Activity of the Glutathione Peroxidase-2. Differences in the Selenium-dependent Expression between Colon and Small Intestine

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Abstract. Background: Selenium (Se) is an essential element which is involved in various biological processes in nearly all tissues of animals and human, e.g. protection against oxidative stress in the cardiovascular system, and may play a role in cancer protection. It is incorporated in the proteome in the form of the genetically encoded amino acid selenocysteine, which is the characteristic component of the selenoproteins. Materials and Methods: We investigated the expression of the selenoenzyme GPx-2 which is predominantly present in the tissues of the gastrointestinal tract such as the small intestine and therefore named gastrointestinal glutathione peroxidase. Rats were fed with a Se-adequate or Se-deficient diet and GPx-2 was assessed by means of enzyme activity with respect to the Se concentration in tissues of the colon and small intestine. Se quantification was carried out by means of graphite furnace atom absorption spectrometry and 2D-gel electrophoresis was applied to investigate the expression of the proteins of the small intestine tissue samples. Results: Twenty-eight differences could be distinguished in the protein spot distribution of the 2D-gels of the homogenates. The GPx-2 activity in the Se-deficient rat colon samples was 6.8 fold lower than in the Se-adequate rats in contrast to 1.2 fold lower levels between the corresponding samples in the small intestine. Conclusion: This finding might explain the different susceptibility of the colon and the small intestine to cancer and support the theory of the protective effect of selenium in the gastrointestinal tract.

Selenium (Se) was shown to be an essential trace element which plays crucial roles in the protection against several diseases such as rheumatoid arthritis, Alzheimer’s disease and cancer (1, 2, 3). Its deficiency is related to a loss of immunocompetence, a higher susceptibility to developing viral diseases such HIV, or Keshan disease, and loss of male fertility (4-6). Se is incorporated in the proteome in the form of the genetically encoded amino acid selenocysteine, which is the characteristic component of selenoproteins. To date, 25 mammalian Se proteins have been identified (7), but there is still a lack of knowledge about their regulation and function. The family of the glutathione peroxidases is known to reduce organic and inorganic hydroperoxides to protect the cell from oxidative stress. It is also thought, that DNA damage, induced by oxidative stress, is one possible initiator of tumorigenesis (8). Low molecular weight selenocompounds (9) as well as selenoproteins (10, 11) are claimed to be responsible for the protective effect of Se in carcinogenesis.

The small intestine is much less susceptible to cancer than is the colon, although both have similar anatomy (12). The number of new occurring cases per year and the number of deaths from small intestinal cancer is much lower in comparison to colon cancer, which is one of the most common kinds of cancer. Hong et al. presumed that this is caused by these tissues’ different response to oxidative stress-induced DNA damage (13), but they did not show which part of the antioxidant defence system is involved. The distribution of selenium in the different organs under Se-deficient conditions is strongly dependent on the kind of tissue itself (14). There is a hierarchy in organs to keep Se levels adequate as long as possible to fulfil essential functions (e.g. in reproductive organs). If there is any influence of Se on the oxidative stress response of the colon and small intestine, colons susceptibility for cancer would indicate a lower position in this hierarchy then small intestine.

In the tissues of the gastrointestinal tract, a specific form of glutathione peroxidase is expressed, mainly gastrointestinal glutathione peroxidase (15) (GPx-2; EC: 1.11.1.9; MW: 21.9 kDa; pI: 7.60). In order to elucidate if this selenium-dependent enzyme family is a possible...
candidate to explain the differences between colonic and small intestinal cancer, the enzyme activity of the glutathione peroxidases in both tissues were investigated with special emphasis on the selenium status of the animals. Low selenium status is claimed as a general factor in the risk for developing cancer (3), therefore changes of the proteome of selenium-deficient animals might uncover other proteins involved in mechanisms of cancer protection. In the second part of this study, the proteome of the small intestine with respect to the Se-status of the animals was investigated by 2D-electrophoresis.

Materials and Methods

Adult male albino rats of the Wistar strain *Rattus norvegicus* with a body mass between 400-450 g were used in this study. One group of rats was fed with a diet low in selenium (5-10 µg kg⁻¹), the other group was fed with the same diet but at a sufficient Se concentration (300 µg kg⁻¹), (MP Biomedicals, Solon, OH, USA). Exact conditions for this animal model and the composition of the diet are described elsewhere (16).

The 6-months-old rats of both groups were killed by cardiac puncture under appropriate anaesthesia, and the small intestine and colon were removed, transported on dry-ice and frozen at ~80°C until usage. After thawing, samples were washed with Tris-HNO₃ buffer (25 mmol L⁻¹; pH 7.4), freeze-dried, homogenised in a mortar, dissolved by microwave-assisted digestion (~100 µg sample in 3 mL HNO₃ + 1 mL H₂O₂, using a MARS 5) (CEM GmbH, Kamp-Lintfort, Germany) and Se was determined by graphite furnace atomic absorption spectrometry with Zeeman background correction (AAnalyst 600; Perkin Elmer, Rodgau, Germany). Results from three animals per group were calculated as mean values ± standard deviation in mg Se per kg tissue dry weight.

