Antioxidant activity and leukemia initiation prevention *in vitro* and *in vivo* by N-acetyl-L-cysteine

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Abstract. *N*-acetyl-L-cysteine (NAC) is the most abundant water-soluble component of garlic. No study to date has studied the leukemia prevention ability of NAC in mouse systemic leukemia model. The current study aimed to investigate the leukemia initiation prevention potential of NAC in a mouse model. The cytotoxic concentration of NAC was determined first in HL-60 cells, and its *in vivo* activity was studied in a mouse acute myelocytic leukemia model with WEHI-3 leukemia cells. The results showed that a non-toxic concentration of NAC efficiently scavenged free-radicals, lowered lipid peroxidation and reduced DNA damage induced by hydrogen peroxide in a cultured HL-60 leukemia cell line. NAC also elevated the cellular antioxidant enzyme activity significantly. Furthermore, NAC prevented mouse death induced by injection of murine WEHI-3 leukemia cells and reduced organ damage, as well as activated antioxidant mechanisms. The results of this study provided strong evidence that NAC may have potential benefits in terms of elevating antioxidant activity and preventing leukemia initiation.

Introduction

Garlic has traditionally been used both for culinary reasons and for the treatment of diseases in many cultures for thousands of years, as evidenced in written histories (1). Studies have shown that garlic can reduce the risk of heart disease (2), and also exerts anticarcinogenic activity against several types of cancer through similar mechanisms (3,4). Garlic is predominantly rich in organosulfur compounds, which are known to contribute to its aroma and potential biological activities (5). The organosulfur compounds derived from garlic have been under investigation for many years, and accumulated evidence has indicated that they have health benefits. However, the composition of raw garlic is variable, and garlic extracts prepared by different methods have different properties (6). Therefore, it is still unclear which components are key to its potential medical use. For example, alliin is one of the main organosulfur compounds found in whole garlic cloves, and accounts for the majority of cysteine sulfoxides in garlic; however, although alliin is known to have antioxidant properties and many bioactivities, there exists very little direct evidence to support its beneficial effect *in vivo*. More in-depth studies are still required in order to reveal the components of garlic that are key contributors to its medical effects.

N-acetyl-L-cysteine (NAC), a thiol-containing antioxidant, is known to prevent cell damage in disorders caused by oxidative stress (7). In our previous study, it was demonstrated that NAC is the most abundant compound in the water-soluble extract of garlic (8). The antioxidative property of NAC enables it to directly interact with reactive oxygen species (ROS) and nitrogen species. ROS play a major role in the pathogenesis of many diseases (9,10). Under normal conditions, cells express a low level of ROS to regulate intracellular signaling pathways and organismal homeostasis (11,12). ROS regulation of signaling pathways contributes beneficial results to normal cellular functions. However, excessively high levels of ROS are detected in almost all types of cancer, suggesting that ROS play a major role in cancer development (13).

Antioxidants could prevent certain reactive species in cells from damaging cellular components, including proteins, lipids and DNA (14). As DNA damage has been linked to cancer, it is reasonable to assume that reducing DNA damage might prevent or slow the progression of cancer. Decreasing the ROS level by administering an antioxidant, such as NAC, to cancer cells has been shown to cause cancer cell damage and reduce invasion and invadopodia formation (15,16), indicating that antioxidants play an important role in alleviating metastasis. However, several recent studies have demonstrated that administration of high dosages of antioxidants such as NAC or vitamin E in a mouse model or in cell culture promoted tumor growth (17-19). This evidence raises the possibility that an optimal level of antioxidant that allows maintenance of homeostasis of oxidative stress is critical for cancer treatment or prevention. Regulating cellular antioxidant activity is therefore thought to represent an important approach for cancer

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prevention, and is of critical clinical importance for the treatment of cancers such as acute myelocytic leukemia (AML).

AML is the most common type of leukemia in adults (20), and the second most common type of leukemia affecting children (21). Chemotherapy is still the first-line treatment for AML, though the treatment outcome is still poor. With an increasing understanding of AML biology and genetics in recent years, several emerging therapies have been proposed (22,23). Progress reports suggest that development of novel agents against AML is still necessary, especially novel agents with less toxicity.

