Additive antitumor effect of arsenic trioxide with exposure to ionizing radiation to human acute promyelocytic leukemia HL-60 cells

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Received December 31, 2023; Accepted May 1, 2024

DOI: 10.3892/or.2024.8768

Abstract. Arsenic trioxide (ATO) is expected to be a chemical drug with antitumor activity against acute promyelocytic leukemia (APL), a type of acute myeloid leukemia. In Japan, its antitumor effects were confirmed in clinical trials for APL, and it has been approved in various countries around the world. However, there have been no reports on ATO's antitumor effects on radioresistant leukemia cells, which can be developed during radiotherapy and in combination with therapeutic radiation beams. The present study sought to clarify the antitumor effect of ATO on APL cells with radiation resistance and determine its efficacy when combined with ionizing radiation (IR). The radiation-resistant HL60 (Res-HL60) cell line was generated by subjecting the native cells to 4-Gy irradiation every week for 4 weeks. The half-maximal inhibitory concentration (IC_{50}) for cell proliferation by ATO on native cell was 0.87 μM (R^2=0.67), while the IC_{50} for cell proliferation by ATO on Res-HL60 was 2.24 μM (R^2=0.91). IR exposure increased the sub-G1 and G2/M phase ratios in both cell lines. The addition of ATO resulted in a higher population of G2/M after 24 h rather than 48 h. When the rate of change in the sub-G1 phase was examined in greater detail, the sub-G1 phase in both control cells without ATO significantly increased by exposure to IR at 24 h, but only under the condition of 2 Gy irradiation, it had continued to increase at 48 h. Res-HL60 supplemented with ATO showed a higher rate of sub-G1 change at 24 h; however, 2 Gy irradiation resulted in a decrease compared with the control. There was a significant increase in the ratio of the G2/M phase in cells after incubation with ATO for 24 h, and exposure to 2 Gy irradiation caused an even greater increase. To determine whether the inhibition of cell proliferation and cell cycle disruptions is related to reactive oxygen species (ROS) activity, intracellular ROS levels were measured with a flow cytometric assay. Although the ROS levels of Res-HL60 were higher than those of native cells in the absence of irradiation, they did not change after 0.5 or 2 Gy irradiation. Furthermore, adding ATO to Res-HL60 reduced intracellular ROS levels. These findings provide important information that radioresistant leukemia cells respond differently to the antitumor effect of ATO and the combined effect of IR.

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Key words: arsenic trioxide, radioresistant leukemia, HL-60, cell cycle distribution, reactive oxygen species

Introduction

Human acute myeloid leukemia (AML) is a cancer of hematopoietic stem/progenitor cells that causes rapid growth of abnormal cells in the bone marrow and circulating blood, interfering with normal blood cell production through the accumulation of immature myeloblasts (1). According to the World Health Organization classification system, there are >20 subtypes of AML, which are classified based on genetic abnormalities (gene or chromosome changes) in myeloblasts and the percentage of myeloblasts in bone marrow and blood. This type of leukemia tends to worsen quickly if not treated (2).

AML treatment consists of chemotherapy and hematopoietic stem cell transplantation (3,4). In general, leukemic cells and hematopoietic tissues are more sensitive to external stress than other tissues (5), so they are removed with chemotherapy or whole-body ionizing radiation (IR) before hematopoietic stem cell transplantation. However, serious side effects such as graft-vs.-host disease and infections caused by immunodeficiency are possible (6). Furthermore, leukemic cells exposed to IR can develop a radiation resistance population, which reduces the therapeutic effects of radiotherapy. To reduce damage to healthy tissues while effectively targeting cancer cells, fractionated irradiation is commonly used in radiotherapy. Furthermore, it is noninvasive and widely accepted, even by patients with limited treatment options. According to the radiotherapeutic guidelines for AML by the International Journal of Radiation Oncology (7), the most common total body irradiation schedules include twice-daily 2 Gy fractions given over 3 days (total dose, 12 Gy); twice-daily 1.5 Gy fractions over 4-4.5 days (total dose, 12-13.5 Gy); and three-times-daily

1.2 Gy fractions over 4 days (total dose, 12 Gy). It is known that fractionated radiation exposure of cancer cells can result in radioresistant cells in rare cases (8). Our group previously established a radioresistant leukemic cell model with HL60 and its characteristics were determined (9-12). However, the pharmacological effect of various already approved chemicals in these cells has remained elusive.

Recently, arsenic trioxide (ATO) has been proposed as a chemical drug with antitumor properties against acute promyelocytic leukemia (APL), a type of AML. ATO was found to have antitumor effects on lymphoma and liver carcinoma in China in the 1970s, and Niu *et al* (13) described clinical trials on APL. Soignet *et al* (14) then confirmed its antitumor effect in patients with APL. Furthermore, the antitumor effects of APL were confirmed in clinical trials in Japan, and it has been approved by pharmaceutical regulations in several countries (15). ATO easily binds to thiol groups, and when it interacts with intracellular mitochondria, reactive oxygen species (ROS) are produced, causing cell damage (16,17). ROS activated by ATO inhibits cell proliferation and death via a cascade of active caspase families in the mitochondrial pathway (18). However, there have been no reports on the antitumor effects of ATO on radioresistant leukemia cells or when combined with radiation.

The present study sought to clarify the antitumor effect of ATO on leukemia cells that have developed radiation resistance, as well as to determine its efficacy when combined with IR.

Materials and methods

Cell preparation and culture. The human leukemia cell line HL60 (native cells) was purchased from the RIKEN BioResource Center. The radiation-resistant HL60 (Res-HL60) cell line was generated by exposing the cells to 4 Gy irradiation per week for 4 weeks. Native cells and Res-HL60 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Japan Bioserum) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Inc.) in a saturated humidified atmosphere at 37˚C with 95% air and 5% CO_2. The characteristics of Res-HL60 (a higher cell proliferative capacity and smaller cell size) were reported in previous studies by our group (9-12).

Irradiation. X-ray irradiation (150 kVp, 20 mA with 0.5-mm aluminum and 0.3-mm copper filters) was performed with an X-ray generator (MBR-1520R-3; Hitachi Medical Co., Ltd.) at a 45-cm distance between the focus and target. The dose was monitored using a thimble ionization chamber set next to the sample during irradiation. The dose rate was 1 Gy/min. The exposure of cultured cells to X-rays was performed in the same manner as previously described (9-12).

