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Effect of *N*-acetylcysteine on Radiation-induced Genotoxicity and Cytotoxicity in Rat Bone Marrow

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Irradiation-injury/*N*-acetylcysteine/WR-2721.

The aim of this study is to evaluate the potential radioprotective effects of *N*-acetylcysteine (NAC) against genotoxicity and cytotoxicity. The effect of WR-2721, as a representative of clinically used radioprotector, was compared with that of NAC, using the chromosomal aberration (CA) and micronucleus (MN) test systems in the irradiated rat's femoral bone marrow cells. We also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). The rats (n = 16) were divided randomly and equally into four groups: Control (C), Radiation (R), R+NAC (received irradiation and 1000 mg/kg NAC) and R+WR-2721 (received irradiation and 200 mg/kg WR-2721) rats. All the irradiated groups received whole-body gamma irradiation as a single dose of 6 Gy. Group R showed higher CA and MN formation when compared to C. Group R showed higher frequency of MN formation when compared to both R+NAC and R+WR-2721. The mean MI and PCE/NCE ratios were lower in Group R when compared to those of Group C. The mean MI and PCE/NCE ratios of both R+NAC and R+WR-2721 groups were lower when compared to those of Group C. The MI in Group R was lower when compared to that of both R+NAC and R+WR-2721 groups. In this study, the results give clues about the beneficial effects of NAC against radiation-induced genotoxicity and cytotoxicity in rat bone marrow and its effect may be comparable to that observed for WR-2721.

INTRODUCTION

Ionizing radiation interacts with mammalian cells and produces different cytotoxic effects and it is also a major therapeutic agent for cancer treatment. Radiation produces two main species that combine to kill cells in a clinical setting; these are reactive oxygen species (ROS), and DNA double-strand breaks (DSB).[1)] ROS is a product of normal cellular metabolism, which is a mainly oxidative metabolism in mitochondria and pathologic processes such as inflammation. Overproduction of ROS can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA.[2)]

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Radiation can cause various lesions by direct interaction with DNA or indirectly through damage induced by free radicals mainly resulting from the radiolysis of water molecules, the most abundant molecule in the cell.[2)] The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone.[2,3)] Finally, irradiation induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage as well as DNA–DNA and DNA–protein cross-links. Misjoined or unrepaired DNA DSBs can produce deletions, translocations, and acentric or dicentric chromosomes, thereby leading to significant cellular damage and cell death.[4,5)] Damage to chromosomes is also manifested as breaks and fragments, which appears as micronuclei in the rapidly proliferating cells.[6)]

Many of the regulatory changes in cells after irradiation may be mediated through the production and interaction of classical signal transduction, ROS, and DNA DSBs.[2,7)] It is reasonable to assume that agents, capable of scavenging free radicals, would play a significant role in modulating cell damages caused by ionizing radiation. The protection of normal tissues may provide an increase in tumor control by providing an increase in the radiation dose.[8)]

Several chemicals have been shown to provide radiation

protection in experimental animals; however, their clinical utility is limited by drug toxicity.[9,10] Among the large number of tested compounds, cysteamine derivatives, i.e. aminothiol radioprotectors (for example cysteine, cystamine, WR-2721, glutathione) were the most examined ones.[8,11,12] The suggested mechanisms of sulfhydryl compounds are free-radical scavenging and the facilitation of direct chemical repair at sites of DNA damage by hydrogen atom donation. Reduced glutathione (GSH) is a multifunctional intracellular non-enzymatic soluble antioxidant. GSH is highly abundant in the cytosol, nuclei, and mitochondria. It is considered to be the major thiol-disulphide redox buffer of the cell and plays a crucial role in the detoxification of xenobiotics.[2,13]

N-acetylcysteine (NAC), an aminothiol and synthetic precursor of intracellular cysteine and GSH, has been used as a mucolytic agent and the drug of choice in paracetamol intoxication without major side effects.[14,15] In addition, it has been shown to prevent radiation-induced DNA breaks and to have a place in cancer prevention.[16,17]

The aim of this study is to evaluate the potential radioprotective effects of NAC against genotoxicity and cytotoxicity. The effect of NAC was also compared with that of WR-2721, as a representative of clinically used radioprotector, in the oxidative damage caused by gamma-irradiation in rat's femoral bone marrow cells after single dose of whole body irradiation. Two conventional cytogenetic assays, CA and MN, were used for detecting the fixed unrepaired fraction of the radiation induced DNA damage. The measurement of MN is widely used in radio-and chemotoxicity and has been proven to be a valid marker for quantization of oxidative damage to DNA.[18–20] MI and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) were also evaluated in order to determine the cytotoxic effects of gamma radiation in femoral bone marrow. MI reflects the frequency of cell divisions and it is an important parameter in determining the rate of cytotoxicity.[18,19] The PCE/NCE ratio is an indicator of the proliferation rate, and a decline in the post-irradiation ratio is an expression of the known early effects of radiation on cell cycle, which indicates a mutagen-induced bone marrow cytotoxicity or suppression of erythropoiesis.[6,19]

