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# EFFECT OF A THIAZOLE DERIVATIVE ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN MURINE LYMPHOMA CELLS

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Previous *in vitro* studies have demonstrated a pronounced cytotoxic effect of the thiazole derivative N-(5-Benzyl-13-thiazole-2-yl)-35-dimethyl-1-benzofuran-2-carboxamide (BF1) on tumor cells. Additionally, it has been determined that scavengers of reactive Oxygen species (ROS) significantly reduce the cytotoxic effect of BF1. In this study, the influence of BF1 on the activity of superoxide dismutase (SOD) and catalase (CAT), both normally and in the presence of ascorbic acid, in mouse Nemeth-Kellner lymphoma (NK/Ly) cells has been studied to evaluate the possible role of antioxidant activity during the action of this substance.

The experiments were performed using nonlinear male mice weighing 20–30 g. Intraperitoneal inoculation of 10–15 million cancer cells into the mice induced the ascites form of lymphoma. The thiazole derivative (BF1) was dissolved in dimethyl sulfoxide and added to the test samples at 1, 10, and 50 μM final concentrations. Superoxide dismutase (SOD) and catalase (CAT) activities were determined spectrophotometrically in a homogenate of the lymphoma cells after incubation with the drug for 30 minutes.

The baseline level of SOD in the lymphoma of the mice was 0.33±0.02 activity units/min×mg protein. BF1 significantly increased the enzyme activity by 35 % and 29 % at concentrations of 10 ($p<0.01$) and 50 μM ($p<0.05$), respectively. The baseline level of CAT activity was 4.61±0.17 nmoles $H_2O_2$/min×mg protein, and this significantly decreased by 15 % ($p<0.05$) and 20 % ($p<0.01$) following the action of the thiazole derivative at a concentration of 10 and 20 μM, respectively. The increase of SOD activity, coupled with a decrease or absence of changes in CAT activity, may be cytotoxic to cancer cells. Simultaneously, upon the addition of ascorbic acid as a scavenger of ROS to the environment, the activities of SOD and CAT did not change under the action of BF1 at any of the investigated concentrations.

Therefore, the effect of the thiazole derivative BF1 has been canceled in the presence of ROS scavengers in the environment. This may indicate the dependence of the cytotoxic effect of BF1 on the presence of ROS in tumor cells.

*Keywords:* tumor, thiazole derivative, antioxidant enzymes, ROS scavengers

It is well known that disturbances in redox homeostasis can lead to the development of various pathologies in the organism. Under these conditions, the ratio of lipid peroxidation (LPO) and the activity of antioxidant defense enzymes are often disturbed. The antioxidant defense system of the body controls all stages of free radical reactions, from their initiation up to the formation of end products, such as hydroperoxides and malondialdehyde. The intensity of free radical processes is determined by the balance of prooxidant and antioxidant reactions in cells. This balance can be affected by various factors: substances containing thiol groups and vitamins (A, E, P, and C), chelating agents (e.g., deferoxamine), inhibitors of superoxide ions (e.g., xanthine

oxidase), superoxide scavengers (e.g., flavonoids), scavengers of hydroxyl radicals, and drugs of plant and synthetic origin. Catalase (CAT) and superoxide dismutase (SOD) are key conjugated enzymes of antiradical defense, for which hydroperoxide ($H_2O_2$) is both a substrate and a product, respectively.

Oxidative stress-enhancing chemotherapeutic agents are toxic to cancer cells, as they are involved in biological processes such as cell cycle arrest, DNA damage, and induction of apoptosis. Free radicals, which negatively affect the effects of anticancer drugs, can cause oxidative stress in cells, damage DNA, and induce cell death [8].

At the same time, antioxidant enzymes can be targets for anticancer drugs, since changes in enzymatic activity affect the levels of primary and secondary LPO products, which can be toxic to cancer cells. Meanwhile, the level of reactive Oxygen species (ROS) and superoxide radical products is regulated by the activity of antioxidant defense enzymes, it was important to determine the effect of the thiazole derivative BF1 on the activity of key enzymes of the antioxidant defense system in lymphoma cells, both normally and in the presence of the ROS scavenger – ascorbic acid.

