras de sangre del seno coronario del receptor en 3 momentos: basal, isquemia y reperfusión. Se determinó la concentración de malonildialdehído como marcador de peroxidación lipídica y de varios antioxidantes: glutatión peroxidasa, glutatión reductasa, superóxido dismutasa, α-tocoferol, retinol y estado de antioxidantes totales.

Resultados. Durante la isquemia-reperfusión aumentó la producción de malonildialdehído y la actividad de los antioxidantes enzimáticos, mientras que el retinol disminuyó. El incremento de malonildialdehído y de la actividad de la glutatión peroxidasa entre el momento basal y la reperfusión fue significativamente mayor en el grupo A.

Conclusiones. Durante el trasplante se incrementó progresivamente el nivel de peroxidación lipídica y se activaron los sistemas antioxidantes intracelulares. La TMZ ejerció un efecto citoprotector y limitó el daño por isquemia-reperfusión generado por los radicales libres, además de modificar el patrón de reacción de parte de los sistemas de defensa.

ABBREVIATIONS

HT: heart transplantation.
IRI: ischemia-reperfusion injury.
MDA: malondialdehyde.
OFR: oxygen free radicals.
TMZ: trimetazidine.

INTRODUCTION

Heart transplantation (HT) has revolutionized the natural history of patients with end-stage heart failure and is currently associated with a 10-year survival rate of 54%.[1] However, the procedure is not free of complications that are partly responsible for the high mortality rate. While the long-term survival and quality of life of transplant recipients has improved significantly owing to advances in immunosuppression and better management of donors and recipients, the surgical technique, strategy for myocardial protection and operative and hospital mortality rates have not undergone substantial changes over the last 25 years. Twenty-four percent of the transplant recipients in Spain die during the first posttransplantation year and, of these, 50% do so within the first month.[1] The most common cause of in-hospital death is primary graft failure, a syndrome that is associated with a number of clinical variables,[2] but their pathophysiological mechanisms have yet to be clarified.

Although modifications are being made in the classical surgical technique, such as the bicaval technique or whole organ transplantation, aside from reducing the degree of atrioventricular valve insufficiency and the incidence of atrial arrhythmias, these techniques do not appear to lower the rates of primary graft failure or early posttransplantation mortality.[3] Another possibility under investigation is the attempt to optimize the myocardial preservation technique for the purpose of attenuating the ischemia-reperfusion injury (IRI) mediated by oxygen-derived free radicals (OFR), which is known to be involved in the development of primary graft failure.[4-11] In the experimental setting, the use of antioxidants has been shown to diminish the damage produced by OFR and improve graft function and survival.[4-6,8-11] Nevertheless, to date, none of these agents has generated any clinical benefit whatsoever in human HT.

Experimental studies have shown that trimetazidine (TMZ) exerts a cytoprotective effect by reducing the generation of OFR and the damage they induce, conferring on the cells a greater resistance to hypoxia and capacity for functional recovery during reperfusion.[12-16] There is clinical evidence that it limits IRI in the heart after acute myocardial infarction, when administered in combination with conventional therapy,[17] primary angioplasty[18] or thrombolysis,[19] as well as after coronary revascularization surgery.[20,21] In experimental kidney[8,9] and lung[10] transplantation models, the addition of TMZ to the cardioplegic solution or its administration to the recipient has been associated with a lower level of OFR-induced cytotoxicity and with better postoperative graft function. The objective of the present report is to determine whether or not TMZ exerts a cytoprotective effect against IRI in HT.

MATERIAL AND METHOD

Study Population and Definition of Study Groups

In this study, we employed 42 2-month-old female Landrace Large-White pigs weighing between 18 and 25 kg. The animals were supplied by an industrial farm where they had been bred for human consumption, and had been vaccinated against Aujeszky's disease and porcine parvovirus and dewormed with oxibendazole against roundworm. Upon their arrival in our hospital, they were stabled, observed over a 1-week period and fed ad libitum with barley flour (Pig Starter 90 Plus, Purina).

