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Influence of low-dose X-ray on plasma membrane properties of erythroleukemia cell lines (K562, K562/adr)

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ABSTRACT

Background: Low-dose X-ray in medical use for diagnosis and therapy can result in cellular biology either directly or indirectly. In cell biology, the interaction of low-dose radiation generates many radical molecules that interact with cellular organelles, such as the plasma membrane.

Objectives: This study aimed to evaluate the effect of low-dose X-ray on both drug-sensitive (K562) and drug-resistant (K562/adr) erythroleukemic cell lines.

Materials and methods: Cells were exposed by using an X-ray at 135 kVp to obtain the absorbed dose of 0.05, 0.1, and 0.2 Gy. The intracellular reactive oxidant species (RS), malondialdehyde, membrane fluidity, drug uptake, and drug accumulation were instantly observed after radiation.

Results: The result showed a significant increase in RS in both cell lines as a function of radiation dose. In K562, the malondialdehyde (MDA) value increased in a radiation dose manner, while membrane fluidity was significantly modified at 0.1 and 0.2 Gy. In K562/adr, the uptake rate of pirarubicin (THP) and IC_{20} were altered but not significantly different from sham control.

Conclusion: Low-dose X-ray significantly increased the intracellular RS in both cell lines and decreased the membrane fluidity at 0.1 Gy of K562. There are alterations of anticancer drug uptake rate in both cell lines, but they are not significant.

Introduction

X-ray is classified as a low-linear energy transfer that is generally used for diagnosis and radiotherapeutic planning at a dose range of 0.0002-40 mGy.[1] However, it is still being used as low-dose radiation therapy (30-100 cGy) in regenerative medicine to increase cell proliferation and in pneumonia by suppressing inflammatory response.[2,3] Past studies on cellular effect have mostly focused on the degeneration of DNA. At low doses of X-ray, the studies showed the induction of DNA double-strand break.[4] In fact, the cellular effects of radiation are not selective. The cell membrane is the principal organelle that acts as a cell's boundary and is semipermeable for molecules. Certain substances can pass through the membrane depending on not only their properties but also the membrane's properties. When X-rays pass through the cell, the ionising radiation causes a breakout of molecules, and then free radicals such as reactive oxygen species (ROS)

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are generated. The consequential reaction of those molecules on unsaturated fatty acids, which is called lipid peroxidation, respectively provides products such as lipid hydroperoxides (LOOH), malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE). Those oxidative productions eventually lead to membrane fluidity alteration.[5,6]

Alteration of membrane fluidity relates to biological alteration in the sensitivity of drugs.[7] The previous observation of low-dose X-ray (up to 0.2 Gy of 120 kVp) on human peripheral blood mononuclear cells shows an augmentation of the intracellular ROS.[8] However, the instant effect of X-ray at 50, 70, and 100 kVp indicates an insignificant increase of osmotic fragility, and the alteration of membrane fluidity is observed at 4 hrs (100 kVp).[9] As mentioned, previous studies focus on normal haematopoietic cells but not on cancer cells. Moreover, the homogeneity of lipid composition between normal cells and cancer cells is different.[10] Hence, this study aimed to investigate the instant effect of low-dose X-ray on cancer cell membrane properties. Two erythroleukemic cell lines, drug-sensitive (K562) and drug-resistant (K562/adr), were exposed to low-dose X-ray up to 0.2 Gy.

Materials and methods

Chemicals

The 2',7'-Dichlorofluorescin diacetate (DCHF-DA; Sigma-Aldrich, USA) solution and 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma-Aldrich, Japan) were prepared at high concentration by solubilising them in DMSO and then diluting them till the appropriate concentration in phosphate buffer solution (PBS). The thiobarbituric acid reactive substances (TBARS) working reagent contained 2 mg/mL of 2-Thiobarbituric acid (Sigma-Aldrich, Japan), which is solubilised in a mixture of 50 mM NaOH (RCl Labscan, Thailand) and Glacial acetic acid (Fisher Scientific, UK) with a ratio of 1:1. Pirarubicin (THP, Sigma-Aldrich, Japan) was freshly prepared in DMSO, and then working solution (1 µM) was prepared in the physiological buffer (Luckoff-Na^+) of pH 7.3 that contained 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 20 mM HEPES, and 5 mM glucose. Then 0.1 mg/mL of resazurin sodium salt (Sigma-Aldrich, Japan) was sterilely prepared in PBS.