Determination of the half maximal inhibitory concentration (IC_{50}) of ATO. Crystallized ATO (Kanto Chemical Co., Inc.) has a low solubility in pure water. Thus, after dissolving ATO in a 20% sodium hydroxide solution (Nacalai Tesque Inc.), hydrochloric acid (Nacalai Tesque Inc.) was added to neutralize it. The ATO solution (4.8 mM) was sterilized by passing it through a 0.45-μm filter to then it was added to cell culture medium (RPMI1640) to reach final concentrations of 0.39 to 25 μM. Native cells and Res-HL60 cells were seeded in a 24-well plate (Corning, Inc.) with 0.5 ml of culture medium at $1x10^5$ cells/ml. The cultures were incubated at 37˚C in a humidified atmosphere with 95% air and 5% CO_2. ATO was added to the culture medium after 24 h and the total number of viable cells was counted after 48 or 72 h using the trypan blue dye exclusion method (Merck KGaA). ATO concentrations that reduced the number of viable cells by 50% (IC_{50}) were calculated by plotting the cell viability against the log concentration of ATO and fitting the concentration. The statistics of the Boltzmann function were used to calculate the IC_{50}. The percentage of viable cells was calculated using the trypan blue exclusion assay, and viable cells were counted with a Burker-Turk hemocytometer.

Cell cycle distribution analysis. Native cells and Res-HL60 cells were seeded in a 60-mm culture dish with 4 ml of medium and $2x10^5$ cells/ml. After being irradiated at 4 Gy and/or administered ATO, the cells were incubated for 24 h (early phase) and 48 h (late phase). The harvested cells ($5x10^5$ cells) were treated with pre-cooled (-20˚C) 70% ethanol for 10 min on ice, and RNase I (5 μg/ml; Merck KGaA) was also added. These cells were stained with propidium iodide (50 μg/ml) for 30 min in the dark at room temperature. Cell cycle distribution analysis was performed with a Cell Lab Quanta™ Sc MPL (Beckman Coulter, Inc.). To calculate the proportion of cells in the sub-G_1, G_0/G_1, S and G_2/M phases, the Kaluza analysis software (version 2.1; Beckman Coulter, Inc.) was used.

Measurement of intracellular ROS levels. The ROS fluorescent probe dichloro-dihydro-fluorescein diacetate (DCFH-DA; Dojindo Laboratories, Inc.) was used to measure intracellular ROS levels. The prepared cells ($2x10^5$ cells) were harvested from the same dishes as those used for cell cycle measurements. The cells were washed twice with Hanks' balanced salt solution (HBSS) and then incubated with DCFH-DA working solution for 30 min at 37˚C in a humidified atmosphere of 95% air/5% CO_2. The cells were washed twice more with HBSS. The ROS levels were then measured using a flow cytometer (Cell Lab Quanta™ Sc MPL). The excitation and fluorescence wavelengths were set to 488 and 530 nm, respectively.

Statistical analysis. Statistical analysis was performed using OriginLab software version 9.1 (OriginLab Corp.) and Office 365 (Microsoft Corp.) with an add-in software (OMS Publishing, Inc.). The Boltzmann function was used to calculate the IC_{50} and the coefficient of determination (R2 value) was calculated. Following the Kruskal-Wallis test to assess group differences, the Steel test was performed as a non-parametric post-hoc test to identify significant differences in the cell damage analysis (surviving fraction, cell-cycle distribution and ROS detection). All data in this study were nonparametric. $P<0.05$ was considered to indicate statistical significance.

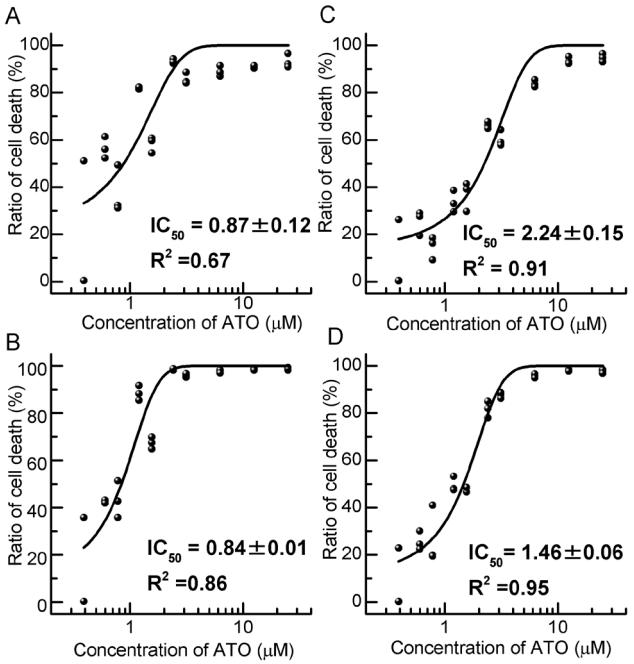

Figure 1. Dose-response curve of ATO. The proliferation activity during the administration of ATO in native and Res-HL60 cells was investigated. After incubation for 48 or 72 h, viable cells were identified using the trypan blue exclusion assay. The figures show native cells with ATO after (A) 48 h and (B) 72 h of incubation, as well as Res-HL60 cells with ATO after (C) 48 h and (D) 72 h. R^2 value, coefficient of determination; IC_{50}, half maximal inhibitory concentration; ATO, arsenic trioxide; Res-HL60, resistant HL60 cells.

