Tyrophostin 4-Nitrobenzylidene Malononitrile Reduces Chemotherapy Toxicity without Impairing Efficacy

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ABSTRACT

In mice, 4-nitrobenzylidene malononitrile (AG1714), which belongs to the tyrophostin family, reduced toxicity induced by doxorubicin and cisplatin without impairing their antitumor efficacy. AG1714 reduced mortality induced by doxorubicin and cisplatin. It prevented, in a dose-dependent manner, cisplatin-induced nephrotoxicity as assessed by measurement of serum creatinine and blood urea nitrogen levels. The protective effect of AG1714 was most pronounced on its administration 2 h before cisplatin. AG1714 also prevented doxorubicin-induced myelosuppression as assessed by the scoring of bone marrow nucleated cells and colony-forming units. Cisplatin-induced small intestinal injury was also protected by AG1714 as assessed by histopathological analysis. In vitro, AG1714 reduced cisplatin-induced apoptosis in a murine fibroblastic cell line (A9) and did not affect doxorubicin-induced apoptosis of B-16 melanoma cells. In contrast to its protective effect against mortality and injury of normal tissues induced by chemotherapy, AG1714 did not impair its antitumor activity and in some tumor models enhanced it. This was evident by using the murine tumors B-16 melanoma, Lewis lung carcinoma, and methylcholanthrene-induced fibrosarcoma and the human tumors SK-28 melanoma and human ovary carcinoma xenografts in nude mice. Experiments in which low and high doses of cisplatin and doxorubicin were administered to tumor-bearing mice demonstrated that AG1714 reduced mortality of high-dose chemotherapy and increased its therapeutic index. AG1714 could provide a novel, useful tool to improve chemotherapy by allowing dose intensification.

INTRODUCTION

Most of the commonly used antineoplastic drugs produce toxicity in organs composed of self-renewing cell populations such as the bone marrow (1) and gastrointestinal tract epithelium (2). Infertility can also follow chemotherapy (3). Some antitumor agents are associated with more specific toxicity effects, such as the cardiomyopathy of doxorubicin (4), nephrotoxicity of cisplatin (5), and pulmonary fibrosis associated with bleomycin (6). A major challenge for medical oncology is to develop therapeutic modalities that will prevent toxicity induced by antineoplastic treatments without impairing their antitumor effect.

Different strategies have been developed previously to prevent toxicity of anticancer drugs. Some of them were based on thiol compounds such as 2-mercaptopetethanol sulfonic acid, which is used to prevent nephrotoxicity induced by ifosfamide (7), and amifostine (WR-2721), an aminophosphonothioate compound that in vivo selectively protects normal tissues against chemotherapy toxicity (8). Its selective effect is attributed to the cell surface phosphatase in normal cells that generates a free thiol, which is the active form of this drug. Protection of anthracycline-induced cardiac toxicity could be prevented by the iron-chelator ICRF-187 (9) and by the antioxidant prophylactic without interfering with its antitumor effect (10). These drugs reduce the generation of and the effects of free radicals that are involved in doxorubicin-induced cardiomyopathy. Alterations in pharmacokinetics of anticancer drugs may affect their toxicity and efficacy (11). The toxicity of selected chemotherapeutic agents is decreased by the use of continuous infusion chemotherapy without loss of antitumor efficacy (12). Another strategy to reduce chemotherapy-induced toxicity, mainly its suppression of bone marrow, is the clinical application of hematopoiesis growth factors such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (13).

Recent data indicate that the antitumor effects of a variety of structurally unrelated chemotherapeutic agents are attributed to their ability to induce apoptosis (14, 15). The toxicity of these agents may also be related to the induction of apoptosis in normal cells (16) including small intestinal epithelium (17), proximal tubular epithelial cells (18), and thymocytes (19).

