Impact of ascorbic acid concentration on the survival rate of *saccharomyces cerevisiae* under gamma irradiation

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ABSTRACT

Ionizing radiation induces severe oxidative stress, leading to cytotoxicity. This study investigates the radioprotective mechanism of Ascorbic Acid (AA) on Saccharomyces cerevisiae exposed to Cobalt-60 gamma irradiation. Yeast cells were treated with AA concentrations (300 µM) and subjected to radiation doses ranging from 25 to 100 Gy. Survival rates were quantified via colony formation assays and analyzed in comparison with previous studies on DNA single-strand breaks (SSB). While prior research demonstrated that AA consistently mitigates DNA damage, the current cellular results reveal a dose-dependent limitation. Specifically, 300 µM AA exhibited significant radioprotective efficacy at a low dose (25 Gy). However, at higher doses (75 - 100 Gy), AA paradoxically reduced survival rates compared to controls, diverging from its protective trend on DNA. The data may suggest a trend towards a functional transition of AA from an antioxidant to a pro-oxidant role, particularly when combined with high radiation doses. Consequently, the study implies that radioprotection observed at the molecular DNA level might not necessarily guarantee cellular survival under extreme oxidative conditions.

Keywords: Ascorbic acid; Saccharomyces cerevisiae; Gamma irradiation; Radiation protection.

1. INTRODUCTION

Ionizing radiation (IR), originating from both natural background sources and anthropogenic activities, represents a persistent environmental and occupational health hazard. The potential for accidental radioactive release - exemplified by historical nuclear incidents - underscores the critical need for effective radiation countermeasures. The biological toxicity of IR is primarily mediated through the radiolysis of water, which generates a surge of reactive free radicals. These chemical species, characterized by unpaired valence electrons or open electron shells, act as highly unstable intermediates possessing dangling covalent bonds. Consequently, they initiate indiscriminately oxidative attacks on vital biomolecules, including cell membranes, lipids, proteins, and nuclear DNA, ultimately leading to cytotoxicity or mutagenesis [1].

Among the spectrum of radiation induced lesions, DNA damage is the most significant determinant of cell fate. Free radicals can induce single-strand breaks (SSBs) at phosphodiester bonds, base damage, and protein-DNA crosslinks. More critically, they cause double strand breaks (DSBs) lesions formed by opposing or closely spaced breaks on the DNA helix. DSBs are considered the most deleterious form of damage; due to the complexity of repair mechanisms involved, failure to repair or misrepair of DSBs frequently results in chromosomal aberrations, genomic instability, and cell death [2, 3].

To mitigate these deleterious effects, the deployment of natural compounds as radioprotectors has been a subject of extensive research [4]. The efficacy of these antioxidants is conventionally evaluated through their capacity to scavenge free radicals quantified via assays such as 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as well as their ability to reduce DNA fragmentation and preserve cellular morphology [5, 6]. Theoretically, these agents protect biological systems by neutralizing free radicals before they interact with target biomolecules or by inhibiting their uncontrolled propagation.

AA is a potent antioxidant ubiquitous in citrus fruits and vegetables [7, 8]. Its molecular architecture, characterized by four hydrogen bond donor groups and six hydrogen-bond acceptor groups, renders it an exceptional electron donor. This structural property allows AA to stabilize reactive free radicals by donating electrons, thereby restoring their chemical stability [9]. Historically, AA has been documented as a promising radioprotector capable of shielding living organisms from IR-induced injury [10].

However, the correlation between the molecular antioxidant capacity of AA and its actual radioprotective efficacy at the cellular level remains complex, particularly under varying radiation intensities. While previous research has established the ability of AA to protect DNA structure, its impact on cell survival requires further elucidation. In this study, we evaluate the radioprotective mechanism of AA on *Saccharomyces cerevisiae* cells exposed to gamma irradiation. By analyzing cellular survival rates in comparison with established molecular DNA damage data, this research aims to provide a comprehensive understanding of AA's behavior from DNA protection to its effects on cellular viability under oxidative stress.

