

Selenium status in relation to reduced glutathione peroxidase activity in aspirin-sensitive asthma

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Summary

Selenium status and its relationship to lowered platelet glutathione peroxidase activity was investigated in 18 subjects with aspirin (ASA)-induced asthma and in asthmatic and non-asthmatic ASA-tolerant subjects. Mean serum selenium concentration in ASA-tolerant asthmatics ($1.25 \mu\text{m/l}$; $98.5 \mu\text{g/l}$) was significantly higher than that in ASA-induced asthma subjects ($1.14 \mu\text{m/l}$; $89.7 \mu\text{g/l}$) and than that in healthy controls ($1.15 \mu\text{m/l}$; $91 \mu\text{g/l}$). Although there was a correlation between serum selenium concentration and platelet glutathione peroxidase activity, enzyme activity was significantly lower in the ASA-induced asthma group compared to other groups even after correcting for selenium status. These results indicate that lowered platelet glutathione peroxidase activity in ASA-induced asthma is a product of both selenium availability and an unidentified syndrome-specific (possibly genetic) factor.

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Introduction

Aspirin (ASA) hypersensitivity is important both as a relatively common and potentially fatal clinical problem, and as an intriguing pharmacological model of allergic inflammation.

There are at least two distinct clinical forms of ASA sensitivity: ASA-induced asthma (ASA-asthma) and non-asthmatic acute urticarial/angioedematous reactions (which can occur in asthmatics). We also believe that ASA-induced asthma itself is clinically heterogenous (and possibly heterogeneous). It occurs in the 'aspirin triad' syndrome when it is associated with chronic upper and lower airway inflammation, progressive severe chronic asthma, and recurrent nasal polyps, and it occurs as an isolated phenomenon in otherwise mild asthmatics who never develop any further problems.

The pathogenesis of ASA-asthmatic states is elusive. Abnormal eicosanoid metabolism may well be involved, but the state(s) can clearly not be attributed simply to NSAID-induced blockade of cyclo-oxygenase. ASA fails to trigger attacks in most asthmatics and while there is considerable crossreactivity between ASA and other cyclo-oxygenase inhibitors in ASA-asthma, it is incom-

plete [1]. No consistent abnormality of eicosanoid generation has been demonstrated in tissue from ASA-sensitive individuals. Little attention has been paid to non-eicosanoid pathways in the condition, although all the drugs capable of triggering the reaction are known to have other pharmacological actions.

Ameisen *et al.* [2,3] have produced strong evidence that an abnormal platelet reactivity occurs in, and is specific for, ASA-asthma. This involves ASA/NSAID-induced release of oxygen radicals as well as the H_2O_2 -dependent generation of other mediators. An increased frequency of low whole blood peroxidase activity had been observed in patients with ASA intolerance by Malmgren *et al.* [4]. We subsequently showed lowered levels of intracellular glutathione peroxidase (GSH-Px) activity in the platelets of ASA-sensitive asthmatics [5].

GSH-Px (EC.1.11.1.9) is a selenoprotein, the enzyme activity of which is dependent on a selenocysteine residue in each of its four polypeptide chains [6]. Enzyme activity is limited by selenium (Se) availability in deficiency states, but platelets with adequate repletion [17]. Platelet glutathione peroxidase activity is proportional to liver Se content in Se-depleted and re-fed animals and in Se-deficient human populations [8,9]. The present work was therefore undertaken to elucidate the relationship of lowered GSH-Px levels in ASA-asthma to selenium status.

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Subjects and methods

Subjects

The study population consisted of 178 out-patients presenting with asthma and/or other atopic disease, selected simply on their willingness to provide a blood sample and being over 16 years of age, and of 73 healthy comparison subjects.