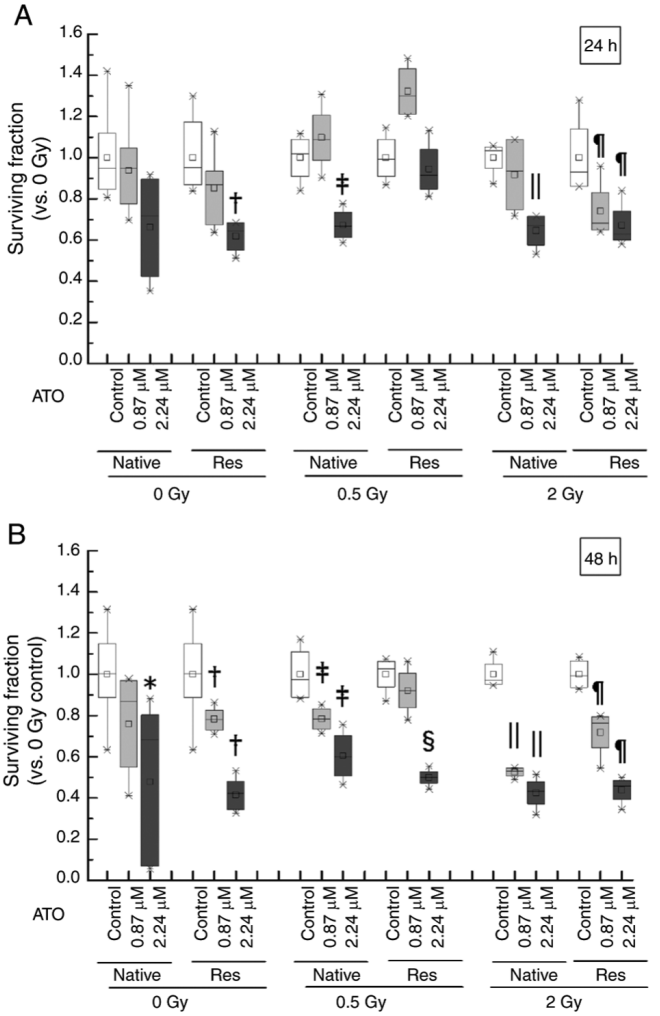

Figure 2. Viability of native and Res-HL60 cells after treatment with ATO and/or IR. Cell viability was assessed at (A) 24 h and (B) 48 h following irradiation with 0.5 or 2 Gy and/or addition of 0.78 or 2.24 μM ATO. The non-ATO-treated group was used as the control group. The results of the Steel test show significant differences (P<0.05) between the control and various other groups. *P<0.05 vs. nonirradiated native cell; †P<0.05 vs. nonirradiated Res-HL60 control; ‡P<0.05 vs. 0.5 Gy irradiated native cell; §P<0.05 vs. 0.5 Gy irradiated Res-HL60 control; ||P<0.05 vs. 2 Gy irradiated native cell; ¶P<0.05 vs. 2 Gy irradiated Res-HL60 control. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells.

Results

Cell toxicity of ATO. To determine the IC_{50} of ATO for HL60 cells, the number of viable cells after culture with various concentrations of ATO was calculated (Fig. 1). The IC_{50} for native cell was 0.87±0.12 μM after 48 h and 0.84±0.01 μM after 72 h of incubation (Fig. 1A and B). Furthermore, the IC_{50} for Res-HL60 was 2.24±0.15 μM after 48 h and 1.46±0.06 μM after 72 h of incubation (Fig. 1C and D). Thereafter, the viable cell count, cell cycle distribution analysis and intracellular ROS level analysis were performed at the IC_{50} concentration of ATO (native: 0.87±0.12 μM; Res: 2.24±0.15 μM).

Analysis of viable cells after exposure to IR and/or ATO. As the combination of ATO and 4 Gy was found to be too toxic, conditions similar to the clinical dose (0.5-2 Gy) were used in the present study. The inhibition potency of cell proliferation in HL-60 cells exposed to ATO and/or IR was assessed in the early phase and late phase. The survival rate due to the addition of ATO showed a significant decrease in the late phase in native cells {native control: Median [interquartile range (IQR)]=1.00 (0.89-1.15); 2.24 μM ATO: Median (IQR)=0.68 (0.07-0.80), P<0.05}, but the viability of Res-HL60 cells started to decrease in early phase {control in early phase: Median (IQR)=0.95 (0.87-1.18); 2.24 μM ATO in early phase: Median (IQR)=0.64 (0.55-0.68), P<0.05; control in late phase: Median (IQR)=1.00 (0.89-1.15); 0.87 μM ATO in late phase: Median (IQR)=0.78 (0.73-0.83), P<0.05; 2.24 μM ATO in late phase: Median (IQR)=0.42 (0.34-0.48), P<0.05} (Fig. 2). After exposure of the native cells to 0.5-Gy IR in the late phase, a significant decrease in the surviving cell fraction in the ATO concentration dependency in comparison with the nontreatment control was observed [control: median (IQR)=0.97 (0.89-1.11); 0.87 μM ATO: Median (IQR)=0.78 (0.74-0.83), P<0.05; 2.24 μM ATO: Median (IQR)=0.60 (0.51-0.70), P<0.05] and 2 Gy [control: Median (IQR)=0.97 (0.95-1.05); 0.87 μM ATO: Median (IQR)=0.53 (0.51-0.54), P<0.05; 2.24 μM ATO: Median (IQR)=0.43 (0.37-0.48), P<0.05]. Furthermore, Res-HL60 cells with additional ATO at the IC_{50} concentration (2.24 μM) and 2 Gy IR exposure in the early phase were significantly decreased [median (IQR)=0.63 (0.60-0.74), P<0.05] compared to the control cells (without ATO) [median (IQR)=0.93 (0.86-1.14)]. Native cells exposed to 2-Gy were also similarly decreased in early phase [control: Median (IQR)=1.04 (0.95-1.05); 2.24 μM ATO: Median (IQR)=0.67 (0.57-0.72), P<0.05]. A similar trend at 2-Gy was continued until the late phase [native control: Median

