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Qualitatively determine the possible radioprotective ability of the amino acids N-acetyl-L-cysteine and N,N,N-trimethyl glycine (betaine)

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ABSTRACT

Ionizing radiation exposure induces severe cellular injuries, such as single- and double- strand breaks, base lost, base's changes, chromosomal aberrations, etc. Double strand breaks are the most significant and dangerous for the cell. As a result of action of the ionizing radiation the level of free radicals (more specific reactive oxygen species) increases many times and leads to severe cellular injuries. Oxidative stress has appeared. Double strand breaks could lead to formation of genomic instability that could become a stable change and to mutate the cell. High doses of ionizing radiation led to destroying of the whole DNA-genome and to formation of DNA-fragmentation. The aim of the currents study is to qualitatively determine the possible radioprotective ability of the amino acids N-acetyl-L-cysteine and N,N,N-trimethyl glycine (betaine). DNA-ladders method has been used for conformation of the radioprotective activity of analyzed two amino acids (betaine and N-acetyl-L-cysteine). The obtained result of the conducted research showed a possible high potential for radioprotective ability of both metabolites and set the stage for additional studies in an experimental cell model to confirm or reject the result.

***Keywords*:** DNA-ladders, γ-H2AX assay, DBS, ROS, N-acetyl-L-cysteine, NNN-trimethyl glycine (betaine)

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1. INTRODUCTION

Ionizing radiation induces a variety of DNA lesions, including single- and double-strand breaks, base breaks, and sugar residues [1]. Double-strand breaks are the most dangerous for the cell. Cells can adapt to small DNA damage by repair [11], but a single double-strand break can be essential for cell death [19]. As a result of the action of ionizing radiation, the level of free radicals and active forms of oxygen in the cell increases many times. Reactive oxygen species (ROS) are widely produced in living organisms, but their levels in the cell are kept relatively low. Therefore, the body has developed an antioxidant defense system that limits their production. Disturbance of the balance between the processes producing ROS and the antioxidant defense systems leads to the occurrence of oxidative stress, causing cellular damage [6, 7, 18]. The development of oxidative stress and its consequences depend on the ability of the organism to restore the physiological balance, independently or with help from the outside [10, 23]. Reactive oxygen species induce changes in DNA molecules as a result of single- and double-strand breaks (SSB and DSB), in the form of base substitutions, chromosomal aberrations (insertion, deletion, inversion, translocation), gene amplifications, micronuclei formation and genomic instability [5, 12, 14-17, 24].

Double-strand breaks can lead to formation of stable chromosomal aberrations, genomic instability and carcinogenesis. Accurate counting of the number of double-strand breaks is accomplished using a γ-H2AX assay. H2A is a family of conserved, histone proteins with three main members: H2A1-H2A2, H2AZ and H2AX. H2AX is a heteromorphic isoform of H2A and is found in mammalian cells and tissues in varying percentages, between 2-25% [2].

H2AX is a highly conserved protein and contains a distinctive C-terminal SQ (E/D) (I/L/F/Y) motif [13]. Like other histone proteins, H2AX can undergo phosphorylation, acetylation and ubiquitination and participate in cellular regulation [22]. Histone H2AX is associated with DNA repair, is involved in the regulation of cell division and in immuno-receptor transduction and signal transduction. Based on the important functions it performs in the cell, it has been established that H2AX is a major participant in the process of tumorigenesis by causing genomic instability in human cells [8]. Has been found that the formation of specific DNA bands requires H2AX phosphorylation and correlates with the formation of nuclear γH2AX-foci [9]. The amount of H2AX that is γ-phosphorylated per double-strand break corresponds to 2 Mbp of chromatin or thousands of nucleosomes [20]. The maximum amount of phosphorylated H2AX molecules can range from several hundred to several thousand per double-strand break in DNA [3]. The process of phosphorylation of H2AX molecules is related to the process of cell apoptosis. Initiation of DNA fragmentation during apoptosis induces double-strand breaks that stimulate γ-H2AX formation [21]. The accumulation of γ-H2AX could be detected as soon as double-strand breaks occur in DNA.

Electrophoretic separation and UV-visualization of obtained DNA-fragments (DNA ladder) leads to the formation of bands (bands) differing in degree of staining and distribution in the agarose gel, which are distinguished from the heavy, saturated colored line of the genomic DNA. This method efficiently separates the resulting nucleosomal DNA fragments containing γ-induced phosphorylated H2AX molecules from the overall genomic DNA. The formation of DNA fragments directly correlates with the presence of apoptotic cells and severe γ-induced DNA damage. The damaged cell will have destroyed genome and could be visualized by performing of the DNA-ladders electrophoretic separation on agarose gel. If effective radioprotector against the action of ionizing radiation is used, the result could be less destroyed

total DNA genome. That is mostly quality effective method that could significantly show the protection role of the used radioprotector substance. It is known that natural metabolites with antioxidant activity could perform good radiation protection activity against the harmful effects of the ionizing radiation. For the current study two main metabolites with antioxidant activity have been chosen (betaine and N-acetyl-L-cysteine), as potential radioprotectors.

