Changes of the glutathione enzymatic redox system in human gastrointestinal tract tumours

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Abstract

Introduction: Glutathione (GSH) and GSH-dependent enzymes - glutathione peroxidase (GSHPx), glutathione S-transferase (GST) and glutathione reductase (GSHR) - play a significant role in protecting cells from reactive oxygen species (ROS), which are implicated in tumour disease. The aim of this study was to evaluate reduced glutathione level and GSH-dependent enzyme activities in different types of gastrointestinal tract tumours.

Material and methods: We investigated GSH level and GSHPx, GST and GSHR activities in cancer and cancer-free adjacent tissues from patients with gastric (n = 15), liver (n = 30) and colorectal cancer (n = 65), and colorectal cancer liver metastases (n = 50). As a marker of oxidative stress the lipid peroxidation level (expressed as thiobarbituric acid-reactive substances level – TBARS) was determined.

Results: Increased level of lipid peroxidation was observed in most of the studied tumours. A significant increase in GSH level was observed exclusively in benign and malignant liver tumours compared to control liver tissue ($p \leq 0.05$). Activity of studied GSH-dependent enzymes was unchanged in colorectal cancer. Statistically significant differences of those enzymes' activity in gastric and liver tumours, and colorectal cancer liver metastases compared with control tissues were observed ($p \leq 0.05$).

Conclusions: Changes of lipid peroxidation and GSH levels, and GSH-dependent enzyme activities, suggest that gastrointestinal tract tumours are under the influence of oxidative stress. These results indicate that the glutathione enzymatic redox system is an important mechanism for protection of cancer cells against increased levels of ROS and may point to its role in facilitating development of gastrointestinal tract tumours.

Key words: gastrointestinal tract tumours, oxidative stress, lipid peroxidation, glutathione, GSH-dependent enzymes.

Introduction

Gastrointestinal tract tumours are one of the most often occurring type of tumours in humans and are a serious clinical problem [1].

The aetiology of gastrointestinal tract tumours is a multifactorial process. Growing evidence indicates that reactive oxygen species (ROS) and their reactive derivatives may contribute to initiation and promotion of carcinogenesis (DNA damage, interaction with oncogenes and tumour suppressor genes, immunological mechanisms) [2, 3].

The gastrointestinal tract is particularly exposed to the action of environmental factors in food (chemical compounds, bacteria, viruses,

parasites, alimentary antigens). Their action could lead to the formation of chronic inflammatory states, which are accompanied by oxidative stress. Oxidative stress is potentially harmful to cells and ROS are known to make an important contribution to pathology of diseases, including neoplastic disease [4, 5]. Reactive oxygen species are generally cytotoxic, due to oxidative damage they might cause to cellular components such as DNA, proteins and lipids, affecting enzyme activity and membrane function [6]. Therefore, cells must be protected from oxidative injury by antioxidant enzymes, e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione S-transferase (GST), glutathione reductase (GSHR) and non-enzymatic antioxidants, e.g. glutathione (GSH) [7, 8].

Cellular GSH and its related enzymes are among the principal protective mechanisms against endogenous and exogenous toxic substances, and free radical mediated damage in gastrointestinal tract mucosa as well as in other tissues [9].

Glutathione has many physiological functions including antioxidant defence, as a direct scavenger of ROS. It is a co-substrate in reactions catalysed by GSH-dependent enzymes (GSHPx, GST) and it may contribute to antioxidant defence by networking with other antioxidants such as vitamins E and C. Glutathione plays a crucial role in regeneration of vitamins C and E from their oxidised by-products [10-12].

Glutathione peroxidases (selenium-dependent and selenium-independent) catalyse the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides, thereby simultaneously oxidising glutathione [9].

Glutathione S-transferase is implicated in the protection of cells against many cytotoxic exogenous and endogenous carcinogenic chemicals [13, 14].

Glutathione reductase in the presence of NADPH catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (2 GSH) and maintains an appropriate level of co-substrate for GSHPx and GST [9].

The important role of glutathione and GSH-dependent enzymes in the defence of cells against oxidation by free radicals and reactive oxygen intermediates has been reported in many diseases [8, 11, 14, 15], but the participation of GSH and activity of its related enzymes in human gastrointestinal tract tumours has been poorly investigated.

Therefore, the aim of this study was to evaluate reduced glutathione level and GSH-dependent enzyme activities in cancer and cancer-free adjacent tissues obtained from patients with different types of gastrointestinal tract tumours.

