Reduced selenium status of patients with asthma

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SUMMARY

1. Selenium is an essential component of glutathione peroxidase (GSH-Px, EC 1.11.1.9), an enzyme which helps protect cells against damage caused by free radicals and hydroperoxides.

2. We report the plasma, whole blood and platelet concentrations of selenium, and whole blood and platelet activities of GSH-Px, in 49 patients with asthma, 23 of whom had coexisting eczema, and 76 healthy control subjects.

3. The asthmatic patients had significantly lower concentrations of selenium measured in plasma \((P<0.01)\) and whole blood \((P<0.001)\), but not in platelets. When the data were summarized as odds ratios there was a highly significant 3.54- and 5.08-fold increased probability of asthma observed for the lower range of plasma and whole blood selenium concentrations, respectively.

4. No overall decrease in platelet or whole blood GSH-Px activity was found when the asthmatic and control groups were compared.

5. Although patients with symptomatic asthma have a reduced selenium status, this does not appear to influence the antioxidant capacity of their circulating blood cells.

Key words: asthma, glutathione peroxidase, selenium.

Abbreviation: GSH-Px, glutathione peroxidase.

INTRODUCTION

Although asthma is a disease of complex aetiology, airways inflammation is recognized as being fundamental to its pathophysiology [1]. Among the mediators which may contribute to this abnormal airways response are oxygen radicals such as superoxide, hydroxyl anion and hydrogen peroxide, all of which have the potential to damage cell membranes through lipid peroxidation [2]. Selenium is an essential component of the enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9) [3], which, along with the other cellular antioxidant defence mechanisms, helps protect the cell against damage that may be caused by free radicals and hydroperoxides [4]. It is possible that low selenium levels will exacerbate mucosal inflammation in asthma by reducing the activity of GSH-Px and therefore antioxidant protection.

Another possible mechanism by which GSH-Px may be involved in the asthmatic process is in its putative role as a regulator of the lipoxygenase pathway of arachidonic acid metabolism. In both animal and human studies, GSH-Px has been linked to the reduction of 12-hydroperoxyeicosatetraenoic acid to 12-hydroxyeicosatetraenoic acid [5–7], a platelet-derived lipid with chemotactic activity for eosinophils and neutrophils [8]. The 12-hydroperoxide has an inhibitory effect on the cyclooxygenase pathway of arachidonic acid metabolism [9] and has also been shown to stimulate the synthesis and release of leukotriene B\(_4\) by human leucocytes [10]. Leukotriene B\(_4\) and the sulphidopeptide leukotrienes are considered to be important inflammatory mediators in asthma [11]. Although there are some studies which suggest that reductions of 5-hydroperoxyeicosatetraenoic acid and 12-hydroperoxyeicosatetraenoic acid to their respective mono-hydroxyeicosatetraenoic acids may occur by pathways not requiring GSH-Px [12–14], recent studies on the activity of human platelet 12-lipoxygenase [15] and the synthesis and release of leukotriene B\(_4\) by peripheral blood leucocytes [16] indicate an important role for this enzyme activity.

A recent study has reported lowered whole blood GSH-Px activity in patients with asthma who demonstrated food and aspirin intolerance [17]. The purpose of the present study was to assess the selenium status of patients with asthma in order to determine whether they were at risk of selenium deficiency. Selenium concentrations were measured in plasma, whole blood and platelets, and GSH-Px activity in whole blood and platelets, from asthmatic patients. Since selenium concentrations are affected by diet [18], historical control data cannot be used and therefore matched non-asthmatic control subjects were also studied.

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METHODS

Subjects

Patients. Forty-nine patients, 24 women and 25 men, aged 15–61 (mean 33.3) years, who attended a chest clinic for symptomatic asthma, were studied. Of these, 23 patients also had active eczema. All subjects were asked to complete a questionnaire which included details of their medication, alcohol consumption and smoking habits [19]. All patients used inhaled \( \beta \)-adrenoceptor agonists on an 'as required' basis for symptomatic relief, but were asked not to use these 4 h before blood sampling. Twenty-eight were using inhaled corticosteroids regularly and 18 were taking systemic corticosteroids daily. All patients had >15% variability in their forced expiratory volume in 1 s recorded over the previous 2 months and therefore conformed to the CIBA clinical description of asthma [20]. All those taking systemic corticosteroids were also using preparations for inhalation. Three of the women were taking combined oral contraceptives. None had taken aspirin within 2 weeks of the study day and none was following any exclusion diet regimens. Three admitted to smoking cigarettes and 36 took alcohol.

