Selenium Concentrations in Plasma of Patients with Arteriographically Defined Coronary Atherosclerosis

Julie Anne Moore,1 Robert Nolva, and Ibert C. Wells2

A prospective epidemiological study (Lancet II: 175–179, 1982) implicates low concentrations of selenium in plasma in coronary atherosclerosis. We examined this relationship more directly by fluorometry of selenium in the plasma of 91 hospitalized patients who were being examined by coronary arteriography for clinical evaluation of chest pain. We observed a significant, inverse correlation between the plasma selenium and severity of coronary atherosclerosis. These results confirm those of the epidemiological studies, but the role, if any, of selenium in atherogenesis still is unclear. Its concentration in plasma is decreased by ethanol and cigarette use; possibly this is the mechanism of its relation to hypertension and atherosclerosis.

Additional Keyphrases: trace elements · fluorometry · heart disease · effects of smoking and ethanol intake · nutritional status

In a recently reported, prospective epidemiological study, a decreased concentration of total selenium in serum was associated with increased risk for coronary heart disease, cardiovascular disease, and myocardial infarction, both fatal and non-fatal (1).

Reportedly (2), patients with congestive cardiomyopathy also have a significantly lower selenium concentration in their serum than do healthy controls. That in these cases the low concentrations may have resulted from a dietary deficiency of selenium is suggested by a fatal cardiomyopathy in a patient on home parenteral nutrition whose diet was considered to have been selenium deficient (3). Moreover, Keshan disease, a congestive cardiomyopathy affecting mainly children and young women living in selenium-deficient areas of China, is principally attributed to the low dietary intake of selenium (4, 5).

In recent years, patients requiring cinearteriography because of chest pain have been studied in attempts to correlate biochemical changes with the severity of coronary atherosclerosis. Some of these patients have normal coronary arteries, and serve as controls for the remaining patients, in whom the occurrence and severity of coronary atherosclerosis are objectively defined. This former group of patients is considered to be a better control than a "normal, healthy" population, some of whom may be in an early, symptomless stage of coronary atherosclerosis. We studied a group of such patients to ascertain the relation, if any, between the concentration of selenium in plasma and the severity of the disease. We found a significant inverse relationship between total selenium and severity of coronary atherosclerosis.

Materials and Methods

Plasma was sampled from 106 hospitalized patients (Table 1) who were to undergo coronary arteriography—six women and 100 men.

The plasma samples were promptly stored at −20 °C until they were analyzed, in the same order as they were collected, without knowledge of the clinical diagnosis of the donor.

On arteriography, "zero vessel" disease was defined as no narrowing as great as 50% of any coronary arterial lumen visible on the arteriogram; "one-", "two-", or "three-vessel" disease was defined as a narrowing of 50% or more in the corresponding number of the three major coronary arteries or their branches. On the basis of these definitions, each patient was assigned to a group by clinical specialists who were independent of this research project.

Total selenium was measured in plasma by converting it to Se4+, which then was complexed with 2,3-diaminonaphthalene (DAN), to form the fluorescent derivative 4,5-benzopiazaselenol. Selenium occurs in plasma as low-molecular-mass compounds, such as the seleno homologs of the sulfur amino acids, and bound to some proteins as seleninic or selenenic acid derivatives. The procedure, adapted from those of Lalonde et al. (6) and Hasunuma et al. (7), was as follows.

To 0.25 mL of plasma, add 1.0 mL of an equimol mixture of 15.8 mol/L nitric acid and 11.8 mol/L perchloric acid. Heat this mixture for 2 min at 150 °C, then for 2 h at 190 °C, and finally for 2 h at 210 °C. After the mixture cools, add 0.2 mL of 6 mol/L hydrochloric acid and heat at 150 °C until nitric oxide fumes are no longer evolved. Add 1 mL of a solution containing, per liter, 20 mmol of EDTA, 7 mol of ammonia, and 10 mg of bromcresol purple. Heat at 140 °C

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*An arbitrary value was assigned to each of several atherosclerosis risk factors as follows: age > 50 years, 1; plasma total cholesterol > 1800 mg/L, 2; ratio (very-low-density lipoprotein cholesterol + low-density lipoprotein cholesterol) to high-density lipoprotein cholesterol > 4.1; cigarette use, 1; moderate ethanol consumption, −1; hypertension, 2; diabetes, 1; hypothyroid, −1; hyperthyroid, −1; body weight > 23 kg above ideal, 1. The total weighted risk for each individual was calculated and the mean (and SEM) of the totals in each classification is given.

b Different from zero-vessel disease, p < 0.05 by Student's t test.

