EXAMINE OF THE POTENTIAL RADIATION PROTECTION ABILITY OF AMINO ACID BETAINE (N, N, N-TRIMETHYL GLYCINE) AGAINST THE CELLULAR HARMFUL EFFECT OF THE IONIZING RADIATION IN ANIMAL EXPERIMENTAL MODELS

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Keywords: *radioprotector, radiation protection, acute radiation syndrome, betaine, oxidative stress, Cu/Zn SOD, experimental animals.*

Abstract: The serious harmful effect of the ionizing radiation is one of the most dangerous professional circumstances for the pilots and astronauts. Have been found that normal cellular antioxidant metabolites, could be effective radioprotectors. The antioxidants' potential to reduce the cellular damage in animal models have been studied for more than 50 years. The amino acids are highly active metabolites that effectively neutralize reactive oxygen species (ROS, free radicals) and have antioxidant activity.

AIM: Follow up of the after-radiation exposure recovery and radioprotector activity of betaine (N, N, N-trimethyl glycine), applied to experimental animals (mice).

MATERIALS AND METHODS: Have been used 120 experimental animals (white male mice C3H, with body weight 23 gr), supplied by the "Certified vivarium for experimental animals", Military Medical Academy - Sofia. Divided in four experimental groups. Two of them were exposed to radiation by ^{137}Cs-source with power 2,05 Gy/min (The Institute of PPG "Acad. D. Kostov", BAS, Sofia). The amino acid was applied peroral as a food supplement to the animals in the experimental groups. It was monitored Cu/Zn SOD protein expression index in three radiation sensitive organs (liver, spleen and testicles). The protein expression is confirmed by Western blot analysis.

RESULTS: The obtained results confirm the positive radiation protection effect of the researched substance.

CONCLUSION: Betaine (N, N, N-trimethyl glycine) showed positive effect to the survival rate in all examined experimental animals' groups. The application of Betaine induced increase of the expression of Cu/Zn SOD protein in the researched experimental groups.

ИЗСЛЕДВАНЕ НА ПОТЕНЦИАЛНАТА РАДИОПРОТЕКТИВНА СПОСОБНОСТ НА АМИНОКИСЕЛИНАТА БЕТАИН (ТРИМЕТИЛ ГЛИЦИН) В ЕКСПЕРИМЕНТАЛНИ ЖИВОТИНСКИ МОДЕЛИ

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Ключови думи: *радиопротектор, радиационна защита, остър радиационен синдром, бетаин, оксидативен стрес, Cu/Zn SOD, опитни животни.*

Резюме: Вредното въздействие на йонизиращата радиация е много опасно и застрашаващо живота обстоятелство в работната среда на летците и космонавтите. Установено е, че нормалните клетъчни метаболити, функциониращи като антиоксиданти, могат да бъдат ефективни радиопротектори. Изследването на антиоксиданти с потенциална радиопротективна активност е започнало още в средата на миналия век. Аминокиселините са високо активни метаболити, които участват в различни анаболни и катаболни процеси. Високата им метаболитна активност определя активната им роля в неутрализирането на активните кислородни видове (свободни радикали) и съответно високата им антиоксидантна активност.

ЦЕЛ: Проследяване на възстановяването, след облъчване с йонизираща радиация на използваните експериментални животни (мишки) и потенциалната радиопротективна способност на бетаин (триметил глицин), приложен върху тях.

МАТЕРИАЛИ И МЕТОДИ: Използвани са опитни животни (бели мъжки мишки C3H, с тегло 23 гр.), доставени от сертифициран вивариум за опитни животни, ВМА – София. Те бяха разделени на четири експериментални групи. Две от експерименталните групи бяха облъчени с 137Cs с мощност 2,05 Gy/min (ИФРГ "Акад. Д. Костов", БАН, София). Аминокиселината бетаин се прилага като перорална хранителна добавка към таргетните експериментални групи. За целите на настоящото изследване е изследвана експресия на Cu/Zn SOD протеин в три радиационно чувствителни органа (черен дроб, далак и тестиси). Протеиновата експресия се потвърждава чрез Western blot анализ.

РЕЗУЛТАТИ: Получените резултати потвърдиха положителният радиопротективен ефект на изследваното вещество.

ЗАКЛЮЧЕНИЕ: Бетаинът (триметил глицин) показва положителен ефект върху степента на преживяемост в групите от експериментални животни. Прилагането на бетаин индуцира повишаване на експресията на Cu/Zn SOD протеин в изследваните експериментални групи.