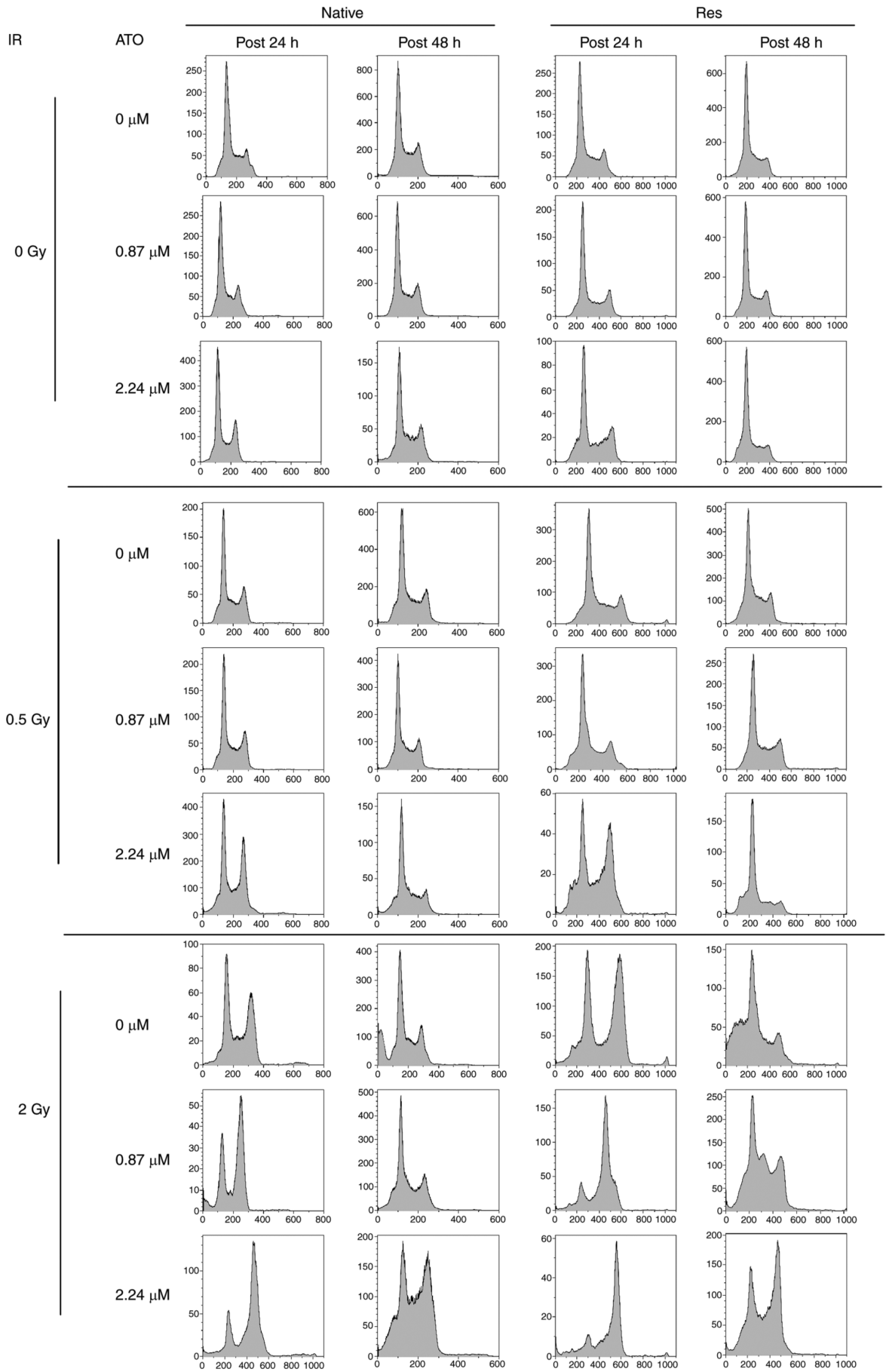

Figure 3. Representative flow cytometry histograms for cell cycle analysis. Native and Res cells treated ATO and/or IR for each duration were shown. The horizontal and lateral axes show the DNA content and cell count in each channel, respectively. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells; IR, ionizing radiation.

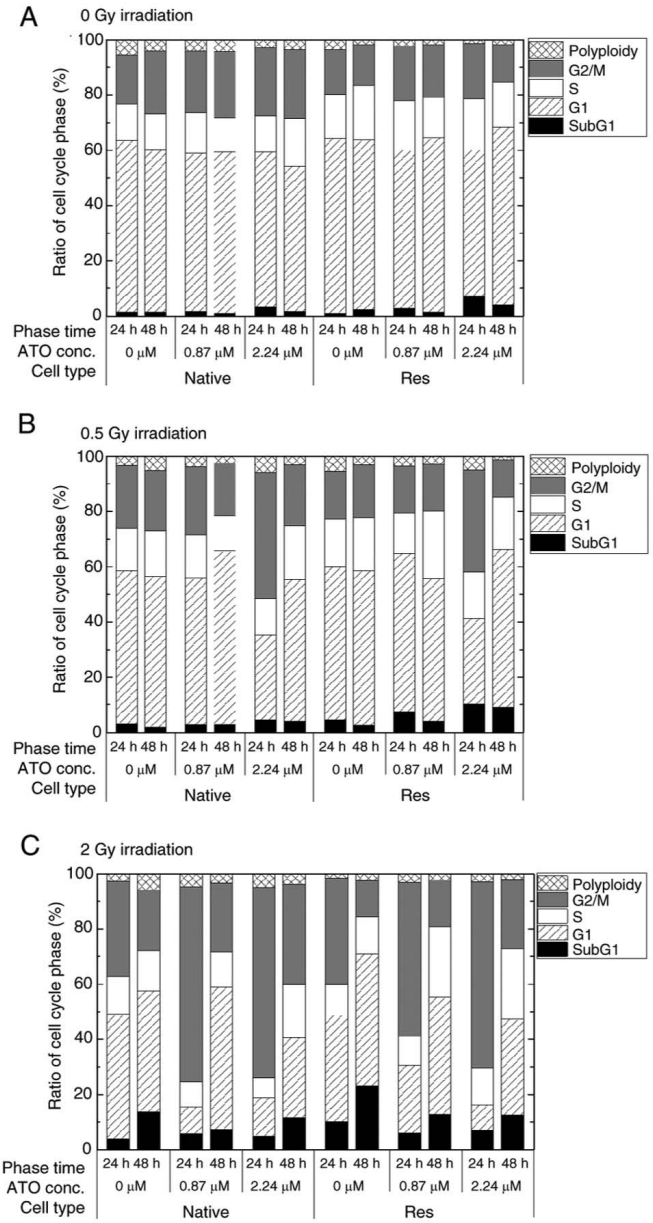

Figure 4. Cell cycle distribution analysis was performed on native and Res-HL60 cells treated with ATO and/or IR. Each phase was examined at 24 and 48 h after treatment with ATO and/or IR. (A) Nonirradiated conditions, (B) exposure to 0.5 Gy and (C) exposure to 2 Gy with or without ATO. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells; IR, ionizing radiation.

