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MECHANISMS OF MODULATING ACTION OF THYMOQUINONE (COMPONENT OF BLACK CUMIN, *Nigella sativa*), AFFECTING THE ACTIVITY OF SOME NUCLEAR AND MITOCHONDRIAL GENES IN MICE TISSUES AFTER EXPOSURE TO X-RAY RADIATION

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Abstract. The paper discusses a promising herbal preparation – thymoquinone, a component of black cumin (*Nigella sativa*), studied in experimental animals (mice, rats) in many pathologies, characterized by a positive effect and lack of toxic effect. The drug has been studied in a wide range of doses for injection and oral administration. Thymoquinone has antimicrobial, antiviral, anti-inflammatory, and radioprotective properties. The main damaging component of ionizing radiation is oxidative stress. For this reason, radioprotectors have recently been evaluated based on the drug ability to reduce oxidative stress. As markers of oxidative stress, we used parameters of changes in the expression of nuclear and mitochondrial DNA genes that perform essential functions in the cell. *C57Bl/6* mice were administered thymoquinone (10 mg/kg) after 30 min irradiation was performed (6 Gy). After 6 and 24 hours, gene expression in brain and spleen cells was studied using real-time PCR. It was shown that the activity of nuclear genes increased after exposure to radiation, but was normalized if thymoquinone was administered to mice 30 minutes before irradiation. Mitochondrial genes were also modified to target the activity of control cells. The test results show that thymoquinone has protective properties and may be promising as a radioprotector.

Keywords: *radioprotectors, thymoquinone, nuclear and mitochondrial genes*

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INTRODUCTION

The need to study radiation effects on humans is associated with a wide range of its applications in medicine for diagnostic or therapeutic procedures, contact with various radiation exposures in industrial settings, as well as possible consequences of atomic or nuclear use [1]. An increase in the duration and length of space flights is expected, which may be accompanied by increased exposure to radioactive background, therefore additional preventive measures are needed to preserve the health of astronauts. For example, some herbal preparations with a wide spectrum of protective effects, including radioprotective properties, can be used for preventive purposes [2].

Special attention is paid to the effects of radiotherapy as one of the methods for treating cancer, as the side effects of radiation in some cases may exceed the positive results of treatment. This is associated with oxidative

stress accompanying radiation exposure, as the impact of free radicals plays an important role in the pathogenesis of certain diseases, and consequently leads to an imbalance between oxidative and antioxidant parameters. For instance, oxidative stress can cause radiation-induced pneumonia, which may develop even 6 months after radiotherapy for lung cancer. Radioprotectors with antioxidant properties can protect cell membranes and DNA from the side effects of radiotherapy [3]. Complications during radiotherapy for prostate cancer may include cystitis, rectitis, and in breast cancer – fibrosis, which also dictates the need for protection of tissues surrounding the tumor. One way to protect normal tissues during radiotherapy may be the use of low doses of radiation, which stabilize normal cells, making them more resistant to high doses of radiation [4].

