USE OF MICROMOLECULAR COPPER COMPLEXES OF THIOSEMICARBAZIDES AS AN ENDOGENOUS CATALASE INDUCER/ACTIVATOR

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Introduction*. Oxidative stress is a major mechanism in the pathogenesis of many diseases, including severe multifactorial ones. Catalase (CAT), along with superoxide dismutase, is one of the first-line antioxidant defense enzymes. The development of methods/compounds that can increase/induce CAT will provide new possibilities to strengthen antioxidant protection and prevent oxidative damage to cells and tissues.*

Material and methods. *The ability of three copper coordination compounds from the class of transition metal thiosemicarbazides to enhance/induce CAT in erythrocytes of healthy white laboratory rats has been studied.*

Results. *Dichloro-(methyl-N-(prop-2n-1-yl)-2-(pyridin-2-ylmethylidene)hydrazine-carbimido-thioate)copper was found to show the highest induction and/or activation of CAT, exceeding 2.71 times the values of the control group and 1.80 times the values produced by vitamin D3. This reveals increased synthesis of CAT after exposure to this compound, a phenomenon that we have established for the first time.*

Conclusions. *The compound can be used as a therapeutic agent, which, by activating the production of CAT in the body, can prevent and/or reduce the occurrence of multifactorial diseases, due to prevention of the damage of cells associated with excessive accumulation of hydrogen peroxide. The obtained data open perspectives for the research of biologically active synthetic compounds, which will diversify the arsenal of effective tools for preventing/treating various diseases.*

Cuvinte-cheie: *compus coordinativ al cuprului, derivați de tiosemicarbazidă, producerea sau activarea CAT, boli multifactoriale.*

UTILIZAREA COMPLEXELOR MICROMOLECULARE DE CUPRU ALE TIOSEMICARBAZIDELOR CA INDUCTORI/ACTIVATORI ENDOGENI AI CATALAZEI

Introducere. *Stresul oxidativ este un mecanism major în patogeneza multor boli, inclusiv a celor multifactoriale severe. Catalaza (CAT), împreună cu superoxid dismutaza, este una dintre enzimele de apărare antioxidantă de primă linie. Dezvoltarea de metode/compuși care pot crește/induce CAT va oferi noi posibilități de a spori protecția antioxidantă și de a preveni deteriorarea oxidativă a celulelor și a țesuturilor.*

Material și metode*. A fost studiată capacitatea a trei compuși de coordonare a cuprului din clasa tiosemicarbazidelor metalelor de tranziție de a crește/induce CAT în eritrocitele șobolanilor albi de laborator sănătoși.*

Rezultate. *S-a constatat că dicloro-(metil-N-(prop-2n-1-il)-2-(piridin-2-ilmetiliden)hidrazin-carbimido-tioat)cuprul exercită cea mai potentă inducție și/sau activare a CAT, depășind de 2,71 ori valorile lotului martor și de 1,80 ori valorile produse de vitamina D3. Aceste date relevă sinteza endogenă crescută a CAT după expunerea la acest compus, fenomen care a fost stabilit pentru prima dată.*

Concluzii. *Compusul poate fi utilizat în medicină ca agent terapeutic care, prin activarea/inducerea CAT în organism, poate preveni și/sau reduce apariția bolilor multifactoriale cauzate de deteriorarea celulelor și a țesuturilor, asociate cu acumularea excesivă de peroxid de hidrogen. Datele obținute deschid noi perspective pentru cercetarea compușilor sintetici biologic activi care vor diversifica arsenalul de instrumente eficiente pentru prevenirea sau tratarea diferitor boli.*

INTRODUCTION

CAT plays a central role in the detoxification of oxygen peroxide (H_2O), which is crucial in defending cells against oxidative damage caused by H_2O_2. Hydrogen peroxide is toxic due to its ability to form other reactive oxygen species (ROS), such as the hydroxyl radical in the Fenton reaction. Additionally, H_2O_2 can act as an important signalling molecule, being involved in multiple physiological and pathological processes (1). CAT can also act as a peroxidase, thus contributing to the metabolism of micromolecular substrates such as methanol, ethanol, azide, and hydroperoxides. In the case of ethanol, the enzyme is able to oxidize it to acetaldehyde, contributing to its metabolism in the liver. Thus, CAT may have additional roles, such as detoxification or activation of toxic and antitumor compounds.

