Effect of Double-Blind Crossover Selenium Supplementation on Biological Indices of Selenium Status in Cystic Fibrosis Patients

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Twenty-seven cystic fibrosis patients received selenium supplementation (2.8 μg of sodium selenite per kilogram of body weight per day) or a placebo. This 5-month trial was conducted as a double-blind, placebo-controlled study. After an interval of 2 months, treatments of the two groups were interchanged (crossed over) for another 5-month period. A group of healthy subjects, living in the same area, was investigated simultaneously. No selenium deficiency was found either in plasma or in erythrocytes before the supplementation. This result was inconsistent with a previous study performed in 1988 in our laboratory. This change in selenium status can be explained by progress in the nutritional nursing care of children and by the addition of selenium to the diet. During the study, selenium concentrations in plasma decreased when patients received placebo treatment and increased during selenium intake. In one of the two groups a similar variation was found for glutathione peroxidase activities in plasma and erythrocytes, whereas erythrocyte selenium was normal and did not change in any group. Nowadays, in the Grenoble area, the selenium status of cystic fibrosis patients is close to normal. Nevertheless, this study indicates a fragile equilibrium, given that selenium concentrations can be lowered by placebo or mildly increased by supplementation.

Indexing Terms: glutathione peroxidase • nutritional status • trace elements

Although most of the genetic mutations responsible for cystic fibrosis have been identified, the relations between these mutations and clinical manifestations of the disease (e.g., pancreatic insufficiency, abnormalities in the concentration of sweat electrolytes, and chronic pulmonary infection) have not yet been established (1). Malnutrition observed in cystic fibrosis has been associated with poor dietary intake, malabsorption, and malabsorption of food, and increased nutrient requirements secondary to chronic infection (2). Although it has been suggested that cystic fibrosis might be caused by a nutritional deficiency in the trace element selenium (3), this is unlikely, and it is now believed that patients with cystic fibrosis are at risk for clinical selenium deficiency, in part because of malabsorption (2).

Selenium is part of the active site of glutathione peroxidase (GSH-Px), located in cytosol, plasma (4), or membranes (5). All of the GSH-Px isoenzymes have a tetrameric structure with one atom of selenium per subunit. GSH-Px catalyzes the reduction of organic hydroperoxides and hydrogen peroxides by glutathione and is thus an important component in the mechanism for protecting tissues from oxidative damages. Aberrant free-radical activity has been observed in cystic fibrosis (6), accompanied by an increase in lipid peroxidation (7, 8).

Several authors have reported a correlation between selenium and GSH-Px activity in patients with cystic fibrosis, particularly among patients with low selenium concentrations in blood (9–11). In our laboratory, previous studies (7, 8) were designed to investigate antioxidant status in cystic fibrosis. Results have shown that cystic fibrosis patients have significantly lower plasma selenium concentrations and lower GSH-Px activities than normal individuals.

Acute diseases involving infectious or inflammatory processes may lead to the abnormal metabolism of trace elements because of qualitative or quantitative variations in cytokine or hormone secretion. For this reason, the most valuable index of selenium deficiency is the repletion observed after selenium supplementation.

Among four severely affected patients receiving cyclic parenteral nutrition, a temporary increase in GSH-Px activities was observed. This increase was the outcome of a preliminary trial in which the patients received 3 months of supplementation with sodium selenite (200 μg/day selenium) (7).

The purpose of this study was to investigate the selenium status of 27 cystic fibrosis patients supplemented with a dose of selenium close to the recommended nutritional requirement (2.8 μg per kilogram of body weight per day).

Materials and Methods

Patients

We studied 27 patients with cystic fibrosis: 12 females and 15 males, ages 7 to 20 years (mean age, 12 years). Diagnosis was confirmed by two positive tests with high electrolyte concentrations in sweat. All patients receiv-

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6 Nonstandard abbreviations: GSH-Px, glutathione peroxidase; SP, patients receiving first selenium, then placebo; PS, patients receiving first placebo, then selenium.
ing pancreatic extracts and vitamins maintained their
treatment during the study. Their anthropometric cri-
tera presented a mild decrease in comparison with
Sampe’s curves: % body weight, −0.6 SD; % height, −0.2
SD; % mid-arm muscle circumference, −0.6 SD; and %
triceps skinfold, −0.3 SD. Shwachman scores have a
median of 83 (40 to 95) and Brasfield scores a median of
8 (1 to 22).