MATERIAL AND METHOD

Animals

Sixteen healthy adult female Wistar rats (Gaziantep University, Faculty of Medicine, Experimental Medicine Research Unit; 8 weeks of age, with average body weight of 170 ± 20 g) were used. The rats were randomly selected and housed in polycarbonate cage with free access to tap water and rat chow with a dark/light cycle of 12:12 h. The temperature value of animal laboratory, where the animals were kept, was 22 ± 2°C and the relative humidity was 50–70%. All procedures in this study were performed in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals and were also approved by the Institutional Animal Care and Use Committee in the Faculty of Medicine at Gaziantep University.

Experimental design

The rats were acclimatized for 1 week to our laboratory conditions prior to experimental manipulation. After the stabilization period, the rats were divided randomly into four equal-size groups (4 rats per group), namely, Control (C), irradiation (R), irradiation + NAC (R+NAC) and irradiation + WR-2721 (R+WR-2721) groups. C rats received neither radioprotector nor irradiation, but 2.2 ml of saline was injected intraperitoneally (i.p.). All rats of the study groups (R, R+NAC and R+WR-2721) received whole-body gamma irradiation as a single dose of 6 Gy. Besides irradiation, R rats received 2.2 ml of saline (i.p.) while the R+NAC and R+WR-2721 rats received 1000 mg/kg, (i.p.) NAC (containing 300 mg of *N*-acetylcysteine, Asist ampul, Hüsnü Arsan İlaç, İstanbul) and 200 mg/kg, (i.p.) WR-2721 (containing 500 mg of amifostine, Ethyol flacon, Er-Kim İlaç, İstanbul) respectively. Saline, NAC and WR-2721 injections in study groups were performed 15 minutes prior to irradiation. A cobalt-60 teletherapy unit (Shandong Xinhua SCC-8000F, China) was used for all irradiations. The dose rate was 1.80 Gy/min at a distance of 80 cm.

The study was terminated by sacrificing the rats under Ketalar (Eczacıbası, Turkey) anesthesia (35 mg/kg, intramuscularly) 72-h after irradiation. At termination, femurs of each rat were bilaterally harvested and cleaned of any adhering muscle. Bone marrow cells were bilaterally collected from rats' femurs. Contents of bone marrow were utilized for the cytogenetic tests (CA, MN) by separating the same amount of the specimens. After cytogenetic tests had been measured, cytotoxicity tests (MI, PCE/NCE ratio) were performed by the preparation concerned.

Chromosome aberration assay

Cytogenetic analysis was performed on bone marrow cells according to conventional cytogenetic techniques. Bone marrow cells were collected from the femurs by flushing in 3 ml of RPMI 1640 (Biochrom AG, Germany) medium and incubated at 37°C for 35 min with 35 μl of colcemid solution (10 μg/ml, Biochrom AG, Germany). After incubation, the material was centrifuged at 1200 rpm for 10 min, fixed in aceto-methanol (acetic-acid: methanol, 1:3, v/v). Centrifugation and fixation (in the cold) were repeated five times. The material was resuspended in a small volume of the fixative, dropped onto chilled slides, flame-dried and stained on the following day in 10% buffered Giemsa (pH 6.8). At least 100 well spread metaphases containing 42 ± 2 chromosomes were examined per animal to score for different types of aberrations.

Micronucleus test

The frequency of micronucleated erythrocytes in femoral bone marrow was evaluated according to the procedure of Schmid[20] using the modifications of Agarwal and Chauhan.[21] The bone marrow was flushed out from femurs using 1 ml of RPMI 1640 and centrifuged at 1200 rpm for 10 min. The supernatant was discarded. Evenly spread bone marrow smears were stained using the May-Grunwald and Giemsa protocol. Slides were scored at a magnification of 1500 × using a light microscope.

Mitotic index determination

The mitotic index was used for determining the rate of cell division. The slides, prepared for the assessment of chromosomal aberrations, were also used for calculating the mitotic index. Randomly selected views on the slides were monitored to determine the number of dividing cells (metaphase stage) and the total number of cells. At least 1000 cells were examined in each rat. Mitotic index values are expressed as % in this study.

Scoring

In analyzing the frequencies of MN formation, 2000 PCEs per animal were scored to calculate the MN frequencies. Two hundred erythrocytes (immature and mature cells) were examined to determine the ratio of PCEs to normochromatic erythrocytes (NCEs).

Statistical analyses

Descriptive values of data were represented as means ± standard deviation (SD.). The one-way analysis of variance (ANOVA) test was used for determining significant differences in the frequencies of CA, MN, MI values and PCEs/NCEs ratios between groups followed by the

Table 1. Frequency of chromosome aberrations and the value of mitotic index in bone marrow cells of female Wistar rats.