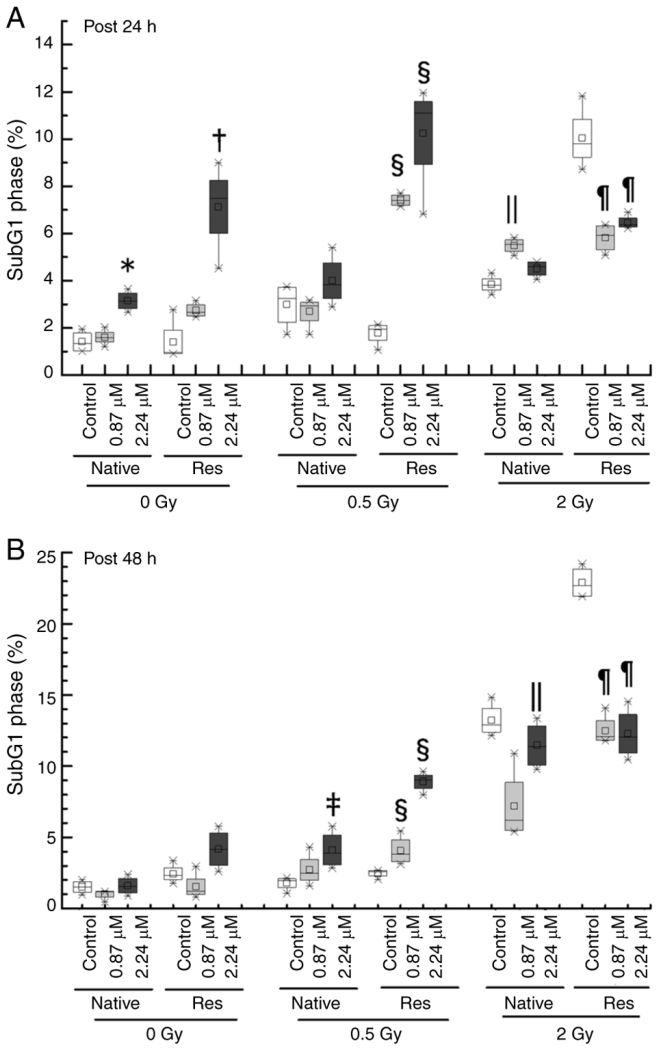

Figure 5. The apoptotic population, sub-G1, of native and Res-HL60 cells was studied using flow cytometry. The conditions at (A) 24 h and (B) 48 h after exposure to 0.5 and 2 Gy with or without ATO were investigated. The non-ATO-treated group was designated as the control group. The Steel test indicated significant differences (P<0.05) between the control cells and various other groups. *P<0.05 vs. nonirradiated native control; †P<0.05 vs. nonirradiated Res-HL60 control; ‡P<0.05 vs. 0.5 Gy irradiated native control; §P<0.05 vs. 0.5 Gy irradiated Res-HL60 control; ‖P<0.05 vs. 2 Gy irradiated native control; ¶P<0.05 vs. 2 Gy irradiated Res-HL60 control. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells.

(IQR)=0.97 (0.95-1.05); native with 0.87 μM ATO: Median (IQR)=0.53 (0.51-0.54), P<0.05; native with 2.27 μM ATO: Median (IQR)=0.43 (0.37-0.48), P<0.05; Res control: Median (IQR)=0.99 (0.94-1.06), Res with 0.87 μM ATO: Median (IQR)=0.76 (0.64-0.79), P<0.05; Res with 2.24 μM ATO: Median (IQR)=0.46 (0.39-0.49), P<0.05].

Alteration of cell-cycle distribution by IR with or without ATO. Analysis of the cell-cycle distribution of native and Res HL60 cells was conducted using flow cytometry (Fig. 3). Exposure to IR increased the ratio of the sub-G1 phase and G2/M phase in both cell lines in comparison to the non-irradiated control (Fig. 4). Furthermore, the addition of ATO resulted in a larger G2/M-phase population at early phase in comparison to the group with no additional ATO. To provide a detailed cell cycle population analysis, statistical analysis was performed. When the rate of the change in the sub-G1 phase population was examined in greater detail, the subG1 phase population in both control cells without ATO was significantly increased by exposure to IR, but only the conditions of 2-Gy in late phase exhibited a marked increase {native in early phase [0 Gy, 1.32 (1.01-1.78)%; 0.5 Gy, 3.24 (2.24-3.72)%, P<0.05 vs. 0 Gy; 2 Gy, 3.82 (3.60-4.18)%, P<0.05 vs. 0 Gy], Res in early phase [0 Gy, 0.95 (0.90-1.90)%; 0.5 Gy, 1.95 (1.48-2.10)%, P<0.05 vs. 0 Gy; 2 Gy, 9.80 (9.21-10.84)%, P<0.05 vs. 0 Gy], native in late phase [0 Gy, 1.50 (1.10-1.89)%; 0.5 Gy, 1.95 (1.48-2.10)%; 2 Gy, 12.90 (12.38-14.05)%, P<0.05 vs. 0 Gy], Res in late phase [0 Gy, 2.32 (2.03-2.84)%; 0.5 Gy, 2.60 (2.29-2.65)%; 2 Gy, 22.70 (21.92-23.85)%, P<0.05 vs. 0 Gy]} (Fig. 5). Res-HL60 supplemented with 2.24-μM ATO showed a higher rate of change

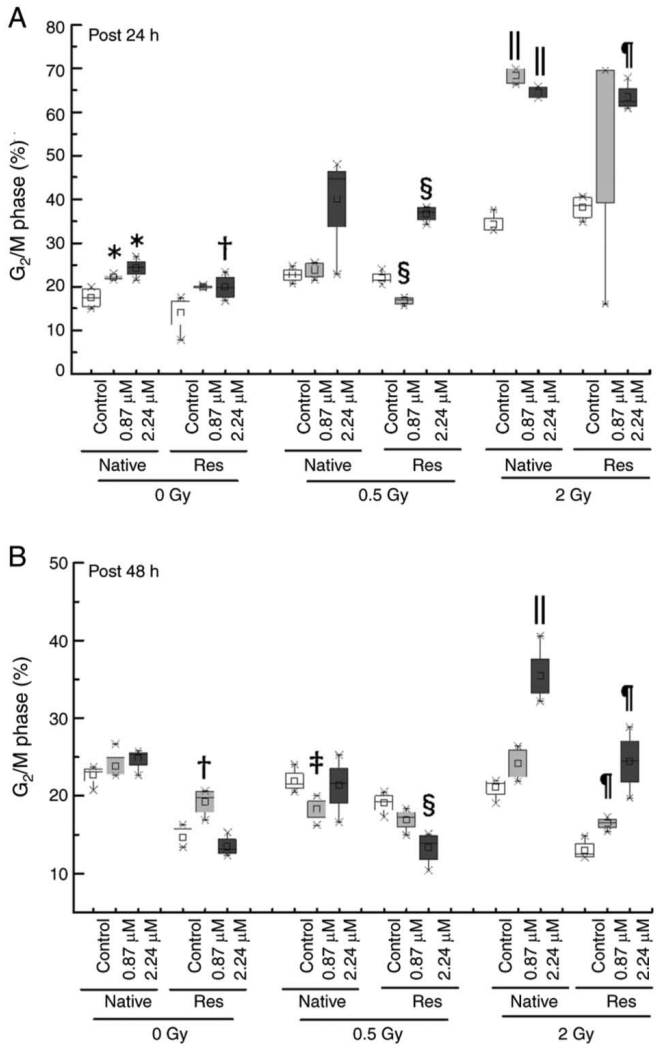

Figure 6. The G2/M cell cycle arrest population of native and Res-HL60 cells was examined using flow cytometry. The conditions at (A) 24 h and (B) 48 h after exposure to 0.5 and 2 Gy with or without ATO were investigated. The non-ATO-treated group was used as the control group. The Steel test indicated significant differences (P<0.05) between the control cells and certain other groups. *P<0.05 vs. nonirradiated native control; †P<0.05 vs. nonirradiated Res-HL60 control; ‡P<0.05 vs. 0.5 Gy irradiated native control; §P<0.05 vs. 0.5 Gy irradiated Res-HL60 control; ‖P<0.05 vs. 2 Gy irradiated native control; ¶P<0.05 vs. 2 Gy irradiated Res-HL60 control. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells.

