EFFECT OF Cu(II) COORDINATION COMPOUNDS ON THE ACTIVITY OF ANTIOXIDANT ENZYMES CATALASE AND SUPEROXIDE DISMUTASE IN PATIENTS WITH COLORECTAL CANCER

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Colorectal cancer (CRC) is a serious medical and economical problem of our times. It is the most common gastrointestinal cancer in the world. In Poland, the treatment and detection of CRC are poorly developed and the pathogenesis is still unclear. One hypothesis suggests a role of reactive oxygen species (ROS) in the pathogenesis of CRC. Experimental studies in recent years confirm the participation of ROS in the initiation and promotion of CRC.

The aim of the study was to examine the effect of the following coordination compounds coordination compounds: dinitrate (V) tetra(3,4,5-trimethyl-N1-pyrazole-κN2) copper(II), dichloro di(3,4,5-trimethyl-N1-pyrazole-κN2) copper(II), dinitrate (V) di(1,4,5-trimethyl-N1-pyrazole-κN2) copper(II), dichloro di(1,3,4,5-tetramethyl-N1-pyrazole-κN2) copper(II) on the activity of antioxidant enzymes superoxide dismutase (SOD, ZnCu-SOD) and catalase (CAT) in a group of patients with colorectal cancer (CRC) and in the control group consisting of patients with minor gastrointestinal complaints.

Material and methods. The study was conducted in 20 patients diagnosed with colorectal cancer at the age of 66.5±10.2 years (10 men and 10 women) versus the control group of 20 people (10 men and 10 women) aged 57.89±17.10 years without cancer lesions in the biological material – hemolysate prepared in a proportion of 1ml of water per 1 ml of blood. CAT activity was measured by the Beers method (1952), while SOD activity was measured by the Misra and Fridovich method (1972).

Results. We found that patients with CRC showed a statistically significant decrease of SOD and CAT activity (CAT – 12.75±1.97 U/g Hb, SOD – 1111.52±155.52 U/g Hb) in comparison with the control group (CAT – 19.65±2.17 U/g Hb, SOD – 2046.26±507.22 U/g Hb). Simultaneously, we observed that the investigated coordination compounds of Cu(II) significantly increased the antioxidant activity of CAT and SOD in patients with CRC (mean: CAT 25.23±4.86 U/g Hb, SOD – 3075.96±940.20 U/g Hb).

Conclusions. Patients with colorectal cancer are characterized by reduced activity of antioxidant enzymes catalase and superoxide dismutase which suggests impaired antioxidant barrier. Therefore, coordination compounds of Cu (II), which enhance the activity of CAT and SOD, may prove useful in the prevention and treatment of colorectal cancer.

Key words: colorectal cancer, catalase, superoxide dismutase, coordination compounds

Antioxidants may be classified into physiological (natural) ones and synthetic compounds. They protect both cells and extracellular spaces against harmful effects of an excess of free radicals formed in pathological processes. They also maintain pro- and antioxidant balance in diseases with increased production of oxygen free radicals. These groups include antioxidant enzymes, preventive antioxidants and free radical scavengers.

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The best known antioxidant enzymes include superoxide dismutase (SOD, ZnCu-SOD), catalase (CAT) and glutathione peroxidase (GPX). Superoxide dismutase is the main cytoplasmatic enzyme catalysing the reaction of dismutation superoxide anion radical to hydrogen peroxide and molecular oxygen. SOD is a metalloenzyme which occurs in two forms – an intracellular and extracellular one (1). Another enzyme participating in ROS elimination is catalase. This enzyme catalyses the breakdown of hydrogen peroxide to water. Catalase is found mainly in peroxysomes of mammal cells, in hepatic and renal cells, in red and white blood cells, and in nervous tissue cells. The third enzyme – glutathione peroxidase – is a metalloenzyme which participates in hydrogen peroxide reduction with concurrent transformation of reduced glutathione into its oxygenated form. At the catalytic site, this enzyme contains four selenium atoms contained in selenocysteine.

