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Effects of *N*-acetylcysteine on life shortening induced by chronic low dose-rate gamma-ray exposure in mice

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ABSTRACT

Background: The development of methods to alleviate radiation-induced health effects is important for the practical use of radiation therapy and for understanding the molecular mechanisms mediating these effects. Here, we examined the protective capability of *N*-acetylcysteine (NAC) on life-shortening effects induced by continuous low dose-rate gamma-ray exposure in mice. ***Materials and Methods***: Female B6C3F1 mice were exposed to gamma-rays for 400 days at a dose rate of 20 mGy/day beginning at 8 weeks of age. Control unexposed mice and exposed mice were divided into two groups; the first was provided with regular water, and the second was administered 40 mM NAC during the exposure period. ***Results:*** Although NAC administration did not affect the life span of non-irradiated mice ($p = 0.232$), a 59-days life extension was observed in the exposed group ($p = 0.0177$). Moreover, radiation exposure and NAC treatment affected body weight. The reduction of body weight observed in NAC treated mice was associated with a reduction in water intake. ***Conclusion:*** Our data demonstrated that the life-shortening effects of chronic low dose-rate radiation exposure in mice were alleviated by NAC administration.

Keywords: *low dose-rate radiation, chronic exposure, life-shortening effect, N-acetylcysteine, mouse model.*

INTRODUCTION

Rapid increases in the use of radiation for medical diagnostics and in radiation exposure resulting from the nuclear power plant accidents at Chernobyl and Fukushima have attracted much attention to the health effects of low-dose and low dose-rate radiation [1-4]. Unfortunately, however, studies on the effects of low-dose and low dose-rate radiation are quite limited [5]. Previously, we examined the lifespan-shortening effects of chronic low dose-rate irradiation using mice as a model system and found that about 400 days of continuous exposure at a dose rate of 21 or 1.1 mGy/day reduced life span, whereas a dose rate of 0.05 mGy/day had no effect on life span [6].

N-Acetyl-L-cysteine (NAC) is a potent chemical that reduces the effects of radiation by scavenging of radiation-induced radicals. In fact, NAC and other radical scavengers have been shown to reduce radiation-induced DNA damage, chromosome abnormalities, cell death, tissue damage, and individual lethality [7-12]. In some cell types, however, the protective effects on cell killing were not observed [13-16]. This could be explained by the reduction of radicals by scavengers and concomitant reaction of NAC with cellular proteins, resulting in modified protein activities and cellular radio-sensitivities [13]. Reduction of radiation effects with scavengers has also been shown in late effects, such as neoplasm induction and life shortening, although the effects were found to vary

depending on sex, genetic background, and radiation dose [17-20]. Furthermore, all of these studies have been carried out using acute high-dose radiation, and no studies have yet evaluated the effects of low dose-rate exposure. Recently, Miller et al. reported that weekly exposure with 50 mGy of 100 kVp X-rays from a clinical CT scanner four times (a total dose of 200 mGy) in 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)-pretreated mice elevated lung tumor incidence, which was suppressed by NAC administration during the irradiation period [21].

Accordingly, based on these previous studies showing the strong and persistent, long-term effects of NAC, we evaluated the effects of NAC treatment on lifespan in mice treated with low dose-rate continuous irradiation.

MATERIALS AND METHODS

Mice and irradiation

Female B6C3F1 mice were purchased from CLEA Japan Inc. (Tokyo) at 6 weeks of age and divided randomly into four groups: (i) control, (ii) NAC-treated, (iii) exposed to chronic low dose-rate radiation, and (iv) NAC-treated during the radiation exposure period. Each group consisted of 60 mice. Mice were housed at 4 mice/cage. The irradiation and NAC treatment started at 8 weeks (56–59 days) of age. The radiation was gamma ray from Cs-137, and the dose-rate was 20 mGy/day. This dose-rate was similar to the dose-rate of 21 mGy/d that was shown previously to reduce lifespan by 120 days after 400 days of continuous exposure [6]. The exposure was interrupted for 2 h everyday (10:00–12:00), when the health of the mice was evaluated. During the same period, cages, feed, and drinking water were renewed on schedule. The renewal rates were once a week for cage and feed and twice a week for water. Mice were fed FR-2 (Funabashi Farms Co., Japan), and NAC (Sigma-Aldrich, St. Louis, MO, USA) was administered through drinking water at a concentration of 40 mM. This dose of NAC was shown to reduce lymphoma incidence by half and extend life span 18 weeks in Atm-deficient mice [22]. The radiation exposure period was 400 days, yielding a total dose of 8 Gy. NAC administration was also carried out for 400 days, beginning on the first day of radiation exposure. After the end of exposure, the mice were moved to no radiation room and maintained until death. The body weight of each mouse was monitored every week. All mice were maintained under specific pathogen-free (SPF) conditions throughout the experiment. The details of mouse maintenance and irradiation conditions were described previously [6, 23]. The experiments were conducted according to legal regulations in Japan and following the Guidelines for Animal Experiments of the Institute for Environmental Sciences.