The following conditions and therapy were bases for
rejection because active oxygen species were implicated:
diabetes, hepatitits, cirrhosis, corticotherapy, and insu-
lin therapy. To take part in the study, patients already
receiving selenium supplementation were asked to stop
such intake 2 months before the beginning of the ex-
periment.

A double-blind, crossover study was performed, with
an interval of 2 months before crossing over. Each
patient received 2.8 μg of sodium selenite (Aguettant,
Lyon, France) per kilogram of body weight per day for 5
months and placebo during another 5-month period (I2,
I3). The subjects were chosen at random from a group of
eligible patients. Thirteen patients (6 females and 7
males) underwent the treatment in the order selenium/
placebo (SP group), and the remaining 14 patients (6
females and 8 males) participated in the reverse order:
placebo/selenium (PS group). Seventeen healthy indi-
viduals living in the same geographical area (8 females
and 9 males, ages 8 to 19 years, mean 11 years) were
selected as control subjects. Blood samples were col-
lected just before the beginning of the study (M0), after
5 months (M5), and at the end of the treatment (M12).
Control subjects were investigated simultaneously at
M0 and at M5. One patient in the SP group died during
the study, so only investigation during the M0 period
was carried out.

Informed consent was obtained from all subjects and
their families. This study was approved by the ethical
committee on human experimentation of the Michallon
Grenoble Hospital.

Methods

Tests were carried out with 10 mL of blood collected in
trace-element-free heparinized tubes (Sobioba, Grena-
oble, France). Samples were immediately centrifuged for
10 min at 1600 × g. Plasma was subdivided and frozen
until analysis and erythrocytes were washed with iso-
tonic, trace-element-free Tris-HCl buffer, 400 mmol/L,
pH 7.15.

Electrothermal atomic absorption spectrometry was
used to determine the selenium content of plasma with
a Perkin-Elmer Model 5100 spectrometer fitted with an
HGA 600 graphite furnace (Perkin-Elmer, Norwalk,
CT), an AS 60 autosampler, and transversal Zeeman
background correction (I4). The accuracy of the tech-
nique was tested by analysis of Seronorm trace element
serum, batch 112 (Nycowes Pharma, Oslo, Norway:
measured value, 1.08 ± 0.04 μmol/L; certified value, 1.1
μmol/L; n = 38).

Erythrocyte selenium was measured by stable-isoto-
pe-dilution gas chromatography–mass spectrometry.

Isotope ratio measurement (10°Se/11°Se) was made on a
Nermag R10-10C quadrupole mass spectrometer cou-
ped to a DN 200 gas chromatograph (Delsi-Nermag
Instruments, Argenteuil, France) under the following
conditions: glass needle injector 250 °C, capillary col-
umn CPSiI 5 CB (Chrompack, Middelburg, the Nether-
lands) 9 m × 0.32 mm (i.d.), film 1-μm thick and helium
pressure 500 kPa, purge flow-rate 20 mL/min. The
precision of the technique in red blood cells was 8.4%, n
= 10 (I5).

Selenium-dependent GSH-Px isoenzymes in plasma
and erythrocytes were evaluated by a modified method
of Gunzler, with tert-butyl hydroperoxide (Sigma Chem-
ical Co., via Coger, Paris, France) used as substrate
instead of hydrogen peroxide (I6). Results were ex-
pressed as micromoles of NADPH (Boehringer-Man-
heim, Germany) oxidized per minute per gram of
hemoglobin (U/g Hb) for erythrocyte GSH-Px and as
units per liter for plasma (U/L) GSH-Px.

After precipitation of serum proteins, serum tocoph-
erol was extracted into hexane and then quantified by
measuring relative fluorescence at specific activation
(285 nm) and emission (330 nm) wavelengths (I7) with
a Model LS 50 fluorimeter (Perkin-Elmer Ltd., Bucks,
UK).

