DECREASED SULFHYDRYL GROUPS OF SERUM ALBUMIN IN CORONARY ARTERY DISEASE

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We hypothesized that oxidative processes in myocardial tissues initiated by ischemia also cause the same kind of changes in blood proteins and lead to oxidation of sulfhydryl groups (SH). To test this hypothesis, the ratio (%MA) of mercaptalbumin to total albumin (=mercaptalbumin+nonmercaptalbumin) and the total SH contents of serum were measured in patients with coronary artery disease. There was a positive correlation between %MA and total SH contents and both values were reduced in proportion to the severity of the coronary artery disease. Furthermore, these values were lower early hours after the onset of acute myocardial infarction than 4 weeks later. These results support our hypothesis, and may provide a basis for the administration of agents which can counteract oxidative stress in order to protect myocardium in patients with coronary artery disease.

FREE radicals, especially oxygen radicals, have recently been implicated as important mediators of ischemic myocardial injury^{1,2} since they interact with a variety of cell components such as polyunsaturated fatty acids and structural and functional proteins.³⁻⁵ Sulfhydryl groups (SH) in proteins are easily oxidized when exposed to oxidative stress. There is experimental evidence that the SH content of the myocardium is decreased by ischemia⁶, suggesting a linkage between ischemia and oxidation of SH in tissue proteins. Such an oxidation process may also nonselectively affect proteins in the blood as well, changing their structures. Therefore, we hypothesize that myocardial ischemia may cause oxidative changes in blood proteins.

To test our hypothesis, we chose to investigate serum albumin, since its oxidation

can be evaluated by separating the two components of albumin, reduced form mercaptalbumin (MA) and oxidized form nonmercaptalbumin (NA) with high performance liquid chromatography (HPLC)? In this study we present data that the ratio of MA to NA is decreased in patients with coronary artery disease, suggesting an oxidative alteration in blood proteins in this disorder.

METHODS

Study Population

Forty nine patients aged 44 to 75 years were studied for evaluation of mercaptalbumin and serum total SH content and were classified into four groups. Group 1: 21 patients with normal coronary arteriograms and normal cardiac functions who had undergone cardiac catheterization due to chest pain, and served as controls. Group 2: 15 patients with stable exertional angina pectoris of 4.0 ± 2.8 months' duration. Group 3: 6 patients with unstable angina pectoris occurring in a crescendo pattern during both

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Fig.1. Separation of serum albumin by HPLC. Human serum was applied to HPLC system as described in the methods section to separate serum albumin into the two forms. Two peaks of mercaptalbumin (MA) and nonmercaptalbumin (NA) appeared after the first major peak of globulin rich fractions. Representative profiles of control (upper panel) and acute stage of myocardial infarction (lower panel). ABS=absorbance at 220 nm

mild effort and rest within 2.8 ± 1.9 days. Group 4: 7 patients with acute myocardial infarction without pulmonary edema. There were no significant differences in mean age (Group 1: 60.5 ± 6.8 , Group 2: 61.5 ± 7.6 , Group 3: 62.5 ± 9.1 Group 4: 63.1 ± 7.1) or in the male to female ratio (Group 1: 14/7, Group 2: 11/4, Group 3: 4/2, Group 4: 5/2) among the four groups. No patient had renal or hepatic disease. Patients with acute infection or chronic infection and those who had undergone any surgical procedure within the month preceding the study were excluded. No drugs had been given to patients in groups 1 and 2. Sublingual nitroglycerine was administered to relieve chest pain in group 3. Patients in group 4 were treated with nitrates and calcium channel blockers (nifedipine or diltiazem) after admission. Drugs known to affect SH availability (such as captopril, penicillamine, and ethacrynic acid) were not administered to any patient.

Fig.2. The ratio (%MA) of mercaptalbumin to total albumin in coronary artery disease. %MA in each group is shown. In group 4, %MA was determined early hours (acute) after the onset of infarction and 4 weeks later (chronic). Group 1 represents controls, group 2 exertional angina pectoris, and group 3 unstable angina pectoris. * $p < 0.05$, ** $p < 0.01$, NS=not significant.

Blood Sampling

Patients in groups 1 to 3 underwent cardiac catheterization after 12-h fast, and blood was sampled from the ascending aorta through the catheter before the injection of contrast medium. In group 4, blood was drawn 7.6 ± 3.6 h (acute stage) after the onset of myocardial infarction through an indwelling cannula inserted into the brachial artery for blood pressure monitoring, and through the catheter from the ascending aorta upon cardiac catheterization 4 weeks later (chronic stage). Serum was prepared after blood sampling, and stored at -80°C until use.

Fig.3. Total SH contents of serum.
Total SH contents of serum was measured in each group.
* $p < 0.05$, ** $p < 0.01$, NS=not significant.

Determination of Mercaptalbumin (MA) and Nonmercaptalbumin (NA)

Human serum albumin consists of a reduced form, mercaptalbumin (MA), and an oxidized form, nonmercaptalbumin (NA). MA, the predominant form of human serum albumin has one SH group at the 34th amino acid residue, cysteine, while NA has mixed disulfide with cysteine or glutathione at the same site.

