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Evaluation of oxidant-antioxidant balance and DNA damage in blood of patients with cancer of the head and neck under the influence of copper(II) complex: Preliminary studies

Równowaga oksydacyjno-antyoksydacyjna oraz uszkodzenia DNA oceniane we krwi pacjentów z rakiem głowy i szyi poddanej działaniu kompleksu miedzi(II) – badania wstępne

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

Katarzyna Malinowska[1,A,B,E], Alina Morawiec-Sztandera[2,B,D], Małgorzata Majczyk[2,B,D], Dariusz Kaczmarczyk[3,B,D], Anna Merecz-Sadowska[4,E,F], Radosław Zajdel[4,C], Hanna Zielinska-Blizniewska[1,A,G]

[1]Department of Allergology and Respiratory Rehabilitation, 2nd Chair of Otolaryngology, Medical University of Lodz, Lodz, Poland
[2]Department of Head and Neck Neoplasms Surgery, Medical University of Lodz, Lodz, Poland
[3]Department of Human Physiology, Medical University of Lodz, Lodz, Poland
[4]Department of Economic Informatics, University of Lodz, Lodz, Poland

Summary

Introduction: The primary aim of this research was to evaluate the oxidative stress markers and the level of oxidative DNA damage in the pathogenesis of head and neck cancer.

Materials/Methods: Sixty-two subjects matched for age and gender, including 31 patients with head and neck cancer and 31 control patients without cancer symptoms, were enrolled in our study. In our work, the activity of antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as a total antioxidant status (TAS), were estimated. Additionally, an alkaline comet assay was used to measure the level of DNA damage in the group of patients with head and neck cancer and the group of healthy control patients. These tests were performed on a blood sample with and without prior incubation of dinitratebis (1-phenyl-5-(2-hydroxyphenyl)-3-methyl-N1pyrazol-κN2)cooper(II).

Results: Significant increases of SOD, GPX CAT, TAS ($P < 0.001$) were seen in blood from patients with head and neck cancer and prior incubation of cooper (II) component compared to blood from healthy controls without prior incubation of analyzed chemical. Moreover, we did not observe any relationship between the level of DNA damage and the studied component dinitratebis (1-phenyl-5-(2-hydroxyphenyl)-3-methyl-N1pyrazol-κN2)cooper(II) in the group of patients with head and neck cancer or in healthy controls.

Discussion: Free radicals such as reactive oxygen species, which induce oxidative stress, may contribute to head and neck carcinogenesis. Therefore, we suggest that modulation of pro-oxidant /antioxidant status might be a relevant target for both prevention and therapy.

Keywords: CAT, SOD, GPX, head and neck cancer, copper complex

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Author's address: dr n. farm. Katarzyna Malinowska, Department of Allergology and Respiratory Rehabilitation, 2nd Chair of Otolaryngology, Medical University of Lodz, ul. Żeligowskiego 7/9, 90-725 Łódź; e-mail: katarzyna.malinowska@umed.lodz.pl

INTRODUCTION

Squamous cell carcinoma is a type of a cancer arising from the non-keratizing stratified epithelium. It represents a great majority of the neoplasms of the upper respiratory and digestive tract. This group of neoplasms is more commonly known as head and neck squamous cell carcinoma (HNSCC) and it represents 90% of all head and neck neoplasms. The remaining 10% of head and neck neoplasms are adenocarcinomas, lymphatic malignancies and tumors of the salivary glands [1, 32, 33]. HNSCC is one of the most burdensome types of neoplasms for suffering patients. The deterioration of the upper aero-digestive tract that results from HNSCC has a negative influence on the psychological and the sociological sphere of life. Despite displaying various clinical courses, HNSCCs share many common features. Patients affected by cancer of the head and neck region have a 12–35% risk of developing a second form of cancer, unrelated to the primary tumor in this location. Patients suffering from HNSCC are the sixth most commonly diagnosed group of patients with newly diagnosed neoplastic diseases [17, 37, 41].