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until the solution becomes yellow; let it cool, then add 1.5 mL of 66 mmol/L hydrochloric acid, which adjusts the pH to between 1.0 and 2.0.

Perform the following steps under subdued lighting to avoid possible inactivation of the fluorescing derivative by visible light (6). Prepare freshly 20 mL of a 1.0 g/L solution of DAN hydrochloride in 0.1 mol/L hydrochloric acid, heat at 50 °C for 20 min, and extract the cooled mixture twice with equal volumes of cyclohexane (AR grade). Add 0.5 mL of the extract to each solution of digested plasma and incubate the mixture in a 50 °C water bath for 20 min. Extract the resulting fluorescent complex, 4,5-benzopiazelenol, into 1 mL of cyclohexane by vigorous shaking. Measure the fluorescence of the extract with a spectrophotofluorometer (we used a Model 204 instrument from Perkin-Elmer Corp., Norwalk, CT), with the excitation wavelength set at 360 nm and the emission wavelength at 520 nm.

Prepare a calibration curve from data obtained by treating known amounts of sodium selenite according to the above procedure, and use the curve to convert measured fluorescence intensities of plasma samples to selenium concentrations.

Results

Table 2 presents data values for total plasma selenium for the four groups of patients.

Values in each group beyond the 95% confidence level were eliminated according to acceptable statistical criteria, which removed three plasma samples from consideration. The 91 remaining values for the four groups were compared by Student’s t-test and by analysis of variance. The mean plasma selenium concentrations of the patients with two- and three-vessel disease were significantly less (p < 0.05) than that of the patients with zero-vessel disease, the control group. The mean value for patients with “one-vessel” disease did not differ significantly from that of the control patients, although the value was less than that for the control group.

Discussion

Thus, total selenium in plasma appears indeed to be inversely related to the severity of coronary atherosclerosis. However, such a relation does not tell us whether the decrease is a etiologic factor in, or a result of, atherosclerosis or the result of some side effects of atherogenesis with no direct relevance to it. The independent information already mentioned (2–5) makes the last possibility the least probable.

We measured only the total concentrations of the selenium in the plasma. More insight might have been gained had we independently measured the concentrations of “organic” or “inorganic” selenium, or the selenium in the erythrocytes, or the activity of glutathione peroxidase (EC 1.11.1.9), an important enzyme that contains selenium. On the other hand, because arterial endothelial cells are thought to be involved in atherogenesis and are undergoing constant, rapid turnover, the total selenium in plasma may be the best measure of the selenium most available for incorporation into these cells.

Were decreased selenium concentrations in the plasma of the patients with severe coronary atherosclerosis due to a dietary deficiency of the element or to a metabolic process? The diet of each patient was, to the best of our knowledge, essentially the ordinary American diet, which is adequate with respect to selenium, so this possibility seems unlikely. More probable is deterioration of liver function, which, whether due to ethanol abuse, or other causes, results in a decrease in plasma selenium (8). The mechanism for this remains obscure but it may be the consequence of decreased concentrations of α- and β-globulins in the plasma; these globulins are thought to be the carriers of selenium in the plasma (9) and their production in the liver is thought to be depressed in liver disease. However, liver deterioration is currently recognized as neither a cause nor a consequence of atherosclerosis.

