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Antioxidant and Oxidative Stress Changes during Heart Failure Subsequent to Myocardial Infarction in Rats

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Antioxidant enzyme activities and oxidative stress were evaluated in the myocardium in relation to hemodynamic function subsequent to myocardial infarction in rats. One week after the coronary ligation, the left ventricular peak systolic pressure, left ventricular end-diastolic pressure, and aortic pressures remained near control values and there were no differences in lung and liver wet/dry weight ratios between experimental and control animals. In the 4-, 8-, and 16-week experimental animals, there was a progressive drop in left ventricular peak systolic pressure and an increase in left ventricular end-diastolic pressure. Aortic systolic pressure was depressed at 8 and 16 weeks. In myocardial infarct rats, there was a significant increase in wet/dry weight ratio of lungs at 8 weeks and at 16 weeks; this ratio was increased for lungs as well as liver. Based on the hemodynamic data as well as other observations, animals in the 1-, 4-, 8-, and 16-week groups were arbitrarily categorized into nonfailure and mild, moderate, and severe failure stages, respectively. In the nonfailure stage, there was a marginal increase in superoxide dismutase, glutathione peroxidase, and catalase activities as well as vitamin E levels. The redox state in these hearts, assessed by the reduced/oxidized glutathione ratio, was significantly increased. Superoxide dismutase activity was unchanged in mild and moderate failure stages but significantly depressed at 16 weeks. Glutathione peroxidase and catalase activities showed progressive decreases through mild, moderate, and severe failure stages. Vitamin E levels were significantly depressed at moderate and severe failure stages. There was a progressive increase in lipid peroxidation at mild, moderate, and severe stages of heart failure and the redox ratio was significantly depressed in the severe failure stage. These data suggest that heart failure subsequent to myocardial infarction may be associated with an antioxidant deficit as well as increased myocardial oxidative stress. (Am J Pathol 1996, 148:291–300)

Congestive heart failure (CHF) after myocardial infarction (MI) is a common clinical syndrome with grave prognosis. Loss of contracting myocardium due to MI results in a chronic increase in the work load of the remaining viable myocardium. The heart responds with an increase in muscle mass and this process of heart hypertrophy represents a fundamental compensatory mechanism that permits the ventricle to sustain normal perfusion pressure.[1] However, if this increased load on the heart is allowed to continue for a prolonged period, cardiac pumping function is compromised and heart failure supervenes despite the presence of hypertrophy. Research on heart failure has resulted in several postulates to explain the pathophysiology of this disorder. In this regard, data from animal experiments have suggested that increases in free radical formation and subsequent oxidative stress associated with the occurrence of a relative deficit in the endogenous antioxidant reserve may be one of the mechanisms for the development of CHF.[2,3] Evaluation of the pentane content in exhaled air in patients has been used as an index of free radical injury and lipid peroxidation.[4] Increase in pulmonary pentane excretion in heart failure patients with acute MI,[5] coronary artery disease,[6] angioplasty,[7] and other

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conditions[8] have been documented. Although data from patients[4–8] as well as animal experiments[2,3] provide a strong indication of the involvement of increased free radical production and lipid peroxidation in the pathogenesis of myocardial dysfunction subsequent to MI, direct information on myocardial antioxidant changes is lacking.

MI subsequent to coronary ligation in rat hearts has been reported to be accompanied by hypertrophy of the viable myocardium that in prolonged conditions is followed by different stages of failure.[9,10] Heart hypertrophy due to chronic pressure overload in guinea pigs and rats is accompanied by an increase in the endogenous antioxidant reserve with a concomitant decrease in lipid peroxidation and oxidative stress.[2,11] On the other hand, heart failure in a variety of animal models was accompanied by a decrease in the antioxidant reserve and increased lipid peroxidation.[11–13] However, changes in myocardial antioxidants as well as oxidative stress in CHF subsequent to MI have not been described. In the present study, we examined changes in the myocardial antioxidant defense systems as well as oxidative stress in relation to the hemodynamic function at 1, 4, 8, and 16 weeks after the coronary ligation in rats.

Materials and Methods

Experimental Model

MI was produced in male Sprague-Dawley rats weighing 150 ± 10 g via occlusion of the left coronary artery.[14] In this procedure, the animals were anesthetized with ether, the skin was incised along the left sternal border, and the third and fourth ribs were cut proximal to the sternum with the subsequent insertion of retractors. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated approximately 1 to 2 mm from its origin with a 6–0 silk thread. After ligation, the heart was repositioned in the chest. Closure of the incision was accomplished by a purse-string suture. Throughout this surgical procedure, rats were maintained on a positive pressure ventilation delivering a mixture of 95% O_2 and 5% CO_2 mixed with ether. The mortality of all animals operated upon in this fashion was 35% within the first 24 hours. Control animals were treated in a similar fashion with the exception that the suture around the left coronary artery was not tied. No mortality was observed in the sham control animals within 24 hours of the operation. After the operation, animals were allowed to recover. Animals were provided food and water *ad libitum* and were used at 1, 4, 8, and 16 weeks for different studies.

Hemodynamic Studies

Animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). A miniature pressure transducer (Millar Micro-Tip) was inserted into the right carotid artery and then advanced into the left ventricle. Left ventricular end-diastolic (LVEDP), left ventricular peak systolic (LVPSP), and aortic diastolic (ADP) and aortic systolic (ASP) pressures were recorded on a computer with the Axotape acquisition data program. After hemodynamic recordings, animals were sacrificed and the heart and other organs were removed for additional studies.

