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COMBINED EFFECT OF RADIATION AND YM-881 (SMANCS) ON MURINE TUMORS AND BONE MARROW

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Abstract

The combined effect of radiation and YM-881 (SMANCS) was studied in vitro and in vivo. When 0.25 μg/ml of YM-881 was simultaneously combined with radiation, during and after irradiation for 30 min in total, D_q decreased from 3.3 Gy to 1.4 Gy without changing D_0 in the dose-survival curve of exponentially growing SCC VII tumor cells. Five or ten times administrations of 0.1 mg/kg YM-881 at an interval of 24 h did not inhibit tumor growth. However, administration of 0.1 mg/kg YM-881 just before every irradiation which was repeated five times at an interval of 24 h yielded dose modifying factors (DMFs) of 1.8–1.2 when the tumor response to treatment was evaluated by the time for the tumors to regrow to three times the original volume. Administration of YM-881 ten times just before every irradiation yielded DMFs of 1.3–1.2. Adverse effects of the combination on bone marrow were examined by spleen colony assay. After five injections of 0.1 mg/kg YM-881, the mean number of CFU-S per femur decreased to 77% of the pretreatment level, but this was not significant statistically ($0.1 > p > 0.05$). The slope of radiation response curve for CFU-S per femur was not affected by the combination.

Key words: Radiation effects, YM-881, murine tumors, bone marrow.

Clinical radiotherapy data show that an increase of local control rate usually improves the survival rate of cancer patients (1). Radiotherapy and chemotherapy are frequently combined in order to improve the local control rate and to eradicate distant micrometastases (2). Two conditions are necessary to the success of this combination; The first is that the increase of the tumor response to radiation has to be larger than that of surrounding normal tissues. The second condition is related to fractionation of clinical radiotherapy: the increase of tumor response to radiation must also occur when the dose per fraction is low.

YM-881 (SMANCS) is a conjugate of neocarzinostatin (NCS) and a copolymer of stylene maleic acid (SMA) (3, 4). Both YM-881 and NCS cause cell killing mainly by induction of single and double strand breaks in DNA (5–8), similar to ionizing radiation (9). Therefore, an additive response of this damage may occur when radiation and YM-881 are combined. Furthermore, selective phenomena may occur as YM-881 seems to accumulate more effectively in tumors than in normal tissues (10). To examine the clinical applicability of combined YM-881 and radiation therapy, we have investigated the tumor and bone marrow response to this combination.

Material and Methods

In vitro studies. Before the in vivo studies, we investigated the combined effect of radiation and YM-881 on cell cultures. SCC VII tumor cells, i.e. squamous cell carcinoma cells syngenic in C3H/He mice, were used. This tumor line was kindly supplied by Dr R.F. Kallman, Stanford University. In order to secure good tumor growth in mice, the tumor was maintained by alternating passages in C3H/He mice and in Eagle's minimum essential medium (MEM) supplemented with 12.5% fetal calf serum (FCS), 292 mg/l L-glutamine, and antibiotics. Twenty-four h prior to the experiments, $2–3 \times 10^5$ exponentially growing tumor cells were seeded into 20-cm^2 plastic Petri dishes containing 4.5 ml of the complete medium.

YM-881 was dissolved in the complete medium at a concentration of 2.5 μg/ml just before use. YM-881 solution (0.5 ml) was added to the dishes with 4.5 ml medium

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to obtain a final concentration of 0.25 μg/ml. The cell killing effect of YM-881 reached a plateau level after 30 min in preliminary experiments. In the combination experiments with YM-881 and radiation, we therefore started treatment with the drug just before irradiation and continued during and after irradiation for 30 min in total. After the treatment with YM-881, the cells were rinsed five times with 5 ml phosphate buffered saline to completely remove the drug. Tumor cells were irradiated using an x-ray source (50 KVp, 4 mA) with a 0.25-mm Al filter at a dose rate of 1.6 Gy/min. After treatment, cells were trypsinized (0.05% trypsin, 0.02% EDTA, 37°C, 3–5 min), counted, diluted, and replated in appropriate numbers for colony formation. Eight to nine days later, colonies were fixed, stained, and counted to determine the surviving fraction.

Tumors and mice. Eight-week-old C3H/He male mice were obtained from the Animal Center of Kyoto University. They were caged in groups of 10 or less at a constant temperature with food and drinking water available ad libitum. Exponentially growing tumor cells (5×10^4) in culture were inoculated subcutaneously into the right thigh of each mouse. The volume of tumors was estimated by daily caliper measurements of 3 perpendicular diameters, assuming an ellipsoid shape. The tumors were irradiated when they reached a size of 9–10 mm in diameter 12 days after inoculation. Some tumors which were smaller or larger than the size desired were not used for study.

