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The activity of antioxidant enzymes in colorectal adenocarcinoma and corresponding normal mucosa

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Oxidative stress is one of several factors which contribute to the development of colorectal carcinogenesis. The aim of the study was an assessment of the activity of antioxidant enzymes in tumour and corresponding normal distal mucosa in a group of patients with colorectal adenocarcinoma. Samples of tumour and corresponding normal mucosa were obtained during a resection of colorectal cancer from 47 patients aged between 26 and 82 years. The average distance of corresponding normal distal mucosa from the tumour was 4.49 cm. Activities of antioxidant enzymes: superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione *S*-transferase (GST), and catalase (CAT) were measured in tissue homogenates. The patients were grouped according to the tumour stage (Duke's staging), grading, localization, and size of tumour, as well as age and sex. Statistical analysis was performed. The activity of SOD and GPx was considerably increased, while the activity of GST decreased significantly in tumour as compared with normal mucosa. GR activity in colorectal cancer was evidently higher in tumours of proximal location compared with the distal ones. The distance of corresponding normal distal mucosa from the tumour was analyzed and related to all assayed parameters. A decreased GST activity was observed in corresponding normal mucosa more than 5 cm distant from the tumour in patients with CD Duke's stage. The higher activity of superoxide dismutase and glutathione peroxidase in tumour compared to corresponding normal mucosa could indicate higher oxidative stress in colorectal adenocarcinoma cells.

Key words: oxidative stress, antioxidant enzymes, colorectal adenocarcinoma, corresponding normal mucosa

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INTRODUCTION

Oxygen is an element essential for life, however, owing to its potent chemical activity, some by-products of oxygen metabolism are toxic to living organisms. These include reactive oxygen species (ROS), some are free radicals (Abele, 2002; Valko *et al*., 2007). Reactive oxygen species include the superoxide anion radical $^{\bullet}O^{-}_{2}$, hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^{1}O_{2}$), hydrogen peroxide (H_2O_2), and hypochlorous acid (I) (HOCl) (Halliwell & Gutteridge, 1999; Zasadowski *et al*., 2004).

Oxidative stress is defined as a lack of equilibrium between the oxidative agents (pro-oxidants) and antioxidants, compounds that protect biomolecules against the harmful effects of pro-oxidants (Klaunig & Kamendulis, 2004). When ROS production exceeds their utilization capacity, that leads to damage of nucleic acids, proteins and lipids, which in turn results in a disruption of cell, tissue or organ functions (Evans *et al*., 2004; Tanaka *et al*., 2007; Valko *et al*., 2007).

The cells of eukaryotic organisms have developed defence mechanisms that limit the level of ROS and the damage caused by their activity. Those defence mechanisms include antioxidant enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidise (GPx), glutathione S-transferase (GST), and catalase (CAT). These enzymes are present in various isoforms, in the intracellular and extracellular compartments, and their activity creates an integrated antioxidant defence system. Cells are also protected against oxidative stress by a system of non-enzymatic free radical scavengers, such as ascorbic acid, reduced glutathione, β-carotene, α-tocopherol, and cytochrome *c* (Zasadowski et al., 2004; Valko *et al*., 2007).

It has been shown that increased oxidative stress plays a role in the pathomechanism of many diseases, including senile cataract, atherosclerosis, diabetes, and degenerative brain disorders (Craghill *et al*., 2004; Singh *et al*., 2004; Dalle-Donne *et al*., 2005; Violi & Cangemi, 2005; Ceriello, 2006; Lin & Beal, 2006). Excessive synthesis of reactive oxygen species or inadequacy of the antioxidant defence system mechanisms are also aetiological factors in carcinogenesis (Cerutti, 1994; Ray *et al*., 2000; Liu *et al*., 2003; Klaunig & Kamendulis, 2004; Lu, 2007). The strongest ROS involvement, resulting from the exposition of organs to the activity of environmental pro-oxidants, has been demonstrated in the aetiology of bronchial and gastrointestinal tract cancers (Vanisree & Shyamaladevi, 1999; Kamp *et al*., 2001; Skrzydlewska *et al*., 2003; Dincer *et al*., 2007; Inayama *et al*., 2007; Rainis *et al*., 2007).