Statistical data shows that worldwide more than 650.000 new cases of HNSCCs are diagnosed and 300.000 deaths are reported, annually. The regions with the highest cancer incidence are South and Southeast Asia [14]. The incidence of head and neck cancer is five times higher among male, smokers and alcohol drinking individuals. Moreover, affected patients are most commonly 50–70 years old [8, 20, 21, 29, 48].

Epithelial tumors of the head and neck region constitute between 5.5 to 6.2% of all malignant tumors registered in Poland, including about 8% men and about 2% women, which is approx. 5.500 to 6.000 new cases per year according to National Cancer Registry. Moreover, in Poland, head and neck neoplasms arise mainly in the larynx. Less common localizations are the tongue and the tonsils, rarely the pharynx, the nose and the paranasal sinuses [9, 39, 44]. Copper(II) plays an important biological role in all living cells. Cu(II) complexes with organic ligands are used as analgesics, antipyretics, anti-inflammatory, agents, and anti-platelet aggregation compounds. Because of the redox properties and interaction between Cu(II)/Cu(I), copper complexes with complexes of copper O2 biomimetic. Ions were biologically interesting ligand studied in detail [11].

The aim of the study was to analyze the antioxidant properties of Cu(II) coordination compound in head and neck tumors and their impact on DNA. These coordination compounds play a significant role in removing and reducing the creation of free radicals, which defends the body from the negative effects of their actions. These coordination compounds also aid the antioxidant system, which includes among other antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), peroxidase glutathione (GPx), low molecular weight antioxidants like reduced glutathione (GSH), vitamins A and E, melatonin, and many other compounds. Metal elements do not naturally occur in living organisms; however, their complex compounds are widely used for medical as well as biological purposes [16, 18, 24]. The specific objective of our project is to investigate the antioxidant enzymes: SOD, CAT, GPx. Additionally, an alkaline comet assay was used to determine the extent of blood lymphocyte DNA damage under the influence of copper(II). The study also tested TAS in the plasma of patients.

MATERIALS AND METHODS

Material

Patients

Sixty-two patients with head and neck cancer (group A), 40 men and 22 women, mean age 51.8 ±11.1 years were enrolled in this study. The study group was subdivided into subgroups A1 (n = 31) and A2 (n = 31). The control group (B) consisted of sixty-two subjects, 32 men and 30 women, mean age 62.1 ±17.7 years, with no symptoms of head and neck cancer. The control was also divided into subgroups B1 (n = 31) and B2 (n = 31). Blood from A1 and B1 group were incubated with dinitratebis (1-phenyl-5--(2-hydroxyphenyl)-3-methyl-N1pyrazol-κN2)cooper(II), group A2 and B2 were not subjected to prior incubation.

All patients from both groups were hospitalized in the Head and Neck Tumors Surgical Ward of the Second Otolaryngology Department of the Medical University in Lodz. Patients in

subgroups A1 and A2 of the study were required to have histopathologically diagnosed head and neck cancer and no family history within relatives of the first and second degree. The control group was recruited from patients without head and neck cancer syndromes and other forms of cancer. All patients and control subjects were Caucasian. The study was conducted based on permission from the Bioethics Committee (No. RNN/142/09/KB). Blood samples were collected in 5 ml EDTA tubes. Before collection, each patient was informed of the whole procedure and the aim of the project. All the patients and controls were age and sex matched (no difference calculated, P > 0.05).

Methods

Synthesis of complex 1C

Copper(II) nitrate Cu(NO3)2·H2O (22.5 mg, 0.93 mmol) was dissolved in 2 ml ethanol and was added dropwise at room temperature to a stirred solution of ligand 1 B 1-phenyl-5- -(2-hydroxyphenyl)-3-methylpyrazole (44.3 mg, 0.18 mmol) in ethyl acetate (10 ml). The reaction solution was stirred for 48 hours at room temperature. The resulting green solid 1C was obtained by the diffusion of diethyl ether into the mixture. Yield: 28.9 mg, mp 257.5–263.0°C. Anal. Calcd. for 1C C30H28N6O8Cu·3H2O (718.1 g/mol) C, 66.84%; H, 4.73%; N, 11.69%; Cu 8.84%. Found: C, 66.18%; H, 4.15%; N, 11.13%; Cu, 9.25%. FTIR (KBr, cm–1): ν(O–H) 3286; ν(C–CH3) 3089; ν(C N) 1545, 1618.