However, the molecular mechanisms regulating the expression of CAT, the oldest known antioxidant enzyme, have not yet been fully elucidated. Identifying these mechanisms will allow us to find new approaches to modulate antioxidant status in a variety of pathological conditions (2, 3).

Deficiency or impaired functioning of CAT is linked to the pathogenesis of a number of age-related diseases, such as diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia (4).

Several known compounds have been identified to increase CAT activity in tissues during various pathological processes. Notable examples include the elevation of CAT activity in erythrocytes of patients with type II diabetes through metformin treatment (5). Additionally, CAT production and/or activity in the mouse macrophage-like cell line RAW264 is increased by treatment with sodium nitroprusside (SNP) and 1-hydroxy-2-oxo-3,3-bis-(2-aminoethyl)-1-triazene (NOC18). At the maximum concentration of 300 μmol/L, NOC18 statistically and conclusively increases CAT activity by up to 50%, thereby protecting macrophages from apoptosis induced by H_2O_2 (6). Furthermore, adding coffee to the feed of animals with liver cancer increases CAT activity almost to the values specific to healthy animals (7). Melatonin has been shown to increase CAT activity in peripheral blood mononuclear cells (PBMC) by 80% compared to untreated cells (8), while Fullerenol ($C_{60}(OH)_{36}$) increases it in red blood cells by 24% ($p<0.05$) compared to untreated ones (9). However, these methods have the disadvantage of being ineffective, as they do not provide stable, sufficiently high induction and/or activation of CAT.

The method closest in technical essence and result to increasing the production or activity of CAT in the body is based on the administration of vitamin D3 or its derivatives (10). However, this method has several disadvantages, namely that it does not provide sufficient stimulation of the production and/or activity of CAT, and the simultaneous use of vitamin D3 with its analogues increases the risk of developing hypervitaminosis D and its toxic effects.

The problem that our method solves is the generation of new copper coordination compounds from the class of thiosemicarbazides, thereby expanding the range of synthetic compounds with high CAT induction/activation potential.

MATERIAL AND METHODS

Three new local copper coordinating compounds (CC), derivatives of thiosemicarbazide, were included in the study:

1) Acetato-2-(({(metilsulfanil)((prop-2-en-1-il)amino)metiliden}hidraziniliden) metil) fenolatoaquacupru (coded (Cu(OAc)2);
2) Bromo-2-{(2-(prop-2-en-1-ylcarbamothioyl)-hydrazinylidene) methyl}phenolatocopper, coded (Cu(HL)Br);
3) Dichloro-(methyl-N-(prop-2n-1-yl)-2-(pyridin-2-ylmethylidene)hydrazine-carbimidothioate)copper, coded (Cu(L-H)Cl2).

These compounds were synthesized at Moldova State University in the “Advanced Materials in Biopharmaceutics and Technology” Laboratory, by the team of professor Aurelian Gulea, PhD, academician (11, 12, 13).

The effect of the local CC on erythrocyte CAT was evaluated in experiments on *Rattus norvegicus domestica albino*. The experiments were conducted in accordance with contemporary principles in the biological standardization of experiments and the Helsinki Declaration with subsequent amendments (Somerset West Amendment, 1996). The study was approved by the Research Ethics Board of Nicolae Testemițanu State University of Medicine and Pharmacy (approval no. 81 of 19.09.2020).

The experiments involved 50 rats weighing 180-230 g, divided into 5 groups of 10 animals each. The first group, the control, consisted of animals kept on a regular vivarium diet and injected with physiological saline 3 times a week for 30 days. CC were administered in a dose of 1.0 µM/kg, 3 times a week for 30 days in the following way: group 2 – (Cu(OAc)2), group 3 – (Cu(HL)Br), group 4 – (Cu(L-H)Cl2). The rats in group 5 (prototype) were administered vitamin D3 in a dose of 20.0 µM/kg in the same way as CC. After 24 hours from the last administration of the tested compounds, the blood was collected for the evaluation of CAT. The erythrocyte mass, obtained after decanting the blood serum, was washed 3 times with physiological saline.

