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THE SENSITIVITY OF CELLS WITH THE VARIOUS LEVEL OF NAD(P)H:QUINONE OXIDOREDUCTASE 1 TO CYTOTOXIC ACTION OF QUINONIMINES AND α-TOCOPHEROL SYNTHETIC DERIVATIVES

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The effects of α-tocopherol with shortened to 6 carbon atoms side chain (α-Toc-C_6), α-tocopherol succinate (α-TS) and quinonimine 2,6-dichlorophenolindophenol (DCPIP) on DT-diaphorase activity and viability of rat thymocytes, splenocytes and hepatocytes were investigated. It was shown that the lowest basal activity of the enzyme is inherent in splenocytes. In comparison to splenocytes, DT-diaphorase activity was 1.4 and 5 times higher in thymocytes and hepatocytes, respectively. It was found that the sensitivity of cells to the cytotoxic effect of DCPIP was inversely proportional to the basal level of DT-diaphorase activity and accompanied by its activation with subsequent inhibition at non-toxic and toxic concentrations, respectively. Hepatocytes were least sensitive to the cytotoxic effect of α-Toc-C_6. In thymocytes and splenocytes α-Toc-C_6 exerts inhibitory effects on DT-diaphorase, whereas in hepatocytes an increased activity of the enzyme was observed, which probably caused their high survival rate. Simultaneous induction of cytochrome P450 enzyme expression by α-Toc-C_6 in hepatocytes is also possible. Cytotoxic effect of α-TS does not depend on the basal level of DT-diaphorase activity in cells, is not accompanied by its induction and it is most likely determined by the non-specific esterase activity.

K e y w o r d s: α-tocopherol with shortened to 6 carbon atoms side chain (α-Toc-C_6), α-tocopherol succinate (α-TS), quinonimines, thymocytes, splenocytes, hepatocytes, DT-diaphorase.

NAD(P)H:quinone oxidoreductase 1 (DT-diaphorase, NQO1, 1.6.99.2) is a homodimeric cytosolic flavoprotein. It is involved in at least three systems of biochemical pathways, that is, single-step two-electron reduction of quinones to hydroquinones (without formation of intermediate toxic semiquinone), maintenance of endogenous antioxidants in reduced active form, regulation of the stability of the tumor suppressor and pro-apoptotic p53 factor. Such a wide range of biological activities enables DT-diaphorase to participate in the regulation of antioxidant defenses, detoxification of xenobiotics and programmed cell death [1].

DT-diaphorase is expressed almost in all organs and tissues of mammalians, its activity is modulated rather easy in vivo and in vitro, at that both the enzyme activation and inhibition lead to pronounced physiological response. Participation of DT-diaphorase in the catabolism of heterogeneous components, as well as its antioxidant and cytoprotective properties provide better cells survival in case of its increased activity. Thus, DT-diaphorase activators are traditionally considered as the compounds that reduce the risk of oxidative stress, apoptosis and carcinogenesis [1, 2]. On the other hand, inhibition of the enzyme in cancer cells, in most types of which its activity is significantly higher compared to that in cells of corresponding normal tissue, leads to both reduction of cell resistance to chemotherapeutic agents [3] and reversion of their malignant phenotype [4].

Participation of DT-diaphorase in various biochemical pathways, which support cell homeostasis, determined the search for compounds which biological effects are mediated by modulation of the enzyme activity. These compounds may include components of natural vitamin E and synthetic tocopherol derivatives, which exhibit pro-apoptotic ef-

fects on different cell types [5]. Thus, we have previously found that the reduction of the rat thymocytes viability upon the presence of α-tocopherol with a side chain shortened to 6 carbon atoms (α-Toc-C_6) and α-tocopherol succinate (α-TS) is accompanied by proteolytic degradation of the molecule of DT-diaphorase [6]. At the same time, α-TS inducing apoptosis of PC3 prostate cancer cells, is able to increase simultaneously their resistance to pro-apoptotic effect of quinones. This effect diminished upon inhibition of endogenous DT-diaphorase, although α-TS did not induce the enzyme expression [7]. These data allow us to suggest the existence of dependence of the cytotoxic effects of quinone and α-tocopherol derivatives on the metabolism peculiarity of cells of various origins, in particular, on the basal level of DT-diaphorase activity. It is known that the expression of DT-diaphorase in mammalians has pronounced organ specificity, whereby its activity in the cells of various tissues substantially differ [8].

The aim of this work was a comparative study of the ability of quinonimine and synthetic derivatives of α-tocopherol (α-TP) to induce the death of cells of various origins, differing in the basal levels of DT- diaphorase activity.

Materials and Methods

White female rats (100-150 g body weight) were used in the experiments. All experiments were performed in compliance with the general ethical principles for animals use in experiments (protocol #1 20.03.2015 Committee for control of handling of experimental animals of Palladin Institute of Biochemistry, NAS of Ukraine).

