

PROOXIDANT AND ANTIOXIDANT PROCESSES IN THE LIVER HOMOGENATE OF HEALTHY AND TUMOR-BEARING MICE UNDER THE ACTION OF THIAZOLE DERIVATIVES

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Thiazole derivatives were shown to have toxic effects *in vitro* on cancer cells of different origin and can be considered as potentially antineoplastic, but their effect on the normal tissues needs to be studied. In this research the newly synthesized thiazole derivatives of *N*-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) and 8-methyl-2-Me-7-[trifluoromethyl-phenylmethyl]-pyrazolo [4,3-*e*] [1,3] thiazolo [3,2-*a*] pyrimidin-4 (2H)-one (PP2) were used and their effect on the pro- and antioxidant processes after adding in a 1, 10 and 50 μ M concentrations to the liver homogenate of healthy and NK/Ly lymphoma-bearing mice was estimated. The level of superoxide radical and TBA-active products as well as catalase, SOD and glutathione peroxidase activity were measured. It was shown that superoxide radical and TBA-active products level and catalase activity were significantly higher in the liver of tumor-bearing mice than in the liver of the healthy mice. Neither BF1 no PP2 influenced the studied indices in the liver homogenate of healthy and tumor-bearing animals with the exception that PP2 significantly reduced the level of TBA-positive products in both cases. The data obtained showed that the studied thiazole derivatives did not cause severe liver toxicity in both healthy and tumor-bearing mice.

Key words: thiazole derivatives, NR/Ly lymphoma, liver, lipid peroxidation, antioxidant system.

Almost all the chemotherapy agents have negative side effects when antitumor drugs damage healthy cells, in particular hepatocytes. The liver is the major organ for the detoxification of drugs in human and animals and plays an important role in the excretion of drugs from an organism. Damage of free radical processes and activity of antioxidant system under the effects of anti-tumor agents may lead to negative side effects.

A cytotoxic effect of investigated thiazole derivatives on cell lines of melanoma, glioblastoma, hepatocarcinoma and leukemia cells has been established [1, 2]. However, thiazole derivatives did not affect the human pseudonormal embryonic kidney cells and keratinocytes [1]. It was found that investigating compounds changed prooxidant-antioxidant metabolism in Nemeth-Kellner lymphoma (NK/Ly)

cells [3]. In particular, the studied thiazole derivatives activated superoxide dismutase and inhibited the catalase and superoxide dismutase activity in lymphoma cells. Presumably, the mechanism of action of these compounds on tumor cells is associated with direct or indirect effects on the enzymes of the antioxidant system [4]. On the other hand, the products of tumor development during its growth in the body can affect the balance of prooxidant and antioxidant processes in other organs, including the liver [5]. Therefore, studying these processes in the liver of tumor-bearing mice is also relevant for planning comprehensive treatment.

To better establish the level of safety of newly synthesized substances, the functional state of liver cells has to be studied. Particularly, the changes in free radicals and antioxidant processes in liver cells

may suggest the possible mechanisms of negative side effects that often occur after using antitumor drugs. The purpose of this work was to investigate the effect of thiazole derivatives on the level of primary and secondary products of peroxide lipid oxidation (LPO), superoxide radicals and the activity of enzymes of the antioxidant defense in the mouse liver with or without NK/Ly lymphoma and to compare the studied parameters in the liver of healthy mice and tumor-bearing mice.

Materials and Methods

The study was performed on white (outbred) wildtype male mice (20–30 g) with or without grafted NK/Ly lymphoma. The first group (A), which included healthy mice, served as a positive control. Animals were kept in standard vivarium conditions at constant temperature on a mixed ration. Manipulations with animals were carried out under the principles of the “General Ethical Principles of Experimentation on Animals” approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, France, 1985).

Mice were anesthetized with chloroform, then decapitated, abdominal dissected and the liver was rapidly excised. The cooled and washed from the blood liver was crushed through a metal press and homogenized in a Potter-Evelheim homogenizer. The liver from mice with lymphoma was extirpated for 28 days after cancer cells inoculation.

The concentration of protein in liver homogenate was determined by the method of Lowry et al. [6]. Thiazole derivatives were synthesized at the Department of Organic Chemistry of Ivan Franko National University of Lviv (the head of a department – Dr. M. Obushak). The steps of synthesis are described in details in our previous publication [1].

N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) and 8-methyl-2-Me-7-[trifluoromethyl-phenylmethyl]-pyrazolo-[4,3-e]-[1,3]-thiazolo-[3,2-a]-pyrimidin-4(2H)-one (PP2) were used as a potential antitumor compounds.