Orthotopic HT was performed in 21 animals and the other 21 were used as donors. The experiments were divided into 2 groups and the animals were randomly assigned to one or the other. Group A consisted of 11 donors and 11 recipients that were to undergo HT according to an approach in which both the surgical technique and the myocardial protection strategy were similar to those employed in humans in our hospital. Prior to their harvest, the donor hearts were arrested using one liter of a cardioplegic solution with an elevated potassium concentration. Once the graft had been sutured and prior to unclamping the aorta, 250 mL of saline were infused antegradely via aortic root. Group B was composed of the animals to be used in the 10 remaining HT in which we employed a different myocardial protection strategy based on the administration of TMZ to both donors and recipients. In donors, it was added to the liter of cardioplegic solution (TMZ: 10^{-5} mol/L) employed to arrest the heart prior to harvest. The recipients were pretreated with intravenous TMZ (2.5 mg/kg body weight) 10 minutes prior to aortic clamping. Later, the drug was added to the 250 mL of saline (TMZ: 10^{-5} mol/L) that were infused antegradely via aortic root after the graft had been sutured and immediately prior to the unclamping of the aorta.

Anesthetic and Surgical Techniques

All the pigs were treated according to the guidelines of the American Physiological Society for research in

laboratory animals. On the day of the study, they were premedicated with intramuscular ketamine (20 mg/kg body weight), diazepam (1 mg/kg body weight) and atropine (0.04 mg/kg body weight). Anesthesia was induced with 5% isoflurane in oxygen at 2 Vmin and maintained with continuous infusion of fentanyl (10 µg/kg body weight/h) and pancuronium (0.2 mg/kg body weight/h), and 1% to 1.5% isoflurane in oxygen at 2 Vmin.

The donor heart was harvested according to a technique similar to that employed in human HT, after cardiac arrest induced with a crystalloid cardioplegic solution at 4ºC, introduced via aortic root, and irrigation of the pericardial sac with saline at 4ºC. The heart was stored until implantation in a bag containing saline at 4ºC, which, in turn, was kept on ice to maintain the organ at a temperature of 5ºC to 8ºC. Implantation into the recipient was performed according to the classical technique. First, the animal was anticoagulated with heparin sodium (3 mg/kg body weight), and cardiopulmonary bypass was established with hypothermia at 28ºC. We used a Stöckert model 10-00-00 heart-lung machine (Stöckert Instrumente GmbH, Munich, Germany), connected to a cardiotomy reservoir; a low priming volume, polypropylene, hollow-fiber oxygenator with a Bentley® Spiraloxy™ heat exchanger; and a Bentley® BMR 1900 venous reservoir (Baxter Healthcare Corporation, Bentley Division, Irvine, CA, USA).

Variables Studied and Sample Collection

The variables analyzed are shown in Table 1. The analyses were performed in blood samples obtained from the recipient coronary sinus at 3 time points: prior to heparinization and initiation of cardiopulmonary bypass (baseline); at the moment of maximal cold ischemia (once the graft had been sutured, just before the aorta was unclamped); and after 30 minutes of reperfusion.

Determination of Lipid Peroxidation Products

Free radical-induced lipid peroxidation is a well-established mechanism of cell damage that leads to the breakdown of the polyunsaturated fatty acids of the cytoplasmic membrane into lipid peroxides and aldehydes, such as malondialdehyde (MDA). The determination of these reactive aldehydes in blood or tissue is an appropriate index of lipid peroxidation and, thus, an indirect measure of the OFR concentration. In this study, we assessed the MDA concentration in serum. The analysis was carried out with the Bioxytech® LPO-586 colorimetric method (Oxis International, SA, France), using a Philips spectrophotometer (model PU8620, Cambridge, UK).

TABLE 1. **Variables Analyzed**

Intraoperative variables: ischemia time, cardiopulmonary bypass time, need for inotropic drugs
Markers of cell necrosis: creatine kinase, lactate dehydrogenase
Lipid peroxidation products: malondialdehyde
Enzymatic antioxidants: glutathione peroxidase, glutathione reductase, superoxide dismutase
Nonenzymatic antioxidants: vitamin A (retinol), vitamin E (alpha-tocopherol)
Other antioxidants: total antioxidant status

Determination of Glutathione Peroxidase and Glutathione Reductase

Glutathione peroxidase inhibits *de novo* formation of OFR by neutralizing the peroxides that react with transition metals, a reaction that produces OFR. This enzyme catalyzes glutathione oxidation by fatty acid hydroperoxide. The activity of glutathione reductase is complementary to that of glutathione peroxidase. It catalyzes the reduction of oxidized glutathione, resulting in the recovery of the glutathione peroxidase substrate. For their determination, we employed a spectrophotometric method, using the Ransel® Glutathione Peroxidase and Glutathione Reductase kits (cat. no. GR2368; Randox Laboratories Ltd., Crumlin, Antrim, UK), run on a Hitachi 717 automated analyzer from Boehringer-Mannheim.