In addition to ROS, cell oxidants include reactive forms of nitrogen (RNS, reactive nitrogen species) such as the nitric oxide radical ($NO^{\bullet}$) and peroxynitrite (III) $ONOO^{-}$, which are also thought to contribute to the

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Abbreviations: CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione *S*-transferase; ROS, reactive oxygen species; SOD, superoxide dismutase.

development of cancer (Klatt & Lamas, 2000; Hofseth *et al*., 2003; Dalle-Donne *et al*., 2006; Beevi *et al*., 2007; Patel *et al*., 2009). Zafirellis *et al.* (2010) examined the inducible nitric oxide synthase (iNOS) expression in colorectal cancer and found that it could be a prognostic factor.

It is postulated that ROS are crucial factors in the initiation, promotion and progression of the carcinogenic process (Ray & Husain, 2002; Evans *et al*., 2004; Pervaiz, 2006; Laviano *et al*., 2007). As early as 1984, Zimmerman & Cerutti (1984) demonstrated that exposure of mouse fibroblasts to reactive oxygen species may lead to neoplastic transformation. An increased level of oxidative damage to neoplastic cells may be a result of: (I) increased ROS production in the absence of antioxidant system dysfunction, (II) a stable ROS level combined with a reduced efficiency of the antioxidant system, (III) errors in the DNA oxidative damage repair system, (IV) a combination of the above (Klaunig & Kamendulis, 2004; Halliwell, 2007; Karihtala & Soini, 2007).

Studies have shown that high production of ROS and the resulting oxidative stress are characteristic for neoplastic cells, both *in vivo* and *in vitro* (Szatrowski & Nathan, 1991; Toyokuni, 1998; Zhou *et al*., 2003; Hileman *et al*., 2004; Pelicano *et al*., 2004; Bechtel & Bauer, 2009) and the results of studies by Kondo *et al.* (1999) have demonstrated an increased level of ROS in adenocarcinoma compared to colon adenoma. Epithelial cells that line the intestine are in direct contact with oxygen radicals originating from diet or those produced by intestinal microflora (Skrzydlewska *et al*., 2001). Excessive ROS production, connected with the phagocyte 'oxidative burst', as well as a rising level of ROS in the defective vascular system in the vicinity of the neoplastic lesion, are the main causes of an increased level of ROS in neoplastic cells when compared to adjacent "healthy" tissues (Ray & Husain, 2002). Furthermore, the intensified metabolic activity of neoplastic cells also translates into increased production of the superoxide anion radical (Spitz *et al*., 2000). There are limited reports on the prooxidant-antioxidant balance in cancerous tissue of patients with colorectal cancer (Kanbagli *et. al*, 2000) and the precise mechanisms of oxidative stress and the role of ROS are still not fully understood (Skrzydlewska *et al*., 2005). Therefore, the aim of our study was to assess the activity of antioxidant enzymes in tumour and in corresponding normal distal mucosa tissues in patients with colorectal adenocarcinoma.

MATERIALS AND METHODS

Patients. The study group consisted of 47 patients aged 26-82 years (mean age 65 years) with a preoperative diagnosis of colorectal cancer, based on imaging and histopathological examination of specimens. The group consisted of 20 women and 27 men, that is 43% and 57% of the studied cases, respectively.

Patients with diabetes, diseases of the gastrointestinal tract, pancreas or liver, lipid metabolism disorders or acute infections, as well as tobacco smokers, were excluded from the study group. Hereditary and familial factors associated with the development of colorectal cancer were also eliminated. Patients did not receive preoperative radio-chemotherapy.

Specimens. Tissue specimens from both tumour and corresponding normal mucosa were obtained during resection of neoplastic lesions at the Clinic of Oncological and Reconstructive Surgery of the Maria Sklodowska–Curie Memorial Cancer Center & Institute of Oncology in Gliwice. The normal mucosa was obtained from a distal segment of resected colon, at a distance of at least 2 cm from the tumour, the average distance of that distal margin was 4.49 cm. The specimens were placed in ice and transported promptly to the laboratory, where they were washed twice with cold 0.9% NaCl. Subsequently, they were divided into two smaller fragments of which one was frozen at –80°C, and the second was examined histopathologically.

