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GALLIUM CITRATE, A NEW SENSITIZER OF CELLS TO HYPERTHERMIA

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The killing effects of heat were studied on cultured mammalian cells (L5178Y) pre-incubated with gallium (Ga) citrate, which is a popular tumor-imaging diagnostic agent. The cells showed higher sensitivity to heat when they were pre-incubated with Ga-citrate. The pre-incubated cells showed decreased ATP levels, and this may be responsible for the heat-sensitizing effect.

Key words: Cellular ATP level — Gallium citrate — Hyperthermia — L5178Y cells — Sensitizer

Hyperthermia is an attractive method of cancer therapy. Decrease in the treatment temperature markedly reduces discomfort to the patient, and thus the search for sensitizing agents is very important.

Gallium citrate has been demonstrated to accumulate in tumors[1,2] and in inflammatory lesions.[3] Since the antitumor activity of Ga-nitrate was first demonstrated in an animal study by Hart and Adamson[4] in 1971, the possible role of gallium in cancer treatment has been studied extensively.[5–11] These investigations have shown that cancer therapy by Ga-nitrate alone is not successful except for malignant lymphoma, although the side effects were very mild and well tolerated.[5] The drug has been suggested for combination therapy. We have examined the cytotoxic effects of Ga-citrate in combination with heat.

The methods for culture and the colony formation assay of mouse leukemic L5178Y cells have been described elsewhere.[12,13] The cells were pre-incubated at 37° in the presence or absence of 1.0m*M* Ga-citrate for 24 hr and washed with medium without Ga-citrate. The surviving fraction of the cells determined by colony formation assay was 0.379 ± 0.123 when the cells were treated with Ga-citrate. Then, the pre-incubated cells were transferred to a water bath for heating at various temperatures. Treatment times represent the total times of immersion in the water bath. ATP levels were measured just before heating by the luciferase method as described elsewhere.[14] The fraction of the cells stained with eosin Y was estimated by counting the cells after mixing two parts of cell suspension and one part of 1% eosin Y in 0.15*M* NaCl. The counting was performed not less than 2 minutes and not more than 10 minutes after mixing.[15]

Figure 1 shows the effects of heat (41.0°) on the cells with or without pre-treatment with 1.0m*M* Ga-citrate for 24 hr. The killing effects of heat at 41.0° were much higher on the cells pre-incubated in the presence of Ga-citrate. The survival of the gallium-treated cells at 41.0° was close to that of the cells without gallium at 42.5°, although the shapes of the curves were not the same.

Figure 2 shows the effects of heat alone or heat in combination with Ga-citrate on cell death determined by means of a dye-exclusion test. The cells pre-incubated with gallium and heated at 41.0° were stained with eosin Y much earlier than the cells without gallium heated at 41.0° or even at 44.0°.

Since it has been demonstrated that a decrease in cellular ATP levels results in higher sensitivity of the cells to hyperthermia,[16–20] we determined the cellular ATP levels. Table I shows that the ATP level of L5178Y cells pre-incubated with Ga-citrate was only 21.6 ± 11.1% of the control.

The present results showed a remarkable sensitizing effect of Ga-citrate on killing of cultured L5178Y cells by hyperthermia. The cause may be the decrease in cellular ATP levels (Table I) due to the pre-treatment with Ga-citrate, although there is a possibility of involvement of lysosomes, which accumulate gallium.[21,22]

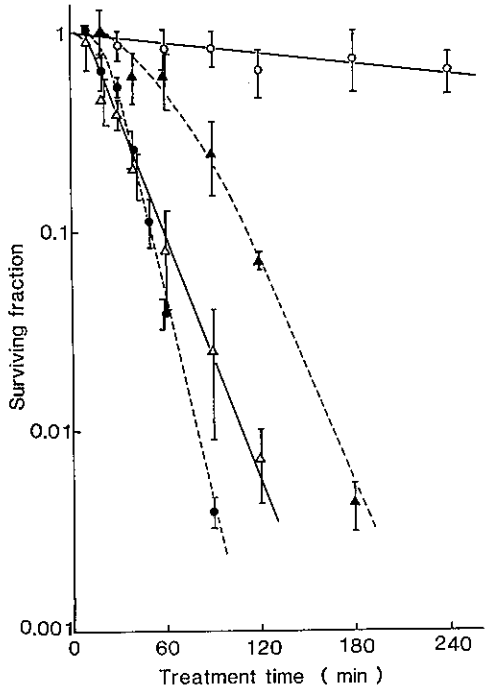

Fig. 1. Effects of heat treatment on colony-forming ability of L5178Y cells pre-incubated in the presence or absence of Ga-citrate. Cells were pre-incubated in the presence (△) or absence (○, ▲, ●) of 1.0m*M* Ga-citrate for 24 hr and heated in a water bath at 41.0° (○, △), 42.0° (▲), or 42.5° (●). Correction has been made for the toxicity of Ga-citrate (0.379±0.123) in the figure. The numbers of experiments were 5 and 3 for the experiments at 41.0°, and others, respectively. The plating efficiency of the control cells was 0.689±0.186.

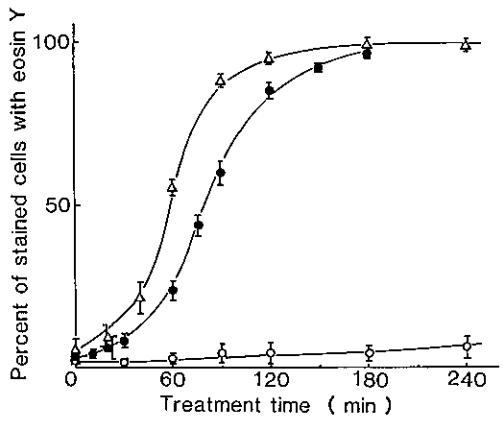

Fig. 2. Effects of heat-treatment on the lethality of L5178Y cells determined by means of the dye-exclusion test. Cells were pre-incubated in the presence (△), or absence (○, ●) of 1.0m*M* Ga-citrate for 24 hr and heated in a water bath at 41.0° (○, △) or 44.0° (●). No cell growth was observed during the heating. Total cell numbers were 80–90% of the control for the cells heated alone and 70–80% for the cells treated with Ga-citrate. The numbers of experiments were 4 in all cases.

Table I. Cellular ATP Levels of L5178Y Cells Treated with Ga-citrate

	Intracellular ATP	
	(fmol/cell)	(%)
Control	3.26±0.334	100
Ga-citrate treated[a)]	0.726±0.3805	21.6±11.06

a) The cells were treated with 1.0m*M* Ga-citrate at 37° for 24 hr.

The results of the dye-exclusion test showed that the cytotoxic effects of heat at 41.0° were higher on the cells pre-incubated with Ga-citrate than those of heat at 44.0° on the cells without gallium (Fig. 2), whereas those determined from the colony formation assay showed similar survival between the gallium-treated cells heated at 41.0° and the cells without gallium heated at 42.5° (Fig. 1). This discrepancy was observed when the cells were pre-treated with gallium citrate, though the cell death by heat alone measured in terms of colony-forming ability has been shown to be well correlated with that determined by means of the dye-exclusion test.[23] In addition to the above-mentioned difference, there was a difference in the shapes of the survival curves determined from the colony formation assay between the two conditions, heat at 42.5° alone and heat at 41.0° in combination with gallium (Fig. 1). Therefore, the mode of sensitization by gallium citrate may not be simply an increase in the killing efficiency of heat itself. The present results suggest the feasibility of clinical application of gallium citrate as a sensitizer.

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