in the median (IQR)% [0 Gy, 7.50 (6.02-8.25)%; 0.5-Gy, 11.1 (8.91-11.58)%; 2 Gy, 6.36 (6.28-6.65)%] than the native cells [0 Gy, 3.15 (2.82-3.48)%; 0.5 Gy, 3.84 (3.22-4.78)%; 2 Gy, 4.59 (4.22-4.80)%] in the early phase (P<0.05) (Fig. 5A). However, 2-Gy irradiation with 0.87- and 2.24 μM ATO resulted in downregulation compared with the control (P<0.05) (Fig. 5B). By contrast, a significant increase in the ratio of the G2/M phase in both cell lines was observed after the early phase with ATO [native with 0.87 μM ATO, 21.97 (21.81-22.28)%; native with 2.24 μM ATO, 24.30 (22.82-25.85); Res with 2.24 μM ATO, 19.74 (17.87-22.11)%] in comparison to the control [native, 17.50 (15.40-19.50)%; Res, 15.20 (11.11-16.81)%)] (P<0.05), and exposure to 0.5 and 2 Gy irradiation with 2.24 μM ATO induced a further increase in the median (IQR) [native with 0.5 Gy, 44.63 (33.80-44.80)%; Res with 0.5 Gy, 37.11 (35.22-38.10)%; native with 2 Gy, 68.44 (66.76-69.95)%; Res with 2 Gy, 62.47 (61.40-65.21)%] in comparison to control cells [native with 0.5 Gy, 22.70 (21.81-23.90)%; Res with 0.5 Gy, 21.40 (20.90-22.83)%; native with 2 Gy, 33.20 (32.90-35.60)%; Res with 2 Gy, 38.65 (35.90-40.50)%] (P<0.05) (Fig. 6A). In addition, Res-HL60 cells exposed to 2-Gy irradiation and 2.24-μM ATO maintained a higher G2/M phase population even in the late phase than the control group however it was comparatively lower than early phase [control of native, 21.80 (20.02-21.90)%; native with 2.24 μM ATO, 34.51 (33.20-37.51)%; control of Res, 12.54 (12.24-13.38)%; Res with 2.24 μM ATO, 24.54 (21.86-27.19)%] (P<0.05) (Fig. 6B).

Intracellular ROS. To determine whether the inhibition of cell proliferation and cell cycle disruption is related to ROS activity, the intracellular DCFH-DA reaction, a ROS marker, was measured the fluorescence intensity using a flow cytometer (Fig. 7). Although the ROS levels of Res-HL60 were significantly higher than those of native cells under nonirradiation conditions without ATO [median (IQR) at the early phase, 3.5 (1.75-5.25) for native vs. 134.56 (101.31-227.24) for Res; median (IQR) at the late phase, 11.38 (7.70-20.71) for native vs. 61.39 (52.01-75.62) for Res)] (P<0.05), they did not significantly differ or change after exposure to 0.5 or 2 Gy irradiation at the early phase (Fig. 8A). However, there were similar responses in ROS levels after exposure to 0.5 or 2 Gy irradiation at the late phase in comparison to non-irradiated conditions (in other words, Res-HL60 cells were detected to have higher levels of ROS than native cells) (Fig. 8B). Furthermore, adding ATO at the IC_{50} concentration to Res-HL60 reduced intracellular ROS levels and the differences were more pronounced in the late phase.

Discussion

An *in vitro* cell culture model was used in the present study to clarify the antitumor effect of ATO on radiation-resistant leukemia cells and/or to determine its efficacy when combined with IR. In our established model (Res-HL60), the IC_{50} of ATO was higher than that of native cells, and a higher percentage of G2/M phase was observed after exposure to 2 Gy with ATO in the early phase compared to a single 2 Gy IR. This combination (exposure to 2 Gy with ATO) also showed a significantly decrease of surviving fraction (~60%). These effects may be the result of additive effects between ATO and IR.

Exposure to IR causes apoptosis in cells by targeting DNA (19). Flow cytometry can identify the sub-G1, G1, S and G2/M phases of the cell cycle, with apoptotic cells included in the sub-G1 phase (20). A higher population in the sub-G1 phase in ResHL60 and native cells was also determined following administration of ATO and/or IR. Furthermore, in Res-HL60 cells supplemented with ATO, the effect on the sub-G1 phase, which is induced by IR, was increased, implying that ATO promotes apoptosis when exposed to IR. However, the production of intracellular ROS in Res-HL60 cells differed from previous reports on leukemia cells. A previous study by Ho *et al* (17) found that ATO induces apoptosis via the mitochondria-mediated caspase 3 pathway by producing intracellular ROS. In native cells, ROS production responses were similar to previous reports following the addition of ATO; however, in Res-HL60 cells, the concentration of ATO