Treatment Groups	Fixation time (h)	Metaphase per 1000 Cells		Total number of examined metaphases (n)	Chromatid		Chromosome		Fragment	Dic	TSCA	Mean ± SD % SCA (excluding gaps)
					Gap	Break	Gap	Break				
		1	5.26									
		2	4.84									
C	24	3	4.92	400/4	3	4	3	4	–	–	14	1.00 ± 0.82
		4	5.1									
		Mean ± SD	5.03 ± 0.19									
		1	2.23									
		2	2.62									
R	24	3	3.05	400/4	16	17	18	12	2	2	67	3.00 ± 0.82[a‡]
		4	2.51									
		Mean ± SD	2.60 ± 0.34[a*]									
		1	3.24									
		2	3.5									
R+WR-2721	24	3	4.02	400/4	13	14	15	7	–	–	49	1.75 ± 0.50
		4	3.25									
		Mean ± SD	3.05 ± 0.37[a*,b†]									
		1	3.75									
		2	4.1									
R+NAC	24	3	3.8	400/4	13	12	14	7	–	–	46	1.75 ± 0.96
		4	3.55									
		Mean ± SD	3.80 ± 0.23[a*,b*]									

n: number of animals; TSCA: total structural chromosome aberrations; Dic: dicentric chromosome; WR-2721: Amifostine; NAC: *N*-acetylcysteine; C: Control rats that were treated with 2.2 ml of saline; R: Radiation exposed rats that were exposed to 6 Gy, single dose, whole-body gamma irradiation and treated with 2.2 ml of saline; R+WR2721: rats that were exposed to same irradiation procedure as R rats and treated with 200 mg/kg-BW WR-2721; R+NAC: rats that were exposed to same irradiation procedure as R rats and treated with 1000 mg/kg-BW NAC. Each group consists of ten rats. All values are given as the mean ± standard deviation (SD.). Statistical analysis was performed by using the ANOVA test followed by the Bonferroni post hoc test. [a] Compared to C rats, [b] Compared to R rats; * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$. The cases, where statistically significant differences were found, are given in the Table.

Bonferroni post-hoc test. A value of P less than 0.05 was considered significant, and in all calculations, the SPSS (v 11.5; Lead Technologies, Inc., IL USA) program was used.

RESULTS

The metaphase analysis of the bone marrow cells revealed various types of chromosomal aberrations consisting of chromatid and isochromatid types of gaps and breaks, double minute (including isochromatid breaks) exchanges, dicentric chromosomes and fragments (Table 1). Numerical aberrations were not scored in this study. Tables 1 and 2 summarize the results of the chromosome aberration assay (CA), mitotic index (MI), PCEs/NCEs ratios and micronucleus formation (MN) in bone marrow cells after gamma irradiation alone and with radioprotectors (NAC and WR-2721).

When the mean CA value of R rats was compared with that of the C rats, the mean CA values of R rats was significantly higher than that of C rats after irradiation ($p = 0.023$). With respect to the CA analyses, no statistically significant differences were found between the C rat samples and both R+NAC and R+WR-2721 rat samples, indicating the protective effect of each of NAC and WR-2721 treatment on radiation induced damage ($p > 0.05$) (Table 1 and Fig. 1).

The results of the MI (used on a measure of evaluation of cell cycle kinetics) are summarized in Table 1. The outcomes have shown that the MI of bone marrow cells was significantly decreased after irradiation in R, R+NAC and R+WR-2721 rats in comparison to the C rats ($p = 0.0001$,

Table 2. Micronucleus induction and the ratio of PCE in bone marrow cells of female Wistar rat.

Treatment Group	Rat number	Micronucleus per 2000 PCEs	Fixation Time (h)	PCEs per 200 erythrocytes
C	1	1		102
	2	3		95
	3	2	24	105
	4	2		110
	Mean ± SD	2.00 ± 0.82		103.00 ± 6.27
R	1	5		42
	2	4		53
	3	6	24	47
	4	5		57
	Mean ± SD	5.00 ± 0.82[a]†		49.75 ± 6.60[a]*
R+WR-2721	1	3		55
	2	3		61
	3	4	24	64
	4	3		59
	Mean ± SD	3.25 ± 0.50[b]‡		59.75 ± 3.78[a]*
R+NAC	1	4		62
	2	3		56
	3	3	24	54
	4	2		51
	Mean ± SD	3.00 ± 0.82[b]‡		55.75 ± 4.65[a]*

PCEs: polychromatic erythrocytes; WR-2721: Amifostine; NAC: *N*-acetylcysteine; C: Control rats that were treated with 2.2 ml of saline; R: Radiation exposed rats that were exposed to 6 Gy, single dose, whole-body gamma irradiation and treated with 2.2 ml of saline; R+WR2721: rats that were exposed to same irradiation procedure as R rats and treated with 200 mg/kg-BW WR-2721; R+NAC: rats that were exposed to same irradiation procedure as R rats and treated with 1000 mg/kg-BW NAC. Each group consists of ten rats. All values are given as the mean ± standard deviation (SD.). Statistical analysis was performed by using the ANOVA test followed by the Bonferroni post hoc test. [a] Compared to C rats, [b] Compared to R rats; * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$. The cases, where statistically significant differences were found, are given in the table.