Determination of Superoxide Dismutase

The antioxidant function of this enzyme involves the catalysis of the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (superoxide dismutase reaction). The concentration of superoxide dismutase in peripheral blood was determined by spectrophotometry, using the Ransod® Superoxide dismutase kit (cat. no. SD 125; Randox Laboratories Ltd., Crumlin, Antrim, UK), run on a Hitachi 717 automated analyzer from Boehringer-Mannheim.

Determination of Vitamin Levels

The antioxidant mechanism of action of vitamin E is double: it neutralizes lipid peroxyl radicals, leading to the formation of tocopheroxyl radical, a relatively stable compound that alone is incapable of initiating the lipid peroxidation chain; and reduces the polymorphonuclear leukocyte infiltration and the tissue injury it produces through the inhibition of P-selectin and intercellular adhesion molecule-1 expression on the endothelial surface. Vitamin A has in vivo and in vitro antioxidant activity, as shown by its capacity to reduce the level of lipid peroxidation produced by OFR and potentiate cell membrane resistance to oxidative damage. The serum

vitamin A (retinol) and vitamin E (alpha-tocopherol) concentrations were determined by reverse-phase high-performance liquid chromatography.

Determination of Total Antioxidant Status

The "total antioxidant status" reflects the overall antioxidant potential of a given solution. The specificity of this parameter is limited since it does not distinguish between enzymatic and "nonenzymatic" antioxidants. It was measured in plasma by a spectrophotometric method using the Total Antioxidant Status® kit (cat. no. Nx 2331; Randox Laboratories Ltd., Crumlin, Antrim, UK), run on a Hitachi 717 automated analyzer from Boehringer-Mannheim.

Statistical Analysis

The study was designed as a clinical trial involving 2 groups of animals, one that was treated with TMZ and another that was not. The objective was to compare them in terms of the changes in a series of quantitative variables measured at 3 time points: baseline, during ischemia, and during reperfusion. The between-group differences in the variables at all 3 times were also analyzed. The Shapiro-Wilk test was employed to evaluate the normal distribution. The statistical analysis was performed using repeated measures analysis of variance involving a within-subject factor (time) and a between-subject factor (treatment), and the effect of the interaction between the 2. The Tukey multiple comparison test was used for within-group analysis. Statistical significance was set at $P<.05$ (two-sided test). The SPSS statistical software package, version 10.0.7 for Windows (SPSS, Inc, Chicago IL, USA), was employed.

RESULTS

The transplanted heart was successfully disconnected from the heart-lung machine in every case. Thus, the measurements corresponding to reperfusion were taken after cardiopulmonary bypass had been discontinued. Tolerance to TMZ was excellent and there were no cases of arterial hypertension or significant changes in heart rate during its intravenous administration. The organ ischemia and cardiopulmonary bypass times were similar in the 2 groups (149±24 minutes in group A vs 157±14 minutes in group B and 100±15 minutes in group A vs 105±14 minutes in group B, respectively; $P>.05$). Inotropic agents were necessary during the reperfusion period in order to wean the animals from cardiopulmonary bypass in 13 cases, 6 in group A and 7 in group B ($P>.05$). The analytical data are presented in Table 2.

