

Lowered platelet glutathione peroxidase activity in patients with intrinsic asthma

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Platelet glutathione peroxidase (GSH-Px) activity and serum selenium (Se) levels were determined in 20 patients with intrinsic asthma. Nine of the patients had NSAID-intolerance. The mean value of GSH-Px activity in the patients was 47.0 ± 7.1 U/10¹¹ platelets, which is significantly lower than that of 56.4 ± 12 U/10¹¹ platelets in the controls ($P < 0.01$). There was also a tendency towards lowered Se levels in the patients compared with controls. The results are discussed in view of the protective role of GSH-Px against oxidative stress and the tentative regulatory function of GSH-Px in arachidonic acid metabolism.

Key words: arachidonic acid; asthma; GSH-Px; NSAID-intolerance; platelets.

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An intrinsic abnormality of arachidonic acid (AA) metabolism has been postulated in the pathogenesis of asthma. Many of the metabolites that are generated from AA have been implicated in asthmatic reactions, and particularly the leukotrienes that are produced along the 5-lipoxygenase pathway (8). The increased knowledge of cell-to-cell interactions in inflammatory responses has, however, created a mounting interest concerning the role of platelets and platelet-derived AA metabolites in asthma. An increased platelet 12-lipoxygenase flow has been reported in asthmatic patients (23), which may be of significance considering that the initial 12-lipoxygenase metabolite 12-hydroperoxyeicosatetraenoic acid (12-HPETE) has been shown to enhance the production of 5-lipoxygenase metabolites by leukocytes (9) and mast cells (3).

12-HPETE is reduced to the corresponding hydroxyeicosatetraenoic acid (12-HETE) by the selenoenzyme glutathione peroxidase (GSH-Px; glutathione:H₂O₂ oxidoreductase, EC 1.11.1.9) (4). 12-HPETE has been shown to influence the platelet AA metabolism by stimulating 12-lipoxygenase and inhibiting cyclooxygenase

activity (6, 18), and by catalyzing 12-HPETE elimination GSH-Px can thus be considered a modulator of the two AA-metabolizing pathways.

Besides the lipid hydroperoxide 12-HPETE, GSH-Px is able to reduce several other organic hydroperoxides as well as hydrogen peroxide (19), and, accordingly, GSH-Px has an important role in the cellular protection against oxidative damage (15). In its capacity of antioxidant, GSH-Px may also promote an adequate regulation of AA metabolism, since oxidative stress enhances AA metabolism and thereby creates greater demands on the regulatory systems.

In view of our previous finding of a lowered GSH-Px activity in whole blood in asthmatic patients (10), and of the tentative regulatory function of GSH-Px in the platelet AA metabolism, we carried out an investigation of platelet GSH-Px activity in asthma.

MATERIAL AND METHODS

Twenty patients (11 men, 9 women, age range 20-70 years) suffering from intrinsic asthma

participated in the study. Nine of the patients had intolerance to non-steroid anti-inflammatory drugs (NSAIDs) verified by challenge test or typical history. Patients with atopic disease were excluded from the study; the criteria for atopy were positive skin test or RAST to common inhalant allergens or a serum IgE level above 100 U/ml. Patients with acute or recent (< 3 weeks) infections were also excluded.

The control sample consisted of 40 healthy volunteers, 16 men and 24 women, age range 20–70 years, recruited from the hospital staff and their acquaintances and with no history of asthma or allergy.

There was an even age distribution in the control sample but not in the patients, since 19 of them were aged 41–70 years and there was only one patient within the age range of 20–40 years. Therefore, in addition to a comparison between the whole patient group and the whole control sample, the 19 asthmatic patients in the age range of 41–70 years were also compared with 19 controls of the same age-range and equal sex distribution (matched control group).

None of the subjects had taken NSAIDs or oral corticosteroids less than ten days prior to blood sampling. Treatment with theophyllines and inhaled corticosteroids was discontinued 24 h before blood sampling, but the patients were allowed to continue their habitual oral or inhaled β_2 -agonist treatment. None of the subjects had been taking any selenium (Se) preparation for at least the last 6 months.

Non-fasting blood-samples were collected between 8.00 and 9.00 in the morning. Blood was drawn from an antecubital vein with a 1.4 mm stainless steel cannula into prechilled 10 ml plastic centrifuge tubes (duplicate samples) containing 1/10 volume of 4.5% Na_2EDTA -solution as anticoagulant. 5 ml of blood was used to prepare serum.

GSH-Px assay. Platelet preparations were made according to Levander et al. (7) and modified as follows. The blood was centrifuged at 4°C for 15 min at $190 \times g$. Also the remaining steps were taken at 4°C and the samples were kept on ice during preparations. The platelet-rich plasma (PRP) was transferred into a conical

plastic tube and diluted with an equal volume of cold wash buffer (135 mM NaCl, 5 mM glucose, 3 mM EDTA, 12 mM Tris-HCl, pH 7.4) and centrifuged at $800 \times g$ for 15 min. The supernatant was decanted and discarded, and the pellet was resuspended in 10 ml of cold wash buffer and centrifuged again at $800 \times g$ for 15 min. The supernatant was decanted and the platelet pellet was resuspended in cold KH-buffer (150 mM NaCl, 5 mM glucose, 6 mM KCl, 32 mM NaHCO_3 , 15 mM KH_2PO_4 , 1.5 mM MgSO_4 , pH 7.4) to a volume equivalent to the original PRP volume. The suspension was put on ice for 30 min before determination of platelet count to allow disaggregation of remaining platelet aggregates. The platelet count was determined with a Linson 431 A cell counter and the suspension was transferred to a polypropylene centrifuge tube and frozen at -70°C .