In recent years, natural products such as plants and their components have begun to be used as radioprotectors or compounds that reduce the toxic effects of radiation during radiotherapy [5]. An increasing number of studies are devoted to investigating the preventive and therapeutic properties of thymoquinone (TQ) – a component of *Nigella sativa*, which does not possess toxic properties and positively affects various endogenous and exogenous human pathologies [6]. Black cumin and its active component – TQ, exhibit various pharmacological activities against many diseases (viral, bacterial, as well as in cardiopathology and tumor formation), due to their antioxidant properties. Cell protection from oxidative stress and inflammation is regulated through *Nrt2* and *NFkB* pathways. This drug is characterized by neuroprotective, nephroprotective, gastroprotective, hepatoprotective, and anticarcinogenic effects [7]. TQ inhibits the proliferation of tumor cells, which is associated with increased activity of phosphatase and tensin homolog, ultimately leading to the suppression of *P13K* (AKT pathway). It is known that cell cycle arrest under the action of TQ is accompanied by effects on the activity of *P53*, *STAT3* genes, as well as on the mitochondrial pathway of apoptosis. Several authors consider TQ promising for the prevention and treatment of various pathologies [8]. TQ also possesses radioprotective properties, as it can reduce oxidative stress indicators in brain cells of rats subjected to total irradiation. This is associated with increased superoxide dismutase activity and scavenging of free radicals formed during radiation exposure. Such a mechanism protects cell membranes, which helps to protect brain cells from radiation-induced damage [9]. TQ promoted the activation of antioxidant enzymes (glutathione peroxidase, glutathione S-transferase, superoxide dismutase, etc.) in rats after cranial irradiation. TQ prevented the increase in malondialdehyde levels caused by radiation exposure [10]. As is known, the damaging effect of ionizing radiation on tumor cells is also accompanied by effects on normal cells of the body [11]. In rats that were administered TQ at a concentration of 10 mg/kg 30 minutes before whole-body irradiation (6 Gy), liver cells, parotid glands, brain, and testes were examined based on the criterion of malondialdehyde content, which is a marker of oxidative stress, as well as glutathione peroxidase. In irradiated cells with pre-administered TQ, a decrease in malondialdehyde levels and activation of antioxidant enzymes were observed. In rats that received *Nigella sativa* oil during head irradiation, the histological structure of the parotid glands was restored, fibrosis around the ducts was reduced, and the level of *TGF-β* decreased [12]. The authors believe that TQ is an ideal protector that protects normal cells during tumor irradiation. Thus, it has been shown that TQ reduces the effect of radiation, protecting normal cells from damaging effects and enhancing the immune status of the organism.

The authors, studying the radioprotective activity of TQ, used indicators of oxidative stress. They also investigated the effects of TQ on the activity of several genes, including mitochondrial ones, which were previously characterized by us as sensitive to radiation [4, 13, 14]. Mitochondrial genes are more sensitive to damaging factors. For example, when a cell is exposed to radiation or chemical mutagens, mitochondrial genes suffer 3–50 times more damage than nuclear genes [15]. For this reason, we investigated the activity of several genes and both nuclear and mitochondrial DNA (n- and mtDNA), after radiation exposure and TQ administration.

MATERIALS AND METHODS

The study used male *C57Bl/6* mice at 2 months of age weighing 22–25 g, obtained from the experimental animal breeding facility of the Branch of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Pushchino, Moscow region). All animal experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Directive 2010/63/EU. During the experiment, the animals were kept in standard vivarium conditions at the FSBI SSC FMBC named after A. I. Burnazyan of FMBA of Russia The animals were managed in polycarbonate cages on an IVC (individual ventilation cages) system with sterile air supply (Pharmbioline, Finland). The animals were acclimatized for 1 week before the start of experiments. Mice were fed standard pelleted laboratory animal food (LLC "Mest",

Moscow) with free access to clean drinking water. The animals were housed five per cage under a standard 12-hour light/dark cycle at a temperature of 22±2°C and humidity of 45±5%.

Animal irradiation was carried out at the FSBI SSC FMBC named after A. I. Burnazyan of FMBA of Russia using the RUST-M1 X-ray biological unit at a voltage of 200 kV, tube current of 2.5 mA, and a 1.5 mm aluminum filter. The X-ray irradiation dose rate was 1 Gy/min. Mice in plastic containers (5 specimens together) were exposed to irradiation at a dose of 6 Gy.

Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) (Merck, Darmstadt, Germany) was administered to animals intraperitoneally at 10 mg/kg body weight. The substance was administered 30 minutes before irradiation.

To isolate brain and spleen tissues, mice were euthanized by decapitation 6 and 24 hours after irradiation. Control groups included both non-irradiated and irradiated mice that did not receive TQ. Brain and spleen were isolated and frozen at −80°C until analysis.