Histology and Tissue Weights

Four hearts from four animals in each of the respective post-surgery durations were excised and fixed in formalin-calcium solution and processed for routine paraffin embedding. The ventricles in paraffin were sectioned transmurally and 5-μm-thick sections were stained with either hematoxylin and eosin or Masson's trichrome. The left ventricular scar as well as the junctional region between the scar and healthy myocardium were examined.

Lungs and liver were removed and weighed. For the determination of dry weight, the lungs and liver were placed in the oven at 65°C until a constant weight was reached. Wet/dry weight ratios were calculated for lungs and liver.

Biochemical Assays

Connective tissue, atria, and the scar tissue were carefully removed from all hearts and only the viable portion of the ventricular tissue was used for biochemical studies.

Superoxide Dismutase (SOD)

SOD activity in the myocardium was determined by the method previously described by Marklund.[15] Myocardium was homogenized (1:10) in 50 mmol/L Tris-HCl, pH 8.20, containing 1 mmol/L diethylenetriamine penta-acetic acid. The homogenate was centrifuged at 20,000 × *g* for 20 minutes. The supernatant was aspirated and assayed for total SOD activity by following the inhibition of pyrogallol auto-oxidation. Pyrogallol (24 mmol/L) was prepared in 10 mmol/L HCl and kept at 4°C before use. Catalase, (30 μmol/L stock solution) was dissolved in an alka-

line buffer (pH 9.0). Aliquots of supernatant (150 μg of protein) were added to Tris-HCl buffer containing 25 μl of pyrogallol and 10 μl of catalase. The final volume of 3 ml was made up with the same buffer. Changes in absorbance at 420 nm were recorded at 1-minute intervals for 5 minutes. SOD activity was determined from a standard curve of percent inhibition of pyrogallol auto-oxidation with commercially available SOD. Data are expressed as total SOD units per milligram of protein derived from a SOD standard curve. The assay was highly reproducible and the standard curve was linear up to 250 μg of protein with a correlation coefficient of 0.998.

Glutathione Peroxidase (GSHPx)

GSHPx activity was determined in myocardial tissue by a method previously described by Paglia and Valentine.[16] Tissue was homogenized 1:10 in 75 mmol/L phosphate buffer, pH 7.0. The homogenate was centrifuged at 20,000 × *g* for 25 minutes and the supernatant was aspirated and assayed for total cytosolic GSHPx activity. GSHPx activity was assayed in a 3-ml cuvette containing 2.0 ml of 75 mmol/L phosphate buffer, pH 7.0. The following solutions were then added: 50 μl of 60 mmol/L glutathione, 100 μl of glutathione reductase solution (30 U/ml), 50 μl of 0.12 mol/L NaN_3, 100 μl of 15 mmol/L Na_2 EDTA, 100 μl of 3.0 mmol/L reduced nicotinamide adenine dinucleotide phosphate (NADPH), and 100 μl of cytosolic fraction. The reaction was started by the addition of 100 μl of 7.5 mmol/L H_2O_2 and the conversion of NADPH to nicotinamide adenine dinucleotide phosphate (NADP) was monitored by a continuous recording of the change of absorbance at 340 nm at 1-minute intervals for 5 minutes. GSHPx activity was expressed as nanomoles of NADPH oxidized to NADP per minute per milligram of protein, with a molar extinction coefficient for NADPH at 340 nm of 6.22×10^6.

Catalase

Catalase activity in the myocardial tissue was determined by the method previously described by Clairborne.[17] Tissue was homogenized in 1:10 50 mmol/L potassium phosphate buffer, pH 7.4. Homogenate was centrifuged at 40,000 × *g* for 30 minutes. Supernatant (50 μl) was added to a 3-ml cuvette that contained 2.95 ml of 19 mmol/L H_2O_2 in 50 mmol/L potassium phosphate buffer, pH 7.4. Changes in absorbance at 240 nm were continuously followed for 5 minutes. Catalase activity was expressed as units per milligram of protein.

Vitamin E

Myocardial tissue was freeze clamped and stored in liquid nitrogen until analyzed by the high performance liquid chromatography (HPLC) method described by Lang et al.[18] Tissue was weighed and homogenized in a solution containing 1 ml of 0.1 mol/L aqueous sodium dodecyl sulfate, 1 ml of water along with 50 μl of ethanolic butylated hydroxytoluene (10 mg/ml). The sample was transferred to a 10-ml test tube fitted with a teflon-lined screw cap. The homogenizer was rinsed with 2 ml of reagent alcohol, which was combined with the homogenate. The mixture was vortexed for 30 seconds and 2 ml of hexane were added. Tubes were capped and vortexed for 2 minutes and were then centrifuged for 5 minutes at 1000 × *g* to separate the layers. A 1-ml volume of the hexane (top) layer was transferred to a small vial and dried under nitrogen. The residue was redissolved in 0.25 ml of reagent alcohol. Immediately after preparation, samples were analyzed by a Beckman Gold HPLC system. Different components of the Beckman Gold HPLC system used included a model 116 programmable solvent delivery system, model 166 programmable detector module, Altex injector system, and a Brownlee Silica Spheri-5 (25 cm × 4.6 mm) column. The mobile phase consisted of methanol/reagent alcohol (1/9, v/v) containing 20 mmol/L lithium perchlorate. The solvent flow rate was 1.0 ml/minute and the injected volume was 20 μl. With an UV detector at 275 nm, the area under the peak was recorded and compared with an external standard of α-tocopherol (Sigma Chemical Co., St. Louis, MO).