Introduction

The potential radiation protection effect of the antioxidants has been researched in animal experimental models for more than 50 years. Natural metabolites with antioxidant activity could provide effective preventive protection against low-dose-rate irradiation [15]. Many antioxidant nutrients have antimutagenic properties and their ability to modulate the long-term radiation effects need further examinations [5]. The exposure with over threshold doses (≥ 1 Gy) of ionizing radiation causes development of acute radiation syndrome. Measurable effects can be detected in many organs and tissues. Could be detected persistent and transient alterations in protein expression, growth factor activity, and normal cell and tissue function [3]. The severity of the disease depends on various factors, such as the impact of the environment and the presence of protective agents against reactive oxygen species action (antioxidants). Reactive oxygen species (ROS) have significant role in the intracellular signalling and redox regulation. The balance of the ROS generation and removal is regulated by the presence of effective antioxidants (antioxidant substances and antioxidant enzymes). Any disturbance of the balance leads to increase of the ROS formation (oxidative stress). [8, 14, 17].

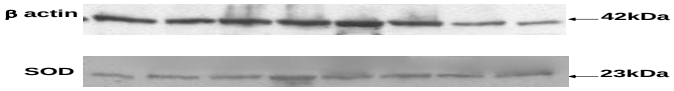
The aim of the present study was to examine the antioxidant radioprotective effects of natural metabolite betaine (N, N, N-trimethyl glycine) to oxidative stress induced by ionizing radiation.

Materials and methods

Have been used 120 experimental animals (white male mice C3H, with body weight 23 gr), supplied by the "Certified vivarium for experimental animals", Military Medical Academy - Sofia. They were divided in four experimental groups. Two of the experimental groups were exposed to radiation by 137Cs-source with power 2,05 Gy/min (The Institute of Plant Physiology and Genetics "Acad. D. Kostov", Bulgarian Academy of Science, Sofia). The amino acid betaine has been applied peroral as a food supplement. The control group received *betaine (N, N, N-trimethyl glycine)* in max effective non-lethal dose of 100 mg/kg body weight (calculated with MTT assay) The dose was supplied to every mouse of the group peroral for 15 consecutive days. The mice in the group were non irradiated. The second control group of experimental mice were whole body exposed to dose of 7.5Gy and without feeding. The third group of experimental animals included mice without feeding and irradiation. The fourth group of experimental animals were irradiated and fed with the food supplement contained *betaine*. The liver, spleen and testicles tissue samples were accurately weighed and homogenized (Soniprep 150 MSE) for 30 seconds in ice-cold 1,15% KCl buffer, pH 7.4. The homogenates were used for Western blot analysis. Statistical significance of the data was analyzed using Student's t-test and data are shown as means ±SD. The accepted statistical significance was $p<0.05$.

Results

Ionizing radiation generates ROS as a result of water radiolysis [6]. The mice irradiation leaded to decrease the protein expression of the antioxidant enzyme Cu/Zn SOD (SOD1). It was measured by the Western blot analysis of homogenates of the three sensitive organs – liver, spleen and testicles. The assays were implemented 24 hours after the whole-body radiation exposure (Fig. 1,2 and 3). The data were presented as an integral optical density (IOD). The implementation of Betaine increased the protein expression of SOD1 in all examined probes of all organs.



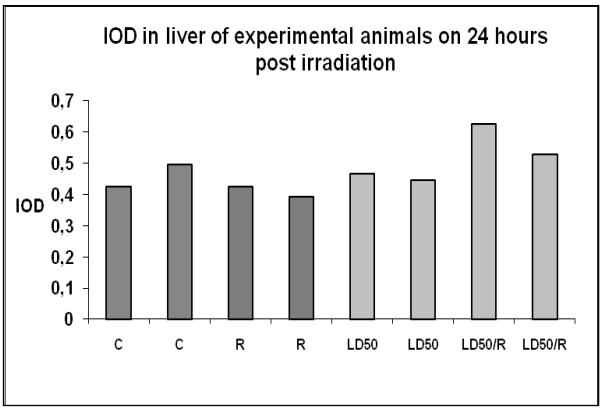
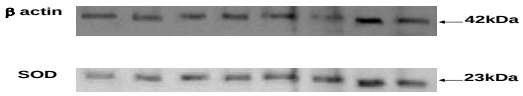


Fig. 1. Integral optical density of Cu/Zn SOD protein expression in liver on 24 hours post irradiation. Experimental animals were irradited with a single dose of 7.5Gy from gamma source Cs-137. C-nonirradiated controls, R-nonirradited controls with administration of betaine, LD50-irradiated with a single dose of 7.5Gy, LD50/R-irradiated with 7.5Gy and administered betaine.