$p = 0.0001$ and $p = 0.0001$, respectively). On the other hand, with the applications of NAC and WR-2721, there was a marked increase in MI in both R+NAC and R+WR-2721 groups when compared to R group ($p = 0.0001$ and $p = 0.005$, respectively).

The effects of gamma irradiation on the frequency of MN formation are shown in Table 2. Irradiation induced a significant increase in MN formation in Group R when compared with the C rats ($p = 0.001$) (Fig. 2). It was assigned that the MN averages of R+NAC and R+WR2721 rats were considerably and statistically lower than those of the R rats ($p = 0.016$ and $p = 0.038$, respectively). No statistically significant differences were found between the C rats and both R+NAC and R+WR-2721 rats.

The effects of gamma irradiation on the PCEs/NCEs ratios (used as a measurement of cytotoxicity) are shown in Table 2. Results obtained by the PCEs/NCEs ratios have shown that the averages of R, R+NAC and R+WR-2721 rats were considerably and statistically lower than those of the C rats ($p = 0.0001$, $p = 0.0001$ and $p = 0.0001$, respectively). The mean PCE/NCE ratio of R rats was lower than that of R+NAC and R+WR-2721 rats; however, these differences were not significant.

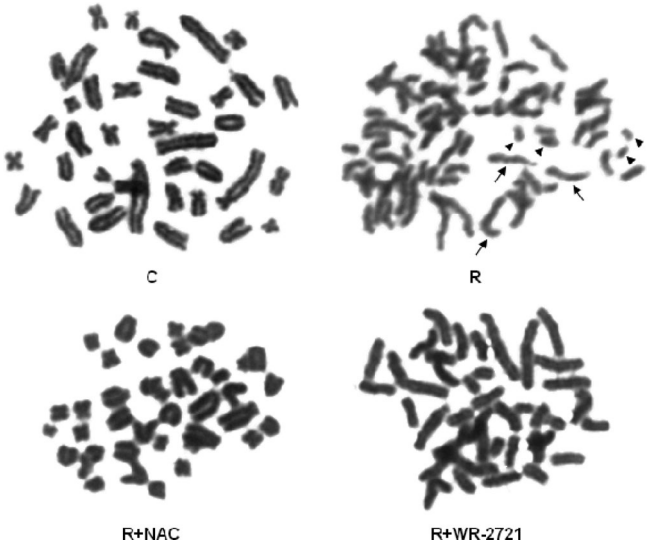

Fig. 1. **(C)** The normal chromosome structure in numerical and morphological senses is observed on a metaphase plate of C rat (× 100). **(R)** Chromatid separations, chromosome breaks and numerical chromosomal anomalies after irradiation on a metaphase plate of R rat (× 100). (R+NAC and R+WR-2721) It is seen respectively that the chromosome morphology is slightly worse in R+NAC and R+WR-2721 groups than the metaphase plates obtained from the control group, but no or less number of breaks or other anomalies were observed in comparison to group R (× 100). The arrows show the aberrated chromatids whereas the arrow tips show breaks.

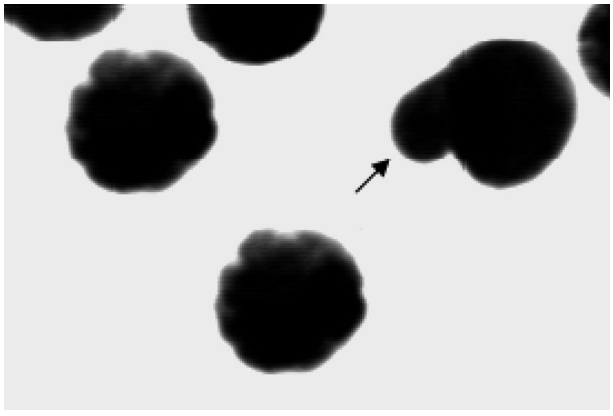

Fig. 2. Typical view of micronucleus in rat bone marrow cells (arrows) 72 h after irradiation (× 100).

DISCUSSION

In the present study, we particularly investigated the protective effect of NAC on radiation induced DNA damage and this effect was compared with that of WR-2721, as a representative of clinically used radioprotector. In order to obtain an assessment of DNA damage induced by whole-body gamma irradiation, we used cytogenetic tests (CA, MN, MI, PCE/NCE ratios) that detect biological effects resulting from unrepaired DNA damage. In the present study, as it was observed in previous studies, radiation produced a significant increase in the frequency of CA with MN confirming the clastogenic effect of irradiation.[6,9,22–24] In addition, there was a marked decrease in MI and the mean PCE/NCE ratio.

