Effects of Early Decrease in Oxidative Stress After Medical Therapy in Patients With Class IV Congestive Heart Failure

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It has been reported that patients with congestive heart failure (CHF) have increased breath pentane content, conjugated diene levels, and plasma malondialdehyde (MDA) levels, an indirect marker of lipid peroxidation.[1–3] Ghatak et al[4] found that patients with chronic CHF had increased MDA and superoxide levels, which correlated with the severity of the CHF. Low glutathione levels and superoxide dismutase (SOD) activity have also been reported.[5,6] There have been no studies in human refractory CHF to evaluate the impact of acute intensive medical therapy on oxidative stress status and antioxidant enzyme activity. We determined the plasma levels of MDA, SOD, catalase (CAT), and glutathione peroxidase (GSH-Px) activities before and after therapeutic intervention in patients with chronic advanced CHF and refractory symptoms (New York Heart Association functional class IV).

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We enrolled 15 patients admitted to our Coronary Care Unit with the diagnosis of refractory CHF. All patients signed an informed consent approved by our Institutional Review Board and Ethical Committee. Inclusion criteria were: (1) persistence of pulmonary congestion, edema, or worsening of renal function despite optimal treatment with diuretics, digitalis, and vasodilators; (2) evidence of left ventricular dilation and systolic dysfunction as determined by echocardiography, with a left ventricular ejection fraction

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TABLE 1 Clinical Characteristics of Patients With Refractory Heart Failure (n = 15)

Age (yrs)	63 ± 14
Men	13 (90%)
Etiology	
Ischemic	11 (73%)
Idiopathic	3 (20%)
Hypertensive	1 (7%)
History	
Myocardial infarction	11 (73%)
Coronary bypass surgery	6 (40%)
Valvular replacement	2 (13%)
Atrial fibrillation	4 (27%)
Previous 6 mo admission	7 (47%)
Clinical presentation	
Pulmonary congestion	9 (60%)
Pulmonary congestion + right heart failure	6 (40%)
Long-term therapy	
Angiotensin-converting enzyme inhibitors	10 (66%)
Type 1 angiotensin II receptor antagonists	2 (13%)
Hidralazine + isosorbide	3 (20%)
Digitalis	8 (53%)
Diuretics	15 (100%)
Spironolactone	2 (13%)
Left ventricular parameters	
Ejection fraction (%)	26 ± 7
End-diastolic diameter (mm)	73 ± 12
End-systolic diameter (mm)	61 ± 13

≤35%; and (3) treatment with intravenous inotropes or vasodilators for 48 to 72 hours in addition to conventional therapy. We excluded patients with changes in maintenance therapy or who used β blockers in the last 2 months before the study, as well as patients with cardiogenic shock, coronary artery bypass surgery, angioplasty, or myocardial infarction in the last 6 months, patients with chronic angina, uncontrolled hypertension, significant valvular disease, correctable precipitating factors of decompensation (i.e., acute coronary syndrome, acute valvular dysfunction, infection, arrhythmia, severe anemia, hyperthyroidism, or pulmonary embolism), and presence of other conditions that affect determination of oxidative stress status, such as renal insufficiency (plasma creatinine >2 mg/dl), autoimmune diseases, neoplasia, advanced liver or pulmonary disease, and acute or chronic inflammation.

The control group was composed of 14 healthy volunteers matched by age and sex. They were all asymptomatic, with an unremarkable medical history and a normal physical examination. Control subjects were excluded if they had any known coronary risk factor, if they were taking any medications, vitamin supplements, antioxidants, or if they drank alcohol on a regular basis.

Once the diagnosis of advanced refractory CHF was established, patients were admitted to the coronary care unit where the vasoactive drugs were administered. This consisted of dobutamine in 9 patients, dobutamine plus dopamine in 6 patients (doses ranging from 4 to 6 μg/kg/min); 7 patients also received nitroglycerin and 1 patient also received sodium nitropruside. Infusions were maintained for 72 hours. The mean maximal dose of dobutamine and dopamine were 4.5 ± 0.5 and 2.6 ± 0.8 μg/kg/min, respectively. Intravenous diuretics were prescribed when appropriate.

Before treatment and at the end of 5 days, the following protocol was performed. We utilized the Lee clinical score (scale 1 to 13 points).[7] This score is based on a combination of clinical and radiographic observations. In each patient, we determined the value of the score at the time of admission and on day 5. A favorable clinical response was defined as an improvement of ≥3 points in the clinical score. Daily weight and a strict inputs and outputs were registered. Other data registered included detailed evaluations of renal function and electrolytes, medications, and resource utilization. Noninvasive indexes of left ventricular function (left ventricular end-diastolic and end-systolic diameters), as well as left ventricular ejection fractions were determined by Doppler 2-dimensional echocardiography shortly after admission. Pulmonary and visceral congestion indexes were determined by the alveolar-arterial oxygen gradient, the degree of congestion on chest x-ray (scale 1 to 3),[7] and liver function tests at baseline and on day 5.