Determination of the enzyme activity of glutathione peroxidase was achieved by a coupled reaction. Reduced glutathione (GSH) was oxidized with tert-butyl hydroperoxide in the presence of glutathione reductase and then the oxidized glutathione was reduced under presence of glutathione reductase (GR). In brief: Organs were washed and homogenised in Tris-HCl buffer (50 mmol L⁻¹; pH 8.0) containing 0.5 mmol L⁻¹ EDTA, then 0.307 mg GSH and 0.4 u GR were added, where unit means the amount of enzyme needed to reduce 1 µmol of oxidized glutathione per minute at 25°C and pH 7.6. Before starting the reaction, the solution was incubated for 30 minutes at 37°C. NADPH (0.208 mg) and tert-butyl hydroperoxide (0.090 mg) were added and the decrease in absorbance at 340 nm was monitored for 2 minutes (Cary 50; Varian GmbH, Darmstadt, Germany). Enzyme activity was calculated by the slope of time versus absorption and expressed as units glutathione peroxidase activity per mg protein; 1 unit was defined as the amount of enzyme needed to reduce 1 µmol NADPH per minute at 25°C and pH 8.0.

Total protein content was measured by the method of Bradford as described elsewhere (17). Tissue samples for the 2D-electrophoretic investigation were homogenised in Tris-HCl buffer (25 mmol L⁻¹; pH 7.4) containing protease inhibitor cocktail (P8340; Sigma, Deisenhofen, Germany) by means of sonification (Branson Sonifier W-450 with a parabolic beaker resonator; Heinemann, Schwäbisch Gmünd, Germany) using 450 W and 50% pulsation for 10 minutes at 4°C. Proteins were solubilised by adding urea (6 mol L⁻¹), thio-urea (3 mol L⁻¹) and DTT (70 mmol L⁻¹), given as final concentrations. Ampholytes (Servalyt, pH 3-10; Serva, Heidelberg, Germany) were added to a final concentration of 2%. Isoelectric focusing on gel pipes as the first and large SDS-PAGE as the second dimension (18) were used to separate 300 µg of protein on a 22x30 cm gel. After separation, proteins were detected by silver staining according to the method of Blum (19).

Results

The different response to oxidative DNA damage, which may be a cause for the different susceptibility of colon and small intestine to cancer (13), could be induced by redox active proteins. Glutathione peroxidase, an enzyme whose activity is directly dependent on the Se status, is an important factor of the defence system against oxidative stress. We determined the Se concentration and the relative glutathione peroxidase activity of the colon and small intestine of animals fed at different Se levels.

In Table I, the Se concentrations and the GPx activities of the investigated tissues are shown. There are differences in the Se concentrations in the tissues of Se-adequate rats: the value for the colon is 1.5-fold higher than for the small intestine, whereas in the Se-deficient rat the concentration is only half the value of that for the small intestine.

As expected the Se concentrations decreased drastically in the tissues of the Se-deficient rats. In the colon, the Se concentration decreased to 0.04±0.02 mg·kg⁻¹ which represents 4% of the value of the adequately fed animal, while the value in the small intestine decreased to 14% (0.09±0.01 mg·kg⁻¹).

The pattern of results for the activity of glutathione peroxidase in the investigated samples is approximately similar. The activity in the colon is three-fold higher than in

<table>
<thead>
<tr>
<th>Selenium concentration</th>
<th>Glutathione peroxidase activity</th>
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<tbody>
<tr>
<td>Se def. mg·kg⁻¹</td>
<td>u·mg⁻¹</td>
</tr>
<tr>
<td>Colon Adequate</td>
<td>0.97±0.14</td>
</tr>
<tr>
<td>Colon Deficient</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>Small intestine Adequate</td>
<td>0.63±0.18</td>
</tr>
<tr>
<td>Small intestine Deficient</td>
<td>0.09±0.01</td>
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Values are means ± standard deviation (n=3) per kg dry weight, or per mg total protein respectively. The amount of glutathione peroxidase, needed to reduce 1 µmol NADPH per minute at 25°C and pH 8.0 was defined as one unit.
the small intestine of the adequately fed animals. Under Se-deficient conditions, the activity in the colon decreased to 5.6±2.4 u·mg⁻¹ or 15% that of the adequately fed rat, respectively. For the small intestine, only marginal differences between the GPx activity of the rats with different Se-status were found.

Differences in the protein expression pattern of the small intestine of Se-adequate and Se-deficient rats were found by means of 2D-gel electrophoresis. Twenty-eight differently expressed spots from more than 1800 spots per gel could be located. They include 12 up-regulated, 9 down-regulated, as well as 7 spots expressed exclusively under selenium deficiency.

Discussion

Selenium deficiency is discussed as a factor involved in increasing the risk of cancer (3, 20, 21). In the presented study we investigated differences in the proteome of the small intestine of Se-deficient and Se-adequate rats as well as differences of glutathione peroxidase expression between the colon and small intestine of both groups.

The data indicate that colon tissue of animals with an adequate selenium supply contained more Se than did small intestine tissue and had a higher enzyme activity of glutathione peroxidase. The higher GPx levels are consistent with the findings of Sanders et al. who described a higher activity of other antioxidant effective enzymes such as catalase or superoxide dismutase in the colon compared to the small intestine (22). Under selenium-deficient conditions the Se content and the GPx activity decreased in the tissues in the present study. The major difference between the two types of tissues is the degree of the decrease. The Se level and the GPx activity in the small intestine are not as strongly affected as in the colon. The Se level and the GPx activity are higher in the small intestine than in the colon with the Se-deficient diet although both tissue levels fell. These findings suggest that the colon is better protected against oxidative stress under an adequate Se status, but is less protected if additional risk factors, such as Se deficiency, appear. This could be a possible cause for the higher susceptibility of the colon for cancer. Whether there is a correlation between the Se status, the GPx activity and a higher incidence of cancer needs to be investigated by further studies.

Other Se-dependent proteins which are potentially involved in the protection mechanism of the small intestine need to be identified. Here 28 different protein spots were
detected by 2D-gel electrophoresis comparing Se-adequate and Se-deficient tissues. These proteins need to be identified in future work as does their function in the metabolism of the body.

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References