## Materials and Methods

The activity of antioxidant enzyme systems was estimated in non-linear male mice weighing 20–30 g (a total of 10 individuals, 5 for each series of experiments), with implanted NK/Ly lymphoma. The animals were kept under stationary conditions in a vivarium at a constant temperature on a standard diet. All animal manipulations were carried out by the national «General Ethical Principles for Animal Experimentation» adopted by the First National Congress on Bioethics (Kyiv, Ukraine, 2001), which are consistent with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, France, 1985). The ascitic form of lymphoma was induced by the intraperitoneal injection of 10–15 million cancer cells into the mice. Ascitic fluid was collected by draining the abdominal cavity with a sterile syringe under ether anesthesia on the 7th to 10th day after inoculation. Lymphoma samples were immediately frozen in a freezer at -20 °C and later used for research. The protein content in each sample was determined by the method of Lowry et al. [5].

The 2,5-disubstituted thiazoles used in the study were synthesized at the Department of Organic Chemistry of Ivan Franko Lviv National University (head – Dr. of Chem. Sci., Professor M. Obushak). The synthesis steps are described in detail in [6]. From the number of synthesized compounds N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) was used as the most cytotoxic to tumor cells [4].

The derivative of thiazole BF1 was dissolved in dimethyl sulfoxide (final concentration of DMSO did not exceed 5 %) and added to the test sample (liver homogenate or lymphoma homogenate) at active concentrations of 1, 10, and 50 μM and incubated for 10 minutes. Catalase activity was determined spectrophotometrically according to the method of Bolann et al. [2] at an absorbance wavelength of 410 nm. The enzyme activity was expressed in nmol of $H_2O_2$/min × mg protein. SOD activity (U/min × mg protein) was determined according to the method of Bournonville et al. [3].

Statistical analysis of the research results was performed using MS Excel 2013. To assess the significance of the difference between the statistical characteristics of two alternative sets of data, the Student's t-test was calculated. A difference with $p < 0.05$ was considered significant. The distribution of experimental data was normal. A two-way ANOVA is used to estimate the dependance of the effect of BF1 on SOD and CAT activity in the presence or absence of ROS scavenger.

## Results and Discussion

It is known that the levels of SOD and CAT activity differ between cancerous and normal cells due to lower mitochondrial activity in cancer cells [1]. After examining the level of SOD activity in mouse lymphoma, it was found that this activity was 0.32±0.02 U/min×mg protein and significantly increased by 35 % and 29 % under the action of BF1 at concentrations of 10 and 50 μM, respectively (Fig. 1, A). At the same time, upon the addition of ascorbic acid (AA) as a scavenger of ROS, the activity of SOD did not change under the action of BF1 at any of the investigated concentrations (Fig. 1, B).

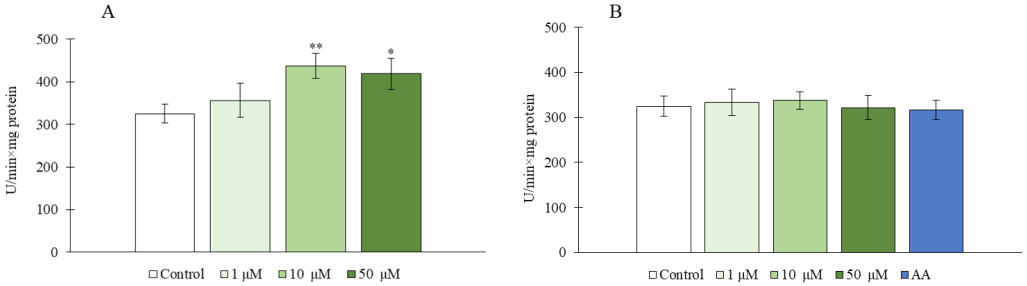


Fig. 1. SOD activity in mouse lymphoma under the action of BF1 without (A) and in the presence of AA (B). M±m, n=5. Significance: * – p<0.05, ** – p<0.01 vs Control

The control level of CAT was 4.61±0.17 nmol $H_2O_2$/min × mg protein (Fig. 2, A). It significantly decreased by 15 % and 20 % under the action of BF1 at a concentration of 10 and 50 μM. In contrast, other concentration of the drug did not cause significant changes in the activity of the investigated enzyme (Fig. 2, A). AA also nullified the effect of BF1 on CAT activity in lymphoma cells (Fig. 2, B).

