

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

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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 M, 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 cardíacos, 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 $20,0 \pm 4,8$ meses. Los valores de MDA en trasplantados y con ICC fueron significativamente mayores que en sujetos normales ($3,35 \pm 0,8$; $3,27 \pm 1,7$, y $0,90 \pm 0,3$ M, respectivamente). La actividad de GSH-Px aumentó en trasplantados respecto al grupo control ($0,40 \pm 0,07$ y $0,33 \pm 0,05$ U/g Hb, respectivamente). La actividad de SOD fue menor en trasplantados respecto al grupo control ICC ($0,44 \pm 0,1$ frente a $0,87 \pm 0,6$ 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.

ABBREVIATIONS

CCI: chronic cardiac insufficiency.
MDA: malondialdehyde.
GSH-Px: glutathione preoxidase.
CAT: catalase.
SOD: superoxide dismutase.

INTRODUCTION

Although there have been important advances in the treatment of chronic cardiac insufficiency (CCI), the morbidity and mortality rates continue to be high.¹ 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.²⁻⁵ Basic and clinical studies suggest that oxygen free radicals may contribute to a deterioration in cardiac function.²⁻⁷ Free radicals include the superoxide anions, hydroxyl radicals, peroxy, and H₂O₂.⁸ 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 the free radicals produces oxidative stress.

Experiments in mice with acute myocardial infection have suggested a slight relationship between the appearance of cardiac insufficiency and oxidative stress.³ *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.⁹ High concentrations of pentane have been found in exhaled air as well as has an increase in the plasma values of malondialdehyde (MDA), an indirect marker for lipid peroxidation in patients with CCI.¹⁰⁻¹² In addition, high concentrations of MDA in plasma correlate with increased seriousness of symptoms and functional compromise in patients with CCI.¹³

Different therapeutic interventions have been used to decrease oxidative stress in patients with CCI. On the experimental level, using models of ischemia and reperfusion, it has been shown that the administration of antioxidants prevents or delays the transition from a state of compensated cardiac insufficiency to one of decompensated cardiac insufficiency.¹⁴ At the clinical level, chronic beta-blocker therapy in patients with CCI decreases oxidative stress.¹⁵ Cardiac transplant is a consolidated therapeutic alternative in patients with CCI and serious functional limitations.¹⁶ Theoretically, cardiac transplant can decrease oxidative stress by im-

proving cardiac function. Nevertheless, animal studies have demonstrated greater metabolism of oxygen free radicals with a consequent increase in oxidative stress.¹⁷⁻¹⁹ Few clinical studies have evaluated the state of oxidative stress post cardiac transplant, and some authors have related it to the presence of infection, rejection, or the use of immunosuppressive medications.²⁰⁻²² Recently, an increase in oxidative stress has been attributed to a decrease in the enzyme antioxidant capacity 1 year post transplant.²³

The purpose of this study was to analyze the state of post cardiac transplant oxidative stress by measuring MDA levels in plasma and the activity of different enzyme antioxidant systems. The measurements were obtained in patients with CCI and in a group of healthy controls, and were then compared.

PATIENTS AND METHODS

Cardiac transplant patients

This study included 11 patients with cardiac transplants (8 men, 3 women), with an average age of 48 years (range 16 years to 62 years). The average amount of time post transplant was 20 months (range 12 months to 26 months). The immunosuppressive protocol consisted of cyclosporin A, azathioprin, and steroids. Endomyocardial biopsies were performed on all patients within 2 weeks of the measurement of the oxidative stress parameters. The biopsies were performed by jugular puncture according to the classic technique and disposable biotopes, from which at least 4 samples were obtained for diagnostic study under the microscope. The biopsies were analyzed following the classification of the Heart Rejection Study Group of the International Society for Heart and Lung Transplantation.²⁴ None of the patients had evidence of cardiac rejection per the biopsies performed or evidence of infection for at least the 3 preceding months. Echocardiograms of all patients showed a normal ejection fraction and Class I NYHA functional capacity (FC).

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, angiotensive enzyme conversion inhibitors [AECI], 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 myocardiopathy 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 de 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 at -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).²⁵ The values were expressed as μM .

SOD

The hemolyzate was extracted using the method described by McCord et al.²⁶ For the measurement of activity, we used Misra method.²⁷ This is based on the determination of the speed of epinephrine auto-oxidation, which is accelerated by SOD. The oxidation level

was determined by photocolormetry 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.²⁸ The reaction was initiated by the addition of 1 mL of 30 mM H_2O_2 . 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 H_2O_2 /minute) per g Hb.

