POLSKI
PRZEGLĄD CHIRURGICZNY
2015, 87, 7, 357–361

10.1515/pjs-2015-0069

DE GRUYTER
OPEN

EVALUATION OF ANTIOXIDANT DEFENSE IN PATIENTS WITH COLORECTAL CARCINOMA

KATARZYNA MALINOWSKA[1], MICHAŁ MIK[2], ŁUKASZ DZIKI[2], ADAM DZIKI[2], IRENEUSZ MAJSTEREK[1]

Department of Clinical Chemistry and Biochemistry, Medical University in Łódź[1]
Kierownik: prof. dr hab. *I. Majsterek*
Department of General and Colorectal Surgery, Medical University in Łódź[2]
Kierownik: prof. dr hab. *A. Dziki*

Cancers are among the most feared diseases of modern civilization. In Poland, colorectal cancer is one of the tumors with the worst prognosis. The ability to cure is primarily dependent on the stage of the disease at the time of diagnosis.
The aim of the study was evaluate antioxidant response in patients with colorectal carcinoma.
Material and methods. Twenty patients (14 men and 6 women) aged 61.9± 11.1 years with colorectal cancer were included in the study. Twenty healthy subjects (4 men and 16 women) aged 64 ± 15.3 years formed the control group. The erythrocyte activities of antioxidant enzymes, superoxide dismutase (SOD), and glutathione peroxidase (GPx),
Results. A significant increase of GPx, and SOD ($p < 0.05$) were seen in patients compared to healthy controls.
Conclusion. The results indicate that the tested antioxidant enzyme activity of glutathione peroxidase and superoxide dismutase is increased in patients diagnosed with colorectal cancer compared to the control group.
Key words: GPx, SOD, colon cancer

Colorectal cancer is a malignant tumor growing in the colon, appendix and rectum. The large intestine and rectum are the end portions of human digestive system. Are primarily responsible for the absorption of water and formation of stool. In terms of the incidence of cancer, colorectal cancer (CRC) is classified in second place among both women and men. Is the second cause of death in men (after lung cancer) and third (after breast cancer and cervical cancer) in women (1-8). It is now believed that certain types of tumors arise via genetic mechanisms, and others – epigenetic. There are also many conditions that both of these mechanisms interact in a complex process of carcinogenesis. Today, there is no doubt that environmental factors participate in the final "decision" or cells with mutations in the DNA will neoplastic transformation. Growing evidence indicates that one of the factors responsible for the induction of malignant transformation of cells are reactive oxygen species (reactive oxygen species – ROS). Because of their substantial reactivity they constitute a serious threat to the integrity and proper functioning of cells.

Regardless of important biological functions of ROS may also be damaging agents cellular components. Imbalance between ROS generation and antioxidant systems performance lead to oxidative stress. The formation of cancer is a multistep process in which distinguished the initiation phase, promotion, and progression. The results of numerous studies in experimental systems in vitro and in vivo studies indicate that ROS are involved not only during the initiation and promotion of carcinogenesis, but also its progression. Oxidative DNA

modifications caused by ROS may be part of the process of initiating tumor. Evidence of this can be detected elevated levels of modified bases in tumor tissue compared to normal tissue surrounding the tumor.

It is also expected that such changes in DNA are converted into a factor in the change of benign malignant and may lead to an increase in metastatic potential (2, 3). Biological strategy of defense against hydroxyl radical precursors: superoxide anion and hydrogen peroxide based on the fact that both are dismutation reaction, which is their weak point. The simplest measure of defense is thus speeding up the process. For this purpose, living organisms have developed a group of enzymes that catalyze the degradation of superoxide anion and hydrogen peroxide. Here ones include catalase (CAT), superoxide dismutase (ZnCu-SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (3).

MATERIAL AND METHODS

Patients

The study involved 20 patients with colorectal cancer including 14 men and 6 women, aged 61.9 ± 11.1. Control group consisted of healthy individuals without cancer lesions, 16 men and 4 women, aged 64 ± 17.7. Tests were carried out on the blood of patients hospitalized in the Department of General and Colorectal Surgery, Medical University in Łódź, research material was blood that was collected into vacuum tubes with anticoagulant – heparin lithium in the amount of 4 ml. Experienced were conducted with the consent of bioethics committee No RNN / 693/14 / KB Medical University in Łódź.

