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Influence of selenium on postischemic brachial artery dilation in patients with peripheral arterial occlusive disease and selenium deficiency: A monocentric prospective controlled open pilot study

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Abstract

In an open prospective phase-IV pilot study the effect of selenium (intravenous bolus injection of 500µg sodium selenite) was investigated on the brachial artery diameter of patients (n=20) with peripheral arterial occlusive disease in stage II according to Fontaine (PAODII) and selenium deficiency. Flow-mediated vasodilation was assessed under resting conditions (control phase) and at 5 and 30 seconds after cuff deflation completing suprasystolic compression of the upper arm. After 30 minutes, 500 µg of selenium was injected as bolus and the above mentioned measurement procedure was repeated.

Taken at rest, the mean brachial artery diameter of the 20 patients with PAOD was 4.14±0.44 mm. Immediately after injection of selenium, the diameter increased to 4.65±0.45 mm, i.e. in average about 0.51 mm or 12.25 per cent ($p<0.0001$). Consequently, this open study showed that under resting conditions selenium has a marked effect on brachial artery diameter.

The respective mean brachial artery diameters measured at 5 and 30 seconds after cuff deflation were significantly larger by 0.418 mm ($p<0.0001$) and 0.42 mm ($p<0.0001$) after selenium injection than before treatment with selenium.

This indicates that despite vasodilation under resting conditions postischemic reactive hyperemia was not negatively influenced. Selenium mediated vasodilation was as pronounced in the phase of reactive hyperemia as under resting conditions.

The brachial artery diameters of the patients were identical before each testing phase ($p=0.945$); homogeneity at baseline was given; a carry-over effect was ruled out.

In all, the study provides clear though not evidential results accountable to the pilot character of the study and biometric specifications. The study will, nevertheless, prove valuable as hypothesis generation and as basis of a data based sample size calculation for a future, prospective placebo-controlled randomized clinical study.

Key words: selenium, reactive hyperemia, brachial artery, peripheral artery disease, vasodilation

Introduction

In western industrial nations atherosclerotic diseases are still the primary cause of death. According to the response-to-injury theory, lesion of the vascular endothelium favours the adhesion of thrombocytes which, by releasing growth factors, promote proliferation of smooth muscle cells [19]. At the same time infiltration of macrophages and accumulation of lipids takes place [21]. In recent years, inflammatory processes – induced either by autoimmune reaction or infective agents – are being discussed in the pathogenesis of atherosclerosis besides the traditional clinical risk factors [21]. In addition, the imbalance between oxidative stress and antioxidative protection mechanisms are in discussion as a relevant factor in atherogenesis. To date, a large number of endogenous and exogenous substances (vitamin C and E, selenium, catalase, etc.) have been investigated [4, 14].

Selenium is an essential trace nutrient required in µg amounts by humans for the synthesis of selenoproteins such as glutathione peroxidase and thioredoxin reductase. These are the major forms of selenium in endothelial cells that have important functions relevant to inflammation and cardiovascular disease. In humans a link between selenium intake and cardiovascular disease was first suggested by the negative correlation between regional cardiovascular disease mortality and the selenium content of drinking water and crops in a cross sectional study [28]. Subsequent prospective human studies have reported that serum selenium is inversely related to risk of progression of carotid artery atherosclerosis [29], ischemic heart disease [30], coronary artery disease [31], and cardiovascular disease mortality [32]. Endothelial dysfunction is an early event in the development of such diseases. The available data support a causative role for oxidative damage in the development of endothelial dysfunction. Hydrogen peroxide and other reactive oxygen species are significant in endothelial dysfunction because of their ability to react with and eliminate nitric oxide (NO), the main mediator of endothelial-dependent vasodilation [5]. In healthy individuals with normal selenium levels the daily intake of 300 µg of selenium for 48 weeks did not influence the brachial artery responsiveness to transient occlusion [Hawkes]. But it is unclear if selenium application influences the vascular resonsiveness in patients with atherosclerotic disease – with endothelial dysfunction – and selenium deficiency.