2,6-dichlorophenolindophenol and (+)-α-tocopherol succinate (Sigma, USA) were used in experiments. α-Tocopherol with a side chain shortened to 6 carbon atoms (2,5,7,8-tetramethyl-2-(4′-methyl-3′-pentenyl)-6-oxychroman) was synthesized in the Department of Vitamins and Coenzymes Biochemistry, Palladin Institute of Biochemistry, NAS of Ukraine [9].

Thymocytes and splenocytes were obtained using standard methods [10]. The number of cells was counted and cell viability was determined by dye exclusion method with trypan blue. Cell viability was found to be at least 97%. Cells (at 10^6 cells/ml) were resuspended in RPMI-1640 medium containing 20 mM Hepes-NaOH buffer (pH 7.3), 0.1% BSA, 100 units/ml penicillin, 100 μg/ml streptomycin and 50 μM β-mercaptoethanol.

Hepatocytes were isolated by three-step liver perfusion method in situ, proposed by P. O. Seglen [11]. Enzymatic solution contained 0.075% dispase II and 75 units/ml collagenase (type IV, Sigma, USA). The hepatocytes were washed three times with phosphate-buffered saline (PBS, 136.9 mM NaCl; 2.7 mM KCl; 8.1 mM Na_2HPO_4; 1.5 mM KH_2PO_4; pH 7.2) by gravitational sedimentation for 10 min (4 °C) and then one time with DMEM medium containing 20 mM Hepes-NaOH buffer (pH 7.3), 5% bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin by centrifugation for 30 s at 200 g. Cells were resuspended in the medium at 10^6 cells/ml. Viability of hepatocytes found to be not less than 87%.

About 1 x 10^5 cells in each well of 96-well plate were incubated with studied compounds at concentrations indicated in the figures for 18 h at 37 °C. Hydrophobic substances were added from concentrated stock ethanol solutions previously diluted by culture medium to the final concentration of organic solvent of less than 0.1%. The corresponding amount of ethanol was added to control cells. On completion of the incubation, the cells were precipitated by centrifugation (200 g, for 10 min) and washed with PBS.

Cells viability in routine experiments were measured using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT assay) according to the manufacturer's instructions (Sigma, USA). The absorption of formazan solution in the control cells was taken as 100%.

NAD(P)H:quinone oxidoreductase activity was determined as described in [12]. The cell precipitate after being washed with PBS was mixed with 25 μl of solution consisting of 0.8% digitonin – 2 mM EDTA-Na – 25 mM Tris-HCl buffer, pH 7.8 and incubated for 10 min at 37 °C and then additionally for 10 min at room temperature upon shaking. 100 μl of medium containing 25 mM Tris-HCl buffer, pH 7.5; 0.7 mg/ml BSA; 0.01% Tween-20; 5 μM FAD; 30 μM NADP; 1 mM glucose-6-phosphate; glucose-6-phosphate dehydrogenase (2 units/ml); 0.3 mg/ml MTT and 50 μM menadione were added to well. Samples were incubated at 37 °C, the reaction was terminated by adding 25 μl of solution containing 0.3 mM dicumarol – 5 mM K_3PO_4 in 0.5% DMSO. Absorption was measured at 570 nm on a microplate photometer Multiscan EX (Thermo, USA) referenced against a sample of intact cells incubated in medium supplemented with 0.15 mM dicumarol. The absorption of control cells was taken as 100%.

Protein concentration was determined using the bicinchoninic acid-$CuSO_4$ reagent according to manufacturer's instructions (Sigma, USA).

The obtained data are presented as mean ± standard error of the mean (M ± SEM). For isolation of each cell type, 3-5 independent experiments were performed. Cell incubation was carried out in four parallels for each concentration of the studied compounds. The statistical processing of the obtained data was performed using software SigmaPlot2000 and a Student's *t*-test. The differences were considered significant at $P < 0.05$.

Results and Discussion

DT-diaphorase activity in rats was unevenly distributed in cells of various tissues. Thus, splenocytes are characterized by the lowest enzyme activity (Fig. 1). DT-diaphorase activity in thymocytes was 1.4-fold ($P < 0.05$) and in hepatocytes was 5-fold ($P < 0.05$) higher than that in splenocytes. It should be noted that currently available published data examining the DT-diaphorase distribution in rats regard various tissues but not individual cells [8]. Furthermore, the absolute values of enzyme activity depend on the method of assessment. That does not allow comparing the data obtained by different authors. However, the researchers repeatedly noted the high activity of DT-diaphorase in the rat liver (as well as minor activity in the spleen) [8, 13], that allows us to consider this data as accurate.

