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Repair of Skin Damage During Fractionated Irradiation with Gamma Rays and Low-LET Carbon Ions

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Isoeffect/Repair/Dose/Top-up/Therapy.

In clinical use of carbon-ion beams, a deep-seated tumor is irradiated with a Spread-Out Bragg peak (SOBP) with a high-LET feature, whereas surface skin is irradiated with an entrance plateau, the LET of which is lower than that of the peak. The repair kinetics of murine skin damage caused by an entrance plateau of carbon ions was compared with that caused by photons using a scheme of daily fractionated doses followed by a top-up dose. Right hind legs received local irradiations with either 20 keV/μm carbon ions or γ rays. The skin reaction of the irradiated legs was scored every other day up to Day 35 using a scoring scale that consisted of 10 steps, ranging from 0.5 to 5.0. An isoeffect dose to produce a skin reaction score of 3.0 was used to obtain a total dose and a top-up dose for each fractionation. Dependence on a preceding dose and on the time interval of a top-up dose was examined using γ rays. For fractionated γ rays, the total dose linearly increased while the top-up dose linearly decreased with an increase in the number of fractions. The magnitude of damage repair depended on the size of dose per fraction, and was larger for 5.2 Gy than 12.5 Gy. The total dose of carbon ions with 5.2 Gy per fraction did not change till 2 fractions, but abruptly increased at the 3rd fraction. Factors such as rapid repopulation, induced repair and cell cycle synchronization are possible explanations for the abrupt increase. As an abrupt increase/decrease of normal tissue damage could be caused by changing the number of fractions in carbon-ion radiotherapy, we conclude that, unlike photon therapy, skin damage should be carefully studied when the number of fractions is changed in new clinical trials.

INTRODUCTION

Carbon-ion radiotherapy using HIMAC synchrotron employs smaller number of fractionation than conventional photon therapy. Dose escalation studies including 18 fractions over 6 weeks and 9 fractions over 3 weeks are applied to patients with lung cancer, and have obtained promising therapeutic outcomes.[1] We have previously reported that high LET carbon ions produced stronger biolological effects against tumors than skin with small numbers of fractionated irradiation.[2] Low LET carbon-ions share a character similar to photons so that both of them produce less damage than high LET carbon ions by a given dose. However, low LET carbon-ions are different from photons in such that an increase of total isoeffect doses along with number of fractions saturates earlier than photons. As patients with deep-seated tumors receive low LET carbon-ions to surface skin, further understandings of skin reaction after fractionated irradiation is required to propose an optimum use of carbon-ion radiotherapy. We have investigated and here report how differs the repair kinetics of skin cells after irradiation with low LET carbon ions from that with photons.

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MATERIALS AND METHODS

Mice

C3H/HeMsNrsf female mice aged 12–18 weeks were used for the skin study. The animals were produced and maintained in the specific pathogen-free (SPF) facilities. Hairs on the right hind leg of female mice were removed by applying a depilatory agent (Shiseido, Tokyo) 7 to 8 days before the first irradiation.

A total of 588 mice for the gamma-ray experiments and of 547 mice for the carbon-ion experiments were used with 5 mice for each irradiation dose point. All of the data collect-

ed from repeated experiments were combined.

Irradiation

Carbon-12 ions were accelerated by the HIMAC synchrotron up to 290 MeV/u.[3] The desired LET was obtained by inserting a given thickness of polymethyl methacrylate (PMMA) upstream of the mice. Carbon beams with 20 keV/μm LET was obtained at the entrance of the plateau of SOBP. A desired irradiation field was obtained by the simultaneous use of an iron collimator and a brass collimator. With pentobarbital anesthesia (50 mg per kg) and taping, five mice were immobilized on a Lucite plate to place their right hind legs in a rectangular field of 28 × 100 mm, and received either a single dose or daily-fractionated doses. The foot was excluded from the irradiation field. Cs-137 γ-rays with a dose rate of 1.6 Gy/min at an FSD (Focus Surface Distance) of 21 cm were used as a reference beam. A doughnut-shaped radiation field with 30 mm-rim was used to collimate the vertical beam. Daily fractionation was given with either the equal daily doses or the fixed daily doses followed by top-up doses, using an interfractional interval of 24 ± 1 hours. For the equal daily doses, several graded doses were