Blood was obtained by venipuncture at baseline and on day 5. The sample was centrifuged and plasma was stored at −20°C. The erythrocytes were washed 3 times with saline solution, homogenized, and centrifuged in the same manner. Cell lysates were prepared by adding 0.1-ml cell suspension to 0.4-ml distilled water and stored at −20°C.

MDA assay: Lipid peroxide formation was determined by examining the content of thiobarbituric acid reactive substances as previously described.[8] A standard commercially available MDA assay (Merck, Darmstad, Germany) was used.

SOD activity: SOD was extracted from the hemolyzed erythrocytes according to the method of McCord and Fridovich[9] and its activity was assayed as described by Misra and Fridovich[10] SOD activitiy was expressed as a unit per milligram of hemoglobin.

CAT activity: The activity was determined by the method described by Beers and Sizer.[11] Activity was expressed as a millimole of hydrogen peroxide per minute per gram of hemoglobin.

GSH-Px activity: The enzymatic activity was determined by the method described by Paglia and Valentine[12] Enzymatic activity was expressed as nanomoles of reduced nicotinamide-adenine dinucleotide phosphate oxidized per minute per gram of hemoglobin.

Results are presented as mean ± SEM. We utilized Student's *t* test for paired samples for the comparison of the oxidative stress parameters on admission and at day 5. Student's *t* test for nonpaired samples was utilized for the comparison of the oxidative stress parameters between patients with heart failure and controls. A p value <0.05 was considered statistically significant.

The clinical characteristics of patients are listed in Table 1. Most patients had ischemic dilated cardiomyopathy, and 6 underwent previous myocardial revascularization surgery. Seven patients had been admitted within the last 6 months for decompensated

TABLE 2 Clinical Evolution of Patients With Decompensated Refractory Heart Failure (n = 15)

	Baseline	5 Days	p Value
Clinical score (1–13 points)	11.4 ± 1.1	6.6 ± 2.9	<0.001
Weight (kg)	72 ± 10	71 ± 11	0.006
Systolic blood pressure (mm Hg)	126 ± 25	112 ± 18	0.02
Diastolic blood pressure (mm Hg)	83 ± 14	63 ± 7	0.001
Heart rate (beats/min)	93 ± 16	82 ± 10	0.07
Blood ureic nitrogen (mg/dl)	30 ± 15	31 ± 19	0.36
Plasma sodium (mEq/l)	134 ±3.5	135 ± 3.3	0.33
Creatinine (mg/dl)	1.4 ± 0.3	1.3 ± 0.5	0.6
Lactic acid (mmol/L)	1.9 ± 1.0	1.4 ± 0.5	0.18
Congestion indexes			
Alveolar-arterial O_2 gradient (mm Hg)	282 ± 151	101 ± 86	<0.001
Pulmonary congestion index (1–3)	2.9 ± 0.4	1.4 ± 0.8	<0.001

TABLE 3 Clinical Evolution of Patients With Decompensated Refractory Heart Failure With Clinical Improvement After Therapy (n = 13)

	Baseline	5 Days	p Value
Clinical score (1–13 points)	11.3 ± 1.1	5.4 ± 0.9	<0.0001
Weight (kg)	72 ± 11	69 ± 11	0.0009
Systolic blood pressure (mm Hg)	131 ± 24	114 ± 19	0.0033
Diastolic blood pressure (mm Hg)	84 ± 15	63 ± 8	0.0033
Heart rate (beats/min)	93 ± 12	80 ± 9	0.01
Blood ureic nitrogen (mg/dl)	25 ± 9	27 ± 12	0.62
Plasma sodium (mEq/l)	133 ± 3	136 ± 3	0.024
Creatinine (mg/dl)	1.3 ± 0.4	1.2 ± 0.3	0.63
Lactic acid (mmol/l)	1.7 ± 0.8	1.1 ± 0.2	0.15
Congestion indexes			
Alveolar-arterial O_2 gradient (mm Hg)	279 ± 130	98 ± 80	<0.001
Pulmonary congestion index (1–3)	2.7 ± 0.4	1.12 ± 0.6	<0.001

CHF. Ten patients were in sinus rhythm, 4 were in atrial fibrillation, and 1 had a predominantly paced rhythm. In addition, 4 patients had left bundle branch block and 4 had right bundle branch block. Ten patients had evidence of previous myocardial infarction by electrocardiographic criteria. The mean left ventricular ejection fraction was 26 ± 7% (range 12% to 35%). The mean end-diastolic diameter was 73 ± 12 mm, the end-systolic diameter was 61 ± 13 mm, and the left atrial maximum diameter was 52 ± 8 mm. Chest x-rays showed cardiomegaly (cardiothoracic index >0.5) and pulmonary congestion in all patients.