Cellular Necrosis

Creatine kinase and lactate dehydrogenase levels increased significantly between baseline and ischemia and between ischemia and reperfusion ($P<.001$). As

TABLE 2. **Results of Analyses Performed at Baseline and at 2 Time Points During Transplantation**

Variable	Group	Baseline	Ischemia	Reperfusion
Creatine kinase, mean±SD (U/L)	A	747.3±308.5	1238.2±732.1*	2297.3±935.8†
	B	1238.1±1109.1	1388.9±907.8*	2365.8±777.3*
Lactate dehydrogenase, mean±SD (U/L)	A	602.3±133.7	781.2±346.6*	1104.8±311.5†
	B	712.4±126.9	872.8±235.8*	1162.6±196.0†
Malondialdehyde, mean±SD (mmol/L)	A	3.61±1.04	7.93±2.52*	9.69±3.36†
	B	3.22±1.45	6.18±1.69*	7.01±1.86†
Glutathione peroxidase, mean±SD (U/g hemoglobin)	A	287.4±71.9	335.5±90.6*	378.5±86.3†
	B	398.1±117.9	419.8±92.3*	442.2±123.6†
Glutathione reductase, mean±SD (U/L)	A	74.9±12.2	125.7±45.4*	130.7±38.9*
	B	85.3±11.9	111.4±37.4*	118.3±36.6*
Superoxide dismutase, mean±SD (U/g hemoglobin)	A	706.8±227.9	796.8±215.1*	917.6±349.2†
	B	983.4±272.8	1106.0±249.3*	1227.3±322.5†
Alpha-tocopherol, mean±SD (μg/dL)	A	135.7±35.6	140.8±39.6	135.5±37.6
	B	136.9±34.8	123.4±41.6	129.7±34.9
Retinol, mean±SD (μg/dL)	A	24.2±7.7	20.2±8.2*	19.2±7.5*
	B	22.8±4.1	16.9±3.3*	17.7±2.7*
Total antioxidant status, mean±SD (mmol/L)	A	0.60±0.07	1.08±0.41*	1.16±0.38*
	B	0.71±0.18	1.10±0.50*	1.09±0.45*

*Statistically significant difference versus baseline value. †Statistically significant difference versus values at baseline and during ischemia.

the 2 groups behaved similarly, there were no between-group differences or interaction effect.

Lipid Peroxidation

Malondialdehyde production increased significantly between baseline and ischemia and between ischemia and reperfusion in both groups ($P<.001$). However, the increase with respect to the baseline value during ischemia-reperfusion was greater in group A (interaction effect; $P<.05$) (Figure 1A). The increase in MDA between baseline and reperfusion was less marked in group B (6.08 ± 2.75 μmol/L vs 3.79 ± 1.73 μmol/L; $P=.03$), and the same occurred when the increases between baseline and ischemia were compared (4.32 ± 1.81 μmol/L vs 2.97 ± 0.94 μmol/L; $P=.04$) (Figures 2A and 2B). Although the increase in the level of lipid peroxidation during the interval between ischemia and reperfusion was greater in group A, the difference was not statistically significant (difference in MDA levels: 1.76 ± 1.28 μmol/L vs 0.83 ± 1.27 μmol/L; $P=.11$).

Enzymatic Antioxidants

The glutathione peroxidase activity increased significantly in both groups during the procedure, between baseline and ischemia and between the latter and reperfusion ($P<.001$). There were differences between the 2 groups, with higher glutathione peroxidase levels in group B ($P=.048$), but there was no interaction effect (Figure 1B). However, the increase in the plasma glutathione peroxidase activity between baseline and reperfusion was significantly more marked in group A (91.09 ± 44.76 U/g of hemoglobin vs 44.10 ± 38.98 U/g of hemoglobin; $P=.019$) (Figure 2C).

The glutathione reductase activity increased between baseline and ischemia-reperfusion in both groups ($P<.01$), although there were no differences between ischemia and reperfusion. There were no significant between-group differences or interaction effect (Figure 1C). The increase in the plasma activity between baseline and reperfusion was greater in group A, with a difference that nearly reached statistical significance (61.82 ± 46.7 U/L vs 33.0 ± 30.45 U/L; $P=.10$).

The superoxide dismutase values increased significantly between baseline and ischemia and between ischemia and reperfusion.

Although the behavior of the 2 groups was similar, the enzyme activity was more marked in group B ($P=.01$) (Figure 1D).