Selective interception of cellular pathways that culminate in apoptosis may provide an effective strategy for increasing the therapeutic index of the anticancer agents. Tyrosine phosphorylation was implicated in the mediation of apoptosis, induced by a variety of means (20–22). On the other hand, inhibitors of tyrosine kinases were reported to induce apoptosis (23). The molecular targets of the tyrosine kinase inhibitors that affect apoptosis are not yet defined. Tyrophostins are specific inhibitors of tyrosine kinases that selectively inhibit distinct signal transduction pathways (24) and thus could selectively modulate apoptosis in different cell types.

We hereby report the protective effect of AG1714,2 which belongs to the tyrophostin family, against toxicity induced by antineoplastic drugs without impairing their antitumor efficacy.

MATERIALS AND METHODS

Materials. Doxorubicin hydrochloride was obtained from Farmatia (Milano, Italy) or from Sigma Chemical Co. Cisplatin was obtained from Sigma. AG1714 was synthesized as described (25) and was obtained from Drs. A. Levituki and A. Gazit (Department of Biological Chemistry, The Hebrew University of Jerusalem, Israel) or from Ouro Chemicals (Jerusalem, Israel). AG1714 was dissolved in propylene carbonate:cremophore (40:60, v/v, at a concentration of 20 mg/ml (stock solution)). Before injection, stock solution was diluted 1:20 with sodium chloride (0.9%), containing sodium bicarbonate (0.8%).

Mice. CD1 female mice and athymic (nu/nu) female mice, all 6–8 weeks of age, were obtained from Harlan, Israel. C57BL female mice, 6–8 weeks of age, were obtained from the Animal Breeding Center at Tel Aviv University.

Histological Sections. Animals were sacrificed by ether. Organs were formalin fixed and paraffin embedded. Sections were stained with H&E-light green.

Tumor Models. Three mouse tumors in C57BL mice were used: B-16 melanoma, 3LL (a Lewis lung carcinoma), and MCA-105 (a methylcholanthrene-induced fibrosarcoma). Two human tumor xenografts in nude mice were used: SK-28 melanoma and OVCAR-3, a human ovarian carcinoma.

B-16, 3LL, and MCA-105 were grown in culture and obtained from a large stock of frozen cells. The stock was prepared from in vivo s.c. growth in C57BL mice, except MCA-105, which was obtained from p.p. growth.

2 The abbreviations used are: AG1714, 4-nitrobenzylidene malononitrile; CFU, colony-forming unit.

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The human tumor cell line SK-28 was propagated in CD1 nude mice by s.c. growth and OVCAR-3 by i.p. growth. B-16 cells were injected (i.v.) at $5 \times 10^4$ cells/mouse, 3LL cells by injection (i.v.) of $2 \times 10^5$ cells/mouse, MCA-105 cells by injection (i.v.) of $2 \times 10^5$ cells/mouse, SK-28 cells by injection (i.v.) of $4 \times 10^6$ cells/mouse, and OVCAR-3 cells by injection (s.c.) of $3 \times 10^6$ cells/site.

**Blood Chemistry.** Serum creatinine levels were determined by the picric acid colorimetric method using reagents from Sigma Diagnostics. Serum urea nitrogen (blood urea nitrogen) levels were determined using a Hitachi analyzer.

**Mouse Bone Marrow Cells.** Bone marrow cells were obtained from femur, suspended in PBS, diluted in Turk solution and counted manually, using a hemocytometer. The number of CFUs were determined using a semisolid agar assay as described (26).

Iscove's modified Dulbecco's medium containing FCS (15%) and 2-mercaptoethanol ($5 \times 10^{-5}$ M) was used for preparation of condition medium. Condition medium was obtained by incubation for 72 h of lungs from mice that had received injections of lipopolysaccharide. Bone marrow cells were incubated in semisolid agar medium containing condition medium (10%), FCS (5%), and 2-mercaptoethanol ($5 \times 10^{-5}$ M). Colonies containing clusters of $>50$ cells were counted after incubation for 12 days.