Of the patient subjects, 18 were classified as having ASA-induced asthma, and 124 asthmatic and 28 non-asthmatic atopics with rhinitis, acute urticaria, gastrointestinal reactions, etc. were classified as ASA tolerant. Eight subjects were excluded from analysis on the basis of questionable and unconfirmable sensitivity to ASA. The diagnosis of ASA-induced asthma was based on clinical criteria, usually those of very clear-cut histories of two or more distinct episodes of acute exacerbations of asthma clearly related to the ingestion of ASA and/or other NSAIDs. Confirmation by oral provocation was only sought in cases giving less definite or atypical histories of sensitivity. Most subjects classified as ASA tolerant reported taking ASA and/or other NSAIDs without ill effect within the preceding 3 years. The healthy comparison group(s) consisted of 25 hospital/laboratory volunteers matched to the ASA-asthma group for smoking status (i.e. all current non-smokers) and 48 subjects donating blood at a single session at the Regional Blood Transfusion Centre.

The mean age of the study population was 37 years and age differences between groups were not statistically significant. As would be predicted, the sex ratio did differ between groups, being approximately equal in healthy and ASA-tolerant groups, but there being a 2:1 excess of females in the ASA-asthma group.

Platelet isolation

Platelet-rich plasma was obtained by centrifuging 10–20 ml of blood anticoagulated with acid citrate dextrose, at 150 *g* for 15 min. The platelets were then washed three times in saline buffer containing 36 mM citric acid, 5 mM glucose, 2 mM calcium chloride, 1 mM magnesium chloride, 0.35% serum albumin and 100 nM prostaglandin E₁ (PGE₁) at 4°C. Platelet lysates were then obtained by resuspending fresh centrifuged platelet pellets in saturated digitonin medium at 4°C for 1 hr [10]. Supernatants subsequently stored at –70°C were found to suffer no appreciable loss of enzyme activity.

Glutathione peroxidase assay

The coupled assay of Paglia and Valentine [11] was used as modified by Levander *et al.* [8]. Briefly, the volume of

lysate was adjusted according to enzyme activity and incubated in a final volume of 1 ml with 2 μ mol glutathione, 1 unit glutathione reductase (EC 1.6.4.2) and 103 μ mol NADPH (Sigma Chemical Co., St Louis, MO) at pH 7.0 and 37°C. The reaction was then initiated by the addition of 15 μ l of 15 mM t-butyl hydroperoxide, and light absorption at 340 nm was followed for 4 min. One milliunit of GSH-Px activity was defined as the oxidation of 1 nmol of NADPH per min and was related to the protein content of the lysate as determined by the Lowry method [12]. The between-batch coefficient of variation for platelet GSH-Px was 8.2%.

Measurement of serum selenium

Selenium in serum was measured by graphite furnace atomic absorption spectrometry with Zeeman background correction by a modification of the method described by Welz *et al.* [13]. The analysis was calibrated against Seronorm 103 lyophilized reference serum (Nycomed, Birmingham, U.K.). Analyses were performed on coded specimens, without information concerning ASA status or GSH-Px activity. The between-batch coefficient of variation for selenium concentration in serum was 7.0%.

Statistics

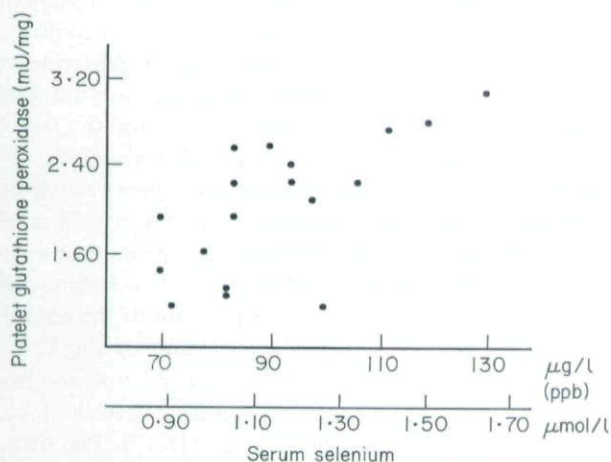
Statistical procedures were performed using the SPSS/PC+, version 3.0, software package on an OPUS PCII computer. The distribution of both GSH-Px and serum Se approximated to a normal distribution, with, in each case, two extreme values outside the predicted range. Results were essentially unchanged by excluding these extremes. There were no significant differences between the sexes within any group for either variable, but sex and age were included as independent variables (without revealing any significant effects) on all relevant procedures. The intergroup differences reported here were tested by analysis of variance (ANOVA) and subsequent pairwise comparisons by the Scheffe procedure. Analysis by Kruskal–Wallis and Mann–Whitney non-parametric procedures gave essentially identical results which are not reported further here. Independent variables examined in multiple regression procedures included age, sex, current medication and other associated medical conditions (including rhinitis, recurrent nasal polyps, food hypersensitivity and skin involvement). Serum only was obtained from the blood donors, and adequate platelet suspensions for reliable GSH-Px activity measurement were not obtained from 64 of the ASA-tolerant asthmatics. The mean and distribution of serum Se values in the former were identical with the other healthy volunteer