Table 1. Skin Reaction Score

score	Developing stage	Decaying stage
0.5	doubtful difference from normal appearance	hair graying (A ≧ 1/2)
1.0	slight reddening	hair graying (A < 1/2)
1.5	definite reddening	no hair and thick skin
2.0	severe reddening or definite dry desquamation	no scab and thin skin
2.5	severe dry desquamation	scab (A ≦ 1/3)
3.0	slight moist desquamation (A ≦ 1/3)	scab (1/3 < A < 2/3)
3.5	definite moist desquamation (A ≦ 1/3) or slight moist desquamation (1/3 < A < 2/3)	scab (A ≧ 2/3)
4.0	severe moist desquamation (A ≦ 1/3) or definite moist desquamation (1/3 < A < 2/3) or slight moist desquamation(A ≧ 2/3)	
4.5	severe moist desquamation (1/3 < A < 2/3) or definite moist desquamation (A ≧ 2/3)	
5.0	severe moist desquamation (A ≧ 2/3)	

A: area of interest

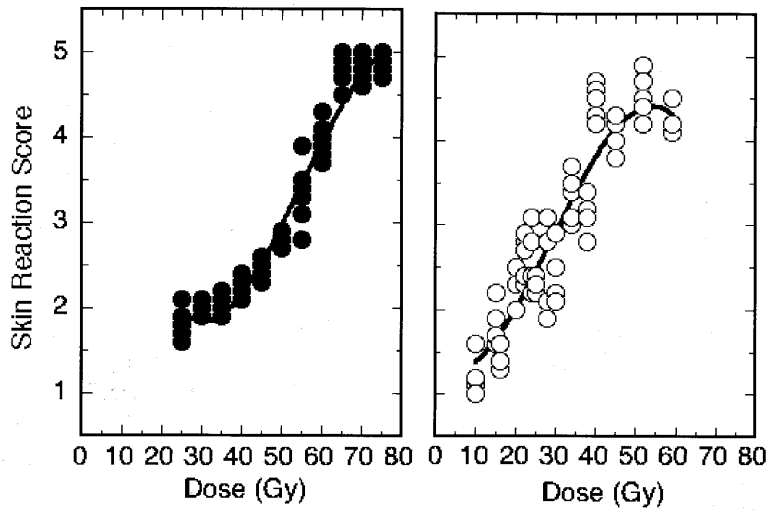

Fig. 1. Dose response of leg skin. Mice received single doses of either γ rays (●) or carbon ions of 20 keV/μm (○). Skin reaction was macroscopically scored using a scale listed in Table. Five largest scores of a mouse were averaged for 5 mice, and here plotted as a symbol.

used to determine an isoeffect dose. The top-up dose scheme can bring non-detectable radiation damage measurable by adding large doses of either neutrons[4] or photons[5–7] to the preceding small doses of photons. The added doses or top-up doses are used for normal tissues such as skin,[4] oral mucosa[5] and spinal cord,[6] and tumors[7] as well. Using the top-up dose scheme here, we measured repair of skin damage caused by small doses. Same radiation qualities were used between small doses and top-up doses irrespective of carbon ions or γ rays.

Endpoints and data analysis

Skin reactions of the irradiated legs were scored every other day, starting from Day 7 after initial irradiation up to the Day 35. Our scoring scale consisted of 10 steps, ranging from 0.5 to 5.0 (Table 1). The five highest scores in an individual mouse were averaged, and this averaged score was designated as the averaged peak reaction.[8]

To analyze the effectiveness of various fractionation schemes, a dose-response curve was constructed by plotting the averaged peak reaction as a function of the radiation dose for each scheme. This dose-response curve was used to obtain an isoeffect dose, that was defined as the radiation dose necessary to produce a skin reaction score 3.0. The data for each dose response curve were fitted to a cubic polynomial function using a least-squares method. The 95% confidence limit around the isoeffect dose for a skin reaction