Glutathione

Concentration of total glutathione ie, oxidized (GSSG) + reduced (GSH), was measured in the myocardium by the glutathione reductase/5,5′-dithiobis-(2-nitrobenzoic acid) recycling assay.[19] The rate of dithiobis-nitrobenzoic acid formation is followed at 412 nm and is proportional to the sum of GSH and GSSG present. Myocardial tissue was homogenized in 5% sulfosalicylic acid. The tissue homogenate was centrifuged for 10 minutes at 10,000 × *g*. Supernatant was stored at 4°C until assayed. GSSG alone was measured by treating the sulfosalicylic acid supernatant with 2-vinylpyridine and triethanolamine. The solution was vigorously mixed and the final pH of the solution was checked to be between 6 and 7. After 60 minutes, the derivatized samples were assayed as described above in the glutathione reductase/5,5′-dithiobis-(2-nitrobenzoic

Table 1. *Ventricular and Blood Pressures in Experimental Rats at 1, 4, 8, and 16 Weeks after Myocardial Infarction as Compared with Sham Controls*

Post-surgical duration (weeks)	LVEDP (mm Hg)		LVPSP (mm Hg)		ADP (mm Hg)		ASP (mm Hg)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	2.3 ± .25	1.8 ± .6	126.3 ± 3.3	133.1 ± 4.3	65.0 ± 1.7	64.5 ± 1.0	108.3 ± 8.3	101.7 ± 3.5
4	2.1 ± .72	7.6 ± .44*	126.9 ± 1.2	114.0 ± .57†	64.6 ± 1.0	72.8 ± 2.4	110.8 ± 4.4	100.1 ± 2.3
8	3.9 ± .43	18.6 ± 1.0†	128.4 ± 2.3	102.4 ± .92†	66.9 ± .2	70.0 ± 1.2	106.1 ± 0.6	93.7 ± 1.0*
16	3.9 ± .53	30.6 ± .96†	127.5 ± 2.5	89.7 ± 1.5†	66.8 ± .6	73.3 ± 1.3	102.0 ± 2.0	91.4 ± 0.6*

Values are mean ± SE of 8 to 10 rats.
*†Significantly different ($P < 0.05$) from respective control groups by *ANOVA and †ANOVA followed by Bonferroni's test.

acid)-GSSG reductase recycling assay. GSH values were calculated as the difference between total (GSSG plus GSH) and GSSG concentrations. Values are reported in GSH equivalents, micromoles per gram of tissue weight.

Thiobarbituric Acid Reacting Substances (TBARS)

Lipid peroxide content in myocardium was determined by using the thiobarbituric-acid-reactive material method as described previously.[20] Tissue was homogenized in (10% wt/vol) 0.2 mol/L Tris/0.16 mol/L KCl buffer, pH 7.4, and incubated for 1 hour at 37°C in a water bath. A 2-ml aliquot was withdrawn from the incubation mixture and pipetted into a 12-ml Corning culture tube. This was followed by the addition of 2.0 ml of 40% trichloroacetic acid and 1.0 ml of 0.2% thiobarbituric acid (TBA). To minimize peroxidation during the assay procedure, 100 μl of 2% butylated hydroxy-toluene was added to the TBA reagent mixture. Tubes were then boiled for 15 minutes and cooled on ice for 15 minutes. A 2-ml volume of 70% trichloroacetic acid was added and tubes were allowed to stand for 20 minutes, at which time the tubes were subsequently centrifuged at 800 × *g* for 20 minutes. The developed color was read at 532 nm on a Zeiss spectrophotometer. Commercially available malondialdehyde was used as a standard.

Proteins and Statistical Analysis

Proteins were determined by the method described by Lowry et al.[21] Data were expressed as the mean ± SEM. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA), and ANOVA followed by Bonferroni's test was used to identify differences between groups. Values of $P < 0.05$ were considered significant.

Results

Hemodynamic Data

At 1 week, there was no change in the LVPSP or LVEDP (Table 1). LVPSP was significantly decreased and LVEDP was significantly elevated in the 4-, 8-, and 16-week groups as compared with their respective sham controls as well as 1-week experimental animals. Between 4 and 16 weeks of MI, these changes in LVPSP and LVEDP were progressive. ASP showed a drop at 4, 8, and 16 weeks, but the decreases were statistically significant only at 8 and 16 weeks (Table 1). Aortic diastolic pressure was not changed in any of the groups (Table 1). These values in sham controls were no different from each other.

Tissue Weight and Histological Observations

At 1 and 4 weeks after MI, there were no differences in lung or liver wet/dry weight ratios in experimental animals relative to controls. However, this ratio for lungs was significantly higher at 8 and 16 weeks whereas the liver wet/dry weight ratio was significantly increased only at 16 weeks (Table 2). At 16 weeks after MI, myocardial tissue from different regions of the left ventricle was examined by trichrome staining. A significant portion of the left ventricular free wall of the heart that suffered infarction showed connective tissue deposition with scanty healthy looking myofibers being still present. In the viable myocardium away from the scar area, there was connective tissue deposition in the perivascular region as well as in the interstitial spaces (data not shown).

