In vivo Effects of Hyperthermia on the Cellular Uptake of Adriamycin

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The cellular uptake of Adriamycin (ADR) by solid tumors consisting of Ehrlich ascites tumor cells (EATC) was investigated. The intracellular levels of ADR in the EATC were measured by flow cytometry. The EATC tumors were heated at various temperatures for 30 min after the in vivo administration of ADR to the mice into which the EATC were inoculated. The amount of intracellular ADR in the EATC excised from the mice treated with 40°C hyperthermia was increased by 80% over that determined for those treated at body temperature. However, the quantity of ADR in the EATC excised from mice which received 43°C hyperthermia was similar to that for those treated at body temperature. These results indicate that combined therapy using hyperthermia and ADR has both synergic and additive effects, depending on the temperatures used for hyperthermia.

INTRODUCTION

The effects of hyperthermia alone or hyperthermia in combination with either radiation or anti neoplastic agents have been studied for possible means of treating cancers. The first two of these are currently used clinically. However, hyperthermia in combination with antineoplastic drugs has not yet been approved for clinical use.

There exists a controversy over the mechanism of action of chemotherapeutic agents in combination with hyperthermia in vivo. This concerns whether the combination of hyperthermia and chemotherapeutic agents produces a potentiating effect or, simply an additive effect1, 2).

Adriamycin (ADR) is a widely used chemotherapeutic agent for treating various types of cancer. The antineoplastic effect of ADR is known to occur after its uptake by cells; therefore, the potency of its effect can be estimated by measuring the intracellular levels of ADR3, 4). The changes in cellular uptake of ADR under the influence of hyperthermia in vivo were studied and are described in the present report.
MATERIALS AND METHODS

Experimental Tumor
Ehrlich Ascites Tumor Cells (EATC), $2 \times 10^6$ cells, were inoculated subcutaneously into the foot pads of 6-week-old ICR strain mice. After two weeks, the tumors which had grown to approximately $220 \pm 40 \text{ mm}^3$ were used in the subsequent experiments.

Drugs
ADR was provided by Falmitalia Carlo Erba Co.. The ADR, 10 mg/kg, was administered intravenously via the tail vein of each mouse prior to hyperthermia. The drug was always handled under a yellow light, because the cytotoxicity and fluorescence of ADR were decreased by fluorescent light.

Hyperthermia Treatment
Water baths were maintained at temperatures within a range of $\pm 0.05^\circ$C for the heat treatments of the mice. The heat treatments consisted of immersing the tumors of mice in the water baths.

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Fig. 1. X-Y plot of incorporated ADR in Ehrlich solid tumor cells. Abscissa: scatter (cell size), and ordinate: a fluorescence intensity of intracellular ADR in a cell. Blood cells were separated by using a gate of scatter.
Measurement of Intracellular Uptake of ADR

After treatment, each mouse was sacrificed by cervical dislocation, then its tumor was excised, minced and suspended in cold phosphate-buffered saline (PBS) for measurement of the intracellular quantity of ADR.

When ADR is excited by a 488 nm laser beam, it emits a characteristic fluorescence spectrum with peaks at 556nm and 582nm\(^5\). Using this characteristic of ADR, flow cytometry (FCM) was adapted to measure the intracellular levels of ADR\(^3,4\). Thus, the EATC were analyzed by excitation at 488nm, with emissions integrated above 530nm, which were selected by gating the scattered light from the cell suspension. Figure 1 shows the X-Y plot of the FCM. The data obtained were displayed in the form of a histogram with cell numbers plotted against fluorescence intensity.

![Graph showing fluorescent profiles of cellular ADR in Ehrlich tumor cells. ADR, 10 mg/kg, were administered intravenously. Profiles 1, 2, 3 and 4 represent those 1, 2, 3 and 4 hrs after the intravenous administrations, respectively. (FCM: Laser power; 900 mW, photomultiplier voltage; 630 V, Gain:16)
RESULTS

Time dependent changes in the EATC intracellular levels of ADR at body temperature

After the intravenous administration of ADR, a time course study of changes in the intracellular levels of ADR in the EATC was conducted, and the results are shown in Fig. 2. The intracellular levels of ADR in the EATC gradually and continually decreased when measured 1, 2, 3 and 4 hours after the ADR administration (100%, 80%, 69% and 51%, respectively). The intracellular levels of ADR were most readily detected one hour after the ADR administration. In the later experiments, changes in the intracellular levels of ADR were measured using the

![Graph showing fluorescent profiles of cellular ADR in Ehrlich tumor cells under hyperthermic conditions.](image)

ADR, 10 mg/kg, were administered intravenously. Hyperthermia was performed immediately after the administration of ADR for 30 min. Thereafter, the mice were kept at room temperature for 30 min, before being sacrificed. Profiles 1, 2 and 3 represent those of hyperthermia at 40°C, no hyperthermia and hyperthermia at 43°C, respectively. (FCM: Laser power; 900 mW, photomultiplier voltage; 630 V, Gain:8)
EATC excised one hour post ADR administration.

Changes in the EATC intracellular ADR levels of ADR after the application of hyperthermia

Fig. 3 shows the intracellular levels of ADR in the EATC, which were heated at various temperatures for 30 min after the in vivo administration of ADR to the mice. The amount of intracellular ADR in the EATC excised from the mice treated with 40°C hyperthermia increased by about 80% over that at body temperature (181%). However, the amount of ADR in the EATC excised from the mice treated with 43°C hyperthermia was similar to that at body temperature (102%).

DISCUSSION

Hyperthermia and antineoplastic drugs should result in synergic effects against cancer, but the agent selected holds the key to optimum anti-cancer effects, with minimum side effects. An abundance of basic research data support this aspect.

Combined hyperthermia and ADR produce synergistic effects in vitro due to increased permeability of the cell membrane, which results in the influx of ADR into the cell and, in turn, increments of intracellular levels of ADR\(^3\). Concerning the in vivo situation, there are two possible mechanisms of action of combined hyperthermia and ADR; one which results in a synergic effect\(^4\) and one which results in an additive effect\(^5\).

The reason for the disparity between the in vitro and in vivo results was clarified. The intracellular uptake of ADR, which is specifically indicative of the antineoplastic effect of ADR, was measured. As shown in Fig. 3, the intracellular uptake of ADR in the EATC excised from the mice treated with 40°C hyperthermia, increased by about 80% over that of those treated at body temperature. Furthermore, the intracellular level of ADR in the EATC treated at 43°C was similar to that of those treated at room temperature.

The discrepancy between the in vivo and in vitro results, can be explained. The rate of blood flow to the tumor at the time of hyperthermia must be considered. Blood flow measured at temperatures below 41°C reportedly showed either no change or a tendency to increase, but blood flow measured above 41°C was less than that at body temperature\(^7\). In the present study, the blood flow to the tumor and the cellular uptake of ADR were considered, as follows: the blood flow to the tumor using a combination of 40°C hyperthermia with ADR was similar to that at body temperature and, as in the in vitro study, the influx of ADR into the cell was increased by heat, resulting in an increase in ADR uptake. The ability of ADR to influx was increased, as in the in vitro study, when combined with hyperthermia at 43°C. However, the hyperthermia of 43°C reduced the blood flow to the tumor, resulting in a reduction of the amount of ADR reaching the EATC. Therefore, the increment in the intracellular levels of ADR did not seem to be attained.

The above observations demonstrated that combined hyperthermia and ADR therapy has both synergistic and additive effects, depending on the temperatures used.
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