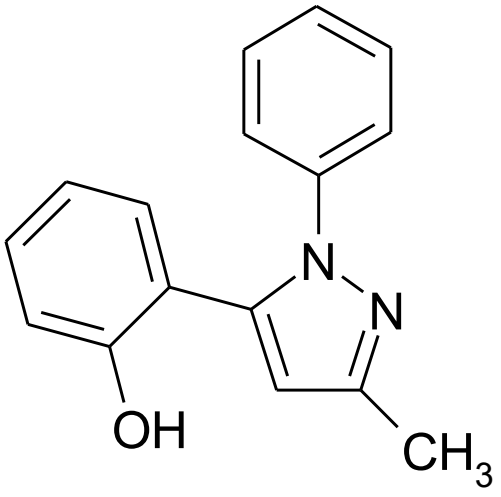

Fig. 1. Ligand 1B; 1-phenyl-5-(2-hydroxyphenyl)-3-methylpyrazole

Blood sample preparation

Peripheral blood lymphocytes from healthy donors and HNSCC patients were isolated by centrifugation (15 min, 280xg) in a density gradient in histopaque-1077 (Sigma, Saint Louis, MO, USA). Viability of lymphocytes was determined by use of trypan blue exclusion and was >97%. The suspension, at a density of 1×106 cells/ml of PBS, was prepared for the comet assay. Erythrocytes were separated from blood plasma by centrifugation (10 min, 710 g) at 4°C and washed three times with 0.9% NaCl before examination.

Comet assay

DNA damage, such as single and double strand breaks and alkali labile sites, was measured by the single-cell electrophoresis method [23, 43]. In this case, the comet assay was performed under alkaline conditions. To assess the level of DNA damage, isolated lymphocytes from patients with head and neck cancer as well healthy control individuals were exposed to different concentrations (20, 30, 40 and 50 μM) of 1C – 30 min at 37°C (longer incubation did not change the level of DNA damage). The control lymphocytes were incubated in-free RPMI1640 medium (Sigma, Saint Louis, MO, USA). After exposure to 1C, the cells were centrifuged and washed twice by PBS. A suspension of cells in 0.75% Low Melting Point (LMP) agarose (Sigma, Saint Louis, MO, USA) dissolved in PBS was spread onto microscope slides (Superior Marienfeld, Lauda-Königshofen, Germany) precoated with 0.5% normal-melting agarose (Sigma, Saint Louis, MO, USA).

The cells were then lysed overnight at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After lysis, the slides were placed in running buffer (300 mM NaOH, 1 mM EDTA, pH > 13) for 20 minutes at 4°C, then electrophoresis was conducted at 4°C (the temperature of the running buffer did not exceed 12°C) for 20 min at the electric field strength of 0.73 V/cm (28mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 μg/ml DAPI, and covered with cover slips. To prevent additional DNA damage, all the steps described previously were conducted under dimmed light or in the dark.

The objects were observed at 200× magnification in an Eclipse fluorescence microscope (Olympus OVK-S5-022-FL attached to video camera) equipped with a UV-1 filter block (an excitation filter of 359 nm and a barrier filter of 461 nm). To imagine analyses, CaspLab Comet Assay Software was used. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells, and the mean percentage of DNA in the comet tail (Tail DNA %) was calculated. This quantity is positively correlated with the level of DNA breakage in a cell and was taken as an index of DNA damage in each sample.