Lloyd et al. (10) studied the effects of smoking and ethanol ingestion on plasma selenium concentrations, finding them to be significantly depressed by chronic ethanol ingestion and cigarette smoking. This prompted us to examine our data with respect to the use of ethanol and cigarettes by our patients. The plasma selenium concentration (µg/L) of our patients were as follows: nonsmokers, nondrinkers, 151.5; smokers, nondrinkers, 125.3; drinkers, nonsmokers, 117.1; smokers, drinkers, 101.1. The values of the last two groups were significantly different from each other (p < 0.05) and from that of the nondrawer/nondrinker group (p < 0.01) by Student’s t-test, results in essential agreement with those of Lloyd et al. Furthermore, ethanol use apparently is a more potent depressant of plasma selenium than is cigarette use, and these two factors apparently act additively in depressing the plasma concentrations of selenium. We emphasize that these data are only qualitative, because our information was confined to whether a patient did or did not smoke cigarettes or drink ethanol and not how much was smoked or drunk.

Our results appear to be even more significantly related to ethanol and cigarette use when each group of arteriographically classified patients is subdivided according to the use of these materials (Table 3). It is apparent that the decreases in plasma selenium (Table 2) that occur with increasing severity of coronary atherosclerosis parallel almost exactly the decreases in the percentages of the patients in each group who neither smoke cigarettes nor drink ethanol. However, the average (±SEM) values for total selenium in plasma of these kinds of patients in the four groups are 143 ± 9.39, 128 ± 14.89, 124.5 ± 21.5, and 112.5 ± 24.5 µg/L for the zero-, one-, two-, and three-vessel disease groups, respectively. These means are not significantly

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No. of patients listed in Table 2.
different, but the trend is the same as that of the values observed for the intact groups. This suggests that decreased values for total selenium in plasma may be associated with coronary atherosclerosis independently of cigarette and ethanol use, but more extensive data will be required to establish this point. Nevertheless, there appears to be little reason to doubt that the use of ethanol and cigarettes does diminish the total selenium concentrations in plasma.

These associations of low plasma selenium with ethanol and cigarette use are of great interest, because cigarette use is an accepted risk factor for atherosclerosis, whereas moderate alcohol use may be initially protective because it may increase the concentrations of high-density lipoproteins in plasma. However, alcohol use is also a risk factor for hypertension, which in turn is a prominent risk factor for atherosclerosis. Is selenium the tissue constituent immediately affected by these two materials in producing their pathological effects?

The manner in which selenium might be involved in atherogenesis can only be conjectured about at present. It is an essential component of glutathione peroxidase (11), an enzyme thought to function in the degradation of hydrogen peroxide and other organic hydroperoxides generated during oxidative metabolism. Selenium may therefore be involved in protecting the coronary endothelium from oxidative damage. This idea has been stated more specifically by Miettinen et al. (12), who observed an association of low selenium concentrations of serum with low concentrations of polyunsaturated fatty acids in serum lipids. They considered the latter to be an independent risk factor for coronary artery disease.

Interestingly, an inverse relationship has also been observed in humans between serum concentrations of selenium and the occurrence of cancer. In a recently reported prospective study (13), serum concentrations of selenium in patients who developed cancer within the following five years were significantly less than those of patients who were still cancer-free after five years. This suggests the possibility of a similar type of relationship between plasma concentrations of selenium and both atherosclerosis and cancer. Thomas and Kim (14) have recently reviewed the data that support the possibility that some atherosclerotic proliferation may be a result of the development of occasional neoplastic foci, a concept first proposed by the Benditts (15). This apparent linkage by a common denominator of the two great disease scourges of modern man lends urgency to the need for defining the true relationships of selenium to atherosclerosis and cancer.

The mean value found for plasma total selenium concentration in the control patients in this study was 136 \( \mu g/L \) (1.72 \( \mu mol/L \)), which is similar to the values obtained in other studies of humans by fluorometry (6) and neutron activation analysis (13). In two studies in which atomic absorption analysis was used, the total selenium concentrations in plasma of controls were 55 \( \mu g/L \) (0.70 \( \mu mol/L \)) (1) and 80.1 \( \mu g/L \) (1.01 \( \mu mol/L \)) (2). In a study in which nickel was used to stabilize selenium in biological samples during atomic absorption analysis, the control group of humans had a mean total selenium concentration of 121 \( \mu g/L \) (1.53 \( \mu mol/L \)) in plasma (16). Obviously, different analytical methods can produce discrepant results; the need for a proven, reliable method of analysis for selenium is evident.

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References