BIOLOGICAL PROPERTIES OF BENZOPYRAN-BASED PLATINUM (II) COMPLEXES

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The aim of the study was to analyze the physicochemical synthesized complex 3 [(1,3- thiazol -2-ylimino) methyl]-4H- chromene -4 -one with tetrachloroplatinate(II) dipotassium and determination peroxidase activity and glutathione (GPX) in red blood cells of cancer patients and healthy subjects.

Materials and methods. Tests were carried out with the approval of the Bioethics Committee No. RNN/260/08/KB. Blood was collected into tubes with anticoagulant (heparin lithium). Determination of glutathione peroxidase activity was performed by methods of Little and O’Brien in 20 person groups hospitalized at the Department of General and Colorectal Surgery Veterans General Hospital in Łódź.

Results. The study was an increase of activity in the control without the compound and after the introduction of the complex relative to the treatment groups. In healthy subjects, without the use of glutathione peroxidase complex averaged 73.25 ± 23.88 U / g Hb after application of the compound corresponds to the reference group 81.01 ± 25.94 U / g Hb. In contrast, in patients without the use of the complex activity amounted to 42.85 ± 27.49 U / g Hb. In the study group, which uses synthesized complex GPX activity corresponds to 67.72 ± 13.44 U / g Hb.

Conclusions. The obtained results underline that the introduction of significant blood antioxidant complex research has a significant impact on the results of the determinations. Statistically significant (p < 0.05) difference occurred in both test and no relation to the administration of the complex in relation to the control of 1. 2.

Key words: flavones, Pt(II) complexes

Reactive oxygen species (ROS) are substances that are present in relatively low concentrations but significantly decrease the oxidation level of other molecules. They form in every living cell of the body during the physiological process of respiration. ROS react with major cell structures and molecules, changing their biological functions. Antioxidants may be classified into physiological (natural) and synthetic compounds. These groups include antioxidant enzymes, preventive antioxidants and free radical scavengers.

The best known antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (1-4). Glutathione peroxidase (GPX) is a metalloenzyme that participates in hydrogen peroxide reduction with the concurrent transformation of reduced glutathione into its oxygenated form. The active site of this enzyme contains four selenium atoms that form part of selenocysteine (5, 6). Human cells contain 4 various forms of glutathione peroxidase. GPX-1 which is present in the cell interior, for example in erythrocytes, has the greatest role in ROS elimination. Any 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 numerous diseases, including cancer and neurodegenerative disorders such as Parkinson’s disease or Alzheimer’s disease (7, 8).

Colorectal cancer is currently the most common gastrointestinal cancer in the world. Incidence has been steadily increasing, which may be partially related to population ageing. This phenomenon can be observed in the United States and Western European countries where the proportion of elderly people is constantly increasing. In these countries, mortality rates associated with intestinal cancer have been decreasing for some time due to early detection and treatment of the disease. In Poland, colorectal cancer is one of the types of cancer with the worst prognosis. The possibility of cure depends primarily on disease progression at diagnosis (9, 10).

Cancer treatment with cytostatics is a difficult process that is not always effective and must include the mechanisms of action, pharmacokinetics and dosage regimens of these drugs. Some chemical compounds have interesting biological properties, such as antioxidant or anticancer properties. This group includes pyrazole, chromone and flavone derivatives which are known for their antioxidant and anticancer properties. These derivatives display a wide spectrum of biological activity and readily form coordination compounds with transient metal ions such as Cu(II), Pt(II). Recent years have seen many trials where complexes of metals with pyrazole and flavone derivatives were obtained. The resulting compounds often displayed excellent biological activity, showing promise of finding potential drugs in the future (11, 12, 13).

MATERIAL AND METHODS

Patients

Studies were conducted on blood samples collected from patients who were divided into 4 groups: control groups 1, 2 and study groups 3, 4. The first and second group comprised patients without neoplastic lesions (20 individuals aged 55.1 ± 10.4 in each group), while the study group was made up of the third and fourth group which comprised patients with diagnosed diseases such as lung cancer, gastric cancer, colorectal cancer and pancreas cancer (20 individuals aged 56 ± 13.9 in each group). The abovementioned patients were hospitalized at the Chest Surgery, General Surgery and Surgical Oncology Clinic of the Military Medical Academy University Teaching Hospital – Central Veterans’ Hospital located on Żeromskiego 113 St. in Łódź. The study material was human blood (4 ml) drawn to evacuated tubes containing lithium heparin as an anticoagulant. Blood was centrifuged at a speed of 3500 RPM for 10 minutes. Plasma was discarded and erythrocyte mass was washed three times with 0.9% NaCl, with the same centrifugation conditions. Subsequently, the supernatant was discarded and 1 ml of glucose plus 1 ml of mixture (containing 80 µl of complex compound and 920 µl of blood at a concentration of 10 micrograms per kg of body weight) were added to the washed erythrocytes at a ratio of 1:1, and the totality was incubated at a temperature of 37°C. A sample was prepared after this period and frozen at a temperature of -70°C. The hemolysate was used for further investigations. The studies had been approved by the Bioethics Committee on 20 May 2008 (No RNN/260/08/KB).

