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INDUCTION OF APOPTOSIS IN S-180 AND S-180R TUMOR CELLS BY ADRIAMYCIN *IN VIVO*

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Apoptosis of tumor cells have become a new standard for chemotherapy. It is useful to demonstrate induction of apoptosis in tumor cells by anti-cancer drugs *in vivo*. We reported the results of apoptosis induction in murine tumor cell line S-180 and it's resistant cell line S-180R by adriamycin in different dose and different time. We found that apoptosis in S-180 cells could be induced by low dose of adriamycin, the apoptosis was started at 24 h. after the administration, and reached to 62.5% of the cells to apptosis until 72 h. Comparison with the parental cell line, only 13% of S-180R cells were apoptosed. At high dose, 20% of S-180R cells were apoptosed, whereas, almost all S-180 cells were killed in the same time. The lymphocytes were appeared in abdominal cavity of the mice after treatment of adriamycin for 24 h. It was very interested to find out that there was no lymphocyte left in the abdominal cavity of the mice with S-180R cells treated at high dose of adriamycin.

Key words: Apoptosis induction, S-180 and S-180R cell lines, Multidrug resistant, Adriamycin.

We have developed an adriamycin resistant murine tumor cell line S-180R *in vivo*.[1] This cell line is resistant to adriamycin for 66 folds, to VP16 for 9 folds, and the drug-pump ability was enhanced nearly 100 folds comparing with it's parental cell line S-180. All of these were majorly due to stable over-expression of mdr1 gene products, p-glycoproteins, P-170, which can pump out the anti-cancer drugs from the cells. We also found the mdr1 gene was amplified in a great extent, it's mRNA was over-expressed in S-180R cell line. Anyhow, from the evidences of DNA RNA, protein of mdr1 gene, showed that the S-180 cell line was stably inherited for more than 140 generations.

In this report, we demonstrated that induction of apoptosis for both cell lines, after treatment of adriamycin, analysis of its time course, comparison of dose response. The results indicated that adriamycin was a good inducer of apoptosis for the parental cell line S-180, but not for the resistanbt cell line S-180R. It must be treated by some other kind of anti-cancer drugs, otherwise, some side-effects may be acquired.

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MATERIALS AND METHODS

Experimental Animals

BABL/c mice (male, about 20 g) were provided

by animal Center of Chinese Academy of Medical Sciences (Beijing).

Cell Lines

S-180 murine tumor cell line was a gift of professor Wang Naiqin in Beijing Institute for Cancer Research. S-180R cell line was developed in our laboratory.

Reagents

Propidium iodide (PI) was purchased from Sigma Chemical CO.; Adriamycin from Farmitalia Carlo Erba, Italy.

Instrument

Flow Cytometer FACS 420 was used in Basic Research Institute of Traditional Chinese Medicine (Beijing).

Treatment of Mice with S-180 or S-180R Tumor Cells

S-180 or S-180R tumor cells (2×10^6) were inoculated into mice by injection abdominally. After 6 days, different dose (3.25 μg/mouse or 6.5 μg/mouse) of adriamycin were injected abdominally four time a day. At the time as indicated in Figure 1 and Figure 2, after the administration, 100 μl of cells were drawed from abdominal cavity, the cells were used to prepare samples for flow cytometry.

Preparation of Cells for Flow Cytometry

S-180 or S-180R cells (100 μl), taking from abdominal cavity, were washed twice with PBS, then fixed in 70% cold ethanol for 24 h. The supernatant was removed after centrifugation (1500 × g, 5 min). The cells were washed another two times with PBS. RNase A (1mg/ml) of 2 μl was added to the cells in 100 μl PBS. the reaction mixture was incubated at 37 ℃, for 45 min. with shaking peridically. PI (500 μg/ml) of 100 μl was added to the cells and PBS was added to 1 ml of total volume, the tubes were put at 4 ℃ in dark for 1 h. Finally, the cells were analyzed by flow cytometer FACS 420 with it's excitation at 488 nm and emission at 620 nm.