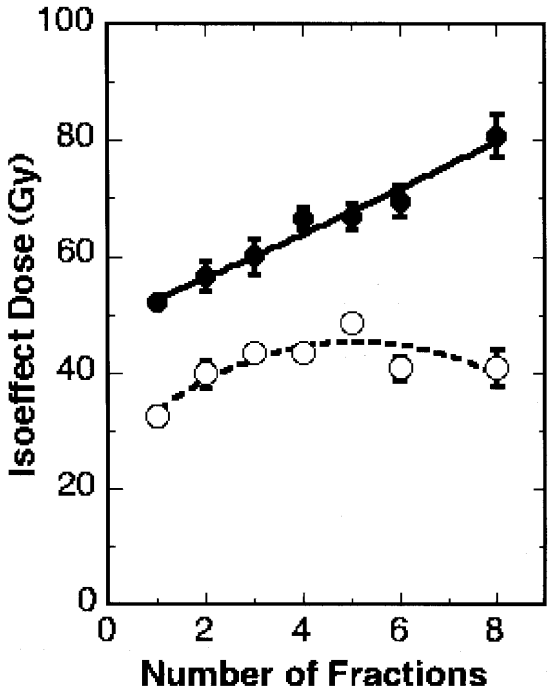

Fig. 2. Isoeffect doses obtained by a scheme of equal dose per fraction. The symbols and bars are the means and the 95% confidence limits for mice irradiated with either γ rays (●) or carbon ions of 20 keV/μm (○).

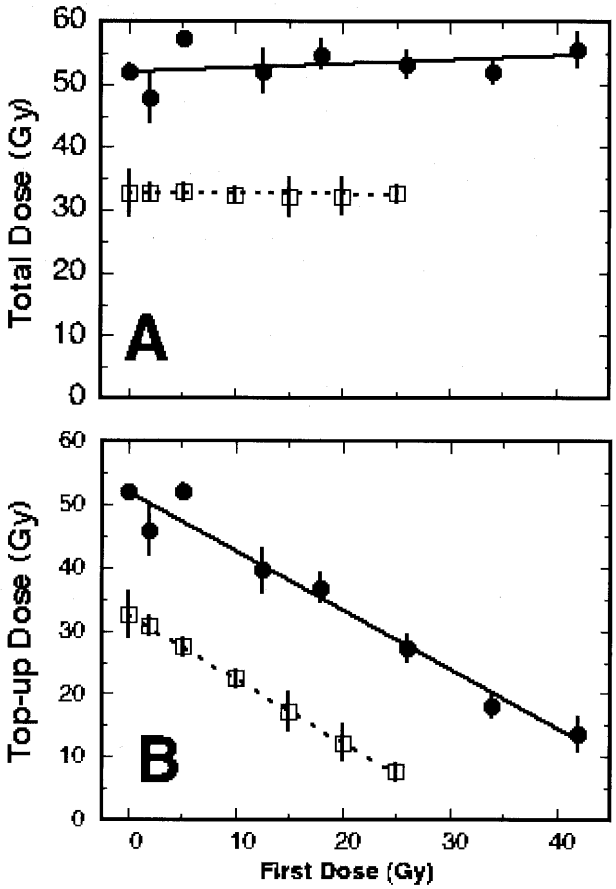

Fig. 3. Dependence on the first dose of total and top-up doses obtained by a scheme of top-up irradiation. Mice received first doses of either γ rays (●) or carbon ions (□) on Day 0, and received top-up doses on Day 1. The mean values with 95% confidence limits for total doses (A) and top-up doses (B) are plotted against the first dose.

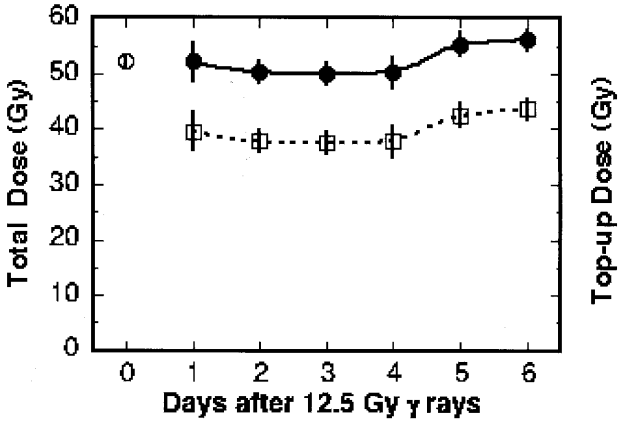

Fig. 4. Dependence on interval time of the total and top-up doses for γ rays. Mice received a first dose of 12.5 Gy on Day 0, and received second top-up doses between Day 1 and Day 6. The mean values with 95% confidence limits for single doses (○), total doses (●) and top-up doses (□) are plotted against interval time.

score 3.0 was calculated using the Maharanobis distance.[8)]

Data obtained by top-up experiments were used to analyze repair capacity of skin during fractionated irradiation. We plot isoeffect doses against number of daily doses on normal scale. A slope of a linear regression line was used to measure the magnitude of repair. A simple method was used to calculate a theoretical regression line for full, 100% repair in such assumption that any dose per fraction did not contribute to a total isoeffect dose. For no, 0% repair, we assumed that all dose per fraction accumulated to a total isoeffect dose.