Given facts allow us to suggest that the sensitivity of cells with various levels of basal DT-diaphorase activity to apoptosis inducing toxic quinones will also differ. We investigated the cells viability and the DT-diaphorase activity under the action of 2,6-dichlorophenolindophenol (DCPIP) – benzoquinonimine, which is a synthetic substrate of DT-diaphorase. According to the published data, DCPIP induces apoptosis on account of pro-oxidant effect and its cytotoxicity to various melanoma cell lines correlates inversely with the level of DT-diaphorase expression in them [14], providing reduction of DCPIP to its nontoxic leukoform (*p*-aminophenol hydroquinone), thereby preventing the development of oxidative stress.

Splenocytes exhibited the highest sensitivity to the action of DCPIP (Fig. 2, *A*). A pronounced decreasing of their viability was observed at apoptogene concentration of as low as 20 μM. At concentration of 40 μM DCPIP, cell survival decreased by 2.2-fold ($P < 0.05$, hereinafter with respect to control). Toxic DCPIP concentration for thymocytes was 40 μM (1.6-fold, $P < 0.05$), whereas the hepatocytes under these conditions retained full viability (100%).

Assessment of the DT-diaphorase activity in thymocytes (Fig. 2, *B*) revealed a significant activation of the enzyme (1.95-fold, $P < 0.05$) with subsequent inhibition (2.4-fold, $P < 0.05$) under action of DCPIP at a non-toxic and toxic concentrations, respectively. Less pronounced activation of the enzyme was observed in splenocytes and hepatocytes (1.2- and 1.5-fold respectively, $P < 0.05$). At the same time gradual decrease of enzyme activity with increasing concentrations of DCPIP occurred in splenocytes, while DT-diaphorase activity in hepatocytes retained the control level up to a concentration of apoptogene of 40 μM, which probably caused their high survival rate.

Activation of DT-diaphorase in response to the action of its nucleophilic substrates is characteristic of cells of various origins [2], however, it should be noted that the degree of this activation in thymocytes significantly exceeded that in both the splenocytes that slightly differed in basal activity and hepatocytes, in which initial activity of DT-diaphorase was much higher. It is likely, this phenomenon is caused by the different ability of various cells to induce DT-diaphorase gene expression. Furthermore, the possibility and degree of DT-diaphorase activation by chemical compounds can be characterized by the value of its basal activity. Thus, according to the published data, activation of the enzyme under the action of the inducers was not observed in both the cells with initial relatively low and with very high levels of the enzyme activity [15].

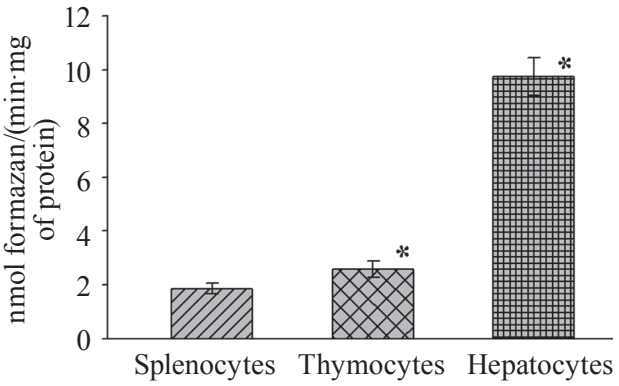

*Fig. 1 Basal activity of DT-diaphorase in cells isolated from different rat tissues (M±SEM, n = 3-5, * P < 0.05)*