Total serum cholesterol was determined by a modifi-
cation of the Liebermann–Burchard reaction (I8), used
in the UNI-KIT III Cholesterol PAP (Roche Diagnostica,
Basel, Switzerland) and adapted to a Cobas Fara cen-
trifugal analyzer (Roche Diagnostica).

Statistical Analysis

All values were expressed as mean ± SD, and statis-
tical analysis was performed with PCSM software (Data
system, Delta soft, Grenoble-Meylan, France). The effect
of the treatment was tested with an analysis of variance
with repeated measures and without covariance for the
two groups of cystic fibrosis patients and for control
subjects. Differences between groups were tested with
an analysis of variance on independent series. Analysis
of variance was followed by a Newman–Keuls test with a
significance limit of P < 0.05. Simple linear correla-
tions were assessed by Pearson’s test with a limit of
statistical significance set at P < 0.05.

Results

Plasma selenium concentrations in cystic fibrosis pa-
patients were very close to those of control subjects, with
no significant difference being found before the trial
(M0) between the two cystic fibrosis groups and the
control group (Table 1).

During placebo periods, plasma selenium concentra-
tions decreased in the two groups of patients. The
decreases in concentration were almost the same: PS,
−0.203 μmol/L; SP, −0.274 μmol/L. At the end of the
placebo periods, only the PS group was significantly
different from the control group (P < 0.0001). The in-
creased concentrations in plasma selenium observed
during the supplementation periods were more substan-
tial: PS, +0.111 μmol/L; SP, +0.39 μmol/L. At the end of
the activity of GSH-Px. The most significant change was observed in GSH-Px activity in M5 compared to M0. In contrast, during the same period, selenium concentration increased in plasma and erythrocytes. In addition, selenium concentrations in plasma and erythrocytes were significantly higher in the control group compared to the placebo group. These findings suggest that selenium supplementation may have a positive effect on the GSH-Px activity and selenium concentrations in plasma and erythrocytes.

Discussion

Our findings suggest that selenium supplementation may be beneficial for patients with cystic fibrosis. Selenium supplementation increased GSH-Px activity and selenium concentrations in plasma and erythrocytes, which may protect against oxidative stress. Further studies are needed to determine the optimal dose and duration of selenium supplementation for patients with cystic fibrosis.
rectly related to selenium deficiency, and similar symptoms have been reported in cystic fibrosis patients. Lesions occur in pancreas, liver, and heart, and both animal model studies and human studies in China suggest that these shared lesions are the result of selenium deficiency (19). In cystic fibrosis, some cases of carcinoma (20) and cardiomyopathy (21) have been reported to result from selenium deficiency. Selenium protects the body against damage from active oxygen species mainly through GSH-Px activity. Nevertheless, selenium deficiency is generally moderate in cystic fibrosis patients; for example, their selenium status is higher than the normal value for healthy children living in New Zealand (22), an area poor in soil selenium content.

Selenium status is generally assessed by measuring selenium concentrations in biological fluids and tissues and the enzymatic activity of GSH-Px (11). Selenium concentration is usually determined in plasma or red blood cells because of easier accessibility than with urinary or tissue selenium. The plasma or serum selenium concentration appears to be a sensitive index of short-term selenium status, whereas erythrocyte selenium concentrations, which have a slower response to changes in intake or status, provide a better long-term index (23). GSH-Px can be measured in serum, plasma, red blood cells, or platelets, and reflects selenium dietary intake. Selenium can be incorporated in newly synthesized erythrocyte GSH-Px only during the bone marrow life of the erythroid precursors (24). In erythrocytes, several authors have shown that increases in the activity of GSH-Px parallel the increase in protein synthesis that occur in the presence of selenium (24, 25).