Hemoglobin (Hb) (9)

The concentration of hemoglobin (Hb) in the blood hemolysate was determined by Drabkin. This parameter has a maximum absorbance at a wavelength of 540 nm. The color intensity of the resulting compound is proportional to the concentration of hemoglobin. This parameter was necessary to calculate the activity of antioxidant enzymes studied (GPx and SOD).

Determination of the activity of glutathione peroxidase (GPx) in red blood cells (10)

As a substrate for the enzyme used cumene. Control samples were prepared and tested in the centrifuge tube by adding 0.1 ml of 50 fold diluted hemolysate and 0.7 ml of Tris-HCl buffer, pH 7.6. Incubated for 10 minutes in a water bath at 37 ° C. At this time, the control were added 0.1 ml of a solution of reduced glutathione in Tris-HCl buffer, and test sample 0.1 ml of a solution of reduced glutathione and 0.1 ml of 0.05% solution of cumene in Tris-HCl buffer. The tubes were once again placed for 5 minutes in a water bath at 37 ° C. After cooling to room temperature, samples were added 1.0 ml of an aqueous solution of 20% trichloroacetic acid, and the control 0.1 ml of 0.05% solution of cumene in Tris-HCl. The tubes were centrifuged for 10 minutes at 1400xG acceleration. After centrifugation, 1.0 ml of the supernatant containing the reduced glutathione, which has not been used in the reduction reaction of cumene by the active enzyme, was added 2 ml of 0.4 M Tris-HCl buffer pH 10.0 and 0.1 ml of an alcoholic solution of DTNB. Test samples were measured against the control, using a spectrophotometer DU-650 at a wavelength of 412 nm. The enzyme activity was calculated after taking into account the dilution and molar absorbance coefficient expressing it in the U / gHb.

Determination of the activity of superoxide dismutase (SOD ZnCu) in red blood cells (11)

Principle of the method determining the superoxide dismutase is based on the phenomenon of inhibition of the enzyme reaction of auto-oxidation of adrenaline. To measure the activity of CuZn-SOD in the test samples used previously diluted hemolysate prepared twice. To 0.1 ml of the hemolysate was added 0.9 ml of distilled water chilled to + 4 ° C, 0.5 ml of 96% ethanol and 0.25 ml of chloroform. The mixture was shaken for two minutes in a closed tube stopper. The tubes were centrifuged for 10 minutes at 4200xG acceleration at + 4 ° C. Then the control sample is added to 2.9 ml of 0.05 M carbonate buffer, pH 10.2, and 0.1 ml of a solution of adrenaline in 0.01 N HCl at pH 2.0.

Assay test contained 2.8 ml of 0.05 M carbonate buffer, pH 10 ml, 2, 0.1 ml of the su-

pernatant containing the superoxide dismutase solution of adrenaline and 0.1 in 0.01 N HCl at pH 2.0. Measurements were made of changes in absorbance at a wavelength of 480 nm on a spectrophotometer DU-650 at 37 ° C against the blank, which was a 0.05 M carbonate buffer, pH 10.2. Superoxide dismutase activity was determined in one-minute intervals on the basis of changes in absorbance of the sample containing the enzyme, the referenced to the same time to absorb changes in the control, where they should be 0.025 absorbance units per min. Superoxide dismutase activity in the test samples were expressed in units of adrenaline (U / gHb / 100 ml).

RESULTS

For those who have not been diagnosed with cancer of the enzyme activity was calculated CuZn-SOD and it was 1114.72 ± 178.59 U / g / Hb / 100 ml and GPx-25.42 ± 7.79 U / Hb Patients diagnosed with colorectal cancer activity of CuZn-SOD was on average 1956.17 ± 671.45 U / g / Hb / 100 ml, and GPx activity-30.01 ± 12.44 U / Hb (fig. 1, 2).