Total RNA from spleen and brain tissues was extracted using ExtractRNA isolation kit (Evrogen, Russia) according to manufacturer's instructions. RNA concentration was determined using NanoVuePlus spectrophotometer (GEHealthcareTM, USA) and adjusted to 400 ng/μl. Two μg of RNA was reverse transcribed to complementary DNA (cDNA) using MMLV reverse transcriptase (Evrogen, Russia) according to manufacturer's protocol in a total reaction volume of 20 μl. Real-time PCR was performed on DT-prime instrument ("DNA-Technology", Russia) using qPCRmix-HSSYBR Low ROX kit (Evrogen, Russia) according to manufacturer's protocol. The reaction mixture contained 4 μl of 20-fold diluted cDNA and 250 nM of each primer. Primer sequences and PCR conditions for nuclear genes were used from our previously published work [16], primer sequences for mitochondrial genes are presented in work [17]. Melting curve analysis was performed for all genes, and specificity and integrity of PCR products were confirmed by the presence of a single peak. The 2-ΔΔCT method was used for analysis. PCR tests were performed in triplicate for each sample. Target gene transcription levels were normalized to the *GAPDH* reference gene. Normalized gene expression values were analyzed using PrismGraphPad 7.0 software. Gene expression in control (intact) mice was taken as 100%. Statistical processing of results was performed using "STATISTICA 7.0" software. Mann-Whitney non-parametric test was used to assess significance of differences. Differences were considered significant at $p < 0.05$.

RESULTS

The expression of several nuclear and mitochondrial DNA genes was studied in spleen and brain tissues of mice that were administered TQ at a dose of 10 mg/kg before irradiation (6 Gy).

In Fig. 1, it can be seen that in the spleen tissue after irradiation of mice, there was a statistically significant increase in the expression of *IAP-1* and *IKBa* oncogenes compared to animals that were injected with TQ only without irradiation. In the brain tissues of irradiated mice, an increase in the expression of genes *NFKB (p50)*, which control cell proliferation and inflammation processes, and the oncogene *iNOS* was observed compared to control animals. From the data in Fig. 1, it can be seen that the administration of TQ to mice led to a decrease in the expression of these genes in both the spleen and brain tissues of mice 24 hours after irradiation. These data may indicate the protective effect of TQ on the studied nuclear genes in terms of modification of their activity.

Fig. 2 presents the results of TQ influence on the expression of mtDNA genes involved in oxidative phosphorylation and energy balance in the spleen and brain tissues of irradiated mice that were administered TQ. These results show that 6 and 24 hours after irradiation, there is a decrease in the activity of all studied genes: the 2nd subunit of NADH-dehydrogenase — a component of complex 1, cytochrome *b* (*CYT-B*) — a component of complex III, the 6th subunit of ATP-synthase — a component of complex 5. The decrease in the expression of these genes was recorded in the tissues of the spleen and brain. A more pronounced decrease in the activity of these genes in the post-radiation period was observed in the spleen tissue compared to the indicators in the brain tissue. However, in irradiated animals that were administered TQ, an increase in gene activity was observed in both studied tissues, which also confirms the ability of TQ to protect not only nuclear but also mitochondrial DNA.

DISCUSSION

One of the effective and common methods of treating cancer is radiotherapy. However, radiation affects not only tumor cells but also normal ones by increasing the level of free radicals. It has been previously shown that such changes can cause damage to lung tissue and lead to the development of radiation-induced pneumonia after 6 months, which is often fatal. In rats with lung irradiation (5 Gy) and administration of TQ 30 minutes before irradiation (50 mg/kg) and for 10 days after irradiation, a decrease in all indicators of oxidative stress was shown [3]. TQ does not have toxic properties, but the variety of its activity is related to the concentration