Cell lines

All experiments were performed on both drug-sensitive (K562) and drug-resistant (K562/adr) erythroleukemic cell lines. Cells were cultivated in a completed medium consisting of RPMI-1640 supplemented with 10% of foetal bovine serum and 1% penicillin-streptomycin for 72 hrs before experiments. The resistance property was maintained by cultivating with 400 nM of doxorubicin. For each experiment, cells were collected and exposed to the X-ray (135 kVp) at 0.05, 0.1, and 0.2 Gy. After irradiation, the intracellular RS, MDA, membrane fluidity, drug uptake, and cytotoxicity of the cells were immediately determined.

Determination of intracellular reactive oxidant species (RS)

Cells (5×10^5) were collected and resuspended in 0.1 mL phosphate buffer saline solution and then exposed to X-ray. After that, cells were added into a DCHF-DA solution (2 µM) and incubated for 30 min at 37 °C, 5% CO_2, and 95% humidity. The fluorescence intensity at 523 nm (excited at 502 nm) of dichlorofluorescin (F_{DCF}), which represented the RS level (mainly H_2O_2), was measured by a spectrofluorometer (Perkin Elmer, LS55, USA).[11,12]

Determination of lipid peroxidation

The TBARS assay was used to directly measure the MDA, which is a product of lipid peroxidation. Firstly, the cells ($4x10^6$) were incubated in the TBARS solution that consisted of 400 µL PBS and 500 µL of TBARS working reagent at 80 °C for 1 hour. Secondly, the solution was cooled down to 25 °C for 15 min, and then the sediment was discarded by centrifugation. Finally, the absorption spectrum of MDA-TBA reaction product was determined by spectrophotometer (Agilent, model 8453, China).[13,14] The MDA level was calculated from the MDA standard curve.

Determination of membrane fluidity

Membrane fluidity of the cell was determined by observing the accumulation of a fluorescence probe DPH that intercalated between lipid bilayers of the membrane. The fluorescence intensity of DPH (F_{DPH}) signified membrane fluidity.[9,15] Cells ($5x10^5$) were incubated in the 0.1 µM DPH solution (1,900 µL) for 10 min. Next, the fluorescence intensity (F_{DPH}) at 430 nm (excited at 350 nm) was determined by a spectrofluorometer at 37 °C.

Drug uptake

The cellular drug uptake assays were performed by using THP.[16] THP is an anticancer drug that passively diffuses across the plasma membrane by its lipophilicity property, and then the fluorescence intensity is quenched by DNA intercalation. The kinetic uptake of THP by cells ($2x10^6$) was performed at 37 °C by determining the fluorescence intensity of 1 µM of THP solution at 590 nm (excited at 480 nm). After cell addition, the fluorescence intensity was decreased by DNA intercalation that represents cellular drug uptake (uptake rate) across the plasma membrane. The uptake rate can be calculated from the slope of tangent by equation 1. The accumulation of the drug by cells (C_n) was calculated from the fluorescence intensity at a steady state (F_n) by equation 2 (Figure 1)

$$\text{Uptake rate} = \frac{\text{Slope}}{F_0} \times 1\mu M \quad (1)$$

$$C_n = \frac{F_0 - F_n}{F_0} \times 1\mu M \quad (2)$$

Cytotoxicity of pirarubicin

After irradiation, cells ($5x10^3$) were cultivated for 24 hrs in 96 wells/plate; each well contained 0.1 mL of the completed RPMI-1640 medium with the various concentrations of THP of 0-300 nM for K562 and 0-3000 nM for K562/adr. The living cells were determined by adding 0.01 mL of resazurin solution into each well and then incubating at 37 °C and humidifying with 5% CO_2 for 4 hrs. The fluorescence intensity at 590 nm was measured on a spectrofluorometer.[17] The cytotoxicity of THP was expressed as the inhibition concentration (IC) at 20% (IC_{20}). The IC value was calculated as follows.

$$IC = \frac{F_c - F_s}{F_c} \times 100 \quad (3)$$

Where F_s is the fluorescence intensity of irradiated cells, and F_c is the fluorescence intensity of control cells.