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**Fig. 2.** AG1714 reduces doxorubicin and cisplatin-induced mortality. CD1 mice received injections (i.p.) of 20 mg/kg doxorubicin (A) or 14 mg/kg cisplatin (B). AG1714 (20 mg/kg) or the vehicle used for its solubilization was injected (i.p.) 2 h before chemotherapy. Mortality was monitored daily. The differences in mortality between mice injected with doxorubicin alone and doxorubicin and AG1714 ($P < 0.001$) and between mice injected with cisplatin alone and cisplatin and AG1714 ($P < 0.005$) were statistically significant.

**Fig. 3.** AG1714 reduces cisplatin-induced nephrotoxicity. CD1 mice received injections (i.p.) of 14 mg/kg cisplatin. AG1714 (10 mg/kg) or the vehicle used for its solubilization was injected (i.p.) 2 h before cisplatin. Serum creatinine and blood urea nitrogen were determined 4 days after cisplatin administration. Eight to 27 mice (in three to eight separate experiments) were used in each group for the determination of creatinine levels, and 3 mice were used in each group for the determination of blood urea nitrogen levels (n. total number of animals used in each group). The results are expressed as the mean ± SD. The differences in creatinine and blood urea nitrogen levels in mice treated with cisplatin alone versus cisplatin and AG1714 were statistically significant ($P < 0.001$ and $P < 0.02$, respectively).
Fig. 4. AG1714 reduces doxorubicin-induced myelosuppression. CD1 mice received injections (i.p.) of 10 mg/kg doxorubicin. AG1714 (20 mg/kg) or the vehicle used for its solubilization was injected 2 h before doxorubicin. After 3 days, the number of femur bone marrow nucleated cells (A) and CFUs (B) was determined as described in “Materials and Methods.” Nine to 21 mice (in three to six separate experiments) were used in each group for the determination of bone marrow CFUs (n, total number of animals used in each group). The results are expressed as the means; bars, SD. The differences between the mice receiving injections of doxorubicin alone and the mice receiving injections of doxorubicin and AG1714 were statistically significant (P < 0.001) for both bone marrow nucleated cells and CFUs.

Statistical Analysis. Results are expressed as means ± SE or ± SD. Differences between means were determined using Student’s t test. The survival of different groups of mice was analyzed by χ² and Fisher’s exact test, depending on the sample size. The results are considered as significant where P < 0.05.

RESULTS

AG1714. Fig. 1 depicts the structure of AG1714 used in this study.

Prevention by AG1714 of Doxorubicin and Cisplatin-induced Mortality. Administration (i.p.) of AG1714 (20 mg/kg) to CD1 mice, 2 h before injection (i.p.) of doxorubicin (20 mg/kg), markedly prevented mortality induced by the antitumor drug. Under the experimental conditions used, 90% of the animals died within 7 days after treatment with doxorubicin alone, whereas only 10% of the animals treated with AG1714 before doxorubicin died within that period (Fig. 2A). The surviving animals were followed for an additional 20 days and did not show any ill effects.

AG1714 was also effective in reducing cisplatin-induced mortality. Under similar experimental conditions, mortality within 9 days after administration of cisplatin (14 mg/kg) was 80%, whereas mortality of the mice pretreated with AG1714 was 10% (Fig. 2B).

AG1714 also reduced mortality induced by repeated administration of doxorubicin at low doses. After 10 injections (i.p.) of doxorubicin at 5 mg/kg over 21 days (cumulative dose, 50 mg/kg), four of five mice died. The mortality of mice treated as above and receiving injections (i.p.) of AG1714 (5 mg/kg) 2 h before each doxorubicin injection was reduced to one of five.

Protective Effect of AG1714 against Cisplatin-induced Nephrotoxicity. An important side effect of cisplatin chemotherapy is nephrotoxicity, which results from injury to renal tubular epithelial cells. We investigated the effect of AG1714 on cisplatin-induced nephrotoxicity.

Nephrotoxicity was induced by administration (i.p.) of cisplatin (14 mg/kg) to mice and was manifested 4 days later by an increase in serum levels of creatinine and urea nitrogen (blood urea nitrogen). Treatment of mice with AG1714 (20 mg/kg) 2 h before injection of cisplatin (14 mg/kg) essentially prevented the nephrotoxic effects of cisplatin (Fig. 3).