Irradiation and drug. Irradiation was performed with 10 MV x-rays from a linear accelerator. The beam was collimated to the tumor-bearing thigh of the mouse and the dose rate 5 Gy/min. During irradiation, the tumor-bearing limb of the unanesthetized mouse was fixed in the field with adhesive tape. The rest of the animal's body received a dose below 1% of the tumor dose. For studies on the combined effect of radiation and YM-881 on bone marrow, the entire body of the mouse was irradiated.

YM-881 was dissolved in sterile physiological saline at a concentration of 5 μg/ml, and 0.1 mg/kg of the drug was administered. LD50 value of the drug in male mice was 1.5 to 2.0 mg/kg (Yamanouchi Pharmaceutical Co., Ltd. personal communication) and five or ten times administration of the drug did not cause any apparent systemic toxicity in our preliminary experiments. The drug solution was injected via the tail veins (11).

Assay for tumor response and bone marrow toxicity. As a measure of the response of tumors to treatement we used the time it took for the tumor to regrow to three times its original volume after treatment. The tumor diameters were measured by a caliper three times per week. The dose modifying factor (DMF) was calculated from the radiation doses necessary to obtain the same biological effect.

The bone marrow toxicity of the treatment was evaluated by spleen colony assay (13). Just before total body irradiation of the mice 0.1 mg/kg of YM-881 was administered. This combined treatment was repeated five times at an interval of 24 h. Twenty-four h after the final treatment, the bone marrow of the femur was washed out with 1 ml physiological saline. Bone marrow suspensions from 3 mice were pooled. After counting of nucleated cells using a hemocytometer, an adequate number of cells were injected via the tail vein into 6–9 new recipient mice, whole body irradiated (9 Gy) 24 h previously. Nine days later, the spleens were removed, fixed with Bouin's solution and the colonies counted. The number of CFU-S (colony forming unit spleen) per femur was calculated from the actual numbers of colonies observed, nucleated cells injected and nucleated cells obtained per femur.

Student's t-test was used for statistical evaluation of the difference in response to the treatment.

Results

In vitro studies. Incubation of SCC VII tumor cells with 0.25 μg/ml YM-881 for 30 min reduced the surviving fraction to 0.47 (Fig. 1). The dose-survival curve of the tumor cells following irradiation was exponential with a shoulder. D_0 and D_q were 1.27 Gy and 3.3 Gy respectively. When

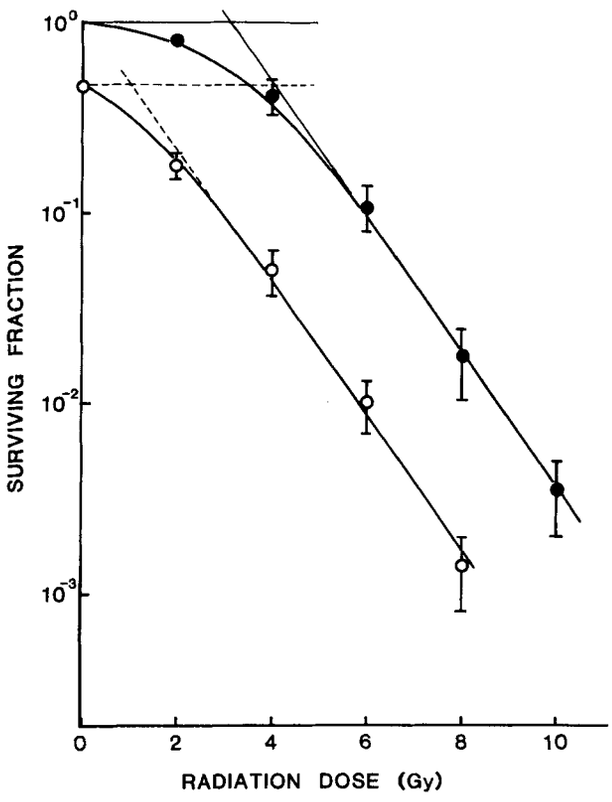

Fig. 1. Dose survival curves of exponentially growing SCC VII tumor cells treated by radiation with (○) and without (●) 0.25 μg/ml YM-881. The treatment of tumor cells with YM-881 started just before irradiation and continued during and after irradiation for 30 min in total. Error bars represent standard deviations.

YM-881 was given during and after irradiation for 30 min in total, D_q decreased to 1.4 Gy with no change in D_0.

In vivo studies. Tumors of untreated mice grew to three times the original volume in 5.0 days (Fig. 2). Large single doses of YM-881 inhibited the tumor growth in a dose dependent fashion (data not shown). Five daily injections of 0.1 mg/kg of the drug had no significant effect on tumor growth (Fig. 2). However, when 0.1 mg/kg of YM-881 was injected just before each irradiation and repeated 5 times at an interval of 24 h, the time necessary for tumor regrowth to three times the original volume was significantly larger than when the tumors were exposed to radiation alone (Fig. 2). DMFs of 1.8–1.2 were obtained in the 2–4 Gy fraction region.

