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# HEPATOPROTECTIVE EFFECT OF UBIQUINONE AND COMPLEX OF PRECURSORS AND MODULATOR OF ITS BIOSYNTHESIS UNDER DOXORUBICIN TREATMENT

*The important part of doxorubicin toxicity is an activation of MMPs, which leads to increased destruction of extracellular matrix. The mitochondrial electron-transport chain function was found to be impaired in animals treated with doxorubicin. The decrease in CoQ radical – ubisemiquinone – level was found in organs of animals treated with doxorubicin in therapeutic dosage. The treatment by ubiquinone and complex of precursors of modulator of ubiquinone biosynthesis in parallel to doxorubicin leads to significant decrease in tissue MMPs activities, increase of ubisemiquinone content and normalization of mitochondrial electron-transport chain function, which leads to notable reduction of doxorubicin toxicity.*

*Key words*: ubiquinone; ubisemiquinone; matrix metalloproteinases

## INTRODUCTION

Doxorubicin is an antracycline antibiotic widely used as antineoplastic agent. The antitumor effect of Doxorubicin correlates with dose-dependent liver toxicity, cardiac toxicity, etc. [13]. The main role in Doxorubicin liver toxicity may belong to increased reactive oxygen spieces (ROS) levels, that lead to oxidative damage to lipids, proteins, nuclear and mitochondrial DNA. It has been suggested that under antioxidant treatment (such as vitamin E and ubiquinone) the antineoplastic activity of Doxorubicin is improved via decreased levels of mitochondrial-produced ROS and the side toxic effects of Doxorubicin treatment are decreased [9, 14]. Ubiquinone (Coenzyme Q, CoQ) plays an important role in mechanisms of cellular functioning not only as an electron transport chain (ETC) component, but also as a regulator of gene expression, signal transduction, apoptosis, etc. [4, 12]. The inhibition of regulation and level of CoQ biosynthesis its dietary intake cannot satisfy the physiological needs of mammal organism fully [4, 8, 12]. Thus, an additional supplementation of CoQ in the form of oral pharmaceuticals is required to satiate the organism's CoQ needs. Nevertheless, the approach based on substrate activation has certain drawbacks. Particularly it is the high cost of therapy course (that frequently requires dosage of 100-350 mg per day for 5-6 months), the continued suppression of endogenous CoQ synthesis (possibly through the substrate-enzyme inhibition mechanism) that limits CoQ applicability in medical pratice [4, 8, 12]. Therefore, the aim of this project was to study the activities of mitochondrial ETC complexies I, II and IV, CoQ content and redox state, and active MMP-2 and MMP-9 content in rat liver under treatment with Doxorubicin, $CoQ_{10}$ medical, and complex preparation of modulators and precursors of CoQ biosynthesis.



## MATERIALS AND METHODS

Male white rats of 180-220 g body mass were assigned into 4 groups: 1) control (intact) animals; 2) animals subjected to Doxorubicin treatment; 3) animals subjected to combined Doxorubicin and α-tocopherol acetate, 4-hydroxybenzoic acid, and methionine treatment ("EPM" complex); 4) animals subjected to combined Doxorubicin and $CoQ_{10}$ treatment ("Qudesan", Akvion Co, the Russian Federation). Doxorubicin (Kyivmedpreparat JSC, Ukraine) was administered intraperitoneally in doses of 2.2 mg/kg of body mass daily for 8 days [7, 11]. The rats of the control group were administered the corresponding volume of normal saline. The animals were sacrificed by decapitation in accordance with intenational regulations on treatment of laboratory animals. CoQ content was assayed after [2]. Ubisemiquinones content was evaluated by EPR on digitalized PE-1307 spectrometer [1]. NADH-CoQ-oxidoreductase (NQR) (EC1.6.5.3) activity (Complex I) and succinate-CoQ-oxidoreductase (SQR) (EC 1.3.5.1) activity (Complex II) were assayed after [15] and [6], cor-