Nonenzymatic Antioxidants

There were no significant differences in the alpha-tocopherol level either between time points or between groups, the 2 of which behaved similarly. The retinol concentration decreased significantly between baseline and ischemia ($P<.001$), although no significant difference was observed between ischemia and reperfusion; nor were there between-group differences or an interaction effect (Figure 1E). However, between the time of maximal ischemia and reperfusion, the 2 groups behaved differently. In group A, the retinol concentration continued to decrease, reaching a minimum after 30 minutes of reperfusion, whereas in group B, not only did it not decrease, it even increased slightly. The difference (retinol during reperfusion minus retinol during ischemia) was close to statistical significance (-0.99 ± 2.49 μmg/dL in group A vs 0.79 ± 2.23 μmg/dL in group B; $P=.10$).

Total Antioxidant Status

The total antioxidant status improved significantly between baseline and ischemia and between baseline and reperfusion ($P<.001$), but not between ischemia and reperfusion. There were no significant differences between the 2 groups; in fact, their behaviors were similar (Figure 1F).

DISCUSSION

Cardiac surgery, and HT in particular, constitutes an ideal setting for the study of IRI since the procedures are reproducible and involve prolonged ischemia and controlled reperfusion. In this study, the possible cytoprotective effect of TMZ, as an agent that attenuates OFR-mediated damage, was analyzed in an experimental HT model.

Ischemia-Reperfusion Injury in Heart Transplantation

Judging by the increase in the plasma creatine kinase and lactate dehydrogenase activities, during HT, there is a progressive loss of cytoplasmic membrane activity and cell viability that commences during the ischemic phase and peaks during reperfusion. In other experimental[22,23] and clinical[20,21,24] models of IRI, a similar increase in the activity of these enzymes was observed during reperfusion. Concerning HT, Bando et al[25] found that creatine kinase MB isoenzyme remained constant during ischemia, but showed a very significant increase during reperfusion.[25] Other authors, however, have demonstrated that creatine phosphokinase activity begins to increase as early as the hypothermic storage phase, reaching a maximum 5 minutes after flow is restored.[26]