Statistical analysis

Statistical analysis was performed by OrigingPro8 Software. All data from independent experiments were described as mean±standard error (SE). All obtained parameters from a variety of radiation-absorbed doses were analysed by one-way ANOVA (Tukey procedure). Significance level was defined at the alpha value of 0.05.

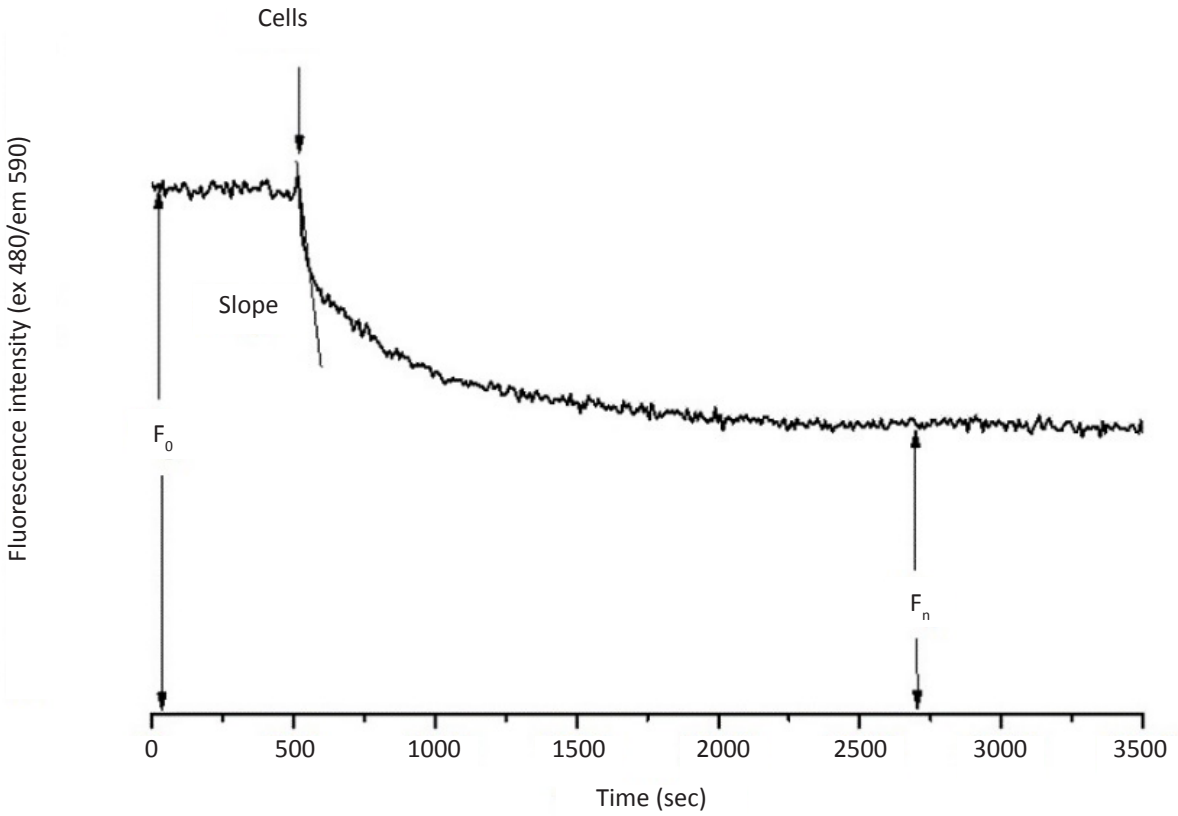


Figure 1. *Kinetic uptake of pirarubicin (THP) show the initial fluorescence intensity (F_0) of 1 µM of THP, slope (after adding cells), and the fluorescence intensity at steady state (F_n).*

Results

The effect of low-dose X-ray on cellular level was instantaneously investigated after irradiation. The fluorescence intensity of dichlorofluorescin (DCF) that represents the intracellular RS was determined. For sham control, the K562/adr cell line showed a fluorescence intensity of DCF lower than the K562 cell line. After irradiation, the RS level significantly increased in an absorbed dose-dependent manner either K562 or K562/adr. In this study, the lipid peroxidation production was evaluated from the MDA level. The MDA levels (mean±SE) of both non-irradiated K562 and K562/adr were equal to 2.12±0.16 and 3.27±0.17 fmole/cell, respectively. The MDA level in irradiated K562 was increased as a function of absorbed doses but not significantly different from non-irradiated K562; besides, no change of MDA level in irradiated K562/adr was detected. The result of membrane fluidity was determined by using the fluoresce probe DPH. Compared to the sham control, the fluorescence intensity of DPH of both irradiated cell lines slightly increased at a dose of 0.05, significantly increased at 0.1 Gy, and then decreased at 0.2 Gy (Figure 2).

