Effects of Beer Administration in Mice on Acute Toxicities Induced by X Rays and Carbon Ions

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Beer/Radioprotection/LD$_{50/30}$/Crypt survival/Chromosome aberration.

We have investigated the tissue specificity of radioprotection by beer, which was previously found for human lymphocytes. C3H/He female mice, aged 14 weeks, received an oral administration of beer, ethanol or saline at a dose of 1 ml/mouse 30 min before whole-body irradiation with ^{137}Cs γ rays or 50 keV/μm carbon ions. The dicentrics of chromosome aberrations in spleen cells were significantly ($p < 0.05$) reduced by beer and ethanol-administration for γ-ray irradiation, but not for carbon-ion irradiation. The number of jejunal crypts plotted against the dose showed that both beer and ethanol significantly increased D$_0$ (slope of a dose-survival curve) for γ rays and carbon ions as well. Beer administration significantly ($p < 0.05$) increased LD$_{50/30}$ (radiation dose required to kill 50% of mice within 30 days) for γ rays and carbon ions. Ethanol-administration also significantly ($p < 0.05$) increased the LD$_{50/30}$ value for γ rays, but not for carbon ions. It is concluded that beer administration reduces the radiation injury caused by photons and carbon ions, depending on the tissue type. Radioprotection by beer administration is not solely due to OH radical-scavenging action by the ethanol contained in beer.

INTRODUCTION

A search for the chemical agents that are able to protect human beings from ionization radiation is a key issue in radiation biology[1]. Because radioprotective substances may produce severe side effects, radioprotective agents with low toxicities have been searched for many years[2,3]. Several studies concerning radioprotection have been conducted on foods, including vitamins, garlic extract, squalene, caffeine and miso (fermented soybean paste)[4–11]. We previously found that drinking beer reduces radiation-induced chromosome aberrations in human lymphocytes[12]. Beer increases the plasma antioxidant capacity in humans[13], and effectively inhibits salmonella mutation caused by heterocyclic amines (HAs), 2-chloro-4-methylthiobutanoic acid (CMBA) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)[14–16]. In the present study using mice, we investigated and reported on the tissue specificity of beer-drinking induced radioprotection.

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MATERIALS AND METHODS

Mice

Fourteen-week-old C3H/HeMsNrsfICR female mice were used. These mice were produced and maintained in the specific pathogen free (SPF) facilities at the National Institute of Radiological Sciences (NIRS). Mice irradiated with γ rays were kept in the SPF facilities, while mice receiving carbon ions were transported to an accelerator facility shortly before irradiation. They were housed four or five per cage. Food and water (pH 2.3) were supplied ad libitum, but only water was supplied for a period of 24 h before irradiation. After radiation, food and water were supplied until the termination of experiments.

Irradiation

Mice were placed in a Lucite box and given whole-body irradiation at 0.5 Gy/min with a ^{137}Cs γ ray unit under unanesthetized conditions. Carbon ions were accelerated up to 290 MeV/u with the HIMAC synchrotron at the National Institute of Radiological Science (NIRS, Chiba, Japan). Lucite absorbers with 81 mm thickness were used to select a LET of 50 keV/μm within a spread-out-bragg-peak (SOBP) of 6 cm wide. The mice were orally administered 1 ml of either beer (ethanol content, 40 mg/ml or 5.5% (v/v)), 5.5% (v/v) ethanol or saline via an orogastric tube 30 min before irradiation.

Plasma ethanol concentration

The plasma ethanol concentration was determined using a

Sigma ethanol kit (Sigma). To each vial, 3.0 ml of glycine buffer reagent was added, capped and gently inverted several times. Ten microliters of blood plasma or deionized water was then put into each vial. The vial solutions were incubated for 10 min at room temperature, and then transferred to cuvettes for measuring the absorbance at 340 nm.

Splenocyte cultures

Irradiated mice were killed by cervical dislocation. The spleen was dissected aseptically from each animal. Splenocytes were cultured in RPMI 1640 medium (Gibco-BRL, N.Y., USA) containing 20% (v/v) foetal calf serum, 100 U/ml Penicillin (Sigma Chemical Co., St. Louis, MO, USA), 3 µg/ml Con A (Sigma Chemical Co., St. Louis, MO, USA), 10 µg/ml LPS (Sigma Chemical Co., St. Louis, MO, USA) and 0.05 µg/ml colcemid (Gibco-BRL, N.Y., USA) under 5% CO_2 at 37°C for 48 h.