Selenium deficiency in our patients. Until 1983, most authors agreed that cystic fibrosis patients had lower plasma selenium concentrations than did healthy control subjects living in the same area (Table 6). Only Castillo et al. (9) found decreased concentrations when cystic fibrosis patients were vitamin E-deficient (<7 mg/L). They were also the only ones to observe a decrease in erythrocyte selenium content, but these results could partly be due to the control values they refer to. In a previous study in the area of Grenoble (France) during 1987–1988, a net biological deficiency in plasma selenium concentration was observed in cystic fibrosis patients (7). In the area of Nancy (France), a less severe deficiency was observed, but the selenium concentrations were significantly lower in the plasma and erythrocytes of cystic fibrosis patients (8). This defect was supposed to result from pancreatic insufficiency that creates a situation of general malabsorption and malnutrition. Malnutrition can also be secondary to chronic infections that decrease appetite and induce the secretion of cytokines, such as tumor necrosis factor, and thus modify trace element metabolism.

Therefore, it was surprising to observe, before starting the supplementation (in 1991), that selected patients presented a plasma selenium status very close to normal. This result was explained during discussions with parents and children. Parents conceded retrospectively that since the first study in the area of Grenoble most of them had given selenium to their children by self-medication, a fact they had not stated at the first interview. This hypothesis was confirmed by the decrease in plasma concentration observed during the placebo period for the group receiving placebo first (PS), as well as for the group receiving selenium first (SP). In fact, selenium status of cystic fibrosis patients decreased when they were not selenium-supplemented. Antibiotic therapy, e.g., against Pseudomonas aeruginosa, can help to reduce the metabolic disturbances caused by tumor necrosis factor and might also help to maintain selenium status. It is also worth noting that our patients presented a mild evolution of the disease with only one-half SD according to Sampe’s curves. More serious clinical situations have led to increased loss and decreased intake of trace elements.

Data on erythrocyte selenium content confirms the normal status observed in our cystic fibrosis patients. Values of other indicators of selenium deficiency such as plasma or erythrocyte GSH-Px were not significant.

Effect of supplementation on selenium status. To our

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<th>Table 6. Indicators of Selenium Status in Cystic Fibrosis Patients and Control Subjects (Values from Literature)</th>
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D, decreased value for cystic fibrosis. ND, not determined.

Cystic fibrosis values.
knowledge, no trial of selenium supplementation in cystic fibrosis patients has been published previously. In a preliminary attempt, four patients undergoing cyclic parenteral nutrition at the terminal stage of the disease received selenium supplementation. Net increases in blood selenium and GSH-Px activity were observed, as well as a decrease in thiobarbituric acid reactants, which are lipid peroxidation markers (26). Since then, a significant inverse correlation between the concentration of serum thiobarbituric acid reactants and whole-blood selenium has been observed (27). These first results led us to perform this trial. Only in the group that received the supplement first (SP group) did the 2.8-μg dose result in a significant increase in plasma selenium concentrations. This rise was not observed similarly in red blood cells. GSH-Px activity variations were observed in patients as well as in control subjects, but results were significant only in the SP group, for plasma and erythrocyte enzymes. The variable effect of the supplementation in the two cystic fibrosis groups cannot be explained by differences in age, anthropometric, or clinical conditions. However, when patients were taken as one group (PS and SP), a positive linear correlation was found between plasma selenium and GSH-Px activities at M6. Similar observations were reported by others (9–11) but only with selenium-deficient patients. In our study, correlations also exist after supplementation.

Because of the enhanced selenium toxicity in vitamin E deficiency, we first investigated the tocopherol status of our patients. We saw no significant difference between the cystic fibrosis patients and the control group, even when we related tocopherol concentrations to cholesterol. Low blood concentrations of vitamin E in children with cystic fibrosis reflect tissue depletion and not defective transport (28). All patients treated daily with tocopherol continued their treatment during the study.

In conclusion, improvement in nutrition and the development of selenium self-medication may actually lead to an apparently normal selenium status in patients with cystic fibrosis. Nevertheless, our investigations show that their selenium status is not well established and that the equilibrium is easily modified when placebo is given. The markers used to investigate selenium status in cystic fibrosis patients seem appropriate, but, with regard to the differences in response between the two groups, selenium status in cystic fibrosis does appear peculiar. In this case, as opposed to subjects receiving parenteral nutrition (24) or New Zealand residents (29), no direct relation can be systematically established between dietary intake and selenium status. Evaluation of selenium status for each cystic fibrosis patient still remains necessary, and decreased concentrations of selenium must be the determining factor for a selenium supplementation strategy.

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