There was a marked improvement (≥3 points) in the clinical heart failure score from day 1 to day 5 in 13 of the 15 patients. This was associated with a reduction in body weight, a significant decrease in the alveolar-arterial oxygen gradient, and a decrease in the radiologic pulmonary congestion index. Liver function tests did not change significantly (Table 2).

Table 3 shows the changes observed in the clinical and laboratory parameters in the 13 patients who had an improved clinical condition after therapy. These patients had a significant decrease in heart rate and an increase in plasma sodium concentrations.

Two patients had no significant improvement in their clinical score (13 to 11 and 11 to 11 points, respectively). One of them died and the other continued to be dependent on intravenous inotropic drugs; these patients eventually underwent heart transplantation.

Table 4 shows the changes observed in oxidative stress parameters from admission to day 5 and the comparison with the control subjects. Patients with CHF had a significant elevation of MDA levels on admission and less activity of the enzyme GSH-Px compared with the control group. All of the patients with CHF did not have significant changes in oxidative stress after therapy.

Table 5 shows the changes in oxidative stress parameters in the subset of patients that improved with therapy. There was a significant reduction in MDA levels on day 5 that did not reach normal values. There were no significant changes in the activity of antioxidant enzymes, although there was trend toward decreased activity in the enzyme CAT. In the 2 patients without clinical improvement with therapy, MDA levels increased from 2.2 to 3.1 and from 2.1 to 3.3 nmol/L, respectively.

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In the present study, we found that patients with refractory CHF had an increase in oxidative stress, as determined by higher plasma MDA levels and lower GSH-Px activity, when compared with age-matched controls. After intensive therapy, the MDA levels decreased in patients with significant clinical improvement and without changes in the activity of antioxidant enzymes. This finding could reflect a decrease in oxygen radical species with therapy. Few studies have explored the short-term neurohormonal changes associated with intensive therapy of chronic decompensated heart failure. Missouris et al[13] studied 9 patients with decompensated heart failure. After treatment, there was a significant reduction of plasma brain and atrial natriuretic peptides and noradrenaline plasma levels.

Despite the significant decrease of MDA, levels did not reach normal values. This finding could be related to a persistent neurohormonal activation after therapy. These findings agree with previous investigations, in which we determined noradrenaline plasma levels at baseline and after 5 days of inotropic support.[14] Our patients had more advanced CHF than the patients studied by Missouris et al[13] with a higher plasma levels of noradrenaline at baseline. After inotropic support, we did not demonstrate any significant changes in noradrenaline plasma levels. This observation suggests that a longer period of intensive or conventional therapy might be necessary to obtain a more pronounced and sustained decrease in oxidative stress.

Our data do not show the tissue origins of MDA; both poorly perfused peripheral tissues (i.e., skeletal muscles, guts, kidneys) and the myocardium could

TABLE 4 Evolution of Oxidative Stress Parameters in Patients With Refractory Heart Failure Compared With Control Group

	Baseline (n = 15)	5 days (n = 15)	Controls (n = 14)	p Value*	p Value†	p Value‡
MDA (μmol/L)	2.7 ± 1.8	2.0 ± 0.9	0.9 ± 0.3	0.19	0.014	0.004
SOD (U/mg hemoglobin)	2.0 ± 1.5	1.7 ± 1.3	1.3 ± 0.4	0.34	0.97	0.26
CAT (U/g hemoglobin)	186 ± 46	161 ± 63	193 ± 28	0.36	0.65	0.10
GSH-Px (U/g hemoglobin)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.46	0.011	0.04

*Baseline versus 5 days; †baseline versus controls; ‡5 days versus controls.

TABLE 5 Evolution of Oxidative Stress Parameters in Patients With Refractory Heart Failure With Clinical Improvement After Intensive Therapy Compared With Control Group

	Baseline (n = 13)	5 Days (n = 13)	Control (n = 14)	p Value*	p Value†	p Value‡
MDA (μmol/L)	3.1 ± 1.7	1.9 ± 1.0	0.9 ± 0.3	0.02	0.014	0.001
SOD (U/mg hemoglobin)	2.1 ± 1.6	2.0 ± 1.3	1.3 ± 0.4	0.51	0.97	0.08
CAT (U/g hemoglobin)	189 ± 47	173 ± 66	193 ± 28	0.06	0.65	0.35
GSA-Px (U/g hemoglobin)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.37	0.011	0.09

*Baseline versus 5 days; †baseline versus controls; ‡control versus 5 days.

have contributed to this result.[2,15] Poor nutritional status and increased metabolic rate may also be factors.[16] Finally, we did not address the mechanisms of increased lipid peroxidation, but this mechanism has been attributed to an increase in catecholamines, cardiac sympathetic tone, microvascular reperfusion injury, inflammatory response, and cytokine stimulation.[2,17]

In summary, we demonstrated that clinical improvement after intensive medical therapy in patients with decompensated CHF is associated with a decrease in patients' oxidative stress status without changes in antioxidant enzyme activities.

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