The therapeutic effect of ionizing radiation depends on the generation and use of ROS to eradicate tumor cells, and non-target cells are also damaged in this process.[25,26] Therefore, application of ionizing radiation to 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.[8,27] The development of radiation protectors is not only for raising the effectiveness of cancer treatment, but also for studying the underlying mechanisms of radiation cytotoxicity.[28] Thiol supplementation to maintain tissue redox balance has been studied by various researchers. Their application, however, has been limited by their toxic side effects in both animal and cell models.[29,30] It is an extremely slow and a very expensive project to discover new drug molecules and it also has a high rate of failure.[24] The aim of identifying the present drugs, having minimal or no toxicity, for new uses may be more reasonable.

One of the mechanisms of protection is free-radical scavenging, and it is based on the supposition that free radicals formed from the radiolysis of water are the main cause of radiation damage to cells.[2,8] It is known that GSH plays a crucial role in the detoxification of drug-or radiation-induced oxygen free radicals.[2,31] NAC, an aminothiol and synthetic precursor of intracellular cysteine and GSH, has

been used for many years as a mucolytic drug. When orally administered, it is well-tolerated and has no clinically significant adverse effects. It is a reducing agent and has proven anti-oxidant, anti-inflammatory and cytoprotective effects.[32–34] NAC has been considered to have a place in cancer prevention, too.[33,34]

The evidence from both in vitro and in vivo studies suggests that NAC is capable of replenishing intracellular GSH by reducing extracellular cystine to cysteine,[35] or by supplying sulfhydryl (–SH) groups that can stimulate GSH synthesis and enhance glutathione-S-transferase activity.[34,36,37] NAC is a potent free radical scavenger in consequence of its nucleophilic reactions with ROS.[5,38] Thus, NAC treatment may be beneficial for conditions of GSH depletion and free radical formations during oxidative stress.[5,14,16,39] It is suggested that NAC may protect cell membranes against lipid peroxidation and protein oxidation, and helps maintain the integrity of cellular organelles.[16,40,41] However, it remains to be determined whether and how NAC influences basic cellular processes such as apoptosis.[32]

The present study was designed on the basis of the effect of NAC as an antioxidant agent shown in the previous studies. On the grounds of the result of our study, it may be suggested that NAC is capable of decreasing irradiation-induced genotoxicity and cytotoxicity in rat bone marrow. Our observation on the protective effect of NAC in the study is in accordance with the results of some other studies, where different parameters are used. In the study of Reliene *et al*., it was shown that dietary supplementation with NAC suppressed carcinogenesis-associated biological markers in Atm-deficient mice, such as DNA deletions and oxidative DNA damage.[39] Numerous studies have shown that NAC inhibits both the apoptotic process induced by ROS and the imbalances of the redox potential.[17,31,42] Interestingly, NAC was able to induce apoptosis in transformed cells but not in normal cells.[5] In another study by Oda *et al*., ricin-induced apoptotic cell death was strongly inhibited by NAC.[43] Campain *et al*. have reported that nicotine was considerably less effective in inducing either oxidative damage or formation of MN in the cells pretreated with two antioxidants, NAC and catalase.[44] It has been suggested that, NAC, by virtue of its free radical scavenging capacity and replenishment of glutathione stores, reduced the cellular and DNA damage caused by nicotine. NAC has been shown as one of the most promising chemopreventive agents against lung tumorigenesis and an efficient antioxidant.[33] In our study, the administration of NAC significantly improved the radiation-induced increase in CA and frequency of MN formation. In addition, R+NAC rats showed higher MI in comparison to R rats.

The similar role of NAC as a radioprotective agent against oxidative damage induced by UV and ionizing radiation has been shown in experimental studies.[16,39,40,45] Morley *et al*. have reported that NAC supplementation can slow down DNA damage induced by UVA, UVB and visible radiation. They suggested that, although the specific mechanism of NAC protection has not been clarified yet, their results support the hypothesis that NAC may protect the cells directly, by scavenging ROS induced by UVA and visible radiation, and indirectly by donating cysteine for GSH synthesis.[45] On the contrary, He and Hader have documented that NAC did not demonstrate an influential effect on lipid peroxidation and DNA strand breaks induced by UVB.[46] In the study of Mansour *et al*, pretreatment with NAC showed a protection against gamma-radiation induced cellular and DNA damage.[16] They suggested that NAC was effective in protecting against radiation-induced damage by replenishment of glutathione stores as well as scavenging of ROS and inhibition of NO(x). As also observed in our study, although NAC reduces the cellular and DNA damage caused by irradiation, arguments are going on about its effect mechanism.[47,48]

In the present study, radiation decreased the PCE/NCE ratio of the bone marrow in group R. Through an addition of each radioprotector, the PCE/NCE ratio increased slightly; however, this difference was not statistically significant. In some experimental studies, where different radioprotectors were used, it was observed that the PCE/NCE ratio may be of close values with the control group or may display fluctuations depending on drug dose or time.[22,49]

