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Original Article

Evaluation of Radio-Protective Effects of N-Acetylcysteine on Radiation-Induced Lethality in Mice

Ashkan Salajegheh[1], Mehdi Hoseini[2]*, Mina Nouri[3], Rasool Dehghani Soltani[4], Yaser Masoumi-Ardakani[5]

Abstract

Introduction
It has long been known that ionizing radiation can lead to detrimental effects in normal cells. In this light, Radioprotective chemicals have been used to decrease morbidity or mortality caused by ionizing irradiation. This study aimed to evaluate the radio-protective effect of N-acetylcysteine against radiation-induced mortality in male mice.

Materials and Methods
52 healthy male mice were divided into four groups including NAC before irradiation (1), irradiation (2), NAC after irradiation (3) NAC before irradiation (4) and control. Three groups were treated orally with 100 mg/ kg of NAC. Gamma irradiation was performed at 8 Gy using a Co-60 machine. Kaplan-Meier method and the log-rank test were performed, using SPSS version 16. The significance level was considered to be 0.05.

Results
The statistical analysis revealed a significant difference between the test and control groups ($P<0.05$). The percentage of survival after 30 days was 46.2% for the irradiation group (1). In addition, the percentage of decreased lifespan was calculated at 5.90%, 23.60% and 17.93% for the first-third groups, respectively.

Conclusion
Results revealed lack of effectiveness of treatment with NAC after lethal dose. These results suggested that application of NAC for mice before irradiation protected them from the lethal effects of whole-body irradiation.

Keywords: Gamma-Irradiation, Radioprotective, N-Acetylcysteine, Radiation Sickness

1-Department of Radiology, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2-Medical Physics Department, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
*Corresponding author: Tel: +985138828247; E-mail: hoseini.mehdi98@gmail.com

3-Department of Radiology, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
4- Student Research Committee, Kerman University of Medical Sciences, Kerman- Iran
5- Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

1. Introduction

Ionizing radiation is frequently used in various fields of medicine, including radiation therapy and radiopharmaceuticals for diagnostic and therapeutic purposes [1]. In addition, ionizing radiation transfers energy to the vital biological macromolecules (e.g. DNA, proteins and membrane fats) by penetrating into the body tissues, which leads to the interaction with living cells and generating various cytotoxic effects, also a main therapeutic factor for cancer treatment [2]. Radiation can produce two major lesions, namely DNA double-strand breaks (DSB) and reactive oxygen species (ROS), which combine to destroy cells in a clinical setting [3].

Radio-protector is a compound prescribed to human or animals before radiation exposure or shortly after exposure in order to reduce the harmful effects of radiation [4]. More than 60 years ago, the first in vivo studies on protection by antioxidants versus ionizing radiation were performed by Patt et al., reported that thiol-containing amino acids and cysteine protected rats from a lethal dose of X-rays [5].

Radio-protective compounds are mainly applied for two groups of individuals; first, for patients with cancer treated with radiation therapy. Radiation can cause severe damage and death in tumor cells through creating free radicals or direct action. Given the high growth and proliferation rate of tumor cells, radiation can cause more severe damage in such cells, compared to normal cells. However, due to non-specificity of radiation on tumor cells and normal cells, this damage can be seen in normal cells. Therefore, cells with high growth and multiplication rate are more vulnerable to radiation. According to the literature, use of a drug as a radioprotector will protect the normal cells more than tumor cells because it quickly floods normal tissues but penetrates more slowly into tumors [2]. This is a major step toward the treatment of cancer patients, which reduces the adverse effects of radiation on patients. The second application of radiation protectors is during radiation accidents [6-8]. Given the real risk of the radiation accidents to the healthcare community, it is important to protect hospital staff from the deadly effects of radiation, which causes morbidity and mortality [9]. In this regard, many compounds have been developed and evaluated [10-13].

