Change in Ascorbate Radical Production in an Irradiated Experimental Tumor with Increased Tumor Size

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ABSTRACT

We have reported that ascorbate radical (Asc •• ) could serve as an indicator of the amount of hydroxyl radical and superoxide produced by irradiation in vivo. Using this method, we investigated the relationship between tumor size and Asc •• production after irradiation (10 Gy) and between tumor size and the radical-scavenging ability of WR-2721 (300 mg/kg). Asc •• was measured in normal muscle and SCC-VII tumors transplanted into mice (n = 6). In tumors, the increase in Asc •• significantly decreased with increasing tumor size (r = —0.483; P < 0.05). The increase in Asc •• production after irradiation was more inhibited by WR-2721 in normal muscle tissue than in tumor tissue at various sizes. In tumors, the increase in Asc •• was less inhibited by WR-2721 with increasing tumor size. These results demonstrate that the increase in radical production after irradiation and drug distribution decreased with increasing tumor size and that WR-2721 has excellent differential protection. This method is expected to measure changes in the amounts of local hydroxyl radical and superoxide modified by a change of tumor environment or drug administration.

INTRODUCTION

In radiotherapy, the efficacy of treatment depends upon P02 distribution (1), malignancy grading (2), intrinsic cellular radiosensitivity (3), and tumor regression (4). Numerous previous studies have demonstrated that hypoxic cells are radioresistant (1, 5). The antitumor effect of irradiation decreases in larger tumors because the hypoxic fraction increases with growth in tumors (6, 7).

It is widely known that ionizing radiation, such as X-rays and γ-rays, induces irreversible damage to DNA, mainly by indirect mechanisms, reactions of free radicals that are produced by the cell by irradiation. This can lead to cell death (8, 9). Thus, the radiation damage increases with the increase in free radicals formed by ionizing radiation. The production of free radicals by irradiation may decrease with increased tumor size, but there are no data on the production of free radicals in various tumor sizes.

We have shown that Asc •• produced by the reaction of ascorbic acid with HO•• or O2•• can serve as an indicator of the amount of HO•• and O2•• produced by irradiation in vivo (10). In this study, we initially investigated increases in Asc •• production after irradiation of tumors of various sizes to clarify the mechanism of radioresistance with increased tumor size. Then we investigated the change in Asc •• production after irradiation and administration of WR-2721 as a radical scavenger to clarify the mechanism of differential protection of WR-2721 and compare the radical-scavenging ability in tumors of various sizes with that in past reports.

MATERIALS AND METHODS

Experimental Animals and Tumors. The animals used were C3H/He mice (male, 6 weeks old; obtained from Shizuoka Experimental Animals). They were kept in metabolic cages, and food and water were freely available during the course of the study. For the experimental tumor, we used SCC-VII tumor cells, which were originally derived from a SCC and maintained by s.c. transplantation into mice. The tumor was used when it reached a mean diameter of 8 ± 1, 15 ± 1, and 22 ± 1 mm. All experimental procedures were performed under anesthesia by injecting pentobarbital sodium (50 mg/kg) i.p.

Irradiation Technique. Normal muscle and tumors were locally irradiated with 60Co γ-rays (40 mm in diameter; dose, 10 Gy; dose rate, 1.6 Gy/min; SSD, 55 cm).

Method for Collection of Asc ••. Fig. 1 depicts the method used to collect radicals. A dialysis membrane (length, 6 mm; diameter, 0.2 mm) was connected between the glass tubes (outer diameter, 0.15 mm; inner diameter, 0.075 mm). The tube for collecting Asc •• was inserted so that the dialysis membrane was in contact with the normal thigh or the center of the tumor. The concentration of the Ringer’s solution (4 mM K+, 147 mM Na+, 3.1 mM Ca2+, and 157.2 mM Cl−), obtained by bubbling with nitrogen gas, was perfused in the tube at a flow rate of 1 μl/min. Asc •• was collected from interstitial fluid around the membrane through the dialysis membrane. The perfusion fluid was collected in a capillary tube at 15-mm intervals at room temperature.