Table

**CoQ AND UBISEMIQUINONE CONTENT, NQR (COMPLEX I), SQR (COMPLEX II), AND CYTOCHROME C OXIDASE (COMPLEX IV) ACTIVITIES, AND MMP2 AND MMP9 ACTIVITIES (CONCENTRATION OF ACTIVE FORMS OF THE ENZYME) IN LIVER TISSUE AND MITOCHONDRIA OF ANIMALS UNDER DOXORUBICIN ADMINISTRATION AND TREATMENT WITH EPM COMPLEX AND $CoQ_{10}$ (M ± m, n = 6)**

| | Control | Doxorubicin | Doxorubicin + EPM | Doxorubicin + $CoQ_{10}$ |
|---|---|---|---|---|
| CoQ content in liver tissue, μg/g of protein | 190,70 ± 44,24 | 99,01 ± 18,46* | 207,15 ± 37,99# | 176,37 ± 29,91# |
| CoQ content in liver mitochondria, μg/g of protein | 69,70 ± 9,41 | 114,74 ± 4,67* | 83,85 ± 13,52# | 63,02 ± 2,74# |
| Ubisemiquinone level in liver tissue, c.u. | 1,5 ± 0,14 | 0,7 ± 0,08* | 1,7 ± 0,1# | 1,3 ± 0,01# |
| Complex I, mmol NADH × (min × mg of protein)$^{-1}$ | 12,26 ± 1,41 | 7,45 ± 1,01* | 15,93 ± 2,42# | 12,62 ± 1,99# |
| Complex II, mmol of succinate × (min × mg of protein)$^{-1}$ | 16,38 ± 0,83 | 12,66 ± 1,29* | 19,71 ± 2,96# | 17,18 ± 1,28# |
| Complex IV, mmol of cytochrome *c* × (hr × mg of protein)$^{-1}$ | 1,69 ± 0,26 | 1,18 ± 0,13* | 1,43 ± 0,04# | 1,34 ± 0,09 |
| MMP2, μg/g of fress tissue | 0,05 ± 0,003 | 6,6 ± 0,5* | 4,2 ± 1,8* | 0,1 ± 0,008*# |
| MMP9, μg/g of fress tissue | 0,02 ± 0,001 | 0,6 ± 0,3* | 7,2 ± 4,8*# | 0,12 ± 0,003*# |

* – the difference with Control group is statistically significant; # – the difference with Doxorubicin group is statistically significant; $P < 0.05$.

respondingly. Cytochrome c oxidase activity (EC 1.9.3.1) (Complex IV) was assayed spectrophotmetrically after intesity of oxidation of reduced cytochrome c by the enzyme [5]. Protein content was determined with Lowry method [10]. Active MMP-2 and MMP-9 content was determined with zymography on PAA gel [3]. The results were processed with variational statistics methods. The numerical data are presented as median with standard deviation (M ± m). Reliability of inter-median differences was assayed after Student's t-test.

## RESULTS AND DISCUSSION

Doxorubicin administration correlates with twofold decrease in CoQ levels of liver tissue if compared to control (Table). Concurrently to this, CoQ content in liver mitochondria increases in nearly 1.6 times. The levels of CoQ in tissue and mitochondria of liver are normalized under treatment with EPM complex and $CoQ_{10}$ in addition to Doxorubicin administration. The established consensus is that CoQ interacts with ETC enzyme complexes not directly, but through its semi-reduced free-radical form – ubisemiquinone. The high liver levels of ubisemiquinones are characteristic for normal functioning of CoQ cycle in mitochondria [12]. The ubisemiquinone content in liver under Doxorubicin administration was observed to decrease in 2.1 times, accordingly, if compared to control (Table). The increased CoQ content in liver mitochondria (Table) that coincedes with decreased ubisemiquinone content prompts us to suppose the transition of the available CoQ in liver into fully reduced or fully oxidized state. Treatment of experimental animals with EPM and CoQ in addition to Doxorubicin administration leads to increase in ubisemiquinone levels in liver tissue in comparison to Doxorubicin-only group.

Our studies demonstrate the decrease of Complex I, II and IV activities in liver mitochondria under Doxorubicin administration (Table). Administration of EPM Complex and $CoQ_{10}$ leads to increase in activities of these mitochondrial ETC complexies to levels of control animals.