In addition to the results discussed above, the effect of NAC was compared with that of WR-2721 in the present study. The findings about NAC in this study were comparable to those of WR-2721. WR-2721, a member of phosphorothioates, is thought to be a broad-spectrum cytoprotective and radioprotective agent. WR-2721 and related compounds that cover the SH with a phosphate group reduce toxicity as well as genotoxicity.[8,11,50] There are many aspects of WR-2721 that limit its use clinically such as its toxicity and its requirement to be present at the time of irradiation in order to be effective.[9,51] It is not clear how WR-2721 induces radioprotection.[10,12] The structural similarity of the metabolites of WR-compounds to endogenous polyamines suggests that they may interact with DNA and influence its protection, repair, and synthetic processes.[10] The radioprotective effect of WR-2721 observed in this study is in parallel to the results of similar studies in the literature.

There are a few number of comparative studies involving WR-2721 and NAC together among the investigations with radioprotectors. The study of Mantovani G *et al*. assessed, in 120 advanced cancer patients, the biological parameters relevant to cancer cachexia, such as oxidative stress and ROS. Each of WR-2721 and NAC significantly increased the progression of the peripheral blood mononuclear cells through the cell cycle, thus providing evidence of their potential role in the functional restoration of the immune system in advanced cancer patients.[52] Klingler *et al*. reported the observation and the improvement of granulocyte/macroph-

age colony-forming cells cloning efficiency after irradiation. They pointed out that only NAC was able to recruit progenitor cells in the cell cycle whereas WR-1065 possibly inhibited cell cycle progression by S and G2/M arrest.[53] Despite some arguments, it can be stated that the observed cytoprotective effects of NAC and WR-2721 in our study are in parallel to most of the results of studies mentioned.[53,54]

In conclusion, despite WR-2721, NAC has not been used clinically for radioprotective purpose yet. Due to the low toxicity and considerable history of clinical use for NAC, as being an agent of sulfhydryl compounds, further experimental studies are needed for proving this result and for ruling out the potential protection of tumor cells and for exploiting the clinical advantage of NAC.