No study has evaluated the leukemia-preventive effect of NAC. As NAC is the major compound of the water-soluble extract of garlic, we investigated whether NAC has a free-radical scavenging effect in leukemia, and whether it can prevent leukemia *in vivo*. The present study was therefore designed to determine the antioxidant activity of NAC in human leukemia cells and to examine its role in the cells under oxidative stress. The effect of NAC on initial occurrence of leukemiaprevention in an AML mouse model was also examined.

Materials and methods

Cell culture. A human acute promyelocytic leukemia HL-60 cell line was kindly provided by Dr Tzou-Chi Huang (National Pingtung University of Science and Technology, Pingtung, Taiwan). HL-60 cells were grown in Iscove's modified Dulbecco's medium (Hyclone, Logan, UT, USA) supplemented with 4 mM L-glutamine (Gibco; Invitrogen, Carlsbad, CA, USA), 15% fetal bovine serum (Hyclone), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco; Invitrogen). HL-60 cells from passages 20-40 were used in the experiments. A WEHI-3 mouse myelomonocytic, macrophage-like leukemia cell line purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan) was grown in Iscove's modified Dulbecco's medium with 4 mM L-glutamine, supplemented with 0.05 mM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) and 10% fetal bovine serum. Both cell lines were cultured in a 95% humidity atmosphere under 5% CO_2 in air at 37˚C.

Determination of 10% cytotoxic concentration. Cultured HL-60 cells were treated with different concentrations of H_2O_2 (ranging from 0-100 mM) or NAC (ranging from 0-50 mM) for 24 h. The cytotoxicities of H_2O_2 and NAC towards HL-60 cells were determined by trypan blue exclusion. This method is based on the principle that live cells possess intact plasma membranes that exclude the trypan blue dye. The CC_{10} indicated the cytotoxic concentration that kills 10% of treated cells (24,25). The 10% cytotoxic concentration (CC_{10}) after 24 h of treatment was calculated using Microsoft Excel software.

Cellular reactive oxygen species detection assay. In order to evaluate the radical scavenging effect of NAC, the intracellular ROS level was analyzed. A DCF-DA assay was performed to detect the intracellular production of hydroxyl, peroxyl and other ROS within the cells. HL-60 cells were incubated for 30 min with 10 μM DCF-DA and washed with PBS, followed by treatment with H_2O_2 at the CC_{10} concentration alone or in combination with various concentrations of NAC for 2 h. The fluorescence intensities of DCF were quantified using a microplate reader (Turner Biosystems, Sunnyvale, CA, USA), with an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

Determination of malondialdehyde (MDA), TAC, SOD and GSH/GSSG levels in cultured HL-60 cells. To further understand the antioxidant effects of NAC, the levels of MDA, TAC, SOD and GSH/GSSG in cultured HL-60 cells treated with H_2O_2 at the CC_{10} concentration were measured. Upregulation of the MDA level indicates oxidative degradation of cellular major components; however, enhancement of the total antioxidation capacity (TAC) represents a cellular protective response to oxidative stress (26). HL-60 cells were treated with H_2O_2 alone or in combination with various concentrations of NAC for 8 h and then subjected to MDA assay. For measurement of the TAC, SOD and GSH/GSSG levels, well-grown HL-60 cells were incubated with various concentrations of compounds for 12 h. Cells were washed twice with PBS, harvested and analyzed following the instructions of the kit manufacturers. The OD value was determined using a spectrophotometer (Bio-Rad Model 680).

Quantification of oxidative DNA damage. HL-60 cells were treated with 20 mM H_2O_2 alone or in combination with various concentrations of NAC for 16 h. The assay protocol followed the instruction manual provided with the kit. Briefly, cellular DNA was extracted and converted to single-stranded DNA at 95˚C for 5 min, and rapidly chilled on ice, then DNA nucleosides were digested by incubating the denatured DNA with nuclease P1. The unknown sample or 8-OHdG standard was incubated with a 8-OHdG conjugate-coated plate followed by reaction with anti-8-OHdG antibody. After washing and substrate reaction, the absorbance of the microwells was determined using a spectrophotometer (Bio-Rad Model 680) at a wavelength of 450 nm.