Serum MA and NA were separated by high performance liquid chromatography⁷ (Fig. 1). The peaks eluting at 43 min and 48 min were identified as MA and NA respectively with amino acid analysis, electrophoresis, and measurement of SH contents⁸. The system consisted of two columns of Asahipack GS-520 (7.5×500 mm), a JASCO UVIDEC 100-IV UV monitor, a JASCO TRIROTOR-V HPLC pump, and a Shimadzu

Fig.4. Relation between %MA and total SH contents of serum.
There is positive correlation between %MA and total SH contents of serum.

SPD-2A spectrophotometric detector. The carrier buffer was 0.03M sodium phosphate buffer, pH 7.0, 0.30M Na_2SO_4 , with flow rate of 0.8 ml/min. Five μl of patients' sera was injected, and the elution was monitored at 220 nm. The peak areas of MA and NA were measured, and the ratio of MA to total HSA (%MA) was defined as follows:

$$\%MA = [MA / (MA + NA)] \times 100.$$

Measurement of SH Contents of Serum

Total SH contents in patient's serum were measured by a modified method of Ellman⁹ employing 5,5'-dithiobis (2-nitrobenzoic acid) purchased from Wako Chemical Co. Osaka, Japan.

Statistics

Values are given as means $\pm$ SD. Statistical analysis was performed by paired or unpaired Student's t-tests. Linear regression analysis was performed to examine the relation between %MA and total SH con-

Fig.5 Changes of %MA in the acute phase of myocardial infarction. Changes of %MA in 10 patients with myocardial infarction are shown. %MA decreased rapidly from the admission ($60.4 \pm 4.9\%$) to 6 hours after onset of myocardial infarction ($50.7 \pm 4.7\%$). Then %MA slowly increased within 24h. The time of admission was 3.5 ± 1.3 h after the onset of infarction.

** $p < 0.01$, * $p < 0.05$

tents of serum. Significance was defined as $p < 0.05$.

RESULTS

Oxidation of Serum Albumin in Coronary Artery Disease

The %MA in each group is shown in Fig. 2. The %MA in group 2 (63.7 ± 3.4) was significantly lower than that in group 1 (68.2 ± 4.6) and, likewise, the %MA in group 3 (52.5 ± 5.8) was lower than that in group 2. The %MA in group 4 was lower in the early hours (51.9 ± 4.3) after the onset of infarction than that in groups 1 and 2, but became higher 4 weeks (59.2 ± 7.3) after the onset.

Total SH Contents of Serum

Total SH contents were 438.2 ± 67.5 , 393.3 ± 56.2 , and $291.5 \pm 63.9 \mu\text{M}$, in groups 1, 2, and 3, respectively. In group 4 they were $314.3 \pm 37.9 \mu\text{M}$ early after the onset

of infarction, and $403.9 \pm 92.8 \mu\text{M}$ 4 weeks later. Relations similar to those in %MA were observed among each group (Fig. 3).

Relation between %MA and Total SH Contents of Serum

There was a significant positive correlation between %MA and total SH contents of serum ($r = 0.475$, $p < 0.01$, Fig. 4).

DISCUSSION

In the current study, there was a positive correlation between the %MA and total SH contents of serum. Both values were lower in patients with coronary artery disease than in controls, and became smaller with an increase in the severity of coronary artery disease. Furthermore, in patients with acute myocardial infarction, they were higher 4 weeks after the onset than during acute phase. The changes of %MA in the acute phase of myocardial infarction were examined in 10 patients (age: 59.5 ± 11.0 y.o., male: 7, female: 3) as shown in Fig. 5. The %MA decreased rapidly from the admission (3.5 ± 1.3 h) to 6 hours after the onset of infarction. It increased to the nearly same level as chronic stage by 24h after the onset. Although we cannot directly demonstrate myocardial tissue alterations due to ischemia in the studied patients, the present data support the hypothesis that myocardial ischemia can induce oxidative changes in blood proteins.

Radical scavengers such as superoxide dismutase or SH-containing substances (N-2-mercaptopropionyl glycine, captopril) have been shown to attenuate reperfusion injury which is considered to be mainly caused by free radicals¹⁰⁻¹². Our findings showed that endogenous SH groups, which may act as radical scavengers, decreased in coronary artery disease ranging from exertional angina to myocardial infarction. In this context the administration of radical scavengers may provide a new therapeutic approach for myocardial protection in patients with decreased %MA or total SH contents.

Another important consideration is the role of SH in the action of the nitrates^{12,13} that are frequently administered to patients with coronary artery disease. It has been reported that either intracellular or extracellu-

lar SH mediates or potentiates the action of nitrates^{13,14}. The administration of SH-containing substance can reverse the tolerance to nitrates¹⁵⁻¹⁷. It is unlikely that the decrease in total SH contents of serum is due to consumption of SH secondary to nitrates administration, since SH contents in patients with acute myocardial infarction recovered 4 weeks after the onset despite continuous administration of nitrates. However, decreased serum SH levels may attenuate the effects of nitrates, especially in patients with unstable angina pectoris. Under these conditions, N-acetylcysteine or methionine (precursor of cysteine) may be beneficial to elevate SH contents and enhance the action of nitrates in local myocardial vascular beds.

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