Anticancer drug uptake and cytotoxicity

To evaluate the pharmacological properties of the membrane, the uptake rate of pirarubicin (THP) was used to indicate the passive diffusion of the drug across the plasma membrane. The kinetic uptake of THP, which is dependent on the membrane fluidity, was performed under the physiological buffer solution at 37 °C. In this condition, the result showed the drug uptake rate into the intact cells. In the sham control (0 Gy), the uptake rates of K562 and K562/adr are equal to 6.1±0.4 and 9.2±1.0 pM/sec, respectively. The uptake rate of irradiated cells is increased in the absorbed dose manner in both K562 and K562/adr cell lines, but not much differently from the sham control setting. The accumulation of THP (C_n) in both cell lines is calculated from the extinction of fluorescence intensity at a steady state. The C_n values of both irradiated cell lines were not different from the sham control. The C_n value of K562 is about 0.48-0.49 µM, but the value of K562/adr is about 0.22-0.25 µM. The cytotoxicity of THP was presented in term of IC_{20} values, which show the augmentation in K562/adr as 66.2±18.6, 84.3±17.3, 112.2±38.5, and 155.8±36.6 nM for 0, 0.05, 0.1,

and 0.2 Gy, respectively. In the case of K562, the IC_{20} values are 19.8±3.0, 23.3±6.1, 19.3±3.1, and 21.0±2.6 nM for 0, 0.05, 0.1, and 0.2 Gy, respectively (Figure 2). Finally, the mean±SE value of uptake rate, IC_{20}, and membrane fluidity were replotted in biplot as showed in Figure 3.