For chromosome preparation, cultured cells were treated with a hypotonic solution of 75 mM KCl for 20 min at 37°C under humid conditions, and then fixed with methanol-acetic acid (3 : 1). Chromosome slides were stained with 4',6-diamino-2-phenylindole (DAPI; 1 µg/ml) (Roche Diagnostics Corporation, Indianapolis, IN, USA). Mitotic metaphase figures were examined for 150 through 300 cells, and the unstable type of chromosome aberrations of dicentrics was counted under a fluorescence microscope.

The dose-response of the chromosomal aberration frequencies was established by fitting the experimental data to the following quadratic (γ rays) or linear-regression (carbon ions) curves: $Y = \beta D^2$ or $Y = \alpha D$, where Y is the yield of induced chromosome aberrations per cell, D is the dose in Gy, and α and β are fit coefficients. The background value for dicentrics was negligibly small, and set to zero for the practical convenience of calculating and figure drawing.

Intestine

Four mice were used for each irradiation-dose point. The microcolony technique[17] was used for the intestine. The mice were sacrificed at 3.5 days after exposure. The jejunum was removed, fixed in 10% neutral formalin and then stained with haematoxylin and eosin. The number of crypts per transverse circumference was counted microscopically for 10 through 13 histlogical sections of each mouse. The averaged number of crypts was plotted on a semilogarithmic scale against the dose. The crypt survivals were fitted to an exponential function. The D_0 and D_{10} values were calculated from the dose-survival curve; D_0 is the reciprocal slope of the survival curve, while D_{10} is the dose at which the curve meets 10 crypts. The dose reduction factor (DRF) was determined by the formula,

$$\text{DFR} = \frac{D_0(D_{10})\text{ with beer or ethanol}}{D_0(D_{10})\text{ with the salne}}, \quad (1)$$

$LD_{50/30}$

Irradiated mice were observed to determine the mortality for 30 days after exposure. Ten mice per dose point were used for the experiments. All of the data collected from repeated experiments were combined for an analysis.

RESULTS

Plasma ethanol concentrations after beer-administration

Figure 1 shows the time course of the plasma ethanol concentration at 0.5, 1, 2, 3, 4 and 5 h of beer administration. The ethanol concentration was highest at 0.5 h of beer administration, 146 ± 20 mg/dl. Ethanol administration showed a time course similar to that of beer-administration (data not shown). If radioprotection by beer administration was due to ethanol radical scavenging, the maximum protection would be achieved 30 min after beer administration. Therefore, all experiments using whole-body irradiation were conducted 30 min after any oral administration.

Chromosome aberrations in splenocytes

The dicentric frequency is plotted as a function of the radiation dose in Fig. 2. For γ rays, the β value for beer or ethanol-administrated mice was significantly smaller ($p < 0.05$) than that for saline-administrated mice (Table 1). For carbon ions, however, the α value for beer or ethanol-administrated mice was not different from that for saline-administered mice.