Myocardial Antioxidant Enzymes

Various endogenous antioxidant enzyme activities were examined in the remaining, viable myocardium

Table 2. *Lung and Liver Wet/Dry Weight Ratios in the Animals at 1, 4, 8, and 16 weeks after Surgery*

Post-surgical duration (weeks)	Lung		Liver	
	Control	Experimental	Control	Experimental
1	4.8 ± .01	4.8 ± .07	2.7 ± .5	2.7 ± .48
4	4.8 ± .09	5.1 ± .4	2.9 ± .5	3.1 ± .2
8	4.7 ± .01	5.5 ± .04†	2.9 ± .5	3.3 ± .33
16	4.7 ± .1	6.5 ± .14†	2.8 ± .2	3.9 ± .4*

Values are mean ± SE of 8 to 10 rats.
*†Significantly different ($P < 0.05$) from respective control groups by *ANOVA and †ANOVA followed by Bonferroni's test.

at 1, 4, 8, and 16 weeks after MI. At 1 week after MI, antioxidant SOD showed a trend toward increase relative to respective controls, followed by a decrease at 4, 8, and 16 weeks (Figure 1). The depression in the SOD activity at 16 weeks was approximately 45% as compared with its control values and the change was statistically significant. In the 1-week experimental group, GSHPx activity was slightly higher than the control value, but the change was not statistically significant (Figure 2). At 4, 8, and 16 weeks, GSHPx activity was significantly decreased as compared with respective sham controls as well as the 1-week experimental group (Figure 2). A similar trend was seen with respect to catalase activity (Figure 3). At 1 week, catalase activity was 115% as compared with its control, but the change was not significant. At 4, 8, and 16 weeks, catalase activity was 86, 69, and 45% of the respective control values and these changes were significant.

Myocardial Vitamin E Content

Myocardial vitamin E content was analyzed by quantitating α-tocopherol by the HPLC method and these data are shown in Figure 4. At 1 and 4 weeks after surgery, vitamin E levels were no different relative to respective controls. However, at 8 weeks, there was a 25% depression and at 16 weeks the decrease was approximately 38% as compared with controls. These changes were statistically significant.

Lipid Peroxidation

The amount of lipid peroxidation was determined by evaluating myocardial TBARS. At 1 week after MI, TBARS remained near that of control values (Figure 5). At 4, 8, and 16 weeks after MI, TBARS were increased by approximately 20, 40, and 64%, respectively, as compared with their controls.

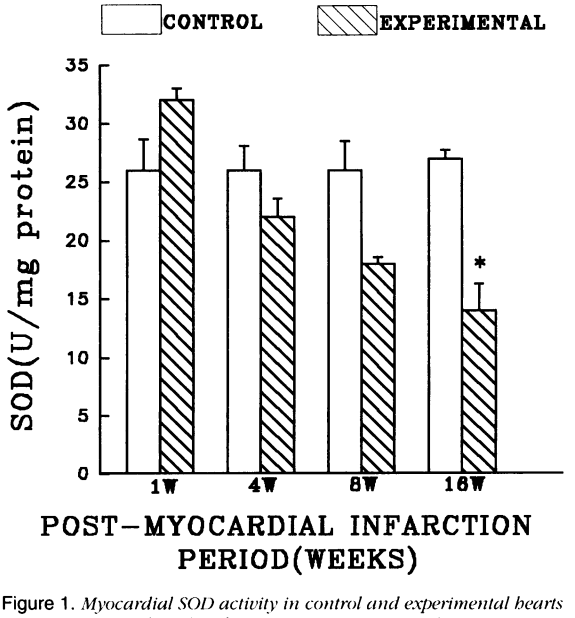

Figure 1. *Myocardial SOD activity in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different ($P < 0.05$) from respective controls as well as 1W experimental group.*

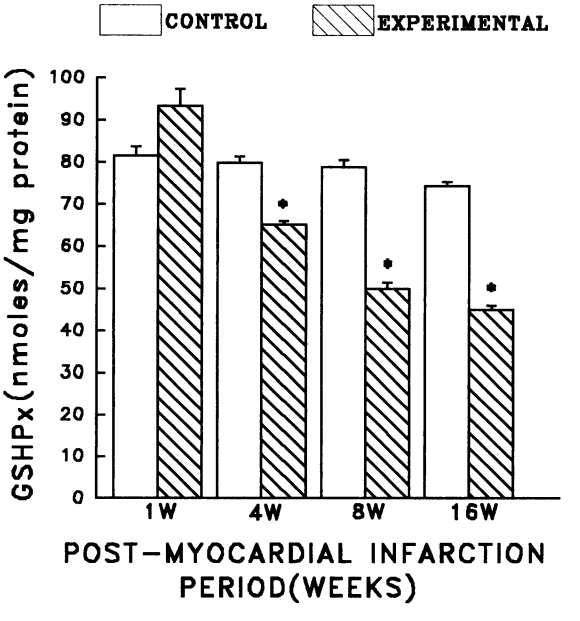

Figure 2. *Myocardial GSHPx activity in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different ($P < 0.05$) from respective controls.*