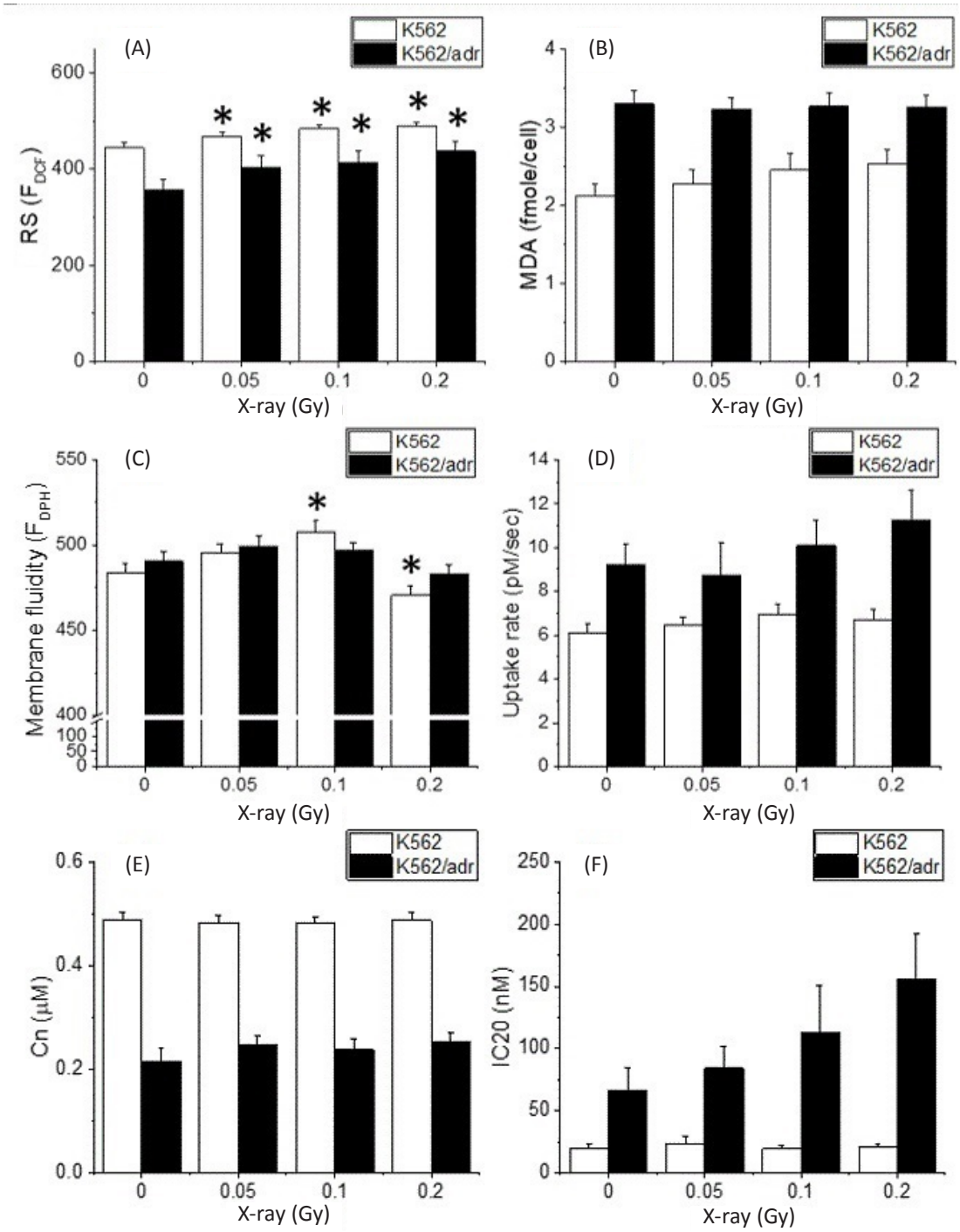


Figure 2. *Mean±SE value of Reactive oxidant species (RS). (A): MDA, (B): membrane fluidity, (C): uptake rate, (D): C_n (E) and IC_{20} of THP, (F) of K562 (□) and K562/adr (■) that exposed to X-ray at absorbed doses of 0, 0.05, 0.1 and 0.2 Gy (the asterisks represent the significantly different from 0 Gy at p<0.05).*