Controls. A control group of 76 normal healthy volunteers, 46 women and 30 men, aged 18–63 (mean 38.9) years were studied over the same period of time as the asthmatic patients for comparison. The normal volunteers came from the same city population as the asthmatic patients and consisted of medical and laboratory personnel. Eighteen of the subjects were cigarette smokers and 56 drank alcohol. Only two women were taking oral contraceptives. None of the control subjects was on a vegetarian diet or taking any medication.

The study had the approval of the Joint Ethical Sub-Committee of the University of Southampton and South West Hampshire Health Authority.

Methods

Venous blood (12 ml) was collected and samples of whole blood, plasma and platelets were stored at \(-20^\circ\)C until analysed, the length of storage being the same for both groups of subjects. After wet-ashing the samples, selenium was determined by a standard method using hydride generation and atomic absorption spectroscopy [21]. GSH-Px activity was measured by rate reaction kinetics using butylhydroperoxide as substrate [22], which provides selectivity for the selenium-containing GSH-Px. Protein determination was by the method of Lowry et al. [23]. The between-batch coefficients of variation for selenium concentrations in plasma, whole blood and platelets were 4.9%, 5.7% and 5.9%, respectively, and for GSH-Px activity in whole blood and platelets, 7.3% and 7.9%, respectively.

Statistical analysis

Since the scatter of results obtained approximated a normal distribution, multiple regression analysis was used to assess the effect of independent variables on a particular variable and the unpaired Student's \( t \)-test for between-group comparison. The results for whole blood plasma and platelet selenium concentrations, and for whole blood and platelet GSH-Px activity, in the asthmatic and control subjects were also analysed by calculating odds ratios. Odds ratios (relative risks) of asthma according to various levels of selenium and GSH-Px activity were determined by unconditional logistic regression. The 95% confidence intervals for the differences between arithmetic means are also given.

RESULTS

There were no significant effects of age, sex, cigarette smoking or alcohol on any of the indices measured in the control population. For patients with asthma and with asthma plus eczema, there was no significant effect of topical inhaled or systemic corticosteroid use on any of the measurements.

Comparison of patients with asthma and patients with asthma plus eczema (Table 1) showed no significant difference for any of the blood indices measured. The 95% confidence intervals for the difference between population means show that patients with asthma plus eczema had a tendency towards lower plasma selenium levels than patients with asthma alone.

The concentration of selenium in plasma, whole blood and platelets, and GSH-Px activity in whole blood and platelets, in the total group of patients with asthma and the control group are summarized in Table 2. A significant decrease in the concentration of selenium in plasma \((P<0.01)\) and whole blood \((P<0.001)\) was demonstrated in the patient group when compared with the control population. There were no significant differences between these two groups for any of the other blood measurements shown in Table 2.

When the data were summarized as odds ratios (Table 3) 3.54 (95% confidence interval 1.51–8.30) and 5.08 (95% confidence interval 1.93–13.39) fold increased probabilities of asthma were observed for the lower range of plasma and whole blood selenium concentrations, respectively, which were highly significant \((P<0.01)\). A similar observation for platelet selenium values was not found. When the odds ratios were calculated for the GSH-Px activity, values of 1.39 (95% confidence interval 0.50–3.90) and 2.57 (95% confidence interval 0.84–7.90) were obtained for the lower range of whole blood and platelet enzyme activities, which, while showing a trend towards an increased probability towards asthma, failed to reach statistical significance \((P=0.53\) and \(P=0.1\), respectively).

DISCUSSION

In this study we have demonstrated that when compared with a matched control group, patients with asthma have lower concentrations of selenium in their whole blood (consisting mainly of erythrocytes) and in plasma, and that this tended to be more pronounced in those with concurrent eczema. In the lower range, both plasma and
whole blood selenium concentrations demonstrated highly significant 3.54- and 5.08-fold increased probabilities, respectively, of asthma being present. In this study reduced selenium levels were not accompanied by a significant reduction in the mean GSH-Px activity measured in whole blood or platelets. Thus, in the population of asthmatic patients studied, it is unlikely that reduced selenium status contributes towards any impaired protection against the inflammatory effect of reactive oxygen intermediates.

In this study, cigarette smoking and alcohol consumption were shown to have no significant effect on plasma and whole blood selenium levels for the control population. A previous report from this unit [24] found significantly decreased levels of selenium in whole blood and serum in male cigarette smokers aged 30–50 years when compared with non-smokers. In the present study it is most likely that the failure to confirm an effect of smoking on selenium status reflects the relatively small number of subjects investigated. In the patient group, the concomitant use of corticosteroids had no significant effect on selenium concentrations, a finding that is in agreement with our previous work [25].