Homogenisation. The tissue fragment (30–80 mg) was homogenised using a PRO 200 homogeniser (PRO Scientific Inc, USA) for 60 seconds at 10 000 RPM (5 times with 2-minute intervals) in nine volumes of ice-cold homogenisation buffer. For SOD assaying, 20 mM Hepes buffer, pH 7.2 (PAA, Austria) was used. Phosphate-buffered saline solution (PBS without Ca and Mg, BIOMED, Poland) containing 0.5% Triton® X-100 (Sigma-Aldrich®, USA) was used for total protein, GR, GPx, GST, and CAT activity determinations. Subsequently, the obtained homogenates were centrifuged at 12 000 × *g* for 15 minutes at +4°C, the supernatants were divided into appropriate portions and frozen at –80°C until required for further testing.

Enzyme activity. The activities of superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione *S*-transferase and catalase were estimated in units/g protein. The total protein content was determined with the pyrogallol-red method using a set of reagents for direct colorimetric measurements of total protein (Sentinel Diagnostics, Italy). The coefficient of variation for the total protein determination assay was 2.07%.

Superoxide dismutase — SOD (cytosolic Cu/Zn-SOD isoenzyme and mitochondrial Mn-SOD isoenzyme, EC 1.15.1.1) activity was measured spectrophotometrically using the Superoxide Dismutase Assay Kit (Cat. No. 706002, Cayman Chemical Company, USA) in accordance with the kit protocol. Absorbance readings were obtained using an ELISA PowerWave XS™ reader (BioTek®, USA) at 450 nm wavelength. Assays were performed in duplicate and the coefficient of variation between duplicate samples ranged from 3.1% to 9.6%. Glutathione reductase (GR, EC 1.6.4.2) activity was measured at 37°C according to the Bioxytech S.A. (France) assay kit instructions using a kinetic–spectrophotometric method. The coefficient of variance for the GR assay was 4.21%. Glutathione peroxidase (GPx, GSH-Px, EC 1.11.1.6) activity was determined using a kinetic–spectrophotometric method in accordance with the Bioxytech S.A. (France) assay kit instructions. The coefficient of variation for the GPx assay was 1.82%. Total (cytosolic and microsomal) glutathione *S*-transferase (GST, EC 2.5.1.18) activity was determined using a kinetic–spectrophotometric method in accordance with the Cayman Chemical Company (USA, Cat. No. 703302) assay kit instructions. The assay is based on a method according to Habig *et al.* (1974), with 1-chloro-2,4-dinitrobenzene as a substrate. The coefficient of variation for the GST assay was 2.73%. The determinations of glutathione peroxidise, reductase and *S*-transferase activities and total protein concentration were done at 37°C with use Technicon RA-XT™ biochemical analyser (Technicon Instruments Corporation, USA). For the determination of catalase (CAT, EC 1.11.1.9) activity, a spectrophotometric method was used with purpald reagent (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) based on the method of Johansson and Borg (1988). Absorbance readings were obtained with ELISA PowerWave XS™ (BioTek®, USA) at 550 nm wavelength.

Readings were taken at 20°C. Calibration was performed using formaldehyde standards (POCH, Poland) at concentrations ranging from 8 μmol/L to 512 μmol/L. The coefficient of variation for the CAT assay was 6.8%.

Histopathology. Histopathological evaluation of the samples was performed at the Tumour Pathology Department of the Maria Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, Gliwice Branch, and histopathological confirmation of colorectal adenocarcinoma was obtained in all patients while the presence of neoplastic cells in the distal margins was excluded. In 37 (79%) of cases the tumour was located in the distal section of the colon while 10 (21%) of tumours were located in the proximal section (the dividing line being the splenic flexure). The average size of the tumour in the study group was 4.67 cm. Depending on the tumour staging according to Dukes, patients were divided into two subgroups. The first subgroup consisted of 27 (57%) of patients with A and B Dukes' staging; the second subgroup comprised 20 (43%) of patients with C and D Dukes' staging. Histopathological evaluation revealed 11 (24%) cases of well/highly differentiated (G1) cancer, 33 (70%) cases of moderately differentiated (G2) cancer, and 3 (6%) poorly/lowly differentiated (G3) cancer. The histopathological examination did not reveal the presence of any lymphocytic infiltration in the area around the tumours, thus eliminating the possibility of local inflammation. The study project was approved by the Bioethics Committee of the Maria Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, Gliwice Branch (No. D0/DGP/493-10/05 and No. KB/493-54/07). The patients were acquainted with the protocol after which they signed an informed consent document to participate in the study.