Any specific feature or action which reduce the adverse effects of radiation must be taken into consideration in order to develop an appropriate medication with adequate radiation protection. These features include tissue protection, convenient method of administration (oral or intravenous), minimal toxicity and appropriate effectiveness, and desirable stability and compatibility with drugs used for patients or healthy individuals [1, 7, 14]. Nevertheless, it seems difficult to discover an ideal radio-protector agent with all the above properties. In this regard, Amifostine (WR-2721) has been approved by American Food and Drug Administration (FDA) for protection against radiation and chemotherapy in humans. However, application of this compound is limited due to its side effects, such as hypotension, vomiting and its short-term effectiveness [6, 15]. Hosseinimehr demonstrated the antioxidant and DPPH free radical scavenging (2, 2-diphenyl-1-picrylhydrazyl) activities of sour orange peel extract, which reduced the H_2O_2 in vitro [16].

NAC is a thiol (sulfhydryl-containing) compound with a molecular weight of 163.19 g/mol and chemical formula of $C_5H_9NO_3S$. This compound is the acetylated form of L-cysteine, which is more effectively absorbed and metabolized by the liver [17]. NAC is a derivative of cysteine with an acetyl group attached to its nitrogen atom. Therefore, NAC can be oxidized by a large diversity of radicals and serve as a nucleophile (electron pair donor), similar to most thiols (RSH) [18]. Moreover, NAC is a naturally occurring compound found in various vegetables (e.g., garlic, onion, peppers and asparagus). Many studies have demonstrated the ability of NAC to inhibit chemically induced oxidative stress and DNA damage [19]. Despite its relatively mild side effects (e.g., nausea, vomiting, rhinorrhea, pruritus and tachycardia), NAC has antioxidant, antiangiogenic and anticarcinogenic properties [18].

With this background in mind, this study aimed to evaluate the radiation protection effect of NAC against radiation-induced mortality. It is recommended that this compound be used as a radiation protector for those who are exposed to ionizing radiation.

2. Materials and Methods

2.1. Medication

NAC in the form of effervescent tablet was produced by the Avicenna pharmaceutical company, Iran. This drug was purchased from a local drug store in Kerman, Iran. The compound was dissolved in double distilled water, followed by the administration of a confirmed dose of NAC to mice in one step by gavage.

2.2. Animals

In total, 52 healthy one-month-old male mice weighing 30±5 gr were purchased from the Animal House of Physiology Research Center of Kerman University of Medical Sciences. Animals at the animal house were kept in appropriate temperature (22±2°C), relative humidity of 50-70% and light conditions. All procedures in this study were performed in accordance with the guidelines of the National Institutes of Health, approved by the Institutional Animal Care and Use Committee in Kerman University of Medical Sciences. The samples were acclimatized for one week in laboratory conditions before the experiment. Tails of the mice were colored in order to distinguish between the groups. After the fixation step, the mice were randomly divided into four equal-size groups, each composed of thirteen animals. The study groups were as follows: 1) irradiation and medication (getting the NAC before irradiation), 2) only irradiation and two ml of saline instead of NAC, 3) drug and irradiation (getting the medicine after irradiation), and 4) control. The control group did not receive any medication and radiation, only two ml of saline was intraperitoneally administered.

2.3. Medication Administration and Irradiation

600 mg of the medication was dissolved in 250 mL of distilled water and 0.5 mL of that was administered to the mice using gavage. Total body irradiation was carried out with a Cobalt-60 teletherapy unit (Theratron 780, Canada) in Shafa Hospital, Kerman, Iran. The dose rate of machine was 94 cGy/min at a distance of 80 cm. Irradiation was conducted in 35 × 35 cm field size, and the mice were irradiated with a total dose of 8 Gy in one fraction. To increase the precision and ease of the experiments, the animals were placed in an encasement at a distance of 80 cm from source.

2.4. Survival Analysis

During 30 days after $\gamma-$irradiation, the survival fraction of animals in each group was separately recorded and finally analyzed using SPSS software (v 16; Lead Technologies, Inc., USA) through Kaplan-Meier and log rank test. The percentage of increased lifespan (ILS%) was calculated according to this formula: "ILS%= (T-C)/C×100". In this regard, T represents the average survival time of the animals receiving the medication and C is the average survival time of the control group [20].

3. Results

In this study, the effects of radiation protection of NAC drug in total body irradiation of mice at lethal doses were studied. Results of Log rank test were used to compare the survival fraction of the mice in different groups, which rejected the hypothesis of identical survival in the four groups. According to the results, using the medication before irradiation increased the survival fraction of the mice (Figure 1). According to Kaplan-Meier graph of the data, higher rate of survival fraction was observed in the first group, compared to the other groups. The relevant lines of the survival of the second and third groups were almost parallel, and it could be concluded that the survival rate of the two groups was roughly equal. In other words, medication had no effect on the survival of the mice after irradiation.