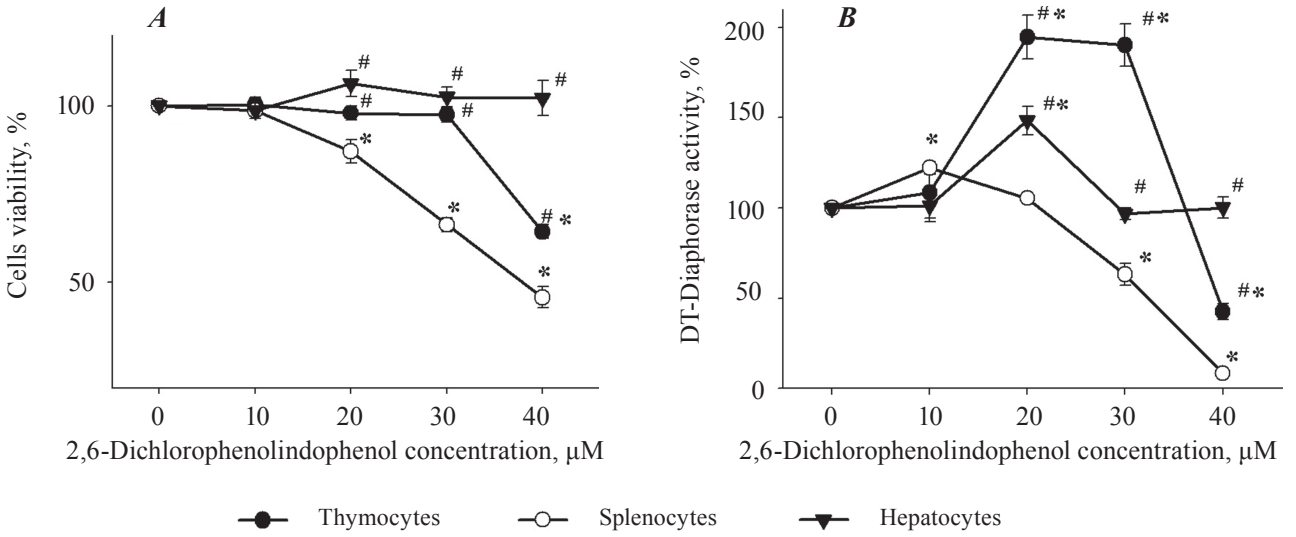

*Fig. 2 Cells viability (A) and DT-diaphorase activity in these cells (B) under the action of 2,6-dichlorophenolindophenol at various concentrations. Here and Fig. 3–4: M±SEM; n = 3-5, * P < 0.05 with respect to the corresponding cell type control, # P < 0.05 with respect to the splenocytes*

The obtained data allow us to conclude that the sensitivity of cells of various organs in rats to the action of cytotoxic DT-diaphorase substrates is inversely related to the basal level of the enzyme activity. From this perspective, it should be noted that the distribution of DT-diaphorase is characterized by not only the organ specificity, but the species one. Thus, the highest enzyme activity in rats is inherent in the liver and lungs [8]. The highest DT-diaphorase activity in humans is observed in the gastrointestinal tract and in the adipose tissue, while levels of the enzyme expression in the heart and liver are extremely low [8, 16]. This phenomenon may cause the high hepatotoxicity of quinones to humans and should be taken into account while interpreting the experimental data obtained for experimental animals.

As it has been noted, there is evidence of a possible involvement of DT-diaphorase in the cytotoxic effect of α-TP derivatives. Previously, we have found that α-TP with a side chain shortened to 6 carbon atoms (α-Toc-C_6) causes the death of thymocytes via necrotic way, accompanied by a decrease in the DT-diaphorase activity and proteolytic degradation of its molecule [6, 17]. The comparative study of the cytotoxic effect of α-Toc-C_6 on cells with different levels of DT-diaphorase activity (Fig. 3, *A*) revealed almost the same negative effect of α-Toc-C_6 on the viability of thymocytes (3.6-fold, $P < 0.05$) and splenocytes (4.1-fold, $P < 0.05$), while survival rate of hepatocytes under these conditions was twice as high (2.2-fold, $P < 0.05$). That is, cells with higher basal levels of DT-diaphorase activity were less sensitive to the cytotoxic effects of both DCPIP and short-chain α-TP analogue.

However, unlike DCPIP, α-Toc-C_6 at nontoxic concentrations did not activate DT-diaphorase in thymocytes or splenocytes (Fig. 3, *B*). At the same time, a decrease in the DT-diaphorase activity was observed in both types of cells at analogue concentrations at which it did not exhibit an effect on cell viability, although this effect in splenocytes was less pronounced (at analogue concentration 20 μM, 1.5 and 1.2-fold, respectively, $P < 0.05$). This fact allows us to suggest that the decrease in the DT-diaphorase activity in thymocytes and splenocytes under α-Toc-C_6 action is one of the causes of its cytotoxicity.

We found that activation of the enzyme (1.46-fold, $P < 0.05$, Fig. 3, *B*) occurred in hepatocytes only, which was unexpected, since, unlike DCPIP, α-Toc-C_6 is not a substrate of DT-diaphorase. It should be noted that hepatocytes physiologically are better adapted to inflow of various natural tocopherols, most of them (except α-TP) exhibit cytotoxic effects on cultured cells. Specific α-TP-transfer protein (TTP) is localized in liver cytosol of mammalians and humans. Complex formation with it facilitates α-TP incorporation into serum lipoproteins, providing its further transport to organs and tissues [18]. It is still unknown whether TTP binds α-Toc-C_6. Though, if the biological binding occurs through the chroman nucleus (which is identical for