At the time of the enzyme assay the samples were left to thaw at 4°C and then put on ice and disrupted by sonication. The platelet sonicates were centrifuged at $49,000 \times g$ for 20 min, and GSH-Px activity was determined in the supernatants.

GSH-Px activity was determined mainly according to the modification by Levander et al. (7) with the coupled assay of Paglia & Valentine (14). We transferred into a plastic tube: 0.820 ml phosphate buffer (50 mM NaH_2PO_4 , 3 mM EDTA, pH 7.4) containing 1 unit of glutathione reductase (Boehringer Mannheim) and added 100 μl of platelet supernatant, 20 μl of 0.1 M GSH dissolved in water (final conc. 2 mM), and 30 μl 0.01 M NADPH dissolved in water (final conc. 0.3 mM). The mixture was incubated for 10 min at 37°C. The reaction was initiated by adding 30 μl of 0.01 M t-butylhydroperoxide (final conc. 0.3 mM), and after stirring the mixture was transferred by a sipper system into a quartz cuvette with 1-cm light path. The conversion of NADPH to NADP was followed at 37°C by measuring of the change in absorbance at 340 nm between 1.5 and 3.5 min after the initiation of the reaction, using a Hitachi 150–20 spectrophotometer. The non-enzymatic oxidation of NADPH was determined by an identical assay system, apart from that the platelet supernatant was replaced by an equal

volume of phosphate buffer. The delta absorbance of the latter system was used as blank and subtracted from that of the former to determine the true enzyme activity. The GSH-Px activity was calculated on the basis of molar absorptivity for NADPH and expressed as units (U; moles of oxidized NADPH per second) per 10¹¹ platelets.

Serum Se assay. Determination of serum Se was made by Analytica AB (Täby, Stockholm) by atomic absorption spectrometry using a Pye Unicam PU-9000 supplied with a graphite furnace. The Se concentration was expressed as µmol Se/l serum.

RESULTS

The mean platelet GSH-Px activity in patients was significantly lower than that of the total control sample (Table 1 and Fig. 1). There was no change in significance when the mean value of the 19 patients aged 41-70 was compared with that of the matched control group.

The mean serum Se in patients tended to be lower than the corresponding value for the whole control sample, but the difference did not reach statistical significance (Table 1). The mean serum Se of the 19 patients aged between 41-70 years was significantly lower than that of the matched control group.

Patients with and patients without NSAID-intolerance did not differ either in platelet GSH-Px activity or in serum Se.

Serum Se and platelet GSH-Px were significantly correlated in the total control sample ($r = 0.51, P < 0.001$) and in the matched control group ($r = 0.57, t = 2.84$; for $P < 0.01 t = 2.90$), but in the patients this correlation failed to reach statistical significance ($r = 0.39, P < 0.1$).

DISCUSSION

The present finding of a lowered platelet GSH-Px activity in patients with intrinsic asthma and NSAID-induced asthma is in conformity with our previous observation of a lowered whole blood GSH-Px activity in this patient category (10). A lowered GSH-Px activity has also been observed, by Bibi et al. (1), in erythrocytes from children with atopic asthma. In contrast, an increased blood GSH-Px activity was observed in asthmatic children by Ward et al. (20), but since there are clinical as well as methodological differences between the studies it is premature to speculate regarding the discrepant findings. Pharmacological influences must always be considered, but the observations in this field are still scant (1, 20), and it remains to be elucidated whether the GSH-Px enzyme activity may be direct-

Table 1
Platelet GSH-Px activity and serum Se concentration in patients with intrinsic asthma (with/without NSAID-intolerance) and healthy controls

	Serum Se	Platelet GSH-Px
	µmol/l mean±SD	U/10 ¹¹ platelets mean±SD
Control sample		
All (n = 40)	0.91±0.19	56.4±12
aged 20-40 years (n = 21)	0.85±0.16	56.0±12
aged 41-70 (n = 19; matched control group)	0.99±0.19	56.8±11
Asthma		
All (n = 20)	0.83±0.18 (t = 1.58, NS)	47.0±7.1 (t = 3.27, P < 0.01**)
aged 41-70 (n = 19)	0.85±0.17 (t = 2.29, P < 0.05*)	47.1±7.2 (t = 3.14, P < 0.01**)
without NSAID intolerance (n = 11)	0.85±0.17	46.0±5.6
with NSAID intolerance (n = 9)	0.81±0.19	48.5±8.6