GSH-Px

GSH-Px was determined using the method described by Paglia et al.²⁹ 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 \pm standard deviation (SD).

RESULTS

Table 1 shows the clinical characteristics of the transplant patients and of the patients with cardiac in-

TABLE 1. Characteristics of the study population

Characteristics	Transplant patients	CCI	P
General			
Number	11	19	
Age	48 \pm 20	61 \pm 10	NS
Sex (M/F)	8/3	19/0	
Risk factors (%)			
Hypertension (%)	2 (18)	5 (26)	
Diabetes mellitus	-	3 (16)	
Dyslipidemia	2 (18)	4 (21)	
AMI	-	6 (32)	<.05
Smoking	-	-	
Medications, no. (%)			
AECI	3 (27)	13 (68)	<.05
Diuretics	2 (18)	16 (84)	<.05
Digitalis	-	14 (74)	<.05
Statins	11 (100)	4 (21)	<.05
Hydralazine	-	6 (32)	<.05
NYHA Functional Class (%)			
I	11 (100)	-	
II	-	2 (10)	
III	-	17 (90)	
IV	-	-	
EF	60 \pm 10	33 \pm 12	<.001
Time post-transplant (months)	20	-	

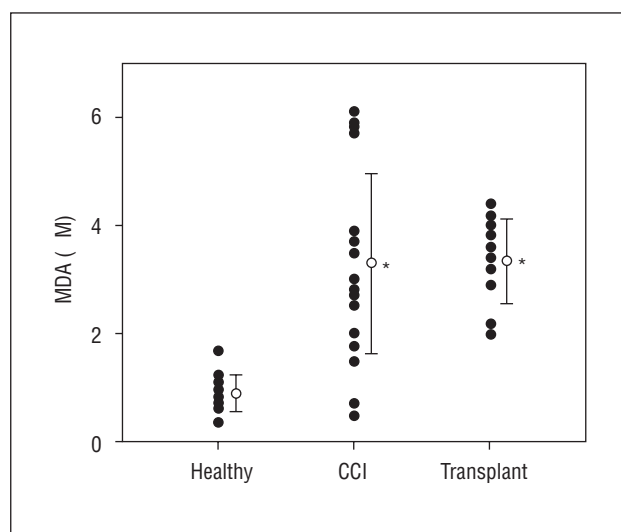


Fig. 1. Malondialdehyde (MDA) plasma values control subjects (n=14), CCI patients (n=19), and cardiac transplant patients (n=11). The values correspond to the average±standard deviation. * $P<0.05$ versus control.

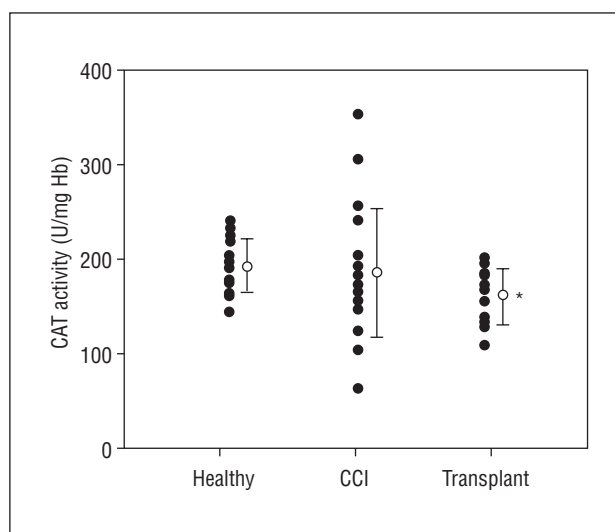


Fig. 3. Catalase (CAT) plasma activity in control subjects (n=14), patients with CCI (n=19), and cardiac transplant patients (n=11). The values correspond to average±standard deviation. * $P<0.05$ versus control.