Feed and water intake

In an attempt to estimate the amount of food and water consumed during chronic radiation exposure and/or NAC treatment, four groups of mice were established, as described above. In this experiment, 6–12 mice were examined in each group. Both food and water were provided *ad libitum*. The amounts of food and water consumed by mice were checked once a week for feed (Monday) and twice a week for water (Tuesday and Friday). The amounts of feed and water intake during 1 week were divided by the number of mice in the cage to estimate the average amount consumed by each mouse. Occasionally, some food or water was spilled from the feeders or waterers. In order to estimate these amounts, we used a statistical method. Outliers at each data point were analyzed by Smirnov-Grubbs test performed using the R program Ver 3.3.1 [24] and were eliminated from the data plotted in the figures.

Statistics

Survival curves were analyzed by log-rank tests for average lifespans. Difference in body weights, feed intake, and water consumption among different groups were analyzed by t-tests. The level of significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 6 (GraphPad, San Diego, CA, USA). Means and standard errors (SEs) were calculated using Excel 2011.

RESULTS

Figure 1 shows the effects of NAC administration on the survival of mice with or without chronic low dose-rate radiation exposure. Mean lifespans of these mice are shown in table 1. Without exposure, the mean lifespans of control and NAC-treated mice were 927 and 959 days, respectively. NAC treatment extended the lifespan of unexposed mice for 32 days; however, this difference was not statistically significant (p = 0.232, Log-rank test). Chronic low dose-rate exposure reduced the lifespan of mice by 176 days from 925 to 751 days (p < 0.0001). The mean lifespans of chronically exposed mice and NAC-treated exposed mice were 751 and 810 days, respectively. Thus, NAC administration increased lifespan by 59 days (p = 0.0177), indicating that NAC treatment was effective for alleviating chronic low dose-rate radiation-induced life-shortening effects.

During the course of the experiment, the body weight of each mouse was monitored every week (figure 2). From about 200 days of age, the irradiated NAC-free group showed an accelerated age-dependent body weight gain when compared with the unexposed group. A similar tendency was observed after 260 days of age in the NAC-treated groups. These differences disappeared at about 650 days of age in the NAC -free group and at about 800 days in NAC-treated mice. Moreover, the administration of NAC reduced the age-dependent body weight increase in both control and exposed mice beginning soon after the start of the treatment.

Next, to elucidate the reason for these changes in body weight, we examined the amounts of food and water consumed during the irradiation and/or NAC treatment period. We limited the observation period to 456 days of age because some exposed mice started to die around this age, which suggested the appearance of diseases in some mice that may influence food and water consumption. Figure 3A shows the amount of food consumed by each mouse in 1 week. The control and radiation-exposed mice consumed similar amounts of feed until 290 days of age, after which the control mice showed slightly higher intake. In contrast, feed intake in NAC-treated mice was similar to that in other groups up to 5 weeks (91 days of age) after the start of treatment, but became slightly reduced after that, irrespective of radiation exposure, until about 280 days of age. Thereafter, feed intake was elevated slightly and reached the level observed in exposed mice. After 370 days, the intake of mice treated with both NAC and radiation decreased slightly.

Time-dependent changes in water consumption are shown in figure 3B. Control and radiation-exposed mice without NAC treatment consumed similar amounts of water until 140 days of age and a slight age-dependent decline was observed in the control group, whereas exposed mice showed sharper decline until 360 days of age, after which a slight increase was observed. Administration of NAC markedly reduced water intake from the beginning of the treatment, i.e., 1 week after the start of treatment (63 days of age) in both the unexposed and exposed groups. In unexposed NAC-treated mice, intake increased with time at a slow rate and then reached a level comparable to that of the untreated control at 380 days of age. In the doubly treated group, the level remained constant until the end of the observation period.

