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Mutation Research 538 (2003) 93–99
Genetic Toxicology and Environmental Mutagenesis
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β-Pseudouridine, a beer component, reduces radiation-induced chromosome aberrations in human lymphocytes

Manami Monobe [a,b,*], Sakae Arimoto-Kobayashi [c], Koichi Ando [a,b]

[a] *Division of Heavy-ion Radiobiology Research Group, National Institute of Radiological Sciences, 9-1 Anagawa-4-chome, Inage-ku, Chiba 263-8555, Japan*
[b] *Graduate School of Science and Technology, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[c] *Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima, Okayama 700-8530, Japan*

Received 24 December 2002; received in revised form 8 April 2003; accepted 8 April 2003

Abstract

We previously found that drinking beer reduces chromosome aberrations in blood lymphocytes that were collect and irradiated in vitro. In this study, human whole-blood was in vitro exposed to 200 kVp X-rays or 50 keV/μm carbon ions in the presence or absence of beer, ethanol and β-pseudouridine (one of the beer components). All three agents reduced the chromosome aberrations (dicentric) caused by either X-rays or carbon ions of 4 Gy. The maximum protection for X-rays by ethanol, beer and β-pseudouridine was 64, 26 and 34%, respectively, while the maximum protection for carbon-ion by ethanol, beer and β-pseudouridine was 22, 26 and 32%, respectively. It is concluded that β-pseudouridine, a nucleoside present in tRNA, is a potent protector against damage caused by radiation with direct and indirect actions.

Keywords: Beer; Chromosome aberration; X-rays; Carbon ions; β-Pseudouridine

1. Introduction

The search for chemical agents able to protect human beings from ionization radiation is a key issue in radiation biology [1]. Because most radioprotective substances produce severe side effects, radioprotective agents with low toxicity have been searched for many years. Radioprotective activities of foods have been reported for vitamins, garlic extract, squalene, caffeine and miso (fermented soy bean paste) [2–9]. We have found and reported that drinking beer reduces radiation-induced chromosome aberrations in human lymphocytes [10]. Beer is effective to inhibit salmonella mutation, and β-pseudouridine in beer could inhibit the mutagenicity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) in *Salmonella* [11]. In this study, we investigated whether β-pseudouridine is effective to reduce chromosome aberrations after radiation with X-rays and charged particles.

2. Materials and methods

2.1. Irradiation

Whole-blood samples were irradiated in air at room temperature with either X-rays (200 kVp, 20 mA, 0.5 mm Cu + 0.5 mm Al filters, focus center distance 55 cm) at a dose rate of 1 Gy/min or ^{12}C ion beams

* Corresponding author. Tel.: + 81-43-206-3235; fax: +81-43-206-4149.
E-mail address: monobe@nirs.go.jp (M. Monobe).

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doi:10.1016/S1383-5718(03)00094-9

(LET: 50 keV/μm) at a dose rate of 3 Gy/min. Carbon ions were accelerated up to 290 MeV/μm by the HIMAC synchrotron at the National Institute of Radiological Science (NIRS, Chiba, Japan). Lucite absorbers with 141 mm thickness were used to define a LET of 50 keV/μm, which is a dose-averaged value including the contribution of fragments. All of the irradiations were carried out at room temperature.

2.2. Blood sampling

Blood was collected in heparinized tubes (Becton Dickinson Co., NJ, USA) from a healthy, non-smoking female volunteer either before drinking a 700 ml beer (ethanol content; 40 mg/ml), within 15 min or 3 h afterwards. The ethanol concentration in the plasma was measured by gas chromatography at the KOTO microorganism laboratory (Tokyo, Japan).

2.3. Treatment with ethanol or beer

Ninety-nine (99)% ethanol was added to whole-blood samples in vitro to provide final ethanol concentrations of up to 1600 mM. The beer used here contained 5.5% ethanol, and was added to whole-blood samples to provide final ethanol concentrations of up to 25 mM. The samples were kept at room temperature for 1 h before irradiation.

2.4. Treatment with β-pseudouridine

β-Pseudouridine (Sigma) was dissolved in 0.9% saline to prepare a stock solution with 25 mg/ml β-pseudouridine. The stock solution was diluted, and added to whole-blood samples with final concentrations of up to 20 mM. The samples were kept at room temperature for 1 h before irradiation.