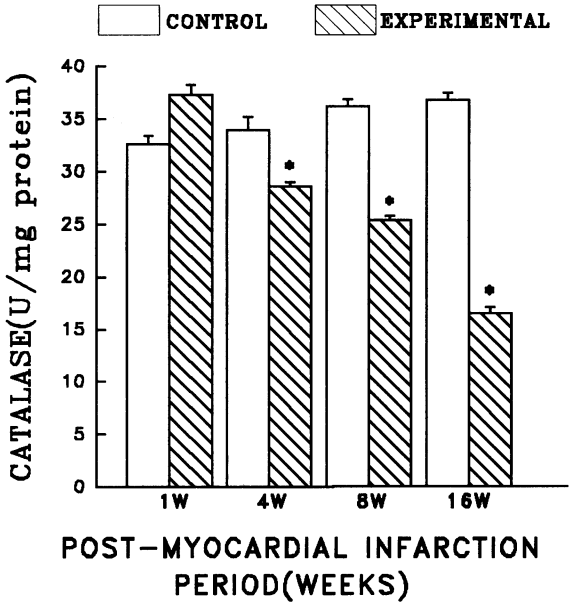

Figure 3. *Myocardial catalase activity in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different* ($P < 0.05$) *from respective controls.*

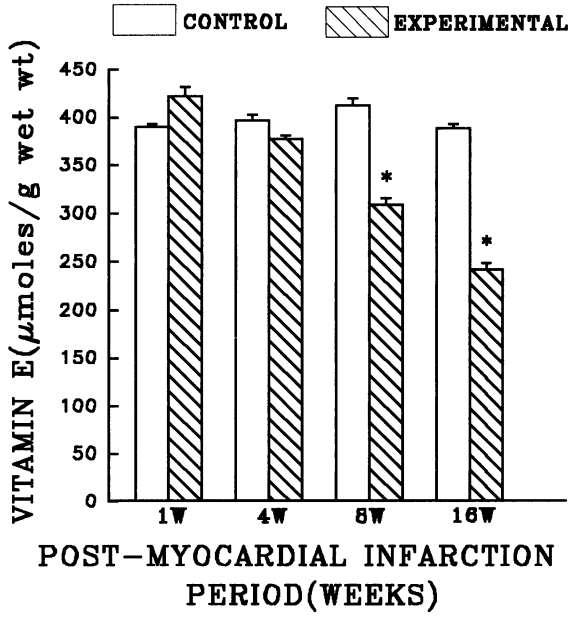

Figure 4. *Myocardial vitamin E content in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different* ($P < 0.05$) *from respective controls.*

Redox State

Myocardial GSH and GSSG contents were evaluated in control and experimental hearts and these data are shown in Table 3. GSH content in experimental hearts showed no significant change at 1 and 4 weeks after MI relative to respective controls. At 8 weeks, GSH content was decreased by

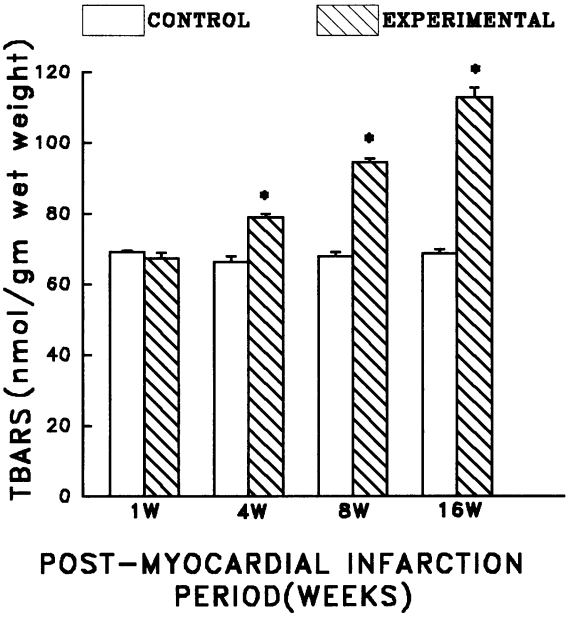

Figure 5. *Lipid peroxidation as indicated by TBARS in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different* ($P < 0.05$) *from respective controls.*

24% and a decrease of approximately 40% was seen at 16 weeks. GSSG content in experimental hearts at 1 and 4 weeks after MI was significantly less than controls. At 8 weeks, GSSG content in experimental hearts was slightly increased compared with control hearts. At 16 weeks, the GSSG content was significantly increased (>100%) relative to controls.

Redox state (GSH/GSSG) was evaluated in control and experimental hearts (Figure 6). The redox state was significantly higher at 1 week after MI but began to decrease at 4 and 8 weeks. A significant reduction in GSH/GSSG ratio was seen at 16 weeks after MI.

Discussion

Despite the remarkable improvements in strategies for treating acute MI and subsequent heart failure, understanding of the pathogenesis of heart failure remains a problem. In animal experiments, occurrence of antioxidant deficit has been reported as one of the mechanisms for the development of heart failure[2,3,11] and patient data supporting these findings have also begun to emerge. In patients with CHF, pentane, a product of lipid peroxidation, was significantly increased as compared with healthy age-matched subjects.[5–8] Furthermore, treatment of heart failure patients with captopril, a converting enzyme inhibitor with a sulfhydryl group, resulted in a reduction in the pentane levels, which appeared to

Table 3. *Myocardial Reduced (GSH) and Oxidized (GSSG) Glutathione Levels in Control and Experimental Rats at 1, 4, 8, and 16 weeks after Surgery*