Material and methods

Patients and tissues

Materials for this study were obtained from 160 patients with gastrointestinal tumours (mean age 60 ±9.7; range 26-82), diagnosed by routine histopathological examination: 15 with gastric cancer - adenocarcinoma (mean age 67 ±9.4; range 23-80), 10 with benign liver tumours - hepatocellular adenoma (mean 48 ±7.2; range 29-68), 20 with malignant liver tumours - hepatocellular carcinoma (mean age 43 ±15.6; range 26-67), 65 with colorectal cancer - adenocarcinoma (mean age 65.8 ±11.4; range 44-82), and 50 with metachronous colorectal cancer liver metastases (mean age 57.1 ±11.2; range 31-81). Patients were hospitalised in the Department of General, Gastroenterological Surgery and Nutrition, in the Department of General, Transplantation and Liver Surgery, and in the Department of General and Transplantation Surgery at the Medical University of Warsaw. None of them had received radiotherapy or chemotherapy prior to surgery. In patients with gastric cancer *Helicobacter pylori* infection was detected by histological diagnosis.

Studies were approved by the Bioethics Committee of the Warsaw Medical University (decision KB/164/2001), and informed consent was obtained from all patients.

Methods

Immediately after surgical removal, the tumour and normal adjacent tissues were washed in 0.9% NaCl and frozen at –80°C. Normal (control) tissues were taken from the resected stomach, liver and colon (respectively) where they appeared macroscopically healthy, about 7-8 cm distant from the tumour. Normal colon was a control for both colorectal adenocarcinoma and colorectal cancer liver metastases. The frozen tissues were cut into small pieces and homogenized on ice in 10 vol. of 50 mmol/l Tris-HCl buffer (pH 7.5) containing 1 mmol/l $MnCl_2$, 0.2 mol/l KCl, 0.1% (v/v) Triton X-100 and PMSF (phenylmethylsulfonyl fluoride) using a Heidolph Diax 900 blender at low speed, five times for 2-min periods at 3-min intervals. After 30 min extraction on a magnetic stirrer, the homogenates were centrifuged at 120 000 × g for 30 min at 4°C.

The obtained supernatants were used for glutathione and lipid peroxidation levels, and glutathione-dependent enzymes' activity determination.

The level of lipid peroxidation (as a marker of oxidative stress) was determined by measurement of the final lipid peroxidation products, which react with thiobarbituric acid (thiobarbituric acid-reactive substances – TBARS). Results were expressed as nmols of TBARS per mg of protein [16].

Reduced GSH level was measured as described by Sedlak and Lindsey [17]. Glutathione was expressed as μmol/mg protein.

Measurement of GSHPx activity was based on the method described by Paglia and Valentine [18]. GSHPx catalyzes the oxidation of glutathione by cumene hydroperoxide (for total activity of GSHPx) or hydrogen peroxide (for selenium-dependent peroxidase, Se-GSHPx).

GST activity was measured according to the method of Habig *et al*. [19] using chloro-dinitrobenzene (CDNB) as a substrate. Glutathione reductase activity was assayed by using oxidized glutathione as a substrate as described by Goldberg and Spooner [20].

The activity of studied enzymes was expressed as U/mg of protein. Total protein was measured in all samples by the Bradford procedure [21], using bovine serum albumin as a standard.

All data were expressed as mean ± S.D. Statistical analysis of obtained data was conducted using Statistica software (StatSoft 6.1). Student's *t* test, Wilcoxon test, and Pearson's correlations were applied for comparison of variables. Differences were considered significant if the *p* value was smaller than or equal to 0.05 ($p \leq 0.05$).

Results

Results are expressed as mean ± SD and summarised in Figures 1, 2 and Table I. Lipid peroxidation indicated by TBARS level was significantly higher in gastric tumours and considerably decreased in hepatocellular cancer compared to cancer-free tissue ($p \leq 0.05$). In colorectal cancer, colorectal cancer liver metastases and control colon tissue the differences in TBARS level were insignificant (Figure 1).

Reduced glutathione level was significantly higher in benign and malignant liver tumours as compared to adjacent normal tissue ($p \leq 0.05$). There were no differences in concentration of GSH in gastric and colorectal cancer compared with control tissues and in colorectal cancer liver metastases with respect to both healthy colon and colorectal cancer (Figure 2).