Electron Spin Resonance Equipment and the Method for Measuring Asc ••. An X-band electron spin resonance spectrometer (JES-RE1X; Japan Electron Co., Ltd.) was used to measure Asc ••. The sample was measured at room temperature at 9.5 GHz, microwave power 20 mW, and modulation amplitude 1.0 gauss. The g-factor of Asc •• was 2.0054, and the hyperfine coupling constant was 1.70 gauss. The signal intensity of Asc •• was compared using a manganese marker as the standard.

Measurement of Asc ••. The collection of Asc •• was started 1 h after placement of the tube. We investigated the following points using this method.

Change in Asc •• Production after Irradiation in Normal Muscle and Tumor at Various Sizes. For normal tissue, tissue of the contralateral thigh was used. Asc •• was measured at the center of the tumor. Starting 45 min before irradiation, the perfusion fluid in the capillary tube was collected three times at 15-min intervals as nonirradiated control samples. The irradiation consisted of 10 Gy. Asc •• was collected once during irradiation and six times at 15-min intervals for 90 min after irradiation. Asc •• is presented as a percentage relative to the mean of three preirradiation measurements, taken as 100%.

Change in Asc •• Production after Irradiation when a Radical Scavenger Is Injected. WR-2721 (obtained from Yamanouchi Co., Ltd.) was dissolved in distilled water to the required concentrations and injected i.p. For control samples, Asc •• was collected three times at 15-min intervals for 45 min before injection of the drug. WR-2721 (300 mg/kg) was injected i.p. Irradiation (10 Gy) was performed 30 min after injection of WR-2721. Asc •• was measured once during irradiation and six times at 15-min intervals for 90 min after irradiation. Asc •• is represented as the percentage relative to the mean of three values obtained before injection of WR-2721. To contrast the ability of WR-2721 to scavenge OH and O2••, the algorithm calculates the protection ratio as follows:

Protection ratio = maximum increases in Asc •• after irradiation with WR-2721 / maximum increases in Asc •• after irradiation only

A smaller protection ratio indicates more effective scavenging of the...
Change in Asc⁻ Production after Irradiation with Radical Scavenger. Table 1 compares the maximum Asc⁻ production after irradiation with 10 Gy with and without WR-2721 (300 mg/kg) in normal muscle and tumors at various volumes. When radical scavenger was supplied before irradiation, the maximum increases in Asc⁻ were significantly less than when the radical scavenger was not supplied in normal muscle and smaller tumors (8 ± 1 mm), but not significantly in larger tumors (15 ± 1 mm and 22 ± 1 mm). The increase in Asc⁻ production after irradiation was more inhibited by WR-2721 in normal muscle tissue than in tumor tissue; the protection ratio was 0.681 for normal muscle and 0.893 for the 8-mm tumor. In tumors, the increase in Asc⁻ was less inhibited by WR-2721 with increasing tumor size. The increase went from 33.8% without the supply of radical scavenger to 19.5% with the scavenger for the smaller tumor (8 ± 1 mm) and 19.0–15.6% for the larger tumor (22 ± 1 mm). The protection ratio was 0.893 for the 8-mm tumor, 0.933 for the 15-mm tumor, and 0.971 for the 22-mm tumor.

DISCUSSION

Tumor oxygen content is a significant independent factor in determining radiosensitivity (1, 5). It is known that the oxygen effect is large and important in the case of ionizing radiation (11). The damage produced by free radicals in DNA may be fixed (made permanent and irreparable) if molecular oxygen is available. Thus, the damaging effects are aggravated by the high oxygen tension.

In tumor tissue, necrotic and hypoxic cells are present due to...
vascular insufficiency with increased cell proliferation. Several investigators have shown that IFP increases with growth in tumors (12, 13). Interstitial hypertension may reduce blood supply. It is known that growth in tumors induces an increase of necrotic and hypoxic cells and a reduction of blood supply and pO2 (5). Radioresistance with growth in tumors can be attributed to these histological changes. Gatenby et al. (1) used CT-guided needle electrodes to obtain pO2 measurements in 31 lymph node metastases and found that the mean pO2 was higher (20.6 ± 4.4 mm Hg) in the complete responders than in the noncomplete responders (4.6 ± 3.0 mm Hg). They suggested that human tumor oxygen content was a significant independent factor in determining response to radiation therapy. Roh et al. (14) measured IFP in human patients with SCC of the uterine cervix. They reported that mean IFP ranged from 10–26 mm Hg in tumors and from 0–3 mm Hg in normal cervix, and oxygen tension inversely correlated with IFP. When IFP decreased during radiation therapy, a positive clinical responder was observed. When IFP increased or remained unchanged, clinical response was poor. They suggested that IFP values provided an indication of tumor oxygenation and that IFP modifications could be prognostic indicators of radiation response. Shibamoto et al. (15) reported that the hypoxic fraction in SCC-VII tumors increased markedly as the tumor grew from 5 to 10 mm (0.86% for the 5-mm tumor and 8.5% for the 10-mm tumor). We reported that the antitumor effect of irradiation decreased with SCC-VII tumor size (7).