Human cells contain 4 various forms of glutathione peroxidase. GPX-1 present inside the cells, for example in erythrocytes, has the greatest role in ROS elimination (2). An imbalance between the quantity of produced ROS or the amount of reactive nitrogen species (RNS) and their effective elimination causes oxidative stress. Oxidative stress has been documented to play an important role in the pathophysiology of multiple diseases, including cancer or neurodegenerative disorders such as Parkinson’s disease or Alzheimer’s disease (3). Colorectal cancer is currently the most common gastrointestinal tract cancer in the world. Its incidence has been steadily increasing, which may be partially related to population ageing. This phenomenon can be observed in the United States and Western Europe countries where the percentage of elderly people has been constantly increasing. Owing to early detection and treatment of intestinal cancer in these countries, mortality rates associated with this disease have been decreasing for a certain time. In Poland, colorectal cancer is one of the cancer types with the worst prognosis (4-7). The possibility of cure depends above all on disease advancement at diagnosis.

The main treatment method of malignancies is chemotherapy. It involves administration of natural or synthetic anticancer medicines which are commonly called cytostatics. Cancer treatment with cytostatics is a difficult process, not always effective, which must take into account the mechanisms of action, pharmacokinetics and dosage regimens of these drugs. The factors which contribute to the complexity of the chemotherapy process are our still incomplete understanding of the cancer causes, only minor biological differences between a cancer cell and a healthy cell, very low selectiveness of action of the cytostatic agents, the narrow therapeutic index of anticancer medicines, high toxicity for healthy cells and dissatisfactory anticancer activity (8, 9). Many compounds have interesting biological properties, including antioxidant ones.

In cancer treatment, synthetic compound with antioxidant properties are considered to be very promising. This group includes pyrazole, chromone and flavone derivatives which are known from their antioxidant, anticancer, antispasmodic, diuretic and cholagogue properties (10). The derivatives of this group such as khellin (5,8-dimetoxy-2 methyl-4’,5’-furan6,7-chromone) have been used as antispasmodic agents active in the bronchi, gastrointestinal tract, uterus and urinary tract and dilating coronary vessels. One of the main applications of these derivatives is the treatment of cardiovascular disorders and cancer (10). Another example is diosmin which acts on blood vessels. It reduces oedema owing to restoration of normal permeability of capillaries and an improvement of venous circulation. It is contained in Detralex. Pyrazole derivatives also display a wide spectrum of biological activity and also have high ability to create coordination compounds with transient metal ions such as Cu(II), Ru(III), Pt(II), Co(II), Pd(II) (11, 12, 13, 14). In the recent years, there has been much chemical research as a result of which complexes of metals with pyrazole derivatives were obtained. In our study, we attempted to investigate the effect of pyrazole-copper ion complexes on the activity of antioxidant enzymes CAT and SOD (15).

MATERIAL AND METHODS

Patients

The study was conducted in 40 unrelated caucasian age-matched patients (p=0.32) who were divided in two study groups. The first group included patients with diagnosed CRC aged 66.5±10.2 years (10 men and 10 women)
and the second group included persons without detectable cancer (10 men and 10 women) aged 57.89±17.10. The purposefulness and extent of the necessary tests and the rules of patient selection and the methodology applied were approved by the Ethics Committee, Resolution No. RNN/260/08/KB. The study subjects were selected from patients treated in the General and Colorectal Surgery Department of the Medical University in Łódź and did not take any vitamin products.

Material

The test material was peripheral blood from 20 CRC patients and 20 patients without cancer. The biological material was collected during routine testing, from a cubital fossa vein, in a quantity of 4 ml, to vacuum test tubes with an anticoagulant (EDTA).

Conditions of incubation with Cu(II) coordination compounds

The material obtained was centrifuged in an Eppendorf centrifuge 5804 R at 0°C to 4°C, to separate plasma from the morphotic elements. Erythrocyte mass was used for further studies, after its triplicate washing with 0.9% NaCl, retaining the same centrifugation conditions. After supernatant removal, the following were added in the specified sequence to the washed blood cells of CRC patients at a proportion of 1:1: 1 ml of glucose solution and 1 ml of a mixture consisting of 80 µl of the Cu(II) coordination compound, 1) dinitrate (V) tetra(3,4,5-trimethyl-N\(^1\)-pyrazole-κN\(^2\)) copper (II), 2) dichloro di(3,4,5-trimethyl-N\(^1\)-pyrazole-κN\(^2\)) copper (II), 3) dinitrate (V) di(1,4,5-trimethyl-N\(^1\)-pyrazole-κN\(^2\)) copper (II), 4) dichloro di(1,3,4,5-tetramethyl-N\(^1\)-pyrazole-κN\(^2\)) copper (II), and 920 µl of blood at a concentration of 2·10\(^{-3}\) mol/l (20 mg of compound per 1 kg body weight). The whole was incubated on a water bath at 37°C for 15 min. After this time, the biological material was centrifuged and the supernatant was discarded. The residue was used for the preparation of hemolysate (1 ml of water and 1 ml of blood). The whole was frozen at -70°C.