Spectrophotometric analysis

The analyzed material was comprised of 4 mL blood samples collected in vacuum tubes containing anticoagulant (EDTA). Part of the blood samples were subjected to prior incubation with 1C (A1 and B1), the other part were not (A2 and B2). The blood was centrifuged for 10 minutes at 3.500 revolutions per minute. Plasma was removed, and the remaining red cells were rinsed three times with 0.9% NaCl solution. The conditions for centrifugation were stable. Next, after removing the supernatant, 1ml of water and

1ml of mixture (80 µl of 1Ccomplex and 920 µl of blood) were added to the rinsed erythrocytes in a 1:1 ratio, the whole mixture was later incubated at 37°C for 15 min, then hemolysis was performed (1ml of mixture: 1 ml of water) and the solution was frozen at –7°C. These prepared solutions were used for later tests.

Hemoglobin assay

Hemoglobin (Hb) concentration in erythrocyte hemolysates was estimated at 540 nm using a spectrometer (UV/VIS Spectrometer Lambda 14P, Perkin Elmer, USA) after conversion into cyanmethemoglobin with Drabkin reagent (Aqua-Med, Poland). Hb concentration was needed to calculate the activity of antioxidant enzymes [47].

Catalase activity

Catalase activity in erythrocytes was determined according to spectrophotometric procedure by Beers and Sizer [2] and calculated as Bergmeyer units (BU/g Hb). CAT activity was measured at 25°C by recording H2O2 decomposition at 240 nm with a spectrometer (UV/VIS Spectrometer Lambda 14P, Perkin Elmer, USA). One Bergmeyer unit (BU) of this activity is defined as the amount of enzyme decomposing 1 g of H2O2 per min.

Glutathione peroxidase activity

Organic cumene hydroxide was used as a substrate for the enzyme. The control and test samples were placed into centrifuge tubes, adding 0.1 ml of 50-fold diluted hemolysate and 0.7 ml of 50 mM Tris – HCl buffer solution with pH = 7.6. The whole solution was incubated at 37°C for 10 minutes, then 0.1 ml of reduced glutathione in buffer solution was added to control solutions, while 0.1 ml of reduced glutathione and 0.1 ml of 0.05% cumene in Tris – HCl buffer were added to the test sample. Both specimens were placed in a water bath at 37°C. After that, the samples were cooled down to room temperature and 1.0 ml of aqueous 20% ACA solution was added, while 0.1 ml of 0.05% cumene solution in Tris – HCl were added to control samples. In the next stage, the specimens were centrifuged for 10 minutes at 1400xg. Following centrifugation, the solution was added to 1.0 ml of supernatant containing reduced glutathione unused in reduction of cumene by the enzyme, which contained 2.0 ml 0.4M Tris – HCl buffer with pH = 10.0 and 0.1 ml of 1C alcohol solution. These samples were analyzed, measuring the absorbance with respect to the control at a wavelength of 412 nm. The activity of the enzyme was given in U/gHb [28].

Superoxide dismutase activity

Superoxide dismutase activity in erythrocytes was measured according to a procedure by Misra and Fridovich [34] and is expressed in adrenaline units [U/g Hb/100 ml]. SOD activity was determined at 37°C by an increase in the absorbance at 480 nm with a spectrometer (UV/VIS Spectrometer Lambda 14P, Perkin Elmer, USA) followed by the auto-oxidation of adrenaline inhibited by CuZn-SOD. One unit of SOD activity is defined as the amount of enzyme inhibiting the adrenaline auto-oxidation at 50%.

Total antioxidant status determination

Determination of total antioxidant status in blood plasma was performed via the spectrophotometric method of procedure no. NX2332 by Randox (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co Antrim, United Kingdom, BT29 4QY). In brief, ABTS (2,2'-Azino-di-[3ethylbenzthiazoline sulphonate]) was incubated with peroxide (metmyoglobin) and H2O2 to produce the radical cation ABTS with a relatively stable blue-green colour. Antioxidants, when added in examined sample, suppressed this colour production, measured as a decrease of absorbance with a spectrometer (UV/VIS Spectrometer Lambda 14P, Perkin Elmer, USA) at 600 nm. The total antioxidant status was calculated as concentration of antioxidants [mmol/l].