RESULTS

Examples of dose response relation for skin reaction are shown in Fig. 1. An isoeffect dose for equal daily fractions (see Fig. 2) and total and top-up doses (see Fig. 3-Fig. 7) were calculated from a given dose response.

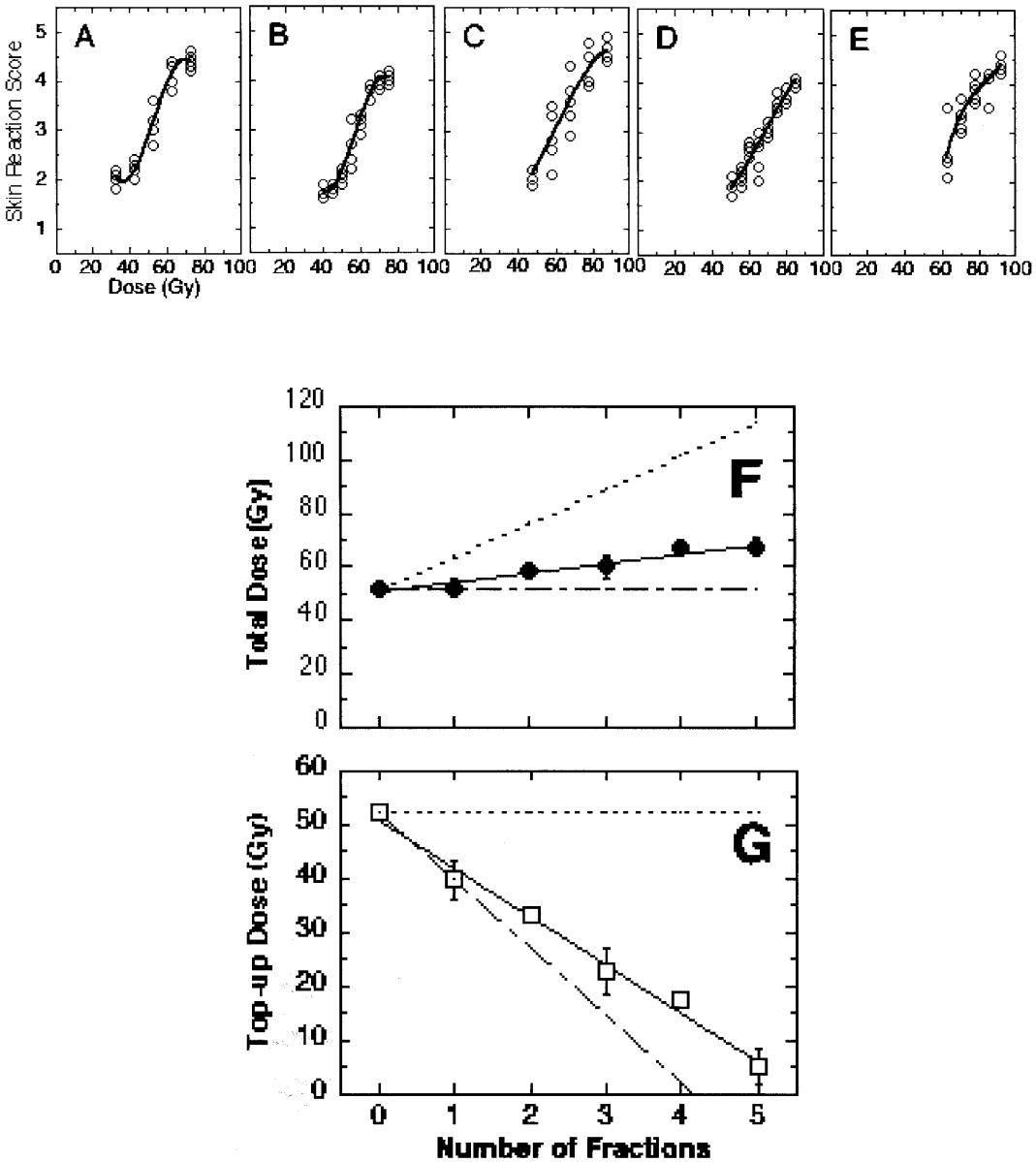

Fig. 5. Total and top-up γ-ray doses for the daily irradiations with 12.5 Gy followed by a top-up irradiation. Panels A through E show dose responses for daily irradiation of once (A), twice (B), 3 times (C), 4 times (D) or 5 times (E), and followed by graded top-up doses 1 day after final 12.5 Gy. A total dose (F) and a top-up dose (G) to produce a skin reaction score 3.0 for a given fractionation schedule was calculated from a dose response. A top-up dose was obtained by substituting daily doses from a total dose. The mean values with 95% confidence limits for total doses (●) and top-up doses (□) are plotted against the number of daily doses. Dotted and dash-dotted lines are for theoretical values of 100 and 0% repair, respectively, while a solid line is for experimental data.

