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Effect of copper(II) on glutathione peroxidase activity in patients with head and neck cancer

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT:

Introduction: Head and neck squamous cell carcinoma (HNSCC) accounts for about 6% of all malignant cancers. The most important risk factors of oral cancers include tobacco smoking, alcohol abuse, bad oral hygiene, papilloma virus infection, riboflavin and iron deficiency.

Objective: The objective of the investigation was to synthesize the Cu(II) complex and to evaluate the antioxidative enzymatic barrier in red blood cells of patients with head and neck tumors as well as in a control group.

Materials and methods: We obtained a consent from a Bioethics Committee (number RNN/142/09/KB) . Blood samples for the examination were obtained from patients of the Department of Head and Neck Neoplasms Surgery, Medical University of Lodz. The experiment was conducted in 40 patients with HNSCC and 40 healthy people. With the use of a spectrophotometric method, glutathione peroxidase was determined.

Results: The investigation was conducted on the hemolysate obtained from the patients who were divided into two groups -study group (1 and 2), which consisted of patients diagnosed with head and neck cancer, and a control group (1 and 2) – healthy people. A significant statistical result for GPX occurred in the control group-1 and test 2 with the complex-pyrazole Cu (II), $p < 0.001$).

Conclusions: Our research proves that the complex compound compound Cis-dichloro-bis(N1-hydroxsymethyl-3-methylpyrazol- κN2) copper (II) has an impact on the activity of the antioxidative GPX enzyme.?

KEYWORDS: pyrazole complex, glutathione peroxidase, head and neck cancer.

INTRODUCTION

The increasing incidence of cancer made it one of the major challenges for molecular biology in the 21st century. Despite multiple studies, new experiments are still conducted worldwide in order to discover mechanisms responsible for creation and development of cancer. Molecular basis of cancerous transformation has become the main direction for development of more effective diagnostic and therapeutic methods. Head and neck squamous cell carcinoma (HNSSC) is a group of malignant tumors stemming from the squamous epithelium and developing in the upper alimentary and respiratory tract. They constitute 90% of all head and neck carcinomas (the other 10% include adenocarcinomas, lymphoadenocarcinomas and salivary gland carcinomas) [1,2,3]. The statistical data show that those tumors constitute 5-6% of all malignant neoplasms in less economically developed countries; every year, 440,000-650,000 new cases are reported, and the peak incidence is observed in the 50-70 age group [4,5]. Due to HNSCC, 350,000 patients die every year [6]. Factors contributing to development of HNSCC may be divided into two groups, namely exogenous and endogenous. Exogenous factors mainly include cancerogenes and oncogenes [7,8]. At this point, smoke should be mentioned, which contains approx. 4,000 chemical compounds such as polycyclic aromatic hydrocarbons, highly toxic for human organism. Those factors also include oncogenic viruses, e.g. human papillomavirus (HPV) [9,10]. HPVs are a group of viruses that lack the envelope and have a double-stranded circular DNA molecule containing 7.9 kbp. [11]

It has been proven in many studies that risk factor for HNSCC also include poor dental hygiene and ill-fitting dental pros-

theses [12]. Among endogenous factors, the most important factor is genetic predisposition [13,14]. The main therapeutic modalities for HNSCC are: surgery, radiotherapy (RTH) and, to a lesser extent, chemotherapy (CHTH), which can be used in monotherapy or combined therapy [15,16]. RTH is a method based on ionizing radiation. It utilizes the difference in radiosensitivity between tumor and the surrounding healthy tissue. Radiotherapy as a single modality can be applied in early stages of HNSCC [16]. CHTH is based on cytostatic effect of chemical compounds on cancer cells.

CHTH it often accompanied by adverse reactions of various degree, which result from damage to healthy cells such as bone marrow, intestinal and oral mucosa, skin, hair and nails [17,18]. Protective mechanisms against free radicals include enzymes such as superoxide dismutase, catalase and glutathione peroxidase. For proper function of those enzymes, the appropriate amount of minerals for their activation is necessary in the body, including zinc, copper, ruthenium, manganese, iron and selenium.

Reactive oxygen species (ROS) production is a fundamental element of aerobic cellular metabolism. The imbalance between ROS production and antioxidant mechanism efficiency leads to oxidative stress, which, in turn, results in many diseases, e.g. oral cancer.

The aim of this study was to assess the possible use of antioxidant properties of Cu(II) complexes in prevention of cancer.

MATERIALS AND METHODS:

Patients

The tests were conducted on blood samples from patients who had been divided into two group: test group 1 and 2, as well as control group 1 and 2. Patients diagnosed with HNSCC were assigned to the test group, the control group consisted of healthy individuals. All patients from the test group were admitted to the Head and Neck Tumors Surgical Ward of the Second Otolaryngology Department of the Medical University in Lodz. The study was conducted based on the permission No RNN/142/09/KB of the Bioethics Committee. The study involved 80 people, 40 of them in the test group (33 men and 7 women aged 48 ± 17.90) and 40 in control group (20 men and 20 women aged 48 ± 13.50).