The thiazole derivatives were dissolved in the dimethylsulfoxide (DMSO, final concentration 5%) before their addition to the liver homogenate at final concentrations of 1, 10 and 50 μ M. Duration of incubation was 15 min. An equal volume of incubation solution was added to the control samples. The

level of lipid hydroperoxides was measured using the method of Myronchuk in the modification of Gavrilov [7] with an absorption wave-length of 480 nm and was presented in conventional unit/min \times mg of protein. The level of TBA-positive products was measured by the method of Timirbulatov & Seleznev [8] with an absorption wavelength of 532 nm and was expressed in μ moles/mg of protein. The level of superoxide radical was measured by the method of Denisenko & Kostenko [9] with an absorption wave-length of 540 nm and was expressed in nmoles/g \times s.

Catalase activity was measured by the method of Korolyuk et al. [10] with an absorption wave-length of 410 nm and was expressed in nmoles of H₂O₂/min \times mg of protein. Superoxide dismutase (SOD) activity was measured by the method of Kostyuk et al [11] and was expressed as unit SOD/mg protein. Glutathione peroxidase activity was measured by the method of Moin [12] with an absorption wave length of 412 nm and was expressed in G–SH/min \times mg of protein.

The MS Excel-2013 program was used for statistical analysis of obtained results. Student’s and Wilcoxon coefficients were calculated to assess the significance of the difference between the statistical characteristics of alternative data sets. The difference of the parameters was found to be significant when $P \leq 0.05$.

Results and Discussion

Fig. 1 shows the changes in the content of primary products of lipid peroxidation in the liver of healthy and tumor-bearing mice under the action of compounds BF1 and PP2. The control levels of hydroperoxides in the liver of healthy mice in both series of experiments did not differ: 2.98 ± 0.19 μ m. units/mg protein and 2.72 ± 0.16 μ m. units/mg of protein. In the liver of tumor-bearing mice, control level were higher by 13.0% and 14.7%, respectively ($P = 0.05$ by Wilcoxon test). However, both substances did not significantly change the levels of hydroperoxides in the liver of healthy and tumor-bearing mice.

These results indicate that the tested substances do not affect the level of primary products of lipid peroxidation. Because hydroperoxides rapidly enter into other redox reactions and become a source of secondary products of LPO, the next step of the study was to test the content of second LPO products in the livers of mice in both groups under the action of the tested substances.

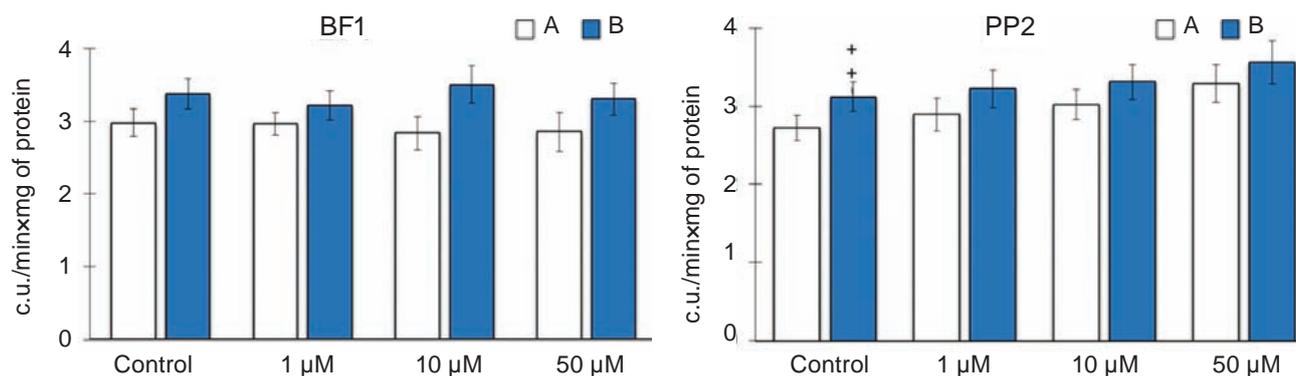


Fig. 1. The level of hydroperoxides in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives. $^{\ddagger}P = 0.05$ (healthy liver vs. liver of tumor-bearing mice) $M \pm m$; $n = 5$