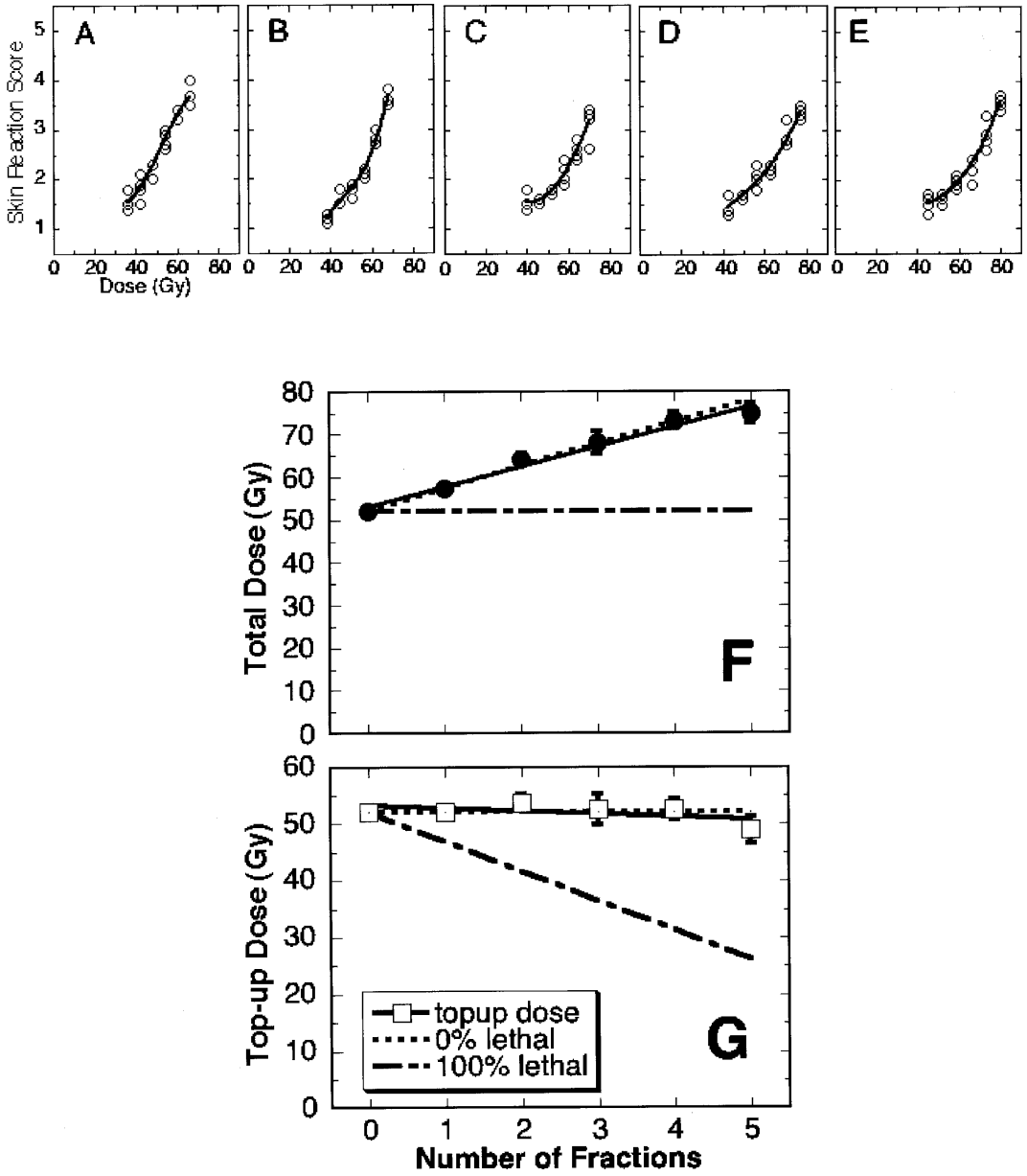

Fig. 6. Total and top-up γ-ray doses for the daily irradiations with 5.2 Gy followed by a top-up irradiation. Panels A through E show dose responses for daily irradiation of once (A), twice (B), 3 times (C), 4 times (D) or 5 times (E), and followed by graded top-up doses 1 day after final 5.2 Gy. A total dose (F) and a top-up dose (G) were calculated from a dose response as stated above. The mean values with 95% confidence limits for total doses (●) and top-up doses (□) are plotted against the number of daily doses. Dotted and dash-dotted lines are for theoretical values of 100 and 0% repair, respectively, while a solid line is for experimental data.

Figure 2 shows skin reactions against the number of equal daily fractions for γ rays or carbon ions of 20 keV/μm. The isoeffect single dose for γ rays was 52.1 ± 1.0 Gy, and significantly ($p < 0.05$) larger than that for carbon ions, i.e., 32.5 ± 1.5 Gy. Isoeffect doses increased with an increase in the number of fractions from 1 to 4, irrespective of radiation quality. When the number of fractions increased from 4 to 8, the isoeffect dose for γ rays continued to increase linearly whereas the isoeffect dose for carbon ions became constant. This implies that the capacity of skin to repair damage caused by carbon ions is reduced when the number of fractions exceeds 4. A question is: which is responsible for the reduction of repair; the number of fractions or dose per fraction? As the dose per fraction and the number of fractions inversely link together in the equal daily fractionation experiment, we employed the top-up dose experiment that could measure damage caused by each fraction without changing the dose per fraction.