2.5. Lymphocyte culture and slide preparation

One milliliter of irradiated whole-blood was cultured in 9 ml of a RPMI 1640 medium (Gibco-BRL, NY, USA) supplemented with 20% (v/v) fetal bovine serum, 1.25 mg/ml sodium bicarbonate, 90 μg/ml PHA-M (Murex Biotech Ltd., UK) and 0.05 μg/ml colcemid (Wako pure Chemical Industries Ltd., Osaka, Japan). Colcemid was added to the medium at the beginning of the culture in order to obtain a sufficient number of cells at the metaphase in the first cell division [12]. After 53 h at 37 °C, the cultured cells were treated with a hypotonic (75 mM) KCl solution for 20 min at 37 °C, and fixed in methanol acetic acid (3:1). Air-dried slides were made under warm and humid conditions. The slides were stained with 4% Giemsa in phosphate buffered saline (pH 6.8), embedded in Eukitt (O. Kindler, Germany), and finally observed under a light microscope with the highest magnification (1000×). One hundred mitotic metaphase figures were examined for each experimental point to detect dicentrics, i.e. unstable type of chromosome aberrations. The experiments were repeated twice.

2.6. Curve fit

The dose-response of the chromosomal aberration frequencies was established by fitting the experimental data to a linear-quadratic regression, $Y = \alpha D + \beta D^2$, where Y is the yield of induced chromosome aberrations per cell, D the dose in Gy, and α and β are fitted coefficients. No dicentric aberration was observed in 1000, at least, non-irradiated cells.

3. Results

3.1. Dose-response relationships for dicentric induction in lymphocytes exposed to X-rays or carbon-ions

The frequencies of radiation-induced dicentrics are plotted as a function of the radiation dose in Fig. 1.

In some experiments, either 10 mM ethanol [10] or beer containing 10 mM ethanol was added to whole-blood before irradiation. Ethanol concentrations of 10 mM correspond to the blood ethanol concentration 3 h after beer drinking [10]. For X-rays, ethanol-addition in vitro was effective to reduce the aberrations (Fig. 1a). This effectiveness is similar to that observed for blood after drinking beer [10]. The reduction of aberrations by in vitro beer-addition was more prominent than that by ethanol-addition or beer drinking. For carbon ions; however, the drinking beer [10] was the most effective to reduce chromosome aberrations, and superior to the in vitro addition of either ethanol or beer (Fig. 1b).

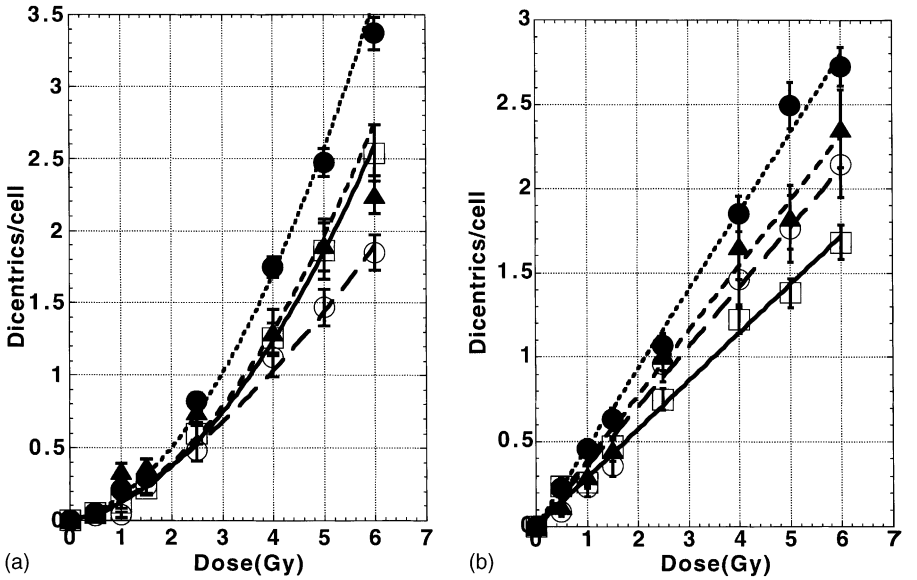

Fig. 1. Dose-response of chromosome aberrations in lymphocytes exposed to either 200 kVp X-rays or LET 50 KeV/μm carbon ions. (a) X-rays; (b) carbon ions. The values represent means ± S.E.M. ($n = 100$). Data was fitted to a linear-quadratic dose-response function. Symbols are: (●) blood was collected before drinking beer [10]; (○) beer was addition to blood in vitro (10 mM ethanol equivalent) (present study); (□) blood was collected 3 h after drinking beer (ethanol concentration is 10 mM) [10]; (▲) ethanol was added to blood in vitro (10 mM ethanol equivalent) [10].

