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REDUCED GLUTATHIONE CONTENT AND ACTIVITY OF GSH-RELATED ACTIVITY IN THE CASES OF COLORECTAL CANCER*

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The gastrointestinal system is particularly susceptible to constant and potentially injurious free radical chemical attack what can lead to carcinogenesis. An important role in the overall antioxidant defence strategy utilised by cells to defend against free-radical-induced oxidative stress is played by glutathione and enzymes of glutathione metabolism, glutathione peroxidase and glutathione reductase. The aim of this study was to examine these parameters in the 49 patients with colorectal cancer. In these cases the reduced glutathione level was decreased while glutathione peroxidase and glutathione reductase activities were increased in tumour tissues in comparison to morphologically unchanged tissues. The glutathione concentration in the blood serum of patients was slightly decreased in comparison with glutathione concentration in the blood serum of healthy people. The level of this parameter was higher in the week following surgery. However the antioxidant enzyme activities were decreased in the cancer tissues. The activity of glutathione peroxidase had been significantly decreased before surgery in comparison to control group and insignificantly decreased after surgery. However, activity of glutathione reductase had been decreased significantly before surgery and increased during next week after surgery but did not reach the control value. The obtained results indicate significant changes in antioxidant capacity of tumour tissues and serum from patients with colorectal cancer. These changes may be important for destruction of tumour tissues.

INTRODUCTION

Reactive oxygen species (ROS) are continuously generated in cells exposed to aerobic environment. Antioxidant defence systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS (Yu, 1994). Despite the antioxidant defence ROS-related damage of proteins and DNA accumulates during life and they have been postulated to lead to such diseases as atherosclerosis, arthritis, neurodegenerative disorders and cancer (Ames, Shigenaga & Hagen 1993). Also a number of exogenous cancer risk factors generate ROS *in vivo* (Amas, 1983; Nakayama, Nagata, Kodama & Kaneko, 1985). Therefore, there was much hope that the increase of cancer incidence could be reversed by removing avoidable source of ROS and/or by enhancing the antioxidant systems.

In the recent years, convincing evidence has been accumulated that ROS are indeed a relevant class of carcinogens (Cerutti, 1994). Cancer development is now commonly recognised as a microevolutionary process that requires the cumulative action of multiple events (Dreher & Junod, 1996). ROS can stimulate cancer development at all stages: initiation, promotion and progression (Dreher & Junod, 1996). In view of the ubiquity of ROS in aerobic organisms the carcinogenic potential of ROS gives rise to concern. It is especially important when the above conditions are accompanied by insufficient capacity of protective antioxidant system. This occurs frequently during inflammation or in the presence of deleterious substances (metals, smoke products and other chemicals). ROS-mediated lipid peroxidation and DNA damage are supposed to play an important role in the process of carcinogenesis. An important

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role in the overall antioxidant defence strategy utilised by cells to defend against reactive oxygen species is played by glutathione (GSH), especially in the gastrointestinal system which is particularly susceptible to constant and potentially injurious ROS attack. GSH levels and GSH-dependent enzymes are important for the metabolism of reactive oxygen species (Brigelis-Flohe, 1999; Sies, 1999). Moreover this peptide is also an essential compound for the intestinal function (Martensson, Jain & Meister, 1990). Jejunal and colonic epithelial cells are dependent upon the availability of GSH, since cellular degeneration occurs when GSH is depleted. GSH is regarded to be necessary for the protection of intestinal epithelia. In the intestinal mucus, GSSG reductase and GSH peroxidase are present to detoxify lipid hydroperoxides that may be present in the lumen (Aw, Williams & Gray, 1992). The mucus may act, as a source of protective thiols needed to maintain intestinal function.

The aim of this study has been to examine the reduced glutathione concentration and glutathione peroxidase and reductase activities in the tissues and the serum in human colorectal cancer.

MATERIAL AND METHODS

The examinations included 49 patients operated due to the colorectal cancer. The tissue samples were collected intraoperatively. In each case, sites of the tumour, histological type as well as the staging of cancer were assessed according to TMN classification. In the all examined cases, we dealt with adenocarcinoma of medium degree of histological differentiation (G_2) and the lesion infiltrated the whole muscular layer (T_3). The control group included tissues far from tumour, in which neoplastic changes were not detected histopathologically.

The blood samples was taken two days before and one week after the surgery. The control blood samples were collected from 20 healthy people. All these samples were centrifuged and the serum was frozen.

Preparation of samples

Tissues were removed quickly and placed in iced 0.15 M NaCl solution, perfused with the same solution to remove blood cells, blotted on filter paper, weighed and homogenised in 9 ml ice-cold 0.25 M sucrose and 0.15 M NaCl with addition of 6 µl of 250 mM butylated hydroxytoluene (BHT) in ethanol, to prevent formation of peroxides during the assay. Homogenization procedure was performed under standardized conditions; 10% homogenates were centrifuged at 10000 × *g* at 4°C for 15 min and the supernatant was kept on ice until assayed.