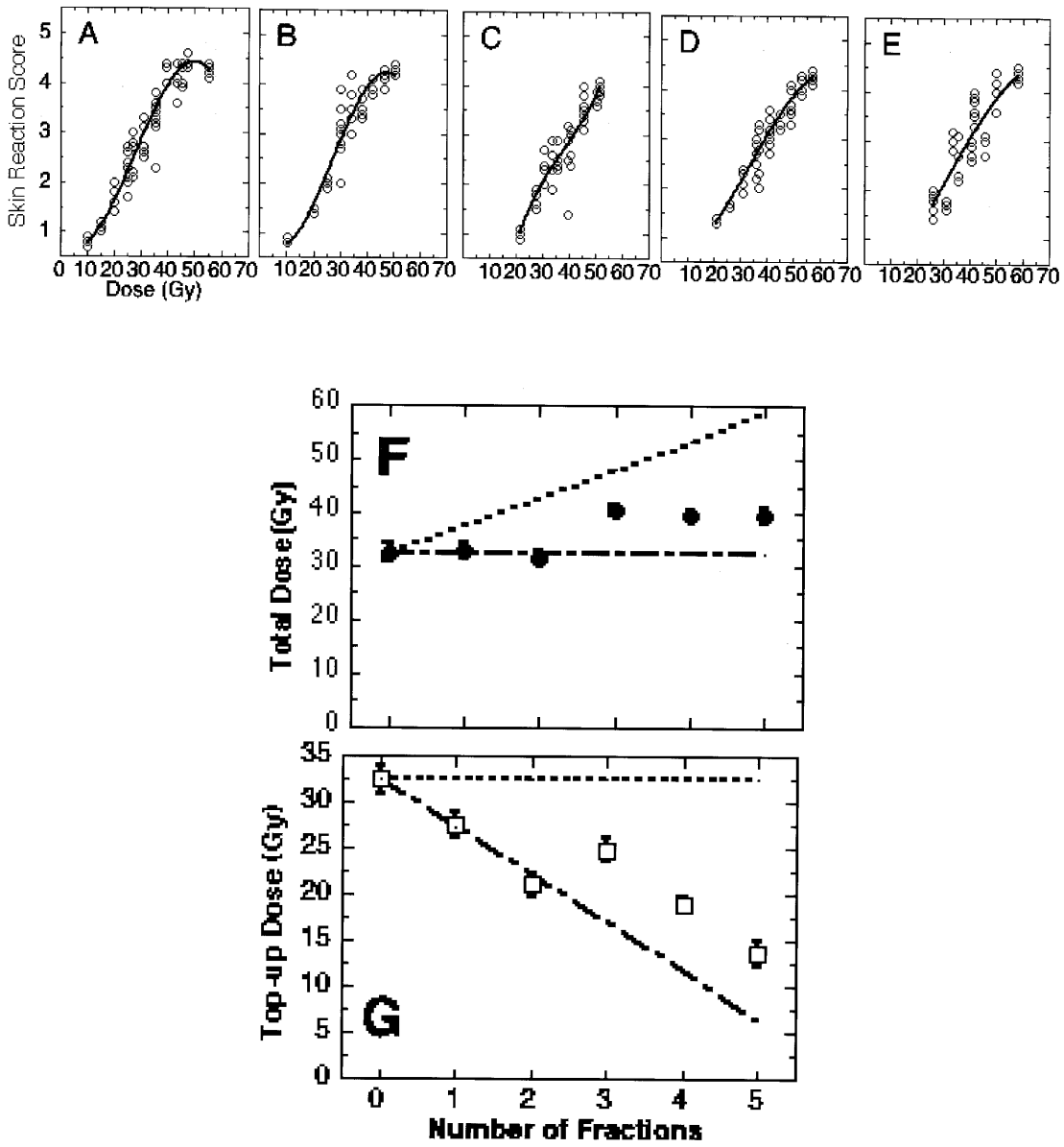

Fig. 7. Total and top-up carbon-ion doses for the daily irradiations with 5.2 Gy followed by a top-up irradiation. Panels A through E show dose responses for daily irradiation of once (A), twice (B), 3 times (C), 4 times (D) or 5 times (E), and followed by graded top-up doses 1 day after final 5.2 Gy. A total dose (F) and a top-up dose (G) were calculated from a dose response as stated above. The mean values with 95% confidence limits for total doses (●) and top-up doses (□) are plotted against the number of daily doses. Dotted and dash-dotted lines are for theoretical values of 100 and 0% repair, respectively.

We examined dose linearity in the top-up experiment. Mice legs were first irradiated with fixed doses of γ rays at Day 0, and followed by graded top-up doses at Day 1. Total isoeffect doses did not depend on the first dose, and were almost identical to each other when the first doses increased from 0 to 42 Gy for γ rays and from 0 to 25 Gy for carbon ions (Fig. 3 A). Top-up isoeffect doses linearly decreased with the first dose for γ rays and carbon ions as well (Fig. 3 B). As the coefficient of slope was near 1.0 for both γ rays and carbon ions, the damage caused by a first dose remained and was not repaired by Day 1.