Statistical analysis. The statistical analysis was performed using Statistica 7.1PL software. Statistical comparisons were made using Student's *t*-test. Values of $p < 0.05$ were considered significant.

Table 1. Activity of antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT), and glutathione peroxidase (GPx) in homogenates of tissues obtained from colorectal tumours and distal margins in patients with colorectal adenocarcinoma

Assayed parameter	Tumour $\bar{X} \pm$ S.D.	Distal margin $\bar{X} \pm$ S.D.	*p*-value
SOD [U/g protein]	88.9 ± 40.69	58.4 ± 29.23	<0.001
GR [U/g protein]	71.3 ± 44.84	58.0 ± 42.20	0.159
GST [U/g protein]	31.0 ± 22.11	42.5 ± 23.45	0.021
CAT [U/g protein]	114.2 ± 63.48	103.4 ± 53.13	0.386
GPx [U/g protein]	54.5 ± 66.96	22.8 ± 23.99	0.004

$\bar{X}$, means; S.D., standard deviation

RESULTS

The activity of SOD and GPx was considerably higher in homogenates obtained from tumours compared to the activity in distal margins. Conversely, GST activity was lower in tumour homogenates, than in distal margin homogenates. No significant differences were found between tumour and distal margin for GR or CAT activity (Table 1). Furthermore, our findings revealed there were no significant differences between the activity of the majority of the enzymes tested in relation to the tumour staging (according to Dukes' classification), grading, their localisation or size, as well as the patients' age or sex (Table 2, 3, and 4). Only the GR activity was found to be statistically significantly higher in tumours with proximal location than in those of distal location ($p = 0.013$; Table 4). All assayed parameters were also analysed for any relation to the distance between the tumour and distal margin (Table 2, 3, and 4). The only significant

Table 2. Activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in homogenates of tissues obtained from colorectal tumours and distal margins in relation to clinical parameters in patients with colorectal adenocarcinoma

			SOD [U/g protein] $\bar{X} \pm$ S.D.		CAT [U/g protein] $\bar{X} \pm$ S.D.	
		N	Tumour	Distal margin	Tumour	Distal margin
Women		20	94.7 ± 43.8	63.1 ± 29.8	109.4 ± 51.5	113.6 ± 66.3
Men		27	84.6 ± 38.4	55 ± 28.8	117.6 ± 71.7	95.6 ± 40.1
Age	<65	17	88.7 ± 35.1	55 ± 29.8	115.6 ± 86.4	115.3 ± 63.2
	>65	30	88.9 ± 44.0	60.3 ± 29.2	113.4 ± 48.2	97.2 ± 47.1
Tumour localisation	Proximal	10	93.3 ± 43.3	57.7 ± 30.7	104.4 ± 59.1	110 ± 44.7
	Distal	37	87.7 ± 40.5	58.6 ± 29.2	116.6 ± 65	101.7 ± 55.5
Tumour staging (Dukes' classification)	A, B	27	90 ± 38.5	56.9 ± 26.9	113.8 ± 60.3	97.6 ±50.5
	C, D	20	87.3 ± 44.4	60.5 ± 32.7	114.7 ± 69.2	111 ± 56.8
Grading	G1	11	85 ± 53.4	60 ± 33.3	95 ± 19.2	112.8 ± 60.4
	G2	33	91.7 ± 40.5	57 ± 31.8	122.1 ± 70.3	100 ± 54.5
	G3	3	130 ± 14.1	75 ± 7	70 ± 0	120 ± 14.1
Distance from distal margin to tumour	≤5 cm	14	84.7 ± 38	55.8 ± 27.9	102.9 ± 56.5	103.3 ± 48.5
	> 5 cm	33	106 ± 47.8	70 ± 32.3	145 ± 76.1	104 ± 71.3

$\bar{X}$, means; S.D., standard deviation

Table 3. Activity of glutathione peroxidase (GPx) in homogenates of tissues obtained from colorectal tumours and distal margins in relation to clinical parameters in patients with colorectal adenocarcinoma