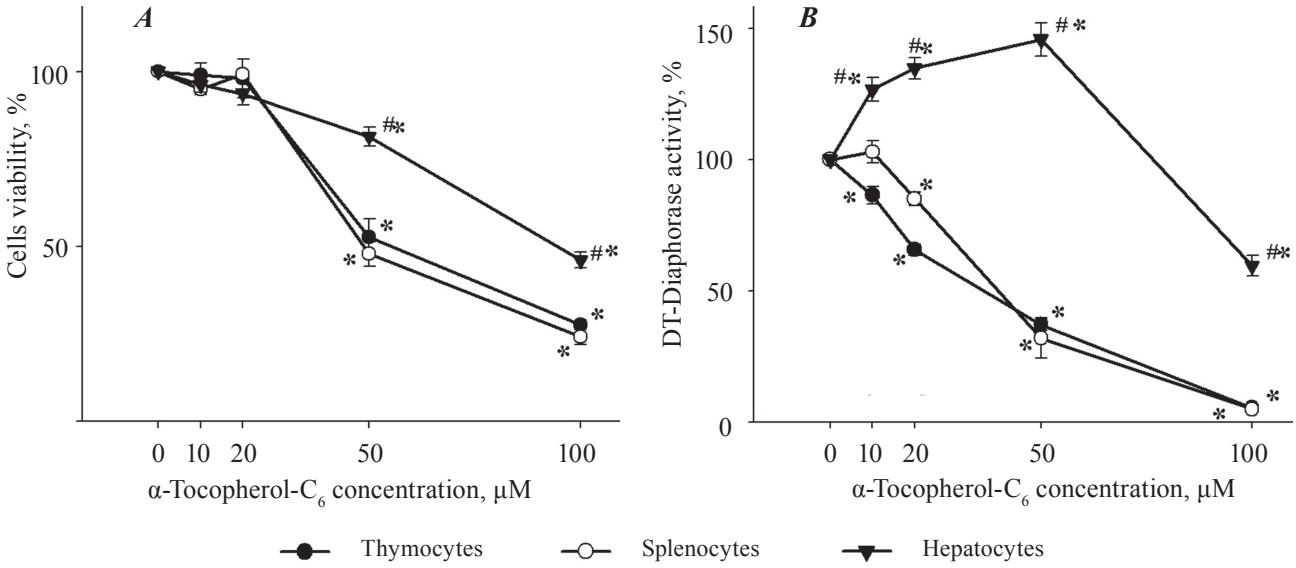

Fig. 3. Cells viability (A) and DT-diaphorase activity in these cells (B) under the action of α-tocopherol-C_6 at various concentrations

both compounds) the probability of this event is relatively high. If it is so, the binding of α-Toc-C_6 with TTP can lead to a reduction of the concentration of free analogue in hepatocytes and consequently to it cytotoxicity. TTP existence in other tissues is less studied, although a recent report on the TTP expression in sheep spleen [19] allow us to suggest its occurrence also in rat splenocytes, which may explain their slightly lower sensitivity (compared to thymocytes) to the inhibitory effect of α-Toc-C_6 at nontoxic concentrations on DT-diaphorase activity (Fig. 3, *B*).

Moreover, activity of cytochrome P450 enzymes, which catalyze the detoxification of xenobiotics, including biological degradation of tocopherols, is the highest in the liver [20]. It is also recognized the ability of vitamin E components to increase gene expression of cytochrome P450 enzymes [21, 22], providing in that way their own biodegradation. From this perspective, an increase of DT-diaphorase activity in hepatocytes under the action of α-Toc-C_6 may be the result of the increase in enzyme expression coordinated with the expression of cytochrome P450 enzymes.

Synthetic α-TP derivative such as α-tocopherol succinate (α-TS) is known as a compound inducing apoptosis of cells of various origin. Pro-apoptotic effect of α-TS is well studied in cancer cell cultures mainly due to the works of J. Neuzil et al. [23] that found the basis for the use of this compound as an antitumor agent. There are numerous and varied assumed mechanisms of α-TS action [24, 25]. According to one of them, DT-diaphorase may be involved in the apoptotic effects of α-TS [7].

Like α-Toc-C_6, α-TS induced death of thymocytes and splenocytes (Fig. 4, *A*), although the latter, despite the lowest basal DT-diaphorase activity, exhibited a higher resistance to the cytotoxic effect of α-TS at a concentration of 50 μM (1.7- and 1.3-fold, respectively, $P < 0.05$). At that, the viability of hepatocytes did not change. In contrast to α-Toc-C_6, no significant increase in the DT-diaphorase activity in hepatocytes under the action of α-TS was observed, and the enzyme inhibition in splenocytes and thymocytes occured only at such analogue concentrations which caused cell death (Fig. 4, *B*).

It is known that hepatocytes are insensitive to the cytotoxic effects of α-TS. Moreover, during induction of hepatocyte death by various toxins such as alkylating agents, iron, doxorubicin, rotenone, etc., α-TS does not enhance their toxicity, but also exhibits cytoprotective property [26] that is characteristic of α-TP. It should be noted that pro-apoptotic effect of α-TS is determined by the intactness of its molecule. Hydrolysis of the ether bond, leading to the formation of α-TP and succinate, neutralizes the cytotoxic effect of the analogue [27]. Therefore, hepatocytes insensity to α-TS is to a smaller degree determined by the high DT-diaphorase activity, and to a greater extent by the high activity of non-specific esterase, which catalyses formation of α-TP, that does not exhibit cytotoxic properties. DT-diaphorase inhibition in thymocytes and splenocytes, in our

