Erythrocyte Selenium-Glutathione Peroxidase Activity Is Lower in Patients with Coronary Atherosclerosis

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SUMMARY

To obtain further insight into the role of erythrocyte antioxidant systems in the development of atherosclerosis, intraerythrocyte enzyme activities and selenium levels in erythrocytes were determined in 37 patients with angiographically proved coronary artery stenosis and 15 subjects with normal coronary angiograms as controls. In a preliminary study, the enzymatic activities of glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR) and selenium-dependent glutathione peroxidase (Se-GPx) were measured in both venous and arterial blood samples obtained from patients before angiography. The data of the preliminary study, which showed that only the Se-GPx decreased in the patients, led us to concentrate on the Se-GPx and Se levels to determine the changes in these variables. Our results showed that there was a decrease in both the activity of Se-GPx and Se levels in erythrocytes parallel to the increase in the severity of coronary artery disease. It was concluded that these parameters might be used as determinants in the assessment of the severity of the disease. (Jpn Heart J 1997; 38: 793–798)

Key words: Atherosclerosis, Coronary artery stenosis, Selenium-dependent glutathione peroxidase, Selenium

ONE of the human conditions in which lipid peroxidation does appear to play a major role in the pathology is atherosclerosis.[1] During the formation and development of atherosclerosis, the intensity of lipid peroxidation and the activity of the antioxidant defense system significantly change.[2] It is known that erythrocytes contain powerful preventative and chain-breaking antioxidants which limit lipid peroxidation[1]; perhaps depletion or failure of these protective mechanisms is also involved in the pathogenesis of atherosclerosis.

Selenium (Se) is known as an essential component of glutathione peroxidase (GPx), an enzyme that protects cells by reducing intracellular peroxides. Im-

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paired Se status and Gpx activity seem to be associated with increased risk of atherosclerotic vascular diseases.[3] Recent studies have reported that there is a correlation between low serum selenium levels and atherosclerotic heart disease.[4-6] However, there is not much written about the relationship between the erythrocyte antioxidant defense systems and coronary artery disease.

The aim of the present study was to evaluate the antioxidant enzymes of erythrocytes that might be an indicator of the protective mechanisms involved in atherosclerosis. For this purpose, the enzymatic activities of some antioxidant defences were assessed in patients in whom coronary angiography was indicated. In a preliminary study, we measured the enzymatic activities of glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR) and selenium-dependent glutathione peroxidase (Se-GPx) in venous blood samples obtained from patients before performing angiography. The data of the preliminary study, which showed that only the Se-GPx decreased in the patients, led us to concentrate on the Se-GPx and Se levels to determine the changes in these variables.

Materials and Methods

Subjects: The study comprised 37 patients with angiographically proved coronary artery stenosis and 15 subjects with normal coronary angiograms who were used as controls. All subjects (37 patients + 15 controls) underwent coronary angiography in the study period because of possible ischemic heart disease, based on either a history of myocardial infarction (MI) or a positive or a suspected treadmill test result. Patients having less than 30% stenosis in all three vessels and having no atherosclerotic lesion seen in their eye-fundus examination were classified as control subjects. Of 52 subjects who had coronary angiography, 9 patients received nitrite and calcium-antagonist therapy because of post-MI angina, 31 patients who had clinical complaints and electrocardiographic manifestations, received a mild calcium-antagonist and/or nitrite and acetyl salicylic acid therapy, and 12 patients with suspicious treadmill test results received no therapy. Subjects receiving any other antioxidant therapy were not included in this study. None of the subjects were diabetic and 6 were smokers (4 patients and 2 control subjects). Three of the patients were hypertensive and 1 control subject had arterial pressure above 140/90 mmHg. Hypercholesterolemia and/or hypertriglyceridemia were observed in 6 patients and 2 control subjects.

The patients were divided into 3 subgroups according to the number of arteries with stenosis and to the presence of further aneurism of the heart. These groups were:

1) Mild coronary artery disease (CAD) (Group I): Only one coronary artery was stenosed. 12 patients were included in this group.

2) Moderate CAD (Group II): Stenosis was detected in two arteries and 12 patients were in this group.

3) Severe CAD (Group III): Three arteries were stenosed and further aneurisms were present; 13 patients were included in this group.

Venous blood samples were collected on heparin just before the angiographic intervention was performed. They were immediately centrifuged at 2000xg for 10 min and the supernatants and the buffy coats were discarded to obtain the erythrocytes.