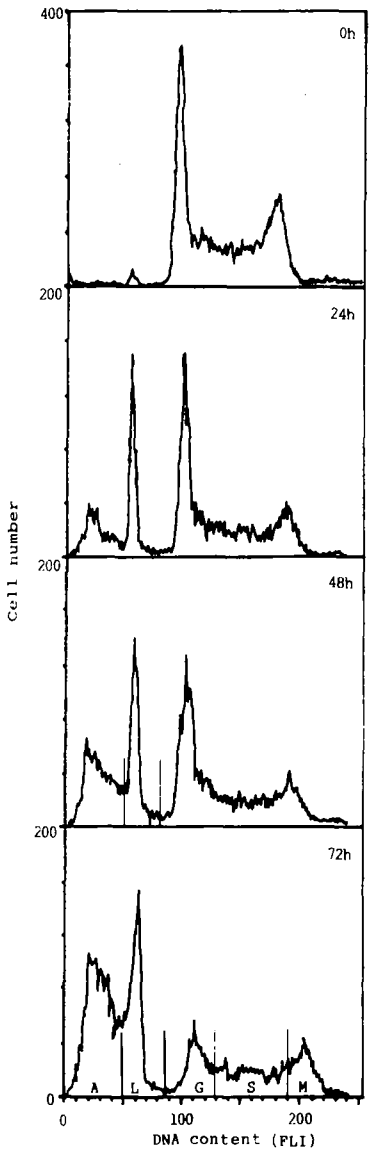

Fig. 1. Time course of apoptosis induced by adriamycin for S-180 cell line *in vivo* analyzed with cytometer FACS 420.

0, 24, 48, 72 present the hrs. after treatment.

A: apoptosis; L: lymphocyte; G: G_0/G_1 phases: S: sphase; M: G_2/M phases.

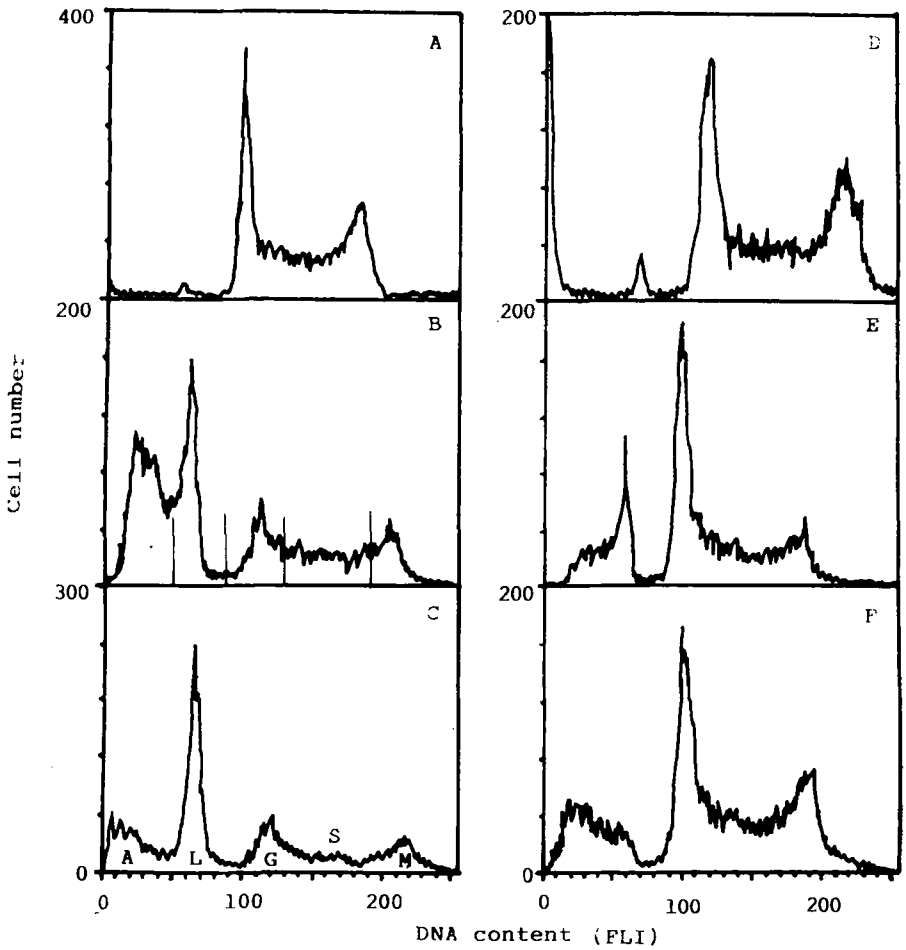

Fig. 2. comparison of apoptosis for S-180 cell line (A–C) and S-180R cell line (D–F).
A, D, for controls; B, E, for 3.25 μg/mouse adriamycin treated for 72 h. C, F, for 6.5 μg/mouse adriamycin treated for 72 h. A, L, G, S, M, as same as Figure 1.