Following 10 daily administrations of 0.1 mg/kg YM-881, no significant delay of tumor growth was observed (Fig. 3). However, 10 daily simultaneous combinations with 0.1 mg/kg YM-881 significantly prolonged the time necessary for the tumors to regrow compared with radiation alone (Fig. 3). DMFs of 1.3–1.2 were obtained in the 2–3 Gy fraction region.

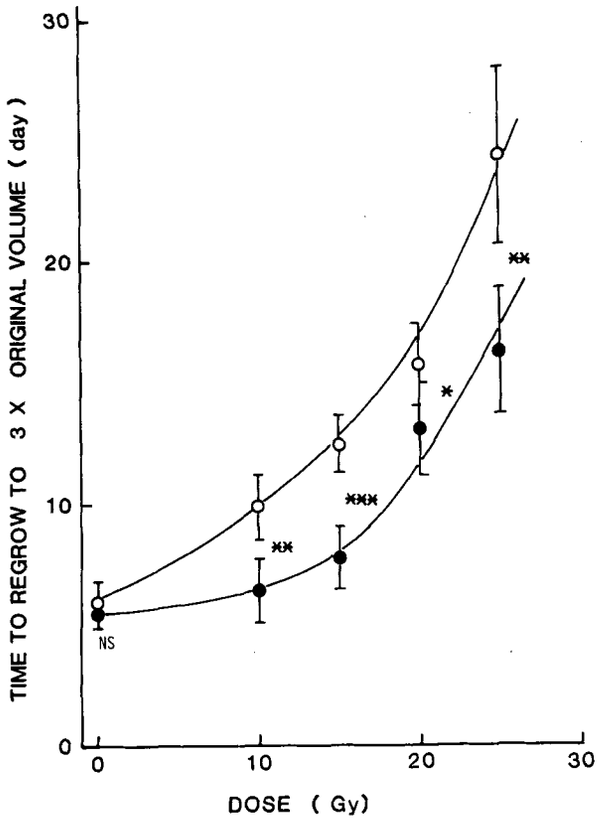

Fig. 2. The time necessary for the tumor to regrow to 3 times its original volume as a function of accumulated radiation doses in 5 fractionated irradiations. (○): the combination of radiation and 0.1 mg/kg YM-881. The drug was administered just before irradiation. (●): radiation alone. NS: $p > 0.5$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Error bars represent standard deviations of 12 tumors.

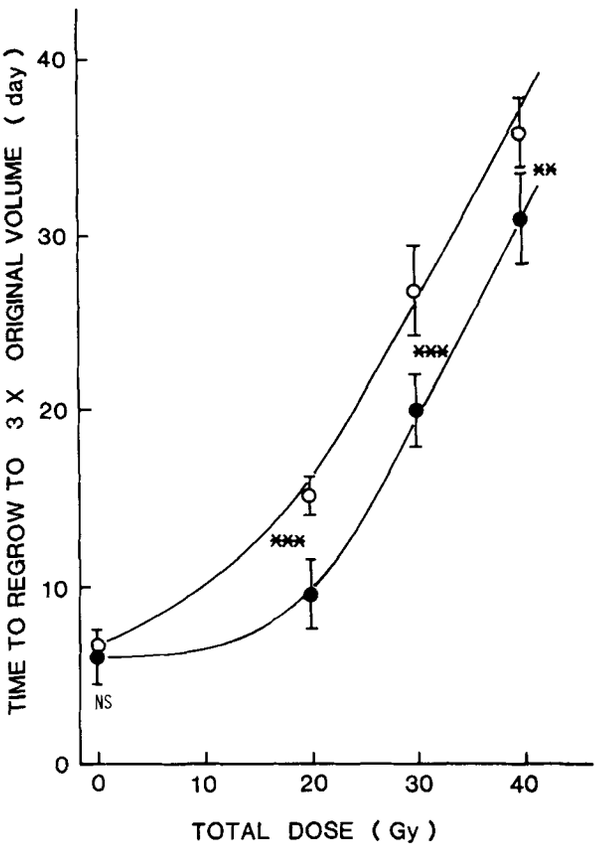

Fig. 3. The time necessary for the tumor to regrow to 3 times the original volume as a function of accumulated radiation doses in 10 fractionated irradiations. (○): the combination of radiation and 0.1 mg/kg YM-881. The drug was administered just before irradiation. (●): radiation alone. NS: $p > 0.5$, **: $p < 0.01$, ***: $p < 0.001$. Error bars represent standard deviations of 13 tumors.