			GPx [U/g protein] $\bar{X}$ ± S.D.	
		N	Tumour	Distal margin
Women		20	60.3 ± 88.9	22.1 ± 22.3
Men		27	50 ± 45.2	22.3 ± 24.5
Age	<65	17	49 ± 55.6	28.2 ± 28.8
	>65	30	57.5 ± 73.4	19.7 ± 20.6
Tumour localisation	Proximal	10	67.7 ± 98.6	14.8 ± 12.9
	Distal	37	51.5 ± 59.2	24.6 ± 25.6
Tumour staging (Dukes' classification)	A, B	27	55.1 ± 65.9	24.8 ± 23.6
	C, D	20	53.4 ± 70.3	20 ± 24.8
Grading	G1	11	63.7 ± 100	25.8 ± 27.4
	G2	33	45 ± 43.9	23 ± 25.5
	G3	3	55 ± 35.3	22 ± 25.4
Distance from distal margin to tumour	≤5CM	14	46.5 ± 53.3	21 ± 24.3
	>5CM	33	76.2 ± 101.5	26.6 ± 22.9

$\bar{X}$, means; S.D., standard deviation

relation found was in GST activity in margin samples, it depended on the distance from the tumour and on Dukes' staging. In the group of patients with the CD Dukes' staging, the GST activity was lower in the distal margins separated from the tumour by more than 5 cm than in the less distant distal margins (50.7 ± 25.86 *vs.* 20.0 ± 10.00; $p = 0.049$).

DISCUSSION

Oxidative stress is considered to be of vital importance among factors playing a role in the process of carcinogenesis in the colon (Kanbagli *et al.*, 2000; Bartsch *et al.*, 2002; Skrzydlewska *et al.*, 2003; Rainis *et al.*, 2007). One of the indications of oxidative stress is lipid peroxidation, a process where polyunsaturated fatty acids, which make up phospholipids, undergo an oxidative reaction resulting in the formation of their peroxides. The end products of lipid hydroxylation, such as aldehydes and alcohols, impair protein synthesis and may have an adverse effect on membrane permeability, hemotactic activity and immunological response (Klaunig & Kamendulis, 2004; Zasadowski *et al.*, 2004; Karihtala & Soini, 2007). Lipid peroxidation is considered to be an important factor responsible for endothelial function and vasotonus regulation (Zasadowski *et al.*, 2004). It has been demonstrated that elderly people have an elevated lipid peroxide content in the intestinal lumen, and a positive correlation has been found between fat consumption and the incidence of malignant breast, ovarian and colon cancer in elderly people. Reac-

Table 4. Activity of antioxidant enzymes glutathione reductase (GR) and glutathione S-transferase (GST) in homogenates of tissues obtained from colorectal tumours and distal margins in relation to clinical parameters in patients with colorectal adenocarcinoma

			GR [U/g protein] $\bar{X}$ ± S.D.		GST [U/g protein] $\bar{X}$ ± S.D.	
		N	Tumour	Distal margin	Tumour	Distal margin
Women		20	72.6 ± 43.3	59.0 ± 32.2	28.9 ± 23.9	46.1 ± 25.4
Men		27	70.2 ± 46.9	57.3 ± 48.5	32.5 ± 21	39.9 ± 22.1
Age	<65	17	58 ± 43.4	51.2 ± 35.7	24.2 ± 16.6	43.3 ± 23.5
	>65	30	78.3 ± 44.7	61.8 ± 45.6	34.6 ± 24	42 ± 23.8
Tumour localisation	Proximal	10	96.2 ± 26.69	63.6 ± 46.4	35.8 ± 27.5	37.5 ± 30.1
	Distal	37	65.5 ± 46.44	56.5 ± 41.6	29.7 ± 20.7	43.5 ± 22
Tumour staging (Dukes' classification)	A, B	27	63.2 ± 42.8	54.8 ± 42.4	31 ± 22.6	40.3 ± 22
	C, D	20	83.5 ± 46.3	62.2 ± 42.6	31 ± 22	45.5 ± 25.7
Grading	G1	11	51.2 ± 18.8	55 ± 37.4	21.8 ± 15.5	32.5 ± 12.8
	G2	33	67.6 ± 48.2	47.4 ± 34	30.8 ± 21.17	44.6 ± 23.9
	G3	3	120 ± 14.1	95 ± 21.2	36.5 ± 33.7	50 ± 14.1
Distance from distal margin to tumour	≤5 cm	14	68.5 ± 48.8	61.2 ± 44	31.2 ± 22.5	43.4 ± 21.5
	> 5 cm	33	81 ± 32.1	51.3 ± 37.1	31.4 ± 22.8	38.1 ± 31.8