DISCUSSION

Danger to the structure and function of cells, posed RFT, could not remain without an evolutionary response. Aerobic organisms, the possibility of using very energy efficient use of oxygen in the breathing process (and other metabolic processes) must also develop defense mechanisms to protect them largely from the effects of ROS formation and their reaction with cellular components. Biological strategy of defense against hydroxyl radical precursors: superoxide anion and hydrogen peroxide based on the fact that both are dismutation reaction, which is their weak point. The simplest measure of defense is thus speeding up the process. (12, 13).

In our work, we study the activity of superoxide dismutase and glutathione peroxidase. Superoxide dismutase include in their active centers metal ions that participate in the reactions dismutation of superoxide anion. In mammals there are three types of superoxide dismutase superoxide.

In mammals there are three types of superoxide dismutase superoxide. The cytoplasmic enzyme is in its composition comprising copper and zinc (Cu, ZnSOD, SOD 1) (12, 13, 14).

This protein molecule is composed of two identical subunits. In the mitochondrial matrix is present containing manganese superoxide dismutase (MnSOD, SOD_2). It is composed of four subunits. On the outside of the cells is present extracellular superoxide dismutase (EC-SOD). The second enzyme described antioxidant is glutathione peroxidase (GSH-Px) is a key enzyme which in the presence of reduced glutathione (GSH) catalyzes the reduc-

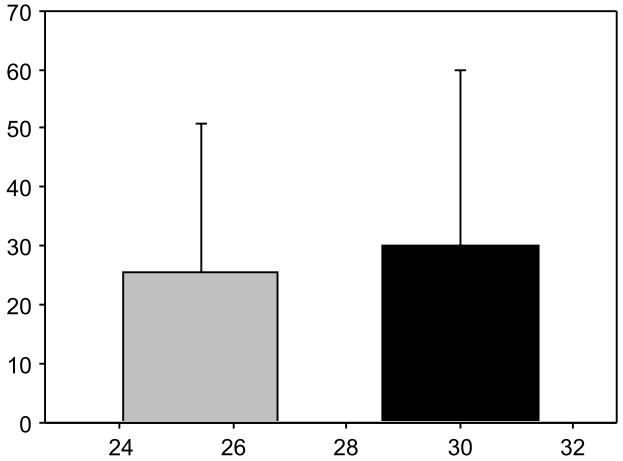

Fig. 1. Graph showing the glutathione peroxidase activity measured in units U / gHb in the study group (black area) as compared to the control group (clear area), p<0.05

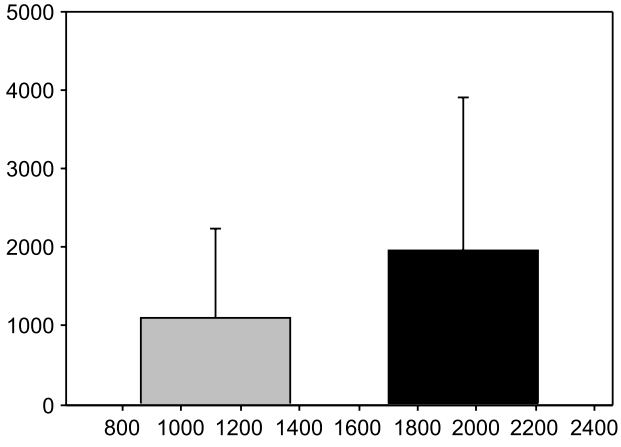

Fig. 2. A graph showing the activity of superoxide dismutase, measured in units U / gHb / 100 ml in the test group (black area) as compared to the control group (clear area), p<0.05

