Persistence of Oxidative Stress After Heart Transplantation: a Comparative Study of Patients with Heart Transplant Versus Chronic Stable Heart Failure

Osvaldo Pérez, Pablo Castro, Guillermo Díaz-Araya, Danniel Nettle, Francisco Moraga, Mario Chiong, Jorge Jalil, Ricardo Zalaquett, Sergio Morán, Pedro Becker, Ramón Corbalán and Sergio Lavandero

Departamento de Enfermedades Cardiovasculares, Hospital Clínico, Pontificia Universidad Católica de Chile.
Departamentos de *Química Farmacológica y Toxicológica y de *Bioquímica y Biología Molecular.
Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.

Introduction and objective. Chronic heart failure (CHF) is associated with oxidative stress. Heart transplantation, an important therapeutic alternative in these patients, could reduce oxidative stress by improving cardiac function. Our aim was to evaluate post-heart transplantation oxidative stress.

Patients and method. We studied three experimental groups: a) heart transplant recipients without evidence of rejection (n = 11); b) NYHA class III CHF patients (n = 19), and c) healthy control subjects (n = 14). Oxidative stress was assessed by measuring plasma malondialdehyde levels (MDA), and determining the enzymatic activities of glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD).

Results. The demographic characteristics of the three groups were similar. Mean time from transplantation was 20.0 ± 4.8 months. Mean MDA plasma levels in heart transplantation and CHF patients were significantly higher than in normal subjects (3.35 ± 0.8; 3.27 ± 1.7 y 0.9 ± 0.3 µM, respectively). GSH-Px activity increased after transplantation compared to control subjects (0.40 ± 0.06 and 0.33 ± 0.05 U/g Hb, respectively), but not the CHF group. A significant decrease in SOD activity was found in the heart transplant vs. CHF group (0.44 ± 0.1 vs. 0.87 ± 0.6 U/mg Hb). There were no differences in CAT values between heart transplant and CHF patients.

Conclusion. These findings demonstrated the presence of permanent oxidative stress in patients who have undergone heart transplantation, characterized by an increase in MDA and a decrease in SOD activity, despite an increase in GSH-Px activity.

Key words: Stress. Transplantation. Heart failure.

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Persistencia del estrés oxidativo postrasplante cardíaco: estudio comparativo entre pacientes con trasplante cardíaco y con insuficiencia cardíaca crónica estable

Introducción y objetivo. Existe estrés oxidativo en pacientes con insuficiencia cardíaca crónica (ICC). El trasplante cardíaco, alternativa terapéutica importante en estos pacientes, podría disminuir el estrés oxidativo al mejorar la función cardíaca. Nuestro objetivo fue evaluar el estrés oxidativo postrasplante cardíaco.

Pacientes y método. Fueron estudiados 3 grupos experimentales: a) trasplantados cardiacos, sin evidencia de rechazo (n = 11); b) pacientes con ICC capacidad funcional III de la NYHA (n = 19), y c) sujetos controles sanos (n = 14). El estrés oxidativo se evaluó determinando valores plasmáticos de malondialdehído (MDA), y actividades de glutatión peroxidasa (GSH-Px), catalasa (CAT) y superóxido dismutasa (SOD).

Resultados. Las características demográficas fueron similares entre los grupos. El tiempo postrasplante fue 20,0 ± 4,8 meses. Los valores de MDA en heart transplantation and CHF fueron significativamente mayores que en sujetos normales (3.35 ± 0.8; 3.27 ± 1.7 y 0.9 ± 0.3 µM, respectivamente). La actividad de GSH-Px aumentó después de transplante comparado con control sujetos (0.40 ± 0.06 y 0.33 ± 0.05 U/g Hb, respectivamente), pero no el CHF grupo. Un descenso significativo en SOD actividad fue encontrado en el transplante vs. CHF grupo (0.44 ± 0.1 vs. 0.87 ± 0.6 U/mg Hb). No hubo diferencias en las actividades de CAT entre trasplantados y pacientes con ICC.

