Increased Accumulation of Vincristine and Adriamycin in Drug-resistant P388 Tumor Cells following Incubation with Calcium Antagonists and Calmodulin Inhibitors

Takashi Tsuruo, Harumi Iida, Shigeru Tsukagoshi, and Yoshio Sakurai

Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Toshima-ku, Tokyo 170, Japan

ABSTRACT

Some calcium antagonists and calmodulin inhibitors enhance the intracellular levels of vincristine and Adriamycin in vincristine- and Adriamycin-resistant P388 leukemia cells by inhibiting their outward transport. The high intracellular drug accumulation was directly related to the enhancement of the cytotoxicity of the antitumor agents, and the vincristine and Adriamycin resistance in these cells was circumvented.

INTRODUCTION

The effect of some antitumor agents is related to the extent of their penetration into and their accumulation and retention within tumor cells (20). The acquired resistance of tumor cells to some agents, a crucial problem in cancer chemotherapy, is also related to intracellular drug accumulation and retention. For example, in VCR- and ADM-resistant tumor cell sublines, these agents can be shown to enter the cell but are actively transported to the outside. This results in a relatively low intracellular level of drug and thus to low cytotoxicity. Inhibition of drug efflux without interfering with internalization may enhance the cytotoxicity of antitumor agents and provide a mechanism to overcome this form of drug resistance in some tumor cells. We have reported that verapamil, a calcium antagonist, inhibits VCR efflux and enhances the cytotoxicity of VCR (24).

The present report concerns our finding that some calcium antagonists and calmodulin inhibitors enhance the intracellular levels of VCR and ADM in VCR- and ADM-resistant P388 leukemia cells which lead to circumventing drug resistance.

MATERIALS AND METHODS

Drugs. VCR and ADM formulated for clinical use were obtained from Shionogi Co., Ltd., Osaka, Japan, and Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan, respectively. [G-3H]VCR sulfate (6.3 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, England. [G-3H]ADM-HCl (76 mCi/mmol) was kindly contributed by Kyowa Hakko Kogyo Co., Ltd. Verapamil (5) and caroverine (10) are coronary vasodilators with calcium antagonist action and were supplied by the Eisai Co., Ltd., Tokyo, Japan, and Mitsubishi Chemical Industries Ltd., Tokyo, Japan, respectively. The other modifiers are calmodulin inhibitors.

Received December 4, 1981; accepted August 5, 1982.

The intracellular VCR and ADM levels in P388/VCR and P388/ADM cells increased following 60-min incubation with VCR or ADM in the presence of the modifier (Table 1). At a nontoxic dose of 6.6 μM of the modifier, the uptake of VCR and ADM was enhanced 2- to 5-fold and approximately 2-fold, respectively. During 10- to 60-min incubation, the uptake of VCR or ADM was linear.

The intracellular accumulation of VCR or ADM in the presence of the modifiers in growth medium or in the presence of sodium azide in glucose-free growth medium was rapidly reduced upon reincubation in the absence of modifiers. This outward efflux of the preloaded drugs was inhibited when the cells were reincubated with the modifiers (Chart 1). We suggest that the increased intracellular accumulation of VCR or ADM is at least partly due to the inhibition of extracellular (outflow) transport of the antitumor agents.

The increased intracellular levels of VCR and ADM were directly related to the marked enhancement of drug cytotoxicity (Table 2). It is important to note that, under the present experimental conditions, the modifiers were not directly cytotoxic to the tumor cells. Trifluoperazine exhibited a relatively small effect as compared to the other modifiers. This was due to the fact that its concentration was low due to cytotoxicity. IC50 of VCR against parent P388 cells was approximately 1.4 nM. Our data demonstrate that in vivo resistance to VCR was circumvented when P388/ADM cells were simultaneously exposed to VCR or ADM inhibition of extracellular (outflow) transport of the antitumor agents.

All of the modifiers, when used at the same concentration,

Table 1

<table>
<thead>
<tr>
<th>Modifier</th>
<th>Cellular accumulation of VCR in P388/VCR cells (pmol/10^5 cells)</th>
<th>Cellular accumulation of ADM in P388/ADM cells (pmol/10^5 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>1.12 (4.6)</td>
<td>33.6 (2.4)</td>
</tr>
<tr>
<td>Caroverine</td>
<td>0.66 (2.9)</td>
<td>24.2 (1.7)</td>
</tr>
<tr>
<td>Prenylamine</td>
<td>0.80 (3.5)</td>
<td>32.3 (2.3)</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>1.16 (6.5)</td>
<td>27.6 (2.0)</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>1.04 (4.5)</td>
<td>23.9 (1.7)</td>
</tr>
<tr>
<td>No. 233</td>
<td>0.37 (1.6)</td>
<td>16.6 (1.2)</td>
</tr>
<tr>
<td>Control</td>
<td>0.23</td>
<td>13.8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, increase (x-fold) in the intracellular amount of the antitumor agents as compared to the control (without modifier).