2. AIM

The aim of the currents study is to qualitatively determine the possible radioprotective ability of the amino acids N-acetyl-L-cysteine and N,N,N-trimethyl glycine (betaine).

3. MATERIALS AND METHODS

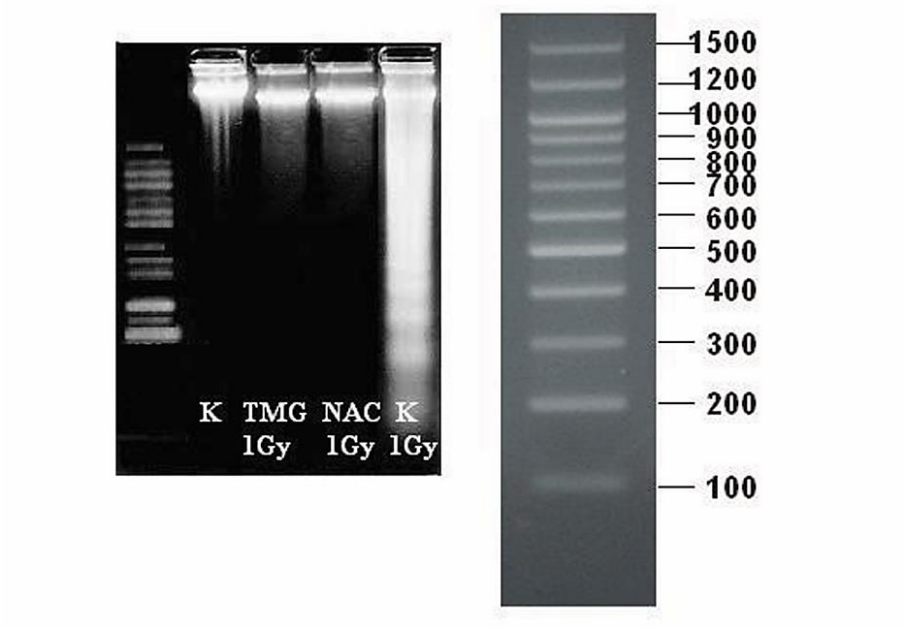
DNA-ladders method has been used for conformation of the radioprotective activity of analyzed two amino acids (betaine and N-acetyl-L-cysteine). The principle of the method determines that DNA from apoptotic cells will form clearly distinguishable DNA-bands distributed by molecular weight of the resulting fragments along the agarose gel, while genomic DNA from viable, undamaged cells will remain at the beginning of the gel because it is the most -high molecular weight. The bands corresponding to the DNA fragments are further away from the start in the agarose gel, the lower the molecular weight of the corresponding DNA fragments. If necessary, the exact mass of the resulting fragments can be determined by comparison with the bands of the molecular mass marker used.

For DNA isolation, cell cultures were used from peripheral blood of 20 healthy donors with preventive treatment (two hours before irradiation) with radioprotector and irradiation with 1 Gy and 3 Gy absorbed dose. To conduct the study, four experimental groups were defined for each radiation dose.