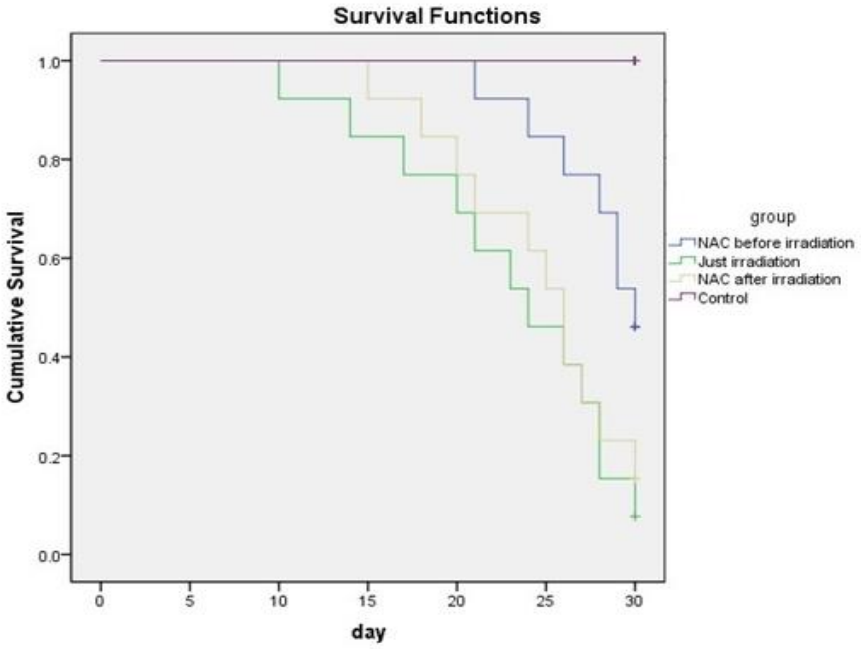

Figure 1. Kaplan-Meier graph of survival fractions of mice after 30 days in all groups

Table 1. Survival rate and percentage of decreased lifespan in all groups after 30 days

Group	Total N	N of Events	Censored		Percentage of decreased lifespan (DLS %)
			N	Percent	
N-acetylcysteine before irradiation	13	7	6	46.2%	5.90
Just irradiation	13	12	1	7.7%	23.6
NAC after irradiation	13	11	2	15.4%	17.93
Control	13	0	13	100.0%	0

Table 2. Comparison of mean and standard deviation of survival time between groups in days

Group	N	Mean±SD	95% Confidence interval		Minimum
			Lower bound	Upper bound	
N-acetylcysteine before irradiation	13	28.23±2.862	26.50	29.96	21
Just irradiation	13	22.92±6.278	19.13	26.72	10
NAC after irradiation	13	24.62±4.822	21.70	27.53	15
Control	13	30.00±.000	30.00	30.00	30
Total	52	26.44±4.972	25.06	27.83	10

Survival fraction of the mice after 30 days in all groups and percentage of decreased lifespan are presented in Table 1.

According to the percentage of increased lifespan (ILS%), obtained by the previously mentioned formula, the percentage of decreased lifespan (DLS%) was calculated for the study groups. The results revealed that decreased lifespan for the first group was greatly lower, compared to the second and third groups. Therefore, application of NAC before irradiation could have a significant effect on reduced possibility of mortality.

On the other hand, results of this study indicated that mean and minimum time of survival was significantly higher in the first group, compared to the second group (Table 2). Nevertheless, no significant difference was observed between the prescription of NAC after irradiation and just

irradiation. This confirms the radiation protection effect of NAC before irradiation.