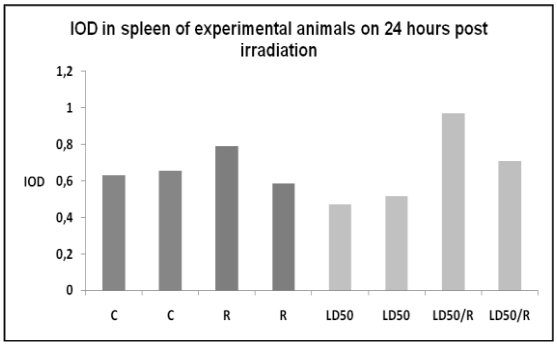


Fig. 2. Integral optical density of Cu/Zn SOD protein expression in spleen on 24 hours post irradiation. Experimental animals were irradited with a single dose of 7.5Gy from gamma source Cs-137. C-nonirradiated controls, R-nonirradited controls with administration of betaine, LD50-irradiated with a single dose of 7.5Gy, LD50/R-irradiated with 7.5Gy and administered betaine.

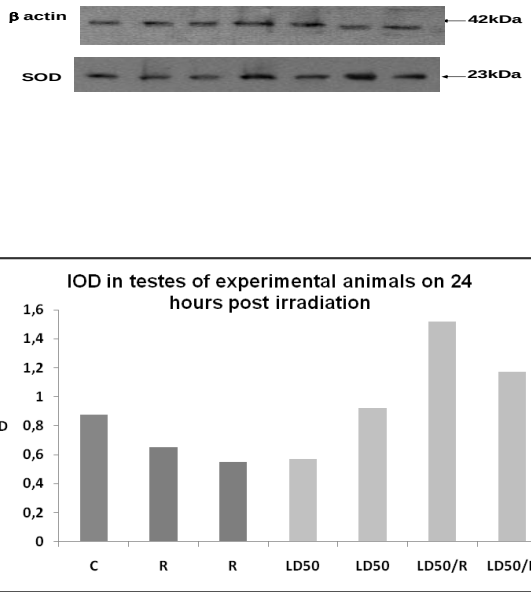


Fig. 3. Integral optical density of Cu/Zn SOD protein expression in testicles on 24 hours post irradiation. Experimental animals were irradited with a single dose of 7.5Gy from gamma source Cs-137. C-nonirradiated controls, R-nonirradited controls with administration of betaine, LD50-irradiated with a single dose of 7.5Gy, LD50/R-irradiated with 7.5Gy and administered betaine.

Discussion

The application of *betaine* (N, N, N-trimethyl glycine) showed a positive effect to 30 days survival mice. The LD_{50} is used to quantify mortality in the population. It is defined as the exposed dose that will cause death in half (50%) of the exposed experimental animals. The time of death depends on the effective dose. For the hematopoietic form of acute radiation syndrome, it is approximately 30-60 days after the exposure. [1]. Medical interventions, such as blood cell replacements, antibiotics and cytokines application and hematopoietic stem cell transplants, could increase survival time and extend the LD50 value [12].

The cell death after radiation exposure is a result of serious DNA damage that occurs because of the oxidative stress [6]. The most common consequences of the harmful effect of the ionizing radiation are chromosomal aberrations existence [9, 10, 2]. Ionizing radiation induces a variety of cellular and tissue damages, such as oxidative stress [35], gene expression influence [16], changes in the signal transduction pathways [11], etc. The current study demonstrates that application of betaine (N, N, N-trimethyl glycine) to whole-body irradiated animals show potential radioprotection activity. In the study was used modulation antioxidant enzyme Cu/Zn superoxide dismutase (SOD1). Superoxide and hydroxyl radicals, generated by ionizing radiation, are rapidly destroyed by SOD1. [7]. The antioxidant *betaine* protects against oxidative stress by modulating SOD1 level in organs. It works cooperatively with other antioxidants (Fig. 1,2 and 3). To be effective radioprotectors, antioxidants must be administered prior to radiation exposure. [7].

In conclusion, *betaine (N, N, N-trimethyl glycine)* showed radiation protection potential against ionizing radiation damage by preventing the serious harmful effect of the oxidative stress.

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