$\bar{X}$, means; S.D., standard deviation

tive oxygen species may be obtained from foods with a high content of polyunsaturated fatty acids, which are a source of lipid peroxides, or may be produced by the intestinal microflora (Skrzydlewska & Stankiewicz, 2001; Dincer *et al*., 2007; Rainis *et al*., 2007). Animal studies have shown that a diet rich in fat is conductive to the neoplastic transformation of colon adenoma (Nicholson *et al*., 1991). The development of some cancers is also connected with chronic inflammatory conditions which are accompanied by the release of significant amounts of ROS and RNS. They are formed through a defence mechanism, created by phagocytes accumulating in the intestinal mucosa in patients with chronic inflammatory diseases. Upon activation, phagocytes produce oxidants whose presence increases the risk of cancer development (Wiseman *et al*., 1996; Seven *et al*., 2000; Roessner *et al*., 2008). Simmonds *et al*. (1992) documented an augmented production of reactive oxygen species in intestinal biopsy specimens of patients with the inflammatory bowel disease. Seven *et al*. (2000) also observed increased lipid peroxidation in rectal biopsy specimens of patients with active ulcerative colitis. Moreover, ROS stool content was increased when a diet rich in fats and low in dietary fibre was consumed (Ehardt *et al*., 1997).

Our analysis of antioxidant enzyme activity revealed a considerable increase in SOD and GPx activity in specimens obtained from tumours when compared with margins. Superoxide dismutase and glutathione peroxidase comprise the first line of the antioxidant defence system and are the main enzymes catalysing ROS decomposition reactions. Therefore, they play an important role in protecting the organism against oxidative damage (Singh, 1997). The presented results are consistent with the data reported by Skrzydlewska *et al*. (Skrzydlewska & Stankiewicz, 2001; Skrzydlewska *et al*., 2003; 2005), Ozdemirler *et al*. (1998), Kanbagli *et al*. (2000) and Satomi *et al*. (1995). Those authors have also recorded a considerable increase in SOD activity in neoplastic tissues compared to margins in a group of patients with colorectal cancers, including adenocarcinoma. Janssen *et al*. (1999) found higher MnSOD activity in colorectal cancer with liver metastases compared to the activity of this enzyme in adenomas and normal colon mucosa. Moreover, other authors have noted higher SOD activity in gastric carcinoma, primary liver tumours and brain tumours (Janssen *et al*., 2000; Czeczot *et al*., 2003; Wang *et al*., 2005; Dudek *et al*., 2004; Kekec *et al*., 2009). GPx activity determinations in colorectal adenocarcinoma specimens performed by Kanbagli *et al*. (2000), Skrzydlewska *et al*. (Skrzydlewska & Stankiewicz, 2001; Skrzydlewska *et al*., 2003) and Erata *et al*. (2005) have shown a statistically significantly higher activity of that enzyme compared to the activity in the tumour margin. Similar differences in GPx activity in colorectal cancer specimens compared to the tumour margin have also been reported by other authors (Ozdemirler *et al*., 1998; Stanczyk *et al*., 2005; Rainis *et al*., 2007; Kekec *et al*., 2009). However, we found in tumour specimens a decreased glutathione S-transferase enzyme activity in comparison to that of the distal margin. Other authors have also observed a decreased GST activity in cancers (Das *et al*., 2007; Sharma *et al*., 2007).

We did not observe any significant differences in glutathione reductase activity between tumour and distal margin. Studies by other authors showed that GR activity was statistically significantly higher in tumour tissue of patients with colorectal cancer as compared to the tumour margin (Skrzydlewska & Stankiewicz, 2001; Stanczyk *et al*., 2005). However, in the case of various types of brain tumours and in a group of patients with cervical cancer, the GR activity in patients' blood was lower than in the control group of healthy subjects (Rao *et al*., 2000; Sharma *et al*., 2007). Our results show a statistically higher GR activity in tumours located in the proximal segment of the colon compared to the GR activity in tumours of distal location. The difference in the GR activity depending on the tumour location may be connected with different levels of functional activity in the respective segments of the colon. Moreover, an evaluation of catalase activity did not reveal substantial differences between tumour and distal margin in analysed cases of colorectal adenocarcinoma. Rainis *et al*. (2007) and Beno *et al*. (1995) noted increased CAT activity in adenoma and colorectal cancer specimens. Hwang *et al*. (2007) observed an increase in CAT expression in gastric adenocarcinoma cells compared to the normal tissue.

