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Cytocidal Effect of Hyperthermia on Tumor Cells *in vivo*

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In vivo 腫瘍細胞에 미치는 溫熱處理의 細胞致死效果

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摘 要

SCK 腫瘍細胞에 대한 溫熱處理의 細胞致死效果는 *in vitro*의 경우보다 *in vivo*의 경우에 顯著하게 컸다. *In vivo*에서 溫熱處理한 후, 腫瘍을 그대로 放置해 두면 腫瘍細胞는 어느 期間동안 계속해서 죽게 되며 腫瘍의 機能的인 血管容積도 마찬가지로 減少한다. *In vivo*에서 X線을 照射하기 前과 後 30分에 溫熱處理한 腫瘍細胞의 放射線生殘曲線은 溫熱處理하지 않은 對照群의 그것에 比해 기울기가 컸다. 結論的으로 腫瘍細胞에 대한 溫熱處理의 細胞致死效果가 *in vitro*에 비해서 *in vivo*의 경우에 크게 되는 것은 腫瘍의 內部環境에 연유하는 것으로 생각된다.

INTRODUCTION

The mechanism and kinetics of heat-induced death of mammalian tumor cells *in vitro* have been studied by a number of investigators (Dewey *et al.*, 1979; Hahn, 1974). However, quantitative information on the kinetics of cell death by heat *in vivo* is still sparse despite the possibility that the heat sensitivity of tumor cells *in vivo* may be different from that of cells *in vitro*, due to differences in environmental conditions (Marmor *et al.*, 1977). In the present study, we compared the heat sensitivity of tumor cells *in vivo* and *in vitro* and the radiation survival curves of tumor cells after combined treatment of X-rays and heat *in vivo*. We also investigated the possible role of blood perfusion on the differential effects of hyperthermia on the tumor cells *in vitro* and *in vivo*.

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MATERIALS AND METHODS

The SCK tumor used in the present study is a mammary carcinoma of female A/J mice which appears to be non-immunogenic. The tumor arose spontaneously in 1974 and was adapted to grow both *in vivo* and *in vitro*. About 5×10^4 cells were injected subcutaneously to the thigh of mice, and we used the tumor grown to 7~9 mm in diameter. For heating, the animals were lightly anesthetized with pentobarbital (0.04 mg/g) and the tumor bearing leg was immersed into water bath. The intratumor temperature, measured by a 29-gauge thermocouple, rose from 34~35°C prior to heating to about 0.5°C lower than the water bath temperature.

For the study of *in vivo* cell survival after treatment, 2 to 3 tumors were excised, pooled, minced and digested with 0.25% trypsin and a small amount of DNase in RPMI 1640 without serum for 20 min at room temperature with continuous stirring. The dispersed cells were washed, counted with trypan blue exclusion method and plated in Falcon Model 3013 plastic culture flasks with 10 ml of RPMI Medium 1640 supplemented with 10% fetal calf serum and antibiotics. The clones were stained and counted 7 to 9 days later. The plating efficiency of the control tumor cells was about 60%. In the study of the effect of heat on tumor cells *in vitro*, the exponentially growing SCK cells in culture flasks were heated in water bath and trypsinized. The single cells were then plated and cultured.

The functional intratumor vascular volume was measured using the method of ^{51}Cr-labelled red blood cells as described elsewhere (Song, *et al.*, 1971).

RESULTS AND DISCUSSION

The heat sensitivities of the tumor cells heated *in vivo* and *in vitro* were compared in Fig. 1. It can be seen that the survival curves of tumor cells heated *in vivo* at 42.5 and 43.5°C were much steeper than the survival curve of *in vitro* cells heated at 43°C. It can be concluded that *in vivo* tumor cells were significantly more heat sensitive than *in vitro* cells.

Bhuyan *et al.* (1977) reported that the 7~10 day old ascitic L1210 cells in stationary growth phase were more heat sensitive than the cells *in vitro*. These investigators concluded the cells in stationary growth phase were more heat sensitive than the cells in exponential growth phase. Bichel and Overgaard (1977) reported that PNJ ascites tumor cells of C3H mice in plateau-phase cells were more heat sensitive than the cells in exponential-phase. In addition, since acidity sensitizes cells to heat, the plateau-phase cells at pH 6.4 were about 1000-fold more heat sensitive than exponential-phase cells at pH 7.2. In the present study, the pH of media for the *in vitro* study was 7.2, and these cells were in

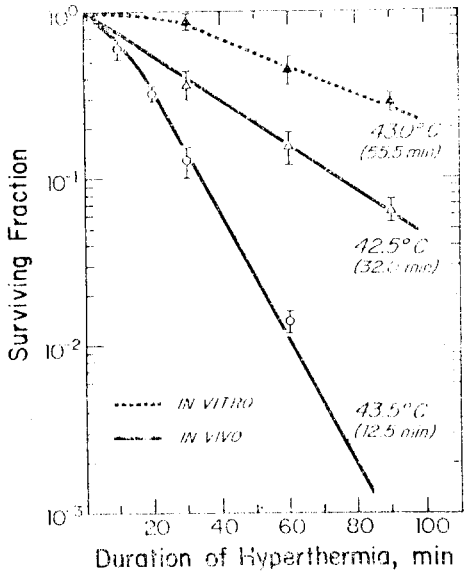

Fig. 1. Comparison of the heat sensitivities of the SCK tumor cells heated *in vivo* and *in vitro*. Times in parentheses are D_0's of the survival curves.

exponentail-phase. On the other hand, the intratumor environment of the heated SCK tumor was acidic, as reported by Song *et al.* (1980), and at least part of the tumor cells might have been in plateau-phase. Therefore, it might be that the greater heat sensitivity of tumor cells *in vivo* than of tumor cells *in vitro* in the present study was in part to a high acidic intratumor environment and/or the presence of plateau-phase cells.