Table 1. Effects of NAC and chronic low dose-rate radiation exposure on life span.

Group	Dose rate (mGy/day)	Total dose (mGy)	Mean life span ± SE (day)	Log-rank test (*P* value)		
				Control versus +NAC	Control versus +IR	+IR versus +IR +NAC
Control	0	0	927 ± 23			
+NAC	0	0	959 ± 23	0.2318		
+IR	20	8,000	751 ± 21		< 0.0001	
+IR+NAC	20	8,000	810 ± 20			0.0177

IR: chronic exposure to gamma-rays

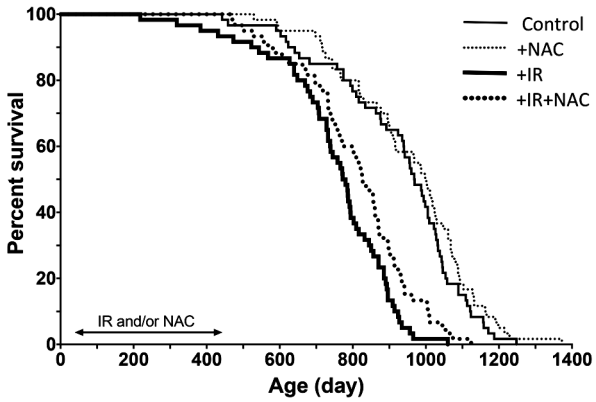

Figure 1. Survival curves of B6C3F1 female mice treated with chronic low dose-rate irradiation and/or NAC. The NAC and/or irradiation treatments were started at 56 days of age. Mice were exposed for 400 days at a dose-rate of 20 mGy/day. The total dose was 8 Gy. NAC was administered through drinking water at a concentration of 40 mM throughout the exposure period. Thin solid line: control mice, thin dotted line: NAC-treated control mice, thick solid line: exposed mice, and thick dotted line: mice treated with both radiation and NAC. The exposure period is shown with a horizontal line with arrowheads at the ends.

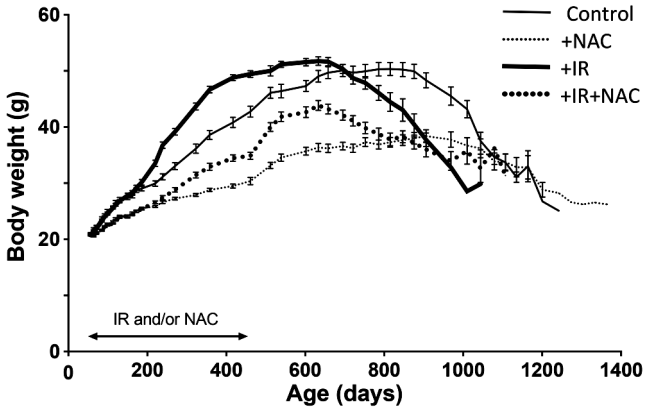

Figure 2. Age-associated changes in body weights. Body weights of the mice were monitored every week from 56 days of age until death, and the averages are plotted. The symbols are the same as those described in figure 1. Data are expressed as means ± SEs.