Whole glutathione peroxidase activity was higher in gastric cancer and considerably lower in benign and malignant liver tumours. The differences in GSHPx between colorectal cancer, colorectal cancer liver metastases and control colon tissue were non-significant. Se-GSHPx was significantly increased only in gastric cancer in comparison with healthy stomach ($p \leq 0.05$) (Table I).

We observed a significantly lower GST activity in benign and malignant liver tumours as compared with adjacent cancer-free liver. In colorectal cancer liver metastases we found similar results with respect to both healthy colon and colorectal cancer ($p \leq 0.05$) (Table I).

Glutathione reductase activity was found to be higher in gastric cancer and hepatocellular carcinoma than its activity in control tissues. The activity of this enzyme was also significantly higher in colorectal cancer liver metastases compared to both colorectal cancer and cancer free tissue ($p \leq 0.05$) (Table I).

A low positive insignificant correlation was found between GSH level and activity of studied enzymes in cancer tissues. Only in colorectal cancer liver metastases was a significant correlation between GSH and GSHPx ($r = 0.649$), GSH and TBARS ($r = 0.552$), and GSHPx and TBARS ($r = 0.362$) observed ($p \leq 0.05$).

Discussion

Increasing evidence has indicated that oxidative stress plays an important role in carcinogenesis. Much of it has come from the fact that antioxidants that scavenge free radicals directly, or interfere with the generation of free-radical mediated events,

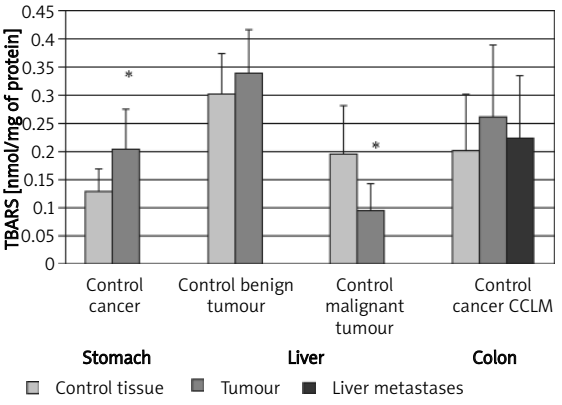

Figure 1. Lipid peroxidation level in gastrointestinal tract tumours
CCLM - colorectal cancer liver metastases
** statistically significant versus control tissue ($p \leq 0.05$)*

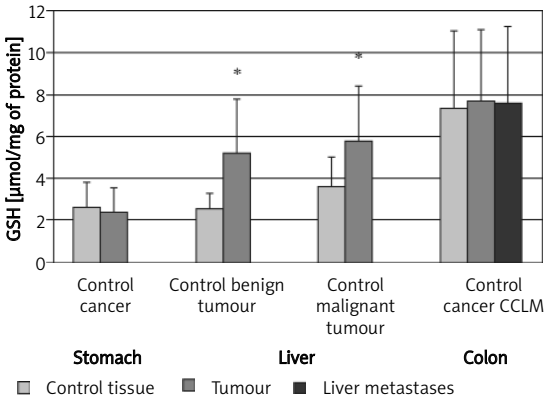

Figure 2. Glutathione level in gastrointestinal tract tumours
CCLM - colorectal cancer liver metastases
** statistically significant vs. control tissue ($p \leq 0.05$)*

Table I. GSH-dependent enzyme activities in gastrointestinal tract tumours

Studied enzymes	Stomach tumour		Liver tumours			Colon tumour		Colorectal cancer liver metastases
	control	cancer	control I	benign	control II	malignant	control	cancer	
[U/mg protein]	(*n* = 15)	(*n* = 15)	(*n* = 10)	(*n* = 10)	(*n* = 20)	(*n* = 20)	(*n* = 65)	(*n* = 65)	(*n* = 50)
GSHPx	0.033 ±0.016	0.104 ±0.039*	0.069 ±0.025	0.049 ±0.006*	0.066 ±0.028	0.034 ±0.016*	0.042 ±0.021	0.041 ±0.019	0.047 ±0.021
Se-GSHPx	0.015 ±0.007	0.024 ±0.008*	0.016 ±0.004	0.017 ±0.008	0.25 ±0.011	0.022 ±0.008	0.019 ±0.008	0.024 ±0.011	0.023 ±0.011
GST	0.018 ±0.008	0.021 ±0.006	0.051 ±0.023	0.032 ±0.016*	0.04 ±0.015	0.024 ±0.009*	0.022 ±0.008	0.021 ±0.01	0.013 ±0.006*/**
GSHR	0.027 ±0.012	0.043 ±0.02*	0.042 ±0.01	0.048 ±0.015	0.041 ±0.017	0.057 ±0.007*	0.036 ±0.014	0.039 ±0.018	0.048 ±0.024*/**