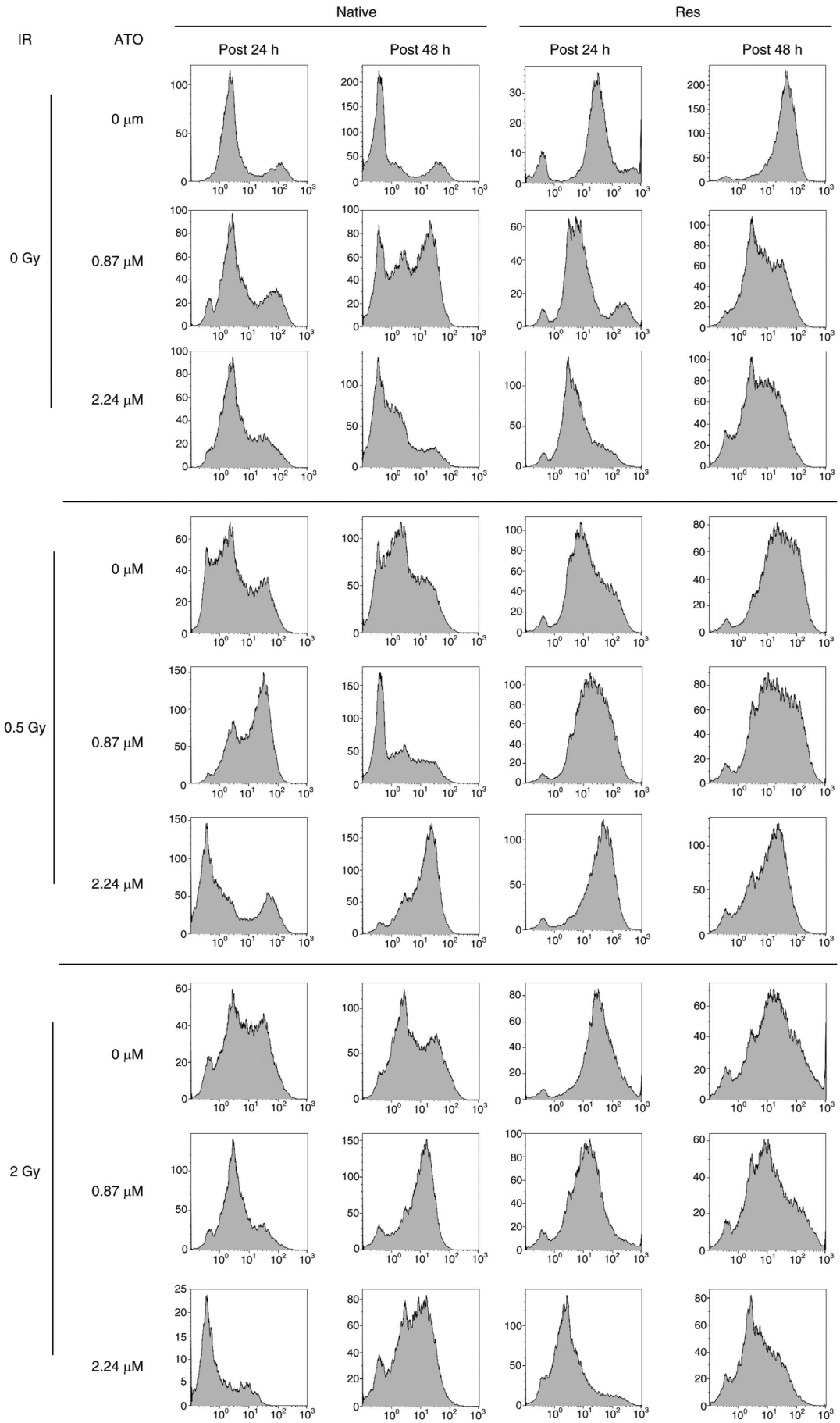

Figure 7. Representative flow cytometry histograms for the analysis of intracellular reactive oxygen species. Native and Res cells treated with ATO and/or IR for each time point are shown. The horizontal and lateral bar are fluorescence intensity of DCFH-DA and the cell count in each channel, respectively. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells; IR, ionizing radiation.

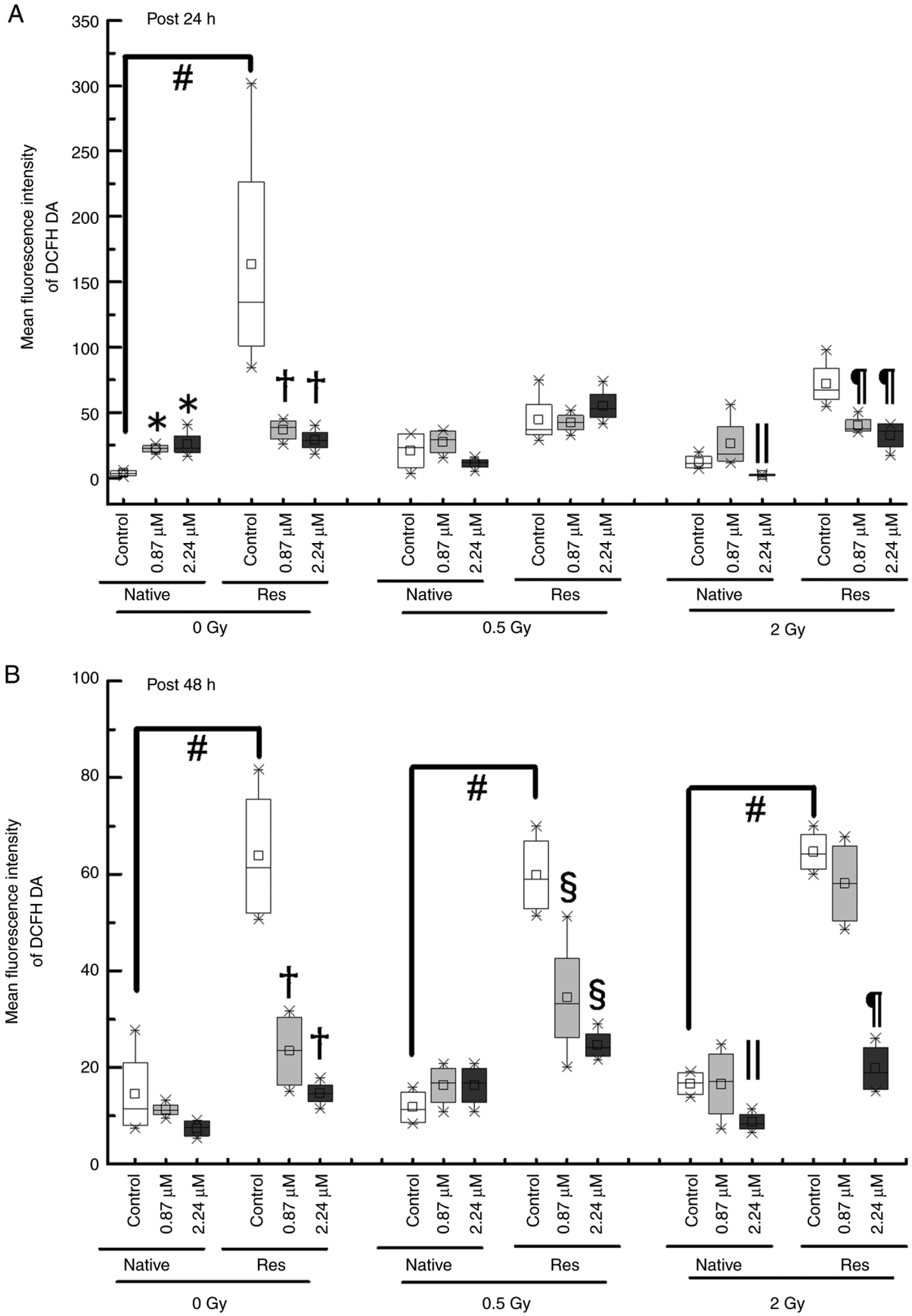

Figure 8. Intracellular ROS levels for HL60 and Res-HL60. ROS production was measured using flow cytometry at (A) 24 h and (B) 72 h after 0.5 or 2 Gy irradiation, as well as exposure to 0.78 and 2.24 μM ATO. The non-ATO-treated group was used as the control group. The Steel test indicated significant differences (P<0.05) between the control cells and various other groups. *P<0.05 vs. nonirradiated native control; †P<0.05 vs. nonirradiated Res-HL60 control; §P<0.05 vs. 0.5 Gy irradiated Res-HL60 control; ‖P<0.05 vs. 2 Gy irradiated native control; ¶P<0.05 vs. 2 Gy irradiated Res-HL60 control. #P<0.05 between native and Res-HL60 cell without ATO. ATO, arsenic trioxide; Res-HL60, resistant HL60 cells; ROS, reactive oxygen species; DCFH-DA, dichloro-dihydro-fluorescein diacetate.