When the tumors were left *in situ* after heating at 43.5°C for 30 min, there was further cell death as shown in Fig. 2, dotted line. The number of clonogenic cells in tumors excised immediately after hyperthermia was about 16% of the control and further decreased to 0.7% of control 5 hr after heating. Following these decreases, the number of clonogenic cells gradually recovered during several days thereafter, but it was still about 40% of control 3 days after heating.

The change in the vascular volume after hyperthermia is shown in Fig. 2, solid line. The vascular volume of the untreated control tumor was 8.80 ± 1.4 ml/100g of tumor. There was no significant change in the vascular volume at the end of heating at 43.5°C for 30 min. Interestingly, the vascular volume began to decrease drastically after the heat treatment. It decreases to 0.84 ± 0.17 ml/100g at 5 hr after heating, which was only about 10% of the control value. The vascular volume started to recover slowly thereafter, but it was about 50% of the control 2 days after heating. A similar result was obtained by 1 hr heating at 43.5°C (Song, *et al.*, 1980).

The delayed death of tumor cells *in vivo* after hyperthermia has been suspected in the past by Crile (1963). More recently, Narmor *et al.* (1979) reported their quantitative study on the kinetics of cell death in EMT6 of Balb/cKa mice following hyperthermia with ultrasound. It was found that a significant additional cell death progressed over a period of 2

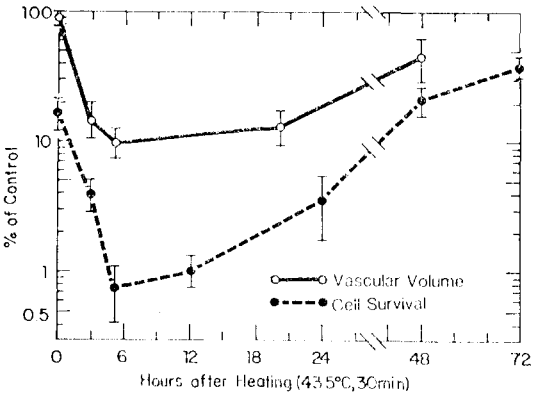

Fig. 2. Changes in the functional intratumor vascular volume and the number of clonogenic cells of SCK tumor after heating at 43.5°C for 30 min *in vivo*.

to 48 hr after hyperthermia at 43.5~44.0°C for 30 min. These investigators suggested that immune reaction and/or vascular damage may be responsible for the delayed cell death after hyperthermia. In view of the fact that SCK tumor is non-immunogenic, it is highly unlikely that immune reaction was involved in the profound death of tumor cells soon after hyperthermia in our study. The similar time course in reduction and recovery of the functional intravascular volume or vascularity and the cell survival shown in Fig. 2 strongly suggests that the additional cell death after hyperthermia may have resulted from the vascular damage. In addition to vascular stasis, a significant decrease in pH may also be responsible for the progressive death of tumor cells after hyperthermia (Song, *et al.*, 1980).

The combined effect of X-rays and heat on the survival of SCK tumor cells *in vivo* is

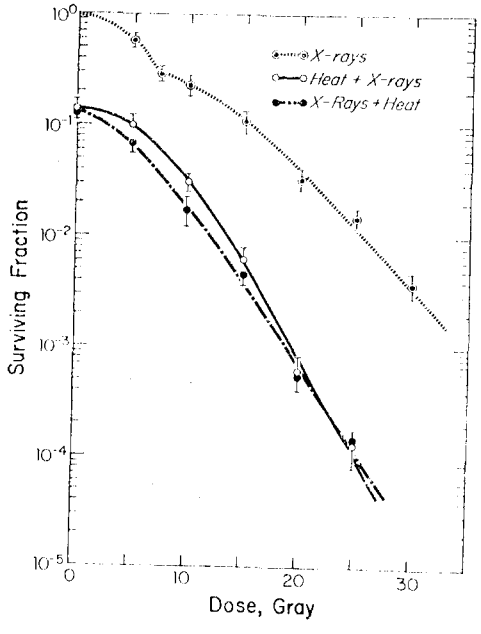

Fig. 3. Combined effect of X-ray and heat on the SCK tumor *in vivo*. Tumors were heated either 30 min before or after X-irradiation at 43.5°C for 30 min.

shown in Fig. 3. The radiation survival curve of the unheated tumor cells was biphasic. The radioresistant terminal portion of the curve is believed to represent the non-cycling hypoxic cells. The radiation survival curves became monophasic when the tumors were heated either before or after irradiation. Furthermore, the slopes of the radiation survival curves of tumor cells heated were steeper than that without heating. The heating of tumors after irradiation resulted in a smaller shoulder than the heating prior to irradiation.

The present investigation demonstrated that the tumor cells *in vivo* are more heat sensitive than are the cells *in vitro* due, in part, to the intratumor environment, such as poor nutritional supply and low pH. These environmental conditions are apparently related to the tumor blood circulation.

ABSTRACT

The cytocidal effect of hyperthermia on subcutaneous SCK tumor cells growing *in vivo* was significantly greater than that on the SCK tumor cells cultured *in vitro*. When the tumors were left *in situ* after heating, the cell survival progressively decreased, and the functional intratumor vascular volume also decreased. The radiation survival curves of tumor cells heated either 30 min before or after X-irradiation *in vivo* were steeper than the radiation survival curves of unheated control tumors. It is concluded that the cytocidal effect of hyperthermia on tumor cells *in vivo* is greater than that *in vitro* due possibly to the intratumor environment.

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