Table 1. Platelet glutathione peroxidase activity (mU/mg)

Group	Mean	95% confidence intervals for mean
ASA-induced asthma (<i>n</i> =18)	2.02	1.73–2.30
ASA-tolerant asthma (<i>n</i> =60)	2.52	2.44–2.61
Non-asthmatic allergies (<i>n</i> =28)	2.53	2.34–2.72
Normal controls (<i>n</i> =25)	2.78	2.67–2.87

Table 2. Serum selenium concentration $\mu\text{mol/l}$ ($\mu\text{g/l}$)

Group	Mean $\mu\text{mol/l}$ ($\mu\text{g/l}$)	95% confidence intervals for mean
ASA-induced asthma (<i>n</i> =18)	1.14 (89.7)	1.04–1.23
ASA-tolerant asthma (<i>n</i> =124)	1.25 (98.5)	1.20–1.29
Non-asthmatic allergies (<i>n</i> =28)	1.15 (91.1)	1.05–1.26
Normal controls (<i>n</i> =73)	1.15 (91.0)	1.12–1.21

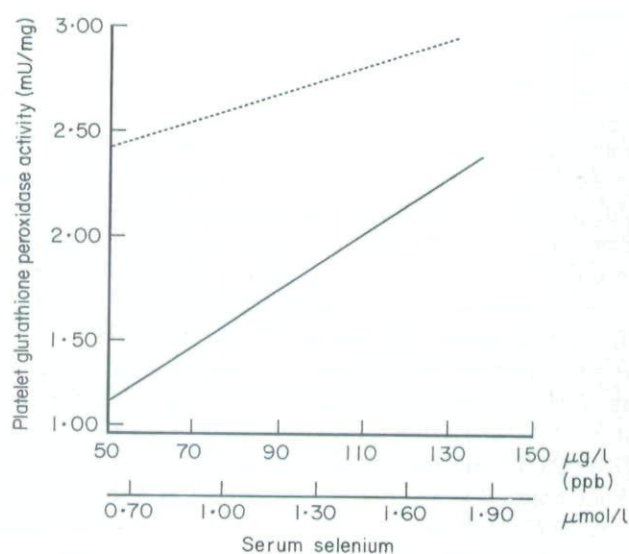
**Fig. 1.** Relationship between platelet glutathione peroxidase activity and serum selenium level in ASA-induced asthma. Pearson correlation coefficient: $r=0.655$; $P=0.005$.

sub-group, and those of the latter not significantly different from those of the rest of the ASA-tolerant asthmatics. The serum Se values given in Table 2 are therefore those of the full population of available values, but analyses restricted to subjects in whom GSH-Px values were obtained did not give significantly different results.

Results

Glutathione peroxidase

Platelet GSH-Px activity for the groups is shown in Table 1. There is a progressive reduction in mean activity from normal controls, through non-asthmatic allergies and ASA-tolerant asthmatics, to ASA-asthmatics. Intergroup differences are statistically highly significant (ANOVA $P=0.0001$). On multiple comparison testing the mean in ASA-asthma is significantly lower than that of each of the other groups and than that of subjects with non-asthmatic reactions to ASA ($P=0.01$).

**Fig. 2.** Relationship between GPX and serum selenium in ASA-asthma and normal subjects. ---, Normal subjects; —, ASA-induced asthma subjects.