The presented study also analyzed the relation between the activity of the studied enzymes and the tumour stage (according to Dukes' classification), grading, the size and localisation of the tumour, as well as patients' age and sex. There were no statistically significant differences. The study results corroborate the conclusions of Erata *et al*. (2005) carried out in a group of patients with colorectal adenocarcinoma, which revealed no connection between the tumour stage and histological tumour differentiation and GPx activity in tumour specimens. Similarly, an absence of a significant relationship between the parameters mentioned above and antioxidant enzyme activity was reported by Dincer *et al*. (2007) in patients with gastric and colorectal carcinoma. A lack of differences in SOD and CAT activity depending on the tumour stage was also demonstrated by Ho *et al*. (2006) in a group of patients with lung cancer and by Dursun *et al*. (2006) between SOD, GPx and CAT activities in patients with oesophageal and gastric carcinoma. However, other studies performed on a group of patients with colorectal cancer noted an increased in antioxidant enzyme activity correlated with the tumour stage as well as histological tumour differentiation (Satomi *et al*., 1995; Stanczyk *et al*., 2005).

The incidence of cancer increases with age, affecting approximately 35% of people over 85 years old. Reactive oxygen species are considered to be a key element of cellular disorganisation occurring in the elderly (Savitha *et al*., 2005; Humphries *et al*., 2006). Studying on rats, Savitha *et al*. (2005) observed a significant decrease in antioxidant enzyme levels with age. The present study did not, however, reveal such differences; this is also consistent with observations of other authors (Dincer *et al*., 2007; Hwang *et al*., 2007). The present study also analysed the relation between the distance from the tumour to the sampled margin and the studied parameters. That analysis revealed only a significant difference in the GST activity and in relation to the tumour staging according to Dukes' classification. Observations in the group of patients with CD Dukes' staging revealed lower GST values in margins more than 5 cm distant from tumours. The results of our study regarding the GST activity also reveal a decrease in the activity of that enzyme in the tumour compared to the distal margin. It is believed that reactive oxygen species may cause enzyme deactivation in the course of carcinogenesis (Dursun *et al*., 2006; Dincer *et al*., 2007). A statistically significant reduction in the activity of these enzymes in patients with advanced clinical stage was also observed by Namyslowski *et al*. (2003) and by Manoharan *et al*. (2005). Assuming that advanced clinical stage is connected with increased oxidative stress, it may affect the activity of enzymes forming the antioxidant barrier.

Numerous studies have reported disturbances in the antioxidant balance, but the data regarding changes in the antioxidant enzyme activity in various types of neoplasms are controversial (Rao *et al*., 2000; Stanczyk *et al*., 2005; Dursun *et al*., 2006; Ho *et al*., 2006; Hwang *et al*., 2007; Sharma *et al*., 2007; Klimczak *et al*., 2009; Pejic *et al*., 2009; Sharma *et al*., 2009). Dincer *et al*. (2007) and Dursun *et al*. (2006) suggest that changes in the activity of antioxidant enzymes may occur in response to metabolic changes and excessive production of free radicals as an expression of impairment of mitochondrial function in neoplastic cells.

The results of our study confirm the hypothesis of increased oxidative stress in the course of the neoplastic process. That is evidenced by high activity of antioxidant enzymes. The increase in the activity of antioxidant enzymes is an outcome of cellular adaptation to conditions of increased oxidative stress and has been described by many authors (Janssen *et al*., 2000; Czeczot *et al*., 2003; Wang *et al*., 2005; Hileman *et al*., 2004; Erata *et al*., 2005; Dursun *et al*., 2006; Dincer *et al*., 2007; Rainis *et al*., 2007). It is thought that the enhancement of the antioxidant enzyme activity is a result of the activation of genes that code for antioxidant enzymes by oxidants (Yokota, 2000; Skrzydlewska & Stankiewicz, 2001; Zhou *et al*., 2003; Skrzydlewska *et al*., 2005).