3.2. Fraction of protection in X-ray-induced chromosome aberrations

When ethanol was added to a blood preparation in vitro before 4 Gy X-rays, radiation-induced aberrations decreased with an increase of the ethanol concentration (Fig. 2a). The dicentric yield was 1.77 per cell without ethanol-addition, and exponentially decreased to 0.64 per cell as the ethanol concentration increased to 800 mM. A further increase of the ethanol concentration up to 1600 mM did not bring about grater protection, such that a plateau was observed. Here, the dependence of the dicentric yield, Y, on the ethanol concentration, x, could be fitted to the formula

$$Y = \theta_n + \theta_p e^{-\delta x},$$

where θ_n is the fraction of no protection or the plateau, θ_p the fraction of protection and δ is the exponential decay constant [13]. Estimates of θ_p, θ_n and δ were 1.13±0.06 (mean and S.D.), 0.64±0.04 dicentrics per cell and 0.0069 ± 0.001, respectively. The maximum decrease or protection, defined as $\theta_p/(\theta_p + \theta_n)$, was 64% (1.13/1.77).

For an in vitro beer treatment, the radiation-induced aberrations again decreased exponentially with an increase of the ethanol concentration in beer until 3.1 mM (Fig. 2b). A further increase of the ethanol in beer again produced a plateau or no further decrease of aberrations at 6.25 mM. This concentration of ethanol as beer was less than one-hundredth of 800 mM, the plateau concentration of a treatment of ethanol alone (Fig. 2a). The estimates of θ_p, θ_n and δ were 0.45 ± 0.04, 1.27 ± 0.02 dicentrics per cell and 1.26 ± 0.28, respectively. Because the aberration frequency without ethanol ($\theta_p + \theta_n$) was 1.72, the maximum protection $\theta_p/(\theta_p + \theta_n)$ was 26% (0.45/1.72).

3.3. Fraction of protection in carbon-ion-induced chromosome aberrations

For an in vitro ethanol treatment, the radiation-induced aberrations again decreased exponentially with an increase of the ethanol concentration until 6.25 mM (Fig. 3a). The estimates of θ_p, θ_n and δ were 0.41 ± 0.06, 1.44 ± 0.04 dicentrics per cell and

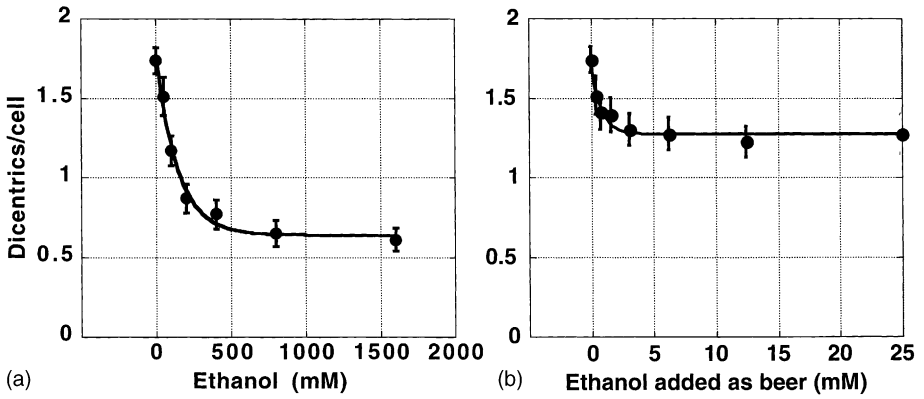

Fig. 2. Dependence of X-ray-induced dicentrics on ethanol concentration. (a) 4 Gy-irradiated dicentrics as a function of ethanol. (b) 4 Gy-irradiated dicentrics as a function of ethanol added as beer.

0.46 ± 0.16, respectively. The maximum protection was 22% (0.41/1.85) at 6.25 mM, or more.