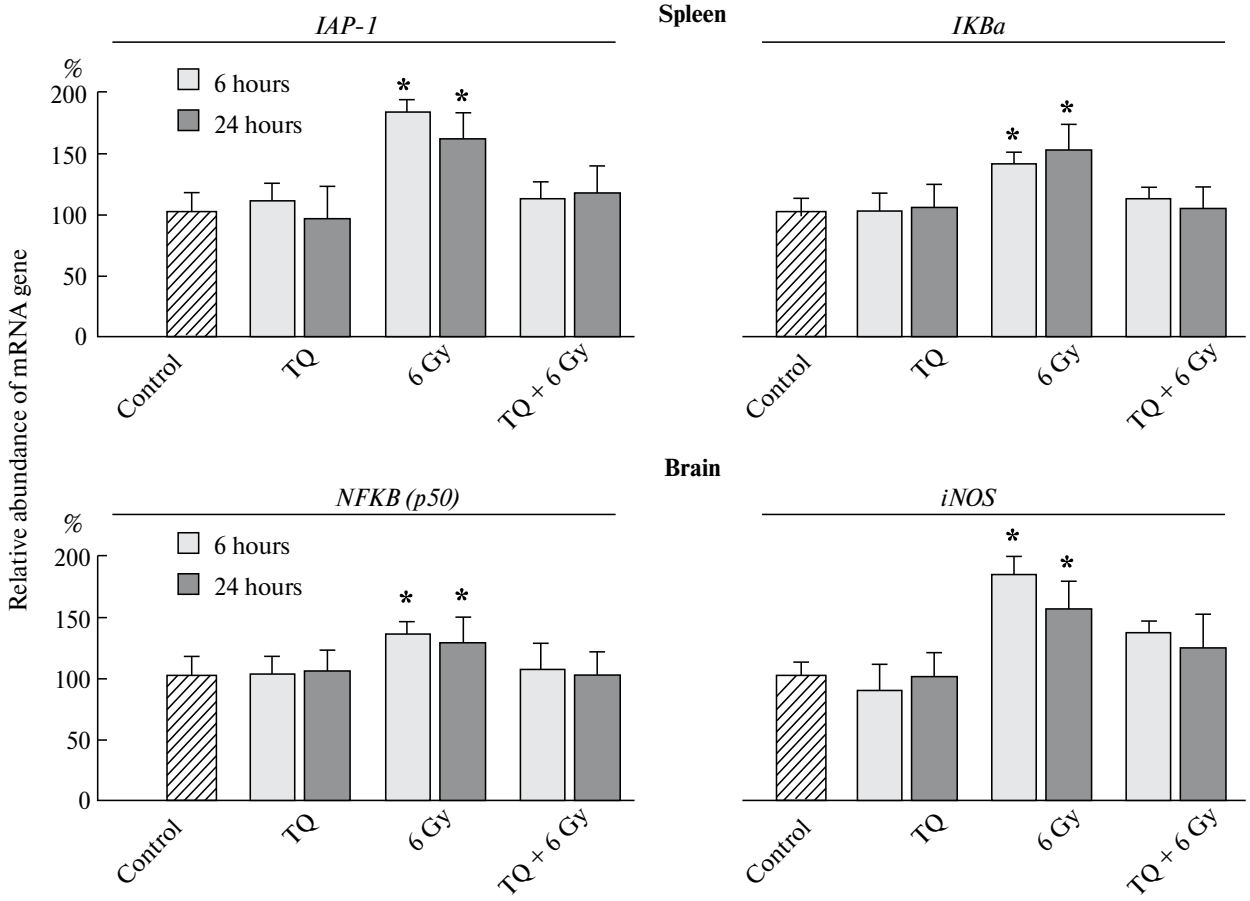

Fig. 1. Effect of TQ on the expression of *IAP-1*, *IKBa, NFKB (p50), iNOS* oncogenes in spleen and brain tissues of mice at 6 and 24 hours after exposure to X-ray radiation at a dose of 6 Gy. The level of gene expression in non-irradiated (control) mice is taken as 100%. Data are presented as mean ± SEM from 5–6 independent experiments. Differences from control are statistically significant at $p < 0.05$ (*)