Assays of enzymatic activities: Erythrocyte selenium-dependent glutathione peroxidase was evaluated by a method of Paglia and Valentine spectrophotometrically, with tert-butyl hydroperoxide as the substrate.[7] Erythrocyte Se levels were assayed with the spectrofluorometric method of Sheehan.[8] G6PD activities were measured by a modified spectrophotometric method of Zinkham.[9] Erythrocyte glutathione reductase activities were evaluated by Sauberlich's method.[10]

Results

Patients with coronary atherosclerosis diagnosed by coronary angiography had similar G6PD and GR activities compared to the control subjects (Table I). However, the Se-GPx activities were decreased in erythrocytes of the atheroscle-

Table I. Glucose-6-phosphate Dehydrogenase (G6PD), Glutathione Reductase (GR) and Selenium-dependent Glutathione Peroxidase (Se-GPx) Activities in Erythrocytes Obtained from the Patient and Control Groups of the Preliminary Study

	G6PD (IU/gHb)	GR (CA)	Se-GPx (IU/gHb)
Control subjects ($n = 5$)	10.92 ± 7.5	1.14 ± 0.4	85.39 ± 36.3
Patients ($n = 6$)	10.12 ± 3.6	1.13 ± 0.4	51.61 ± 33.3*

*$p < 0.05$. Values are means ± SD.

Table II. Selenium levels (Se), Selenium-dependent Glutathione Peroxidase Activities (Se-GPx), Age and Sex of the Patient and the Control Groups

	GPx (IU/gHb)	Se (ug/dl)	Age (years)	Sex (M : F)
Control group ($n = 15$)	80.1 ± 19.7	51.8 ± 9.8	45 ± 10	8 : 7
Group I ($n = 12$)	51.8 ± 15.2**	41.0 ± 13.9*	59 ± 8	9 : 3
Group II ($n = 12$)	47.3 ± 21.1	38.0 ± 15.5	58 ± 6	9 : 3
Group III ($n = 13$)	23.4 ± 11.8#,##	25.3 ± 9.5#,##	59 ± 7	10 : 3
Group I + II + III ($n = 37$)	40.4 ± 20.4**	34.3 ± 14.5**	58 ± 7	28 : 9

* $p < 0.05$, ** $p < 0.001$, # $p < 0.01$ in I/III, ## $p < 0.01$ in II/III.

rotic patients ($p < 0.05$, Table I).

Depending on these data from the preliminary study, we further analyzed the status of erythrocyte Se-GPx activities and in addition we measured the plasma and erythrocyte selenium levels. Se-GPx activities and Se levels are given in Table II. These results showed that the decreases in the activity of GPx and Se levels in erythrocytes of the entire patient group ($n = 37$) were statistically significant ($p < 0.001$ for both) when compared to the control subjects. The data also showed that both the activity of GPx and Se levels in erythrocytes significantly decreased parallel to the increase in the severity of CAD (Table II).

Discussion

The results of the preliminary study are given in Table I. An important aspect of the results was that there was no difference in the antioxidant enzyme activities except one, namely Se-GPx, when the subjects and the controls were compared. Therefore, we concentrated on this particular enzyme in the rest of the study. Moreover, we added the assay of intraerythrocyte Se concentration to our protocol, since this enzyme is selenium-dependent.[3]

Lipid peroxides have been implicated in the initiation of atherosclerosis and in order for the GPx to be able to counteract the adverse effects of peroxides, sufficient amounts of selenium must be available for the formation of the enzyme.[4] Glutathione peroxidase assumes a critical importance in the protection of the red cell against oxidative destruction.[11] The mechanism of action of this enzyme requires the presence of selenocysteine in the active site. Both experimental and clinical Se deficiency result in a decrease in cellular and plasma GPx activity.[12] Our results, given in Table II, show that there is a decrease in both the activity of GPx and Se levels in erythrocytes parallel to the increase in the severity of CAD. In other words, the lowest Se levels and GPx activities were observed in Group III, in which the CAD was the most severe. Wang J, *et al.*[13] showed that GPx activity in arteries of the atherosclerotic quail was lower than that in the control quail. Chen Y, *et al.*[14] reported that selenium-dependent GPx activities in liver, aorta, heart muscle, erythrocyte and platelet in atherosclerotic rabbits decreased significantly and that these tissues suffered from lipoperoxidative damage. Likewise, Buczynski A, *et al.*[15] found that platelet GPx activity of the patients with CHD was significantly lower than that of controls. Chen Y, *et al.*[14] obtained results showing that the Se-dependent GPx activities in RBCs of atherosclerotic rabbits decreased significantly. It was previously reported that mortality from cardiovascular diseases, including coronary heart disease and Keshan disease, was associated with some measures of antioxidant status in blood.[16]

The decrease in Se levels in erythrocytes might be attributed to dietary deficiency in the patient groups,[6] or dietary differences arising from case and control selection may have biased our results. However, both groups had coronary angiography recommended by the cardiologist for reasons of possible ischemic heart disease, which implies equal motivation for dietary changes in both groups. On the other hand, Ricetti, *et al.*[3] have observed that a major degree of regulation for GPx expression by Se is most likely exerted at the posttranslational levels. They concluded that different responses to Se feeding and status might be expected as for GPx.

In conclusion, our results show that Se and Se-dependent glutathione peroxidase are lower in patients with coronary atherosclerosis and the decline increases parallel to the severity of the disease. Therefore, we suggest that these parameters might be used as determinants in the assessment of the severity of the disease.

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