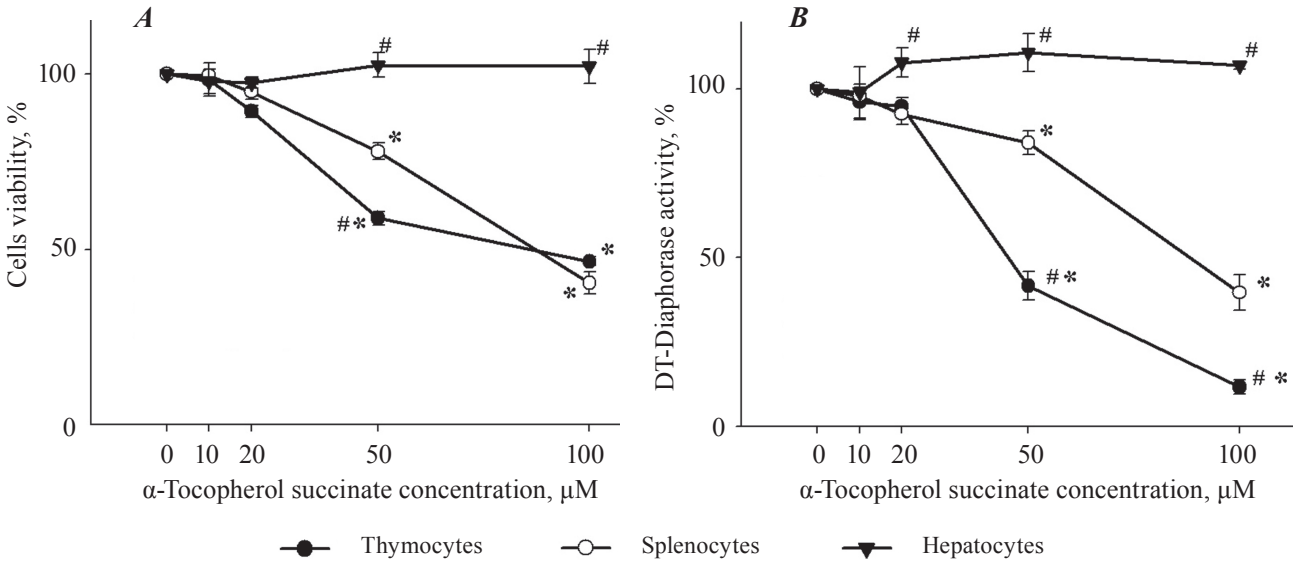

Fig. 4 Cells viability (A) and DT-diaphorase activity in these cells (B) under the action of α-tocopherol succinate at various concentrations

opinion, is not the cause but the consequence of pro-apoptotic effect of α-TS, carried out via mechanisms that do not depend on the basal enzyme activity. Cell death indirectly leads to reduction of activity of key metabolic enzymes, including DT-diaphorase.

The obtained results and their analysis allow us to conclude the following:

1. Sensitivity of cells of various origin to the cytotoxic effect of quinonimine is inversely proportional to the level of basal DT-diaphorase activity.

2. Hepatocytes having high activity of DT-diaphorase are the least sensitive to the cytotoxic effect of α-TP analogue with a side chain shortened to 6 carbon atoms. α-Toc-C_6 exhibits an inhibitory effect on DT-diaphorase in thymocytes and splenocytes, while in hepatocytes an increased enzyme activity was observed, which probably caused their high survival rate. There is also the possibility of simultaneous induction of expression of cytochrome P450 enzymes by α-Toc-C_6 in hepatocytes.

3. The cytotoxic effect of α-TS does not depend on the basal DT-diaphorase activity in the cells, is not accompanied by the enzyme induction and is likely determined by the level of nonspecific esterase activity.

ЧУТЛИВІСТЬ КЛІТИН ІЗ РІЗНИМ РІВНЕМ АКТИВНОСТІ NAD(P)H-ХІНОНОКСИДОРЕДУКТАЗИ 1 ДО ЦИТОТОКСИЧНОЇ ДІЇ ХІНОНІМІНІВ ТА СИНТЕТИЧНИХ ПОХІДНИХ α-ТОКОФЕРОЛУ

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Досліджували вплив α-токоферолу із вкороченим до 6 атомів вуглецю бічним ланцюгом (α-ТФ-C_6), α-токоферилсукцинату (α-ТС) і хініноміну 2,6-дихлорофеноліндофенолу на активність DT-діафорази й життєздатність тимоцитів, спленоцитів і гепатоцитів щурів. Встановлено, що спленоцитам властива найменша базальна активність ензиму. Активність DT-діафорази в тимоцитах у 1,4 раза, а у гепатоцитах – у 5 разів перевищує таку в спленоцитах. Виявлено, що чутливість клітин до цитотоксичної дії хінонімінів обернено