Synthesis of the 3[(1,3-thiazol-2-ylimino)methyl]-4H-chromen-4-one complex containing dipotassiumtetrachloroplatinate(I I) (14)

When the mixed solution of 3[(1,3-thiazol-2-ylimino)methyl]-4H-chromen-4-one (0.13 g, 0.000448 mole) in DMF (5 cm³) was fully dissolved (10 minutes), a solution of 0.1g K₂PtCl₄ dissolved in 2.5 ml water was added at room temperature. The flask was equipped with a pipe containing CaCl₂ and wrapped with aluminium foil to protect from sunlight. The totality was stirred in these conditions for 7 hours. After this period, the solution was allowed to slowly crystallise at a temperature of 4°C.

Haemoglobin (Hb) (15)

Haemoglobin (Hb) concentration in blood hemolysates was determined using Drabkin’s reagent. This parameter was required to determine the activity of the assessed enzyme.
Determination of glutathione peroxidase (GPX) activity in red blood cells (5)

Peroxidase activity was determined using the method established by Little and O'Brien. Organic cumene hydroxide was used as substrate for the studied enzyme. Control and study samples were prepared in spin tubes with 0.1 ml of 50-fold diluted hemolysate and 0.7 ml of 50 mM Tris-HCl buffer (pH=7.6). The totality was incubated for 10 minutes in a water bath at a temperature of 37°C. Subsequently, 0.1 ml of reduced glutathione in buffer solution was added to controls and 0.1 ml reduced glutathione plus 0.1 ml 0.05% cumene solution in Tris-HCl buffer was added to the study samples. The test tubes were once again placed in a water bath at a temperature of 37°C for 5 minutes. Once cooled to room temperature, 1 ml of 20% ACA water solution was added to each sample, and 0.1 ml of 0.05% cumene solution in Tris-HCl to the control samples. The test tubes were later centrifuged for 10 minutes at 1400xG. Following centrifugation, 2 ml of 0.4 M Tris-HCl buffer (pH=10) and 0.1 ml of DTNB alcohol solution were added to 1 ml of the supernatant with reduced glutathione that was not used by the active enzyme in the reduction of cumene. The study samples were measured relative to control samples at the 412 nm wavelength.

RESULTS

In healthy individuals, glutathione peroxidase activity without the use of the complex averaged 73.25 ± 23.88 U/g Hb; when the compound was used, this corresponded to 81.01 ± 25.94 U/g Hb in the reference group. In diseased individuals, enzyme activity without the use of the complex was 42.85 ± 27.49 U/g Hb, respectively. In the study group where the synthesised complex was used, GPX activity corresponded to 67.72 ± 13.44 U/g Hb. In light of these results, the conclusion was drawn that the complex compound, 3[(1,3-thiazol-2-ylimino)methyl]-4H-chromen-4-one with dipotassium untetrachloroplatinate(II) affected enzyme activity and increased activity both in the control group when the compound was administered compared to this group without the complex, and in the study group with the use of the compound compared to the group of diseased individuals without the use of the complex. A statistically significant (p<0.05) difference was observed both in the study group without the compound and following the administration of the complex compared to control groups 1, 2 (fig. 1, 2).

DISCUSSION

Chemical compounds such as chromones, pyrazoles or flavones readily form complexes with transient metal ions (16, 17, 18). One of the primary applications for such derivatives is in medicine where these are used e.g. to treat cancer and circulatory disorders. One example of such drugs is diosmin which acts on blood vessels. Chromono-2(3)-carboxylic acids and their derivatives have antiarrythmic properties. Another derivative of 2-phenylchromone
is highly prevalent in the plant world and displays cardiotonic and hipotensive properties. From the chemical perspective, chromone compounds have been known for their wide spectrum of biochemical activity. They readily form complexes. Scientists from all over the world are eager to experiment with this group of compounds.

There have been reports of studies whereby complexes of metals with flavone derivatives were obtained or with other ligands with a similar structure (19-27). In these studies, the compounds displayed high biological activity which potentially lead to their application in medicine. Studies on cerium (III) complexes with umbelliferone, warfarin and coumachlor deserve attention. These complexes exhibit anticancer activity against two cell lines: P3HR1 and THP-1. The complex of flavone with Pt(II) ions showed 21-fold greater alkylating capacity than cisplatin as well as anticancer activity against L 1210 lymphatic leukemia (28). Further testing of the complex revealed that it induced apoptosis in unchanged, normal lymphocytes to a lesser extent and could become a potential anticancer drug in the future. Interest in compounds with a trans configuration is particularly important since these could display activity against cancerous cells that are cisplatin-resistant. Organophosphorus compounds such as phosphonians (phosphomycin, adefovir) also have diverse biological effects, including antibacterial and antiviral activity.

The biological activity of chromone derivatives as ligands combined with the anticancer effect of Pt(II) complexes could lead to combinations that act in synergy. Recent studies suggest that naturally occurring compounds with a chromone structure display activity against free radicals thereby ensuring protection from oxidative stress. In her paper, Malinowska and Modranka showed that 3(2-aminothiazolyl)methylen)-4H-1-penzopyraz-4-one had a significant effect on the activity of selected antioxidative enzymes: superoxide dismutase (CuZn-SOD) and catalase. As shown, their activity in the study group increased by about 90% compared with the control group (19). I was expecting moreover that the assessment of the biological activity of the compounds with diverse substituents presented here would allow to determine the relationships between the structure of these compounds and their activity. When designing potential drugs that were the subject of this paper I based on data available in literature concerning the relationship between structure and biological activity.

REFERENCES

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