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Amino Acid Betaine (Trimethyl Glycine) As A Factor Of Recovery Of The Hemopoietic Syndrome, Induced By The Ionizing Radiation.

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Abstract
The radioprotection activity of different substances, such as cellular metabolites with antioxidant activity to reduce the cellular damage induced by ionizing radiation has been studied for more than 50 years. Many research studies are focused on the radioprotective efficacy of naturally occurring antioxidants (natural metabolites), and how they might influence various endpoints of radiation damage.
Aim: *in the current study has been research the irradiation recovery and possible antioxidant activity after feeding the experimental animals with trimethyl glycine (betaine), as a food supplement.*
Materials and methods: *in the current study were used experimental modal animals (white male mice c3h, weight 23 gr.) That were divided in four groups. Two of the groups were exposed to radiation from ^{137}cs with power 2,05 gy/min. The food supplement has been administrated to three of the experimental groups of animals, peroral, every day during 15 days before irradiation of those that are exposed. Lipid peroxidation levels in liver, spleen and testis were followed to all of the groups.*
Results: *the research analysis gave significant results of decrease the levels of lipid peroxidation (in liver, spleen and testis), comparing the measurements in four different groups for every organ – control group with irradiation without feeding, control group without irradiation with feeding, experimental group with irradiation and feeding.*
Conclusion: *the researched amino acid has positive effect over survival rate in experimental animals. Administration of the amino acid shows decrease of the oxidative stress in the experimental group in comparison to control group with irradiation.*
Key words: *ionizing radiation, radioprotectors, amino acids, oxidative stress;*

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I. Introduction

The possible potential of natural metabolites with antioxidant activity to reduce the cellular damage, induced by ionizing radiation in animal models, has been noticed in the middle of the 20th century [Weiss, J.F., et al., 2003]. Many research studies are focused on the radioprotective efficacy of the naturally occurred metabolites, specifically amino acids, fatty acids, enzymes, vitamins, microelements (Ko-enzymes) and how they might influence various endpoints of radiation damage [Hall E. J., 2000]. Results from animal experiments indicate that vitamin C, vitamin K, vitamin PP (folic acid), vitamin C, vitamin B_6, nicotinamide, α-lipoic acid, different amino acids (cysteine, glycine, arginine, lysine, etc.), microelements functionating as Ko-enzymes such Zn^{+2}, $Fe^{2+/3+}$, $Cu^{2+/+}$, Mg^{2+}, etc., have radiation protection activity and decrease the irradiation injuries [Weiss, J.F.,et al, 2003]. The effects of whole-body ionizing radiation exposure to animals (mice) have been studied in the laboratory of radiobiology (Military Medical Academy-Sofia, Bulgaria). Data on human exposures have been obtained from the worldwide accidental exposures such as Hiroshima, Nagasaki, Fukushima, etc. Summarizing the research studies on the human whole-body irradiation, three main types of acute radiation syndrome have been defined. The acute radiation syndrome has been developed after threshold exposure ≥ 1 Gy. [Hall EJ and Giaccia AJ, 2006; Steel GG, 2002; Hall EJ and Giaccia AJ., 2006]. In that case a dose-effect relation is observed. The main type syndromes resulting from single-dose over threshold exposure are:

-cerebrovascular syndrome (CNS syndrome), >100 Gy, death within 24-48 h;
-gastrointestinal syndrome (GI syndrome), 5-12 Gy (primarily >10 Gy), death within 3-10 days;
-hematopoietic syndrome (bone marrow syndrome), 2.5 to 8 Gy, death within 1-2 months; survival possible.

Measurable effects of the animal tissues could be detected after 1 to 10 Gy of irradiation. [Barcellos-Hoff M., 1998] For example, radiation has been shown to produce chronic oxidative stress [Mitchell J. B.,

1991]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) persist in all aerobic organisms. In the normal cells, ROS have an important role in the intracellular signalling pathways and redox regulation systems. The ROS/RNS production and removal are in balance, because of the antioxidants' presence (antioxidants and antioxidant enzymes). Any increase of the ROS/ RNS production and disturbing of the balance could create cellular stress. Indicators of oxidative stress could be detected in vitro models after irradiation [Robbins M. E. C.,2002, Leach J. K., et al., 2001].