Crypt survivals

The crypt survivals, which were semi-logarithmically plotted against the dose, were well fitted to a linear-regression line (Fig. 3). The D_0 values of beer- and ethanol-administered mice for γ rays were 1.86 ± 0.13 (mean $\pm$ SD) and 1.87 ± 0.07 Gy, respectively, and significantly ($p < 0.05$) larger than the D_0 value of 1.47 ± 0.14 Gy of saline-administered mice. The D_0 values of beer- and ethanol-administered mice for carbon ions were 1.40 ± 0.08 and 1.28 ± 0.08 Gy, respectively, and significantly ($p < 0.05$) larger than the D_0 value of 1.09 ± 0.05 Gy for saline-
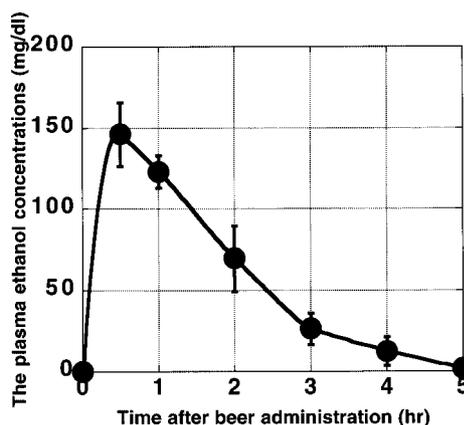
Fig. 1. Time course of the plasma ethanol concentrations after the oral administration of 1 ml of beer. The values represent mean $\pm$ SD ($n = 5$).
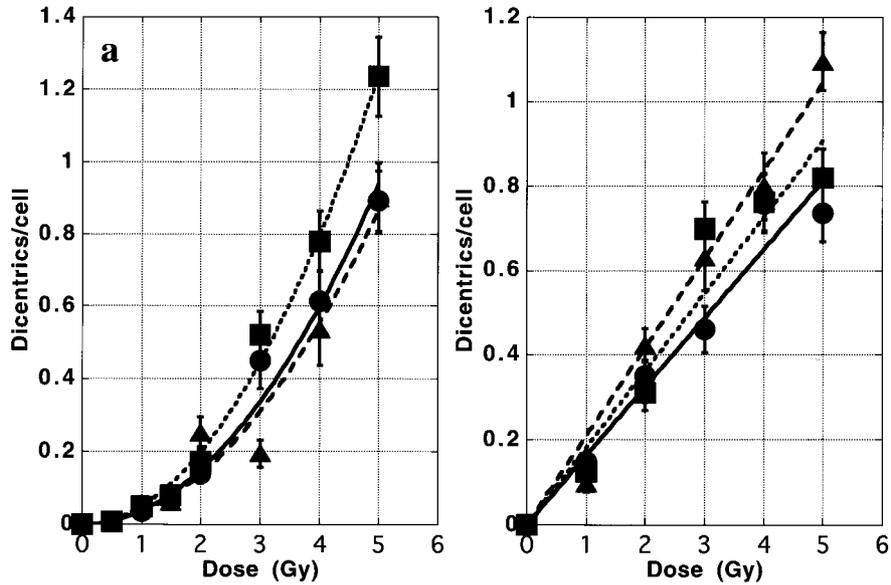
Fig. 2. Dose-response curve of the yield of dicentrics in splenocytes. (a) γ-ray irradiation and (b) carbon-ion. The error bars indicate standard error of the means. The data are fitted to a linear-quadratic dose response function. The symbols are: solid square, saline administration; solid circle, beer administration; solid triangle, ethanol administration.

Table 1. Parameters of dose-response for the induction of dicentrics.

	γ rays	Carbon ions
	$\beta \, (\times 10^2) \pm SD \, (Gy^{-2})$	$\alpha \, (\times 10^2) \pm SD \, (Gy^{-1})$
Saline administration	5.0 ± 0.1	18 ± 1.2
Beer administration	$3.7 \pm 0.1*$	16 ± 0.8
Ethanol Administration	$3.5 \pm 0.2*$	
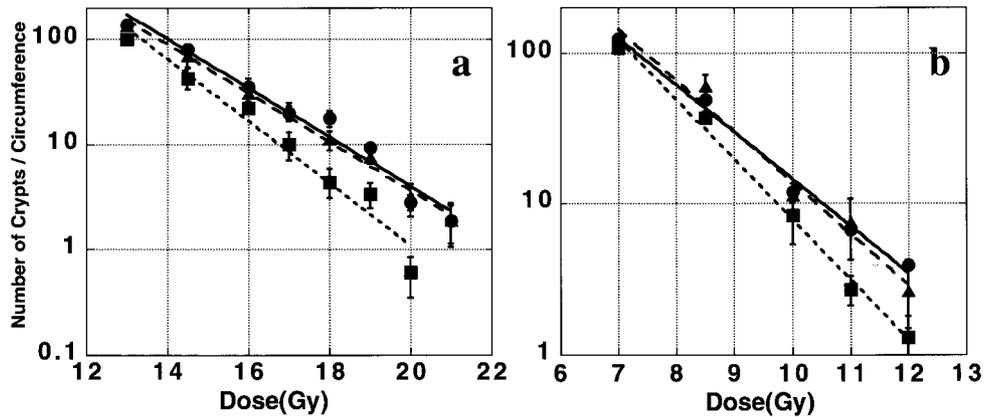
Fig. 3. Survival curves for crypt clonogenic cells in mice. (a) γ rays and (b) carbon ions. The symbols are: solid circle, 1 ml 5.5% beer/mouse; solid triangle, 1 ml 5.5% ethanol/mouse; solid square, 1 ml saline/mouse (mean ± SD). The data are fitted to the curves using a least-squares regression analysis.

administered mice. The D_{10} values of beer-, ethanol- and saline-administered mice for γ rays were 18.3, 18.1 and 16.8 Gy, respectively. The D_{10} values of beer-, ethanol- and saline-administered mice for carbon ions were 9.7, 10.5 and 10.4 Gy, respectively. The DRF (D_0) of beer- and ethanol-administered mice for γ rays was the same as each other, 1.27. The DRF (D_0) values values of beer- and ethanol-administered mice for carbon ions

were 1.28 and 1.17, respectively. The DRF (D_{10}) of beer- and ethanol-administered mice for γ rays were 1.09 and 1.08, respectively. The DRF (D_{10}) values of beer- and ethanol-administered mice for carbon ions were 1.08 and 1.07, respectively.