The dual nature of AA as both an antioxidant and a pro-oxidant has been recognized, particularly in the presence of transition metal ions or under specific concentration thresholds. While our previous research demonstrated that 300 μM AA effectively preserves DNA structural integrity by reducing single strand break (SSB) formation, a critical question remains: does this molecular-level protection translate directly to enhanced cellular survival under high-intensity radiation? This study shifts the focus from molecular shielding to cellular viability, exploring the potential inversion of AA's role from a radioprotector to a radiosensitizer in a living *Saccharomyces cerevisiae* model. By investigating the concentration dependent effects across a range of 25 to 100 Gy, we aim to identify the threshold where AA's protective capacity is overwhelmed by radiation-induced oxidative stress.

2. MATERIALS AND METHODS

2.1. Preparation of yeast cells and ascorbic acid reagent

Saccharomyces cerevisiae cells were cultured in liquid yeast extract peptone dextrose (YPD) medium for 24 hours at 30 °C. For the treatment, AA reagent (analytical grade, 99% purity, Nagara Science Co., Ltd., Japan) was used. To evaluate the radioprotective efficacy of the antioxidant, the growth medium was either maintained without AA (serving as the control group) or supplemented with AA at predetermined concentrations (300 μM). Following the incubation period, cell population density was quantified utilizing a Neubauer counting chamber. The cell suspensions were subsequently diluted and processed through a vacuum filtration unit (Sterifil aseptic system, Billerica, MA, USA). Membrane filters, each retaining an inoculum of approximately 200 cells, were then aseptically transferred into 50 mm Petri dishes in preparation for irradiation.

2.2. Irradiation setup and sample processing

Gamma irradiation was performed using a ^{60}Co isotopic source at the institute of scientific and industrial research (ISIR), Osaka university, Japan. To eliminate errors associated with the samples' biological time variation, all experimental groups were exposed for a constant duration of 30 minutes. The variations in absorbed doses (25 -100 Gy) were achieved by positioning samples at specific spatial coordinates, ranging from 31.01 to 62.01 cm from the source, as detailed in Figure 1 and Table 1.

Absorbed doses were determined through theoretical calculations based on the source's initial activity and decay charts. To ensure high precision (within ± 5%), these calculations were cross verified using an ionization chamber, calibrated according to the university's rigorous radiation safety and dosimetry protocols.

To ensure electronic equilibrium and accurate biological dose delivery to the thin layer of yeast

cells, a tissue-equivalent build-up material was placed over the samples during irradiation. This setup ensures that the maximum dose is deposited at the sample plane, effectively converting the free air kerma into an accurate absorbed dose for the biological specimens. The uniformity of the radiation field at the shortest distance (31.01 cm) was evaluated to ensure consistent dose delivery across the entire surface of the Petri dish (diameter 50 mm). Given that the sample area is small relative to the radiation field, the field non-uniformity was determined to be less than 3%. This level of uniformity ensures that all yeast cells within the dish received a statistically equivalent absorbed dose, maintaining the integrity of the survival rate data.

Figure 1. *Experimental setup for gamma irradiation using a ^{60}Co source, demonstrating the geometric arrangement to achieve targeted absorbed doses.*

Table 1. *Effect of sample positioning and irradiation time on absorbed dose and dose rate using a ^{60}Co source.*

Distance from source (cm)	Exposure time (min)	Dose rate (Gy/min)	Absorbed dose (Gy)
31.01	30	3.333	100
35.80	30	2.500	75
43.85	30	1.667	50
62.01	30	0.833	25

Gamma irradiation was performed using a ^{60}Co source at a fixed exposure time. This setup, providing high-energy gamma rays, aligns with clinical practices as ^{60}Co remains a prevalent source in both brachytherapy and external beam radiotherapy. Such an irradiation system allows for a reasonable simulation of the oxidative stress environment induced by high-energy photon radiation within the human body. To deliver specific absorbed doses (25, 50, 75, and 100 Gy), the source to sample distance was adjusted based on the inverse square law ($I \sim {}^{1}/_{d^2}$) [1]. The overall uncertainty of the absorbed dose encompassing source calibration, spatial positioning, and field non-uniformity was maintained within ± 5%, strictly adhering to international standards for biological research.