For an in vitro beer treatment, the radiation-induced aberrations again decreased exponentially with an increase of the ethanol concentration until 6.25 mM (Fig. 3b). The estimates of θ_p, θ_n and δ were 0.49 ± 0.08, 1.36 ± 0.05 dicentrics per cell and 0.24 ± 0.09, respectively. The maximum protection was 26% (0.49/1.72) at 6.25 mM through 25 mM (Fig. 3b).

3.4. In vitro β-pseudouridine treatment

For X-rays, the radiation-induced aberrations again decreased exponentially with an increase of the β-pseudouridine concentration over a range of 0–3.2 mM (Fig. 4a). The estimates of θ_p, θ_n and δ were 0.58 ± 0.04, 1.15 ± 0.02 dicentrics per cell and 1.22 ± 0.18, respectively. The aberration frequencies without β-pseudouridine ($\theta_p + \theta_n$) were 1.73, and the maximum protection was 34% (0.58/1.73) at 3.2 mM through 25.6 mM (Fig. 4b).

For carbon ions, the radiation-induced aberrations decreased exponentially with an increase of the β-pseudouridine concentration over a range of 0–6.4 mM (Fig. 4a). The estimates of θ_p, θ_n and δ were 0.51 ± 0.10, 1.17 ± 0.06 dicentrics per cell and 0.39 ± 0.23, respectively. The aberration frequency without β-pseudouridine was 1.68, and the maximum protection was 32% (0.51/1.68) at 12.8 mM through 25.6 mM (Fig. 4b).

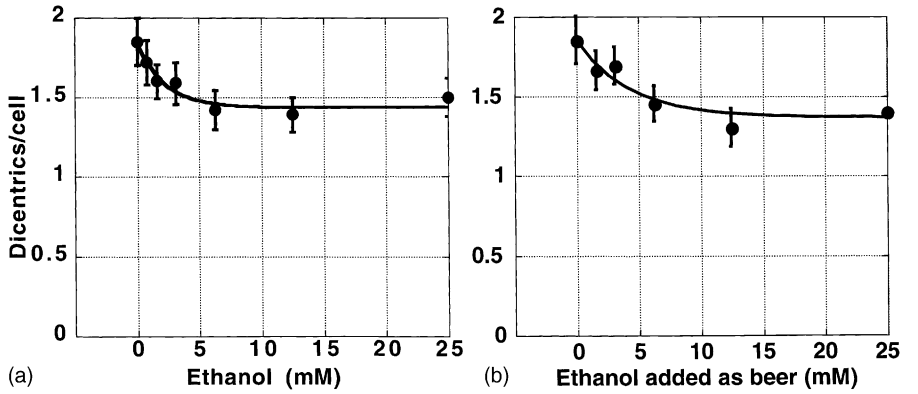

Fig. 3. Dependence of carbon-ion-induced dicentrics on ethanol concentration. (a) 4 Gy-irradiated dicentrics as a function of ethanol. (b) 4 Gy-irradiated dicentrics as a function of ethanol added as beer.

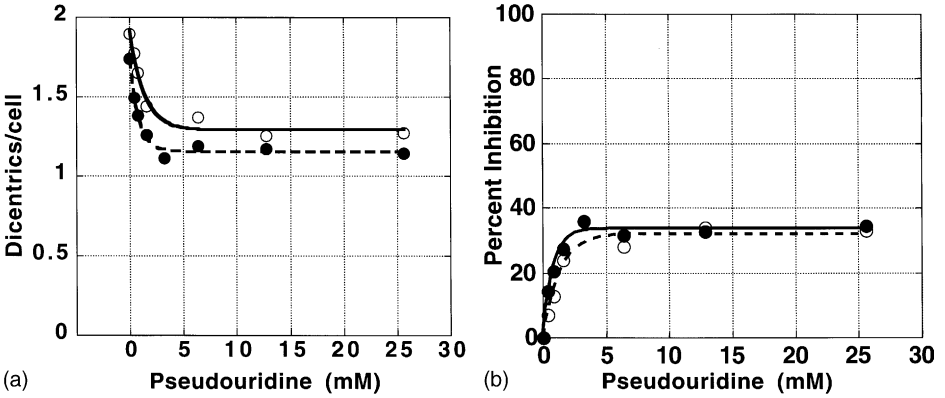

Fig. 4. Dependence of radiation-induced dicentrics on β-pseudouridine concentration. Lymphocytes were irradiated with 4 Gy of either X-rays (●) or carbon ions (○). (a) Number of dicentrics per cell. (b) Percent inhibition calculated from (a).

