

## Brief Reports

# THE TREATMENT EFFECTS AND MECHANISM OF HIGHLY AGGLUTININATIVE STAPHYLOCOCCI, LOCAL THERAPY IN PATIENTS WITH SUPERFICIAL METASTATIC TUMOR

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Highly agglutinative staphylococcin (HAS) is a new biological response modifier used in tumor treatment recently. It has being used in clinic as an effective drug. The superficial metastatic tumor is easy to treat and take samples, so we treated 35 cases of superficial metastatic tumor with HAS, observed the treatment effects and tried to seek the mechanism involved.

## MATERIALS AND METHODS

Thirty-five cases with superficial metastatic tumor which diagnosed by pathology were treated in the First University Hospital from Nov. 1997 through Dec. 1998, including 22 cases of male and 13 cases of female. The ages varied from 35 to 71, 54 year old in average. These patients consisted of 17 lung cancers, 2 thyroid cancers, 2 gastric cancers, 2 ovarian cancers, 3 colonic cancers, 2 hepatic cancers, 2 nasopharyngeal cancers, 1 malignant lymphoma in nasal cavity, 3 esophagus cancers and 1 gall bladder cancer. The pathology types included 19 adenocarcinomas, 12 squamous carcinomas, and 4 the other types.

## Methods of Treatment

HAS (a product of Shenyang Xie He Company) of 1000 U per day was injected into and around the tumors though multiple points for 30 days. To avoid injecting into the blood vessels, it must pay attention to draw back the syringe to make sure there was no blood. If the patient suffered from a severe pain, intramuscular injection of

pethidine or local anesthesia of procaine could be used to release it. Furthermore, patients must be observed closely to prevent the side effects, such as bleeding, allergy, and shock. The changes of the volume of the tumors treated were analyzed by vernier calipers and B ultrasound.

## Flowcytometry

The tissue of the tumor treated was collected by needle biopsy. After that, the tumor tissue was prepared to single cell suspension at a concentration of  $1 \times 10^6$  cell/ml. Tumor cells in 50  $\mu$ l were suspended in 1 ml hypotonic fluorochrome solution of propidium iodide for 30 minutes. Tumor cells in 40  $\mu$ l suspension were incubated with 10  $\mu$ l CD95-PE monoclonal antibodies for 30 minutes, and measured by a flowcytometer (Coulter Company, USA). Blood in vein of the untreated and treated patients was taken, and prepared to  $1 \times 10^7$ /ml lymphocyte suspension. The lymphocyte suspension in 40  $\mu$ l was subsequently incubated with 20  $\mu$ l of fasL monoclonal antibodies (mouse against human antibody, primary antibody) for 1 hour and washed. Then 50  $\mu$ l of IgG-PE (rabbit against mouse antibody, second antibody) and 10  $\mu$ l of CD8-FITC antibody (mouse against human being) were added into it, incubating for 30 minutes. At last, the cells were measured by flowcytometry.

## RESULTS

After 1 week of treatment, a part of the patients suffered from a local response such as mild red, swollen

and pain. They were relieved automatically and needed no special treatment. No patients suffered from the side effects as allergy, shock and bone marrow depression. According to the standard of curative effect of solid tumor established by WHO, after a course of treatment, CR was seen in 1 case, PR in 10 cases, MR in 8 cases, and SD in 6 cases. The remission rate was 31.4% and the effective rate was 82.9%.

The results were analyzed by the software of Coulter Company. Before and after HAS treatment, the average percentage of cell cycle, apoptotic cells, expressions of fas antigen and average fasL expression in CD8<sup>+</sup> T lymphocytes were: G<sub>1</sub> phase (75.4±14.2)%, S phase (9.5±4.5)%, G<sub>2</sub>/M phase (4.6±2.1)%, AP peak (10.5±5.1)%, (0.46±0.11) FI, (0.54±0.14) FI and G<sub>1</sub> phase (45.6±12.6)%, S phase (8.4±3.7)%, G<sub>2</sub>/M phase (3.8±1.8)%, AP peak (41.3±10.3)%, (0.87±0.12) FI, (0.81±0.20) FI, respectively.

## DISCUSSION

HAS is extracted from the metabolite of a variant line of staphylococcus aureus with low toxicity and good effect. It's a new biological response modifier and has direct inhibition effect on the growth of tumor cells. However, the mechanism is not clear yet.

Superficial metastatic tumors are fit for local therapy, because reagents can be injected into or around the tumors accurately, and the tumor tissues can be easily obtained before or after treatment. In addition, observation of the changes of the tumor volume is objective and accurate by using vernier calipers and B ultrasound. The present study confirmed that HAS had remarkable

inhibition effects on tumors. After one month of treatment, the total remission rate is 31.4% and effective rate is 82.9%, having no obvious toxic side effect.

Flowcytometry is a new technique used expeditiously and easily, which can analyze cell cycle and apoptosis quantitatively. Furthermore, flowcytometry has a good correlation with other methods measuring cell apoptosis. In the present study, after treatment with HAS, the average percentage of apoptotic cells increased ( $P<0.01$ ), while percentage of cells in G<sub>1</sub>, G<sub>2</sub> and S/M phase decreased ( $P<0.05$ ), which shows that HAS had induced apoptosis in tumor cells and the apoptotic cells had come from G<sub>1</sub>, G<sub>2</sub> and S/M phase cells in the cell cycle. The mechanism of the regulation of apoptosis has not been clearly elucidated.

A signal molecular of cell death named as fas antigen or Apo-1 has been found recently. When fas binds to fasL, fas antigen can interact with MORT/FADD protein, then touching off the death trigger and opening the death channel. After series changes of signal transmission in cell, the cell apoptosis had happened. FasL is mainly found on the surface of activated T lymphocytes, so that T lymphocytes might attack the target cells expressed with fas antigen. For many tumor cells have low expressions of fas antigen, but high expression of fasL, they can avoid the immune reaction and grow without restriction. In our present study, after patients treated with HAS, the expression of fas antigen on the tumor cell had increased ( $P<0.01$ ) and expression of fasL on the peripheral CD8<sup>+</sup> T lymphocytes had also increased ( $P<0.01$ ). It suggests that the apoptosis of tumor cells may induced by opening the pathway of cell death, which is triggered by combining fasL on lymphocytes with fas antigen on tumor cells.

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