4. Discussion

Increased use of radiation in medical treatment, occupational radiation and radiation accidents force humans to use radio protector agents [21-23]. However, the goal of radiation therapy is to treat cancer with the lowest amount of side effects [2]. According to the literature, two-thirds of biological effects of ionizing radiation are attributed to free radical interactions, which may be related to the damage of cellular macromolecules. Radiation with use of ROS to eradicate tumor cells will also damage the non-target cells during the process. Therefore, application of ionizing radiation for the treatment of malignant tumors has been limited by the need to achieve a therapeutic differentiation between the cancer cell cytotoxicity and normal tissue toxicity.

Free radicals, which are formed from the radiolysis of water, are the main cause of radiation damage to cells. Therefore, the ability of free radical scavengers can play an essential role in inhibiting normal tissue damage and improving cancer treatment with higher dose of radiation [24-26]. In a study, it was demonstrated that level of damage could be controlled by treating animals or cultured cells with antioxidants, such as vitamins or naturally occurring compounds of plants [27]. In the mentioned research, the effects of irradiation on the survival fractions were observed after exposure of total body to $\gamma-$irradiation. According to Reliene et al., given the safety and efficacy of NAC in mammalian, NAC could be effectively used in radiation therapy to prevent radiation-induced genotoxicity without interfering with efficient cancer cell killing [1].

Kilciksiza et al. suggested that the radioprotective effect of NAC against radiation-induced oxidative damage might be similar to that of WR-2721 [14]. Numerous studies have shown that NAC inhibits both the apoptotic process induced by ROS and the imbalances of the redox potential [28]. The radioprotective role of NAC against oxidative damage induced by UV and ionizing radiation has been confirmed in several studies [24-25]. NAC could improve the health of patients treated for head, neck or lung cancers by inhibiting the second malignancy [29]. In this regard, Sridharan marked that ionizing radiation increased the amount of lipid peroxidation, glutathione (GSH), thyroid-stimulating hormone (TSH) and the activity of antioxidant enzymes and enhanced plasma level of vitamins A, E and C. All parameters were maintained at the normal level of NAC pre-treatment [30]. Interestingly, NAC was able to only induce apoptosis in transformed cells and have no impact on normal cells [31]. The development of radio-protective agents is substantial for protecting patients from the side effects of radiotherapy, as well as the population from unsought irradiation. Despite the extensive research performed with regard to the development of radio-protective agents, there is no approved medication to hinder acute radiation syndrome, with the exception of WR-2721 [32].

In the present study, the protective effect of NAC on radiation-induced lethality in the mice was examined. According to the results, NAC increased the resistance of mice against lethal effects of irradiation. This may be due to the antioxidant properties of NAC that by scavenging free radicals eliminates the damage to free radicals induced by ionizing radiation on cells and reduces the total cell damage. Accordingly, administration of NAC before irradiation could be considered as a radiation-protective agent.

Many studies have indicated that NAC is able to inhibit chemically-induced oxidative stress and DNA damage [33]. Our findings are in line with the concept that NAC provides protection against various cellular damages [34-36]. This study was designed using a single-dose irradiation and limited to study of survival fraction. When nuclear accident or occupational accidents occur, people receive a high single dose of radiation; in such cases, NAC can play an essential role in decreasing malignancy and lethal effects. Our findings revealed the potential radio-protective efficacy of NAC. It is

noteworthy that the observed effect in this study was analogous to that of WR-2721. However, in contrast to WR-2721, NAC is almost safe and could be easily used in clinical settings.

5. Conclusion

In the present study, the protective effect of N-Acetylcysteine on radiation-induced lethality in mice was examined. According to the results, NAC increased the resistance of mice against lethal effects of irradiation. This may be due to the antioxidant properties of NAC that by scavenging free radicals eliminates the damage to free radicals induced by ionizing radiation on cells and reduces the total cell damage. Accordingly, administration of NAC before irradiation can be considered as a useful radiation-protective agent.

This study was designed using a single-dose of gamma irradiation and limited to study of survival fraction. In addition, Toxicity of NAC at different doses was not evaluated and the protective effect of NAC in intraperitoneally administration with intravenous was not compared. Therefore, Authors offer to use of NAC in another research So that the protective effect is evaluated holistically. According to increase use of radiation and destructive effects of radiation, using an effective radioprotector can help to improve side effects of radiation in human health. This investigation has shown that NAC can play an essential role against radiation damage. Interestingly, NAC could modulate the direct destructive effect of radiation and reduce malignancy and mortality.

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