REFERENCES

1. Hall, E. J. (1993) Radiobiology for the radiologist. Philadelphia, Pa: Lippincott Williams & Wilkins. p 15–29.
2. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M. and Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. **39**: 44–84.
3. Halliwell, B. (1996) Antioxidants in human health and disease. Annu. Rev. Nutr. **16**: 33–50.
4. Barker, S., Weinfeld, M., Zheng, J. and Li, Murray, D. (2005) Identification of mammalian proteins cross-linked to DNA by ionizing radiation. J. Biol. Chem. **280**: 33826–33838.
5. De Flora, S., Izzotti, A., D'Agostini, F. and Balansky, R. M. (2001) Mechanisms of *N*-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. Carcinogenesis **22**: 999–1013.
6. Hofer, M., Mazur, L., Pospil, M., Weiterova, L. and Znojil, V. (2000) Radioprotective action of extracellular adenosine on bone marrow cells in mice exposed to gamma rays as assayed by micronucleus test. Radiat. Res. **154**: 217–221.
7. Stevenson, M. A., Pollock, S. S., Coleman, C. N. and Calderwood, S. K. (1994) X-irradiation, phorbol esters, and H2O2 stimulate mitogen-activated protein kinase activity in NIH-3T3 cells through the formation of reactive oxygen intermediates. Cancer Res. **54**: 12–15.
8. Weiss, J. F. (1997) Pharmacologic approaches to protection against radiation-induced lethality and other damage. Environ. Health Perspect. **105**: 1473–1478.
9. Uma D. P. (1998) Normal tissue protection in cancer therapy: progress and prospects. Acta Oncol. **37**: 247–252.
10. Weis, J. F. and Landauer, J. F. (2000) Radiaprotection by antioxidants. Ann. N. acad. Sci. **899**: 44–66.
11. Hensley, M. L., Schuchter, L. M., Lindley, C., Meropol, N. J., Cohen, G. I., Broder, G., Gradishar, W. J., Green, D. M., Langdon, R. J., Mitchell, R. B., Negrin, R., Szatrowski, T. P., Thigpen, J. T., Von Hoff, D., Wasserman, T. H., Winer, E. P. and Pfister, D. G. (1999) American Society of Clinical Oncology clinical practice guidelines for the use of chemotherapy and radiotherapy protectants. J. Clin. Oncol. **17**: 3333–3355.
12. Kouvaris, J. R., Koulolias, V. E. and Vlahos, L. J. (2002) Amifostine: The first selective-target and broad-spectrum radioprotector. Mut. Res. **519**: 37–48.
13. Masella, R., Di B., R., Varì, R., Filesi, C. and Giovannini, C. (2005): Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J. Nutr. Biochem. **16**: 577–586.
14. Wanamarta, A., Van Rijn, J., Blank, E., Haveman, J., Van Zandwijk, N. and Joenje, H. (1989) Effect of *N*-acetylcysteine on the antiproliferative action of x-rays or bleomycin in cultured human lung tumor cells. J. Cancer Res. Clin. Oncol. **115**: 340–344.
15. Sjodin, K., Nilsson, E., Hallberg, A. and Tunek, A. (1989) Metabolism of *N*-acetyl-L-cysteine. Biochem. Pharmacol. **38**: 3981–3985.
16. Mansour, H. H., Hafez, H. F., Fahmy, N. M. and Hanafi, N. (2008) Protective effect of *N*-acetylcysteine against radiation induced DNA damage and hepatic toxicity in rats. Bioch. Phar. **75**: 773–780.
17. Solen, G. (1993) Radioprotective effect of *N*-acetylcysteine in vitro using the induction of DNA breaks as end-point. Int. J. Radiat. Biol. **64**: 359–366.
18. El-Habit, O. H. M., Saada, H. N., Azab, K. S., Abdel-Rahman, M. and El-Malah, D. F. (2000) The modifying effect of β-Carotene on gamma radiation-induced elevation of oxidative reactions and geneotoxicity in male rats. Mutat. Res. **4**: 66179–66186.
19. Sudheer, A. R., Muthukumaran, S., Kalpana, C., Srinivasan, M. and Menon, V. P. (2007) Protective effect of ferulic acid on nicotine induced DNA damage and cellular changes in cultured rat peripheral blood lymphocytes: a comparison with *N*-acetylcysteine. Toxicol. Vitro **21**: 576–585.
20. Schmid, W. (1976) The micronucleus test for cytogenetic analysis. In: Chemical Mutagens, Principles and Methods for Their Detection, Eds. Hollaender, A., vol. 4, pp. 31–53, Plenum Press, New York.
21. Agarwal, D. K. and Chauhan, L. K. S. (1993) An improved chemical substitute for fetal calf serum for the micronucleus test. Biotechnol. Histochem. **68**: 187–188.
22. Jagetia, G. C. and Aruna, R. (1997) The herbal preparation abana protects against radiation-induced micronuclei in mouse bone marrow. Mutat. Res. **393**: 157–163.
23. Erdal, N., Gürgül, S. and Çelik, A. (2007) Cytogenetik effect of extremely low frequency magnetic field on wistar rat bone marrow. Mut. Res. **6**: 3069–3077.
24. Kumar, M. S., Unnikrishnan, M. K. and Uma D. P. (2003) Effect of 5-aminosalicylic acid on radiation-induced micronuclei in mouse bone marrow. Mutat. Res. **527**: 7–14.
25. Mishra, K. P. (2002) Fluorescence studies on radiation oxidative damage to membranes with implications to cellular radiosensitivity. J. Chem. Scien. **114**: 705–711.
26. Borek, C. (2004) Antioxidants and radiation therapy. Jour. Nutrition **134**: 3207S–3209S.
27. Prasad, N. R., Srinivasan, M., Pugalendi, K. V. and Menon, V. P. (2006) Protective effect of ferulic acid on γ-radiation-induced micronuclei dicentric aberration and lipid peroxidation in human lymphocytes. Mut. Res. **603**: 129–134.
28. Hahn, S. M., Krishna, C. M., Samuni, A., DeGraff, W., Cuscela, D. O., Johnstone, P. and Mitchell, J. B. (1994) Poten-