As radiation-induced cellular injury depends mainly to ROS activity, it is noticed that antioxidant apply could be used as a preventive action against the development of radiation induced oxidative stress and tissue injury. Therefore, the aim of the present study was to examine the antioxidant radioprotective effects of one amino acid (main metabolite in the cell), used preventively against the development of the oxidative damage and organ injury, induced by the action of ionizing radiation.

II. Materials and methods

In the current study were used experimental modal animals (white male mice C3H, weight 23 gr.) that were divided in four groups. Two of the groups were exposed to radiation from ^{137}Cs with power 2,05 Gy/min. The food supplement has been administrated to three of the experimental groups of animals, peroral, every day during 15 days before irradiation of those that are exposed. Lipid peroxidation levels in liver, spleen and testis were followed to all of the groups.

Mice were randomly divided into four groups. The first group is control group of animals received food supplement at dose of 200 mg/kg body weight for 15 consecutive days and non-irradiated. The second control group of animals is gamma irradiated mice group, exposed to dose of 7.5Gy whole-body radiation without feeding. The third mice group is mice without feeding and irradiation. The fourth group is exposed to dose of 7.5 Gy and administered with betaine at dose of 200 mg/kg body weight for 15 consecutive days before irradiation. Animals that are going to be irradiated were placed in a well-ventilated container and were whole-body exposed to 7.5 Gy, given at a dose rate of 2.05 Gy/min from cesium-137 source, belonging to the Institute of Plant Genetics "Acad. D. Kostov", Bulgarian Academy of Science, Sofia. Animals were sacrificed 24 h after irradiation, following all animal European welfare regulations and requirements. A tissue samples from the liver, spleen and testis were accurately weighed and homogenized (Soniprep 150 MSE) for 30 seconds in ice-cold 1,15% KCl buffer, pH 7.4. The homogenates were subjected to the following biochemical analysis. Lipid peroxidation product, malondialdehyde (MDA), was measured by thiobarbituric acid assay. It is based on MDA reaction with thiobarbituric acid to give thiobarbituric acid reactive substances (TBARS). It is a red color species that absorbs at 535nm (Asakava and Matsushita, 1980). Statistical significance of the data was analyzed using t-test and data are shown as ±SD. The level of the statistical significance is $p<0.05$.

III. Results

Ionizing radiation generates reactive oxygen species as a result of the reaction of hydrolysis of water [Hall E. J., 2000]. Exposure of mice to gamma irradiation resulted in a significant increase in lipid peroxidation, as measured by the formation of MDA in the liver, spleen and testes 24 hours after irradiation (Fig. 1,2 and 3). Increasment occurred at the all analysed organs. The dose (7.5 Gy) caused the 26% increase in liver, 54% increase in spleen and 9% increase in testes, while after use of betaine was found significant difference (6% in liver, 39% in spleen and 2% in testes) of the extent of lipid peroxidation. Application of betaine every day preventively, starting 15 days before acute irradiation, significantly decresed the radiation-induced levels of MDA in the liver, spleen and testes (fig. 1, 2 and 3).

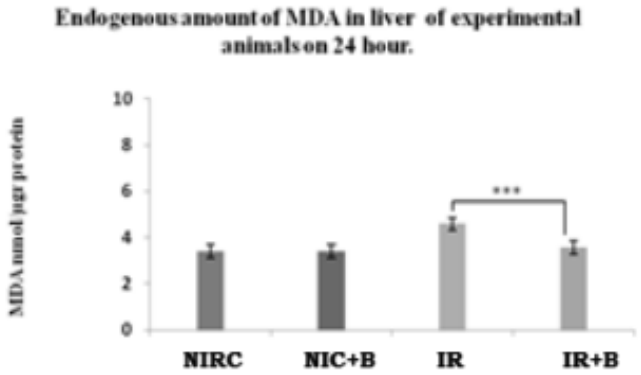

Fig. 1. Levels of endogenous MDA in liver of experimental animals, $p<0.05$.

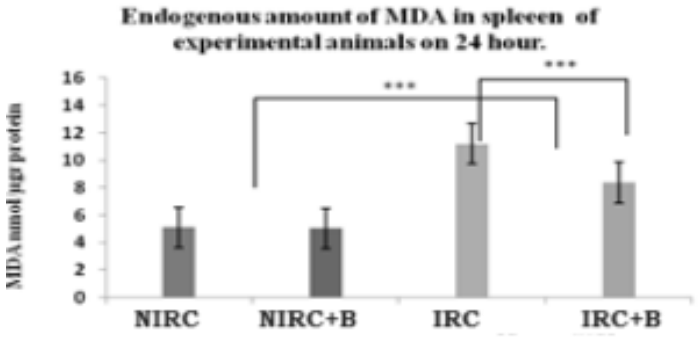

Fig. 2. Levels of endogenous MDA in spleen of experimental animals, p<0.05.