пропорційна рівню базальної активності DT-діафорази й супроводжується її активацією з подальшим інгібуванням у нетоксичних і токсичних концентраціях відповідно. Гепатоцити найменш чутливі до цитотоксичної дії α-ТФ-C_6. У тимоцитах і спленоцитах α-ТФ-C_6 виявляє інгібуючі DT-діафоразу ефекти, у той час як у гепатоцитах спостерігається підвищення активності ензиму, що, ймовірно, і обумовлює їх високу виживаність. Не виключена також можливість одночасної індукції α-ТФ-C_6-м експресії ензимів системи цитохрому Р450 у гепатоцитах. Цитотоксична дія α-ТС не залежить від базальної активності DT-діафорази у клітинах, не супроводжується її індукцією і, найімовірніше, визначається рівнем активності неспецифічної естерази.

Ключові слова: α-токоферол із вкороченим до 6 атомів вуглецю бічним ланцюгом, α-токоферилсукцинат, хініміни, спленоцити, тимоцити, гепатоцит, DT-діафораза.

ЧУВСТВИТЕЛЬНОСТЬ КЛЕТОК С РАЗЛИЧНЫМ УРОВНЕМ АКТИВНОСТИ NAD(P)H-ХИНОН-ОКСИДОРЕДУКТАЗЫ 1 К ЦИТОТОКСИЧЕСКОМУ ДЕЙСТВИЮ ХИНОНИМИНОВ И СИНТЕТИЧЕСКИХ ПРОИЗВОДНЫХ α-ТОКОФЕРОЛА

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Исследовали влияние α-токоферола с укороченной до 6 атомов углерода боковой цепью (α-ТФ-C_6), α-токоферилсукцината (α-ТС) и хинонимина 2,6-дихлорофенолиндофенола на активность DT-диафоразы и жизнеспособность тимоцитов, спленоцитов и гепатоцитов крыс. Установлено, что спленоцитам присуща самая низкая базальная активность энзима. Активность DT-диафоразы в тимоцитах в 1,4 раза, а в гепатоцитах – в 5 раз превышает таковую в спленоцитах. Выявлено, что чувствительность клеток к цитотоксическому действию хинониминов обратно пропорциональна уровню базальной активности DT-диафоразы и сопровождается ее активацией с дальнейшим ингибированием в нетоксичных и токсичных концентрациях соответственно. Гепатоциты наименее чувствительны к цитотоксическому действию α-ТФ-C_6. В тимоцитах и спленоцитах α-ТФ-C_6 выявляет ингибирующие DT-диафоразу эффекты, в то время как в гепатоцитах наблюдается повышение активности энзима, что, вероятно, и обусловливает их высокую выживаемость. Не исключена также возможность одновременной индукции α-ТФ-C_6-м экспрессии энзимов системы цитохрома Р450 в гепатоцитах. Цитотоксическое действие α-ТС не зависит от базальной активности DT-диафоразы в клетках, не сопровождается ее индукцией и, вероятнее всего, определяется уровнем активности неспецифической эстеразы.

Ключевые слова: α-токоферол с укороченной до 6 атомов углерода боковой цепью, α-токоферилсукцинат, хинонимины, спленоциты, тимоциты, гепатоциты, DT-диафораза.

References

1. Dinkova-Kostova A. T., Talalay P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch. Biochem. Biophys.* 2010;501(1):116-123.
2. Nioi P., Hayes J. D. Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat. Res.* 2004; 555(1-2):149-171.
3. Zeekpudsa P., Kukongviriyapan V., Senggunprai L., Sripa B., Prawan A. Suppression of NAD(P)H-quinone oxidoreductase 1 enhanced the susceptibility of cholangiocarcinoma cells to chemotherapeutic agents. *J. Exp. Clin. Cancer Res.* 2014;33:11.
4. Cullen J. J., Hinkhouse M. M., Grady M., Gaut A. W., Liu J., Zhang Y. P., Weydert C. J., Domann F. E., Oberley L. W. Superoxide-mediated mechanism induces growth inhibition of pancreatic cancer via a dicumarol inhibition of NADPH:quinone oxidoreductase. *Cancer Res.* 2003;63(17):5513-5520.