Post-surgical duration (weeks)	GSH (μmol/L)		GSSG (μmol/L)	
	Control	Experimental	Control	Experimental
1	79.4 ± 1.4	82.9 ± 1.7	8.6 ± .66	5.3 ± .27*
4	78.4 ± 1.0	72.3 ± 1.5	10.1 ± .24	7.3 ± .30*
8	79.8 ± 3.3	60.9 ± 1.4†	12.4 ± .24	17.5 ± .39
16	80.2 ± 3.1	47.9 ± 1.9†	11.3 ± .43	28.3 ± 1.8†

Values are mean ± SE of 8 to 10 rats. Significantly different ($P < 0.05$) from respective control group by *ANOVA and †ANOVA followed by Bonferroni's test.

be a consequence of the drug's free radical scavenging properties attributable to its sulfhydryl availability.[8] The present study on rats demonstrates for the first time that the development of CHF secondary to MI correlates with a progressive decrease in antioxidants and a concomitant increase in the oxidative stress as well as lipid peroxidation. Although great caution should be exercised in extrapolating animal data to humans, a reduction in antioxidant reserve in the heart during the failure stages may contribute to the described increased pulmonary pentane content reported in CHF patients.[5–8] However, it is emphasized that increased pulmonary exhaled alkanes reflect total body lipid peroxidation status and to single out heart as the only tissue source of pentane would be inappropriate.

Maintenance of LVPSP, LVEDP, and ASP in the 1-week experimental group indicated sustained cardiac functioning in these animals. In addition, at 1 week after MI, no signs of heart failure were evident

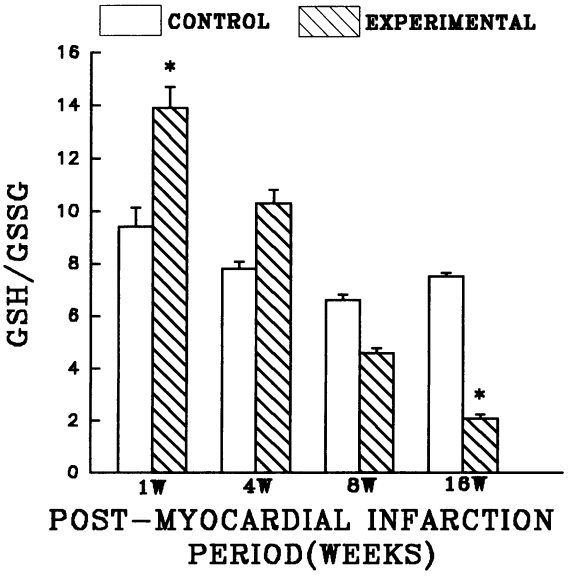

Figure 6. *Ratio of GSH/GSSG (redox state) in control and experimental hearts at 1, 4, 8, and 16 weeks after MI. Data are presented as mean ± SE from 8 to 10 rats, with each assay done in duplicate. *Significantly different (P < 0.05) from respective controls.*

from a wet/dry weight ratio of liver and lungs. Thus, we suggest it to be a stable function or nonfailure stage. In the 4-week experimental group, although LVEDP was elevated and LVPSP was significantly depressed, aortic pressure was maintained and pulmonary edema or lung congestion were not apparent, thereby indicating that these animals were in a stage of mild heart failure. At 8 weeks, a significant reduction in the LVPSP and an increase in LVEDP were accompanied by pulmonary edema without any liver congestion. These animals were considered to be in a stage of moderate heart failure. At 16 weeks, the greatest elevation in the LVEDP and depressed LVPSP were also accompanied by pulmonary edema and liver congestion suggesting a severe heart failure stage in these animals. The proposed segregation of experimental animals into nonfailure and mild, moderate, and severe stages of heart failure at 1, 4, 8, and 16 weeks, respectively, is being adopted here for a more objective comparison of the hemodynamic functional state with the biochemical changes in antioxidants as well as oxidative stress. It is emphasized that this classification is arbitrary.

The present study also shows that sustained cardiac function in nonfailing hearts until 1 week after surgery was accompanied by a mild increase in antioxidants. Increase in the antioxidant reserve at this stage was more clearly evidenced by a significant increase in the redox ratio or reduced oxidative stress. The cellular redox state is considered to be a sensitive index of oxidative stress.[22] An increase in the myocardial redox state has been demonstrated in different conditions and the change is correlated with sustained or better hemodynamic function.[11,22,23] As the post-surgery duration was prolonged further, all stages of failure were accompanied by characteristic changes in antioxidants. Mild failure at 4 weeks was accompanied by a significant depression in GSHPx and catalase activities. Although SOD activity as well as vitamin E content at this stage were not changed, there was a significant

increase in TBARS. Increases in TBARS have also been reported in heart failure due to a chronic pressure overload of the heart[11] as well as in the case of cardiomyopathic hamsters.[12] A close correlation between reduced antioxidant reserve and depressed cardiac function was more apparent at moderate and severe stages of failure in which antioxidants were depressed and oxidative stress as well as lipid peroxidation were higher. These findings may suggest that a relative deficit in the endogenous antioxidants and an increase in oxidative stress may play an important role in the pathogenesis of heart failure. Heart failure in patients with coronary artery disease has been reported to be accompanied by increased lipid peroxidation as indicated by the breath pentane content.[5–8]

Malondialdehyde determination by the assessment of TBARS as an indicator of lipid peroxidation has been shown to lack specificity as it may detect saturated and unsaturated nonfunctional aldehydes as well as other non-lipid-related reactive material.[24] The use of HPLC in quantitating malondialdehyde as a measure of lipid peroxidation has been suggested as a better alternate for estimating lipid peroxidation.[24] However, estimation of the malondialdehyde content in the homogenate that has not been acid heated, as is the case in the HPLC method, could be several orders of magnitude less than compared with the homogenate that has been acid heated.[25] The study underscores the critical importance of the acid-heating stage of the TBA assay.[25] In any case, great caution should be exercised in using the estimation of TBARS alone as an index of lipid peroxidation or oxidative stress in tissues. However, TBARS data become quite meaningful when used in conjunction with direct studies of the redox state as well as enzymatic and nonenzymatic antioxidants, as was done in our study.