of the compound and the method of administration (intravenous, intramuscular, or oral). The protective effect of TQ administered intraperitoneally in studies on mouse liver was effective at doses of 4, 8, 12, 5, 25, and 50 mg/kg, however, increasing the dose to 90.3 mg/kg could lead to the death of animals. Subchronic use of TQ with drinking water for mice for 90 days was not accompanied by any signs of toxicity [6]. The authors prove that the use of TQ before radiotherapy protected rats from side effects. For instance, it was shown that 80% of animals developed cataracts during brain radiotherapy, but its development decreased when TQ was used before or after irradiation. Thus, the authors also believe that TQ has a protective effect. Measurement of the radioprotective effect by several authors is carried out using indicators of induced oxidative stress, which causes an imbalance between the damaging effects and the cell protective mechanisms. Cell protection is closely related to the activity of genes and their regulators — non-coding RNAs. Activation of oncogenes after radiation exposure and reduction of their expression under the action of protectors can undoubtedly serve as a marker of drug effectiveness. We have shown that nuclear DNA oncogenes were activated after radiation exposure, whereas in experiments with radiation and TQ, a decrease in their expression was observed. Mitochondrial DNA genes were also modified after radiation exposure and activated in experiments with TQ. It should be emphasized that recently, mtDNA has been studied most intensively, as its connection with nDNA turned out to be conjugated and influencing its activity. For example, the *P53* gene, which controls cellular homeostasis and a number of other functions, is associated with mitochondrial dysfunction in cardiovascular diseases and affects energy metabolism, oxidative stress, mitochondrial apoptosis, and autophagy [18].

When discussing the action of TQ, it is necessary to focus on the peculiarities of its influence on tumor