An increase in SOD activity may be caused by induction of the corresponding gene, which is regulated by interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α). The activity of these signalling molecules may increase in neoplastic cells (Abele, 2002; Skrzydlewska *et al*., 2003; Hwang *et al*., 2007; Namysłowski *et al*., 2003). The results on GST activity suggest, in turn, an impairment of the enzymatic antioxidant barrier in neoplastic cells. This result may also be explained on the basis of observations made by other authors. In their opinion, changes in the activity of antioxidant enzymes may be a result of enzyme inactivation in the course of the carcinogenic process (Dursun *et al*., 2006; Dincer *et al*., 2007). Reactive oxygen species induce changes not only in nucleic acids, but also in lipids and proteins, including antioxidant enzymes. What is more, proteins that undergo such modification lose their biological activity. This hypothesis is particularly relevant to the actions of the hydroxyl radical (Waszczykowska *et al*., 1999; Evans *et al*., 2004). Stress induced by excessive production of reactive nitrogen species also leads to structural changes in proteins as a result of nitrosylation, which also translates into inhibition of their function (Klatt & Lamas, 2000; Ridnour *et al*., 2004; Pylvas *et al*., 2010). Another hypothesis that aims to explain the causes of the high variability in the activities of the studied enzymes is a change in the expression of some genes in the course of neoplastic transformation, including genes encoding antioxidant enzymes whose expression may be increased or decreased (Stanczyk, 2005; Yokota, 2000).

Genes encoding various antioxidant enzymes, in particular the glutathione transferase isoenzymes, are highly polymorphic and often contain numerous mutations. A connection between the mutations or polymorphisms of those genes and an increase of the cancer development risk has been established in the oesophagus, stomach, large intestine, breasts, lungs and lymph nodes (Katoh *et al*., 1996; Stoehlmacher *et al*., 2002; Ho *et al*., 2006; Holley *et al*., 2006; Lightfoot *et al*., 2006; Kitteringham *et al*., 2007; Landi *et al*., 2007; Yao *et al*., 2010). Studies of the genetic polymorphism of *GSTT1* and *GSTM1* genes have revealed a high differentiation within the respective populations. *GSTT1* has been shown to be polymorphic and is absent in 10–18% of the population of Europe. Similarly, *GSTM1* gene is absent in 38% of individuals (Olędzki & Kędziora-Kornatowska, 2006). The vast majority of ROS-induced damage in cells is removed by repairing enzymes, however, some may accumulate and contribute to the mutagenesis. Moreover, the occurrence of mutations themselves does not result in development of malignancy. It is only when mutations occur in several genes of proliferating cells that the process of carcinogenesis may be initiated (Karihtala & Soini, 2007). Mice with an inactivated *CuZnSOD* gene, in which the incidence of liver cancer has been observed to increase along with age, may serve as an example of the antioxidant defence system as a contributing factor in carcinogenesis (Elchuri *et al*., 2005). In studies by Chu *et al*. (2004), inactivation of two out of four *GPx* genes in mice, namely *GPx1* and *GPx2*, resulted in the development of colorectal cancer; and mice with a decreased catalase activity had a higher incidence of breast cancer (Ishii *et al*., 1996). Furthermore, the gene expression is also affected by epigenetic alterations connected with methylation in their promoter regions. Reactive oxygen species are capable of modifying the pattern of DNA methylation (Klaunig & Kamendulis, 2004). In our earlier studies in a group of patients with colorectal cancer, we observed DNA methylation of *p16* and *MGMT* genes both in tumour and corresponding normal mucosa (Krakowczyk *et al*., 2008). Kim *et al*. (2005) observed hypermethylation of *GSTP1* gene promoter in adenomas and adenocarcinomas of sporadic colorectal cancer. *GSTP1* gene promoter hypermethylation was recognised as a tumour marker for prostate, breast, kidney and gastric cancer (Esteller *et al*., 1998; Gonzalgo *et al*., 2003; Kim *et al*., 2005; Muller & Brenner, 2006).

CONCLUSIONS

The change of antioxidant enzymes activities may result from the fact that neoplastic disease disturbs the balance in both ROS production, as well as in the efficiency of the antioxidant system of the organism. It is difficult to interpret the findings regarding the relation of the distance between the sampled margin and tumour. There have been no reports in the available literature on changes in the activity of antioxidant enzymes depending on the distance from the tumour. Despite finding a significant correlation for only one of the analyzed parameters, it may be surmised that changes in the metabolism of neoplastic cells affect cells of tissues adjacent to the tumour. However, a more in-depth analysis is required.

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