tial use of nitroxides in radiation oncology. Cancer Res. **54**: 2006–2010.
29. Cairnie, A. B. (1983) Adverse effects of the radioprotector WR2721. J. Radiat. Res. **94**: 221–226.
30. Held, K. D. and Melder, D. C. (1987) Toxicity of the sulfhydryl containing radioprotector dithiothreitol. J. Radiat. Res. **112**: 544–554.
31. Atkuri, K. R., Mantovani, J. J. and Herzenberg, L. A. (2007) *N*-Acetylcysteine-a safe antidote for cysteine/glutathione deficiency. Curr. Opin. Pharmacol. **7**: 355–359.
32. Spapen, H. (2004) *N*-acetylcysteine in clinical sepsis: a difficult marriage. Crit. Care. **8**: 229–230.
33. Van Zandwijk, N. (1995) *N*-acetylcysteine (NAC) and glutathione (GSH): antioxidant and chemopreventive properties, with special reference to lung cancer. J. Cell Biochem. **22**: 24–32.
34. De Flora, S., Bennicelli, C. and Camoirano, A., Serra, D., Romano, M., Rossi, G. A., Morelli, A. and De Flora, A. (1985) In vivo effects of *N*-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. Carcinogenesis **6**: 1735–1745.
35. Issels, R. D., Nagele, A., Eckert, K. G. and Wilmanns, W. (1988) Promotion of cystine uptake and its utilization for glutathione biosynthesis induced by cysteamine and *N*-acetylcysteine. Biochem. Pharm. **37**: 881–888.
36. Nakata, K., Kawase, M., Ogino, S., Kinoshita, C., Murata, H., Sakaue, T., Ogata, K. and Ohmori, S. (1996) Effects of age on levels of cysteine, glutathione and related enzyme activities in livers of mice and rats and an attempt to replenish hepatic glutathione levels of mouse with cysteine derivatives. Mech. Ageing Dev. **90**: 195–207.
37. De Vries, N. and De Flora, S. (1993) *N*-acetyl-L-cysteine. J Cellul. Bioch. Supp. **17**: 270–277.
38. Aruoma, O. I., Halliwell, B., Hoey, B. M. and Butler, J. (1989). The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic. Biology and Med. **6**: 593–597.
39. Reliene, R., Fischer, E. and Schiestl, R. H. (2004) Effect of *N*-acetylcysteine on oxidative DNA damage and the frequency of DNA deletions in atm-deficient mice. Cancer. Res. **64**: 5148–5153.
40. Neal, R., Matthews, R. H., Lutz, P. and Ercal. N. (2003) Antioxidant role of *N*-acetyl cysteine isomers following high dose irradiation. Free Radic Biol Med **34**: 689–695.
41. Martinez, M., Hernandez, A. I. and Martinez, N. (2000) *N*-acetylcysteine delays age-associated memory impairment in mice: role in synaptic mitochondric. Brain Res **855**: 100–106.
42. Tirouvanziam, R., Conrad, C. K., Bottiglieri, T., Herzenberg, L. A., Moss, R. B. and Herzenberg, L. A. (2006) High-dose oral *N*-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc Natl Acad Sci USA **103**: 4628–4633.
43. Oda, Y., İWaoka, J., Komatsu, N. and Muramatsu, T. (1999) İnvolvement of *N*-Acetylcysteine –sensitive patways in ricin-induced adoptotic cell death in U987 cell. Biosci. Biotechnol. Biochem., **63**: 341–348.
44. Campain, J. A. (2004) Nicotine: potentially a multifunctional carcinogen? Toxicological Sci. **79**: 1–3.
45. Morley, N., Curnow, A., Salter, L., Campbell, S. and Gould, D. J. (2003) *N*-acetyl-L-cysteine prevents DNA damage induced by UVA, UVB and visible radiation in human fibroblasts. Photochem Photobiol B. **72**: 55–60.
46. He, Y. Y. and Hader, D. P. (2002) UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium Anabaena sp. : protective effects of ascorbic acid and *N*-acetyl-L-cysteine. J Photocem Photobiol B. **66**: 115–124.
47. Kataoka, Y., Bindokas, V. P., Duggan, R. C., Murley, J. S. and Grdina, D. J. (2006) Flow cytometric analysis of phosphorylated histone H2AX following exposure to ionizing radiation in human microvascular endothelial cells. J. Radiat. Res., **47**: 245–257.
48. Tuttle, S., Horan, A. M., Koch, C. J., Held, K., Manevich, Y. and Biaglow, J. (1998) Radiation-sensitive tyrosine phosphorylation of cellular proteins: sensitive to changes in GSH content induced by pretreatment with *N*-acetyl-L-cysteine or L-buthionine-S, R sulfoximine. Int. J. Radiat. Oncol. Biol. Phys. **42**: 833–838.
49. Walichiewicz, P., Przybyszewski, M. W., Jochem, J., Widel, M., Koterbicka, A. and Snietura, M. (2003) Inhibitory effect of local ischaemic preconditioning in total body irradiated rats. Tet. Car. And Mut. Sup. **1**: 195–205.
50. Utley, J. F., Seaver, N., Newton, G. L. and Fahey, R. J. (1984) Pharmacokinetics of WR-1065 in mouse tissue following treatment with WR-2721. Int. J. Radiat. Oncol. Biol. Phys. **10**: 1525–1528.
51. Spencer, C. M. and Goa, K. L. (1995) Amifostine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential as a radioprotector and cytotoxic chemoprotector. Drugs **50**: 1001–1031.
52. Mantovani, G., Macciò, A., Madeddu, C., Mura, L., Gramignano, G., Lusso, M. R., Massa, E., Mocci, M. and Serpe, R. (2003) Antioxidant agents are effective in inducing lymphocyte progression through cell cycle in advanced cancer patients: assessment of the most important laboratory indexes of cachexia and oxidative stress. J. Mol. Med. **81**: 664–673.
53. Klingler, W., Kreja, L., Nothdurft, W. and Selig, C. (2002) Influence of different radioprotective compounds on radiotolerance and cell cycle distribution of human progenitor cells of granulocytopoiesis in vitro. Br. J. Haematol. **119**: 244–254.
54. Murley, J. S., Kataoka, Y., Cao, D., Li, J. J., Oberley, L. W. and Grdina, D. J. (2004) Delayed radioprotection by NFkappaB-mediated induction of Sod2 (MnSOD) in SA-NH tumor cells after exposure to clinically used thiol-containing drugs. Radiat Res. **162**: 536–546.

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