Glutathione peroxidase (GSH-Px; EC.1.11.1.6) activity was measured spectrophotometrically using a method based on that of Paglia and Valentine (1967), whereas GSH formation was assayed by measuring conversion of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to NADP.

Glutathione reductase (GSSG-R; EC.1.6.4.2) activity was measured by monitoring the oxidation of NADPH at 340 nm (Mize & Langdon, 1962).

Reduced glutathione (GSH) was measured using Bioxytech GSH-400 test (Bioxytech S.A., France).

The results were expressed as mean ± SD. Statistical analysis was performed using Student's "t" test for unpaired data and values of $P < 0.05$ were considered significant.

RESULTS

The reduced glutathione level was significantly decreased in the tumour tissue in comparison to morphologically unchanged tissues (Fig.1). However, the glutathione peroxidase and glutathione reductase activities were significantly increased. The glutathione concentration in the blood serum of operated patients was almost unchanged in comparison to glutathione concentration in the blood serum of healthy people (Fig. 2). The concentration of this peptide was significantly higher one week after the surgery. Before operation, however, the activity of glutathione peroxidase was decreased in comparison to the control group and was still lower after the operation. The activity of glutathione reductase was significantly decreased before operation but it significantly increased after the operation in comparison to previous group though it did not reach the control value.

DISCUSSION

GSH, a tripeptide with reactive sulphydryl group, plays an important role in the overall antioxidant defence strategy utilised by cells for defence against free-radical-induced oxidative stress. First of all, it scavenges reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and lipid hydroperoxides (Yu, 1994). It was observed that GSH level in the colorectal tissues and in the

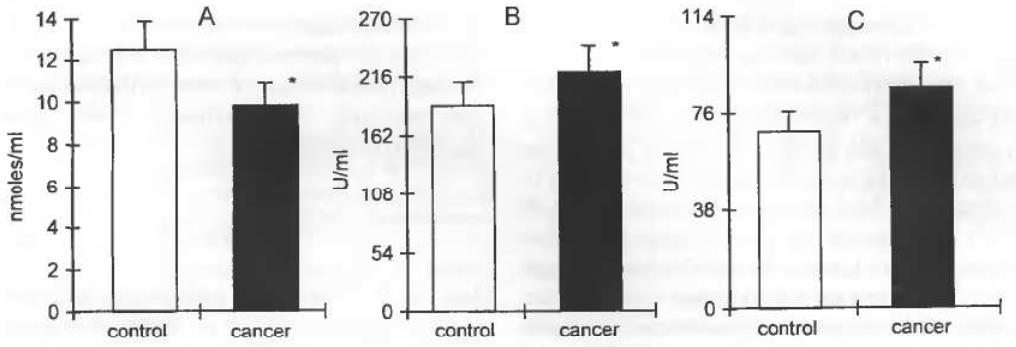

Fig. 1. The GSH content (A) and activity of GSH-Px (B) and GSSG-R (C) in the control and colorectal tissue of 49 patients. Data points represent mean ± SD; (*P < 0.05 in comparison to the control)

serum of patients with cancer was decreased. This can be caused by increased consumption, decreased synthesis or a combination of both. Some evidence has suggested that increased oxidative stress caused by reactive oxygen species is responsible for the effect. It is known that ROS are formed in excess in chronic diseases of the gastrointestinal tract and are likely to play an important role in the increased risk of cancer (Wiseman & Halliwell, 1996). An elevated production of ROS has been shown using colorectal biopsy specimens (Simmonds, Allen, Stevens, van Someren, Blake & Rampton, 1992). Further evidence consistent with damage by ROS is provided by the increase in lipid peroxides in rectal biopsy specimens from patients with active ulcerative colitis and the reports of low levels of GSH, Cu,Zn-SOD and GSH-Px in patients with active inflammatory bowel disease (Simmonds & Rampton, 1993; Inauen, Blizer, Rowedder, Halter & Laureburg, 1988, Berger, Gosky, Zborowska, Wilson & Berger, 1994). The main source of ROS in the gut are probably phagocytes, which are accumulated in the mucus of patients with bowel diseases and generate ROS upon activation. A marked increase in the activity of the inducible form of nitric oxide synthase in the inflamed colonic mucus has been reported, too (Boughtoan-Smith, Evans, Hawkey, Cole, Balsits & Whitte, 1993). In addition, nitrotyrosine (a putative chemical marker for the formation of $ONOO^-$) has been detected by immunohistochemical staining in an animal model of chronic gut inflammation and found to co-localise with NOS (Boughtoan-Smith, Evans, Hawkey, Cole, Balsits & Whitte, 1993). This suggests that both $NO^{\cdot}$ and $O_2^{\cdot-}$ are formed *in vivo* and undergo reaction to give $ONOO^-$. Furthermore, intracolonic instillation of $ONOO^-$ is pro-inflammatory in a rat colitis model (Rachmilewitz, Stamler, Karmeli, Mullins, Singel, Loscalzo, Xavier & Podolsky,

