# Killing effect of Chinese hamster V79 cells exposed to accelerated carbon ions and RBE determination

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**Abstract** Survival curves of Chinese hamster V79 cells exposed to accelerated carbon ions with linear energy transfers of 125.5, 200 and 700 keV/$\mu$m were measured, respectively. Inactivation cross sections corresponding to the irradiation above were deduced from the V79 cell survival curves. They are 7.86±0.17, 10.44±1.11 and 32.32±3.58 $\mu m^2$ in turn. With the surviving response of V79 cells to $^{60}$Co $\gamma$-rays as a reference value, relative biological effectiveness at 10%, 20%, 50% and 80% survival levels were given for the accelerated carbon ions. The results showed that carbon ions with LET of 125.5 keV/$\mu$m had a higher value of RBE at all the four survival levels than the carbon ions with other LETs. It was prompted that the maximum value of RBE for the V79 cell surviving as the biological endpoint emerged at the LET below 200 keV/$\mu$m for carbon ions.

**Keywords** Carbon ions, V79 cells, Inactivation cross section, Relative biological effectiveness

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## 1 INTRODUCTION

The importance of the radiobiology of heavy ions accelerated in laboratories is now getting more and more understood not only for its theoretical significance, but also for its potential application in practice. As we know, with their marked trait in energy deposition,[1] heavy ions provide the most promising tool for tumor radiotherapy. In this strategy, the cancer cells are killed by high dose peak (Bragg peak) at the end of the ion track, while the normal tissues are spared because of low dose plateau in the other part through the track. On the other hand, heavy ions are one of main components of extraterrestrial irradiation field,[2,3] so it becomes an important hazard factor for manned space flight. In this paper, the biological effects of accelerated carbon ions with different LETs on Chinese hamster lung V79 cells were investigated at HIRFL (Heavy Ion Research Facility in Lanzhou) and the RBE of the carbon ions at different survival levels

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were evaluated. This study provided useful data for verification and development of the biophysical model for the heavy ions, for the tumor therapy using heavy ions and for the radiation protection.

## 2 MATERIALS AND METHODS

### 2.1 Cell culture

Chinese hamster lung V79 cells (purchased from Beijing Tumor Institute, the Chinese Academy of Medicine Science) were cultured in RMPI-1640 medium (Gibco Inc.) supplemented with 10% fetal calf serum, 100u/mL penicillin and 100 $\mu$g/mL streptomycin. The cultures were maintained in an incubator at 37°C in humidified atmosphere containing 5% $CO_2$. The cells were inoculated in glass Petri dishes ($\Phi$35 mm) with a density of $5\times10^4$ cells/mL one day before irradiation.

### 2.2 Irradiation

The irradiation was performed at the HIRFL in Institute of Modern Physics, the Chinese Academy of Sciences, Lanzhou. For irradiation, 14.54, 8.18 and 0.86 MeV/u carbon ions were supplied, which corresponded to the LET of 125.5, 200 and 700 keV/$\mu$m, respectively, according to the calculations with TRIM91 code.[4] Before irradiation, the medium was removed and the cells were washed twice with PBS buffer. The Petri dishes were enveloped with 4 $\mu$m Mylar foil under the germ-free condition. The detailed description about the experimental setup was presented in Ref.[5]. The dose rate was kept at about 3Gy/min during the whole irradiation process. The $\gamma$-ray exposures were performed with the $^{60}$Co $\gamma$-ray source at Radiotherapy Department of the First Hospital Attached to Lanzhou Medical College. And the dose rate was about 0.7 Gy/min instead.

### 2.3 Cell analysis after irradiation

The irradiated cells were trypsinized and plated at appropriate dilutions in Petri dishes ($\Phi$60 mm) for survival determination with the method of colony-forming test. After 8 days of further incubation, the cells were fixed with Carnoy and stained with Giemsa (1:20, pH6.8) and then the colonies (>50 cells) were counted.

### 2.4 Acquisitions of inactivation cross section and RBE

Inactivation cross sections $\sigma$ were calculated from the slopes of the survival curves according to the following expression:

$$SF(\Phi) = e^{-\sigma\Phi}$$

where $SF$ is the survival fraction and $\Phi$ is the ion fluence. Parameters were determined by linear regression of the experimental data. The following multi-target model was applied to fit the $\gamma$-ray data because of its validity to this experiment,

$$SF(D) = 1 - (1 - e^{-D/D_0})^m$$

where $D$ is the dose, $D_0$ and $m$ are the extracted parameters through fitting, respectively. By converting the ion fluence into the absorbed dose of the cells for fitting the survival curves, RBE were evaluated at 10%, 20%, 50% and 80% survival levels, respectively.

## 3 RESULTS AND DISCUSSION

The physical characteristics and biological results of the carbon ion exposures with 3 different LETs were listed in Table 1.