The yeast cells were concentrated on a membrane filter, which was then placed in a sterile, dry Petri dish for irradiation. No bulk liquid medium or water was present during the exposure. This dry state irradiation setup was specifically chosen to minimize the contribution of external radiolytic products from the surrounding environment, thereby ensuring that the observed effects of AA were primarily related to its interaction with intracellular oxidative stress and direct radiation-induced damage.

The dose range of 0 - 100 Gy was selected based on the known radioresistance of

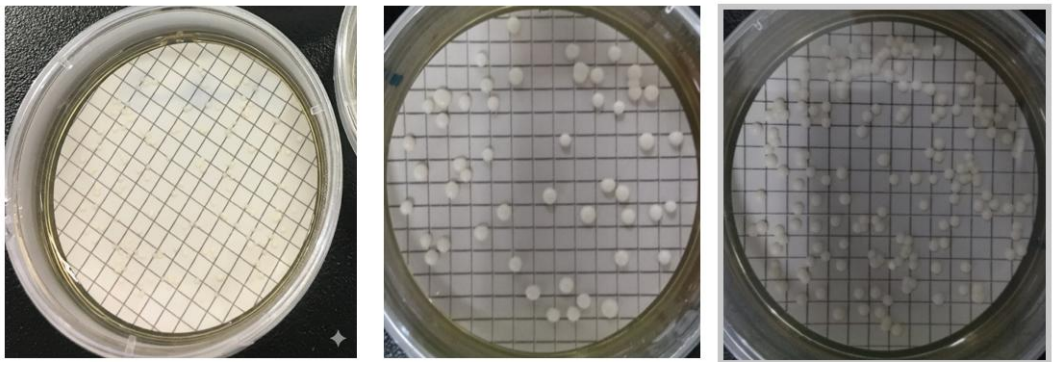
Saccharomyces cerevisiae. Unlike mammalian cells, which typically exhibit lethal effects at doses below 10 Gy, yeast cells possess robust DNA repair mechanisms and a thick cell wall, necessitating higher dose scales (up to 100 Gy or more) to observe significant physiological shifts and survival gradients [11, 12].

Post-irradiation, the membrane filters were aseptically transferred onto solid YPD agar plates and incubated for 48 hours to facilitate colony formation. The surviving colonies were enumerated, and the survival rate was calculated according to equation (1):

$$Survival\ Rate\ (\%) = \frac{Number\ of\ colonies_{\ irradiated}}{Number\ of\ colonies_{\ non-irradiated}} * 100\% \quad (1)$$

3. RESULTS AND DISCUSSION

Figure 2 illustrates the colony formation of *Saccharomyces cerevisiae* on solid YPD agar following incubation for 2 days at 30 °C. Specifically, Figure 2a depicts the post-irradiation growth of cells cultured in an antioxidant-free medium. In contrast, Figure 2b displays the colony density of cells cultured in a medium supplemented with antioxidants:



a) *b)* *c)*

Figure 2. *Representative images of yeast colony development on YPD plates (incubated at 30°C for 48 h) after exposure to radiation. (a) non-irradiated control; (b) 25 Gy irradiation only; (c) 25 Gy irradiation + 300 μM AA.*

The reduction in colony density is clearly visible as the radiation dose increases, while the presence of 300 μM AA at 25 Gy shows a relative maintenance of colony numbers compared to the radiation-only group.

Figure 3 depicts the survival rate of yeast cells as a function of gamma radiation dose, comparing conditions in the presence and absence of Ascorbic Acid (AA). Figure 3 illustrates the survival fraction of *S. cerevisiae* exposed to gamma irradiation doses ranging from 0 to 100 Gy. In the absence of additives (control), cell viability exhibited a consistent, dose-dependent decline. The supplementation of 300 μM Ascorbic Acid (AA) resulted in a distinct biphasic response. At the lower dose of 25 Gy with p-value ~ 0.0015, the AA treated group showed a markedly higher survival rate (~ 0.90) compared to the control (~ 0.70). However, as the absorbed dose increased to 50 Gy with p-value ~ 0.0210, the protective effect of AA diminished, resulting in survival rates comparable to the control group. Notably, at higher doses (at 75 Gy with p-value ~ 0.001; at 100 Gy with p-value ~ 0.001), the presence of AA appeared to reduce cell survival below that of the control, with the survival fraction at 100 Gy dropping to approximately 0.25 compared to 0.35 in the untreated samples. A p-value < 0.05 was considered statistically significant.