Statistical analysis

The data in this study were analysed for three separate experiments from each analysed patient or control sample. Enzyme activity status and total antioxidant status were expressed as mean value ± SD. Blinded replicate samples were used for quality control (QC). If no significant differences between variations were found, as assessed by the Snedecor-Fisher test, the differences between means were evaluated by applying the Student's t-test. Otherwise, the Cochran-Cox test was used. The values from the comet assay were expressed as a mean percentage of DNA damage ±SEM (standard error of the mean). An ANOVA was performed and, where positive, a t-test was used to compare each dose level to the appropriate control. The statistical significance was defined as $p < 0.05$. The data was analyzed using the STATISTICA (StatSoft, Tulsa, OK) statistical package.

RESULTS

Tables 1 and 2 show an antioxidant status in head and neck cancer patients (group A) and healthy subjects (group B) measured as activity of CAT, SOD, GPx as well as TAS. Our data indicates that the addition of Cu(II) compounds (group A1 and B1) leads to statistically significant increases in enzyme activities in comparison to samples without prior incubation of analyzed chemical (group A2 and B2).

Table 1, 2 shows the mean activity of CAT as Bergmeyer units [BU/g Hb], SOD as adrenaline units [U/g Hb/100ml], GPX as enzymatic units [U/g Hb], and TAS, calculated as concentration of antioxidants [mmol/l].

SOD levels increased in the A1 (2533.84 ± 807.31) study group after addition of 1C complex and control group B1 (2606.51 ±471.62) compared to A2 (1276.73 ±563.80) and B2 (1997.42 ±842.78) without Cu(II) complex. Significant results were obtained for groups A1 and A2, $P < 0.001$ and for

Table 1. Effects of the complex Cu (II) on the activity of CAT, SOD, GPX in HNSCC with (A1) and without (A2) exposure to 1C complex

Patient group (A) N = 62			
Enzymes	Study group exposure to 1C (A1) (N = 31)	*Study group does not have exposure to 1C (A2) (N = 31)*	p-value
CAT [BU/g Hb]	16.12 ±7.76	11.88 ±4.02	0.009
ZnCu SOD [U/gHb/100ml]	2533.84 ±807.31	1276.73 ±563.80	<0.001
GPx [U/g Hb]	64.31 ±15.15	42.86 ±27.40	<0.001
TAS	1.49 ±0.33	1.11 ±0.21	<0.001

Table 2. Effects of the complex Cu (II) on the activity of CAT, SOD, GPX controls (B1) and (HNSCC) compared to group (B2)

Control group (B) N = 62			
Enzymes	Study group exposure to 1C (B1) (N = 31)	*Study group does not have exposure to 1C (B2) (N = 31)*	p-value
CAT [BU/g Hb]	20.65 ±6.10	12.64 ±3.41	0.009
ZnCu SOD [U/gHb/100ml]	2606.51 ±471.62	1997.42 ±842.78	<0.001
GPx [U/g Hb]	66.91 ±14.19	60.84 ±14.21	0.098
TAS	1.47 ±0.31	1.44 ±0.38	0.737

Table 3. Relationships between patient groups and controls regarding smoking, alcohol consumption and gender

Variable	**Patients (%)**	**Control (%)**	**OR (95%CI)**	**p – value**
Tobacco consumption				
Yes	36 (58.06)	8 (12.90)	Reference	
No	26 (41.94)	54 (87.10)	9.35 (3.81–22.93) ↑	<0001
Alcohol consumption				
Yes	24 (38.70)	18 (29.03)	Reference	
No	38 (61.30)	44 (70.97)	1.54 (0.73–3.27)	0.34
Gender				
Male	40 (64.51)	32 (51.61)	Reference	
Female	22 (35.49)	30 (48.39)	1.70 (0.83–3.50)	0.20

groups B1 and B2, P <0.001. Furthermore, the results among the groups A2 and B2, without the use of a copper(II) were statistically significant; P <0.001.

Tables 1 and 2 show the mean activity of the enzyme group A1 (64.31 ±15.15) as well as the control group B1 (66.91 ±14.19) after use of the complex compound compared to the activity of the GPX group A2 (42.86 ±27.40) and B2 (60.84 ± 14.21). Statistically significant results were obtained for A1 and A2 groups; P <.001 and for A2 and B2 groups; P <0.002.