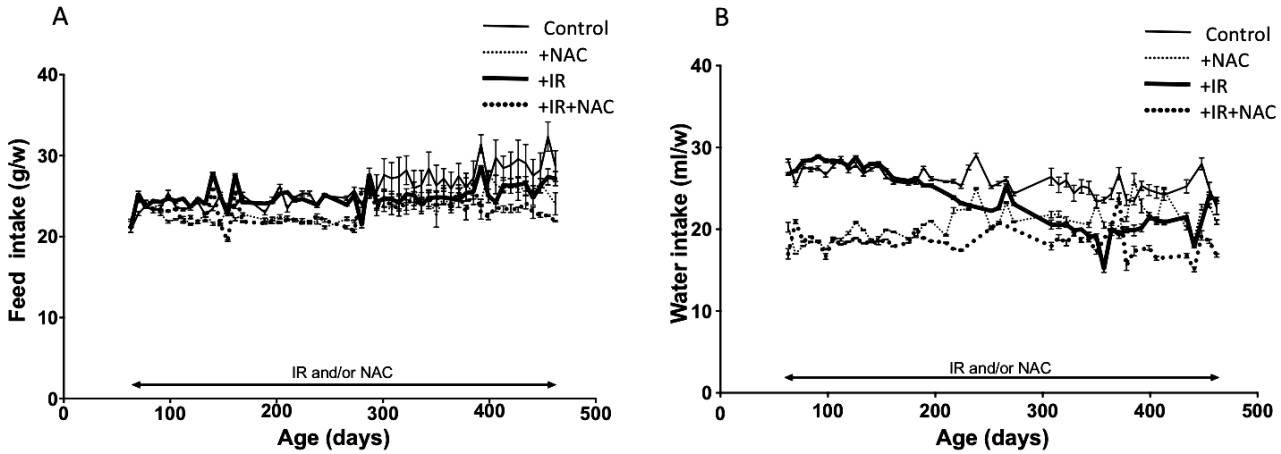

Figure 3. Changes in feed **(A)** and water **(B)** intake during irradiation periods with or without NAC treatment. The symbols are the same as those described in Figure 1. Data are expressed as means ± SEs.

DISCUSSION

In this study, we demonstrated, for the first time, that NAC treatment reduced the life-shortening effects of chronic low dose-rate radiation exposure. The amount of reduction was 59 days, which was approximately one-third of the shortening induced with radiation alone (176 days). This is in contrast to the results of lung tumor induction reported by Miller *et al.* [21]. Lung tumors induced by weekly irradiation with 50 mGy four times in NNK-pretreated mice were suppressed almost completely by NAC administration, although the effect on tumor size was partial. This discrepancy could be based on differences in the endpoints examined (lifespan versus lung tumors), the genetic backgrounds of the mice (B6C3F1 versus AJ), and the method of radiation exposure (chronic low dose-rate versus fractionated high dose-rate), among other factors. We expect that dose rate may be one of the most important of these factors. In this study, we used 20 mGy/day (22 h), corresponding to 0.015 mGy/min, whereas Millar *et al.* used 10–50 mGy/min [21]. At a very low dose-rate, the amount of radicals induced with radiation per unit time would be small, and most radicals could be eliminated with endogenous radical scavengers. If this is the case, additional exogenous supplementation with radical scavengers, such as NAC, could have less effect.

Body weights changed with both radiation exposure and NAC treatment. Irradiation increased body weights in both NAC-free and NAC-treated groups, whereas NAC treatment reduced body weight irrespective of radiation exposure. The former change appeared at 200 or 260 days of age, whereas the latter became obvious soon after the start of the treatment. Previously, Flurkey *et al.* [26] studied the effects of NAC treatment on body weight and lifespan. In their study, body weights were reduced by approximately 10 g after drinking water containing 30.6 or 61.2 mM NAC, similar to the results of the present study. The effects were observed in both males and females; however, lifespan was extended only in males [26]. Our present study in female mice also showed a lack of lifespan extension by NAC treatment, supporting a previous report. Although reduced body weight caused by calorie or food restriction has been shown to be associated with life extension [27-30], it is likely that the NAC-induced reduction in body weight was not associated with lifespan extension in unexposed female mice. The reason for the rapid body weight loss in NAC-treated mice seemed to be related to reduced water intake rather than reduction of food intake because water intake dropped soon after the start of NAC treatment, whereas the reduction in food intake appeared about 120 days after the beginning of the treatment.

Radiation-induced increases in body weight were observed 150-200 days after the start of chronic low dose-rate exposure in both NAC-treated and NAC-free groups. The effect of radiation in NAC-free mice was similar to that observed previously in our laboratory [23, 25]. These changes could be associated with slight decreases in water intake observed in mice of similar ages in both the NAC-free and NAC-treated groups exposed to radiation (figure 3B). Indeed, food intake during this period did not differ appreciably between the exposed and unexposed groups, regardless of NAC treatment.

Further studies are needed to determine the detailed mechanisms mediating these changes in body weight associated with radiation exposure and NAC treatment.

CONCLUSION

The life-shortening effects of chronic low dose-rate radiation exposure in mice were reduced with NAC administration. The level of these effects was limited to about 30% and was associated with changes in body weight and water intake. Further studies are needed to determine the possible associations among these parameters.

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***Conflicts of interest*: Declared none.**

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