Lipid peroxidation, a consequence of the cytotoxic effects of OFR on the cell membrane lipids and an indicator of the increased presence of the latter, also in-
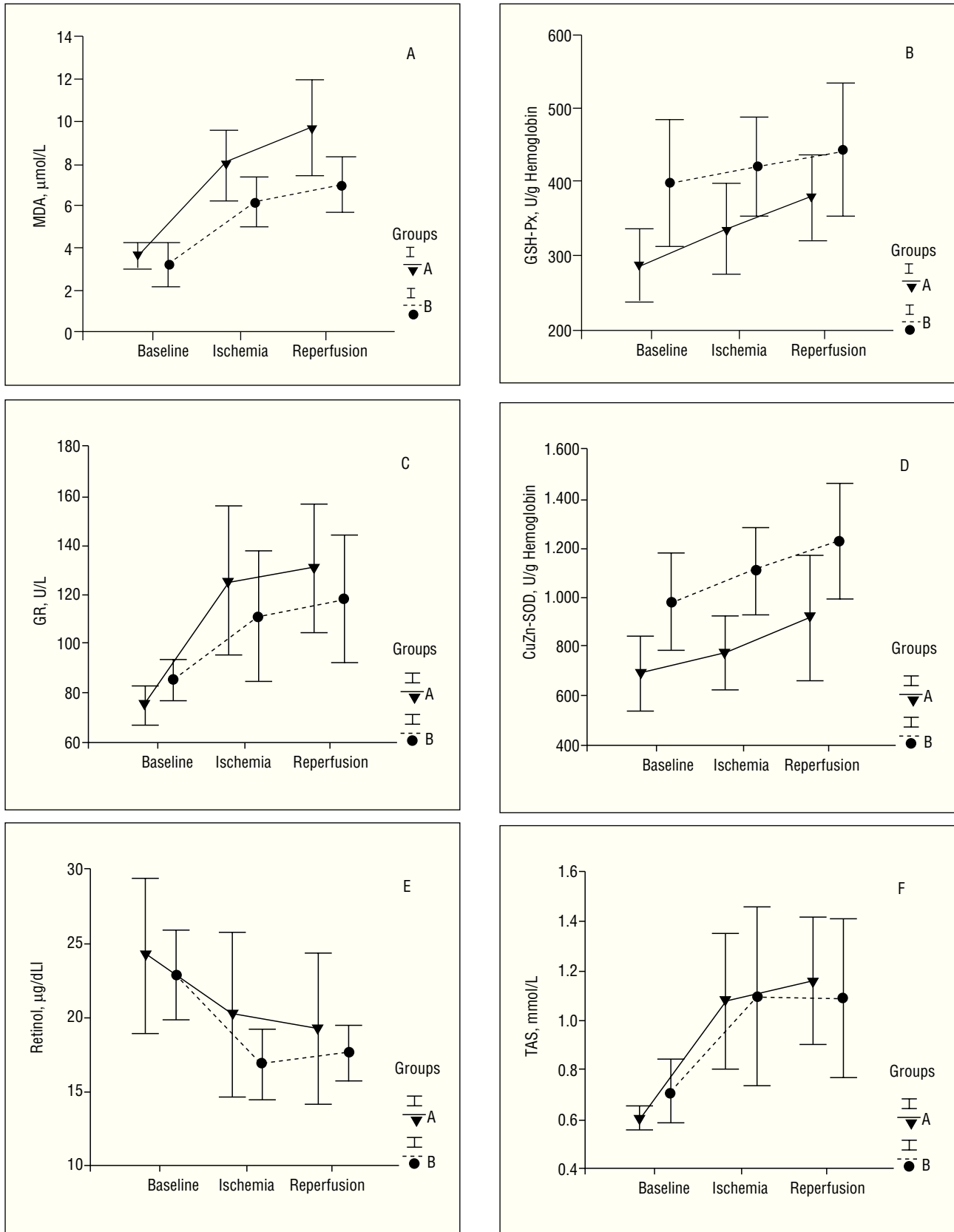
Figure 1. Results of analyses in the 2 groups at baseline and at 2 time points during transplantation. Bar graphs with error bars showing the mean and 95% confidence interval.
A: malondialdehyde (MDA); B: glutathione peroxidase (GSH-Px); C: glutathione reductase (GR); D: superoxide dismutase (CuZn-SOD); E: retinol; F: total antioxidant status (TAS).
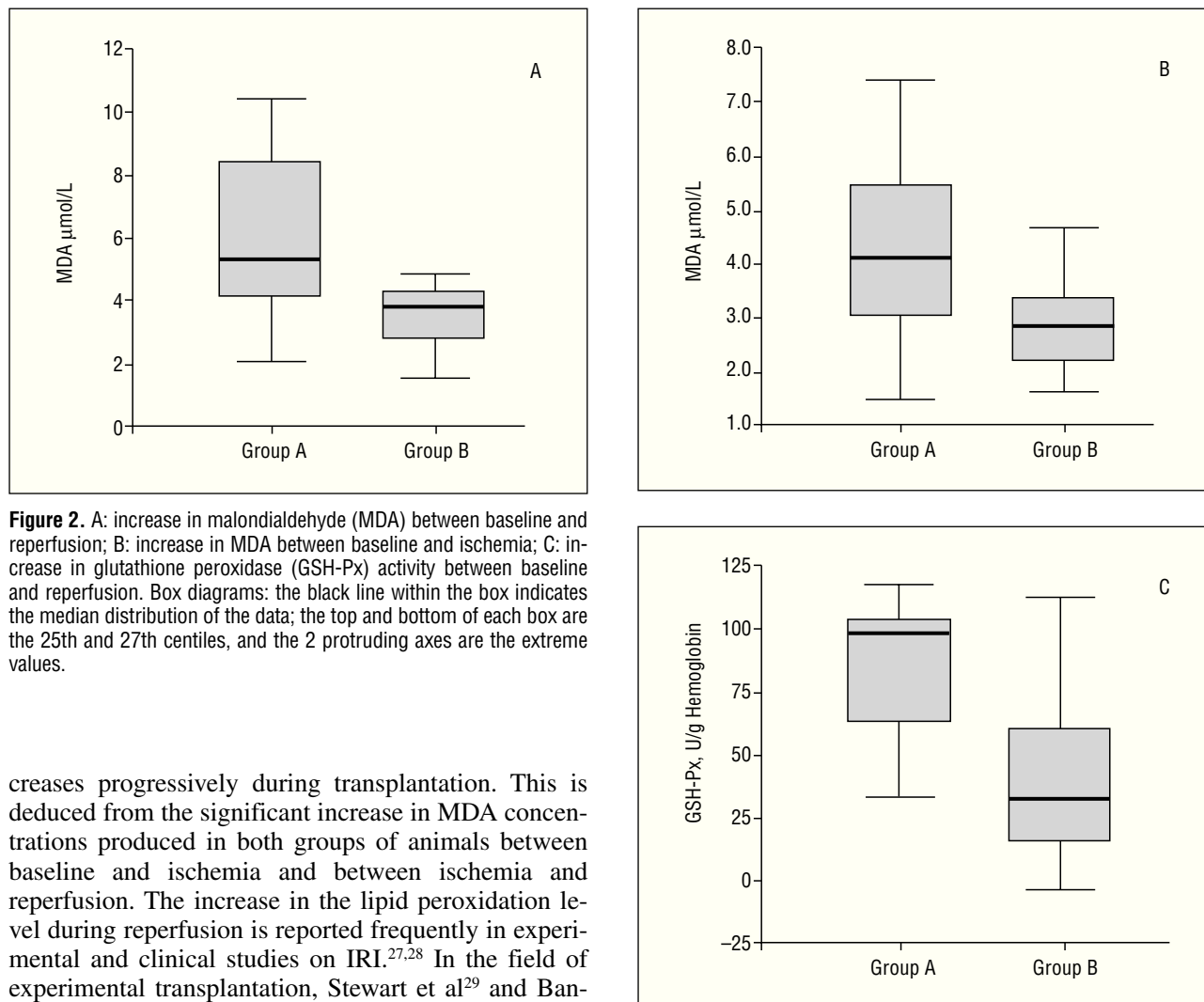
Figure 2. A: increase in malondialdehyde (MDA) between baseline and reperfusion; B: increase in MDA between baseline and ischemia; C: increase in glutathione peroxidase (GSH-Px) activity between baseline and reperfusion. Box diagrams: the black line within the box indicates the median distribution of the data; the top and bottom of each box are the 25th and 27th centiles, and the 2 protruding axes are the extreme values.