Conclusión. Los pacientes sometidos a trasplante cardíaco tienen un aumento del estrés oxidativo, evidenciado por una elevación del MDA y por una disminución de la actividad de SOD, a pesar de una mayor actividad de GSH-Px. Este aumento del estrés oxidativo fue similar al encontrado en pacientes con ICC estable CF III de la NYHA, y se observó en ausencia de episodios reconocidos de infección o rechazo.

Palabras clave: Estrés. Trasplante. Insuficiencia cardíaca.
INTRODUCTION

Although there have been important advances in the treatment of chronic cardiac insufficiency (CCI), the morbidity and mortality rates continue to be high. Various studies have shown an increase in oxidative stress in patients with CCI. The ischemia-reperfusion, increased neurohumoral activity, cytokine stimulation, and the presence of inflammatory cells are stimuli that generate free radicals and modifiers of the state of oxidative stress in CCI. Basic and clinical studies suggest that oxygen free radicals may contribute to a deterioration in cardiac function. Free radicals include the superoxide anions, hydroxyl radicals, peroxyl, and H$_2$O$_2$. These are products of the metabolism and are maintained in low concentrations via the action of diverse enzyme and non-enzyme antioxidant systems. Under normal conditions there is a balance between the production of oxygen free radicals and the defensive antioxidant systems. Any change in this balance in favor of free radicals produces oxidative stress.

Experiments in mice with acute myocardial infarction have suggested a slight relationship between the appearance of cardiac insufficiency and oxidative stress. In vitro studies have revealed that brief exposure to free radicals results in a decrease in high energy phosphates, a loss of contractile function, and structural abnormalities. High concentrations of pentanoyl coenzyme A, hydroxyl radicals, peroxyl, and H$_2$O$_2$. These are products of the metabolism and are maintained in low concentrations via the action of diverse enzyme and non-enzyme antioxidant systems. Under normal conditions there is a balance between the production of oxygen free radicals and the defensive antioxidant systems. Any change in this balance in favor of free radicals produces oxidative stress.

Patients with cardiac insufficiency

Nineteen patients, all male, with cardiac insufficiency were studied, with an average age of 61 years (range 44 to 75 years of age), NYHA FC III, stable, and following the usual treatment regimen (diuretics, digitalis, angiotensin enzyme conversion inhibitors [ACEI], and without beta-blockers at the time of the study). All the patients had dilatation and systolic dysfunction with an ejection fraction (EF) of <40% as determined by echocardiogram. The etiology was ischemia in 7 patients (37%), idiopathic dilated cardiomyopathy in 7 patients (37%), and hypertension in 5 patients (26%). Patients were excluded who had...
a) NYHA FC IV, cardiogenic shock; b) coronary bypass surgery, angioplasty, or myocardial infarct within the last 3 months; c) chronic angina; d) treatment changes or the use of beta-blockers within the last 2 months; e) significant valve disease; f) the presence of decompensatory factors such as acute coronary syndrome, valve dysfunction, arrhythmia, infection, serious anemia, hyperthyroidism, or pulmonary embolism, and g) the presence of conditions that could affect the determination of oxidative stress such as renal insufficiency (plasma creatinine >2 mg/dL), autoimmune disease, neoplasia, advanced pulmonary or liver disease, or acute or chronic inflammation.

Healthy control subjects

For ethical reasons a control group of patients receiving immunosuppressive medication could not be used. The control group was composed of 14 healthy subjects who were age- and sex-paired. All control subjects were asymptomatic, without significant morbid antecedents, and with a normal physical examination. We excluded subjects with known coronary risk factors, or who had been treated with any medication, vitamin supplements, antioxidants, or who regularly ingested alcohol.

All the subjects signed a consent form approved by the Ethics Committee of the Hospital Clínico of the Pontificia Universidad Católica of Chile.