Animal experiment. The animal test procedure examined and approved by the Institutional Animal Care and Use Committee (IACUC) of our University (NPUST-103-052). Co-author Ching-Dong Chang is a veterinary pathologists and in charge of the animal monitoring and minimize the animal distress. A leukemia mice model was established in our laboratory (27). Thirty male BALB/c mice, 6 weeks of age, were obtained from BioLASCO (Taipei, Taiwan). After accommodation for one week, the mice were randomly divided into 5 groups: Group 1 was the negative control with no treatment; group 2 received intraperitoneal (i.p.) injection with NAC 200 mg/kg for 7 days; group 3 was treated with vehicle PBS through the i.p. route for 7 days then injected with WEHI-3 cells via the tail vein; groups 4 and 5 received NAC 50 or 200 mg/kg through i.p. injection for 7 days, following which leukemia was induced by injection of WEHI-3 cells into the tail vein on day 8. The NAC administration dose was cousulted and referred to the literatures (28,29). Following death, blood and major organs were collected. All resting living mice were euthanized by carbon dioxide on day 7 post WEHI-3 injection. The mortality rate and organ weights were recorded. Blood samples were analyzed to assess liver function markers aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as antioxidant parameters, including total antioxidant

capacity (TAC) and levels of SOD, glutathione and lysozyme. Furthermore, blood samples were also subjected to total acid phosphatase (ACP) analysis, a general diagnostic marker of disease condition. Considering the animal welfare, animals inoculated with tumor cells should be euthanized if following conditions is observed and diagnosed by the co-author Ching-Dong Chang veterinarian (1) Animal is unable to present normal activities due to the tumor (2) Animal abdomen appears dark-gray/green or ascites exceed 20% of the animal weight (3). Lethargy, anorexia, dehydration, or other sign of obvious stress or pain (6). Animal is unable to feed or drink normally due to the tumor.

Reagents and kits. 2',7'-Dichlorofluorescin diacetate (DCF-DA), *N*-acetyl-L-cysteine (NAC) and H_2O_2 were purchased from Sigma-Aldrich. A Lipid Peroxidation (MDA) Colorimetric Assay Kit, Total Antioxidant Capacity (TAC) Colorimetric Assay Kit, Superoxide Dismutase (SOD) Activity Assay Kit, Glutathione (GSH/GSSG/Total) Fluorometric Assay Kit, Aspartate aminotransferase (AST) Assay Kit, Alanine aminotransferase (ALT) Assay Kit, Total Acid Phosphatase (ACP) and Lysozyme Activity Assay Kit were purchased from Biovision (Mountain View, CA, USA). An 8-OHdG DNA Damage ELISA kit was obtained from Cell Biolabs, Inc. (San Diego, CA, USA).

Statistical analysis. All cell-based experiments were performed at least 3 times, and data are presented as mean ± standard deviation (SD). Statistical significance was determined between groups using Student's t-test. In the animal experiment, the data were analyzed by one-way analysis of variance (ANOVA), with the post-hoc t-test. A value of $P<0.05$ was considered to be significant.

Results

Cytotoxicity response. First of all, the non-toxic maximum concentration based on our experimental treatment duration needed to be determined. In order to determine the cytotoxicity response, the initial testing concentration range of NAC from 0-50 mM. As the duration of compound treatment in all cell culture assays did not exceed 16 h, we needed to identify the concentration that did not cause the death of more than 10% of cells after 24 h of incubation. Our data indicated that the CC_{10} values of H_2O_2 and NAC were 11.16 and 12.34 mM, respectively. The growth curve showed the higher NAC concentration, the cell viability decreased gradually (data not shown). The working concentration of the next experiments will not over the CC_{10} dose. Thus, we can then investigate the benefit effects under the enough safety prerequisites.