All the asthmatic subjects were receiving regular β2-adrenoceptor agonists for symptomatic treatment, which represents another difference from the control group.

Stimulation of systemic β2-adrenoceptors causes falls in the plasma concentrations of potassium, calcium, magnesium and phosphate as a consequence of enhanced cellular uptake rather than reduced total body stores [26]. To produce these systemic effects with β2-adrenoceptor agonists administered by inhalation, high and frequent dosing is necessary. In all subjects the last dose of inhaled β2-adrenoceptor agonists was at least 4 h before the blood sample was taken. Although as a general rule plasma and whole blood selenium concentrations reflect dietary selenium intake, little is known about the homoeostatic regulation of selenium in the various body compartments. From what is known of the biochemistry of this element there is no particular reason to believe that intermittent use of inhaled β2-adrenoceptor agonists would influence systemic levels of this element, but this factor should be addressed in any future study.

Although there is no reason to suggest that patients with asthma have malabsorption, it is interesting to note that when asthma and eczema occurred together then there was a tendency towards lower levels of plasma selenium when compared with patients with asthma alone. Malabsorption of fat has been reported in patients with eczema [27] and a lowered selenium status has also been reported in patients with this condition [28]. It is therefore possible that the trend towards lower selenium levels in

### Table 1. Comparison of selenium concentration and GSH-Px activity in patients with asthma and patients with asthma plus eczema

Results are shown as mean (SD). There were no significant differences between the groups. Abbreviation: Hb, haemoglobin.

<table>
<thead>
<tr>
<th></th>
<th>Asthma without eczema (x1) (n=26)</th>
<th>Asthma with eczema (x2) (n=23)</th>
<th>95% confidence intervals for the difference between means (x1-x2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium concn.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (μmol/l)</td>
<td>1.03 (0.17)</td>
<td>1.10 (0.14)</td>
<td>-0.16 to 0.02</td>
</tr>
<tr>
<td>Whole blood (nmol/g of Hb)</td>
<td>4.0 (0.9)</td>
<td>4.2 (0.9)</td>
<td>-0.68 to 0.28</td>
</tr>
<tr>
<td>Platelets (nmol/g of protein)</td>
<td>26.5 (5.9)</td>
<td>26.8 (5.5)</td>
<td>-3.64 to 3.04</td>
</tr>
<tr>
<td>GSH-Px activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood (units/g of Hb)</td>
<td>22.4 (6.6)</td>
<td>23.7 (6.0)</td>
<td>-4.99 to 2.39</td>
</tr>
<tr>
<td>Platelets (units/g of protein)</td>
<td>129 (35)</td>
<td>125 (35)</td>
<td>-16.3 to 24.3</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of selenium concentration and GSH-Px activity in all the asthmatic patients and the control subjects

Results are shown as mean (SD). Statistical significance: *P<0.01, †P<0.001 compared with control subjects. Abbreviation: Hb, haemoglobin.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic subjects (x1) (n=49)</th>
<th>Control subjects (x2) (n=76)</th>
<th>95% confidence intervals for the difference between means (x1-x2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium concn.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (μmol/l)</td>
<td>1.07 (0.16)*</td>
<td>1.19 (0.19)</td>
<td>-0.17 to -0.05</td>
</tr>
<tr>
<td>Whole blood (nmol/g of Hb)</td>
<td>4.1 (0.9)†</td>
<td>4.9 (0.9)</td>
<td>-1.11 to -0.49</td>
</tr>
<tr>
<td>Platelets (nmol/g of protein)</td>
<td>26.6 (5.6)</td>
<td>27.5 (6.9)</td>
<td>-3.50 to 1.98</td>
</tr>
<tr>
<td>GSH-Px</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood (units/g of Hb)</td>
<td>23.0 (6.3)</td>
<td>24.0 (6.2)</td>
<td>-3.23 to 1.23</td>
</tr>
<tr>
<td>Platelets (units/g of protein)</td>
<td>127 (35)</td>
<td>133 (31)</td>
<td>-17.7 to 5.7</td>
</tr>
</tbody>
</table>
Table 3. Comparison of selenium status and GSH-Px activity in asthmatic patients and control subjects summarized as odds ratios

