POTENTIATION OF ANTICANCER EFFECT OF CARBOQUONE \textit{IN VIVO} BY GLUCOSE PRETREATMENT

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Anticancer effect of carboquone was notably potentiated in tumor-bearing mice when they were pretreated with 5\% glucose solution in the system of blood flow-interrupting hyperthermic chemotherapy. It was further found by quantitative analysis that the potentiation was much greater than increase in the general toxicity of the drug by administration of glucose. Mitomycin-C, on the other hand, revealed no such marked effect though it is considerably similar to carboquone in chemical structure.

A specific anticancer drug whose action \textit{in vivo} would be greatly potentiated by glucose pretreatment of the host has never been reported until our recent report concerning 5-fluorouracil O-\beta-D-glucuronide. Some authors, however, have speculated about the possible existence of such a drug.\textsuperscript{1, 4, 9}

In the mean time, an \textit{in vitro} screening study previously reported that carboquone is a drug whose anticancer action can be greatly potentiated by glycolysis in cancer cells.\textsuperscript{6} In our succeeding paper on a specially designed \textit{in vivo} experiment, on the other hand, no definite influence of glucose was found when the sugar solution, instead of phosphate buffer, was used as the vehicle for the drug.\textsuperscript{2} Quite surprisingly, however, the anticancer effect of carboquone was found to be greatly potentiated, if glucose is administered to the animals 30 min or more before carboquone treatment in the above \textit{in vivo} system.

Materials and Methods
Preliminary Chemotherapy Experiments: Two-month-old male DDD mice, 25--27 g in body weight, from the Animal Center of Kyushu University were used. Ehrlich tumor cells (10\textsuperscript{6}) were inoculated into the right hind-limb of each mouse. Four groups of 10 mice each, 3 test and 1 control groups, were used for Experiment No. 1, and 3 groups of 10 mice each, 2 test and 1 control groups, for Experiment No. 2. Exactly the same method for temporary occlusion of regional blood flow and for local hyperthermia as described before was employed. Blood-flow interruption for 60 min and local warming at 37\textdegree were applied only once to every test group (Table I, A, B, and C, and Table II, A and B) at 24 hr after tumor transplantation.

As anticancer drugs, carboquone (carbazilquinone or Esquinone, Sankyo Co., Tokyo) was used for Experiment No. 1, and mitomycin-C (Kyowa Hakko Kogyo Co., Tokyo) for Experiment No. 2. A dose of 0.5 mg/kg of each drug, at the rate of 0.4 ml/20 g body weight, was injected intravenously exactly 2 min before the limb-tourniqueting. Sterilized 5\% aqueous solution of glucose was used not only as the vehicle for the drug but also for pretreatment of the mice in specified test groups (Table I, A and B, and the Table II, A), while 0.1M phosphate buffer (pH 7.0) was used as the vehicle in other test groups (Table I, C, and Table II, B). Quantitative Chemotherapy Experiments: For the purpose of testing the change in toxicity of carboquone resulting from glucose pretreatment...
of the host, normal DDD mice of the same sex and weight as mentioned above, 10 animals per each carboquone dose group, were used. The dose of carboquone ranged from 1.5 to 4.5 mg/kg. All 10 groups of mice were observed daily and the percentage death rate 30 days after carboquone iv injection was compared between the groups of control and glucose pretreatment.

As for the quantitative estimation of potentiation of anticancer action of carboquone by glucose pretreatment, 10 DDD mice bearing Ehrlich tumor mentioned above were used for each dose group, which ranged from 0.125 to 1.25 mg/kg of carboquone. Blood-flow interrupting hyperthermic chemotherapy was applied to all 8 groups in the same way as mentioned above. Percentage cure rate of the tumor 4 weeks after carboquone treatment was compared between control and glucose pretreatment.

Results and Discussion

Preliminary Chemotherapy Experiments

Experiment No. 1: As indicated in Table I, the difference in the rate of tumor growth among the 4 groups was striking. In Group A, receiving both glucose pretreatment 4 times and carboquone injection, none of the 10 mice showed tumor growth at all at the end of 4 weeks. Even the effect of a single glucose pretreatment was evident (Group B). Phosphate-buffered saline (PBS) pretreatment (Group C), however, showed a markedly diminished antitumor effect and none of the mice showed complete regression of the tumor at this carboquone dose.

Experiment No. 2: The antitumor effect of mitomycin-C, on the other hand, was not potentiated by glucose pretreatment. As indicated in Table II, no difference in tumor growth inhibition by mitomycin-C was detected between Groups A and B, although the mice in Group A received both 4 times of glucose pretreatment and mitomycin-C, and those of Group B were given only mitomycin-C without glucose pretreatment.

Quantitative Chemotherapy Experiments: Since very interesting results were obtained in the Preliminary Experiment No. 1, experiments were then conducted to determine quantitatively how much the toxicity of carboquone for the mouse changed, as well as how much the anticancer action of carboquone was potentiated when accompanied by glucose pretreatment of the host. Increase in the toxicity of carboquone by glucose pretreatment was found to be slight as shown in Fig. 1. LD₅₀ of carboquone was 3.0 mg/kg in the control group, whereas it was 2.5 mg/kg in the glucose-pretreated group; namely, only 1.2-fold increase in toxicity resulted from glucose pretreatment.