NAC alleviatesthe cellular ROS, lipid peroxidation and oxidative DNA damage induced by H_2O_2. Next, we analyzed the oxidative response induced by H_2O_2 in our system, and characterized the protective dose-response in the presence of NAC. Several experiments were performed. A fluorescent probe, DCF-DA, has been widely-used to measure intracellular oxidant levels. As shown in Fig. 1A, H_2O_2 produced significantly higher levels of ROS. Critically, at the CC_{10}, NAC efficiently blocked the H_2O_2-generated ROS; lower dosages of

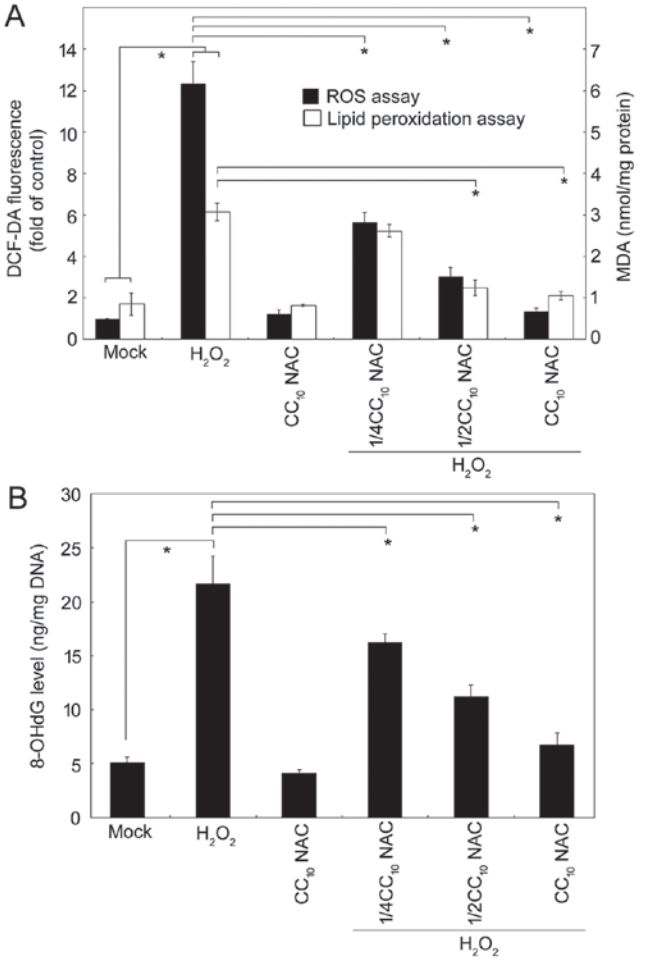

Figure 1. The antioxidant activity of NAC protects HL-60 cells from oxidative stress. Cells were incubated with various treatments as indicated for 2 h (A) ROS (left y-axis) and lipid peroxidation (right y-axis) levels were analyzed by measuring the DCF-DA and MDA contents, respectively. (B) DNA damage was detected by measuring the amount of 8-hydroxydeoxyguanosine (8-OHdG). '*' indicates $P<0.001$.

NAC also exerted significant activity in terms of reducing the ROS level in a dose-response manner. Additionally, MDA is one of the most critical byproducts of lipid peroxidation during oxidative stress, and therefore analysis of lipid peroxidation is essential in the study of pathophysiological processes. The results for ROS and MDA showed an identical trend with very similar response patterns. Furthermore, in order to demonstrate that H_2O_2-elicited ROS affected DNA integrity, we measured the concentration of 8-hydroxydeoxyguanosine (8-OHdG), a critical oxidative DNA damage byproduct. As shown in Fig. 1B, NAC exerted an antioxidant protective activity against H_2O_2-mediated oxidative stress in HL-60 cells.

NAC possesses antioxidant activity. To further confirm the comprehensive antioxidant function of NAC in a HL-60 leukemia cell line, we examined whether NAC elevated cellular endogenous antioxidant mechanisms. There are three different types of antioxidant species, including enzyme systems, small molecules and proteins. The total antioxidant capacity (TAC) assay kit used in this study provided a method by which to measure the combined nonenzymatic antioxidant capacity from culture medium. Thus, both small-molecule antioxidants and protein antioxidants were