Complex synthesis

Synthesis of copper (II) complex compound from pyrazole derivatives was conducted in aqueous solution at room temperature, the molar ratio of ligand to copper(II) chloride being 2:1. The synthesis was conducted in mixed water-ethanol environment [19].

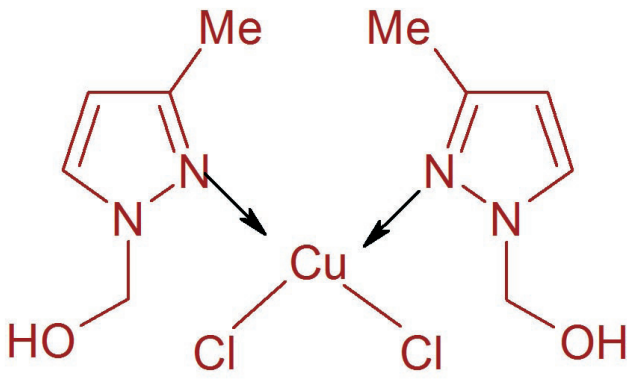

Fig. 1. Copper (II) Cis-dichloro-bis(N1-hydroxymethyl-3-methylpyrazole-κN2).

Spectrophotometric analysis

The studied material comprised blood samples of 4ml collected into vacuum tubes containing anticoagulant (EDTA). The blood was centrifuged for 10min at the speed of 3500 revolutions per minute. Plasma was removed and the remaining red cells were rinsed three times with 0.9% NaCl solution, the conditions for centrifugation being stable. Next, after removal of supernatant, 1ml of water and 1ml of mixture (80 µl of complex and 920 µl of blood) were added to the rinsed erythrocytes in ratio 1:1, the whole mixture was later incubated at 37^{0}C for 15min, then hemolysis was performed (1ml of mixture : 1ml of water) and the solution was frozen at -70°C. Solutions prepared in such way were used for later tests.

Spectrophotometric analysis determining the activity of glutathione peroxidase were performed on the previously prepared hemolysate.

Hemoglobin (Hb) [20]

The hemoglobin concentration (Hb) was measured using the Drabkin's method. Following addition of potassium ferrocynide and potassium cynide, hemoglobin transforms into cyanmethemoglobin, which has maximal absorbance at 540 nm. 20µl of the sample blood were added do 5ml of Drabkin solution (1:251), mixing thoroughly. Then, the solution was kept at room temperature for 30min. After that, the absorb-

ance of the tested and reference sample was measured against the null sample at λ=540 nm.

Glutathione peroxidase (GPX) activity in red blood cells [20]

As a substrate for the enzyme, organic cumene hydroxide was used. The control and test samples were placed into centrifugal tubes, adding 0.1ml of 50-fold diluted hemolysate and 0.7ml of 50mM Tris - HCl buffer solution with pH=7.6. The whole material was incubated at 37*C for 10min, then 0.1ml of reduced glutathione in buffer solution was added to control solutions, while 0.1ml of reduced glutathione and 0.1ml of 0.05% cumene in Tris - HCl buffer were added to test sample. Both specimens were placed in water bath at 37*C. After that, the samples were cooled down to room temperature and 1.0ml of aqueous 20% ACA solution was added, while 0.1ml of 0.05% cumene solution in Tris - HCl was added to control samples. In the next stage, the specimens were centrifuged for 10min at 1400xG. Following centrifugation, the solution was added to 1.0ml of supernatant containing reduced glutathione unused in reduction of cumene by the enzyme, which contained 2.0ml 0.4M Tris - HCl buffer with pH = 10.0 and 0.1ml of DTNB(5,5'-dithiobis- (2-nitrobenzoic acid) alcohol solution. The samples prepared in such way were analyzed, measuring the absorbance with respect to the control at the wavelength of 412nm. The activity of the enzyme was given in U/gHb.

RESULTS:

Fig. 2 It shows the dependence of the control group 1 and test 2 with the complex compound of copper (II) ($p < 0.001$).

Fig. 3 It shows GPX activity in the control group 1 tested on the pyrazole complex as compared to the control group 2 test without use of a compound of copper (II). Cancer patients had lower average GPX activity - test group 1 and the pyrazole complex was 71.77 U / gHb compared to the group without the test compound 2 -90 U / gHb. GPX activity in the control group 1 of the pyrazole complex amounted to 69.8 U / gHb, while the other group without the compound was 101 U / gHb. The results presented are the average activity in patients with HNSCC after application of the complex Cu (II) - (cis-dichlorobis (N1-hydroxymethyl-3-metylopirazol- κN2) copper (II) compared to healthy subjects. Glutathione peroxidase activity was expressed in units of enzyme [U / g Hb]. Significant changes occurred for statistical GPX -1 in the control group and the study-2 complex pyrazole Cu (II) ($p < 0.001$). the presented data show that in the study group patients HNSCC GPX activity is increased (the experiment group-2 without complex)