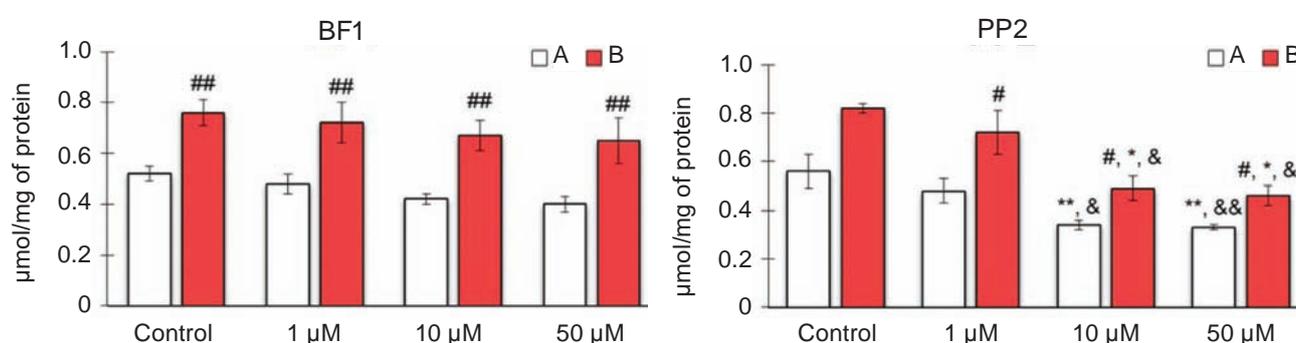


Fig. 2 The level of TBA-positive products in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives. $*P < 0.05$, $**P < 0.01$ (vs Control), $\#P < 0.05$, $\#\#P < 0.01$ (healthy vs tumor-bearing mice), $\&P < 0.05$, $\&\&P < 0.01$ (vs 1 μM)

Fig. 2 shows the changes in the content of TBA-positive products in the liver of healthy mice (A) and mice with lymphoma (B) under the action of the tested compounds. Control levels of TBA-positive products in the liver of healthy mice in two series of experiments were: 0.52 ± 0.03 μmol/mg protein and 0.56 ± 0.07 μmol/mg protein. In the liver of tumor-bearing mice, control levels of TBA-positive products were higher by 46.2% ($\#P < 0.067$) and 46.4% ($\#P < 0.001$), respectively, compared to healthy mice. It was found that the level of TBA-positive products in the livers of mice of both groups did not change under the action of BF1 at all three concentrations. Under the action of PP2 at concentrations of 10 and 50 μM, the level of this product significantly ($*P < 0.01$) decreased by 39.3% and 41% (in the liver of a healthy mouse) and by 40.2% and 43.9% (in the liver of a tumor-bearing mouse), respectively. One-factor analysis established a dose-dependent manner of these effects.

It was found that with the development of Nemeth-Kellner lymphoma, the level of TBA-positive products in the liver of mice increases significantly. However, thiazole derivatives not only do not increase the level of TBA-positive products (BF1), but also reduce it (PP2).

Since the development of the tumor significantly changes the redox balance in the cells, we should expect a change in the level of free radicals. Therefore, the content of superoxide radicals in the liver cells of healthy mice and tumor-bearing mice was studied (Fig. 3). Control levels of superoxide radicals in the liver of healthy mice were: 0.16 ± 0.01 nmol/g \times s and 0.19 ± 0.01 nmol/g \times s. In the liver of tumor-bearing mice, the control levels of the superoxide radicals were higher by 50% ($\#P < 0.01$) and 37% ($\#P < 0.01$), respectively, compared to healthy mice. It was found that BF1 and PP2 in all studied concentrations did not change the level of superoxide radicals in the liver cells of healthy mice and tumor-bearing mice.