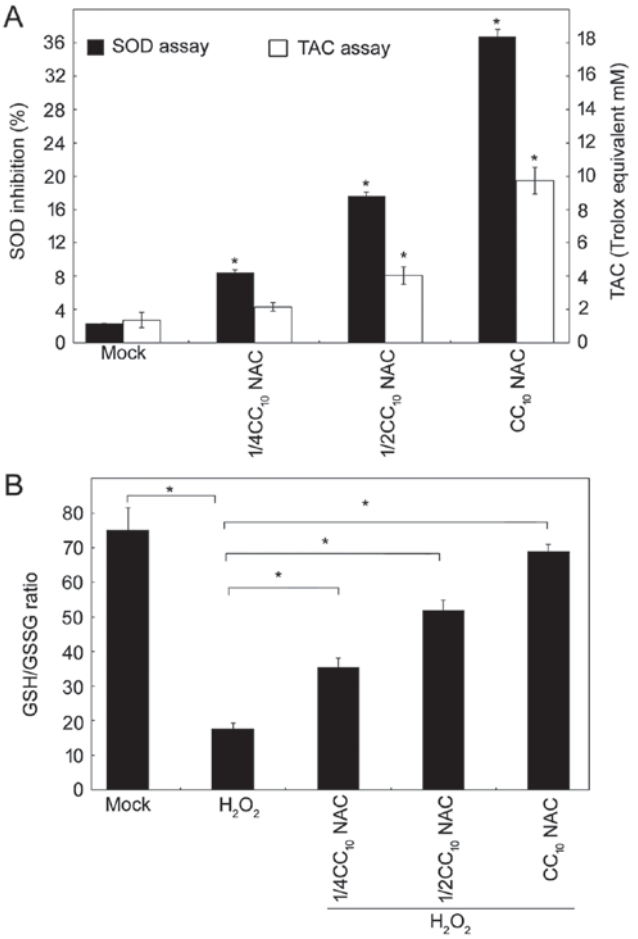

Figure 2. NAC elevated the cellular endogenous antioxidant machinery. Cultured HL-60 cells were treated with NAC at various concentrations as indicated. (A) SOD activity assay is shown on the left y-axis; cellular total antioxidant capacity is shown on the right y-axis. (B) The ratio of GSH/GAAG is shown. '*' indicates P<0.001.

determined in the NAC-treated HL-60 cell system. Another antioxidant enzyme, superoxide dismutase (SOD), which is considered one of the body's most powerful enzymes, was also evaluated. As shown in Fig. 2A, NAC upregulated the endogenous cellular antioxidant activity, and increased TAC and SOD in a dose-response manner. Furthermore, glutathione (GSH) is critical in terms of protecting cells against free-radical damage, and an increased ratio of GSSG to GSH indicates oxidative stress. As clearly demonstrated in Fig. 2B, H_2O_2 reduced the GSH/GSSG ratio, and NAC upregulated the GSH/GSSG ratio by itself; furthermore, NAC reversed the H_2O_2-mediated oxidative effects in HL-60 cells dramatically. Taken together, these results demonstrated that NAC is a good antioxidant for use in this leukemia cell line, not only upregulating the endogenous cellular antioxidant machinery, but also scavenging free-radicals in cells facing oxidative stress.

Leukemia chemoprevention activity of NAC in BALB/c mice. To compare and confirm the antioxidant protective activity of NAC against leukemia *in vivo*, a circulated leukemia mouse model was utilized, created by WEHI-3 injection via the tail vein and resulting in mouse death within one week (30,31). Based on our previous publication, the non-treated-mice died of leukemia by histopathological examination (27). The

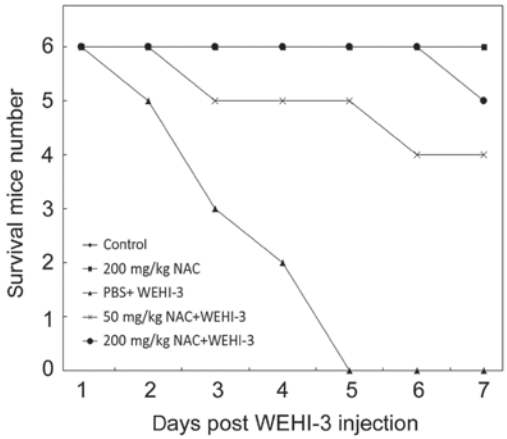

Figure 3. NAC protected cells from leukemia-induced death. Five groups of mice received various treatments, and mouse deaths were recorded each day after administration of WEHI-3 leukemia cells. The mouse survival response was as shown.