Study design

The trial was conducted as a monocentric prospective controlled open phase-IV pilot study [25]. The aim was to examine whether there is an effect of the radical scavenger sodium selenite (Selenase®, biosyn Arzneimittel GmbH, Fellbach, Germany) on the brachial artery diameter of atherosclerotic patients under resting conditions and during postischemic hyperemia. The patients received 500 µg sodium selenite as an intravenous bolus injection. 20 patients with peripheral arterial occlusive disease in stage II and selenium deficiency were included in the study. Due to gender dependence of brachial artery diameters only male probands were chosen.

Inclusion criteria:
- patients with peripheral arterial occlusive disease in stage II according to Fontaine
- patients with a selenium deficiency (plasma concentrations lower than 0.72 µmol/l)
- male subjects
- age between 50 and 60 years

Exclusion criteria:
- female subjects
- age lower than 50 years
- age higher than 65 years
- subjects under current treatment with direct vasodilators

The patients arrived between 9 and 11 a.m. They rested for one hour to ensure adaptation to room temperature after which the

ultrasonic Doppler measurements were started. The time schedule was observed strictly for all patients in consideration of possible individual circadian fluctuations of blood pressure and microcirculation [10].

Eating and drinking were not allowed during the whole examination period so as to rule out interactions of the substance under study with nutrients and/or avoid resorptive disorders [26].

As with all patients, the brachial artery diameter was measured first under resting conditions. Then again, after the lapse of one minute during compression and likewise 5 and 30 seconds after cuff deflation (control phase measurements).

After 30 minutes a bolus injection of 500 µg sodium selenite was administered and – in similar order as above – measurements of the brachial artery diameter before and during compression and of postischemic dilation were taken (test phase measurements). The experimental order is shown in Figure 1.

Patient compliance can be given as 100 per cent as the tested substance was administered directly by the examining physician.

Adverse events occurring in the course of the study were recorded on a questionnaire attached to the test form. The study conformed with the principles outlined in the Declaration of Helsinki/Somerset West, GCP guidelines and standards required according to § 40 – 42 in the Arzneimittelgesetz (AMG), Germany [7]. The local ethic commission of the Landesärztekammer of Saxony, Germany, gave its approval of the study.

Each patient was informed that participation in the study was voluntary and could be withdrawn at any time without statement of reasons. All patients were informed about the test substance and its possible side effects as well as explained the nature, relevance and significance of the study. They all gave their written consent to the study.

Measurement method

Diameter measurements were taken of the right brachial artery using Acuson (Acuson 128 XP/4) Ultrasound (Acuson, Mountain View, California, USA) with a 7.5 MHz linear array transducer [22].

Measurements were performed according to a standardized procedure described by Mannion [12].

Step 1: Measurement under resting conditions
B-mode-ultrasound longitudinal scan of right brachial artery. Measurement setting was in PW-mode (angle correction 60 degrees). The mean vascular diameter was calculated according to the vascular calculation modus of the instrument.

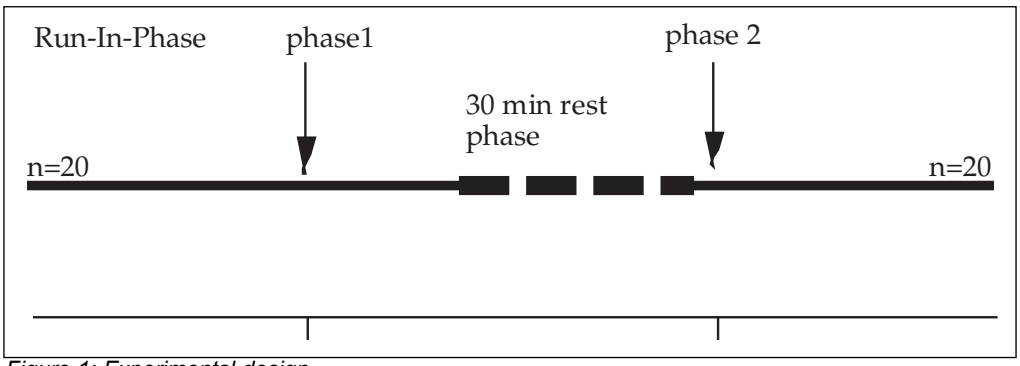

Figure 1: Experimental design

Step 2: Postischemic measurement
After 3 minutes suprasystolic compression the cuff was released (20 mmHg above systolic pressure) and diameter measurements were taken at 5 and 30 seconds after cuff deflation.