Thirty-day lethality

The most prominent radioprotection was found when mice
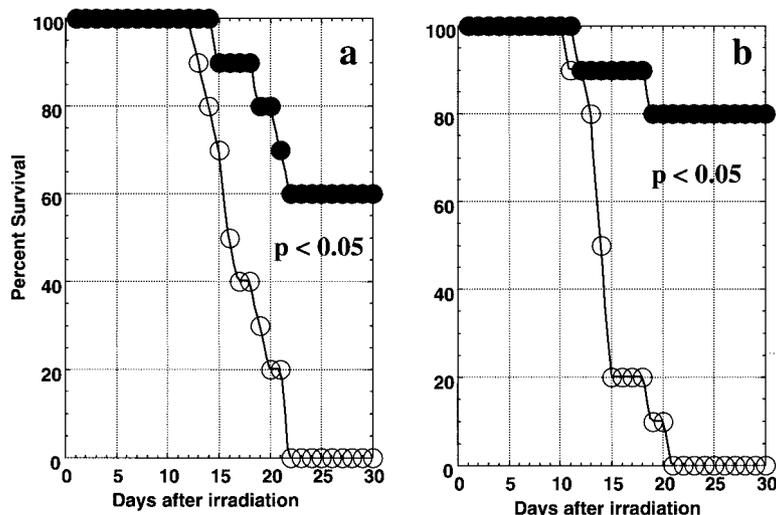
Fig. 4. Survival of beer- and saline-administered mice after whole-body irradiation. (a) γ rays and (b) carbon ions. The percent survival is shown each day following exposure to 7.8 Gy of γ rays and 6.4 Gy of carbon ions. The symbols are: solid circle, 1 ml 5.5% beer/mouse; open circle, 1 ml saline/mouse. Significant differences ($p < 0.05$) were determined by the Wilcoxon and Log-Rank test.
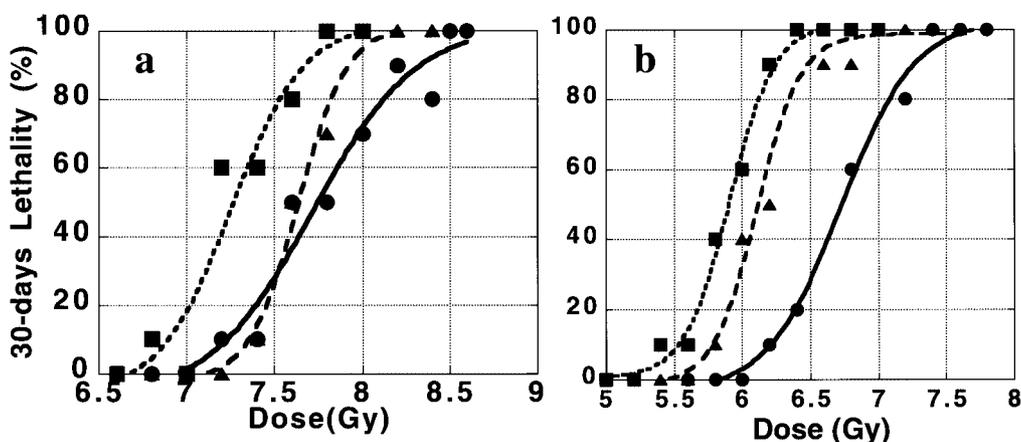
Fig. 5. Percent lethality versus radiation dose. (a) γ rays and (b) carbon ions. The data are fitted to a sigmoidal dose response function: $y = a + b/(1 + \exp(-(x - c)/d))$, where y is the percent lethality, x is the dose in Gy, respectively, and a, b, c and d are constants. The symbols are: solid circle, 1 ml 5.5% beer/mouse; solid triangle, 1 ml 5.5% ethanol/mouse; solid square, 1 ml saline/mouse. $LD_{50/30}$ was calculated by Plobit analysis.

were irradiated with 7.8 Gy of γ rays or 6.4 Gy of carbon ions (Fig. 4). After γ-ray irradiation, the 30-day survivals for beer-administered mice were 60%, whereas the 30-day survival for saline-administered mice was 0%. After carbon-ion irradiation, the 30-day survivals were increased from 0% to 80% by beer administration. These differences were statistically significant ($p < 0.05$ by an analysis of the wilcoxon and Log-Rank test).

