Inhibition of mouse tumors by heavy ion irradiation

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Abstract Inhibition effects, control probabilities and pathology tissue changes of mouse transplanted tumors S_{180} after irradiation with 50 MeV/u $^{12}C^{6+}$ ions are reported. Doses of single irradiation were 0.5, 1, 2, 5, 10, 20, 40 Gy, respectively, at a dose rate of 3 Gy/min. Observing time was 24 days. The results show that each group had significant inhibition action on S_{180} tumors and all inhibitory probabilities were more than 90%; the initial time of inducing tumor inhibition effects were within one week in high dose groups (20, 40 Gy), and after two weeks in medium dose groups (5, 10 Gy) and low dose groups (0.5, 1, 2 Gy); also, inhibitory effects in high dose groups were obviously greater than other groups ($p < 0.05$); tumor control probabilities were different in each group, those in high dose groups (20, 40 Gy) were higher and TCD_{50} (50% tumor control dose) was 20 Gy; the results for curing tumors are different in different doses; pathology inspection presented here were tumor tissue necrosis and degeneration in each dose group and they depended on doses.

Keywords Heavy ion irradiation, Tumor in mouse, Inhibition effect, Control probability, Tumor cure, Pathology change
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1 Introduction

Cancer treatment with heavy ions is a high technique in current radiotherapy. It is one of the most effective methods in the cancer treatment. During the middle 1970 s, the group at Lawrence Berkeley Laboratory (LBL) succeeded in modifying and connecting its existing low-energy heavy-ion accelerator (HILAC) to a high-energy proton accelerator (Bevatron) to obtain heavy-ion beams suitable for radiotherapy. This combined facility was known as the BEVALAC. 450 patients were treated with heavy ions, mostly neon at the Bevalac of LBL[1]. The group reported promising results of radiotherapy with neon ions on advanced macroscopic salivary gland carcinoma, locally advanced paranasal sinus tumors, soft tissue sarcoma, bone sarcoma, prostate cancer and biliary tract cancer as compared with historical conventional radiotherapy[2,3]. In 1994, the Heavy Ion Medical Accelerator in Chiba (HIMAC) of Japan was opened with three treatment rooms with fixed gantries for vertical, horizontal and vertical/horizontal beam lines. The HIMAC was designed to deliver beams of ions, from helium to argon, with energies in the range of 100~800 MeV/u. They decided to start with carbon ions. They have already conducted preclinical studies and initiated phase I and II clinical trials. Until August 1997, a total of 301 patients had been treated with carbon ions[4]. Patient treatment started at GSI, Darmstadt, Germany, on Saturday December 13, 1997. Two patients suffering from tumors at the base of skull were treated with five and four fractions of carbon ions, respectively[5]. It is worthy of note that some experiments of animals such as

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tumor-bearing mice were finished prior to cancer clinical treatment at NIRS (National Institute of Radiological Sciences) of Japan and GSI of Germany. At NIRS, they made experiments on the growth delay and control of the NFSa tumors after single irradiation with 290 MeV/u ^{12}C produced by HIMAC[6]. At GSI, an experimental mouse tumor was used for the first pilot experiment in 1995; its purpose was to survey tumor control after irradiation with 40 Gy of carbon ions by measuring the tumor volume[7]. In 1995, HIRFL (Heavy Ion Research Facility in Lanzhou, China) came into operation for medical studies, i.e. basic research on cancer treatment with carbon ions. In order to collect fundamental data for clinical treatment, we surveyed the S_{180} tumor changes in mice after 50 MeV/u $^{12}C^{6+}$ ion irradiation.

2 Materials and methods

2.1 Animals

Kunming male mice, weight of 20±2 g, were provided by the Lanzhou Institute of Biological Products, Ministry of Health.

2.2 Tumor culture and grouping

S_{180} tumor strain was provided by the Beijing Institute of Medicinals, Chinese Academy of Medical Sciences. The milky white tumor fluids were taken under sterile conditions and diluted to 3.8×10^{10} cells/L according to a certain proportion. Then 0.2 mL of cell fluids was transplanted in the right hind leg of each experimental mouse. Tumor volumes of the mice were measured by caliper 6 days post transplantation and 96 mice with a tumor diameter of approximately 7 mm were selected. They were randomly divided into 8 groups of 12 mice each.