creases progressively during transplantation. This is deduced from the significant increase in MDA concentrations produced in both groups of animals between baseline and ischemia and between ischemia and reperfusion. The increase in the lipid peroxidation level during reperfusion is reported frequently in experimental and clinical studies on IRI.[27,28] In the field of experimental transplantation, Stewart et al[29] and Bando et al,[25] each using a different model of orthotopic HT, and Takeuchi,[30] in a model of heart-lung transplantation, have also observed an increase in MDA after reperfusion.

During HT, there is a reaction on the part of the cellular antioxidant systems that should be interpreted as a response to the progressive increase in OFR production. The augmentation of glutathione peroxidase and glutathione reductase activities results in the neutralization of hydroperoxides that react with transition metals to generate more free radicals, while the erythrocyte superoxide dismutase activity neutralizes the excess of superoxide radicals through the superoxide dismutase reaction. In patients undergoing cardiac surgery with cardiopulmonary bypass, the erythrocyte glutathione reductase activity also increases during reperfusion.[31] In an experimental model involving rats receiving a vitamin B_6-deficient diet, it has been shown that the higher the level of lipid peroxidation, the greater the cardiac glutathione peroxidase and glutathione reductase activities.[32] Other oxidizing agents, such as alcohol, physical exercise and smoking, have been associated with

an augmented activity of this enzyme system.[33,34] The response of superoxide dismutase to oxidative stress is not that uniform and, although an increase has been observed in its activity in the heart that parallels the increase in lipid peroxidation induced by exercise,[33] in other models, a decrease in the activity[34] or in the concentration of this enzyme[28] has been found. Lafont et al[35] have attributed the absence of changes in superoxide dismutase activity following coronary angioplasty for acute myocardial infarction to the short duration of the IRI process in comparison with erythrocyte half-life. This might explain the fact that, in our model, with a longer ischemic time, changes were observed.