tion of hydrogen peroxide (H_2O_2) into water or organic peroxides (ROOH), and further to the corresponding alcohols (ROH). The molecule of glutathione peroxidase contains four identical subunits, forming a flat structure. Glutathione peroxidase does not contain heme or any other prosthetic group outside of selenium. Also acts as storage protein selenium. Besides, there are other forms of classical glutathione peroxidase, including not contain selenium. Selenium contained in the various forms of glutathione peroxidase is approx. 30% of the total pool of this element in the body. In humans, there are four types of glutathione peroxidase (GSH-Px1-4), which serve different physiological functions. Type I Glutathione peroxidase (GSH-Px 1) is defined as classical or cell, as occurs in almost all cells. The second type of peroxidase (GSH-Px 2) is called gastrointestinal glutathione peroxidase. The third type discussed peroxidase (GSH-Px extracellular) is present in the plasma. The type IV glutathione peroxidase (GSH-Px 4) is called. glutathione peroxidase phospholipid peroxides which, like GSH-Px 1 is expressed in most tissues (12-17).

In cancer there are marked differences between the activity of GPx and SOD in human tumor cells in the body and in normal cells (18). Depending on the types of tumors observed decrease in SOD activity, both Cu-ZnSOD and MnSOD in various processes of tumor formation in relation to the activity in normal cells. Studies of SOD and GPx activity in vitro and in vivo studies demonstrated that tumor cells have been transformed and have a different level of superoxide dismutase activity and peroxidase than for normal cells. (19, 20) In colorectal cancers, it was found increased activity of antioxidant enzymes CuZn SOD GPx as compared to the normal epithelium (19-20). Janssen et al. showed that the development of colon cancer may be associated with increased expression of MnSOD. Also shown is the superior activity of epithelial cells in colorectal cancer compared to the activity and amount of MnSOD of healthy epithelium (21-24).

We can therefore conclude that the growth of colon cancer enzyme activity occurs at an early stage of cancer.

Literature describes a positive relationship between MnSOD mRNA and vascular invasion in these types of tumors. These data indicate that over expression of MnSOD mRNA, may be attached to the colorectal cancer with increased tumor aggressiveness. This type of assumption is confirmed by other researchers Satoni et al have also shown that the activity of the enzyme in tumor tissues increases with the various stages and severity of disease (18-29).

In conclusion we can say that our studies confirm the assumptions listed in the work of researchers and determine increase in the activity of antioxidant enzymes people diagnosed with colorectal cancer compared to healthy subjects.

CONCLUSIONS

The results indicate that the tested antioxidant enzyme activity of glutathione peroxidase and superoxide dismutase is increased in patients diagnosed with colorectal cancer compared to the control group.

REFERENCES

1. *Mitura K, Kozieł S, Pasierbek M* i wsp.: Leczenie przepuklin pachwinowych w północnej Ghanie. *Pol Przegl Chir* 2015; 1: 29-40.
2. *Tyczynski JE, Bray F, Aareleid* et al.: Lung cancer mortality patterns in selected Central, Eastern and Southern European countries. *Int J Cancer* 2004; 109: 598-610.
3. *Akkooi AC, Nowecki ZI, Voit C* et al.: Tumor burden according to the Rotterdam criteria is the most important prognostic factor for survival in melanoma patients: a multicenter study in 388 patients 1 with positive sentinel nodes. *Ann Surg* 2008; 248: 949-55.
4. *Kubiak K, Malinowska K, Langner E* i wsp.: Wpływ związków koordynacyjnych Cu(II) na aktywność enzymów antyoksydacyjnych katalazy i dysmutazy ponadtlenkowej u chorych na raka jelita grubego. *Pol Przegl Chir* 2011; 83: 282-92.
5. *Malinowska K, Modranka R*: Wpływ związków chromonu na aktywność wybranych enzymów antyoksydacyjnych u chorych ograniczonych ruchowo. *PML* 2010; 163: 26-28.
6. *Kubiak K, Klimczak A, Dziki Ł*: Synthesis and pro-antioxidant properties of Cu(II) complexes with pyrazole ligands. *PML* 2010;163: 22-25.