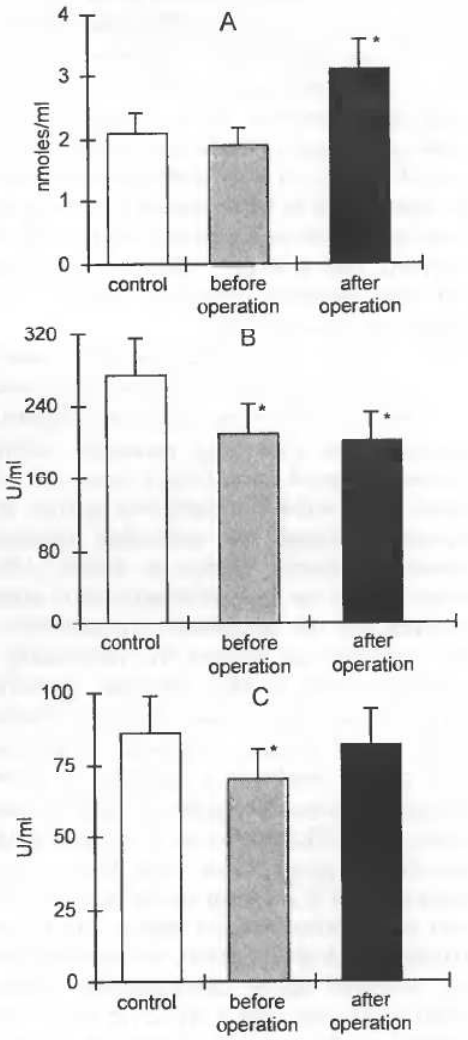

Fig. 2. The GSH content (A) and activities of GSH-Px (B) and GSSG-R (C) in the serum of healthy people and serum from patients with colorectal cancer. Serum was taken before operation and week after operation). Data points represent mean ± SD; (*P < 0.05 in comparison to the control)

1993). Another important ROS in the bowel disease may be HOCl which is produced by the enzyme myeloperoxidase from activated neutrophils and may attack membrane proteins directly (e.g. by oxidising SH groups, destroying methionine and chlorinating aromatic amino acid residues) or indirectly by the formation of chloramines. Both HOCl and chloramines can stimulate colonic secretion (Tamai, Kachur, Baron, Grisham & Gaginella, 1991). A role for ROS has also been described in the stimulation of colonic mucosal proliferation by bile salts. The evidence for increased ROS action in bowel diseases is the increase in the level of oxidative damage. DNA from colon biopsies had significantly increased level of 8-OHG, 2-hydroxyadenine, 8-hydroxyadenine and 2,6-diamino-5-formamidopyrimidine (Wiseman & Halliwell, 1996). These lesions caused by $OH^{\cdot}$ attack, could signify increase DNA damage and/or decrease their repair. The constitutive and ROS-induced activity of poly(ADP-ribose)polymerase has been shown to be decreased in patients with bowel disease including cancer (Markowitz, Rozen, Pero, Tobi & Miller, 1988). It can be associated with decreased, repair of oxidative DNA lesions (von Sonntag, 1982).

Moreover GSH is a co-substrate for the antioxidant enzyme glutathione peroxidase. In this study, an increase in GSH-related enzymes (glutathione peroxidase and glutathione reductase) activities has been observed in the tumour tissue and a decrease in the serum. Oxidants may activate gene expression through the antioxidant responsive element (Rushmore, Morton & Pickett, 1991). However, both the decrease in antioxidant enzyme activities and the unbalanced overexpression of these enzymes can increase the vulnerability of mammalian cells to ROS. Increased peroxidase activity in neoplastic tissues (which was observed in the present study) accompanied by decreased GSH content results in a disability to actively eliminate hydrogen peroxides or lipid hydroperoxides. A significant increase in the level of lipid peroxidation products has been found (unpublished studies). It is known, on the other hand, that lipid peroxidation products such as MDA, cause oxidative DNA modifications, which initiate damage, observed during cancerogenesis (Marnett, 1999). GSH plays also a protective role for proteins of intestine mucus. In the case of its reduced concentration, inflammatory processes are likely to occur more easily. The ability to repair oxidised molecules mainly proteins is also decreased.

In conclusion, the development of colon cancer is connected with a decrease in antioxidant capacity of tumour tissues but the destructive action of ROS may be also recognised as a beneficial and leading to destruction of tumour tissues However after operation the antioxidant defence system begins to normalize.

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