The decrease in the retinol concentration during ischemia-reperfusion may be a consequence of its increased use in the neutralization of OFR. In several reports on experimental IRI[28,36] and in patients subjected to coronary angioplasty[35] or thrombolysis for acute myocardial infarction,[37] a decrease has been observed

in the vitamin E and A concentrations during reperfusion. In cardiac surgery, while Coghlan et al[38] have documented a reduction in alpha-tocopherol following coronary revascularization, other authors have detected no changes.[39] In this report, the fact that the greater production of OFR during ischemia-reperfusion was accompanied by a decrease in the retinol concentration, but not in that of vitamin E may be due to the fact that, despite the augmented use of alpha-tocopherol in the neutralization of a greater quantity of peroxyl radicals, it was rapidly regenerated from the tocopheroxyl radical.

The enhancement, during ischemia-reperfusion, of the total antioxidant status, which represents the overall, nonspecific, antioxidant potential in plasma, is a compendium of the behavior of the remaining antioxidants.

Effect of Trimetazidine

Trimetazidine (1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride; Servier, Courbevoie, France) is a drug substance that was introduced into human clinical practice in 1987 because its antiischemic effects are not accompanied by hemodynamic side effects.[40] Moreover, several experimental and clinical studies have demonstrated that it also exerts a cytoprotective effect, limiting IRI, through several mechanisms of action: potentiation of oxidative glucose metabolism, reduction of the degree of intracellular acidosis and hypercalcemia, and attenuation of the inflammatory response and OFR production.[12-16]

In our experimental HT model, TMZ had no impact on the degree of cell necrosis, but did exert a cardioprotective effect, reducing the level of lipid peroxidation generated by OFR during ischemia-reperfusion. This capacity of the drug to reduce the generation of OFR and the damage they induce in the cytoplasmic membrane has been confirmed in different experimental models involving the heart.[12,13,15] In clinical practice, its beneficial effect, which consists in the improvement of ventricular function following coronary artery surgery, has been related to the reduction of MDA in the coronary sinus during reperfusion.[21] In a porcine model of renal autotransplantation, Baumert et al[8] observed better preservation of mitochondrial integrity and better postoperative renal function after addition of TMZ to the preservation solution. In another study, involving single-lung transplantation in rat, Inci et al[10] obtained better postreperfusion oxygenation, greater cellular energy reserve and a lower level of lipid peroxidation after pretreatment of recipients with intravenous TMZ and its inclusion in the cardioplegic solution.

The lower level of oxidative stress during the ischemia and reperfusion phases may perfectly explain the change in the response pattern of the cellular antioxidant systems that occurred in the treated group. TMZ attenuated the activation of the glutathione-derived enzyme system and the consumption of retinol during reperfusion. In contrast to glutathione peroxidase, in the case of retinol and glutathione reductase, the differences did not reach statistical significance, probably due to the small sample size, but the trend was clear.

Limitations to the Study

The 2 groups of animals differed with respect to the basal glutathione peroxidase and superoxide dismutase activities, which were greater in group B, despite the fact that all the animals came from the same farm, received the same treatment and were fed the same diet, a circumstance that would presumably result in similar degrees of oxidative stress. The explanation may lie in the small sample size and in the wide variability in the level of activity of these enzymes occurring naturally in this species. There was also a considerable difference between the 2 groups in terms of baseline creatine kinase ($P=.08$), which may be a consequence of muscle injuries that the animals provoke in each other during transport and stabling.

HT is not an ideal model for the study of IRI since it involves hyperthermia, cardioplegia and cardiopulmonary bypass, all circumstances that can prove to be confounding factors in the evaluation of the cytoprotective activity of a drug. Hypothermia blocks E-selectin expression on the endothelial surface,[41] which may attenuate polymorphonuclear leukocyte activation and OFR generation. The cardioplegic solution contains antioxidant additives, such as mannitol, hisitidine and reduced glutathione. The contact of the blood with the cardiopulmonary bypass circuit leads to the production of a multitude of mediators that can activate the vascular endothelium and promote the generation of OFR. One way of avoiding this interference would have been to perform heterotopic HT under normothermia and without cardioplegia, but our intention was to reproduce to the greatest possible extent the conditions under which human HT is usually carried out, a setting in which TMZ will probably be employed in the future.

CONCLUSIONS

During the ischemia and reperfusion phases of HT the level of lipid peroxidation increases and the cellular antioxidant systems are activated, a circumstance that indicates an increasing production of OFR. Trimetazidine exerts a cytoprotective effect as it limits the IRI produced by the OFR and attenuates the response of said defense systems.

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