Serum selenium

There is a weak but statistically significant correlation between platelet GSH-Px and serum Se level in the study population as a whole ($r=0.281$; $P=0.001$). The correlation is very much higher in the ASA-asthma group ($r=0.655$; $P=0.005$; Fig. 1) and of similar degree in the normal controls ($r=0.582$; $P=0.05$). The correlation between GSH-Px and serum Se in the ASA-tolerant asthma group was not statistically significant.

Since previous work in experimental animals and man has shown GSH-Px activity to be proportional to Se levels below a critical level of Se, intergroup differences in mean GSH-Px activity might have been due to differences in Se status. That this is not entirely the case is shown by the data in Table 2. Intergroup differences in mean serum Se are statistically significant (ANOVA $P=0.05$), but this is

accounted for by the mean serum Se in ASA-tolerant asthmatics being higher than that in normal controls and than that in ASA-asthmatics. The difference in mean serum Se levels between the ASA-asthma group and the other groups is not significant.

Our wider observations in volunteer subjects and general medical patients [14] have suggested that platelet GSH-Px activity plateaus at serum Se levels of about 1.20–1.27 $\mu\text{m/l}$ (95–100 $\mu\text{g/l}$). The failure to detect a correlation between GSH-Px and Se in ASA-tolerant asthmatics could therefore be related to that group having a mean serum Se of 1.24 $\mu\text{m/l}$ (98 $\mu\text{g/l}$). However, a significant correlation was still not detected in this group when analysis was restricted to cases with serum Se levels < 1.20 $\mu\text{m/l}$ (95 $\mu\text{g/l}$).

The combination of the three observations in the ASA-asthma group is superficially confusing, i.e. a highly significantly lowered GSH-Px activity, which is strongly correlated with serum Se, but in the absence of a reduction in mean serum Se when compared to normal subjects (but not to ASA-tolerant asthmatics). To elucidate this situation the data were analysed further by multivariate and multiple regression analyses. Both procedures confirmed a statistically highly significant relationship between ASA status and platelet GSH-Px activity independent of Se level, age, sex, drug treatment or any other identifiable variable. In other words, as demonstrated in Fig. 2, for a given level of serum Se, platelet GSH-Px in ASA-asthma is lower than would be predicted from normal selenium-deficient controls. The observed platelet GSH-Px activity in individual ASA-asthmatics is a product of both serum Se level and of a factor related to their ASA status.

Discussion

Our results show not only that lowered platelet GSH-Px activity in ASA-asthma is dependent on, and proportional to, lowered serum Se, but also that it is related to another factor the identity of which remains obscure. This factor appears specific for ASA-asthma. It seems unlikely that it can be a result of asthma itself, or of reactions to ASA, since several ASA-asthma subjects were asymptomatic at the time of venesection and most had taken no ASA or other NSAIDs for many years. It therefore seems possible that this factor is in some way related to the predisposition to the condition rather than a consequence of it. Similarly, it also seems more probable to us that the very strong association between the result of the factor's interaction with low Se (i.e. lowered GSH-Px) and ASA-asthma is related to the pathogenesis rather than being a result of the syndrome.

If lowered GSH-Px is a significant factor in the

generation of ASA-asthma, it is clearly not a sole and sufficient condition for the production of that state. There is a significant overlap in platelet GSH-Px values between ASA-sensitive and ASA-tolerant asthmatics. It therefore seems likely that the enzyme modifies (or reduced activity permits) some other initiating stimulus. There are three main groups of process through which this could occur: (i) by direct effects on eicosanoid metabolism, either by generally downgrading both lipoxigenase and cyclooxygenase pathways by removal of the trace peroxy products required for initiation, or by metabolizing specific hydroperoxide mediators or intermediates; (ii) by removal of platelet or leucocyte-produced H_2O_2 , which could be involved in the inflammatory process directly or through the generation of $\text{OH}\cdot$; (iii) by metabolizing some other non-arachidonic organic hydroperoxide(s). Since ASA affects both enzymic and non-enzymic free radical processes, either of the first two possibilities seems equally probable in the present state of knowledge.