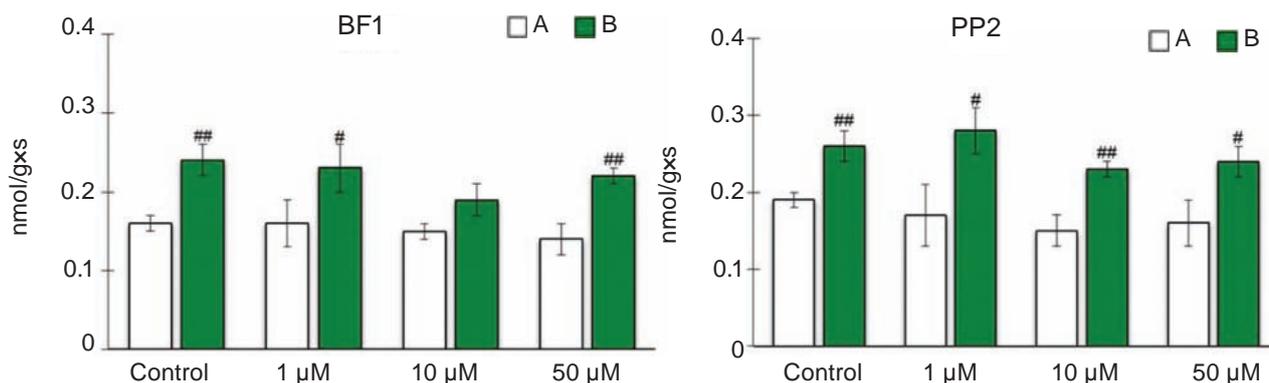


Fig. 3 The level of superoxide radical in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives. # $P < 0.05$, ## $P < 0.01$ (healthy vs tumor-bearing mice)

Fig. 4 shows the changes in the SOD activity under the action of BF1 and PP2. Control levels of the enzyme activity in the liver of healthy mice in two series of experiments were 5.43 ± 1.06 units active/min \times mg protein and 5.32 ± 0.63 units active/min \times mg protein, respectively. In the liver of tumor-bearing mice, control levels of SOD activity were 5.73 ± 0.43 units active/min \times mg protein and 6.02 ± 0.58 u.a./min \times mg protein. Under the action of both tested compounds, the enzyme activity in the liver of healthy mice and tumor-bearing mice was not changed.

Because H_2O_2 is the product of SOD activity, the normal functioning of other enzymes that neutralize hydrogen peroxide is important. Therefore, we tested the effect of the studied derivatives on the activity of CAT and GPO.

Fig. 5 shows the changes in catalase activity under the action of BF1 and PP2. Control levels of enzyme activity in the liver of healthy mice

were 7.33 ± 0.8 nmol H_2O_2 /min \times mg protein and 7.21 ± 0.28 nmol H_2O_2 /min \times mg protein, respectively. In the liver of tumor-bearing mice, control levels of CAT activity were higher by 32% ($P < 0.01$) and 30% ($P < 0.01$), respectively, compared to healthy mice. However, both compounds did not change the activity of the enzyme.

Fig. 6 shows the changes in glutathione peroxidase activity under the action of BF1 and PP2. Control levels of GPO activity in the liver of healthy mice of both groups were 6.23 ± 0.83 nmol GSH/min \times mg protein and 5.86 ± 0.68 nmol GSH/min \times mg protein. In the liver of tumor-bearing mice, the control levels of GPO activity were 5.11 ± 0.92 nmol GSH/min \times mg protein and 5.02 ± 0.68 nmol GSH/min \times mg protein. Under the action of both test compounds, the activity of the enzyme in the liver of healthy mice and tumor-bearing mice did not change.

Many chemotherapeutic drugs have a low selective effect and therefore provoke significant side

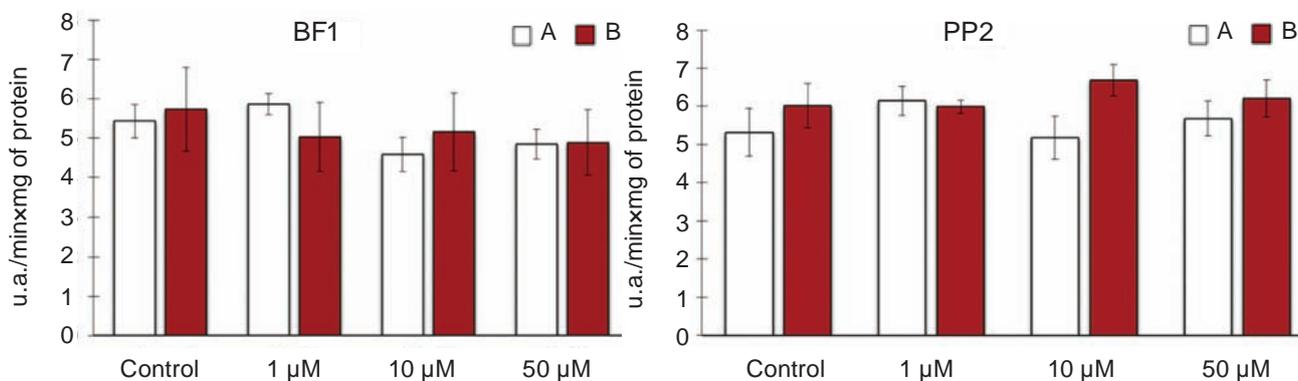


Fig. 4. The level of activity of SOD in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives

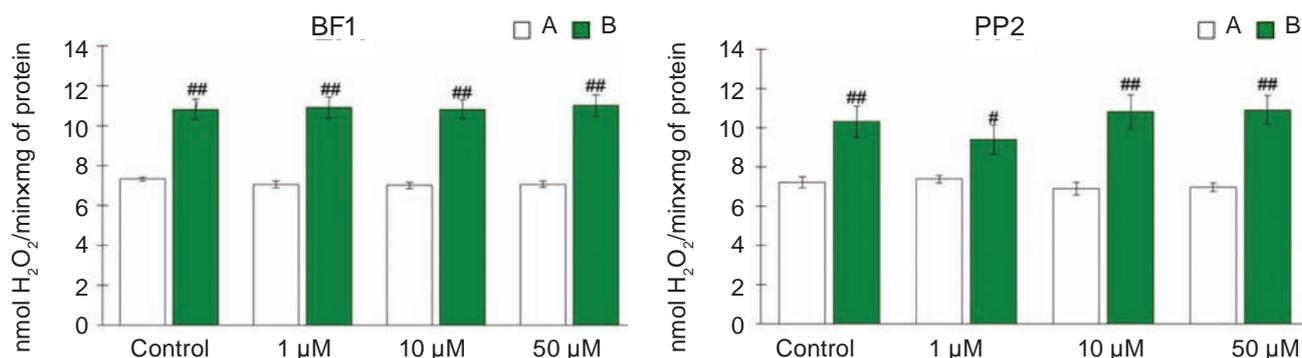


Fig. 5. The level of activity of CAT in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives. [#] $P < 0.05$, ^{##} $P < 0.01$ (healthy vs tumor-bearing mice)

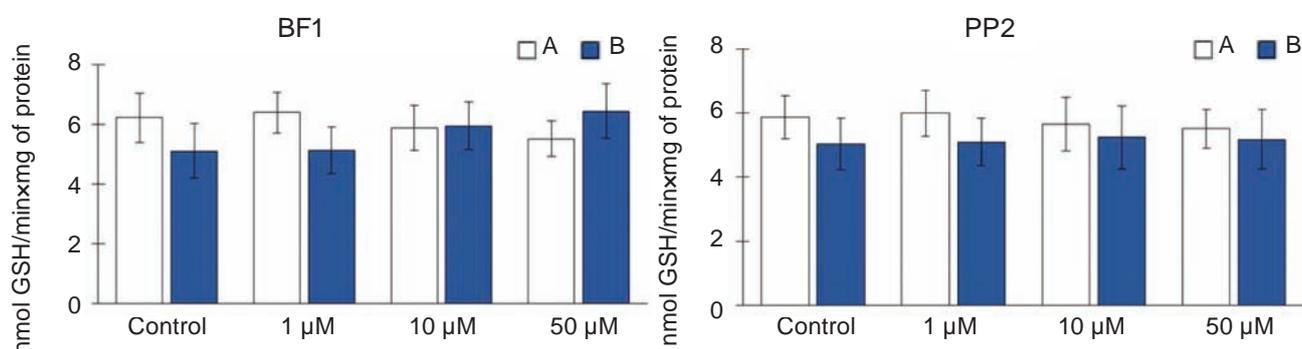


Fig. 6. The level of activity of GPO in the liver of healthy mice (A) and tumor-bearing mice (B) under the action of thiazole derivatives

effects. Particularly dangerous are the side effects of cytostatics, such as cardiotoxicity, hepatotoxicity, neurotoxicity, nephrotoxicity and effects on the immune system. Exposure to anticancer substances can significantly damage the functioning of the body.

With the development of the tumor, the resources of the physiological antioxidant system are depleted [13]. In this status, one could expect a decrease in the antitumor reactivity of the organism, which is a prerequisite for further tumor growth. However, we found that in the liver of tumor-bearing mice, the activity of antioxidant enzymes is balanced, when both SOD activity and CAT activity increase by 13% and 30%, respectively. Such changes in the activity of enzymes in the liver of mice with lymphoma may indicate possible protective mechanisms that are activated in the liver under conditions of intensification of free radical oxidation.