Catalase activity measurement

Catalase activity was measured in hemolysates obtained from erythrocyte suspension by the Berss method (1952) (16). The tested sample was measured against the control which was 0.05 M phosphate buffer at pH=7. In the tested sample, 50 µl of 50-fold diluted hemolysate and 1 ml of 30 mM H\(_2\)O\(_2\) in 0.05 M phosphate buffer at pH=7 were added to 2 ml of 0.05 M phosphate buffer at pH=7. After H\(_2\)O\(_2\) was added, a change in absorbance was measured every 10 s for 1 minute at λ=240 nm. The activity was expressed in Bergmayer units U/g Hb.

Superoxide dismutase activity measurement

Superoxide dismutase activity was measured in hemolysates obtained from erythrocyte suspension by the Misra and Fridovich method (1972) (17). The method is based on using the phenomenon of inhibiting by the enzyme the self-oxidation of adrenalin to adrenochrome. Superoxide dismutase activity is measured at one-minute intervals on the basis of absorbance changes in the sample containing the enzyme, compared with the similar absorption changes in the control sample where they should be 0.025 absorbance unit per minute. Superoxide dismutase activity in tested samples is expressed in adrenalin units U/g Hb.

Statistical analysis

Statistical analysis was performed using the STATISTICA 6.0 software.

RESULTS

In colorectal cancer patients, catalase activity 12.75±1.93 U/g Hb was significantly lower in comparison with the control group 19.65±2.17 U/g Hb (p<0.001). In the tested group, after addition of Cu(II) coordination compounds, the following respective significant increases of enzyme activity were noted for the specified compounds: 1) 24.99±5.53 U/g Hb (p<0.001), 2) 23.34±2.89 U/g Hb (p<0.001), 3) 26.61±4.33 U/g Hb (p<0.001) and 4) 25.99±6.72 U/g Hb (p < 0.001) (fig. 1).
In patients with colorectal cancer, SOD activity 1111.52±155.52 U/g Hb was also significantly lower in comparison with the control group 2046.26±507.22 U/g (p<0.001).

In the tested group, after addition of Cu(II) coordination compounds, the following respective significant increases of enzyme activity were noted for the specified compounds: 1) 3939.09±1645.09 U/g Hb (p<0.001), 2) 2949.61±731.52 U/g Hb (p<0.001), 3) 2538.92±679.82 U/g Hb (p<0.001), 4) 2876.25±704.38 U/g Hb (p<0.001) (fig. 2).

No statistically significant differences were shown for the measured activity of the tested enzymes in the tested group after the use of the individual Cu(II) coordination compounds 1-4 (p > 0.05), the mean values for which were CAT – 25.23±4.86 U/g Hb, SOD – 3075.96±940.20 U/g Hb, respectively.

**DISCUSSION**

According to the World Health Organization, 7.5 million people, representing 1.25% of the world population, die of cancer every year. In Poland, ca. 300 new cancer cases are diagnosed and 22 people die every day. Both the number of cancer cases and mortality increase every year (18). As shown by the World Health Organization’s report Poland has the worst effectiveness of cancer fight of all European countries. Summarising the state of knowledge on epidemiology of malignancies, the WHO Expert Committee stated that many of these cases are a result of lifestyle and environmental factors. Dietary factors are responsible for about 30% of all cancer cases in the world (19). According to Food, Nutrition and the Prevention of Cancer, this rate reaches even 40%. Unhealthy nutrition is the second most common cause of cancer in approximately 20 million people. It is estimated that in the developing countries the incidence of diet-related cancer will increase by 10 million people within 20 years (20, 21).

Many major studies support the correlation between the risk of colorectal adenocarcinoma and levels of antioxidant vitamins and certain microelements in blood plasma. Antioxidant enzymes metabolise free radicals (SOD metabolises O$_2^-$), or intermediate products (GSH-Px and catalase metabolise H$_2$O$_2$) to less toxic or non-toxic compounds. In the process of H$_2$O$_2$ transformation into H$_2$O by GSH-Px, glutathione (GSH) is transformed into a non-oxygenated form – glutathione disulphide (GSSG) which is then regenerated to a form reduced by GSH reductase (GSH-Red). Therefore, the purpose of the present study was to investigate the effect of Cu(II) coordination compounds with pyrazoles on the activity of antioxidant enzymes catalase and superoxide dismutase in patients with colorectal cancer.