**Table 1** Physical parameters of the carbon ions and RBEs at different V79 cell survival levels

| Energy ($MeV \cdot u^{-1}$) | LET(1) ($keV \cdot \mu m^{-1}$) | Penumbra radius $r_p^{(2)}(\mu m)$ | Inactivation cross section $\sigma(\mu m^2)$ | RBE(survival) 10% | 20% | 50% | 80% |
|---|---|---|---|---|---|---|---|
| 14.54 | 125.5 | 6.87 | 7.86±0.17 | 1.50 | 1.60 | 2.02 | 3.17 |
| 8.18 | 200 | 3.16 | 10.44±1.11 | 1.25 | 1.34 | 1.69 | 2.52 |
| 0.86 | 700 | 0.09 | 32.32±3.58 | 1.11 | 1.19 | 1.53 | 2.16 |

(1)*LET was calculated by TRIM91 code*[4]; (2)*Penumbra radius was obtained with the* method in Ref.[6]

The dose-response survival curve for the $\gamma$-ray irradiation, which was taken as a reference, was given in Fig.1. The result showed a survival curve with a clear shoulder and the fitted parameters $D_0$ and $m$ were 2.94±0.68 and 2.02±0.72, respectively. Fig.2 showed the fluence-response curves of the V79 cells exposed to the carbon ions with LETs of 125.5, 200 and 700 keV/$\mu$m. It was clear that the survival fraction decreased exponentially with the increase of fluence. Hence the inactivation cross sections were extracted from the slopes of the survival curves for the three LET ions and plotted as a function of LET in Fig.3. Generally, the cross section of radiosensitive V79 cell nucleus is about 100–110 $\mu m^2$. Therefore, the inactivation cross sections obtained from this experiment were smaller than the geometrical cross section of the cell nucleus. It implied that not every hit on a cell nucleus necessarily led to the loss of reproductive ability of the cell. Even for a nuclear penetration of an ion with LET of 700 keV/$\mu$m, the probability of killing the cell is only 29%–32%. In addition, the inactivation cross sections based on the model calculation of Scholz *et al*[7] exceeded those obtained in our study, which were shown in Fig.3. Here the derivation in the measured data is thought due to the excessively simple assumption about the geometrical structure of the critical

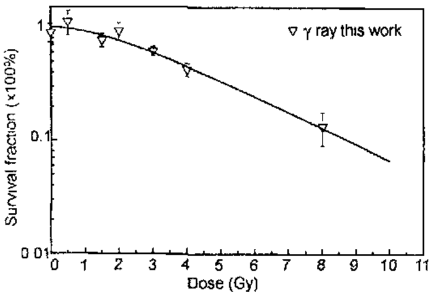


**Fig.1** The survival curve of V79 cells exposed to the reference γ-ray irradiation

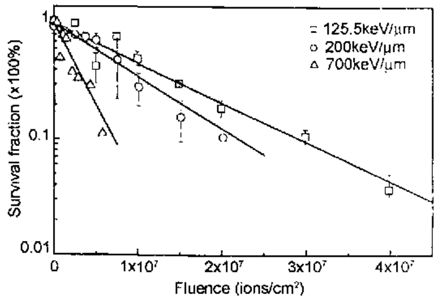


**Fig.2** The survival curves of V79 cells exposed to the carbon ions

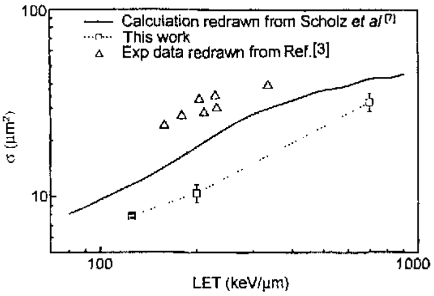


**Fig.3** The relationship between inactivation cross section and LET for V79 cells exposed to carbon ions

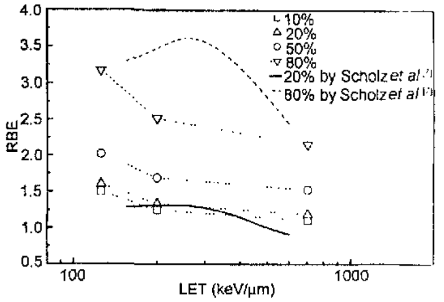


**Fig.4** The relationship between RBE at the different survival levels and LET for V79 cells exposed to carbon ions

targets in the model calculation. On the contrary, the asynchronous Chinese hamster cells were exposed to the carbon ions in this experiment. But a contradiction occurred when the experimental data obtained from Ref.[3] were displayed in the same plot as shown in Fig.3. With the same irradiation dose, all the inactivation cross sections in Ref.[3] exceeded those based on the model calculation[7] and our experiment. So it indicated that a great divergence existed between the model calculation and experiment, even with regard to the simple problem about survival endpoint. This was the reason why we took up this investigation, which seemed to be a preliminary study. And much work is needed to explain the difference between the biophysical model about the biological effect of heavy ions and experiment. It should be pointed out that the range of 0.86 MeV/u carbon ions with LET of 700 keV/$\mu$m is only about 15$\mu$m, but for mammalian cells, most of them are 10 $\mu$m or so in diameters. So the determination of inactivation cross section for 700 keV/$\mu$m ions had a great ambiguity due to the non-track-segment irradiation.

The values of RBE of V79 cells at 10%, 20%, 50% and 80% survival levels were also determined and listed in Table 1 and shown in Fig.4. In this experiment, the maximal RBE were obtained at the LET of 125.5 keV/$\mu$m at all the four levels. Consequently, it can be deduced that the RBE maximum for V79 cells with regard to killing endpoint would appear at the LET below 200 keV/$\mu$m for the carbon ion exposure. When the LET exceeded this limit, the overkill effect would occur so as to lead to the decrease of irradiation-induced biological efficiency. With the same LET, the higher the survival level, the larger the value of RBE. For example, the RBE increased from 1.50 to 3.17 with increasing the survival level from 10% to 80%. The $\gamma$-ray dose-response survival curve with a shoulder was responsible for this phenomenon.

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