Abbreviation: Hb, haemoglobin.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic patients</th>
<th>Control subjects</th>
<th>Odds ratios</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma selenium concn. (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1.2</td>
<td>11</td>
<td>36</td>
<td>1.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1.1 &lt; 1.2</td>
<td>11</td>
<td>15</td>
<td>2.40</td>
<td>0.86-6.70</td>
<td>0.09</td>
</tr>
<tr>
<td>1.0 &lt; 1.1</td>
<td>14</td>
<td>14</td>
<td>3.54</td>
<td>1.51-8.30</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>&lt; 1.0</td>
<td>&gt; 27</td>
<td>&gt; 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood selenium concn. (nmol/g of Hb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5</td>
<td>10</td>
<td>30</td>
<td>1.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4-5</td>
<td>17</td>
<td>33</td>
<td>1.55</td>
<td>0.61-3.90</td>
<td>0.36</td>
</tr>
<tr>
<td>&lt; 4</td>
<td>22</td>
<td>13</td>
<td>5.08</td>
<td>1.93-13.39</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Platelet selenium concn. (nmol/g of protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>14</td>
<td>27</td>
<td>1.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>29</td>
<td>37</td>
<td>1.51</td>
<td>0.67-3.40</td>
<td>0.32</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>6</td>
<td>11</td>
<td>1.05</td>
<td>0.32-3.48</td>
<td>0.93</td>
</tr>
<tr>
<td>Whole blood GSH-Px activity (units/g of Hb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>9</td>
<td>16</td>
<td>1.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>22</td>
<td>37</td>
<td>1.06</td>
<td>0.40-2.81</td>
<td>0.91</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>18</td>
<td>23</td>
<td>1.39</td>
<td>0.50-3.90</td>
<td>0.53</td>
</tr>
<tr>
<td>Platelet GSH-Px activity (units/g of protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 150</td>
<td>16</td>
<td>24</td>
<td>1.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>100-150</td>
<td>19</td>
<td>45</td>
<td>0.63</td>
<td>0.28-1.45</td>
<td>0.28</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>12</td>
<td>7</td>
<td>2.57</td>
<td>0.84-7.90</td>
<td>0.10</td>
</tr>
</tbody>
</table>

the patients with asthma plus eczema may in part be due to an enteropathy associated with the skin disease.

While this study has clearly shown a reduction in whole blood and plasma selenium concentrations in the asthmatic subjects studied, this was not observed in platelets. It has been suggested that platelets may preferentially retain selenium when there is a marginal deficiency [29], since investigation of a group of phenylketonuric children who were receiving a therapeutic diet low in natural protein, and therefore also low in selenium, demonstrated a decrease in platelet selenium concentrations only when plasma and whole blood concentrations of the element were much reduced. The present observations support the hypothesis. It is possible that there is a redistribution of selenium into other tissues in inflammatory conditions which might explain the lowered selenium status of the total asthmatic population but this is unlikely to have a functional correlate, since there was no corresponding decrease in GSH-Px activity.

The role of selenium in GSH-Px may not be the only function of the element. In man, only about 10% of the total selenium in erythrocytes is found in association with GSH-Px [30]. At least 14 distinct selenium-containing proteins have been identified in mammalian tissues, although metabolic roles have not been established for them all [31]. It has been shown recently that selenium may act as a metabolic modulator of phagocytosis [32] and may affect T-lymphocyte activity [33] and the B-cell synthesis of immunoglobulins [34], all functions that could be relevant to the inflammatory and immunological processes known to occur in asthma.

In conclusion, we have demonstrated a reduced circulating selenium status in patients with asthma. Although we are unable to comment on the relationship of these findings to the severity of asthma or to the use of inhaled β2-adrenoceptor agonists, a low concentration of selenium in plasma and whole blood, is associated with a significantly increased risk of having this disease. We have not been able to show a significant association between reduced selenium status in asthma and GSH-Px activity measured in whole blood or separately in platelets. In a number of other studies, blood selenium concentrations below 1.26 μmol/l have been shown to correlate with reduced GSH-Px activity [35] and as a consequence reduced antioxidant protection. It is worth noting that the prevalence of asthma and mortality due to asthma are reported as being higher in New Zealand [36, 37], a country with lower dietary intake of selenium than the U.K. In this report, the biochemical measurements made concerning selenium status and its functional consequences were confined to circulating blood elements. Such measurements may only broadly reflect changes within an organ or tissue of interest and do not exclude greater changes occurring locally. In the context of asthma it would be of particular interest to undertake similar measurements in cells obtained from the surface of the airways by lavage. Although at this time it is premature to attribute any cause and effect relationship between
selenium and asthma, it would be of considerable interest to determine whether greater deficiencies of selenium enhance the severity of this common disease.

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