4. Discussion

We previously reported that drinking beer reduces a radiation-induced chromosome aberration in human lymphocytes [10]. When the present data of in vitro beer treatment was compared with the previous data of drinking beer, the in vitro treatment with beer was more effective than drinking beer for protecting chromosomes from X-ray irradiation (Fig. 1a). We also found that an in vitro treatment with beer was more effective than an in vitro treatment with ethanol for reducing the chromosome aberrations induced by X-ray irradiation in 10 mM ethanol equivalent (Fig. 1a). This implies that beer could contain some non-ethanol elements that protect chromosomes from X-ray and carbon-ion irradiation.

At a low concentration of ethanol equivalent, the radioprotective activity of beer showed 27% inhibition at 25 mM ethanol equivalent, and was higher than that of ethanol, i.e. 13% inhibition at 50 mM (Fig. 2). Beer contains antioxidant substances, such as phenolic compounds [14], which may account for beer's activity to increase any antioxidant capacity of plasma in humans [15]. On the other hand, the radioprotective activity of beer was lower than ethanol at high concentrations of ethanol equivalent. The maximum protection by an ethanol treatment against X-ray irradiation was 64% at 800 mM (Fig. 2a). Ethanol of 1000 mM is known to reduce 70% of the DNA strand scissions in mammalian DNA following exposures to X-ray radiation [16,17]. Because ethanol scavenges hydroxyl radicals [16], protection by ethanol alone in the present study was likely to be due to OH radical interactions. However, the maximum protection by a beer treatment was as low as 26% (Fig. 2b). This value of protection remained, and did not increase when the ethanol equivalent was increased up to 100 mM (data not shown). This follows that some elements in beer may counteract the radical scavenging activity of ethanol. This kind of counteraction has been reported for a chemical mutagen. Glycine betaine in beer can inhibit the mutagenicity of 2-chloro-4-methylthiobutanoid (CMBA) in *Salmonella typhimurium* TA100 and TA1535 [18]. The maximum anti-mutagenic activity of beer is lower than that of glycine betaine alone. This suggests the possibility that beer contains components that antagonize the anti-mutagenic activity of glycine betaine.

The maximum protection by ethanol or beer-treatment against carbon ions was similar to each other (Fig. 3). Carbon ions of ∼50 keV/μm produce DNA damage mainly through direct events [19]. The indirect contribution of OH radicals to DNA strand breaks is 40% after carbon-ion irradiation, and is much less than 70% after X-ray irradiation. The maximum protection achieved by ethanol in the present study was 22% (Fig. 3), and less than an anticipated value of 40%. It should also be noted that the counteraction of beer stated above for X-rays was not detectable here for carbon ions.

β-Pseudouridine is a component of tRNA, and possesses an anti-mutagenic activity against MNNG in *Salmonella* [11]. MNNG is an alkylating agent that

methylates nucleic acid purine and pyrimidine base residues [20–22]. MNNG induces DNA strand breaks as ionizing radiation does. Yoshikawa et al. [11] suggest that the anti-mutagenicity of β-pseudouridine is due to the entrapment of methyl radical derived from MNNG. β-Pseudouridine reduced the chromosome aberration after X-rays and carbon ions (Fig. 4). The maximum protection produced by an in vitro ethanol treatment was 22%, while the maximum protection by an in vitro beer treatment was 26% (Fig. 3). The difference between the two radioprotections was 4%, and showed, no statistic significance. Because the concentration of β-pseudouridine in beer is as low as 4 μg/ml (16 μM) [11], the contribution of β-pseudouridine to protection an in vitro beer treatment would be, if at any, small.

It is concluded that β-pseudouridine, a nucleoside present in tRNA, is a potent protector against the damages caused by radiation with both indirect and direct actions.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan and by the Special Coordination Funds for Research Project with Heavy Ions at the National Institute of Radiological Sciences–Heavy-ion Medical Accelerator in Chiba (NIRS-HIMAC). We would like to thank Dr. Y. Furusawa for his helpful comments.

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