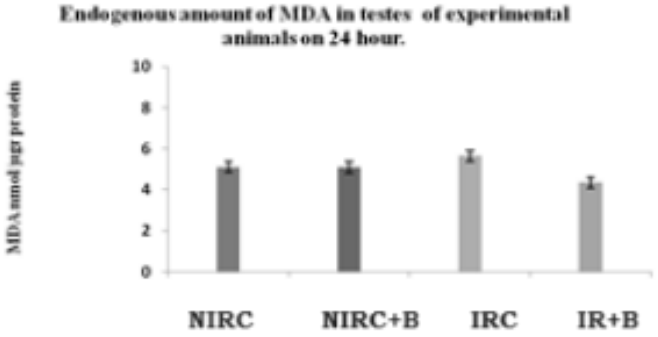

Fig. 3. Levels of endogenous MDA in testes, p<0.05.

IV. Discussion

Administration of betaine has a positive effect over 30 days survival of experimental animals. The LD_{50} is used to quantify mortality in a population. It has been defined as the dose that cause death in half (50%) of the exposed population. As the over threshold exposure show dose-effect relation, the severity of the disease and the lethal period depends on the dose. It is within 30-60 days for the hematopoietic syndrome. Therefore, the term for hematopoietic death is the $LD_{50/30}$ or $LD_{50/60}$, because lethal effect caused of bone marrow failure may occur up to 30-60 days. $LD_{50/60}$ values for humans are estimated to be in a range of 3-6 Gy, based on the experience of the research of the ionizing radiation accidents [Nuclear Technology Publishing, 2001].

The lethal effect of the cells after ionizing radiation is based on DNA damage that occurs directly as DNA strand breaks (DSB or SSB) or indirectly as a result of the hydrolysis of water and action of ROS [Hall E. J.,2000]. If the cell survives, chromosomal aberrations could appear [Lloyd D. C., et al. 2000]. Radiation induces a variety of cellular and tissue damages. The present study shows that application of betaine as a preventive agent against to harmful effect of gamma irradiation has a protective activity against the oxidative stress and tissue damage. The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The present study demonstrates increased concentration of MDA in the liver, spleen and testis, indicating high level of oxidative stress, in irradiated with a single dose of 7.5Gy which markedly enhanced in comparison with controls (Fig. 1,2 and 3). Similar observations are reported on radiation-induced oxidative damage in several organs [7] and mitochondrial membranes [Kamat, J.P., et al., 2000]. Ionizing radiation generates ROS as a result of water radiolysis. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes [Cadet, J., et al., 2004]. Products of lipid peroxidation such as MDA have the ability to interact with and alter macromolecules, possibly resulting in diseases [Petersen, D.R., et al., 2004]. The present results show that 7.5Gy gamma irradiation from cesium-137 source produced significant oxidative damage 24 hours following radiation exposure. It is reported that whole-body exposure of rats to radiation from Co-60 causes tissue damage in several organs, as assessed by increased lipid peroxidation, 2, 12, and 72 h after irradiation [Koc, M., 2003]. Oxidative stress is involved in the pathology of metabolic diseases [Onody, A., et al., 2003]. Therefore, it is known that oxidative stress is linked to the cellular, tissue and organ injuries after exposure to ionizing radiation. It is hypothesized that if the oxidative stress is involved in their damage, then application of correct antioxidant should delay or prevent the

onset of that damage [Benyon R. C. and J. P. Iredale, 2000]. The present result demonstrates that application of betaine, before irradiation, for 15 consecutive days protected against oxidative stress, evidenced by decreased MDA production in the liver, spleen and testis compared to the irradiated mice. Although the prolonged radioprotective effects could be advantageous for post exposure treatment in an environmental radiation exposure. That present a possible use before radiotherapy, where treatments are given daily, and persistent radioprotectors could reduce development of possible secondary effects (secondary leukaemia). The potential of any radioprotective agent for cancer treatment will require attention to dose, schedule and mechanisms of protection and avoidance of tumour protection.

In conclusion, betaine (try methyl glycine) is shown to have effect against gamma radiation by preventing oxidative stress.

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