5. Constantinou C., Papas A., Constantinou A. I. Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. *Int. J. Cancer.* 2008;123(4):739–752.
6. Petrova G. V., Donchenko G. V., Klimenko K. P. Effects of α-tocopherol and its derivatives on the NAD(P)H:quinone oxidoreductase 1 level in rat thymocytes. *Reports NASU.* 2014;(4):156-161. (in Russian).
7. Bellezza I., Grottelli S., Gatticchi L., Mierla A. L., Minelli A. α-Tocopheryl succinate pre-treatment attenuates quinone toxicity in prostate cancer PC3 cells. *Gene.* 2014;539(1):1-7.
8. Rooseboom M., Commandeur J. N. M., Vermeuler N. P. E. Enzyme-catalyzed activation of anticancer prodrugs. *Pharmacol. Rev.* 2004;56(1):53-102.
9. Pat. 21527 UA, ICP A61K 31/355, C07D 311/72. A Technique for Obtaining of the 2,5,7,8-tetramethyl-2-(4-methyl-3-pentenyl)-6-chromanol acetate / Danevich O. I., Kirey Z. M., Andreichuk P. E., Kosenko M. V., Donchenko G. V., Klimenko K. P., Borutska Z. P., Borodina L. O., Svischuk O. A., Makovetsky V. P., Kuzmenko I. V. Publ. 28.02.2000, Bul. N 1. (In Ukrainian).
10. Lymphocytes. A practical approach. Edit by J. Klaus. Moscw.: Mir, 1990. 393 p. (in Russian).
11. Seglen P. O. Preparation of isolated rat liver cells: The enzymatic preparatıon of isolated intact parenchymal cells from rat liver. *Methods Cell. Biol.* 1976;13:29-83.
12. Prochaska H. J., Santamaria A. B. Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells. A screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* 1988;169(2):328-336.
13. Bárta F., Levová K., Frei E., Schmeiser H. H., Arlt V. M., Stiborová M. The effect of aristolochic acid I on expression of NAD(P) H:quinone oxidoreductase in mice and rats - a comparative study. *Mutat. Res. Genet. Toxicol. Environ Mutagen.* 2014;768:1-7.
14. Cabello C. M., Bair W. B. 3rd, Bause A. S., Wondrak G. T. Antimelanoma activity of the redox dye DCPIP (2,6-dichlorophenolindophenol) is antagonized by NQO1. *Biochem. Pharmacol.* 2009;78(4):344-354.
15. Danson S., Ward T.H., Butler J., Ranson M. DT-diaphorase: a target for new anticancer drugs. *Cancer Treatment Reviews.* 2004;30(5):437-449.
16. Siegel D., Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. *Free Radic. Biol. Med.* 2000;29(3-4):246-253.
17. Petrova G. V., Donchenko G. V. The cytotoxicity of troglitazone, a structural analogue of α-tocopherol is mediated by inhibition of NAD(P)H:quinone oxidoreductase. *Ukr. Biokhim. Zhurn.* 2009;81(4):105-111. (in Russian).
18. Niki E., Traber M.G. A history of vitamin E. *Ann. Nutr. Metab.* 2012;61(3):207-212.
19. Zuo Z., Luo H., Liu K.,. Jia H, Zhang Y., Jiao L., Chang Y. Dietary vitamin E affects α-TTP mRNA levels in different tissues of the Tan sheep. *Gene.* 2014;541(1):1-7.
20. Abe C., Uchida T., Ohta M., Ichikawa T., Yamashita K., Ikeda S. Cytochrome P450-dependent metabolism of vitamin E isoforms is a critical determinant of their tissue concentrations in rats. *Lipids.* 2007;42(7):637-645.
21. Mustacich D .J., Leonard S. W., Devereaux M. W., Sokol R. J., Traber M. G. Alpha-tocopherol regulation of hepatic cytochrome P450s and ABC transporters in rats. *Free Radic. Biol. Med.* 2006;41(7):1069-1078.
22. Landes N., Pfluger P., Kluth D., Birringer M., Rühl R., Böl G.F., Glatt H., Brigelius-Flohé R. Vitamin E activates gene expression via the pregnane X receptor. *Biochem. Pharmacol.* 2003;65(2):269-373.
23. Tomasetti M., Santarelli L., Alleva R., Dong L. F., Neuzil J. Redox-active and redox-silent compounds: synergistic therapeutics in cancer. *Curr. Med. Chem.* 2015;22(5):552-568.
24. Zhao Y., Neuzil J., Wu K. Vitamin E analogues as mitochondria-targeting compounds: from the bench to the bedside? *Mol. Nutr. Food. Res.* 2009;53(1):129-139.
25. Angulo-Molina A., Reyes-Leyva J., López-Malo A., Hernández J. The role of alpha tocopheryl succinate (α-TOS) as a potential anticancer agent. *Nutr. Cancer.* 2014;66(2):167-176.
26. Zhang J. G., Nicholls-Grzemski F. A., Tirmenstein M. A., Fariss M. W. Vitamin E succinate protects hepatocytes against the toxic effect

of reactive oxygen species generated at mitochondrial complexes I and III by alkylating agents. *Chem. Biol. Interact.* 2001;138(3):267-284.

27. Farris M. W., Fortuna M. B., Everen C. K., Smith J. D., Trent D. F., Djuric Z. The selective antiproliferative effects of alpha-tocopheryl hemisuccinate and cholesteryl hemisuccinate on murine leukemia cells result from the action of the intact compounds. *Cancer Res.* 1994;54(13):3346-3351.

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