mortality rates of the individual mouse groups are shown in Fig. 3. Additionally, the spleen size of each group was showed in Fig. 4. The result clearly illustrated the larger spleen in mice received WEHI-3 cells. All mice had died by day 5 after WEHI-3 cell injection; however, in mice that received 50 mg/kg NAC, survival was significantly increased, and in mice receiving 200 mg/kg NAC, all mice were alive on day 7 after WEHI-3 administration (Fig. 3). There are nine mice died in our total thirty mice. These nine died mice occurs during the daytime and we isolate the blood and organs immediately.

NAC does not cause organ damage, increases serum antioxidant marker activity and protects mouse organs. Mouse sera and organs were collected and analyzed. First, we evaluated the safety of the dosage of NAC we used. Based on organ weights/10 g body weight (Table I) and serum levels of AST, ALT and ACP markers (Table II), a comparison of group 2 with corn oil-treated healthy mice was performed, and no significant difference was observed in any of these parameters. Thus, 200 mg/kg NAC was sufficiently safe for use in our mouse model. In the group 3 mice, WEHI-3 cells were used to establish leukemia, and the liver, spleen and lung weights were significantly higher than those of the group 1 mice. The food consumption of the WEHI-3-injected mice was significantly lower than that of the control mice. The leukemia mice in group 3 also exhibited higher levels of AST, ALT and ACP, as well as lower antioxidant serum parameters of TAC, SOD, and GSH/GSSG. In the 50 mg/kg NAC administration group, some parameter reversed as compared with group 3, indicated the *in vivo* protective effects of NAC. In the 200 mg/kg NAC treatment groups, the levels of AST, ALT and ACP were not significantly different to those of the control mice. The levels of TAC, SOD and GSH/GSSG indicated that NAC promoted good health. Finally, administration of both 50 and 200 mg/kg NAC reduced mouse death (Fig. 3).

Discussion

Several organosulfur compounds of garlic have been shown to exert multiple pharmacological activities (32-38), and studies have demonstrated that aqueous garlic extract has antioxidant

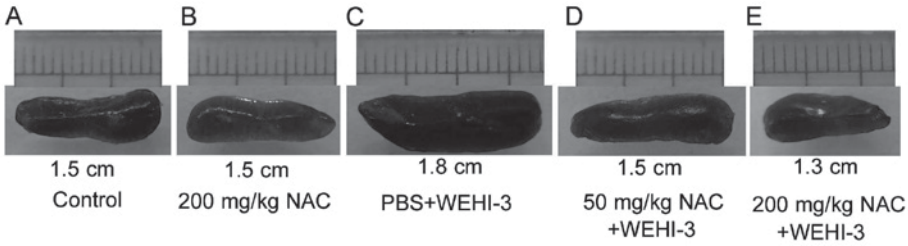

Figure 4. Spleen size of mice received various treatment. Spleen isolated from mice with each group were shown as indicated. The precise size of spleen were measured by ruler and shown.

properties (39,40). It is well-known and well-published that garlic extract possessed the strong antioxidant activities *in vitro* and *in vivo* (41-43). However, there have been no further studies of the active components of garlic extract in terms of the antioxidant effect. In comparison with garlic oil, garlic water-soluble extract has a higher antioxidant activity, and among the identified constituents of garlic water-soluble extract, NAC has been found to be the most abundant (8). In this study, we explored whether NAC has the potential to prevent damage caused by leukemia cells owing to its antioxidant capacity. Further efforts will trying to establish the standard extraction method of garlic containing high amount of NAC and evaluate its possible broad-spectrum cancer-preventive ability.

H_2O_2 is an extremely strong reagent that causes oxidizing damage in cells, as treatment with H_2O_2 leads to high levels of ROS in cells (44). To evaluate the protective effect of NAC against H_2O_2-induced HL-60 cell damage, we used 11.16 μM of H_2O_2 in subsequent assays, as this concentration only caused a ~10% reduction in cell viability after 24 h of incubation; this treatment also caused an increase in the ROS content of ~12-fold and in the MDA content of 3.5-fold.