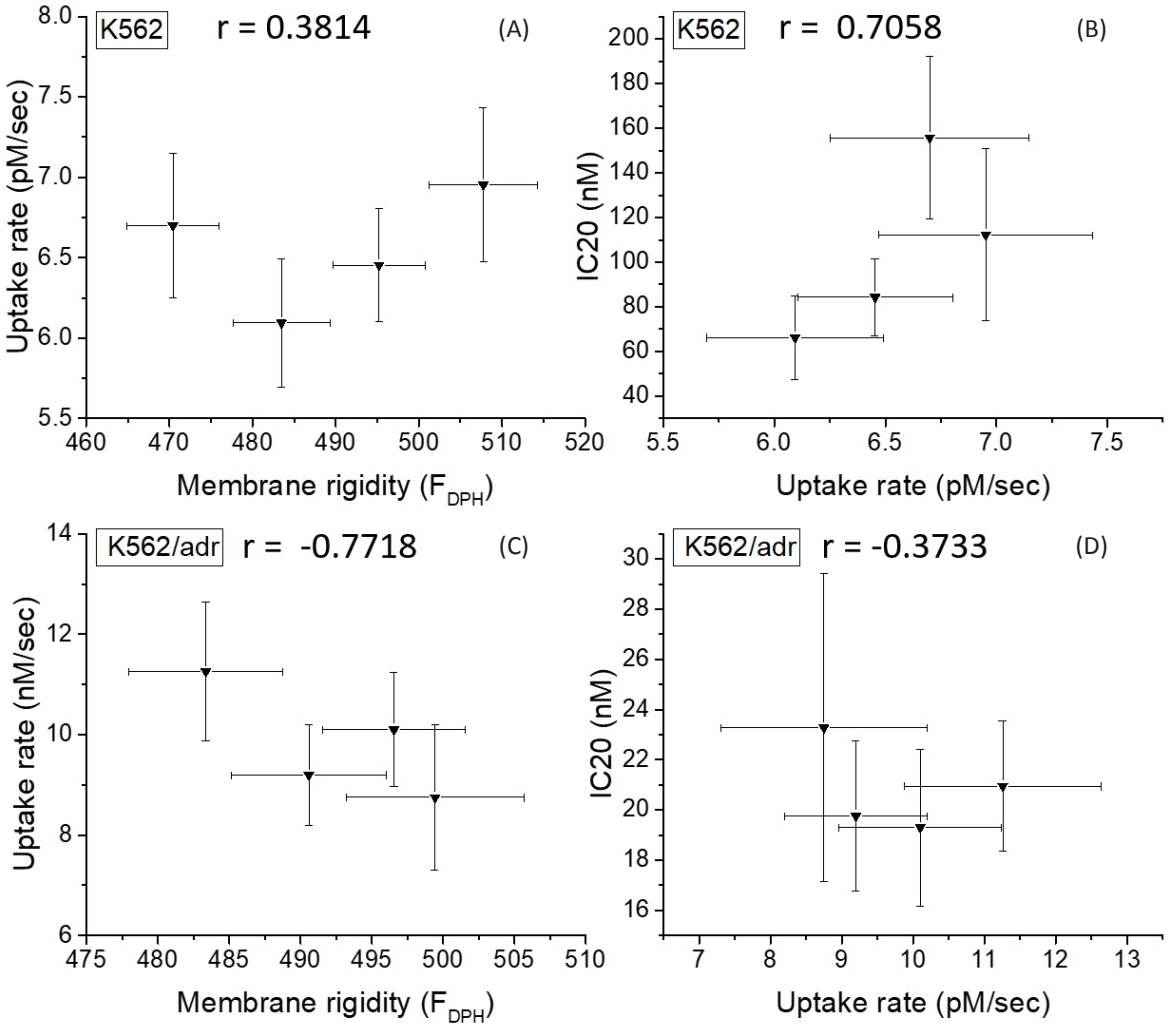
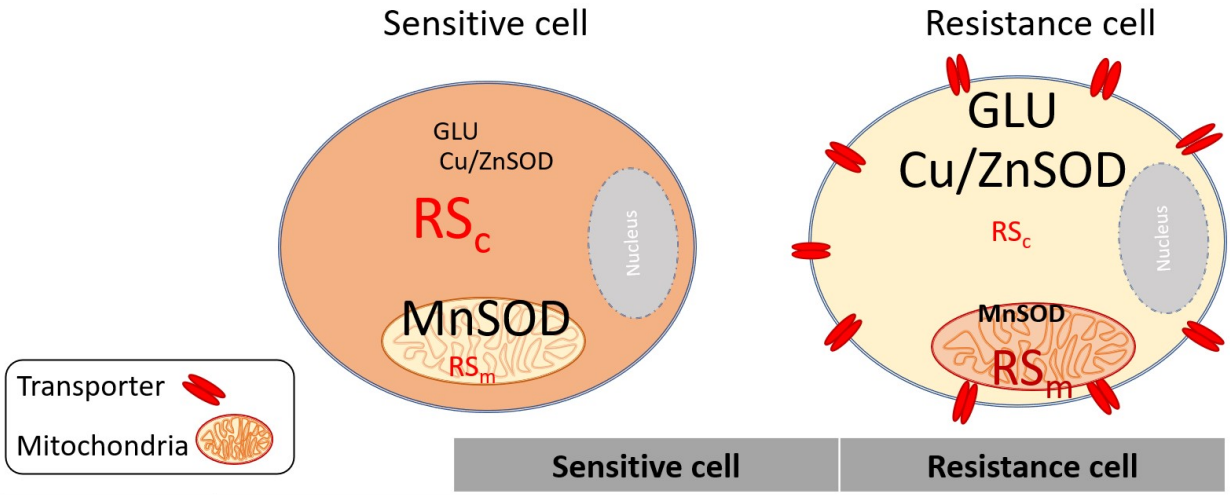


Figure 3. *Biplot (mean±SE) between uptake rate to membrane fluidity (A, C) and uptake rate to IC_{20} of THP (B, D) of K562 (Upper row) and K562/adr (Lower row), and the linear correlation coefficient (r).*