Vitamin E has been shown to be a potent antioxidant and its presence in biological membranes is thought to represent the major defense system against peroxidation of membrane lipids. A relative deficiency in myocardial vitamin E may leave the myocardial tissue susceptible to free-radical-mediated lipid peroxidation and subsequent cardiac dysfunction. Vitamin E deficiency was reported to accentuate adriamycin-induced cardiomyopathy[26] as well as catecholamine-induced cardiomyopathy.[27] Animals with vitamin E deficiency frequently display myopathy of heart muscle.[28] Decreases in vitamin E levels at 8 and 16 weeks may have further compromised the antioxidant protection, particularly in the membrane phase during the moderate and severe stages of failure, thus leaving the myocardium vulnerable to free radical injury. Reduced levels of vitamin E have been correlated with increased lipid peroxidation as well as higher levels of pentane in exhaled air in humans.[4]

Free-radical-mediated damage largely occurs via lipid peroxidation as well as reduction in thiol groups.[29,30] Peroxidation of polyunsaturated fatty acids results in changes in membrane architecture and permeability as well as alterations in membrane-bound enzyme activities.[31] In CHF patients, elevated lipid peroxidation has also been demonstrated.[6,8] Thus, increased lipid peroxidation during the heart failure stages subsequent to MI clearly suggests a shift in the delicate balance that normally exists between free radical production and the antioxidant enzyme defense systems. Myocardial exposure to oxygen radicals in *ex vivo* conditions has been shown to cause increased lipid peroxidation that was correlated with structural damage, loss of high energy phosphates, and contractile failure,[2,32] and the administration of exogenous antioxidants offered protection against contractile dysfunction induced by the oxidative stress.[2,32,33]

Molecular mechanisms for the depressed activities of these antioxidants are not known. Antioxidant enzymes are known to be substrate stimulated as well as inactivated under oxidative stress.[34–37] Changes in antioxidant enzymes under a wide range of physiological and pathological conditions such as age,[38] exercise,[39,40] β-thalassemia,[41] hypertrophy,[2,11,42] heart failure,[11] and hypoxia[43] have been reported. These studies clearly document a dynamic nature of the endogenous antioxidant reserve adjusting to the physiological and pathophysiological conditions imposed. Although the data presented in this study do not explain the mechanism of the depressed antioxidant reserve, these findings do provide a good starting point to formulate more mechanistically oriented investigations.

In conclusion, this study demonstrates that heart failure subsequent to MI is associated with an antioxidant deficit as well as an increase in oxidative stress and lipid peroxidation. Antioxidant adjustments observed during the early stage may serve to sustain cardiac function whereas depressed cardiac function and heart failure may be aided by a decrease in antioxidant reserve. Experiments involving antioxidant therapy are required to test this hypothesis.