In the past few decades, many bioactive natural products with potential therapeutic applications have been widely-studied as sources for new drug development (45). Although numerous compounds derived from plants have been consumed as food for centuries, cytotoxicity is still an important issue in relation to their medical use. Our results showed that at a concentration of 12.34 mM, NAC only caused a decrease in cell viability of 10%, while at this concentration NAC efficiently diminished the number of free-radicals, lowered lipid peroxidation, and reduced the DNA oxidative damage induced by H_2O_2 in an HL-60 leukemia cell line. The results showed that even at low, non-toxic concentrations, NAC significantly reduced ROS and MDA, signs of H_2O_2-induced oxidative stress (Fig. 1).

Increased oxidative stress and ROS levels have been found to be associated with many aspects of tumor development and progression (46). The GSH precursor, NAC, can increase the GSH/GSSG ratio. NAC is an antioxidant that act as ROS-scavenger during stressful conditions (47). To confirm that NAC induced antioxidant activity in HL-60 cells, we investigated whether NAC induced cellular endogenous antioxidant mechanisms. We found that administration of NAC resulted in a significant increase in SOD activity in HL-60 cells, and reversed the reduction in the GSH/GSSG ratio caused by H_2O_2 treatment. The dose response to NAC showed the effects of NAC treatment on the TAC and SOD levels in the cells, indicating upregulation of endogenous cellular antioxidant activity. In addition, incubation of cells with H_2O_2 reduced the GSH/GSSG ratio, while NAC treatment reversed the reduction in the GSH/GSSG level in HL-60 cells. These results also indicated that NAC has a good antioxidant activity in leukemia cells, and also acts as a scavenger of free-radicals when cells face oxidative stress. In order to evaluate whether the antioxidant capacity of NAC has a protective effect *in vivo*, we investigated whether administration of NAC inhibited WEHI-3 leukemia cells in a BALB/c mouse model. The current study did not perform antioxidant experiments on WEHI-3 cells, there is still some procedures points are not totally covered. Peritoneal inoculation of BALB/c mice with WEHI-3 leukemia cells has been reported (48) and widely-used as an *in vivo* mouse leukemia model in many studies examining the anti-leukemia activities of several agents (30,49). In our laboratory, we developed an acute promyelocytic leukemia mouse model by intravenous injection of BALB/c mice with WEHI-3 leukemia cells, and employed this model to study the anti-proliferation and preventive effects of NAC on leukemia development in mice (27). We first tested the safety of NAC by i.p. injection with 50 or 200 mg/kg body weight for 7 days, and no organ abnormalities resulted at either dosage. We then used these doses to study the effect of NAC on WEHI-3-induced ALM mice. Based on the results of the present study, we demonstrated that NAC significantly reduced WEHI-3-induced organ damage in the liver, spleen and lungs (Table I). In addition, mice treated with WEHI-3 alone exhibited increased AST, ALT and ACP serum levels, while in mice pre-treated with 200 mg/kg NCA, these increases were prevented, and in addition, the levels of SOD and GSH/GSSG were returned to the same ranges as those of the control animals (Table II). Although WEHI-3 inoculated mice showed reduced average food intake ~28% as compared to control group mice and reduced body weight ~14% (27), not yet reached the euthanasia criteria. Additionally, WEHI-3 inoculated mice died very soon when symptoms appear, there was no detectable and noticeable suffering which diagnosis by a veterinarian. Taken together, the results indicated that NAC provided a good health promotion function that protected the mice from damage caused by WEHI-3 leukemia cell injection. Lysozyme is present in secretions and body fluid, and is associated with immunity; additionally, it exerts antioxidant activity in terms of clearing free-radicals and hydroxyl molecules. In our mouse model, the role of lysozyme was not evident. On the other hand, as the majority of cancer clinic drugs are oxidative agents, NAC might be a potential cancer treatment drug candidate, however, NAC might also prompt chemoresistance.