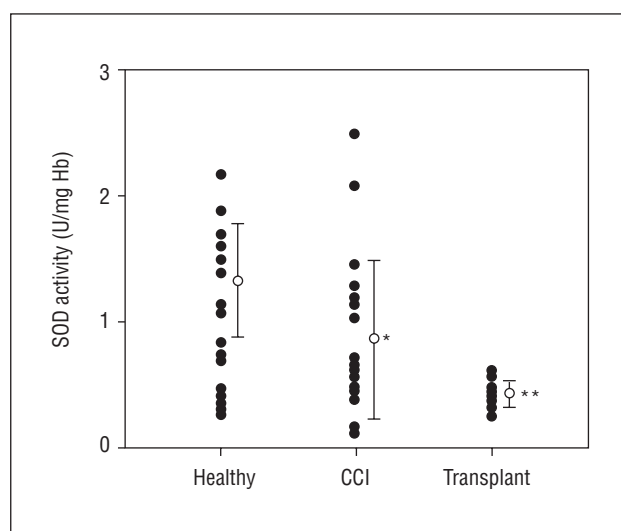


Fig. 2. Superoxide dismutase (SOD) plasma activity in control subjects (n=14), patients with CCI (n=19), and cardiac transplant patients (n=11). The values correspond to average±standard deviation. * $P<0.05$ and ** $P<0.05$ versus the control group and CCI group, respectively.

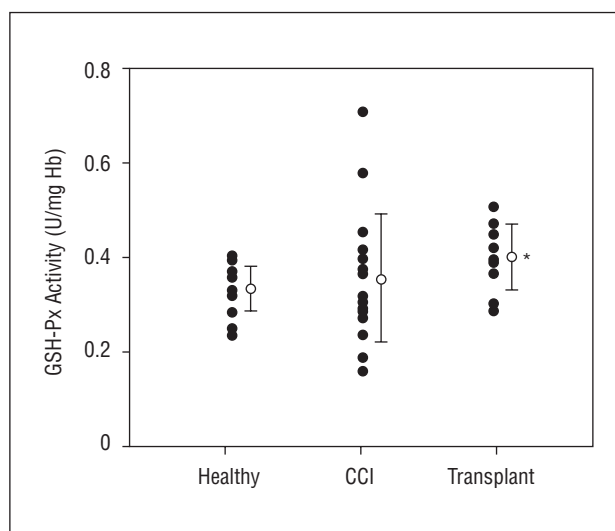


Fig. 4. Glutathione peroxidase (GSH-Px) plasma activity in control subjects (n=14), patients with CCI (n=19), and cardiac transplant patients (n=11). The values correspond to average±standard deviation. * $P<0.05$ versus control.

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 ACEI, 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 measurements 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 $0.9 \pm 0.3 \mu\text{M}$, 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; $P<0.05$). There was no significant difference in CAT activity between the CCI group and the trans-

plant group (187 ± 67 and 62 ± 31 U/mg Hb, respectively), although it was lower in the transplant group as compared to the control group (193 ± 29 U/mg Hb; $P < .05$). On the other hand, the GSH-Px activity was greater in the transplant group with respect to the control group (0.40 ± 0.07 and 0.33 ± 0.05 U/g Hb; $P < .05$), while there was no significant difference between the CCI group and the control group.

DISCUSSION

The principal finding of this study was that patients who underwent cardiac transplant had an increase in oxidative stress, as evinced 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.³⁰ An increase in oxidative stress post cardiac transplant has been described in animals presenting with acute cardiac rejection.¹⁷ Chancerelle et al³¹ observed increased MDA values in transplant patients without evidence of rejection, and increased lipidic peroxidation has been described as one of the mechanisms responsible for accelerated atherogenesis post-transplant.³² Recently, Schimke et al²³ 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).³³⁻³⁵ The membrane changes (lipoperoxidation) due to elevated oxidative stress could be partially responsible for these disease manifestations.

Based on animal studies, Kirshenbaum and Singal³⁶ 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.³⁷ It has been reported that cardiac hypertrophy in mice and guinea pigs is associated with a decrease in the oxidative stress state and an increase in the antioxidant reserve,^{11,12} while CCI under acute and chronic conditions is associated with an increase in oxidative stress and a reduced antioxidant reserve.^{3,7} 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.³⁸ SOD is the first line of defense against damage caused by free radicals, and acts by increasing H_2O_2 values. The principal damage caused by the accumulation of H_2O_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_2O_2 inactivates SOD,³⁹ and decreased values of SOD in plasma have been found in mice post cardiac transplant.¹⁸

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.²³ 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.³² 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_2O_2 , it has been postulated that the GSH-PX system is the principal metabolic route of H_2O_2 in the heart. Nevertheless, CAT allows the cell to decompose H_2O_2 independently of the intracellular glutation concentration, given that it has been shown that the content of plasma thiols, an indicator of the oxidative state of the extracellular medium, is reduced in patients with CCI.⁴⁰ On the other hand, Dieterich et al⁴¹ 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.

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