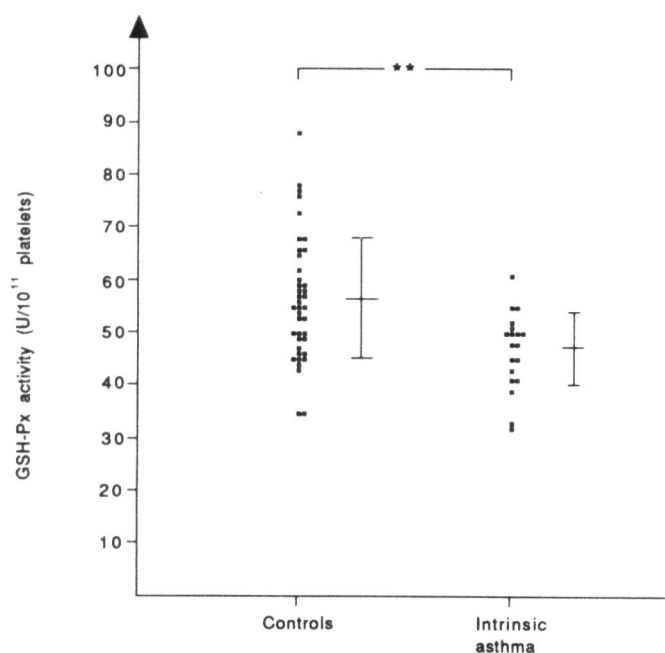


Fig. 1. Scattergram of the distribution of platelet GSH-Px activity in patients with intrinsic asthma and in healthy controls.

ly influenced by pharmacological asthma treatment or indirectly through drug influence on Se nutrition.

An adequate access to Se is a prerequisite for optimal GSH-Px activity. The lowered platelet GSH-Px activity that we found in the present study may thus partly be explained by the lowered Se status, although the reduced Se levels may not be the sole explanation of the lowered enzyme activity. The weaker correlation between serum Se and platelet GSH-Px in the patients compared with controls, may suggest an aberrant Se incorporation into the enzyme or increased enzyme inactivation.

One possibility is that an increased inactivation of the GSH-Px enzyme could occur as a consequence of the asthmatic disease state. GSH-Px, as well as other anti-oxidant enzymes, may be inactivated by excessive levels of oxidants, and an increased production of reactive oxygen species may be anticipated in asthma considering the chronic inflammation and disordered respiration (2, 17, 22). This view is supported by the observations of Bibi et al. (1) that there was a clear correlation between severity of asthma and GSH-Px levels. They found that when compared with asymptomatic state,

the GSH-Px activity was significantly lower during acute asthmatic attacks, when the oxidative stress can be expected to be particularly high. An insufficient free radical scavenging system, as indicated by a lowered GSH-Px activity, may start a vicious circle in more than one way. Oxidant stress leads to an enhanced AA metabolism, during which more reactive oxygen species are formed.

When an impairment of the platelet GSH-Px redox cycle is induced by Se deficiency (5, 16), or by depletion of the reducing equivalent glutathione (4), there is an intracellular accumulation of 12-HPETE and its non-enzymatic rearrangement products. 12-HPETE stimulates platelet lipoxygenase while it inhibits platelet cyclooxygenase (6, 18) and 12-HPETE will thus stimulate its own biosynthesis. A lowered GSH-Px activity in asthmatic patients may thus be one of the prerequisites for an increased flow of AA metabolism along the 12-lipoxygenase pathway. Our results are thus in conformity with the finding of Yen & Morris (23) that platelets from patients with intrinsic and extrinsic asthma converted an increased proportion of AA through the lipoxygenase pathway, estimated by measuring 12-HETE, compared with healthy individuals. Mita et al. (12), who investigated a similar group of patients, found the same pattern in leukocytes. Nizankowska et al. (13), however, found no difference in the production of 12-HETE in a smaller study of aspirin-sensitive asthmatics; however, the inter-individual variations in this study were considerable. Further, it may be relevant to question whether 12-HETE gives adequate information about the metabolic flow via lipoxygenase in general and particularly in this context, when an abnormal lipoxygenase flow may be anticipated.

With normal 12-HPETE reducing capacity only 12-HETE seems to be released from platelets but when the GSH-Px system is impaired, with subsequent 12-HPETE accumulation, also 12-HPETE may be released (4). A lowered GSH-Px activity in asthmatic patients may thus result in an impaired 12-HPETE reducing capacity and an over-flow of 12-HPETE, the full consequences of which remain largely unknown. However, a possible effect may be an increased leukotriene biosynthesis, since platelet-derived

12-HPETE has been found to stimulate the 5-lipoxygenase activity in human blood leukocytes (9) and mast cells (3).

Platelets pertain to the family of inflammatory cells and as such participate in a series of cell-to-cell interactions (21). In this context AA metabolites are crucial (11) and the role of platelets in asthma pathology may very likely be linked to the AA cascade. A lowered GSH-Px enzyme activity may be one of multiple factors behind a dysregulated platelet AA metabolism and future research needs to be done on the relationship between GSH-Px activity and AA metabolic profile in asthma.

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