Step 3: Measurement under selenium treatment
After 30 minutes rest, the drug was administered to the patient and steps 1 and 2 were repeated.

Statistics

To examine the baseline comparability among the patients with respect to test parameters before each measurement phase, homogeneity of the initial parameters was determined for continuous samples by paired t-test and for classified variables by chi-square homogeneity test.

Statistical analysis was in accordance with the guidelines for Good Clinical Practice of the European Union. Completer and intention-to-treat analyses were identical. In case of Gaussian distribution the two-sample paired t-test was used and the Wilcoxon signed rank test for nonnormal distribution.

A significant difference was assumed when the p-value was under 0.05. As the study was explorative, p-values were generated and given descriptively. An a-adjustment according to Bonferoni-Holt was rejected.

Patients

20 patients with peripheral arterial occlusive disease and selenium deficiency were included in the study. All were stage II according to Fontaine, 13 in stage IIa and 7 in stage IIb. All patients had a lowered ankle/brachial pressure index; the mean value was 0.63 ± 0.10. The pain-free walking distance was 345 ± 161m. There were 10 patients with PAOD of lower and 10 with PAOD also of higher extremities; 9 patients had unilateral and 11 had bilateral PAOD. All patients were male; mean age was 66.45 ± 9.5 years; mean body weight 87.0 ± 16.23 [kg] and mean body height 173 ± 5.43 [cm]. The mean systolic blood pressure was 141.5 ± 19.7 [mm Hg], mean diastolic pressure 83.7 ± 8.7 [mm Hg] and mean heart frequency 74.5 ± 11.8 [1/minute].

All 20 patients had arterial hypertension, 10 of them had diabetes mellitus (all type II), 18 of them a lipopathy. 4 of them were current smokers; all others were ex-smokers. 14 of them had a positive family anamnesis.

18 of the twenty patients also had a coronary artery disease. 7 of the patients with coronary artery disease had already suffered a myocardial infarction. 6 had cerebral perfusion disorder. 2 patients had undergone vascular surgery and 5 a peripheral intervention.

The reference range for selenium was taken from analyses of n=100 blood donors in a study by Winnefeld et al. [27]. Accordingly, the mean selenium concentration (in EDTA-anticoagulant blood samples) is 1.28 ± 0.22 µmol/l; the reference range lies between 0.84 – 1.72 µmol/l. Values under the 2-s-threshold of 0.84 µmol/l or 85 µg/l were defined as selenium deficiency.

16 of the 20 patients had values distinctly lower than 85 µg/l; 4 had values just above threshold. However, taking into account the variance of 4.5 % of the measurement method [24], these patients were also included in the study. One patient with normal selenium concentration was excluded. The mean selenium concentration of the patients was 73.77 ± 12.8 µg/l.

Results

Exclusion of a carry-over effect

Since the experimental phases (with and without selenium) were conducted in succession, it was necessary to rule out the possibility of a carry-over effect and determine the comparability for the measurements [22]. Figure 2 shows the mean brachial artery diame-