*GSHPx – glutathione peroxidase, Se-GSHPx – selenium-dependent glutathione peroxidase, GST – glutathione S-transferase, GSHR – glutathione reductase, control I – cancer-free liver tissue adjacent to benign tumours, control II – cancer-free liver tissue adjacent to colorectal cancer liver metastases, *statistically significant vs. control tissue ($p \leq 0.05$), **statistically significant vs. colorectal cancer ($p \leq 0.05$)*

inhibit the neoplastic process [3, 22]. The increased lipid peroxidation in cancer tissues from patients with different types of tumours also supports the involvement of radical reactions in carcinogenesis [23-25].

In our study we found increased TBARS concentration in gastric cancer compared with control tissue. This suggests ongoing lipid peroxidation possibly induced by enhanced generation of oxygen radicals within the influenced stomach tissue. Significant increase of GSHPx and Se-GSHPx activity in gastric cancer seems to counteract oxidative stress and toxicity due to increased antioxidant capacity. Despite high activity of glutathione depleting enzymes, GSH level in gastric cancer was unchanged, probably as a result of increased activity of GSHR.

In hepatocellular carcinoma we observed a significantly decreased level of lipid peroxidation and an increased GSH level. These changes may indicate adaptation of cancer cells for permanent proliferation. An inverse relationship has been observed between lipid peroxidation and the rate of cell proliferation, with highly proliferating tumours showing low levels of lipid peroxidation [26]. In turn, high GSH concentration in benign and malignant liver tumours could play a promoting role in cell proliferation. It has been documented that glutathione has regulatory effects on this process. Glutathione synthesis in tumour tissues was found to be associated with a high rate of cell proliferation [27]. Glutathione is required for inactivation of intracellular ROS which induce apoptosis and cell injury. Elevation of intracellular GSH in tumour cells is associated with mitogenic stimulation, and GSH controls the onset of tumour cell proliferation by regulating protein kinase C activity and intracellular pH [28, 29].

We also observed considerably decreased activity of GSHPx and GST, but increased GSHR activity in liver tumours. Taken together, these results show that a high intracellular level of GSH is sufficient to maintain a low level of lipid peroxidation in cancer cells, despite their decreased detoxification capacity probably caused by inactivation of GSHPx and GST [30]. Glutathione reductase, as a GSH replenishing enzyme, seems to play an important role in adaptation of cancer cells to oxidative stress. Changes observed in the liver may be related to the functions of this organ – it is a place of GSH synthesis, and processes of detoxification of oxygen radicals and their reactive derivatives.

There were no differences in activity or level of any of the examined parameters in colorectal cancer; only lipid peroxidation level was distinctly but not significantly increased. It seems that only mild oxidative stress accompanies colorectal cancer, and normal tissue level of GSH cycle enzymes'

activity is sufficient to allow proper functioning of cancer cells.

However, in colorectal cancer liver metastases, we observed decreased activity of GST and increased activity of GSHR with respect to both healthy colon and colorectal cancer. Other examined activities, lipid peroxidation level and GSH concentration were unchanged. This indicates decreased capacity of cancer cells to detoxify toxic products of their own metabolism [31]. The increased activity of GSHR may suggest a higher ratio of GSH utilization, probably as a direct scavenger of ROS in metastatic cells [10].

Although different changes in GSH redox state homeostasis in tumours have been documented in experimental models, there are few data demonstrating such variations in humans [32-34].

Our results indicate that the glutathione antioxidant system in gastrointestinal tract tumours is imbalanced, which probably arises as a result of enormous production of ROS in the cancer cells (see TBARS level).

The changes in GSH level and GSH-dependent enzyme activities in digestive tract cancers reflect changes in detoxification capacity, which may be an adaptive mechanism by which cancer cells gain a selective advantage over their surrounding normal cells. Antioxidative action of GSH-dependent enzymes provides more substantial protection against increased oxidative stress. The mobilization of the GSH system may be considered as the first line of cellular adaptation to ROS stress.

In conclusion, the glutathione enzymatic redox system is an important adaptation mechanism for protection of cancer cells against increased levels of ROS and may indicate its role in facilitating development of gastrointestinal tract tumours.

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