Tables 1 and 2 also show an average activity of the enzyme CAT in groups A1 (16.12 ±7.76), B1 (20.65 ±6.10), enzyme activity increased in comparison with reference group A2 (11.88 ±4.02) and B2 (12.64 ±3.41). For CAT enzymes, statistically significant results were obtained for A1 compared toA2; P = 0.009, B1 compared to B2; P = 0.009 and for A1 compared to B1; P = 0.013.

The total antioxidant status was significantly higher in patients with head and neck cancer A1 (1.49 ±0.33) with the use of the Cu(II) complex than in A2 (1.11 ±0.21); P <.001, and in control group B1 (1.47 ±0.31) in reference to B2 (1.44 ±0.38). Statistically significant results were obtained for groups B1 and B2; P <0.001.

Relationships between patient groups and controls regarding smoking, alcohol consumption and gender are summarized in Table 3. The study reported that the prevalence of cigarette smoking among patients with HNSCC was higher than in control (P<0001).

Figs. 2 and 3 show the level of DNA damage after incubation with dinitratebis(1-phenyl-5-(2-hydroxyphenyl)-3--methyl-N1pyrazol-κN2)cooper(II) in 20, 30, 40, and 50 μM concentrations, for 30 minutes in 37°C. The results from our studies have not shown statistically significant changes in the level of DNA damage in groups of patients with head and neck cancer or in healthy control patients in each tested concentration.

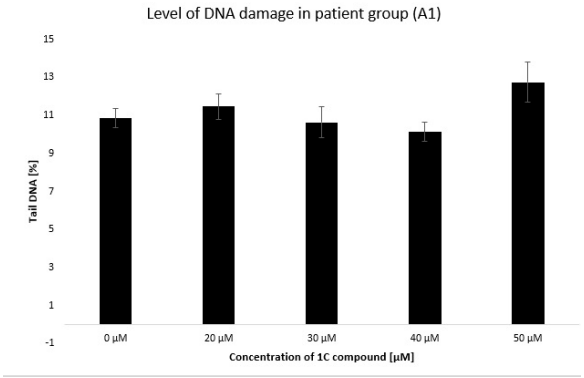

Fig. 2. The level of DNA damage after incubation with different concentration of dinitratebis (1-phenyl-5-(2-hydroxyphenyl)-3-methyl-N1pyrazol-κN2) cooper(II) in group of patients with head and neck cancer (A1)

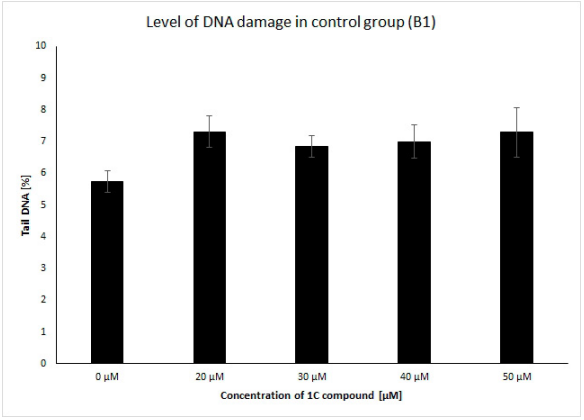

Fig. 3. The level of DNA damage after incubation with different concentration of dinitratebis(1-phenyl-5-(2-hydroxyphenyl)-3-methyl-N1pyrazol-κN2) cooper(II) in control group (B1)

DISCUSSION

The year-on-year increase in cancer incidence makes neoplastic diseases one of the most important problems that molecular biology must deal with in the beginning of the twenty-first century. Despite advancements in treatment options, laboratories all over the world are continuously running experiments that seek to discover mechanisms responsible for the formation and development of cancer. In order to develop more effective diagnostic and therapeutic techniques, it is essential to understand the molecular basics of malignant transformation.

