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

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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 these agents, a crucial problem in cancer chemotherapy, is also related to intracellular drug accumulation and retention (1, 3, 6, 9, 17, 18, 21, 22, 24). 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 (3, 9, 17, 21, 22, 24) 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 vincristine and Adriamycin 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 Radiocchemical Centre, Amersham, England. [G-3H]ADM-HCl (76 Ci/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 Elsai Co., Ltd., Tokyo, Japan, and Mitsubishi Chemical Industries Ltd., Tokyo, Japan, respectively. The other modifiers are calmodulin inhibitors.

1 This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture (65801019) and from the Ministry of Health and Welfare (54-5), Japan.
2 Recipient of an Award from Society for Promotion of Cancer Research, Japan. To whom requests for reprints should be addressed.
3 The abbreviations used are: VCR, vincristine; ADM, Adriamycin; P388/VCR P388 leukemia cells resistant to VCR; P388/ADM, P388 leukemia cells resistant to ADM; ICso, concentration of drug required for 50% inhibition of cell growth or enzyme activity.

Received December 4, 1981; accepted August 5, 1982.
ase (Sigma), 0.2 μg calmodulin (Sigma), and graded modifier concentrations. Modifier and calmodulin (final volume, 0.4 ml) were preincubated for 5 min at 30°C in the presence of buffer, magnesium acetate, and calcium; reactions were started by adding 0.1 ml of the phosphodiesterase and cyclic adenosine 3':5' monophosphate mixture. After a 30-min incubation at 30°C, phosphodiesterase activity was determined as described previously (11, 27), and IC50 was estimated.

RESULTS AND DISCUSSION

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 modifiers (Table 1). At a nontoxic dose of 6.6 μM of the modifiers, the cellular 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 gradually 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 vitro resistance to VCR was circumvented when P388/ADM cells were simultaneously exposed to VCR and a nontoxic dose of modifiers (Table 2). The cytotoxicity mediated by ADM was also enhanced by these modifiers. IC50 of ADM against parent P388 cells was 21 nM. ADM resistance was partially overcome in P388/ADM cells because they are highly resistant (Table 2). The cytotoxicity of VCR and ADM was also enhanced in P388 cells. The degree of enhancement, however, was lower as compared to P388/VCR and P388/ADM cells.

All of the modifiers, when used at the same concentration, 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 exodus 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 exodus 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 at cellular levels is not directly related to their effectiveness as calmodulin inhibitors which was estimated in in vitro enzyme levels.

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Calcium Modifiers on VCR and ADM Accumulation


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