Taken together, the results of the *in vivo* mouse model revealed NAC to be a good antioxidant agent for use in the initial occurrence prevention of leukemia. The longer observation

Table I. Major organ weight and food and water consumption of the BALB/c mice under various treatment protocols.

Parameters	Groups				
	Control	200 mg/kg NAC	PBS + WEHI-3	50 mg/kg NAC + WEHI-3	200 mg/kg NAC + WEHI-3
Heart (g/10g bw)	0.090±0.007	0.093±0.010	0.103±0.023	0.086±0.013	0.093±0.006
Liver (g/10g bw)	0.687±0.074	0.647±0.136	1.608±0.154[a]	1.005±0.079[a]	0.755±0.132
Spleen (g/10g bw)	0.051±0.006	0.078±0.023	0.178±0.032[a]	0.097±0.027[b]	0.064±0.013
Lungs (g/10g bw)	0.101±0.025	0.129±0.030	0.252±0.019	0.168±0.035	0.129±0.027
Kidneys (g/10g bw)	0.381±0.062	0.450±0.085	0.488±0.154	0.408050	0.379±0.060
Food (g/days)	3.098±0.166	3.085±0.149	2.222±0.165[a]	2.750±0.130[b]	2.909±0.193
Water (ml/days)	4.508±0.353	4.366±0.542	4.445±0.464	4.656±0.560	4.550±0.383

All treatment groups were compared with the control group. [a]P<0.001, [b]P<0.01, [c]P<0.05. NAC, N-Acetyl-L-cysteine.

Table II. Serum marker analysis of the BALB/c mice under various treatment protocols.

Parameters	Control	200 mg/kg NAC	PBS + WEHI-3	50 mg/kg NAC + WEHI-3	200 mg/kg NAC + WEHI-3
AST (mU/ml)	63.83±22.06	61.69±16.59	585.4±113.25[a]	210.23±76.61[a]	77.52±26.35
ALT (mU/ml)	70.12±27.52	69.41±28.21	989.50±165.12[a]	378.73±106.69[a]	80.37±21.36
ACP (U/ml)	0.40±0.20	0.37±0.15	0.85±0.23[b]	0.62±0.11[c]	0.41±0.14
TAC (Trolox mM)	14.48±3.18	19.67±2.98[a]	7.34±0.63[a]	16.53±3.35[b]	21.47±4.04[a]
SOD (U/ml)	40.95±8.73	43.09±9.6	19.03±5.42[a]	33.95±4.68	47.58±7.40
GSH/GSSG	7.09±1.88	7.36±1.99	3.17±1.22[b]	3.90±1.07[b]	8.82±2.04
Lysozyme (μg/ml)	1.78±0.91	1.66±0.62	1.65±0.31	2.37±0.55	2.19±0.58

All treatment groups were compared with the control group. [a]P<0.001, [b]P<0.01, [c]P<0.05. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ACP, acid phosphatase; TAC, total antioxidant capacity; SOD, superoxide dismutase; GSH/GSSG, glutathione/glutathione disulfide; NAC, N-Acetyl-L-cysteine.

and complete pathological examination togerther with the involved molecular mechanisms regarding the NAC-mediated effects will be investigated in our next coming study.

In summary, NAC efficiently scavenged free-radicals, lowered lipid peroxidation and reduced DNA damage induced by HL-60 leukemia cells under oxidative stress. NAC prevented death from WEHI-3 leukemia cell-induced AML, and was associated with reduced organ damage, and may be mediated by its activation of antioxidant mechanisms. The results of the present study suggested that NAC is a potential agent for development as a new drug for the prevention of leukemia initiation.

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Availability of data and materials

The analyzed data are available from the corresponding author on reasonable request.

Authors' contributions

CDC, HTC and KKF were involved in data collection. WLS, CDC, HTC and KKF performed analysis and interpretation of data. WLS was involved in study conception and design, drafting of the manuscript, and was the project leader. WLS, CDC, HTC and KKF critically revised the manuscript.

Ethics approval and consent to participate

The Institutional Animal Care and Use Committee (IACUC) of National Pingtung University of Science and Technology approved the animal experiments, which was conducted in accordance with the highest standards of animal welfare and care (NPUST-103-052). All contributed authors were fully informed of the procedures.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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