Caffeine Enhances Adriamycin Antitumor Activity in Ehrlich Ascites Carcinoma-Bearing Mice

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We examined whether caffeine enhances the antitumor activity of adriamycin (ADR), in terms of prolonging survival in Ehrlich ascites carcinoma (ascites-type)-bearing mice. After administration of ADR at a dose of 0.5 mg/kg/d for 5 d together with caffeine (100 mg/kg/d × 5 d), the survival period was increased by 39%. However, caffeine had little effect on this ADR-induced prolongation of survival following administration of ADR at 2.0 mg/kg/d for 4 d.

Although a significant increase in ADR concentration in the ascites tumor was seen after administration of ADR at a dose of 0.5 mg/kg, caffeine failed to increase ADR concentration in the ascites tumor after administration of ADR at a dose of 2.0 mg/kg. The effect of caffeine thus appears to be due to its effect on the tumor distribution of ADR.

Keywords biochemical modulation; caffeine; adriamycin; Ehrlich ascites carcinoma; adriamycin concentration

While chemotherapeutic agents are extremely useful in the treatment of a variety of tumor types, their activity against tumors is limited. For more effective treatment, more powerful agents are needed, as well as biochemical modulators to enhance the effects of known antitumor agents.1–4) Studies of the effects of one such modulator, caffeine, have shown that it has an inhibitory effect on DNA repair5) and it appears to enhance the cytotoxic effects of antitumor agents.6–8) However, most studies have investigated the effects of combinations of cisplatin with caffeine in vitro,9,10) and there are few studies in vivo. Furthermore, the only antitumor agent used in these combinations with caffeine was cisplatin, as it was found that caffeine enhanced the antitumor activity of this agent much more than that of other agents in vitro.6) Accordingly, little work has been done on the effects of caffeine when given with other antitumor agents. We previously investigated the effects of combinations of adriamycin (ADR) and cisplatin with caffeine against Ehrlich ascites carcinoma in vivo, and found that caffeine enhanced the antitumor activity of ADR, rather than that of cisplatin.9) We speculated that these effects of caffeine were caused by its inhibition of efflux from tumor cells, rather than its inhibitory effect on DNA repair.11) As these actions were exhibited to a marked degree in only the tumor, caffeine did not increase the side-effects of ADR.11) In the present study, we have investigated the effects of treatment with combinations of ADR and caffeine on the survival rate of mice with Ehrlich ascites carcinoma. We have also investigated whether changes in the dose of ADR affect the action of caffeine as for as the ADR concentration in the tissues and the survival of the mice are concerned.

MATERIALS AND METHODS

Animals Male CDF1 mice, 6 weeks old and weighing 20–25 g, were obtained from Japan SLC Inc. (Hamamatsu). The animals were housed in a room maintained at 25 ± 1°C and 55 ± 5% relative humidity, and given free access to standard laboratory feed and water.

Reagents ADR (10 mg/vial, Adriacin®), and cisplatin (10 mg/20 ml vial, Randa®) for injection, were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo) and Nippon Kayaku Co., Ltd. (Tokyo), respectively. Caffeine monohydrate was obtained from Wako Pure Chemical Industries Ltd. (Tokyo). Other chemicals were of the highest purity available.

Apparatus Thin-layer chromatography (TLC) densitograms were obtained using a Shimadzu dual-wavelength TLC scanner (CS-910 type).

Animal Experiments To study the effects of caffeine on the ADR-induced reduction in lethality in Ehrlich ascites carcinoma-bearing mice, the male CDF1 mice were divided into 3–6 groups, each consisting of 10 mice. Ehrlich ascites carcinoma (1 × 10⁶ cells/animal) was inoculated intraperitoneally and drug administration was initiated the day after inoculation. The numbers of live and dead animals were recorded daily. Both single and multiple administrations were given, as described previously.4 For single administrations, we used ADR (5.0 mg/kg) or cisplatin (5.0 mg/kg) and caffeine (100 mg/kg/d × 7 d), and for multiple administration, we used ADR (2.0 mg/kg/d × 4 d) and caffeine (100 mg/kg/d × 4 d) or ADR (0.5 mg/kg/d × 5 d) and caffeine (100 mg/kg/d × 5 d).

To study the ADR concentration in tissues, male CDF1 mice were divided into 4 groups, each consisting of 6–7 mice, and Ehrlich ascites carcinoma (1.0 × 10⁶ cells/animal) was inoculated intraperitoneally. ADR (0.5 or 2.0 mg/kg/d) was administered intraperitoneally to the carcinoma-bearing mice 1, 3, 5 and 7 d after tumor inoculation, and caffeine (100 mg/kg/d) was injected intraperitoneally on the day following each of these time points. The animals were killed on the 2nd day after the final ADR administration and liver, heart, kidney, lung and ascites tumor were then rapidly removed. The tissues were homogenized in 10 vol (w/v) of 10 mm phosphate buffer (pH 7.8).