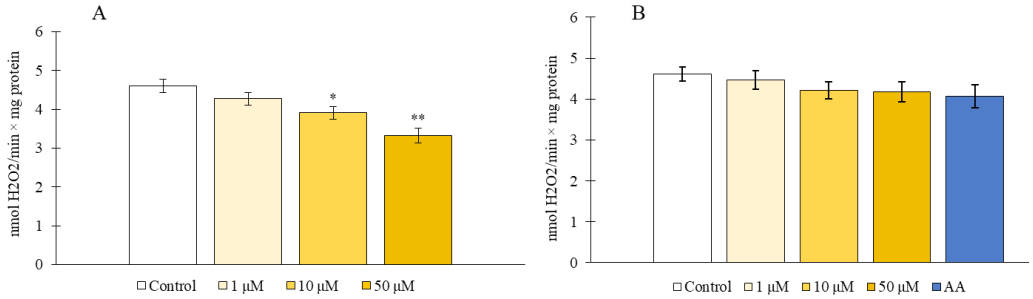


Fig. 2. CAT activity in mouse lymphoma under the action of BF1 without (A) and in the presence of AA (B). M±m, n=5. Significance: * – p<0.05, ** – p<0.01 vs Control

Fig. 3 represents the data of variance analysis of the effect of BF1 on SOD (A) and CAT (B) activity in the presence of ROS scavenger. It was established that in the presence of AA, the share of influence of the scavenger was 72.9 %, and the share of influence of BF1 under investigation was 9.12 % for the study of the activity of SOD. According to the results of variance analysis, it was established that the share of influence of AA was 64.2 % for the study of the activity of CAT. The share of influence of BF1 was 21.2 %. It is important to note that the share of influence of unaccounted factors did not exceed 5.37 %.

The activity of antioxidant enzymes can change under the conditions of tumor growth. It is acknowledged SOD utilizes ROS, forming $H_2O_2$. Cell viability needs to establish a balance between the activity of SOD and enzymes that oxidize $H_2O_2$, such as CAT. In particular, too rapid

growth of SOD activity in the cell without corresponding activation of CAT or peroxidases is in itself cytotoxic [1].

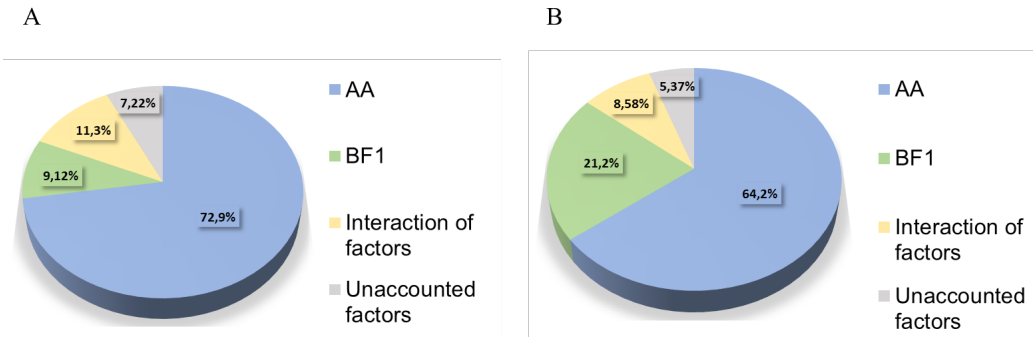


Fig. 3. Variance analysis of the effect of BF1 and ROS scavenger AA on the SOD (A) and CAT (B) activity in NK/Ly cells. M±m, n=5 (for each series of experiments)

It is known that SOD activity in non-Hodgkin lymphoma cells leads to the activation of a mitochondrial type of apoptosis [7]. That is why SOD and CAT, as key enzymes of antioxidant defense, can be targeted for anticancer drugs since changes in enzymatic activity will affect the level of primary and secondary lipid peroxidation products, which can be toxic to cancer cells.