It was found that under the action of substances BF1 and PP2, the content of primary products of LPO, as well as the activity of antioxidant enzymes, did not change. Only the content of TBA-positive products was significantly reduced by the action of

PP2 at concentrations of 10 and 50 μM . One-factor analysis established a dose-dependent manner of these effects. There is a trifluoromethyl group in the structure of PP2, which can increase the permeability of this compound in the cell and determine its high activity [14]. Therefore, the decrease in the level of TBA-positive products may be associated with the action of a trifluoromethyl group in the composition of the compound. These results confirm the hypothesis of the probable interaction of this compound with reactive oxygen species and the possible antioxidant properties in the liver. On the other hand, BF1 does not contain a trifluoromethyl group, but contains benzofuran, which causes the cytotoxic properties of this substance against tumor cells [1-3], but does not affect the pro- and antioxidant processes in non-tumor liver cells. Therefore, the test compounds are different in structure and differently affect the content of LPO products, but both substances do not affect the activity of enzymes of the antioxidant system.

During the development of tumors, systemic damage to the body by the products of tumor decay and changes in the redox balance in tissues and or-

gans are expected. For example, Demkiv et al. [13] found that with the development of adenocarcinoma of the colon in rats, the level of LPO products in the pancreas significantly increased. The liver is also an organ where metastases often occur because one of the most dangerous ways for tumor cells to spread is portal circulation. Metastases can also occur in a lymphogenic way, when cancer cells, in particular carcinomas, spread in the body through lymphatic vessels to regional lymph nodes.

Interestingly, the LPO processes in our studies differed in the liver of control healthy mice and the liver of tumor-bearing mice. It was found that in the liver of mice with lymphoma, the level of TBA-positive products was higher by more than 45% than in the liver of control mice. Thus, the development of lymphoma provokes an increase in the number of secondary products of LPO in the liver, as a consequence of increasing levels of reactive oxygen species. Therefore, thiazole derivatives do not cause severe liver toxicity in both healthy and tumor-bearing mice.

Conclusions. It was investigated that in the liver of tumor-bearing mice, the content of LPO products and the activity of antioxidant enzymes were significantly higher, which may be a consequence of tumor development in the body of a sick mouse. Substances BF1 and PP2 are different in their structure, but none of the substances affected the activity of enzymes of the antioxidant system in the liver of mice of both groups. It was found that the substance PP2 dose-dependently reduced the level of secondary products of LPO in the liver of healthy mice and tumor-bearing mice, while under the action of BF1, this parameter did not change. This may be due to the presence in the structure of the substance PP2 trifluoromethyl group, which determines the toxic properties of the substance.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПРООКСИДАНТНІ ТА АНТИОКСИДАНТНІ ПРОЦЕСИ У ГОМОГЕНАТІ ПЕЧІНКИ ЗДОРОВИХ МИШЕЙ ТА МИШЕЙ- ПУХЛИНОНОСІЇВ ЗА ДІЇ ПОХІДНИХ ТІАЗОЛУ

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Похідні тіазолу *in vitro* виявляють токсичну дію на злоякісні клітини різного походження та можуть розглядатись як потенційно антинеопластичні, проте їх вплив на нормальні тканини потребує дослідження. У роботі було використано новосинтезовані похідні тіазолу N-(5-бензил-1,3-тіазол2-іл)-3,5-диметил-1-бензофуран-2-карбоксамід (BF1) та 8-метил-2-Ме-7-[трифлуорометил-фенілметил]піразоло[4,3-е][1,3]тіазоло[3,2-а]піримідин-4(2H)-он (PP2) та оцінено їх вплив на про- та антиоксидантні процеси після додавання у 1, 10 та 50 μM концентраціях до гомогенату печінки здорових мишей та мишей з прищепленою НК/Лу лімфою. У гомогенатах печінки оцінювали рівень супероксидрадикалу та ТБК-активних продуктів, а також активність каталази, СОД та глутатіон пероксидази. Показано, що рівень супероксидрадикалу, ТБК-активних продуктів та активність каталази були значно вищими в печінці мишей-пухлиноносіїв, аніж у печінці здорових мишей. Встановлено, що сполуки BF1 та PP2 не впливали на величину досліджуваних показників у печінці як мишей-пухлиноносіїв, так і контрольних мишей за винятком того, що сполука PP2 спричинювала значне зниження рівня ТБК-активних продуктів в обох випадках. Одержані результати вказують на те, що досліджувані похідні тіазолу не чинять виразної гепатотоксичної дії на організм здорових мишей та мишей-пухлиноносіїв.

Ключові слова: похідні тіазолу, лімфома NR/Лу, печінка, пероксидне окислення ліпідів, антиоксидантна система.

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