2.3 Beam and irradiation

A 50 MeV/u $^{12}C^{6+}$ beam, produced by HIRFL, was used. Mice were fixed in a special box made of lucite and only sites of tumors were irradiated. The groups were irradiated singly with a dose rate of 3 Gy/min and the doses of 0.5, 1, 2, 5, 10, 20, 40 Gy, respectively, and a control group was unirradiated.

2.4 Endpoints

Tumor diameters were measured by caliper every other day after irradiation up to 24 days. Tumor volumes were calculated by a formula of $a^2 \times b/2$ where a, b represented the length and width diameters of a tumor. Tumor inhibition rate in each group was calculated according to inhibition rate (IR)=(1− the mean value of tumor volumes in an irradiation group/one in control group)×100%. Moreover, the tumor control probability was expressed as a ratio of total of tumor growth stop, contraction and cure in an experimental group to the irradiated tumor number in same group. On the 3 rd day after irradiation, two mice were randomly selected from each irradiation group and killed; the tumors were taken from mice and soaked in 10% formalin for pathology inspection.

3 Results

3.1 Dose-effect on inhibition growth of S_{180} tumors

In Fig.1, it is shown that each radiation group has a greater inhibition effect on S_{180} sarcomas during observation. On the 24 th day after the irradiation, the inhibitory rates (IR) for tumors with doses of 0.5, 1, 2, 5, 10, 20 and 40 Gy are 90.22%, 92.16%, 93.17%,

95.15%, 95.84%, 96.08% and 98.80%, respectively. They all achieved the greatest inhibition effect. Also, the dose-effect in each observing time is obvious. Tumor inhibition effects in high dose groups (20, 40 Gy) are much greater than those in medium dose groups (5, 10 Gy) and low dose groups (0.5, 1, 2 Gy), especially between 6 and 12 days after radiation. However, inhibition rate in each radiated group reaches certain value and increases mildly between 14 and 18 days after radiation

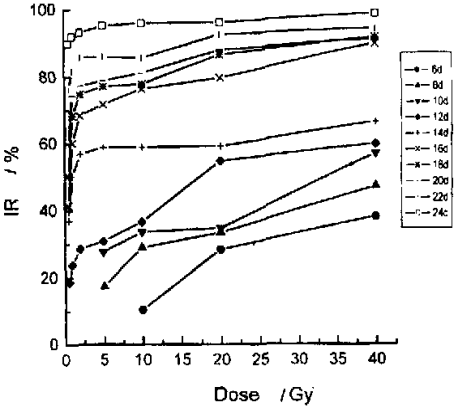

Fig.1 The dose-effect in inhibitory growth of S_{180} tumors irradiated by 50 MeV ^{12}C

3.2 Time-effect on inhibition of S_{180} tumor growth

Fig.2 shows that tumor inhibition effect in every radiated group increases with the extension of observing time, the time-effect is clear, but initial time of inducing effects is different. In 20 and 40 Gy groups (high dose groups), the initial time of inducing effects is earlier and the tumor inhibition effect is greater; the inhibitory rates are 28.37%, and 38.27%, respectively, on the 6th day after radiation and more than 90% on the 16th day (for 40 Gy) and 20th (for 20 Gy). For 0.5, 1, 2, 5 and 10 Gy groups (low and medium dose groups), however, the initial time of inducing effects is later; the inhibitory rates (IR) are 18.64%, 23.84%, 28.61%, 27.67% and 36.55%, respectively on the 12th day after radiation and they all exceed 90% on the 24th day. The inhibitory rate for each group increases slowly after achieving a certain value, finally reaches maximun on the 24th day after radiation.