In this study, the increase in Asc− after irradiation with 10 Gy was greater in normal muscle tissue than in tumor tissue. In tumors, the increase in Asc− decreased with increasing tumor size. There are reports that high pO2 accelerates the production of O2•− and H2O2 (16, 17). And, in the presence of high oxygen, the hydrated electrons formed by ionizing radiation can produce O2•−, which can give more HO•, and increase the damage (18). The higher radical production in smaller tumors as compared to larger tumors can thus be attributed to the higher pO2 in the smaller tumors. Our findings demonstrate that the decrease in Asc− production with increasing tumor size leads to radioresistance in larger tumors.

It is known that WR-2721 has a SH group and scavenges HO• and O2•− formed by irradiation before they cause any DNA damage (6). This compound provided excellent protection for normal tissues but little protection for the transplanted tumor (19, 20). It was reported that injection of WR-2721 increased the resistance of the hematopoietic tissues and skin by a dose-modifying factor of 2.7 and 2.4, respectively, but yielded little protection (dose-modifying factor, 1.15) in cases of transplanted mammary carcinoma in mice (19). The following mechanisms were suggested for the differential protection afforded by WR-2721 (20): (a) the tumor lacks vascularity, particularly in the central portion; hence, the transfer of drug into the tumor is often poor; (b) the protective effects of WR-2721 may be insufficient for hypoxic cells compared with well-oxygenated cells; and (c) cell membrane permeability of WR-2721 differs between normal and hypoxic cells. Our previous study has shown that the increase in Asc− production was more inhibited when WR-2721 was administered before irradiation than when irradiation only was performed. Our findings also indicated that the radical-scavenging ability of WR-2721 was increased as its administered dose was increased. Because our results in this study revealed that the increase in Asc− production after irradiation was more inhibited by WR-2721 in normal muscle tissue than in tumor tissue at various sizes, it seems likely that the ability of WR-2721 to scavenge HO• and O2•− is greater in normal muscle tissue than in tumor tissue. Our results support the idea that WR-2721 has differential protection.

In tumors, the increase in Asc− was less inhibited by WR-2721 with increasing tumor size. Braunschweiger (21) measured blood flow in RIF-1 solid tumor by 86RbCl distribution. They indicated that a strong negative correlation existed between tumor mass and blood flow. Utey et al. (22) measured drug distribution using 35S-labeled WR-2721. They reported that accumulation of 35S-labeled WR-2721 was seen only in the peripheral highly vascularized area of the tumor. Thus, with increasing tumor size, the tumor lacks further vascularity, and a lesser amount of WR-2721 is incorporated into a tumor cell. Therefore, the ability of WR-2721 to scavenge HO• and O2•− decreased with increasing tumor size.

We reported that the method in the present experiment was useful for the following reasons (10): (a) no special treatments, such as freezing of the samples, are necessary because Asc− is relatively stable at room temperature; (b) because Asc− is produced by the reaction between HO•, O2•−, and ascorbic acid, which is intrinsic to living tissues, administration of noxious agents is not necessary; (c) after irradiation of only several grays, Asc− increased in proportion to the radiation dose. This means that Asc− can be quantified even when using a clinical radiation dose; and (d) this method is less invasive because only the dialysis membrane needs to be inserted s.c. This method is expected to measure changes in the amounts of local HO• and O2•− modified by change of tumor environment or drug administration.

In summary, this study has confirmed that the increase in radical production after irradiation decreased with increasing tumor size. We have also demonstrated in terms of radical production that growth in tumors has induced a reduction of drug distribution and that WR-2721 has excellent differential protection.

REFERENCES


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