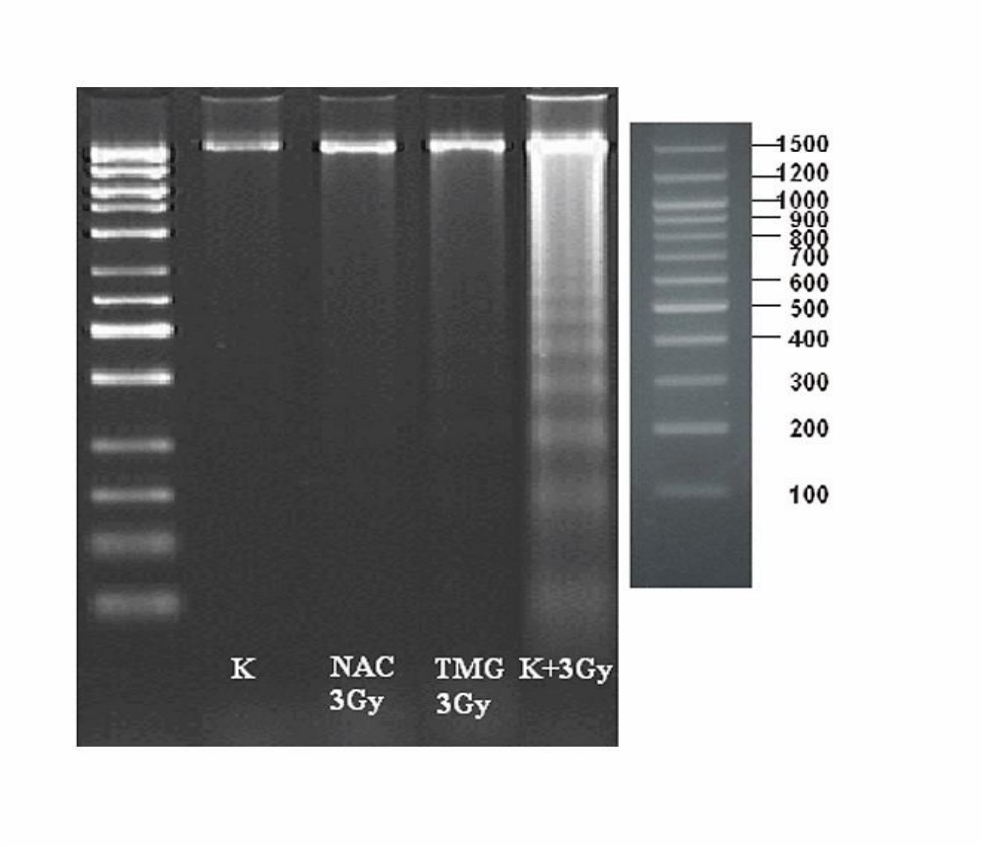
The cultivation period was 24 and 48 hours. The samples with isolated DNA were used for electrophoretic separation of the DNA-fragments (DNA-ladders) according to the method of Bestwick et al. [4]. The results of the analysis are with mostly qualitative characteristics. The presence of potential radioprotection of the investigated substances was determined by the presence and/or absence, as well as by the staining intensity of the formed DNA bands in the agarose gel, visualized under UV light.

4. RESULTS AND DISCUSSIONS

The results for the first cultivation period of 24 hours showed that the two investigated amino acids have a high radioprotective potential. As can be seen in Figure 1 (A) and (B), the DNA samples from the experimental groups cultured for 24 hours and treated with N-acetyl-L-cysteine preventively (two hours) before irradiation with 1 Gy and 3 Gy absorbed dose, showed no formation of colored bands corresponding to broken DNA fragments. At the starts with applied DNA samples from experimental groups with 1 Gy or 3 Gy irradiation and preventive treatment with N-acetyl-L-cysteine, the presence of a dense band located very close to the start was observed. It corresponds to whole, undamaged genomic DNA isolated from viable cells.

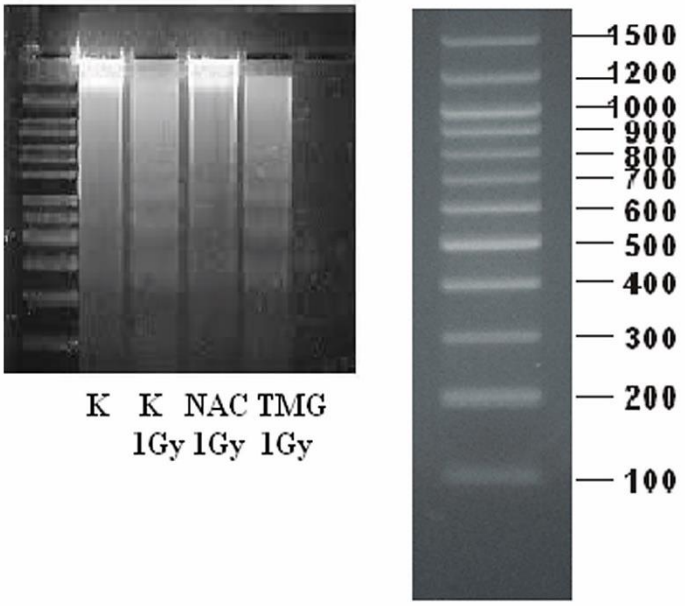


(A)