Regarding cellular viability, 300 μM AA exhibited radioprotective efficacy exclusively at the lower irradiation dose of 25 Gy. In contrast, at higher doses (75 and 100 Gy), the presence of AA tended to diminish survival rates relative to the control. This observed reduction in survival

suggests a potential trend towards a transition to pro-oxidant-like behavior. In this context, AA may act as a radiosensitizer, potentially exacerbating cellular damage under the combined influence of high concentration and elevated radiation doses.

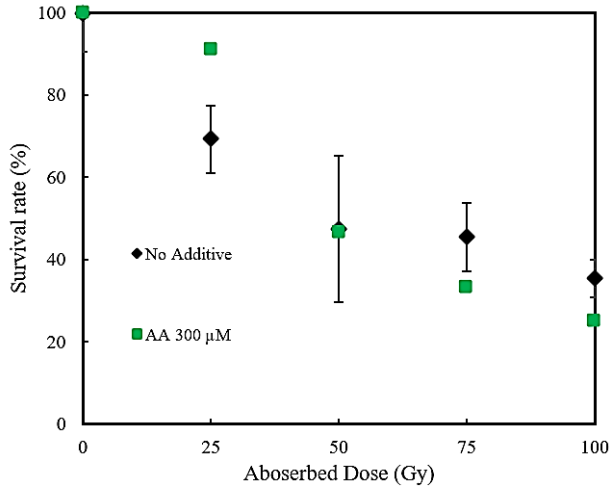


Figure 3. *Cell survival rate as a function of absorbed gamma radiation dose. Data are presented as mean ± standard deviation (SD) (n = 5).*

To elucidate the mechanisms underlying the observed survival patterns, we compared our cellular data (Figure 3) with previous findings on DNA radioprotection reported by Tran Thi Nhan et al. (2022) [13]. Their study demonstrated that AA serves as a potent guardian of genomic integrity at the molecular level, evidenced by a significant reduction in the slope of single-strand break (SSB) formation from 0.1223 in controls to 0.0953 at 300 µM AA. While this confirms that AA effectively mitigates radiation-induced structural damage to DNA, our current biological data reveal a critical divergence at the cellular level. Although 300 µM AA conferred a distinct survival advantage at the lower dose of 25 Gy, this protection was not sustained at higher intensities. Surprisingly, at 75 and 100 Gy, the survival fraction of AA-treated cells declined to levels below those of the untreated control. This discrepancy suggests that while genomic shielding is maintained, it is insufficient to guarantee cellular survival under high-dose irradiation. This implies that other lethal pathways, potentially independent of DNA scission, may become dominant, or that the protective capacity of AA is overwhelmed in a living system.

Furthermore, the correlation between chemical antioxidant potential and biological efficacy provides additional insight into the "dual nature" of AA. As established by Boulmokh et al. (2021) [14], AA exhibits exceptional radical scavenging activity *in vitro*, characterized by rapid saturation in DPPH inhibition assays. This robust chemical reactivity explains the radioprotective effect observed at 25 Gy in our study, where AA likely functions as a primary scavenger, neutralizing reactive oxygen species (ROS) generated by water radiolysis before they can inflict lethal damage. However, the failure of AA to protect cells at doses exceeding 75 Gy despite its proven high antioxidant capacity indicates a non-linear translation from *in vitro* chemistry to *in vivo* biology. It appears that under conditions of extreme oxidative stress induced by high-dose gamma radiation, the redox homeostasis is disrupted.

Collectively, these comparative analyses indicate that the radiation protection effect of AA is strictly dose-dependent. The apparent trend from radioprotection at low doses to a possible radio sensitizing effect at high doses (75-100 Gy) underscores the complexity of cellular responses. This suggests a hypothesis where AA might participate in secondary reactions, such as the Fenton reaction with intracellular transition metals, which requires further direct verification [14].