RESULTS Time Course of Apoptosis Induced by Adriamycin for S-180 Cells

The mice with S-180 cells were treated by low dose of adriamycin (3.25 μg/mouse) four times a day. After 24, 48, and 72 h the S-180 cells, taking from abdominal cavity, were analyzed with flow cytometer FACS 420. as showed in figure, the cells could be divided into three groups, by its fluorescent intensity (channels). The first group of cells was apoptosed cells of S-180 in channels 0–50 (peak A); the second group of cells was lymphocytes of the mice in channels 50–85 (peak L); And the third group of cells in normal cell cycle above channels 85 (peaks G, S, M.). As only comparing the S-180 cells, i.e., only comparing the first and the thrid groups, the apoptosed S-180 cells were 0.5%, 8.3%, 38.1%, and 62.5% after administration of adriamycin at 0, 24, 48, and 72 h respoectively. S-180 cells in normal cell cycle were 99.5%, 91.7%, 61.9%, and 37.5% at 0, 24, 48, and 72 h respectively. In other words, along with the prolongation of the time course of administration of

adriamycin, the number of intact cancer cells were decreased and the apoptosed cells were increased continuously.

Differences of Apoptosis between S-180 and S-180R Cell Line

The 180 cells were very sensitive to adriamycin, even at low dosage, more than half of the cells were in apoptosis (62.5%) at 72 h (Figure 2B). At high dosage of adriamycin, most of the cells were dead, (Figure 2C). On the contrary, the S-180R cells were resistant to adriamycin. At low dosage, about 13% of the cells were in apotposis. At high dosage, about 20% of the cells were in apoptosis. Most of the cells wee still alive in normal cell cycle (Figure 2E, F).

Effects of Adriamycin Treatment on Lymphocytes in Abdominal Cavity of BABL/c Mice

The lymphocytes in the abdominal cavity of mice with S-180 cells were significantly enhanced after the treatment of adriamycin, both low dosage and high dosage, from 24 to 72 h (Fgirue 1, 24–72 h). But in the mice with S-180R cells, the lymphocytes in abdominal cavity were only appeared in low dosage of adriamycin treated mice, and the amount of the lymphocytes were not as much as that in the mice with S-180 cells (Figure 2E). It was very interested to find out that there was no lymphocyte left in the abdominal cavity of the mice with S-180R cells treated with high dosage of adriamycin (Figure 2F). On the contrary, only the lymphocytes were left, almost all the tumor cells disappeared in the abdominal cavity of the mice with S-180 cells with the same treatment. The experiments described above were repeated several times, all the results were similar.

DISCUSSION

In this paper, we represented apoptosis induction by adriamycin for S-180 and S-180R cell lines. We found that the apoptosis is required at least one day treatment with low dosage of adriamycin, and three days were required to reach to maximun. The second result showed that apoptosis response to adriamycin was quite different between S-180 and S-180R cell lines. The results suggested that adriamycin is a good apoptosis inducer for S-180 cell line, but not for S-180R cell line. They are consistent with the results of Ling.[2] However, we did the experiments *in vivo*, they did them *in vitro* and used different cell lines. Although so many papers described several genes involved in apoptosis, for instance, p53 and bcl-xs can stimulate apoptosis, bcl-2 can inhibite apoptosis in some tumor cell lines.[3–5] But the detailed mechanism of apoptosis is still not clear yet. In our case, the S-180R cell line resistants to apoptosis induced by adriamycin, no doubt, it si major because of p-glycoprotein, P-170, over-expression.[1] The third, lymphocytes come into the abdominal cavity to clean the dead tumor cells when treated with adriamycin. It is surprised to find that at high dose treatment, the lymphocytes in abdominal cavity of mice with S-180R cells disappeared, but in mice with S-180 cells, the most tumor cells dead, only the lymphocytes left in abdominal cavity. The S-180R cells might release some substances (including modified anti-cancer drugs) to inhibite the growth of lymphocytes or kill them in abdominal cavity. But what exactly happened? We do not know. If these might be true generally in humanbeings, it would be dangerous for patients, it seems like AIDS, which due to T-lymphocytes are killed by HIV.

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