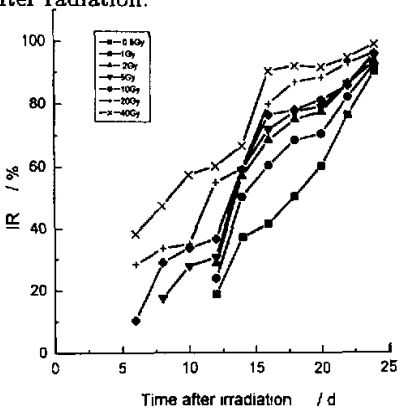

Fig.2 The time-effect in inhibitory growth of S_{180} tumors irradiated by 50 MeV/u ^{12}C

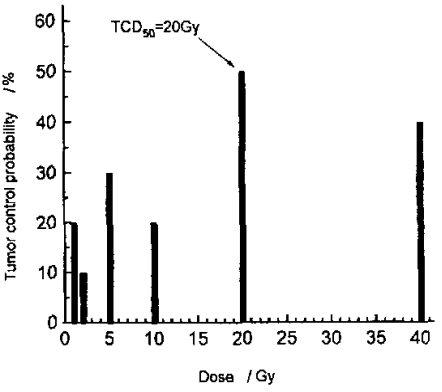

Fig.3 S_{180} tumor control probability 24 d after 50 MeV/u ^{12}C irradiation

3.3 Probabilities of control and cure of S_{180} tumors

It is the relationship between S_{180} tumor control probabilities and doses 24 days

after 50 MeV/u $^{12}C^{6+}$ irradiation in Fig.3. The results show that the tumor control probabilities depend on irradiation doses, i.e. tumor control probabilities in doses of 0.5, 1, 2, 5, 10, 20, 40 Gy are 0, 20%, 10%, 30%, 20%, 50%, 40%, respectively. Also, it is obtained from the above that TCD_{50} (i. e. 50% tumor control dose) is 20 Gy.

From Fig.4 it is found that the results of tumor cure are different in different doses. Observing 30 days after irradiation, tumors for irradiation of 2 Gy still present although they are smaller than control, whereas tumors for irradiation of 20, 40 Gy disappear.

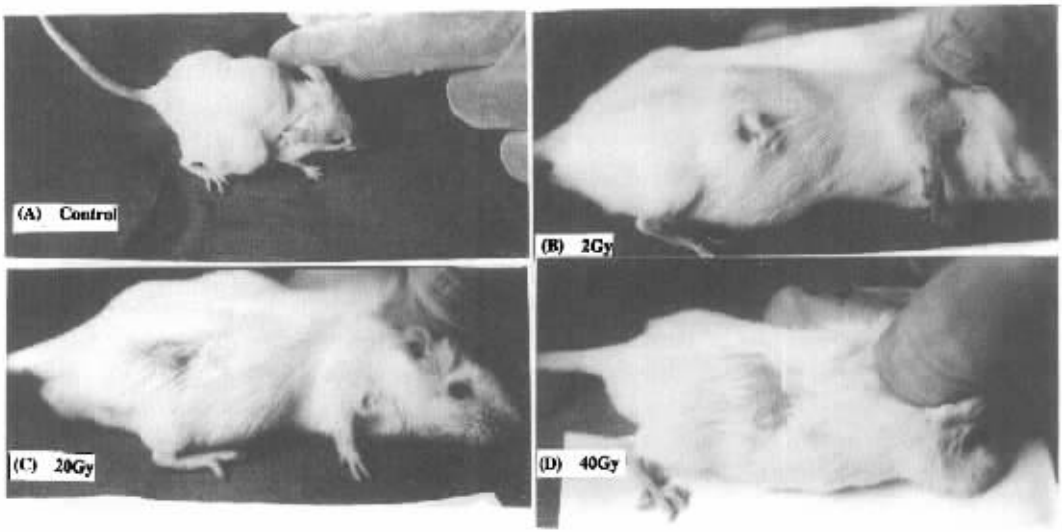

Fig.4 The time-effect in inhibitory growth of S_{180} tumors irradiated by 50 MeV/u ^{12}C

3.4 Pathology changes of S_{180} tumors

Pathology inspection presented that there were tumor tissue necrosis and degeneration in each radiated group and it depended on doses. Necrosis degrees of a tumor tissue are 60% and 65% in high dose groups (20, 40 Gy), 50% and 55% in medium dose groups (5, 10 Gy), 50% in low dose groups (0.5, 1, 2 Gy), respectively. Degeneration degrees of a tumor tissue are "++" in low dose groups, "+++" in high and medium dose groups, respectively. The results show that there are greater killing for tumor cells (Table 1) in high doses.