It was previously established that under the action of BF1, the amount of hydrogen peroxide and superoxide radical in tumor cells increases [4]. It is also known that the cytotoxicity of the substance to tumor cells decreased when scavengers of ROS were added to the medium. In our study, we also observed a decrease in the effect of BF1 on the antioxidant enzymes of lymphoma cells. This indicates a dependence of the cytotoxic effect of BF1 on the presence of ROS in the cells. The results of this study also demonstrate that the activity levels of SOD and CAT differ significantly between cancerous and normal cells, potentially due to the low mitochondrial activity in cancer cells. The variance analysis of the effects of BF1 on SOD and CAT activities in the presence of ROS scavengers provided further insights. These findings suggest that BF1 exerts its cytotoxic effects through modulation of oxidative stress by increasing SOD activity and decreasing CAT activity, leading to an imbalance in ROS homeostasis. The presence of ROS scavengers like AA moderates these effects, indicating the potential therapeutic application of ROS modulation in cancer treatment. The low share of influence of unaccounted factors (<7.22 %) in the variance analysis confirms the significance of the observed effects of BF1 and ROS scavengers.

Overall, this study highlights the critical role of ROS in the cytotoxic mechanism of BF1 and underscores the potential for antioxidant enzymes as targets for cancer therapy. Further research is needed to elucidate the detailed molecular pathways involved and to explore the therapeutic implications of combining BF1 with ROS modulators in the treatment of lymphoma and other cancers.

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## ВПЛИВ ПОХІДНОГО ТІАЗОЛУ НА АКТИВНІСТЬ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ У КЛІТИНАХ МИШАЧОЇ ЛІМФОМИ

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Раніше було досліджено виражену цитотоксичну дію похідного тіазолу N-(5-бензил-1,3-тіазол-2-іл)-3,5-диметил-1-бензофуран-2-карбоксаміду (БФ1) на пухлинні клітини *in vitro*. Крім того, було встановлено, що перехоплювачі активних форм Оксигену (АФО), зокрема, аскорбінова кислота, суттєво знижують цитотоксичну дію БФ1. У цьому дослідженні було вивчено вплив БФ1 на активність супероксиддисмутази та каталази у клітинах мишачої лімфоми Немета-Келнера (NK/Ly) без та за наявності аскорбінової кислоти в середовищі, щоб оцінити ймовірну роль ферментів антиоксидантної системи у механізмі дії речовини.

Експерименти проводили на нелінійних мишах-самцях вагою 20–30 г. Асцитну форму лімфоми прищеплювали внутрішньочеревним введенням мишам 10–15 мільйонів пухлинних клітин. Похідне тіазолу (БФ1) розчиняли в диметилсульфоксиді та додавали до досліджуваних зразків у кінцевих концентраціях 1; 10 і 50 мкМ. Концентрація аскорбінової кислоти становила 50 мкМ. Активність

супероксиддисмутази і каталази визначали спектрофотометрично в гомогенаті клітин лімфоми після інкубації з препаратом протягом 30 хв.

Контрольний рівень активності супероксиддисмутази в лімфомі мишей становив 0,33±0,02 од. активності/хв × мг білка. Речовина БФ1 підвищувала активність ферменту на 35 % і 29 % у концентраціях 10 і 50 мкМ, відповідно. Контрольний рівень активності каталази становив 4,61±0,17 нмоль $H_2O_2$ /хв×мг білка і знижувався на 15 % та 20 % за дії досліджуваного похідного тіазолу в концентрації 10 і 50 мкМ, відповідно. Підвищення активності супероксиддисмутази за зниження або відсутності змін активності каталази може бути токсичним для ракових клітин. Водночас за додавання до середовища інкубації аскорбінової кислоти як перехоплювача АФО активність досліджуваних ферментів не змінювалася за дії БФ1 у жодній із досліджуваних концентрацій.

Отже, дія похідного тіазолу БФ1 нівелюється за наявності в середовищі інкубації перехоплювачів АФО. Це може вказувати на залежність цитотоксичної дії БФ1 від наявності АФО у пухлинних клітинах.

*Ключові слова:* пухлина, похідне тіазолу, антиоксидантні ферменти, перехоплювачі АФО