Discussion

Direct interaction of ionising radiation with matter generates free radical or oxidant species mainly in the form of ROS in a living cell. This leads to biological indirect effects such as lipid peroxidation, protein oxidation, and then DNA damage. In a resting state, the determined RS in drug-resistant cell line K562/adr are less than those of drug-sensitive cell line K562.[12] These values are the summation of RS, located in the mitochondria and cytoplasm. A previous study of cellular energetic state showed the mitochondrial membrane potential of K562 to be higher than K562/adr.[18] Therefore, this study supported that the free radical of the K562 cell is higher than K562/adr, which was generated from the mitochondria and then released in the cytoplasm. In the living cell, the intracellular superoxide dismutase plays an important role in cellular protection against RS. Two antioxidant enzymes, manganese superoxide dismutase (MnSOD) and copper/zinc superoxide dismutase (Cu/ZnSOD), respond to balance the oxidant radical at different locations in mitochondria and cytoplasm, respectively. Studies suggest that the MnSOD activity of K562 is higher than of K562/adr, while the Cu/ZnSOD activity of K562/adr is higher than of K562.[19] In this condition, the amount of cytoplasmic RS (RS_c) of K562 was more important than the amount of mitochondria RS (RS_m). Contrarily in K562/adr, the RS_m was more important than RS_c. Because of the different oxidative regulation in both cell lines, the data showed a higher degree of the MDA level of K562/adr than that of K562. Therefore, these lipid peroxidation productions probably result from RSm rather than RS_c.[20] According to the radiation cause on the increment of the oxidative state of K562 and K562/adr, the cellular response of both cell lines is not similar. Since mitochondria are considered critical organelles that induce cellular oxidative injuries by X-ray, several studies on these enzymes showed that the activity of MnSOD elevated, although Cu/ZnSOD was either reduced or induced by the effect of ionising radiation.[21-23] Other than those two enzymes, the cytosolic antioxidant such as glutathione (GSH) was found to be higher in K562/adr than in K562.[24] The study of GSH redox status also found it to be lessened by the effect of irradiation.[25] In our cases, it is possible that the radiation causes the elevation of RS in both cell lines, but the antioxidant defence systems in both cell lines are different. (Figure 4) These lead to the increase of MDA level in K562 but not

in K562/adr. Even if the MDA level elevation of K562 depends on the absorbed dose, the statistical test was insignificant when compared to the non-irradiation group. The membrane properties were assessed by observing the membrane fluidity and drug uptake. Fluidity of the membrane was determined by a DPH that interacts along the fatty acid path of the lipid membrane. The more fluorescence intensity means the less kinetic motion of the lipid bilayer, so the membrane becomes more rigid. In this study, the fluorescence of DPH of K562/adr was a bit higher than K562, which means that the membrane of K562/adr has less fluidity. These results were supported by the observation of a decrease in membrane fluidity and related with the degree of resistance.[26] The observation in both cell lines showed a biphasic response that is slightly decreased in membrane fluidity at 0.1 Gy and then increased at 0.2 Gy. Furthermore, the drug uptake basically depends on the membrane fluidity. The uptake rate of THP, which demonstrates the passive diffusion across the plasma membrane, did not show a significant modification. Moreover, the drug uptake rate did not show significant modification by the effect of ionising radiation. However, the biplots between uptake rate to membrane fluidity and uptake rate to IC_{20} of K562/adr illustrated a negative correlation.



		Sensitive cell	Resistance cell
Cytosol	MnSOD	Low	High
	Glutathione (GSH)	Low	High
	RS_c	High	Low
Mitochondria	MnSOD	High	Low
	RS_m	Low	High
Before radiation	Malondialdehyde (MDA)	Low	High
After radiation		Change	Not Change

Figure 4. *Model of oxidative balance in cytosol and mitochondria in sensitive and resistance cell: reactive oxidant species in cytosol (RS_c) and in mitochondria (RS_m) the antioxidant in mitochondria (MnSOD) and in cytosol (Cu/ZnSOD and glutathione (GSH)) and lipid peroxidation's product (malondialdehyde (MDA)).*

Conclusion

The findings of the K562 and K562/adr cell lines when exposed to low-dose X-ray of 135 kVp (up to 0.2 Gy) showed a significant increase in the intracellular RS, a mild increase in the MDA level in K562 but not in K562/adr, and a decrease of the membrane fluidity (0.1 Gy) and then increase at 0.2 Gy. The anticancer drug uptake rate in both cell lines is increased by the effect of radiation, but not significantly.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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