Determination of ADR Concentration ADR levels in the tissues were analyzed by TLC, as described by Shinohara et al.12)

Statistical Analysis The significance of any differences was evaluated by using Student’s t-test and the generalized Wilcoxon test.

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RESULTS

Effects of Caffeine on Antitumor Agent-Induced Prolonged Survival in Mice with Ehrlich Ascites Carcinoma

With the single administration schedule, both ADR (5.0 mg/kg) and cisplatin (5.0 mg/kg) significantly (p < 0.001) prolonged survival compared with the control group (Table I). However, caffeine did not enhance the effect of either of these antitumor agents.

Figure 1a shows the effects of caffeine on ADR-induced prolongation of survival in the multiple administration schedule for ADR (2.0 mg/kg/d × 4 d). The median number of survival days in the ADR-only group was significantly higher (p < 0.001) than in the control group, however, caffeine had no effect on this ADR-induced prolongation.

Figure 1b shows the effects of caffeine on ADR-induced prolongation of survival in the multiple administration schedule for ADR (0.5 mg/kg/d × 5 d). The median number of survival days in the control group was 9.8, while, in contrast, the number of survival days in the ADR-only group was increased to 26.5 (p < 0.001, compared with that of the control group); this period was increased significantly (p < 0.05, generalized Wilcoxon test) by caffeine administration (median number of survival days: 33.0), to 139% of that in the ADR-only group.

Effects of Caffeine on ADR Concentration in Tissues

Figure 2 shows the effects of caffeine on the ADR concentration in ascites tumor on the 2nd day after the final administration using the multiple administration schedule of ADR (2.0 or 0.5 mg/kg/d × 4 d). There were no differences between ADR concentrations in both the ADR-only and the caffeine + ADR group at a dose of 2.0 mg/kg of ADR. On the other hand, at a dose of 0.5 mg/kg of ADR, the ADR concentration in the ascites tumor in the caffeine ± ADR group was significantly increased (by 1.6-fold, p < 0.01) compared with that in the ADR-only group.

Following both doses, the ADR concentrations in normal tissues did not differ and there were no differences in these concentrations in the two dosage groups over an 8-d period after ADR treatment.

DISCUSSION

One mechanism by which antitumor agents exert their effects is by causing DNA damage in tumor cells. Thus, agents that inhibit DNA repair and act as biochemical modulators, have been the subject of much interest in recent years. It has been reported that 3-aminobenzamide, \( \beta \)-lapachone, nicotineamide, as well as other agents including caffeine, enhance the effects of antitumor agents. However, some of these results are contradictory and the effects of these modulators on the side effects of these antitumor agents have not been reported. Furthermore, these studies were only carried out \textit{in vitro}, and there are few reports of \textit{in vivo} effects. The antitumor agents used in combination with biochemical modulators were cisplatin and 5-fluourouracil.

There are, however, few reports on combinations with ADR, an agent that is used extensively.

Our previous study showed that caffeine enhanced the antitumor activity of ADR against solid Ehrlich ascites carcinoma \textit{in vivo} using a multiple administration rather than a single administration schedule. We suggested that the effect of this caffeine + ADR combination was due to an increase in ADR concentration in the tumor, and that the ADR-induced inhibition of DNA polymerases activity

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**Table I. Effects of Caffeine on the Prolongation of Antitumor Agent-Induced Survival**

<table>
<thead>
<tr>
<th>Group</th>
<th>Antitumor agent</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADR (5.0 mg/kg)</td>
<td>Cisplatin (5.0 mg/kg)</td>
</tr>
<tr>
<td>Control</td>
<td>12.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Antitumor agent only</td>
<td>29.0((a))</td>
<td>21.3((a))</td>
</tr>
<tr>
<td>Caffeine + antitumor agent</td>
<td>28.0((a))</td>
<td>20.0((a))</td>
</tr>
</tbody>
</table>

Values are median number of survival days. \(a\) p < 0.001 compared with the control group.

**Fig. 1. Effects of Caffeine on Antitumor Activity of ADR**

(a): caffeine 100 mg/kg/d × 4 d, ADR 2.0 mg/kg/d × 4 d; ○: control; O—O, caffeine; ■—■, ADR; □—□, ADR + caffeine. (b): caffeine 100 mg/kg/d × 5 d, ADR 0.5 mg/kg/d × 5 d; ○: control; ---, ADR; □—□, ADR ± caffeine.