7. *Malinowska K, Misiak P, Modranka R* i wsp.: Właściwości biologiczne kompleksu benzopiranu z Pt (II). *Pol Przegl Chir* 2014; 86(4): 299-311.
8. *Misiak P, Wcislo S, Rzepkowska-Misiak B* et al.: Evaluation of the impact of radical resection of the tumor in patients with lung cancer at the level of activity of selected antioxidant enzymes. *Kardiochir i Takochir Pol* 2014; 11(4): 414-20.
9. *Van Kampen E, Zijlstra G*: Standardization of hemoglobinometry. II. The hemiglobincyanide method. *Clin Chim Acta* 1961; 6: 538-44.
10. *Beers RF, Sizer W*: A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952; 195: 133-40.
11. *Misra P, Fridovich J*: The role of superoxide anion in the autooxidation of epinephrine and a simple assay for the superoxide dismutases. *J Biol Chem* 1972; 247: 3170-75.
12. *Salvemini F, Franze A, Iervolino A* et al.: Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression *J Biol Chem* 1999; 274: 2750-57.
13. *Arai M, Imai H, Koumura T*: Mitochondrial phospholipid hydroperoxide glutathione peroxidase plays a major role in preventing oxidative injury to cells. *J Biol Chem* 1999; 274: 4924-33.
14. *Flohe L*: Glutathione peroxidase. *Basic Life Sci* 1988; 49: 663-68.
15. *Burk RF*: Protection against free radical injury by selenoenzymes. *Pharmacol Therap* 1990; 45: 383-85.
16. *Dringer A*: Metabolism and functions of glutathione in brain. *Progress in Neurobiol* 2000; 62: 649-71.
17. *Martin M, Macias M, Escames G* et al.: Melatoin but not vitamin C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. *FASEB J* 2000; 14 (12): 1677-79.
18. *Abdel-Aziz A F, El-Naggar mm*: Superoxide dismutase activities in serum and white blood cells of patients with some malignancies. *Cancer Lett* 1997; 113(1-2): 61-64.
19. *Arivazhagan S* et al.: Erythrocyte lipid peroxidation and antioxidants in gastric cancer patients. *Cell Biochem Funct* 1997; 15(1): 15-18.
20. *Crapo JD* et al.: Copper, zinc superoxide dismutase is a primarily a cytosolic proein in human cells. *Proc Natl Acad Sci USA* 1992; 89: 10405-09.
21. *Hassan HM*: Biosynthesis and regulation of superoxide dismutases. *Free Radic Biol Med* 1988; 5: 377-85.
22. *Izutani R, Asano S, Imano M* et al.: Expression of manganese superoxide dismutase in esophageal and gastric cancers. *J Gastroenterol* 1998; 33(6): 816-22.
23. *Janssen AM, Bosman CB, Kruidenier L* et al.: Superoxide dismutases in human colorectal cancer sequence. *J Cancer Res Clin Oncol* 1999; 125: 327-35.
24. *Janssen AM, Bosman CB, Van Duijn W* et al.: Superoxide dismutases in gasric and esophageal cancer and prognosicimpact in gastric cancer. *Clin Cancer Res* 2000; 6(8): 3183-92.
25. *Malafa M, Margenthaler J, Webb B* et al.: MnSOD expression is increased in metastatic gastric cancer. *Surg Res* 2000; 88(2): 130-34.
26. *Nelson RL:* Superoxide dismutase in cultured benign and malignant tumors of the colon. *Basic Life Sci* 1988; 49: 699-702.
27. *Satomi A, Murakami S, Hashimoto T* et al.: Significance of superoxide dismutase (SOD) in human colorecal cancer tissue: correlation with malignant intensity. *J Gastroenterol* 1995; 30(2): 177-82.
28. *Toh Y, Kuninaka S, Oshiro T* et al.: Overexpression of manganese superoxide dismutase mRNA may correlate with aggressiveness in gastric and colorectal adenocarcinomas. *Int J Oncol* 2000; 17(1): 107-12.
29. *Skrzycki M, Czeczot H*: Rola dysmutazy ponadtlenkowej w powstawaniu nowotworów. *Postępy Nauk Med* 2005; 4: 7-15.

Received: 7.07.2015 r.
Adress correspondence: 90-647 Łódź, Pl. Hallera 1
e-mail: Katarzyna.malinowska@umed.lodz.pl