CAT activity was determined according to the method described by Korolyuk M.A. et al. (1988) (14) with modifications (15). Briefly, 0.01 mL of the erythrocyte mass diluted 1000 times with distilled water was pipetted into the 96-well photometric microplates, and 0.18 ml of 0.03% H_2O_2 was added. In the control samples, instead of H_2O_2, the same amount of distilled water was added. Three parallel reference samples, which contain only H_2O_2 and distilled water, were prepared. The samples were incubated for 10 minutes at 37°C, and afterwards, 0.10 mL of 4% ammonium molybdate solution was added. The solution was shaken, and its absorbance was measured at 410 nm. The difference between the absorbance of the reference sample and the experimental sample was calculated. Enzyme activity was expressed in µmol per s per 1 g Hb (µmol/s·g Hb).

The spectrophotometric method was used to assess the levels of vitamin D in the research samples (16).

The statistical evaluation of the obtained data was conducted using StatsDirect Statistical Analysis Software (StatsDirect Ltd, UK). Mean±standard deviation (X±S) was calculated. To test the significant difference between the studied markers of the compared groups, the non-parametric "U" Mann-Whitney statistical test and the significance threshold "p" ($p<0.05$) were employed.

RESULTS

The results of the assessment of the changes in the CAT activity of erythrocytes due to the administration of certain local CC are presented in the statistical data in Table 1.

Based on the experimental data presented in Table 1, it is evident that the mentioned compounds induce a greater activation of CAT than that produced by vitamin D3, used as a prototype. The studied thiosemicarbazone derivatives increased erythrocyte CAT activity by 1.9-2.7 times compared to the control values and by 1.8 times compared to the values produced by the prototype (vit. D3). Compound Cu(L-H)Cl2 was found to exhibit the highest CAT induction/activation activity, exceeding the control values by 2.72 times and the prototype values by 1.8 times.

Table 1. The impact of local CC, derivatives of thiosemicarbazide, on erythrocyte CAT activity.

Study groups	CAT, µM/s.g Hb (X±S)
Control group	18.80±4.02 (100%)
Acetato-2-(({{(metilsulfanil)((prop-2-en-1-il)amino)metiliden}hidraziniliden) metil) fenolatoaquacupru (0,1 µM/kg) (coded (Cu(OAc)2)	35.7±5.61 *** (190%)
Bromo-2-{(2-(prop-2-en-1-ylcarbamothioyl)-hydrazinylidene)methyl}phenolato copper (0,1 µM/kg), coded (Cu(HL)Br)	50.8±5.66 *** (270%) ## (178%)
Dichloro-(methyl-N-(prop-2n-1-yl)-2-(pyridin-2-ylmethylidene)hydrazine-carbimido-thioate)copper (0,1 µM/kg), coded (Cu(L-H)Cl2)	51.2±7.98 *** (272%) ## (179%)
Vitamina D3 (prototip) (20 µM/kg)	28.6±4.26 **(152%)

Note: statistically significant difference with the control group *- $p<0.05$; ** - $p<0.01$; *** - $p<0.001$; statistically significant difference with the prototype (vitamin D3) - # $p<0.05$; ## - $p<0.01$.

DISCUSSIONS

The established properties of the studied CC are of interest for medicine in terms of expanding the range of synthetic inducers/activators of CAT. Enzyme induction is a process in which a molecule (e.g., a drug) induces an increase in the metabolic activity of an enzyme either by binding to the enzyme and activating it, or by increasing the expression of the gene encoding the enzyme (17, 18). Enzyme induction occurs when the molecule (called an inducer) facilitates gene expression. The specifics of how this occurs depend on control mechanisms as well as on differences between prokaryotic and eukaryotic cells (18).

CAT is one of the most important antioxidant enzymes, present in almost all aerobic organisms. In 1937, CAT was first crystallized from bovine liver in the laboratory of Sumner and Dounce. The gene encoding CAT in humans is located on chromosome 11 (19).