Markers for oxidative stress

The parameters for oxidative stress were determined in peripheral blood. In the 3 groups, we measured the enzyme activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and MDA plasma values, for which we obtained 20 mL of blood via puncture of a peripheral vein. The sample was centrifuged at 3000 rpm for 10 minutes at a temperature of 4 °C. The plasma was separated and stored at –20 °C. The erythrocytes were washed 3 times with a saline solution and the lysates were stored a –20 °C.

**MDA**

The existence of MDA in plasma was made by measuring the contents of the reactive substances to thiobarbituric acid reactive substances (TBARS). The values were expressed as µM.

**SOD**

The hemolyzate was extracted using the method described by McCord et al. For the measurement of activity, we used Misra method. This is based on the determination of the speed of epinephrine auto-oxidation, which is accelerated by SOD. The oxidation level was determined by photocolorimetry at 480 nm. The activity was expressed as units (U) per mg of hemoglobin (Hb).

**CAT**

CAT was determined by the method described by Beers et al. The reaction was initiated by the addition of 1 mL of 30 mM H₂O₂. The change in absorption at 240 nm was measured for 1 minute at 25 °C. In one part of the remaining sample material the hemoglobin was determined and the activity was expressed as U (nm of H₂O₂/minute) per g Hb.

**GSH-Px**

GSH-Px was determined using the method described by Paglia et al and was expressed as U (nm oxidized NADPH/minute) per g Hb.

Statistical analysis

The Student t test was used for non-paired samples, and a value of P<.05 was considered significant. The values were presented as mean±standard deviation (SD).

RESULTS

Table 1 shows the clinical characteristics of the transplant patients and of the patients with cardiac in-
sufficiency. There was no significant age difference. Of the group with CCI, 5 were hypertensive, 3 diabetic, 4 dyslipidemic, and 6 had suffered an acute myocardial infarction (AMI). Thirteen patients (68%) used AECI, 16 diuretics (84%), and 14 digitalis (74%). None of the patients was a smoker at the time of the study. The EF was 33% in the group with CCI and 60% in the transplant group.

Figure 1 shows the results of the measurement of plasma MDA. The MDA values were found to be significantly increased in patients with CCI and cardiac transplant with respect to the control group (3.7±1.7; 3.35±0.8, and 0.9±0.3 µM, respectively), without there being significant differences between transplant patients and patients with CCI. Figures 2 through 4 show the results of the measurement of plasma activity of the SOD, CAT, and GSH-Px antioxidant systems, respectively. The SOD activity in the transplant group was lower with respect to the CCI and control groups (0.44±0.1 versus 0.87±0.6; 1.3±0.4 U/mg Hb, respectively; \( P < .05 \)). There was no significant difference in CAT activity between the CCI group and the transplant group.
The principal finding of this study was that patients who underwent cardiac transplant had an increase in oxidative stress, as evidenced by an increase in the concentration of plasma MDA. This increase in oxidative stress is similar to that found in patients with stable CCI of NYHA FC III, and it is seen in the absence of known episodes of rejection or infection.

SOD activity was found to be decreased in transplant patients with respect to the control group, while GSH-Px activity was found to be increased post-cardiac transplant with respect to the control group. On the other hand, no significant changes were observed in CAT activity between the CCI group and the transplant group. These results show that there is a change in the enzyme antioxidant systems in patients with CCI and transplant patients that causes an overall increase in oxidative stress.