Time dependence of top-up dose was studied using γ rays. A first dose of 12.5 Gy was followed by graded top-up doses with an interval time of 1 through 6 days (Fig. 4). Both total and top-up doses did not change for the time interval between 0 and 4 days. A slight but significant ($p < 0.05$) increase of the total and top-up doses was observed when the time interval further increased from 4 to 6 days. We selected 5 days as the longest overall time of irradiation in the following top-up experiments, by which the participation of cell proliferation to the total and top-up doses could be minimized.

Figure 5 shows the total and top-up doses after multiple fractions with 12.5 Gy γ rays. The total dose was 52.1 Gy for single irradiation or 0 fraction, and gradually increased with an increase in the number of fractions from 2 to 5. Fitting all data including the 1st fraction to linear regression, a value of 3.5 was obtained as the coefficient of slope (Fig. 5 F). The dotted line represents a theoretical regression in case skin full repairs all damage, and possesses a value of 12.5 as the coefficient of slope. Therefore, the magnitude of repair was 3.5/12.5 or 28% of skinís full potential. The top-up dose lineally decreased with an increase in the number of fraction, and possessed a value of –9.0 as the coefficient of slope (Fig. 5 G). The dash-dotted line that possesses a value of –12.5 as the coefficient of slope represents a theoretical regression in case all damage accumulate and are lethal or non-reparable. A ratio –9.0/–12.5 represents a magnitude of non-reparable damage, being 72% of damage are non-reparable.