The dose lethality after γ-ray or carbon-ion irradiation is presented in Fig. 5. The $LD_{50/30}$ for γ rays was 7.8 (95% confidence limit, 7.6–7.9) Gy, 7.6 (7.5–7.7) Gy and 7.3 (7.2–7.4) Gy for beer-, ethanol- and saline-administered groups, respectively. The $LD_{50/30}$ for the beer- and ethanol-administered mice was significantly larger ($p < 0.05$ by analysis of Probit test) than that for saline-administered mice. The $LD_{50/30}$ for carbon ions was 6.6 (6.5–6.7) Gy, 6.2 (6.0–6.3) Gy and 5.9 (5.8–6.0) Gy for the

beer-, ethanol- and saline-administered groups, respectively. The $LD_{50/30}$ for beer-administered mice was significantly larger ($p < 0.05$ by analysis of Probit test) than that for saline-administered mice. The $LD_{50/30}$ of ethanol-administered mice was slightly larger than that for saline-administered mice, even though a statistical significance was not obtained.

DISCUSSION

The chromosome aberrations by radiation have a close correlation to cell death[18]. Stem-cell death in critical organs is also closely related to somatic mortality. Provided that the radiation damage could be measured quantitatively by any end points, a difference in the magnitude of radioprotection between tissues would be a reflection of tissue-specific radioprotection.

Beer contains not only ethanol, but also other antioxidant substances, such as phenolic compounds[19], which may account for beer's activity to increase any antioxidant capacity of plasma in humans[11]. Alcohols reduce radiation-induced DNA scissions by OH radical-scavenging activity *in vitro*. Roots *et al.*[20–22] report that the contribution of OH radicals to the total radiation damage is ~70% for X rays, and ~40% for carbon ions of 52 keV/µm. The difference in the OH radical contribution between X rays and carbon ions well explains the fact that beer and ethanol administration protected splenocytes from γ rays, but not from carbon ions (Fig. 2).

The alpha component of the chromosome aberration in splenocytes was not changed by beer administration (Table 1). This contrasts with our previous study, which showed that beer drinking reduced the alpha term of the peripheral blood by lymphocytes in humans[12]. This may be a reflection of a difference in the tissue- or species- responsiveness to radioprotection[23–25]. The alpha component is the coefficient of a single track-2 hits event in DNA[26]. Slow electrons of 700 eV would produce densely ionizing events over a dimension of ~10 nm, which could be responsible for mammalian cell killing by the single-hit mechanisms of X rays and charged particles as well[27]. A high concentration (1 M) of DMSO protects mammalian cell kill by reducing the alpha component of not only X rays, but also of high-LET charged particles. The mechanism by which DMSO suppresses high-LET radiation-induced cell killing is not yet known.

The radioprotective activity of beer for 50 keV/µm carbon ions was either equal (gut; Fig. 3) or superior (bone marrow; Fig. 5) to γ rays. A general concept is that chemical radioprotectors more effectively modify tissue responses against low-LET radiation than high-LET radiation. However, there are several reports that contradict this general concept. For example, the dose modification factor (DMF) of WR151327, an aminothiol compound, is larger for fission neutrons than X rays for $LD_{50/6}$[28]. The DMF of WR2721, amifostine, for a marrow colony-forming assay was found to be 2.3 for γ rays and 2.4 for 51 keV/µm silicon ions[29]. WR1065 protects radiation damage not only by free-radical scavenging, but also by enhancing the expression of MnSOD in glioma cells[30]. MnSOD plays a role in preventing DNA damage caused by reactive free radicals. This type of intracellar change could also be involved in the gut and bone marrow in the present study. These radical scavenging mechanisms cannot, however, explain the protection by beer against carbon ions with direct actions.

As conclusions, beer administration reduces the radiation injury caused by photons and carbon ions, depending on the tissue type. The radioprotection provided by beer administration is not solely due to the OH radical-scavenging action by the ethanol contained in beer.

ACKNOWLEDGEMENTS

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