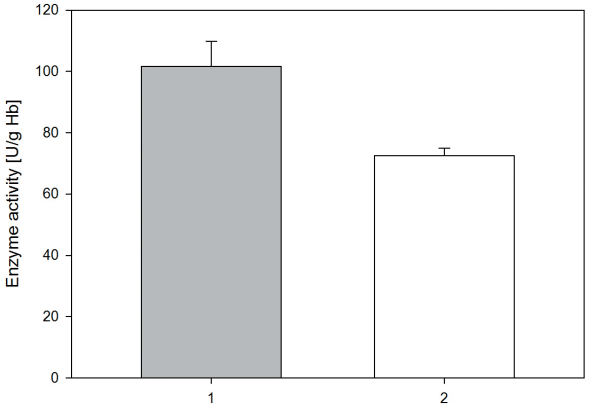

Fig. 2. Glutathione peroxidase activity in the control group 1 and test 2 with a compound ($p < 0.001$)

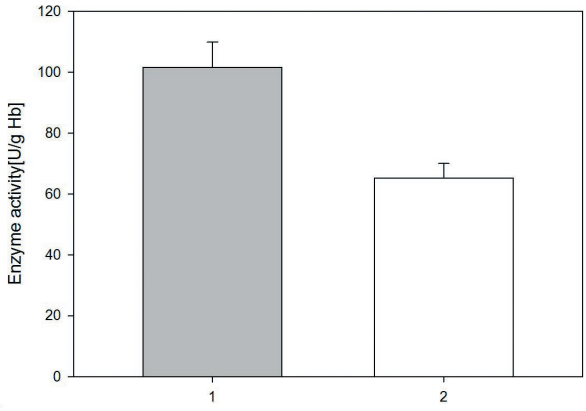

Fig. 3. The activity of glutathione peroxidase in the control group with the test compound 1-, 2-control test without the complex of copper (II)

as compared to those of the same group using a complex pyrazole Cu (II). in the control group, the enzyme activity of GPX is increased (after the addition of the synthesized complex compound) compared to controls without complex pyrazole. In summary, we can conclude that the complex compound containing copper ions (II) exhibits antioxidant properties..

DISCUSSION

One of the most fascinating topics in medicine is current research aiming to determine the pro- and antioxidant activity of new coordinate compounds potentially used as drugs to fight against causes of diseases. Free radicals are, in turn, responsible for development of various diseases, including cardiovascular disorders, stroke, osteoarthritis, neurological disorders and neoplasms. They are considered the main cause of ageing. However, the role of free radicals in development of cancer is of upmost importance. There are three main sources

of free radicals: metabolism and immunological system, environment and other free radicals [21,22]. The main objective of the study was to assess possible application of antioxidant properties of a Cu(II) complex compound in prevention of cancer. Firstly, the compound was synthesized, called copper (II) *Cis*-dichloro-bis(N^1-hydroxymethyl-3-methylpyrazole-κN^2). Next, its potential antioxidant properties were determined by measuring antioxidant activity of GPX in both patient group (1 and 2) and control group (1 and 2) after addition of bivalent metal coordination compound. The pyrazole particle is present in many biologically active compounds, which play an important role in the flow of genetic information. It is a part of purine structure present in DNA and possesses potential coordination sites for platin drugs. Based on the literature data, it can be concluded that such chemicals may have a significant affinity to DNA biding sites. An important argument for synthesizing a Cu(II) complex was that copper (II) complexes can easily be formed [19-24]. They possess very stable N-Cu bonds; the ligands are classified as non-leaving groups [23]. Coordination complexes reach the DNA strand without changing their configuration, which is crucial as they make 'a long way' between the site of administration and the nucleus. From our study, it can be concluded that the Cu(II) complexes we synthesized can show strong anticancer effect. Pyrazoles are compounds that have a broad spectrum of pharmacological activity, including analgesic, spasmolytic, antimicrobial, anti-inflammatory and anticancer effect [23]. In recent years, there has been a number of reports considering synthesis and antioxidant effect of pyrazole compounds containing transition metal ions [23]. In the study in 2013, Malinowska et al. reported significant statistical differences for glutathione peroxidase activity in control group 3 and tested group 2 without using the complex compound and using the product ($p<0.001$) – copper (II) deacon-tetra(N^1,3-triazol,κN^2).The data suggest that in the tested group, which included people with polyps and inflammation, the activity of GPX enzyme increased (tested group 2 without the compound) compared to people from the same group while using the Cu(II) complex. In control group, however, after addition of the complex, a significant rise in the enzyme activity was observed compared to the control group without the complex. Summarizing the studies by the previously mentioned authors, it can be stated that a complex compound containing a transition metal ion shows antioxidant properties [23]. The combination of biological activity, including antioxidant properties of pyrazole complex ligands, and anticancer effect can lead to development of chemicals that show synergic properties. In our research, we concentrate on synthesis of compounds containing pyrazole derivatives and chloromethyl and hydroxymethyl substituents, which will allow to determine the relationship between structure and activity, as well as between structure and toxicity.

CONCLUSIONS

The study proves that the complex compound – copper (II) *Cis*-dichloro-bis N^1-hydroxymethyl-3-methylpyrazole- κN^2) – has an effect on the antioxidant GPX enzyme.

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