Antioxidants are chemical compounds that exhibit protective effects and suppress oxidation reactions, successfully neutralizing the formation of free radicals, which can damage our genetic material. If they are regularly provided to our bodies, they support our health and lower the risk of developing diseases of affluence, including cancer [31]. Head and neck cancer is a major problem and its prevalence has increased significantly in the past few years. Research on the etiopathogenesis of these neoplasms has existed for years; however, it was not until the second half of the past century that scientists became drawn to the inflammatory theory of their origin [3]. There are many various risk factors for the development of head and neck cancers, including carcinogens contained in tobacco smoke, high-proof alcohol, improper diet, and insufficient oral hygiene [45]. In addition to exogenous factors, oncogenic viruses, such as human papilloma virus and Epstein-Barr virus, should be considered. These factors are often accompanied by an excessive production of reactive oxygen and nitrogen species. One of the aftereffects of the overproduction of free radicals is the lipid peroxidation process, resulting in disintegration of polyunsaturated fat. Aldehydes, ketones and hydrocarbons are the products of this process [36]. According to Word Health Organization (WHO), smoking kills more than 8 million people every year [49]. Smokers inhale reactive oxygen species (ROS) present in tobacco

smoke and tar. ROS activity has mutagenic effects, which may result in oxidative damage of biomolecules, such as proteins, DNA and lipids [19, 20, 46]. Drawing on literature data, we conclude that smoking affects developing extrapulmonary malignant neoplasms, including head and neck cancers [49].

The literature describes indicators of prooxidative-antioxidative balance in patients with various diseases, including ulcerative colitis, esophageal cancer, and laryngeal cancer [7, 10, 38]. In this study, the advantage of oxidative reactions over antioxidative reactions and protecting organisms from damaging influence of free radicals was observed. These results prove objectives concerning the intensity of lipid peroxidation process. Under conditions of oxidative stress, which degrade antioxidative protections, cells are not able to keep up with repairing damaged and modified protein and lipid structures. This results in higher occurrence of DNA mutation as well as increased permeability of cell membranes and decreased biological activity of many proteins. These changes lead to the development and multiplication of genetically unstable cells, which are characterized by a partial or complete lack of sensitivity to signals that induce programmed cell death via apoptosis. The cause of increased mitochondrial DNA mutations in head and neck cancer is explained by research that shows mitochondrial DNA, which in contrast to nuclear DNA, is not protected by histones and, thus, has weaker repair systems; mitochondrial DNA repair consists of excision of mismatched bases [5].

HNSCC is strongly associated with tobacco use. The risk for that type of cancer in smokers is approximately 10 times higher than that of never-smokers, additionally 70–80% of new HNSCC diagnoses are associated with tobacco and alcohol use. The number of patients who are active smokers included in this study are statistically significantly larger than controls [45].

ROS are incapacitated by an enzymatic antioxidative barrier, the first line of defense, consisting of the enzymes CAT, GPx and SOD. SOD, which is found in the cellular cytoplasm, catalyzes the dismutation reaction of a superoxide anion radical to hydrogen dioxide and molecular oxygen. Next, it is reduced by CAT and GPx. Based on literature data, cancerous cells in patients with head and neck cancer exhibit decreased activity of antioxidative enzymes compared to control groups. Gokul et al. [12] describe disorders in pro/antioxidative balance in patients with squamous cell carcinomas of the oral cavity. The authors found free-radical reactions to outweigh antioxidative reactions. Decreased antioxidative protection was shown via assessment of superoxide dismutase and catalase activity. The study also stated that an imbalance between pro/antioxidative reactions is acknowledged as one of the factors responsible for pathogenesis of cancer in patients. Elsewhere, increased concentrations of reactive oxygen and nitrogen species were found to result in a decreased presence of components of the antioxidative barrier: total antioxidative status and glutathione concentration were reduced by excessive generation of free radicals in patients with head and neck cancer [26]. Patel et al. [35] compared the concentration of nitrogen oxide and the activity of SOD and CAT in patients with erythroplakia, patients with preinvasive cancer, patients with invasive cancer, and healthy individuals with or without tobacco habits. The level of nitrogen oxide was higher in both stages of carcinogenesis than healthy patients. The lowest erythrocyte SOD and CAT activity was measured in patients with advanced cancer, particularly those with carcinoma of the oral cavity. Interestingly, smokers and patients with erythroplakia had elevated erythrocyte SOD and CAT activity regarding compared to control groups without tobacco habits. Gurudath et al. [15] found a statistically significant decrease in SOD and GPx activity in patients with two different pre-cancerous conditions of the oral cavity, that is, oral submucous fibrosis and leukoplakia, and in patients with invasive cancer, compared to healthy individuals.