Caused about the same degree of accumulation (Table 1) and roughly the same degree of enhancement of cytotoxicity of VCR and ADM (Table 2). However, the calcium antagonists verapamil and caroverine showed rather weak inhibitory activity against calmodulin (Table 3) as determined by in vitro enzyme levels. These results appear to indicate that the effectiveness of all of the modifiers in circumventing VCR and ADM resistance.
at cellular levels is not directly related to their effectiveness as calmodulin inhibitors which was estimated in in vitro enzyme levels.

The actual mechanisms involved in the inhibition of drug efflux remain to be determined; however, we speculate several possible mechanisms to explain our results. First, the efflux of antitumor agents from cells may be controlled, possibly by calcium and calmodulin (calcium-calmodulin complex). Calcium antagonists might lead to a poor formation of calcium-calmodulin complex by lowering the intracellular calcium concentration, and calmodulin inhibitors could directly inhibit calcium-calmodulin complex. In both cases, the efflux of the drug might be adversely affected. A possible explanation for the superior effectiveness of calcium antagonists is that the modification of intracellular calcium environment by calcium antagonists might lead to a prominent inhibition of the formation of calcium-calmodulin complex, since it is well known that intracellular calcium concentration is usually maintained at very low levels when compared to the extracellular concentration (19). Like calcium antagonists, calmodulin is also involved in cellular calcium transport (7, 8, 13, 14). We have not directly measured calcium fluxes, calcium levels, or calmodulin levels; however, we believe that the cellular calcium environment may play an important role in the manifestation of the observed phenomenon.

Secondly, it might be possible that the energy-dependent drug efflux system in the membrane might be perturbed by the modifiers or by the change in calcium environment. The hydrophobic nature of calmodulin inhibitors or calcium antagonists might be related to the inhibition of energy-dependent drug efflux system, thereby potentiating the toxicity of VCR and ADM. Additionally, diMarco (4) reported that the calcium-sequestering agent EDTA caused a marked increase of ADM sequestration in tumor cells. He speculated about 2 possibilities: (a) sustained Ca**2+ deficiency induces damage to the cell membrane and to the mechanism of drug extrusion; (b) calcium deficiency may affect Ca**2+-H**+ exchange on the membrane, which is related to the extrusion of anthracyclines. At the present time, these possibilities regarding the perturbation of the energy-dependent drug efflux system cannot be overlooked.

Thirdly, the affinity of VCR for intracellular targets may be altered by calcium antagonist-induced changes in the calcium environment or by calmodulin inhibitors. The function of microtubules, the target of VCR, is known to be changed by calcium and calmodulin (16, 29, 30). Because the effects we observed with VCR were more pronounced than those with ADM (Table 2), this possibility must be considered at the present.

In any event, the elucidation of the structural and functional interactions between the intracellular calcium environment and calmodulin and the drug transport functions of the cell membrane could be of importance in cancer chemotherapy (25, 26). At present, we can only speculate upon the possibility of identifying clinically effective calcium antagonists and calmodulin inhibitors to achieve promotion of drug responsiveness.

ACKNOWLEDGMENTS

We thank Dr. I. J. Fidler for his critical reading of the manuscript. We are indebted to M. Shimizu for preparing the typescript.

REFERENCES

17. Nishimura, T., Suzuki, H., Muto, K., and Tanaka, N. Mechanism of Adria-


### Increased Accumulation of Vincristine and Adriamycin in Drug-resistant P388 Tumor Cells following Incubation with Calcium Antagonists and Calmodulin Inhibitors

Takashi Tsuruo, Harumi Iida, Shigeru Tsukagoshi, et al.


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: <a href="http://cancerres.aacrjournals.org/content/42/11/4730">http://cancerres.aacrjournals.org/content/42/11/4730</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>E-mail alerts</td>
<td>Sign up to receive free email-alerts related to this article or journal.</td>
</tr>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, contact the AACR Publications Department at <a href="mailto:permissions@aacr.org">permissions@aacr.org</a>.</td>
</tr>
</tbody>
</table>