Concentrating on the prophylaxis and the free radical theory of cancer development, it is worth paying attention to a group of organic substances.
compounds which, owing to their properties, may activate the antioxidant barrier of our body. One of these groups is azoles. A representative of this group is pyrazole, which has found applications in pharmacotherapy (22).

The chemical literature contains many data on the possible practical applications of the compounds with a pyrazole ring, which are used, for example, in agriculture as herbicides, fungicides and pesticides, in the industry as photo-stabilisers and in therapy as antibacterial agents, non-narcotic analgesics, anticancer agents and antipyretic agents (21, 23). The most important of those compounds are: amide of N-dimethyl-3-[(1-(phenylmethyl)-1H-indazole-3-yl)oxy]-1-propanoic acid; amide of 4-[5-(-4-methylphenyl)-3-trifluoromethyl]-1H-pyrazole-1-yl]-benzosulphonic acid; 4-(4-chlorophenyl)-1-(4-fluorophenyl)-1H-pyrazole-3-acetic acid; amide of 2-(dimethylamine)-N-(1,3-diphenyl-1H-pyrazole-5-yl)-propanoic acid; amide of 4-amine-N-(1-phenyl-1H-pyrazole-5-yl)-benzosulphonic acid; 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-acetic acid; 1-(2,4-dichlorophenyl)methyl]-1-H-indazole-3-carboxylic acid (21, 22).

It should be noted that among pyrazole derivatives used in therapy, there is a correlation between their pharmacological activity and chemical structure. The chemical classification corresponds to the pharmacological one. Pyrazole-5-one derivatives have mainly analgesic, antipyretic, less potent anti-inflammatory and antispasmodic properties. On the other hand, pyrazolidine-3,5-dione derivatives exert mainly anti-inflammatory action. In our study, we analysed a group of 20 CRC patients and persons without cancer constituting the control group. We have found that CRC patients are characterised by a significantly reduced CAT and SOD activities (CAT – 12.75 U/g Hb, SOD – 1111.52 U/g Hb) as compared with the control group (CAT – 19.65 U/g Hb, SOD – 2046.26 U/g Hb) which supports the antioxidant barrier disruption hypothesis. At the same time, we have observed that the tested Cu(II) coordination compounds significantly increase antioxidant activity of CAT and SOD in the group of CRC patient (mean: CAT – 25.23 U/g Hb, SOD – 3075.96 U/g Hb).

As shown by prior studies of the dichloro di(3,4,5-trimethyl-N1-pyrazole-N2) Cu(II) complex, this compound causes a 67% increase in catalase and superoxide dismutase activity in a group of patients with colorectal cancer (22, 24, 25). Malinowska et al. (2009), who examined the effect of the complex compound dinitrate (V) di(3,4,5-trimethyl-N1-pyrazole-N2) copper (II) on catalase activity in patients with colorectal cancer, also found that compound to significantly increase catalase activity (25, 26, 27). The researchers from the General and Transplant Surgery Department of the Warsaw Medical University studied the effect of activity of antioxidant enzymes on gastrointestinal tract cancers. The study was conducted in 20 patients with hepatic cirrhosis (8 women and 12 men, mean age 39 years) treated in 2003–2005. The control group were 15 patients (8 women and 7 men, mean age 38 years) who underwent surgery for benign hepatic tumours (angiomas, adenomas). The results obtained indicate that patients with hepatic cirrhosis have antioxidant barrier dysfunction which is manifested by a decrease in the GSH levels and changes in SOD, CAT, GSHPx, GST and GSHR activities as compared with healthy subjects (28, 29). In our study, a similarly significant reduction of the activity of the tested enzymes was found in patients with CRC, which proves disruption of the antioxidant barrier. However, administration of Cu(II) coordination compounds significantly increased the activity of antioxidant enzymes CAT and SOD. In conclusion, we may find that in light of the presented data, the results of our study on the effect of Cu(II) coordination compounds on the activity of antioxidant enzymes in patients with CRC may be of importance for the prevention and treatment of this disease.

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