When we used a small dose of 5.2 Gy γ rays as the dose per fraction, the total dose again lineally increased with an increase in fractions (Fig. 6 F). The magnitude of repair calculated was 92% (4.8/5.2), which was much larger than the magnitude of repair for the dose per fraction of 12.5 Gy, i.e., 28%. The regression line for top-up doses possessed a value of –0.5 as coefficient of slope (Fig. 6 G), and showed that 9% (–0.5/–5.2) of damage was non-reparable. For a dose per fraction of 5.2 Gy carbon ions, the total dose remained constant till 2 fractions (Fig. 7 F). However, the total dose abruptly increased by 7.6 Gy, i.e., 39.9 Gy minus 32.3 Gy, when the number of fraction exceeded 3. The top-up dose lineally decreased with an increase of fractions till 2 (Fig. 7 G). The top-up dose jumped up at the 3rd fraction by 3.9 Gy, i.e., 25.0 Gy minus 21.1 Gy, and decreased again when the number of fractions further increased to 5. It is obvious that the repair of the skin damage is different between carbon ions and γ rays.

DISCUSSION

In the present study, we found that the skin damage caused by low LET carbon ions was repaired in a different manner from one that the damage caused by photons was repaired. A total dose of γ ray increased linearly with an increase in the number of 5.2 Gy per fraction up to 5 fractions (Fig. 6). The linear increase observed in the present study confirms a previous report that the magnitude of skin damage during repeated irradiations is constant for an X ray dose of 2.5 Gy per fraction.[4] We do not exclude, however, other possible explanation to the linear increase such as cell cycle redistribution and proliferation of stem cells. In fact, any change in the magnitude of skin damage is reported for photon irradiations with non-conventional fractionation schemes. Ruifrok *et al.*[9] report that γ -ray sensitivity of mouse skin becomes higher when the inter-fractionation interval increases from 24 hr to 48 hr. It seems that cell cycle redistribution with altered radiosensitivity could be the cause of the high sensitivity. Shirazi *et al.*[10] report that mouse skin rather becomes resistant against X rays by a previous exposure with UB-V radiation, probably due to an increase in the number of epidermal stem cells. No report is found for charged-particle radiation with non-conventional fractionation schemes.

Magnitude of repair depended on the size of dose per fraction for γ-ray radiation. Skin could repair only 28% damage caused by multiple doses of 12.5 Gy (Fig. 5 F, G) while more than 92% damage repaired for multiple doses of 5. 2 Gy each (Fig. 6 F, G). As repair half time of mouse skin irradiated with X rays ranges from 1 and 3 hr,[11] an interval time of 24 hr that we used in the present study should be long enough to expect that skin completely repaired damage caused by each doses. This support a concept of repair saturation[12] so that the capacity of skin to repair radiation damage is limited to a level, and could not repair all damage caused by large doses.

The skin damage caused by carbon ions was abruptly recovered after the 3rd dose of 5.2 Gy per fraction (Fig. 7 F, G). That the recovery from 5.2 Gy per fraction was only once observed for carbon ions (Fig. 7 F, G) but constantly detected for γ rays (Fig. 6 F, G) may share the same mechanisms underlying the increased difference of isoeffect doses between carbon ions and γ rays at large number of fractions in the scheme of equal dose per fraction (Fig. 2). The abrupt recovery could be explained by either cell cycle synchronized to a radioresistant phase, rapid proliferation or induced repair of skin cells. High LET carbon ions induce longer G_2 arrest in human lymphocytes than X rays.[13] A rapid repopulation of tumor cells is induced by low energy neutrons.[14] Cells exposed to a low dose radiation show resistance to a following radiation.[15] The adaptive response is known that a small conditioning dose (generally below 30 cGy) may protect against a subsequent, separate, exposure to radiation that may be substantially larger than the initial dose.[16] As the dose of 5.2 Gy we used in the present study is much larger than 30 cGy, adaptive response may not be relevant to the abrupt recovery of skin receiving carbon ions.

Whatever are mechanisms underlying the abrupt recovery, we conclude that an abrupt increase/decrease of normal tissue damage could be caused by changing the number of fractions in carbon-ion radiotherapy. Skin damage should be carefully studied when number of fractions is changed in new clinical trials.

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