References

1. Cohen J, Shah PM: Cardiac hypertrophy and cardiomyopathy. Circ Res 1974, 35(suppl 2):1–2

2. Gupta M, Singal PK: Higher antioxidant capacity during chronic stable heart hypertrophy. Circ Res 1989, 64: 398–406
3. Singal PK, Kirshenbaum LA: A relative deficit in antioxidant reserve may contribute in cardiac failure. Can J Cardiol 1990, 6:47–49
4. Lemoyne BF, Van Gossum A, Kurian R, Ostro M, Axler J, Jeejeebhoy, KN: Breath pentane analysis as an index of lipid peroxidation: a functional test of vitamin E status. Am J Clin Nutr 1987, 46:267–272
5. Weitz Z, Birnbaum AJ, Sobotka PA, Zarling EJ, Skosey JL: Elevated pentane levels during acute myocardial infarction. Lancet 1991, 337:933–935
6. McMurray J, Mclay J, Chopra M, Bridges A, Belch JJF: Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease. Am J Cardiol 1990, 65:1261–1262
7. Roberts MJD, Young IS, Trouton TG, Trimble ER, Khan MM, Webb SW, Wilson CM, Patterson GC, Adgey AAJ: Transient release of lipid peroxides after coronary artery balloon angioplasty. Lancet 1990, 336:143–145
8. Sobotka PA, Brottman MD, Weitz Z, Birnbaum AJ, Skosey JL, Zarling EJ: Elevated breath pentane in heart failure reduced by free radical scavenger. Free Radical Biol Med 1993, 14:643–647
9. Dixon IMC, Lee S-L, Dhalla NS: Nitrendipine binding in congestive heart failure due to myocardial infarction. Circ Res 1990, 66:782–788
10. Anversa P, Olivetti G, Meggs LG, Sonnenblick EH, Capasso JM: Cardiac anatomy and ventricular loading after myocardial infarction. Circulation 1993, 87(suppl VII):22–27
11. Dhalla AK, Singal PK: Antioxidant changes in hypertrophied and failing guinea pig hearts. Am J Physiol 1994, 266:H1280–H1285
12. Kobayashi A, Yamashita T, Kaneko M, Nishiyama T, Hayashi H, Yamazaki N: Effects of verapamil on experimental cardiomyopathy in the bio 14.6 Syrian hamster. J Am Coll Cardiol 1987, 10:1128–1134
13. Siveski-Iliskovic N, Kaul N, Singal PK: Probucol promotes endogenous antioxidants and provides protection against adriamycin-induced cardiomyopathy in rats. Circulation 1994, 89:2829–2835
14. Selye H, Bajusz E, Grasso S, Mendell P: Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 1960, 11:398–407
15. Marklund SL: Pyrogallol autooxidation. Handbook of Methods for Oxygen Radical Research. Edited by RA Greenwald. Boca Raton, FL, CRC Press, 1985, pp 243–247
16. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967, 70:158–169
17. Clairborne A: Catalase activity. Handbook of Methods for Oxygen Radical Research. Edited by RA Greenwald. Boca Raton, FL, CRC Press, 1985, pp 283–284
18. Lang JK, Gohil K, Packer L: Simultaneous determination of tocopherols, ubiquinols and ubiquinones in blood, plasma, tissue homogenates and subcellular fractions. Anal Biochem 1986, 157:106–116
19. Anderson ME: Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985, 113:548–555
20. Singal PK, Pierce GN: Adriamycin stimulates low affinity Ca^{2+}-binding and lipid peroxidation but depresses myocardial function. Am J Physiol 1986, 250:H419–H425
21. Lowry OH, Rosenbrough NT, Farr AL, Randall AT: Protein measurements with the Folin phenol reagent. J Biol Chem 1951, 193:265–275
22. Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P, Visioli O: Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. Am J Med 1991, 91(suppl 3C):955–1055
23. Shan X, Aw TY, Jones DP: Glutathione dependent protection against oxidative injury. Pharmacol Ther 1990, 47:61–71
24. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R: Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. Am J Physiol 1991, 260:H1057–H1061
25. Fantini GA, Yoshioka T: Use and limitations of thiobarbituric acid reaction to detect lipid peroxidation. Am J Physiol 1992, 263:H981–H982
26. Singal PK, Tong J: Vitamin E deficiency accentuates adriamycin-induced cardiomyopathy and cell surface changes. Mol Cell Biochem 1988, 84:163–171
27. Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS: Role of free radicals in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 1982, 60: 1390–1397
28. Machlin LJ: Vitamin E. Handbook of Vitamins. Edited by LJ Machlin. New York, Marcel Dekker 1984, pp 99–145
29. Singal PK, Dhalla AK, Hill M, Thomas TP: Endogenous antioxidant changes in the myocardium in response to acute and chronic stress conditions. Mol Cell Biochem 1993, 129:179–186
30. Fliss H, Masika M, Eley DW, Korecky B: Oxygen radical mediated protein oxidation in heart. Oxygen Radicals in the Pathophysiology of Heart Disease. Edited by PK Singal PK. Boston, Kluwer Academic Publishers, 1988, pp 71–90
31. Kaul N, Siveski-Iliskovic N, Hill M, Slezak J, Singal PK: Free radicals and the heart. J Pharmacol Toxicol Methods 1993, 30:55–67
32. Gupta M, Singal PK: Time course of structure, function and metabolic changes due to an exogenous source of oxygen metabolites in rat heart. Can J Physiol Pharmacol 1989, 67:1549–1559
33. Brown JM, Grosso MA, Terada LS, Beehler CJ, Toth KM, Whitman GJ, Harken AH, Repine JE: Erythrocytes decrease myocardial hydrogen peroxide levels and reperfusion injury. Am J Physiol 1989, 256:H584–H588
34. Reddy K, Tappel AL: Effect of dietary selenium and

autooxidized lipids on the glutathione peroxidase system of gastro-intestinal tract and other tissues in the rat. J Nutr 1974, 104:1069–1078
35. Kimball RF, Reddy K, Pierce TH, Schwartz LW, Mustafa MG, Cross CE: Oxygen toxicity: augmentation of antioxidant defense mechanisms in rat lung. Am J Physiol 1976, 230:1425–1431
36. Chow CK, Tappel AL: An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. Lipids 1972, 7:518–524
37. Cowan DB, Weisel RD, Williams WG, Mickle DAG: Identification of oxygen responsive elements in the 5′-flanking region of the human glutathione peroxidase gene. J Biol Chem 1993, 268:26904–26910
38. Nohl H, Hegner D: Responses of mitochondrial superoxide dismutase, catalase and glutathione peroxidase activities to ageing. Mech Ageing Dev 1979, 11:145–151
39. Kanter MM, Hamlin RL, Unverferth DV, Davis HW, Merola AJ: Effect of exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. J Appl Physiol 1985, 59:1298–1303
40. Higuchi M, Cartier LJ, Chen M, Holloszy JO: Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J Gerontol 1985, 40:281–286
41. Gerli GC, Beretta L, Bianchi M, Pellegatta A, Agostini S: Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in β-thalassaemia (major and minor). Scand J Haematol 1980, 25:87–92
42. Kirshenbaum LA, Singal PK: Increase in endogenous antioxidant enzymes protects hearts against reperfusion injury. Am J Physiol 1993, 265:H484–H493
43. Dhaliwal H, Kirshenbaum LA, Randhawa AK, Singal PK: Correlation between antioxidant changes during hypoxia and recovery upon reoxygenation. Am J Physiol 1991, 261:H632–H638