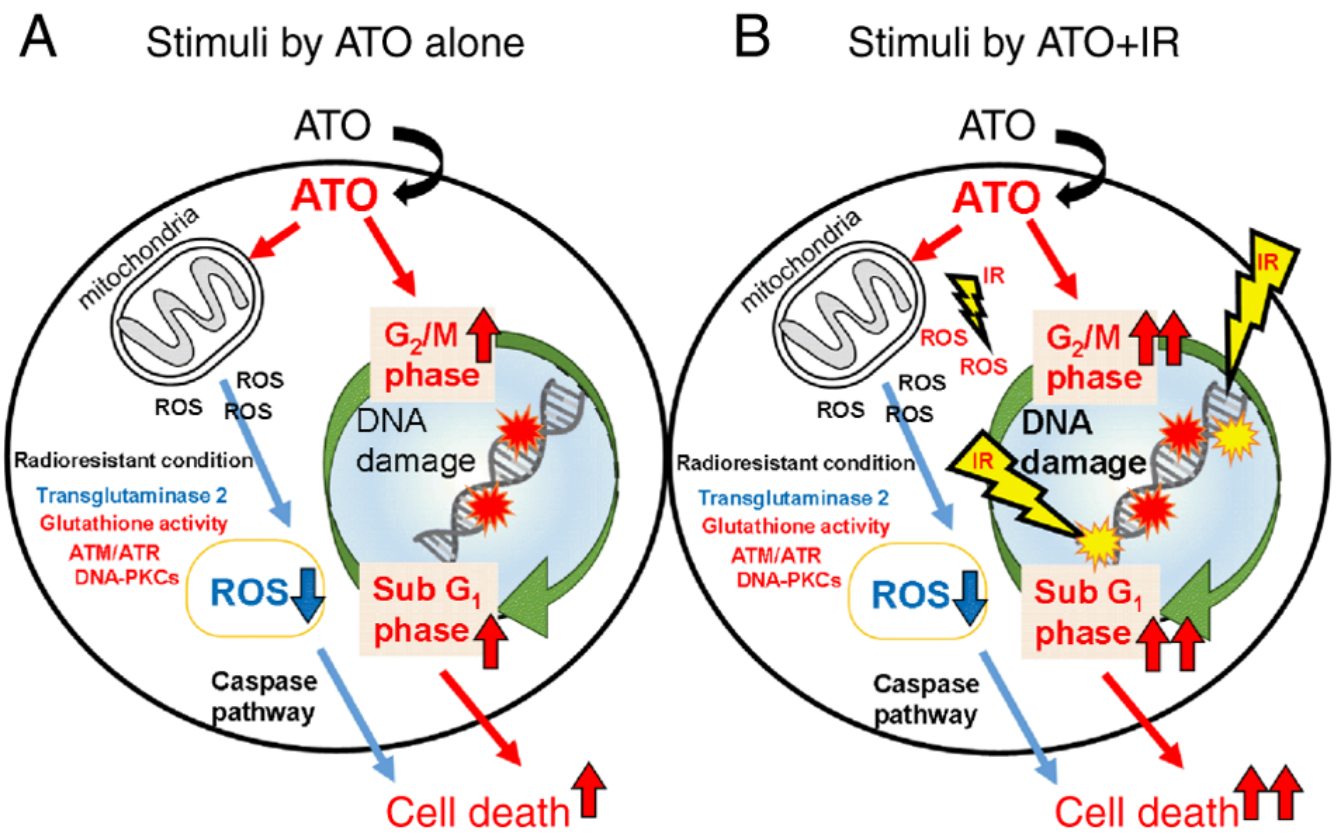

Figure 9. Schematic of the signaling mechanism of (A) ATO alone and (B) ATO + IR in the cell death pathway in the radioresistant HL60 cell model. The red and blue color mean activation and suppression, respectively. ATO, arsenic trioxide; ROS, reactive oxygen species; IR, ionizing radiation; DNA-PKC, DNA-dependent protein kinase catalytic subunit.

and ROS production had no relationship and instead decreased ROS production when compared to native cells. Mitochondrial metabolism contributes to ROS production, which activates the downstream caspase pathway and causes apoptosis (21).

When cells become radioresistant, glutathione activity often increases, resulting in increased antioxidant capacity (22,23). Furthermore, as ResHL60 cells have an active potency of ATM/ATR and DNA-dependent protein kinase than native cells for radiation resistance capacity (9), these combined abilities may suppress ROS-mediated apoptosis. Jambrovics *et al* (24,25) discovered that lacking intracellular transglutaminase 2, a multifunctional enzyme, increases ATO-induced ROS production and cell death. Our identification of IC_{50} concentrations (0.78 μM for native, 2.24 μM for Res) and weaker toxic effect of the ATO concentration in Res-HL60 cells compared to native cells suggest that the antitoxic environment in Res cells is altered in intracellular enzymes, leading to radio- and ATO resistance.

According to numerous reports, the antitumor effect of leukemia cells ranges from 1 to 15 μM (17,26-30) and has a similar ATO concentration to the IC_{50} of Res-HL60, which is noteworthy. In many drug discovery fields, low concentrations are essential for avoiding effects on normal tissue. The concentration of ATO is expected to decrease even further when combined with radiotherapy. From this perspective, it is very significant that in the present study, an additive antitumor effect was produced by combining low concentrations of ATO with radiation on radioresistant cells. Heinke (31) reported that mitochondrial ROS drives cell cycle progression. If ATO causes cell cycle arrest and then cell death, ATO stimuli may be reduced in the production of intracellular ROS. However, determining the cause of the decline in ROS will necessitate a detailed analysis of the intracellular redox state of various types of leukemic cells, including clinical specimens, in the future. These findings (Fig. 9) reveal important information that radioresistant leukemia cells respond differently to the antitumor effect of ATO and the combined effect of IR.

In conclusion, these findings suggest that radioresistant leukemia has distinct redox and cell death signals involving ATO and IR.

Acknowledgements

The authors would like to thank Professor Yoichiro Hosokawa (Department of Radiation Science, Hirosaki University Graduate School of Health Sciences) for his kind assistance with the establishment of Res-HL60.

Funding

This work was supported by JSPS KAKENHI, Grants-in-Aid for Scientific Research (B) (grant no. 21H02861 to SM), Fund for the Promotion of Joint International Research (Fostering Joint International Research; grant no. 17KK0181 to SM), and Grant-in-Aid for Challenging Research

(Exploratory) (grant no. 19K22731 to SM). The Takeda Science Foundation (2022, to SM) also provided support. The funders had no involvement in the study design, data collection and analysis, decision to publish or manuscript preparation.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YM and SM designed the study, drafted the manuscript and contributed significantly to its revision. YM, HS, KY, MK, TY KH, and SM examined biological data. YM and SM checked and confirmed the authenticity of the raw data. SM oversaw the study, critically reviewed the manuscript and gave final approval for the version to be submitted and published. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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