Bone marrow toxicity. The effects of 5 treatments with YM-881, radiation, or simultaneous combination of both on bone marrow are shown in Fig. 4. Five repeated administrations of 0.1 mg/kg YM-881 decreased the mean number of CFU-S per femur to 77% of untreated controls but this was not statistically significant ($0.05 < p < 0.1$). For other radiation doses, except 1.25 Gy, the differences in numbers of CFU-S per femur between the two groups were not statistically significant ($p > 0.5$). The slope of the dose response curve of CFU-S number per femur was also unaffected by the combination of YM-881.

Discussion

The combination of radiation and chemotherapy agents, influences the dose-response curve according to the mechanism of cell killing by the drug, and the sequence and intervals of the treatments (12). When radiation and the cytotoxic agent do not interact in the cell damage, the cell survival curve after combined treatment is parallel to the

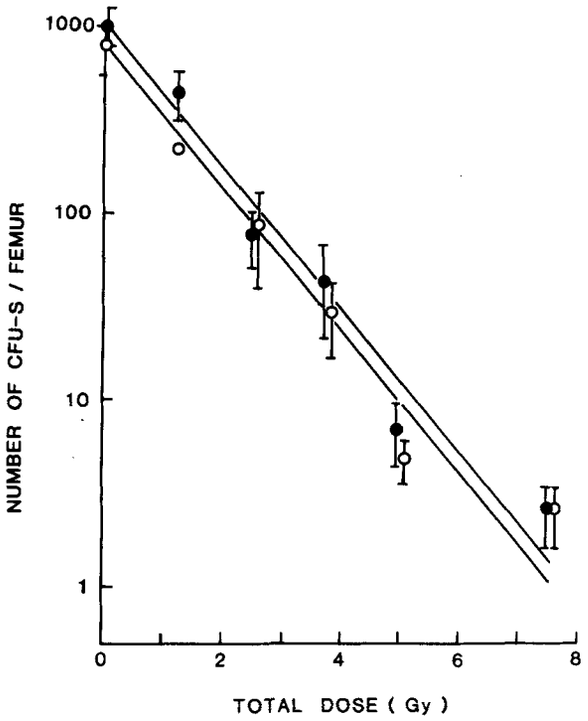

Fig. 4. The dose response curve of CFU-S per femur to 5 fractionated irradiations at an interval of 24 h with (○) or without (●) administration of 0.1 mg/kg YM-881 just before every irradiation. Error bars represent standard deviations.

curve after irradiation alone (13). No changes in either D_0 or D_q are observed in this case. When positive interaction between damages induced by radiation and drug occurs, either D_0 or D_q or both, will change (15). If the damage by the drug is similar to that by the radiation, D_q will be reduced by the simultaneous combination of drug and radiation. NCS, the cytotoxic component of YM-881, generates activated oxygen, and these free radicals break the strands of DNA (14). This reaction is generally accepted as the major mechanism of cell killing by NCS (7) and is similar to that of ionizing radiation (9). Therefore, a decrease of the initial shoulder of the cell survival curve can be expected when YM-881 is simultaneously combined with radiation. Our in vitro results presented in Fig. 1 are consistent with this expectation.

Since D_q represents the extent of recovery from radiation damage between each irradiation (15), our in vitro study showed the possibility that a considerable increase of tumor response to fractionated irradiation could be obtained by combination with YM-881. An extra delay of tumor growth, compared with radiation alone, was observed when we simultaneously combined radiation and YM-881 as could be expected from the in vitro results (Fig. 2). An extra delay of tumor growth was also observed when this combination was repeated at 10 fractions (Fig. 3). In clinical practice the radiation doses are usually fractionated and it seems possible that the simultaneous combination of YM-881 and radiation may offer enhanced response of the tumors.

Since the major adverse effect of NCS is bone marrow suppression (16), we also investigated the combined effect of YM-881 and radiation on bone marrow nucleated cells. Although 5 injections of 0.1 mg/kg of YM-881 decreased mean number of CFU-S per femur, no significant increase in the toxicity of radiation on bone marrow was observed in the combination treatment (Fig. 4). Two explanations are possible for the difference between tumor and bone marrow response. First, YM-881 accumulated in tumors more effectively than in bone marrow, and an increase of the response to radiation took place in tumors fairly selectively. Second, since D_q of the survival curve of bone marrow cells following irradiation is originally very small compared with that of tumors (17), the combined effect of YM-881 to decrease D_q of the cell survival curve might also be small. Therefore, repeated simultaneous combination with YM-881 might not change the response curve of CFU-S per femur obtained after radiation alone.

In conclusion, although YM-881 increased the tumor response to radiation without significantly increasing bone marrow toxicity, more studies on normal tissues especially with a large capacity for recovery are needed before considering the clinical applicability of the simultaneous combination of radiation and YM-881.

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