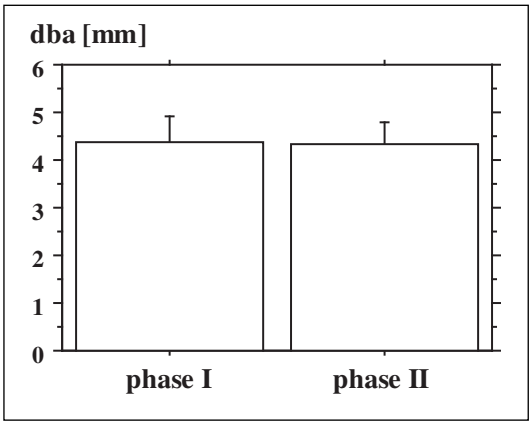

Figure 2: Brachial artery diameter dba in [mm] before each test phase

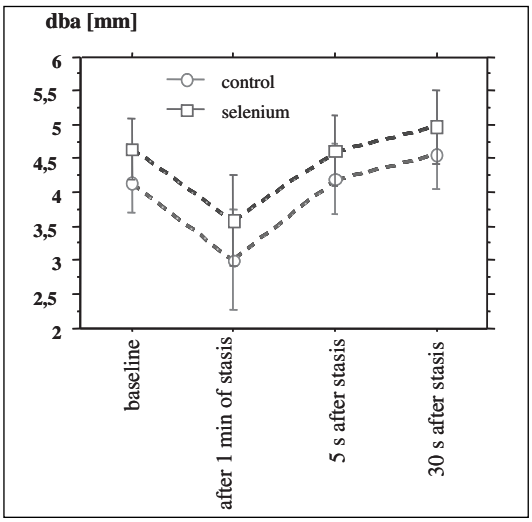

Figure 3: Brachial artery diameters dba in [mm] before and after selenium under rest, after the lapse of one minute during compression and 5 and 30 seconds after cuff deflation.

ter in mm at the beginning of the respective experimental phases. For this purpose diameter measurements under resting conditions were taken 10 minutes after control phase scans without selenium, i.e. before selenium injection.

Artery diameters returned to baseline values after the first experimental phase measurements so that a carry-over effect could be excluded. At the beginning of the second experimental phase – in which selenium was administered – brachial artery diameters were identical with values determined as baseline under resting conditions before the first test phase (p=0.945). Thus homogeneity of baseline diameters was given.

Influence of selenium on endothelial dysfunction in peripheral arterial occlusive disease

Figure 3 shows brachial artery diameters before and after selenium administration measured under rest, after the lapse of one minute during compression and 5 and 30 seconds after cuff deflation.

Taken at rest, mean brachial artery diameter of the 20 patients with PAOD was 4.14 ± 0.44 mm. Measured immediately after injection of selenium, the diameter increased to 4.65 ± 0.45 mm, i.e. in average about 0.51 mm or 12.25 per cent (p<0.0001). Consequently, it was shown that under resting con-

ditions selenium has a marked effect on brachial artery diameter.

Measured 5 and 30 seconds after cuff deflation, the respective mean brachial artery diameters were significantly larger by 0.418 mm ($p<0.0001$) and 0.42 mm ($p<0.0001$) after selenium injection than before treatment with selenium.

Correlation "selenium concentration – brachial artery diameter"

Figure 4 shows the correlation before selenium injection between brachial artery diameters under resting conditions and selenium concentration.

The correlation coefficient is $r=0.255$. This indicates a weak correlation: The lower the selenium concentration the smaller the brachial artery diameters. (A larger dependency can not be expected, because vascular diameters are related highly to body height, for example). The relation between blood selenium concentration and brachial artery diameter is more significant after selenium injection where the correlation coefficient is $r=0.398$. Figure 5 shows this relationship.

Discussion

The patients with PAOD stage II included in the present study – with a typical risk profile and concomitant diseases – showed a marked endothelial dysfunction. Compared to 4% postischemic dilation in healthy subjects, the 0.3% diameter increase after ischemia in the patients is clearly restricted for measurements at 5 seconds after cuff deflation [6, 15, 22].

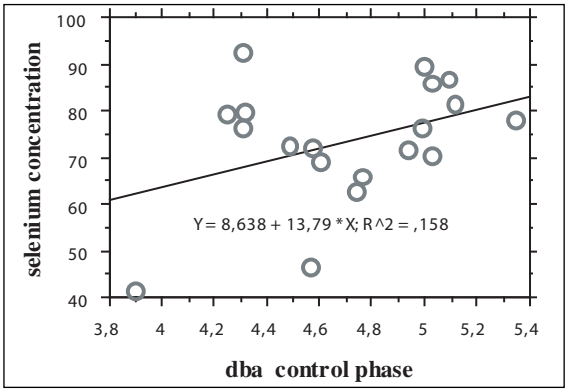

Figure 4: Correlation between brachial artery diameters and selenium concentration

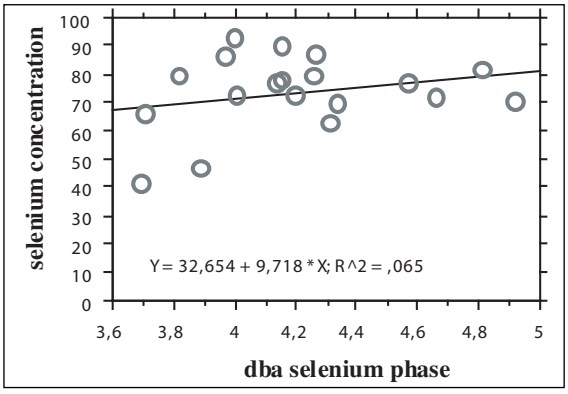

Figure 5: Correlation between brachial artery diameters and selenium concentration