**Fig. 2. Effects of Caffeine on ADR Concentration in the Ascites Tumor**

Ehrlich ascites carcinoma (1.0 × 10⁵ cells/animal) was inoculated intraperitoneally. ADR (0.5 or 2.0 mg/kg/d) was administered intraperitoneally to the carcinoma-bearing mice at 1, 3, 5, and 7 d after tumor inoculation, and caffeine (100 mg/kg/d) was injected intraperitoneally on the day following each of these time points. ADR concentration was determined in the ascites tumor on the 2nd day after the final ADR administration. \(a\) Significant differences from ADR-only values at p < 0.01. ■, ADR; □—□, ADR + caffeine.
and DNA biosynthesis were enhanced by caffeine; this effect was due to the caffeine reducing ADR efflux from the tumor cell.\textsuperscript{11} In that study, we examined the effects of caffeine on the reduction of tumor weight induced by ADR, while, here, we have examined whether caffeine enhanced the antitumor activity of ADR in terms of prolonging the survival of mice with Ehrlich ascites carcinoma. In our previous study,\textsuperscript{4} we found that caffeine enhanced the antitumor activity of ADR against solid tumors using both the single (ADR: 5.0 mg/kg) and multiple (ADR: 2.0 mg/kg/d × 4 d) administration schedules, but that it had no such effects on ascites tumors at the same dose. This difference between solid and Ehrlich ascites carcinoma tumors can be explained by the fact that the ascites tumors were directly exposed to ADR given by intraperitoneal administration hence the effect of ADR in these tumors was greater than that in solid tumors. Therefore, it was difficult to observe the effect of the combination with caffeine. On the other hand, in the multiple administration schedule (ADR: 0.5 mg/kg/d × 5 d, caffeine: 100 mg/kg/d × 5 d), caffeine enhanced the ADR-induced prolongation of survival, increasing the number of days by 39\% (p<0.05). Thus, the combination of caffeine with ADR significantly increases the antitumor activity of ADR in ascites, as well as solid, tumors. The dose of ADR and the administration schedule appear to be important as far as the effects of caffeine are concerned. Furthermore, similar effects were seen with low dose (10 mg/kg/d × 4 d, i.p.) caffeine (data not shown).

Since the mechanism responsible for the action of caffeine may be an increased ADR concentration in the tumor, we examined the effects of ADR dose on caffeine-induced increases in tissue ADR concentrations. In normal tissues, caffeine had no effect on ADR concentration at either dose (0.5 and 2.0 mg/kg) of ADR administered (data not shown). Administration in combination with caffeine had little effect on ADR concentration in the ascites tumor on the 2nd day after the end of ADR (2.0 mg/kg) administration; however, the ADR concentration in the ascites tumor at this time, after ADR (0.5 mg/kg) administration in the ADR + caffeine group, was 1.6-fold (p<0.01) that in the ADR-only group. These results support our previous finding that the caffeine + ADR combination has significant effects in solid tumors in ADR (2.0 mg/kg)-treated mice.\textsuperscript{4} At this ADR dose, there was little effect on the ascites tumors. However, after treatment with caffeine and ADR at 0.5 mg/kg, the ADR-induced prolonged survival of ascites carcinoma-bearing mice was increased. The results of our previous study,\textsuperscript{4,11} as mentioned above, indicated that caffeine increased the antitumor activity of ADR in solid tumors by enhancing reduction of DNA polymerases activity and inhibition of DNA biosynthesis induced by ADR; these phenomena were manifested by increases in ADR concentration in solid tumors.\textsuperscript{11} The results of the present study suggest that the mechanism of the effect of caffeine on ADR-induced antitumor activity in the ascites tumor is the same as that in solid tumors. Previously,\textsuperscript{4} we demonstrated that the combination of caffeine with ADR significantly increased the antitumor activity of ADR without increasing its side-effects. It is noteworthy that a significant increase in tissue ADR concentration in the tumor was observed after administration of a dose of 0.5 mg/kg, but that no caffeine-induced increase in ADR concentration was seen in normal tissues.

In conclusion, our results suggest that caffeine enhances the antitumor activity of ADR in ascites tumors and these effects are brought about by the action of caffeine in increasing the ADR concentration in the tumor cells. Furthermore, the mechanism of the increase in antitumor activity of ADR, without increasing its side-effects, in combination with caffeine and depending on the ADR dose, can be explained by the tumor distribution of ADR.

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REFERENCES