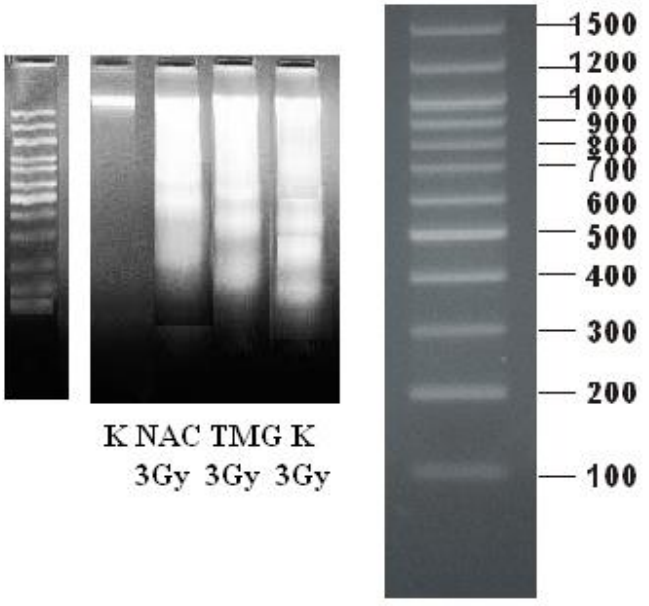


(B)

Figure 1. Formation of DNA fragments (DNA ladders) for a cultivation period of 24 hours: **(A)** experiment conducted with irradiation 1 Gy absorbed dose; **(B)** Experiment conducted with irradiation 3 Gy absorbed dose.



(A)



(B)

Figure 2. Formation of DNA fragments (DNA ladders) for a cultivation period of 48 hours: **(A)** Experiment conducted with irradiation 1 Gy absorbed dose; **(B)** Experiment conducted with irradiation 3 Gy absorbed dose.

The same result was observed for the DNA samples from experimental groups with 1 Gy or 3 Gy irradiation and preventive treatment with N,N,N-trimethyl glycine (betaine). DNA samples from the experimental group with untreated and irradiated with 1 Gy or 3 Gy absorbed dose showed the presence of colored bands (DNA fragments) along the entire length traveled in the gel, which directly correlate with the presence of severe γ-induced DNA damage.

The results obtained for the second culture period of 48 hours were significantly different from the results for the culture period of 24 hours. The obtained results are shown in Figure 2 A) and B). The DNA samples from the experimental group with 1 Gy irradiation and preventive treatment with N-acetyl-L-cysteine did not show the formation of colored bands corresponding to the presence of DNA fragments. Some of the samples from the experimental group with 1 Gy irradiation and preventive treatment with N,N,N-trimethyl glycine (betaine) showed the formation of colored bands (bands). This result correlates with the presence of severe DNA damage and γ-induced apoptosis.

The DNA samples from the experimental group with untreated and non-irradiated lymphocytes did not show the formation of colored bands and the formation of DNA fragments. This result correlates with expectations because in this experimental group the cells were not subjected to ionizing radiation inducing DNA damage, H2AX phosphorylation and formation of free nucleosome fragments (Figure 2 A).

The DNA samples by the experimental groups of 3 Gy irradiation and preventive treatment with N-acetyl-L-cysteine or N,N,N-trimethyl glycine showed the formation of colored bands in the half of the samples in each experimental group. This result correlates with the presence of irreparable DNA-damage and cell death (apoptosis) in a large part of the cells from the cell cultures included in the two experimental groups. According to the results of the study, the radioprotective ability of the two investigated amino acids N-acetyl-L-cysteine and N,N,N-trimethyl glycine, applied preventively in an experimental cell model, decreases with increasing incubation period and radiation dose. Their radioprotective and antioxidant capacity could not compensate existence of severe damages in DNA, induced by relatively high doses of ionizing radiation.

The second tested amino acid N-acetyl-L-cysteine showed significant radioprotective ability in preventive tertization of human, primary lymphocytes isolated from peripheral blood and irradiated with 1 Gy and 3 Gy absorbed dose. The results of the electrophoretic separation and visualization of obtained DNA-fragments (DNA-ladders) showed that the radioprotective ability of N-acetyl-L-cysteine is relatively higher than that of N,N,N-trimethyl glycine (betaine). The better protection of N-acetyl-L-cysteine compared to N,N,N-trimethyl glycine (betaine) correlates with its proven, powerful antioxidant properties. The good protection of both amino acids against the harmful effects of ionizing radiation is probably due to their ability to reduce the concentration of intracellular reactive oxygen species (ROS).

5. CONCLUSIONS

The obtained result of the conducted research showed a possible high potential for radioprotective ability of both metabolites and set the stage for additional studies in an experimental cell model to confirm or reject the result. For this purpose, proven, reliable cytogenetic, biodosimetric analysis, such as Dicentric chromosomal assay (DCA), cytokinesis-

block micronucleus assay (CBMN), should follow the performed study to establish the presence or absence of potential radioprotective properties of both amino acids.

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