THE ENHANCEMENT OF TWEEN-80 ON THE ANTITUMOR EFFECT OF THE HYPERTERMIA 41 °C IN TUMOR-BEARING MICE

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B16 melanoma cells were inoculated into the BALB/C mice to establish melanoma-bearing models. The antitumor effect of Tween-80 in combination with hyperthermia 41 °C was studied. We observed the changes of the mortality of tumor-bearing mice, the tumor growth curves, activities of serum tumor necrosis factor (STNF) and the level of serum sialic acid (SSA) in tumor-bearing mice. The number of pulmonary metastatic tumor foci from blood flow was also detected. The results showed that combined with Tween-80, hyperthermia 41 °C could dramatically suppress the growth of the melanoma in the feet of mice, survive the tumor-bearing mice and decrease the number of pulmonary metastatic tumor foci but no significant effects were observed by treatment with Tween-80 or hyperthermia at 41 °C alone. The activities of STNF and the level of SSA of the melanoma-bearing mice kept at higher levels than those of normal BALB/C mice. Tween-80 combined with heating 41 °C significantly decreased activities of STNF, but the level of SSA still kept high level within 1 to 2 weeks and decreased 10 weeks later with the tumor regression. These results demonstrate that Tween-80 may make hyperthermia exert effective antitumor effect below the critical temperature and increase the safety of hyperthermia in treatment. It is one of the most ideal synergist with hyperthermia. The changes of STNF and SSA suggest that the synergetic effect could involve in facilitating the exposure of tumor antigen and activation of immune system.

Key words: Tween-80, Hyperthermia (41 °C), Tumor growth, Metastasis TNF, Sialic acid.

Hyperthermia has therapeutic potential in the treatment of solid tumors, especially when used in combination with other treatment modalities, such as chemotherapy. The selection of optimal combined drug is important to enhance the antitumor effect of hyperthermia and meanwhile reduce the side effect of hyperthermia. Our past research work on cell level demonstrated that Tween-80 was an ideal medicine combined with hyperthermia to antitumor, it effectively killing tumor cells and being comparatively safe and low in toxicity to normal cells. In this study, we further investigate the antitumor effect and synergistic action of Tween-80 in combination with hyperthermia at 41 °C on the melanoma-bearing mice.

MATERIALS AND METHODS

Experimental Animal

180 BALB/C mice 6–7 week-old, male, were used to establish melanoma-bearing models. All mice were provided by Animal Center of China Scientific Institute, Shanghai.
Cell Culture

B16 mouse melanoma cell line was obtained from cell research unit of China Scientific Institute, Shanghai, and routinely grown in RPMI-1640 supplemented with 10% newborn calf serum, 0.25 mM Hepes, 100 μl/ml penicillin and 100 μg/ml streptomycin. L929 cells (provided by Microbiology Department of Shanghai Second Medical University) were cultured in media as above. Exponential cells were detached by trypsination and harvested in 1640 media. The cell growth and viability were assessed by using a Coulter counter and trypan blue dye exclusion respectively. The cells had a greater than 95% viability in all experiments.

Tumor Growth Curve

5x10^5 B16 cells were inoculated in the rear feet of mice. 10 days later, tumor born could reach mean size of 0.5x0.5 cm^2 and the mice inoculated were randomly divided into 4 groups: (1) for hyperthermia study, the mouse was placed over a water bath (41 °C ±0.1) and the tumor-bearing foot was inserted into the water bath through a 1.5 cm diameter padded opening to a depth that permitted complete submersion of tumor. The tumor was heated for 100 min. (The time required to reach 41 °C was within 5 min.). Rear feet bearing melanoma were put into water bath 41 °C for 60 min.; (2) for Tween-80 study, mouse was given Tween-80 200 µl intravenously with a final concentration of 75 µM. (Tween-80 was purchased from Shanghai Da Zhong pharmaceutical factory.); (3) for Tween-80 combined heating study, 15 min. after Tween-80 injection, mice were followed by heating at 41 °C as above; (4) the control group. The sizes of tumors were estimated by caliper measurement every other day. According to the three dimensions (volume) of tumor, the tumor growth curves were plotted.

Lethal Rate of Tumor-bearing Mice

3x10^4 B16 cells were inoculated to each mouse intraperitoneally. 10 days after inoculation, mice were divided into 4 groups as above. During heating, mouse was immersed vertically into a well stirred, heated water bath (41 °C) until the water reached its chest. The time required to reach 41 °C was within the range of 5 to 10 min. The mice were fed continuously under normal condition and their mortality or survival was observed for 10 weeks after treatment. For serum tumor necrosis factor (STNF) and serum sialic acid (SSA) studies, sterile sera of mice were collected by cardiac punctures 1 week and 2 weeks after treatment, respectively.

Assay of STNF and SSA

The sera were added to 96-well plates at various dilutions, amounting 200 µl/well. The rTNF provided by Beijing Military Scientific Institute with its activity rate 4x10^7 U/mg protein was used as criteria positive control. Negative control was included, too. Exponential L929 cells were seeded at 3x10^4 cells, 75 µl/well and finally add 25 µl Actinomycin D (Act-D. Fluka. Co.) per well and made a final concentration of 1 µg/ml. The cells were cultured at 37 °C in 5% CO₂ for 20 h, fixed in 4% formalin, stained with 0.25% crystal purple dye and dissolved in 1% SDS. Assay of STNF activity was carried out by measuring the OD under the Multiplate Reader. One unit of STNF activity is defined as the dilution of serum, in which 50% target cells were killed. The criteria rTNF were used as calibration. For SSA assessment, CCM method was used as described by Svennerholm L. CCM reagents were provided by Biochemistry Department of Shanghai Second Medical University.

Pulmonary Metastatic Foci of B16 Melanoma

B16 cells were previously treated with Tween-80, heating at 41 °C or both, respectively. Then 4x10^5 cells were injected into each mouse from tail vein. After 3 weeks, the mice were killed and the number of metastatic foci of melanoma in the lungs was counted.

RESULTS

Lethal Rate

In Tween-80 combined with heating at 41 °C group, mortality of mice was much lower than the other groups (P<0.05). No significant difference could be seen between heating group, Tween-80 group and control group (P>0.05). At the same time, we found the mice who had subjected to treatment of both