Table I. Potentiation of Anticancer Effect of Carboquone by Glucose Pretreatment in vivo on Ehrlich Carcinoma in DDD Mouse Limb under Blood-flow Interrupting Hyperthermic Chemotherapy (37°, 60 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatmenta)</th>
<th>Pretreated ip with 1 ml of</th>
<th>Tumor growthb)</th>
<th>Tumor weightc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CQ 5% glucose</td>
<td>5% glucose (×4)</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>CQ 5% glucose</td>
<td>5% glucose (×1)</td>
<td>3/10</td>
<td>0.17±0.09</td>
</tr>
<tr>
<td>C</td>
<td>CQ PB</td>
<td>PBS</td>
<td>10/10</td>
<td>0.51±0.10</td>
</tr>
<tr>
<td>D</td>
<td>Untreated</td>
<td>PBS</td>
<td>10/10</td>
<td>2.28±0.16</td>
</tr>
</tbody>
</table>

CQ=carboquone, 0.5 mg/kg injected iv, PB=0.1 M phosphate buffer solution (pH 7.0), PBS=phosphate-buffered saline (pH 7.0).

a) Treatments were carried out 24 hr after tumor transplantation.
b) Four injections given 3, 2, and 1 hr, and 40 min before CQ injection.
c) Single ip injection given 40 min before CQ injection.
d) Number of tumor bearers/number of mice surviving 4 weeks after treatment.
GLUCOSE POTENTIATION OF CHEMOTHERAPY EFFECT

Table II. Anticancer Effect of Mitomycin-C Resulting from Glucose Pretreatment in vivo on Ehrlich Carcinoma in DDD Mouse Limb under Blood-flow Interrupting Hyperthermic Chemotherapy (37°, 60 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment(a)</th>
<th>Tumor growth(d)</th>
<th>Tumor weight (g, mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug</td>
<td>Vehicle</td>
<td>Pretreated ip with 1 ml of</td>
</tr>
<tr>
<td>A</td>
<td>MMC</td>
<td>5% glucose</td>
<td>5% glucose(b) (×4)</td>
</tr>
<tr>
<td>B</td>
<td>MMC</td>
<td>PB</td>
<td>PBS(c) (×1)</td>
</tr>
<tr>
<td>C</td>
<td>Untreated control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MMC=mitomycin-C, 0.5 mg/kg injected iv, PB=0.1M phosphate buffer solution (pH 7.0), PBS=phosphate-buffered saline (pH 7.0).

a) Treatments were carried out 24 hr after tumor transplantation.
b) Four injections given at 3, 2, and 1 hr, and 40 min before MMC injection.
c) Single ip injection given at 40 min before MMC injection.
d) Number of tumor bearers/number of mice surviving 4 weeks after treatment.

Fig. 1. Increase in general toxicity of carboquone (CQ) in DDD mice by their glucose pretreatment

- ○ 5% glucose solution (1 ml) injected ip at 3, 2, and 1 hr, and 40 min before drug injection.
- ● PBS (1 ml) injected ip at 3, 2, and 1 hr, and 40 min before drug injection.

The potentiation of anticancer action of carboquone by glucose pretreatment, on the other hand, was rather striking (Fig. 2). The dose of carboquone required to obtain 50% cure rate on tumor was as small as 0.175 mg/kg in the glucose pretreatment group, whereas it was 0.685 mg/kg in the control group; namely, the anticancer action of carboquone was potentiated nearly 4-fold when glucose pretreatment of the host was combined with the blood-flow interrupting hyperthermic chemotherapy.

Fig. 2. Potentiation of anticancer effect of carboquone (CQ) by glucose pretreatment of host on Ehrlich tumor of DDD mouse limb under blood-flow interrupting hyperthermic chemotherapy (37°, 60 min)

- ○ 5% glucose solution (1 ml) injected ip at 3, 2, and 1 hr, and 40 min before drug injection.
- ● PBS (1 ml) injected ip at 3, 2, and 1 hr, and 40 min before drug injection.

These experimental results correspond fairly well to those of our in vitro experiment reported previously.6) It should also be pointed out that, in the earlier studies including our previous one, such an excellent result has by no means been obtained with such a low dose of carboquone. Almost the same but somewhat poor therapeutic result as that of the present preliminary experiment was obtained using a double
dose of carboquone (1 mg/kg) in one of our previous experiments in which glucose pretreatment was omitted.\textsuperscript{2)}

It is very important to note that glucose, a simple substance which is available physiologically, can reduce the dose of the toxic drug for obtaining the same magnitude of anticancer action, if the sugar was optimally used. As for the mechanism of the potentiation of antitumor action of carboquone \textit{in vivo} by glucose pretreatment, much remains to be studied. Though we do not know the actual chemical mechanism, we can at least say that carboquone seems to be a unique agent which is excited in an acidic condition brought about in the tumor locus by the tumor cell glycolysis temporarily stimulated \textit{in vivo} by an artificially elevated blood-sugar level. It is well known that the difference in pH between tumor and normal tissues \textit{in vivo} is amplified by glucose administration owing to the selective decrease of pH in the former.\textsuperscript{5)} Furthermore, it has also been demonstrated by us that lactic acid content in a tumor increased about 10-fold when the temporary interruption of regional blood flow with local hyperthermia was applied to a tumor-bearing mouse which had been pretreated with glucose.\textsuperscript{7)} As far as chemical composition is concerned, carboquone consists of a quinone with two aziridines, while mitomycin-C has a urethan in addition to a quinone and an aziridine in its composition.\textsuperscript{8)}

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\textbf{References}