A significant and fascinating area of modern medical research is centered on determining the pro/antioxidative activity of new complex compounds with copper(II) ions, which have the potential to be used as drugs for the fight against agents causing cancer [6, 13, 40]. These copper compounds occur naturally in living organisms, and their synthetic complexes have been applied in medicine and biology.

Azoles are heterocyclic chemical compounds that contain nitrogen atoms. The diazole derivatives have antioxidative properties that provide protection from oxidative stress. Research by Malinowska et al. [31] shows the influence of a copper(II) diaka-tetra (N1,3-triazol,κN2) complex and its antioxidative potential on CAT, GPx, and SOD activity in patients with nasal polyps. This study showed decreased CAT, SOD, and GPx activity in patients group A2, compared to the group A1. It was also shown that enzyme activity increases in control group B1, compared to group B2. This research demonstrated that the copper(II) diaka-tetra (N1,3-triazol-κN2) complex has an impact on antioxidative enzyme activity. Kupcewicz et al. [27] analyzed a number of pyrazole complex compounds and determined their electrochemical and antioxidative properties, as well as toxicity. The authors stated that these compounds, inter alia, lower the concentration of ROS in cancer cells.

The results of total antioxidative status (TAS) confirmed that pyrazole coordination compounds with copper(II) ions have a substantial impact on increasing its level. Kubiak et al. [25] obtained valuable results in their study of the following complexes: 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), and dichloro di(1,3,4,5-tetramethyl-N1-pyrazole-κN2) copper(II) in patients with colorectal cancer. Patients with cancer have statistically significant decreases in SOD and CAT activity compared to the control group ($P < 0.05$). It was observed that the aforementioned compounds considerably increase antioxidative activity of CAT and SOD in patients with colorectal cancer.

Our results confirm those of Malinowska et al. [30]. In a study group of patients with HNSCC, the activity of GPx increases in the study group without complex, compared to patients from the same group after (Cis-dichloro-bis(N1-hydroxymethyl-3-methylpyrazol-κN2) copper(II) complex administration. In the control group, enzyme activity increases after newly synthesized complex compound administration) compared to the control group without the pyrazole complex. Our findings confirm that a significant increase of enzymatic activity takes place in both groups, i.e. patient group A1 and control group B1, compared to the activity of groups A2 and B2 ($P < 0.001$); (Tables 1 and 2).

These features are confirmed by the results of the total antioxidative status for patient group treated with the compound and for the control group with 1-phenyl-5-(2--hydroxyphenyl)-3-methylpyrazole with copper(II). To conclude, we can state that complex compounds with copper(II) ions may have antioxidative properties. The findings show that head and neck cancer cells are characterized by highly disturbed oxidative processes, which suggests pro/antioxidative mechanisms play a major role in HNSCC cancerogenesis [22, 42].

Our study examines also the relationship between studied component and the level of DNA damage. It seems that the use of the organic compounds, which would have the function of the carriers, should minimize the potential toxic effect. The possibility of reducing drug toxicity is the main reason for choosing the derivatives of the compounds from the diazole group.

CONCLUSIONS

Our data shows that the presented cooper(II) complex may increase the level of antioxidant enzymes and probably does not introduce additional lesions to DNA structure, but further studies are needed to confirm these results. We believe that our component may present with activity against cisplatin-resistant neoplastic cells. We took an analysis of influence of the copper complex on DNA structure and activity of antioxidant enzymes. We believe that our future studies will help answer the question whether and how metal – ligand complex may help in the inhibition process of carcinogenesis.

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