MMP2 and MMP9 activity (concentration of active forms) in liver tissue increases significantly under Doxorubicin administration if compared to control (Table). Treatment of experimental animals with $CoQ_{10}$ in addition to Doxorubicin leads to decrease of many times in activity of both gelatinases in liver. Treatment with EPM complex in addition to Doxorubicin administration was also observed to significantly decrease the MMP2 activity and increase MMP9. It has been established that MMP9 is a vascular-specific gelatinase that plays a key part in angiogenesis. The EPM complex, as administered with Doxorubicin, may possibly promote angiogenesis through MMP9 activation as part of oraganism's adaptation to chronic effect of toxic agent. Thus Doxorubicin administration leads to increased gelatinases activity (which are known to be regulated by ROS) associated with decrease in ubisemiquinone content caused by disruption of electron transport.

Treatment of experimental animals with EPM complex and $CoQ_{10}$ in addition to Doxorubicin administration leads to decrease in MMP2 and MMP9 activities assompanied by increased ubisemiquine content in the tissues. Consequently, the inverse correlation between these events reflects the magnitude and trend in oxidative stress development.

## CONCLUSIONS

1. Doxorubicin may deprieve cells of one of the most important preconditions of their normal functioning – namely, the ability to maintain the redox state homeostasis and the pool of oxidative-reductive components. Mitochondrial dysfunction and, primarily, mitochondrial I and III complexes dysfunction, forms

the basis of oxidative stress progression and is the molecular mechanism underlying the disorders of energy exchange that result in all known forms of hypoxia and proteinase activation

2. Treatment of experimental animals with EPM complex and $CoQ_{10}$ in addition to Doxorubicin administration exerts a protective effect on liver cells' mitochondria, evidenced by restoration of electron transport in respiratory chain. It should be stressed that the protective effects of EPM complex on mitochondrial ETC under Doxorubicin administration is on par with those of $CoQ_{10}$. Concurrently, MMP2 and MMP9 activities are decreased, which gives evidence of lessened extracellular matrix destruction. $CoQ_{10}$ appeared to be more efficient at this if compared to EPM complex.
3. The expiremental data obtained may become the basis of development of approaches to correction of liver toxic effects of Doxorubicin by treatment with $CoQ_{10}$ and the complex of precursors and modulators of its biosynthesis. These data may be used to substantiate the application of these biologically active substances within frameworks of complex treatment of different pathologies.

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**ГЕПАТОПРОТЕКТОРНОЕ ДЕЙСТВИЕ УБИХИНОНА И КОМПЛЕКСА ПРЕДШЕСТВЕННИКОВ И МОДУЛЯТОРА ЕГО БИОСИНТЕЗА ПРИ ВВЕДЕНИИ ДОКСОРУБИЦИНА**

Важной составляющей токсического эффекта доксорубицина является активация матриксных металлопротеиназ, что может быть причиной усиления деструкции межклеточного матрикса. При действии на организм животных доксорубицина показано угнетение активности комплексов I, II и IV цепи транспорта электронов в митохондриях, снижение содержания убисемихинона. При одновременном использовании доксорубицина, препарата убихинона и комплекса предшественников и модулятора биосинтеза убихинона значительно снижается уровень активности матриксных металлопротеиназ в тканях, возрастает содержание убисемихинона и нормализуется работа цепи транспорта электронов в митохондриях, что существенно нивелирует токсическое действие доксорубицина.

**Ключевые слова**: убихинон; убисемихинон; матриксные металлопротеиназы

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**ГЕПАТОПРОТЕКТОРНА ДІЯ УБІХІНОНУ ТА КОМПЛЕКСУ ПОПЕРЕДНИКІВ І МОДУЛЯТОРА ЙОГО БІОСИНТЕЗУ ЗА УМОВ ВВЕДЕННЯ ДОКСОРУБІЦИНУ**

Важливою складовою токсичного ефекту доксорубіцину є активація матриксних металопротеїназ, що може бути причиною посилення деструкції міжклітинного матриксу. За дії на організм тварин доксорубіцину продемонстровано пригнічення активності комплексів I, II і IV ланцюга транспорту електронів у мітохондріях, зниження вмісту убісемихінону. При одночасному застосуванні доксорубіцину, препарату убіхінону та комплексу попередників і модулятора біосинтезу убіхінону значно знижується рівень активності матриксних металопротеїназ, зростає вміст убісемихінону і нормалізується робота ланцюга транспорту електронів у мітохондріях, що суттєво нівелює гепатотоксичну дію доксорубіцину.

**Ключові слова**: убіхінон; убісемихінон; матриксні металопротеїнази

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