Measured immediately after the intravenous injection of 500 µg sodium selenite as bolus, there was a marked increase of 12.5% (p=0.0001) compared to the baseline brachial artery diameter (Fig. 3). This was similar for artery diameters in the postischemic phase (Fig. 3). Accordingly, the artery diameters 5 and 30 seconds after cuff deflation were markedly larger than in the control phase without selenium (p=0.0001 respectively). This reaction was the same for all patients, except for one patient who showed no effect.

Of the 21 consecutively tested male patients with peripheral arterial occlusive disease in stage II Fontaine and mean age of 66.45 ± 9.5 years, only one had a normal selenium blood concentration; all others had a selenium deficiency (mean value of 73.8 ± 12.8 µg/l). Selenium concentration values under the 2-s-threshold were referred to as selenium deficiency. According to an investigation by Winnefeld et al., this threshold is 85 µg/l [27]. In an earlier study which included patients with an assumed coronary heart disease, a mean selenium value was given that was approximate to the 2-s-threshold value [24]. However, it was not specified how many of the patients had values under the 2-s threshold, i.e. had a selenium deficiency. Similarly, measurements in patients with myocardial infarction showed no regularity with regard to selenium concentration. Unfortunately, prevalence for selenium deficiency was not given for the relevant group of patients. Therefore, it can not be determined whether the high prevalence for patients with selenium deficiency in the present study is because:

- the reference range (assessed in Thuringia, Germany) is not relevant for patients from Saxony (geographical variability [16]),
- Saxony is generally a selenium deficient area or,
- the primary state of atherosclerotic diseases possibly concurs with a selenium deficiency.

There is quite good argument, however, that patients with atherosclerosis also have "free radical disease" [21] which might be accompanied by low selenium plasma concentrations.

Clinical significance

One cause of endothelial dysfunction is oxygen radicals, which instantly react with NO and thus reduce its bioavailability [17, 18]. In addition, there is clear evidence that oxidized LDL cholesterol inhibits the receptor-mediated effect on Gi-proteins and reduces intracellular bioavailability of L-arginine thus also inhibiting the formation of NO [5, 13, 23]. The antioxidative effect of selenium diminishes both mechanisms so that the brachial artery vasodilation observed in this study after selenium administration is apprehendable. Independent of these findings, there is evidence of a cGMP-independent effect of NO, namely the direct activation of calcium-dependent potassium channels of vascular smooth muscle cells [3].

Endothelial dysfunction reduces blood flow during elevated metabolic demand and a decreased vasodilatation. In extreme cases, this may lead to a paradoxical vasoconstriction, so that under stress situations oxygen supply of the tissue may be insufficient. In consequence, this will easily lead to ischemic microcirculatory disorders, in particular during physical exertion, psychological stress or exposure to cold [20]. These are all factors associated with the occurrence of angina pectoris attacks in patients with coronary artery disease.

Limitations of the study

From the biometrical point of view the following requirements have to be met in a clinical study:

1) observation equivalence
2) random assignment of test drug
3) comparability of test groups

Point 1 was not fulfilled in this study, since the examining physician knew whether his measurements were carried out with or without selenium. It can not be decided in this case, if his expectation of the outcome may have influenced results. Also Point 2 of random assignment is not fulfilled which usually provides comparability of test groups. However, comparability in this open pilot project is given, as it was a cross-over study and conducted on the individual patient on the same day.

Moreover, only the short-term effect of selenium was examined in the present study. For clinical application the long-term effect of selenium remains to be studied.

Conclusion

In patients with endothelial dysfunction on the basis of an atherosclerotic disease with concomitant selenium deficiency the injection of sodium selenite induced a significant vasodilation of the brachial artery under resting conditions and during reactive hyperemia.

In all, the investigations yield quite clear results which, however, can not be regarded as evidential due to the pilot character of the study and the above mentioned biometrical specifications. However, the results may serve as hypothesis generation and as basis of a data based sample size calculation for a prospective placebo-controlled randomized clinical study which is currently in development.

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