In addition to the syndrome-specific factor (which we hypothesize could be related to the known familial predisposition), selenium status is clearly an environmentally dependent influence on GSH-Px in ASA-asthma and presumably also on the clinical expression of the sensitivity. The bioavailability of Se in the soil of the U.K., Scandinavia and some other parts of Europe is poor [15,16]. Clinical Se deficiency diseases are well documented in livestock from these areas [16,17]. The mean U.K. human Se intake in 1976 was only marginally above estimated minimum requirements [18]. A high proportion of the intake at that time was derived from cereal imports, now much reduced, from high Se geographic areas. We have published data showing that at least 25% of apparently healthy U.K. volunteers and blood donors and 50% of general medical patients are of inadequate Se nutritional status for the full expression of GSH-Px [14].

Confirmation of Se deficiency as an aetiological factor in ASA-asthma could explain inconsistencies in reports of the condition's epidemiology [19], since average Se intakes differ by several orders of magnitude worldwide [20]; it could also explain a most intriguing observation concerning disease onset, which is that although ASA-asthma is often considered to be typically a condition of middle aged women, Speer *et al.* [21] reported that rate of disease onset was approximately constant throughout life for men, equal in the sexes before puberty and after 50 years of age, but increased by several fold in women of childbearing years. In our own ASA-asthma patients, we have been struck by the fact that several actually developed asthma for the first time during pregnancy and three previously ASA-tolerant subjects had acute asthmatic reactions to the first aspirin taken after delivery. Serum Se is known to fall in pregnancy, presumably

because of a general transfer of trace elements to the foetus [22]. Chronic menstrual loss may also be a factor, since we have observed low serum Se with low platelet GSH-Px in men who have chronic gastrointestinal blood loss due to congenital vascular malformations.

Our observations of ASA-tolerant asthmatic patients are of interest in two respects: first with regard to the relationship between GSH-Px and Se, and secondly with regard to the higher serum Se in this group.

Both our ASA-sensitive and ASA-tolerant asthmatic groups are abnormal with regard to the GSH-Px/Se relationship when compared both to the normal subjects in this study and to the non-asthmatic medical patients we have studied previously [14]. The expected correlation between platelet GSH-Px and serum Se is evident in the ASA-asthmatics, but the slope of the graph is displaced to the right (Fig. 2). In the ASA-tolerant asthmatics, we failed to detect any significant correlation between these variables. This cannot be accounted for by the higher serum Se of that group. The explanation is obscure. We are presently examining the hypothesis that lowered GSH-Px/Se ratios are related to genetic GSH-Px isotypes with different enzyme activities for a given Se availability. Were this to be the case, one would predict that a left-shifted GSH-Px/Se slope would be detectable if ASA-tolerant subjects with lower serum Se than were available to us in the present study could be examined.

Our observation of a higher serum Se in ASA-tolerant asthmatics than in controls contrasts with other observations from both Britain and New Zealand, in which lowered serum or whole blood Se was observed in asthmatics (whose ASA status was not recorded). The mean serum Se for both our ASA-asthma and normal subjects is very similar to that of the normal control group from Southampton, U.K., studied by Stone *et al.* [23]. The mean for their asthmatic group without eczema was 7% lower than that for the controls, whereas it was 7% higher than controls in our own ASA-tolerant asthmatics.

In the New Zealand study [24], similar plasma Se levels were seen in asthmatic and normal subjects, but a 7% lower whole blood Se value was observed in the asthmatic group. It was concluded from this that the asthmatic group was of lower Se status and therefore that low Se is a risk factor for the development of asthma. Whole blood Se and GSH-Px levels are the sum of those from several compartments, chiefly those of plasma and erythrocytes. While whole blood values in general populations provide useful demographic data, we consider them intrinsically unsafe for the investigation of disease groups, particularly respiratory disorders, where erythrocyte/plasma/platelet and leucocyte Se concentrations and ratios may be predicted to be disturbed. However, despite reservations concerning the inconsistencies between these three sets of

observations, we consider that they justify further research into the role of Se and selenoenzymes in the pathogenesis of both asthma in general and aspirin-induced asthma in particular.

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