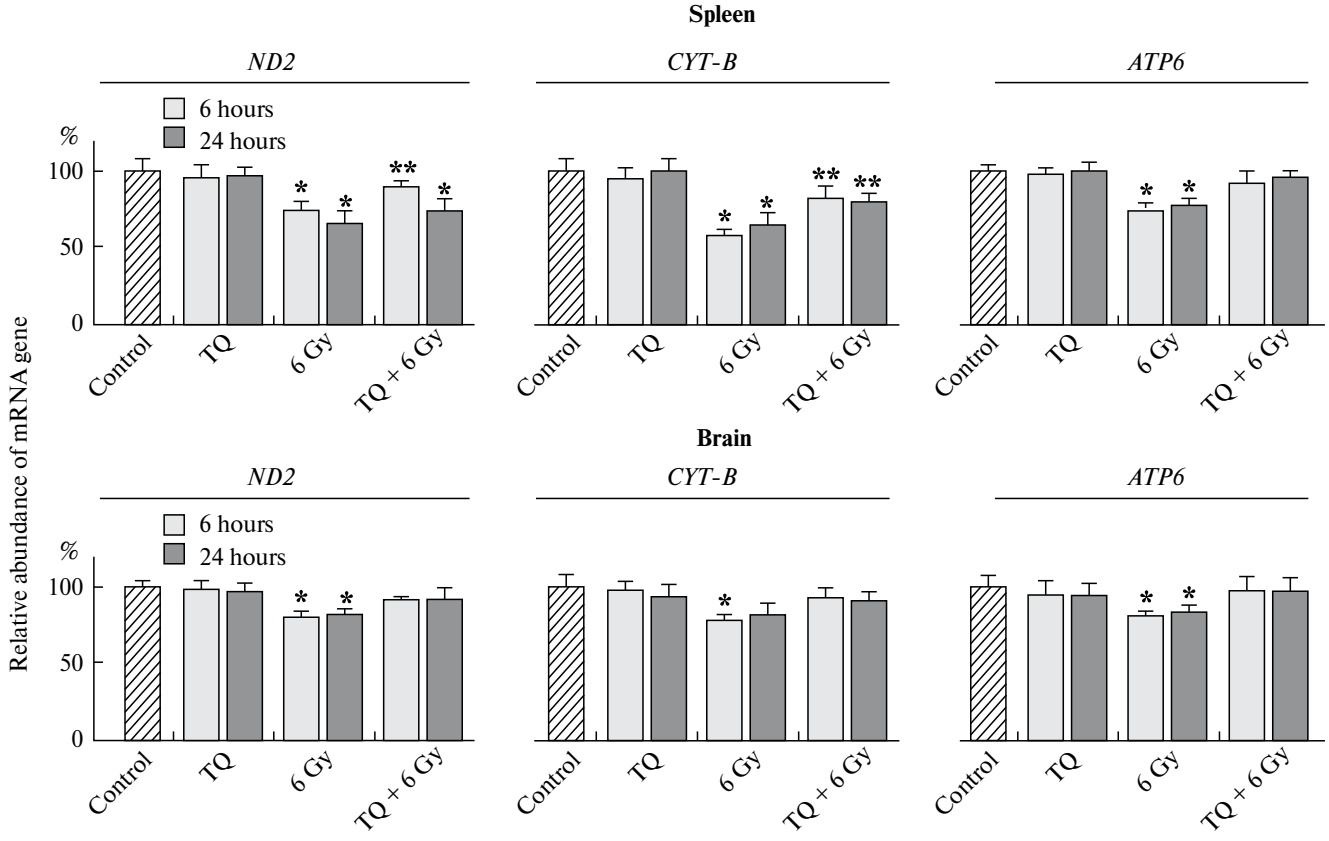

Fig. 2. Effect of TQ on the expression of mtDNA genes involved in oxidative phosphorylation (*ND2*, *CYT-B*, *ATP6*) in spleen and brain tissues of mice at 6 and 24 hours after exposure to X-ray radiation at a dose of 6 Gy. The level of gene expression in non-irradiated (control) mice is taken as 100%. Data are presented as mean ± SEM from 5–6 independent experiments. Differences from control are statistically significant at $p < 0.05$ (*), $p < 0.01$ (**)

cells. A number of authors believe that TQ can inhibit proliferation and induce apoptosis. This involves certain epigenetic mechanisms, including the ubiquitin-like plant homeodomain (*PHD),* RING finger domains gene (*UHRF1*), DNA methyltransferase 1, and histone deacetylase (*HDAC1*). TQ has the ability to bind to *UHRF1* and *HDAC1.* It should be noted that TQ forms a stable bond with zinc located in the active center of the *HDAC1* protein. These data were obtained on MCF-7, HELA, Jurkat cell lines, where TQ acted in a dose-dependent manner, inducing apoptosis [19]. In the experiment on rats that received 50 mg/kg TQ orally 3 times a week, inhibition of the number of metastases in the liver and spleen of animals was shown. In cell cultures, TQ reduced the expression of DNA methyltransferase 1 (*DNMT1*). The authors of work [20] believe that TQ has protective properties against carcinogens that cause breast cancer through epigenetic mechanisms *DNMT1.*

Thus, TQ is a promising drug characterized by multi-vector action on different pathways of cell vital activity, aimed at implementing the body's protective systems. Positive results of the action of TQ or black cumin in numerous diseases of different nature have been shown in animals: antidiabetic, antimicrobial, antiparasitic, anti-inflammatory, anti-hypercholesterolemic, and antihistamine effects, effects on the nervous, cardiovascular, respiratory systems [19, 21]. The above-mentioned authors recommend TQ for the prevention and treatment of many pathologies, based on numerous data obtained in animals and the absence of toxic effects. Population studies are apparently expected. The radioprotective effect can be attributed to the preventive effects of TQ, which is expressed in modifying the activity of genes that restore the balance of protective systems of enzymatic and non-enzymatic nature.

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STATEMENT OF COMPLIANCE WITH ETHICS REQUIREMENTS

All animal experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Directive 2010/63/EU.

ETHICS DECLARATIONS

The study was approved by the Biomedical Ethics Committee of the FSBI SSC FMBC named after A. I. Burnazyan of FMBA of Russia (protocol No. 20 dated February 09, 2024).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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