Therefore, while AA is effective in preserving DNA structure and scavenging radicals at moderate radiation levels, its biological utility is compromised under high-dose exposure, where it may exacerbate cellular toxicity.

The observed divergence between DNA preservation and cell survival at 75-100 Gy marks a significant contribution of this work. While AA maintains its chemical capacity to scavenge radicals and shield the genome, the biological reality in a cellular environment is more complex. At high radiation doses, the excess production of reactive oxygen species (ROS) likely triggers secondary pro-oxidant reactions, such as the Fenton reaction, where AA might facilitate the reduction of intracellular metal ions, leading to further hydroxyl radical generation. This suggests that the radioprotective label of AA is conditional, depending strictly on the balance between radiation intensity and antioxidant concentration. Our findings highlight that at high doses, AA may paradoxically act as a radiosensitizer, a phenomenon that has practical implications for clinical radiotherapy where selective sensitization of target cells is desired.

4. CONCLUSIONS

In conclusion, this study provides insights into the operational limits of AA as a radioprotector, suggesting that protection at the molecular DNA level does not inherently guarantee cellular survival under extreme oxidative conditions. While 300 μM AA significantly enhances viability at a moderate dose of 25 Gy, its efficacy seems to diminish and potentially transitions toward a radio sensitizing effect at higher radiation intensities (75-100 Gy). Due to the high operational complexity and costs associated with high-dose gamma irradiation facilities, the current research focused on establishing a preliminary established the cellular response framework using the 300 μM concentration. To fully resolve the transition mechanism between antioxidant and pro-oxidant roles, our future investigations could expand the experimental matrix to include 500 μM and 1000 μM concentrations. This will be coupled with the direct quantification of intracellular oxidative markers, such as H_2O_2 and lipid peroxidation, and an exploration of the Fenton reaction synergy. These subsequent steps aim to offer a more precise understanding of the tipping point for AA's bioactivity, potentially refining antioxidant-based protocols in radiation medicine.

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TÓM TẮT

Ảnh hưởng của nồng độ Vitamin C lên tỷ lệ sống sót của *Saccharomyces cerevisiae* dưới tác động của chiếu xạ Gamma

Bức xạ ion hóa gây ra oxy hóa nghiêm trọng, nguy hại tế bào. Nghiên cứu này điều tra cơ chế bảo vệ bức xạ của Vitamin C (AA) trên nấm men Saccharomyces cerevisiae khi tiếp xúc với bức xạ gamma Cobalt-60. Các tế bào nấm men được xử lý với nồng độ AA (300 µM) và chịu liều bức xạ từ 25 đến 100 Gy. Tỷ lệ sống sót được định lượng thông qua xét nghiệm hình thành khuẩn lạc và được phân tích so sánh với các nghiên cứu trước đây về đứt gãy chuỗi đơn DNA (SSB). Trong khi nghiên cứu trước đây đã chứng minh rằng AA luôn làm giảm tổn thương DNA, kết quả tế bào hiện tại cho thấy sự hạn chế phụ thuộc vào liều lượng bức xạ. Cụ thể, 300 µM AA thể hiện hiệu quả bảo vệ bức xạ đáng kể ở liều thấp (25 Gy). Tuy nhiên, ở liều cao hơn (75 - 100 Gy), AA lại làm giảm tỷ lệ sống sót so với nhóm đối chứng, trái ngược với xu hướng bảo vệ DNA của nó. Dữ liệu có thể cho thấy xu hướng chuyển đổi chức năng của AA từ vai trò chống oxy hóa sang vai trò gây oxy hóa, đặc biệt khi kết hợp với liều bức xạ cao. Do đó, nghiên cứu này cho thấy rằng khả năng bảo vệ khỏi bức xạ được quan sát ở cấp độ phân tử DNA có thể không nhất thiết đảm bảo sự sống sót của tế bào trong điều kiện oxy hóa cao.

Từ khoá: Vitamin C; *Saccharomyces cerevisiae*; Chiếu xạ Gamma; Bảo vệ phóng xạ.