The significance and importance of the CAT induction phenomenon by CC, derivatives of thiosemicarbazide, stem from the broad range of practical applications of these inducers.

These inducers could potentially be used as agents for the treatment and prevention of renal fibrosis caused by CAT deficiency (20), or for the treatment of some forms of infertility, since CAT is found in mouse oocytes and likely plays a role in genomic protection from oxidative damage during meiotic maturation (21). It has been established that CAT expression is also altered in cancer cells, which favors cell proliferation by inducing genetic instability and activating oncogenes. Regulation of CAT expression is likely controlled mainly at transcriptional levels, although other mechanisms may be involved. In addition to transcription factors such as Sp1 and NF-Y, the transcription factors JunB and RARα are crucial regulators in breast cancer cells by recruiting proteins involved in transcriptional complexes and chromatin remodeling. Therefore, CAT may be an attractive therapeutic target in the context of cancer (22).

CAT was found to significantly decrease chromosomal aberrations and to delay or prevent spontaneous neoplastic transformation in mice fibroblasts and epidermal keratinocytes (23). Similarly, liver CAT activity decreases in the presence of a growing tumor, and after the tumor is removed, this activity returns to normal, demonstrating the importance of this antioxidant enzyme in tumorigenesis (24).

The methods for inducing CAT expression or activity are important for treating retinal oxidative stress associated with diabetic retinopathy (25).

Due to the ability of thiosemicarbazone derivatives to easily penetrate the blood-brain barrier (26) and their stability in the bloodstream, these compounds could be used to develop new effective methods for the early diagnosis of severe brain diseases, such as brain tumors and their metastases, as well as for the visualization of Aβ plaques in Alzheimer's disease.

CAT could be extremely useful for the development of effective therapies for brain and neurological dysfunctions. This is based on the fact that brain CAT activity is extremely low compared to other tissues and organs, such as the liver and kidney. The results of some studies reveal the importance of transient receptor potential (TRP) channels as a key component of the neurological Ca2+ ion entry pathway in response to the harmful action of reactive oxygen species (ROS). Exploratory data suggest that CAT may act effectively by suppressing the TRP channel activated by oxidative stress, exhibiting protective effects on neuronal mitochondrial function and neuronal survival (27). In the future, CAT could be extremely useful for the development of effective therapies for neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, as well as sensory pain, because the decrease of CAT activity due to oxidative stress plays an important role in the pathogenesis of the aforementioned diseases (28).

One of the most severe multifactorial diseases is sepsis, a systemic inflammatory syndrome caused by infections that can lead to organ dysfunction with a high mortality rate (over 25%). Considering that the most susceptible to sepsis are children, people aged over 65 years, as well as patients with immunodeficiency, autoimmune diseases, tumors, kidney, and lung diseases (29), developing treatments using CAT inducers and/or activators would be a desirable and effective option. Septic shock is also one of the frequent complications of COVID-19 (30); therefore, CAT inducers could be an attractive therapeutic target

for the prevention of complications and treatment of SARS-CoV-2 infection. CAT helps regulate cytokine production, protect against oxidative damage, and suppress SARS-CoV-2 replication, as shown in human leukocytes, alveolar epithelial cells, and rhesus macaques, without noticeable toxicity (31, 32).

CONCLUSIONS

1. An effective method of inducing/activating CAT in the body has been developed using biologically active copper coordination compounds from the class of transition metal thiosemicarbazides. These compounds can prevent and/or reduce the occurrence of a variety of multifactorial diseases and the development of cell and tissue damage related to the excessive accumulation of hydrogen peroxide.
2. Therefore, the data obtained mark the beginning, opening up prospects for the development and research of new CCs, which will diversify the arsenal of means to combat various severe pathological processes. Further studies are needed to confirm the therapeutic utility of these bioactive compounds.

AUTHORS' CONTRIBUTION

The authors declare that their authorship complies with the international ICMJE criteria. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published, and agree to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVAL

The research was approved by the Research Ethics Boar of *Nicolae Testemițanu* State University of Medicine and Pharmacy (approval no. 73 of April 26, 2017).

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