Table 1 Pathology changes of S_{180} sarcomas irradiated with heavy ions

Dose/Gy	Necrosis degree/%	Degeneration degree
0.5	50	++
1	50	++
2	50	++
5	50	+++
10	55	+++
20	60	+++
40	65	+++

4 Discussion

As showed in Figs.1 and 2, inhibitory rates (IR) for radiated tumors rise with time escape and/or dose increase, finally reach maximum and approach each other on

the 24th day after radiation. The results may be explained as follows: the higher the radiation doses are, the more seriously the whole tumors are damaged, and the greater the inhibitory rates are. As for time effect, because there are cells with potentially lethal damage (PLD) in the radiated tumors, and the PLD cells appear reproductive death cells and numbers of the reproductive death cells increase with time, thus the radiated tumors grow slowly as compared with control. Then, ±Rs of the radiated tumors become greater. On the 24th day after radiation all ±Rs approximate equal with each other, which results from preliminary percentage of interphase death cells and reproductive death cells between high doses and low doses. The numbers of interphase death cells in high dose are more than those for low doses while numbers of reproductive death cells in high doses are fewer than those for low doses. Therefore, the increase of inhibitory rates (IR) in high doses and low doses becomes slow and fast with time, respectively; to 24 th day after radiation their inhibitory rates approximate equal.

It is found that tumor control probabilities in doses of 20 and 40 Gy, 50% and 40%, are greater than the others (Fig.3) and some of tumors irradiated by 20 and 40 Gy disappear (Fig.4). However, tumor control probability by 40 Gy irradiation is worse than one by 20 Gy irradiation while degree of damaging normal tissue by 40 Gy irradiation is greater than one by 20 Gy irradiation. From the above it is obtained that 20 Gy irradiation is the most efficient dose suitable for the treatment of S_{180} tumors.

In respect of necrosis degree, radiation with doses of 0.5, 1, 2, 5 Gy induced significant necrosis of S_{180} tumors, 50%, although their effects on S_{180} tumors are smaller than radiation with doses of 10, 20, 40 Gy; but there are no differences among those doses of 0.5, 1, 2, 5 Gy (Table 1). All of the radiation doses from 0.5 Gy to 40 Gy have significant effect on the degeneration of S_{180} tumors, the difference is that there are two areas, "++" for 0.5, 1, 2 Gy, and "+++" in 5, 10, 20, 40 Gy (Table 1).

5 Summary

Each radiated group has significant inhibition effect on S_{180} tumors irradiated by heavy ions. Dose-effect and time-effect are all significant. The differences among the radiated groups are that both the dose-effect and time-effect in high dose groups (20, 40 Gy) display earlier than in medium doses (5, 10 Gy) and low doses (0.5, 1, 2 Gy). The TCD_{50}, i.e., the dose of single irradiation with the heavy ions attaining 50% control probability for S_{180} tumors, is 20 Gy. It is found from pathology inspection that there are significant tumor tissue necrosis and degeneration, which is the basic cause for the inhibition, control and cure of the tumor growth.

References

1 Kraft G. Heavy ion therapy at GSI. GSI-Nachrichen, 1993, 11:3
2 Linstadt D E, Castro J R , Phillips T L. Int J Radiat Oncol Biol Phys, 1991, **20**:761
3 Tsujii H *et al.* 5th Workshop on Heavy Charged Particles in Biology and Medicine , GSI, Darmstadt, August 23-25, 1995, 167
4 Tsujii H *et al.* 6th Workshop on Heavy Charged Particles in Biology and Medicine, Bevano, Italy, Sept 29-Oct 1, 1997, H4-1
5 Sisterson J. Particles, No.21, Jan. 1998, 5
6 Nemoto K *et al.* 5th Workshop on Heavy Charged Particles in Biology and Medicine, GSI, Darmstadt, August 23-25, 1995,156
7 Fournier C *et al.* GSI Scientific Report, 1995,139