The lipoperoxidation of the membranes is a relatively slow process. Nevertheless, recurrent cycles of ischemic reperfusion in the heart and the skeletal muscle and the auto-oxidation of catecholamines may increase lipoperoxidation of the membranes. The resulting increase in oxidative stress favors transition to a state characterized by depression of the cardiac function. This increase in oxidative stress has been demonstrated in patients with CCI through an increase in plasma MDA values. An increase in oxidative stress post cardiac transplant has been described in animals presenting with acute cardiac rejection. Chancerelle et al observed increased MDA values in transplant patients without evidence of rejection, and increased lipid peroxidation has been described as one of the mechanisms responsible for accelerated atherogenesis post-transplant. Recently, Schimke et al evaluated oxidative stress in myocardial tissue biopsies during distinct post-cardiac transplant periods in patients without acute rejection. They observed an increase in MDA during the first 3 months post-transplant, a fall in the MDA level, and a later increase after the first year. The increase in oxidative stress during the first period was attributed to the transplant procedure itself (ischemia of the donor heart, ischemia and reperfusion time, viral or bacterial infections). The membrane changes (lipoperoxidation) due to elevated oxidative stress could be partially responsible for these disease manifestations.

Based on animal studies, Kirshenbaum and Singal postulated that when an increase in the generation of oxygen free radicals is produced, the heart, in an adaptive response, increases the enzyme defense systems. Therefore, oxidative stress may serve to prevent or minimize this. Nevertheless, the adaptive response is limited, occurring in very advanced stages, as for example in the case of CCI, a deficit in the antioxidant enzyme systems.

These findings are interesting, as it has been suggested that SOD and GSH-Px are more important in the detoxification of reactive-type metabolites in the heart. SOD is the first line of defense against damage caused by free radicals, and acts by increasing H$_2$O$_2$ values. The principal damage caused by the accumulation of H$_2$O$_2$ is the production of the highly reactive hydroxyl radical, against which no physiological defense systems exist. As a result, CAT and GSH-Px become the most crucial antioxidant enzymes in this group of patients with low SOD activity. Additionally, exhaustion of the enzyme antioxidant systems can be produced by direct damage caused by free radicals, as it has been shown that H$_2$O$_2$ inactivates SOD, and decreased values of SOD in plasma have been found in mice post cardiac transplant.

The study by Schimke et al showed an increase in GSH-Px and SOD activity post transplant. Nevertheless, after the first year, SOD activity tended to fall off, which coincided with the increase in oxidative stress. In our study, together with the increase in MDA concentrations in plasma, we found an increase in GSH-Px activity; nevertheless, SOD activity was reduced, which would translate into an insufficient adaptive response. This increased state of oxidative stress has been demonstrated in patients with CCI by an elevation in MDA plasma values. In a series of patients with refractory CCI, in addition to increased MDA values, we demonstrated a decrease in GSH-Px activity (presented at the XXII Annual Congress of the European Cardiology Society, Amsterdam, August, 2000).

Given that CAT has a lower affinity for H$_2$O$_2$, it has been postulated that the GSH-Px system is the principal metabolic route of H$_2$O$_2$ in the heart. Nevertheless, CAT allows the cell to decompose H$_2$O$_2$ independently of the intracellular glutation concentration, given that it has been shown that the content of plasma thiol, an indicator of the oxidative state of the extracellular medium, is reduced in patients with CCI. On the other hand, Dieterich et al found an increase in the expression of CAT in the hearts of patients with end-stage CCI, and they considered this induction to be a com-
pensatory response of the heart against the elevated oxidative stress in these patients, while SOD and GSH-Px activity remained unchanged.

Study limitations
Among the limitations of our study is the small number of patients studied. This was a comparative study and not a follow-up study, so that there is no demonstration of the changes attributable to cardiac transplant. Oxidative stress was not evaluated in the myocardium but in the plasma, with the limitations of extrapolating the results to what happens in the myocardium or to reflect on what may have occurred in other organs; although blood can reflect the capacity of the entire organism to react against oxidative stress, and its values and enzyme activities may express multiple sources of oxidative stress, among them the striated muscle of cardiac insufficiency.

CONCLUSION
There is a state of increased oxidative stress post-cardiac transplant that is comparable to that of patients with stable CCI of NYHA FC III. Additionally, we observed an increase in GSH-Px antioxidant activity along with a significant decrease in SOD activity.

REFERENCES