The protective effect of AG1714 against cisplatin-induced nephrotoxicity was dose dependent. AG1714, at doses of 5 and 10 mg/kg, was essentially as effective as at 20 mg/kg. Under the experimental conditions outlined in Fig. 3, serum creatinine levels of mice that had
Protection by AG1714 of Doxorubicin-induced Myelosuppression. One of the major toxic effects of doxorubicin is the suppression of bone marrow. We have investigated the effect of AG1714 on doxorubicin-induced myelosuppression in mice. This was assessed by

Fig. 6. AG1714 protects against cisplatin-induced small intestine injury: histopathological analysis. CD1 mice received injections (i.p.) with 14 mg/kg cisplatin. AG1714 (20 mg/kg) or the vehicle used for its solubilization was injected (i.p.) 2 h before cisplatin. Histopathological analysis was done 4 days after cisplatin administration. Segments of the small intestine (ileum) were fixed, and sections were stained as described in "Materials and Methods." Two separate experiments were performed. Three mice were included in each group. Representative sections are presented: A. control; B. cisplatin; and C. AG1714 + cisplatin. ×400.

Fig. 7. Effect of chemotherapy and AG1714 on established lung metastases of murine tumors and human tumor xenografts in nude mice. MCA-105 fibrosarcoma (A), 3LL (B), and B-16 melanoma (C) cells were injected into the tail vein of C57BL mice as outlined in "Materials and Methods." Cisplatin (4 mg/kg) was injected (i.p.) 4 days after tumor inoculation. AG1714 (20 mg/kg) or the vehicle used for its solubilization was injected (i.p.) 2 h before cisplatin. Twenty-four days after tumor inoculation, mice were sacrificed, the lungs were weighed, and the number of metastases were scored. Each group contained five mice, and the results are expressed as the means of the weights of the lungs; bars, SE. The difference between mice treated with cisplatin alone and mice treated with cisplatin and AG1714 was statistically significant (P < 0.05) for 3LL. CD1 athymic mice (nu/nu) were injected (i.v.) with SK-28 human melanoma (D). Doxorubicin (4 mg/kg) was injected (i.p.) 4 days after tumor inoculation. AG1714 (20 mg/kg) or the vehicle used for its solubilization was injected 2 h before doxorubicin. The weights of the lungs and number of metastases were determined 24 days after tumor inoculation. Each group contained five mice, and the results are expressed as means; bars, SE. Each experiment was repeated at least once, and a similar pattern was observed.
and 59%, respectively (Fig. 5A). Pretreatment with AG 1714 resulted in a 47% reduction in the number of nucleated cells and a 55% reduction in the weight of the lungs of mice treated with AG 1714 and doxorubicin, bone marrow cells were reduced by 15% and 22 (dead/total) in mice that were treated with high-dose chemotherapy with AG 1714. The difference in mortality between the two groups was statistically significant (P < 0.05).

The difference between the weight of the lungs of mice treated with doxorubicin (8 mg/kg) + AG 1714 (20 mg/kg) and the weight of the lungs of mice treated with doxorubicin (4 mg/kg) alone or doxorubicin (4 mg/kg) + AG 1714 (20 mg/kg) was statistically significant (P < 0.001).

Mice were weighed. Mice died between 4 and 9 days after doxorubicin administration. AG 1714 was injected (i.p.) 2 h before chemotherapy.

Doxorubicin was injected (i.p.) 4 days after tumor inoculation.

The difference between the weight of the lungs of mice treated with doxorubicin (10 mg/kg × 2) + AG 1714 (20 mg/kg × 2) and the weight of the lungs of mice treated with cisplatin (4 mg/kg × 2) and AG 1714 (20 mg/kg) was statistically significant (P < 0.001).

Mice died between 7 and 10 days after doxorubicin administration. AG 1714 was injected (i.p.) 2 h before chemotherapy.

Doxorubicin was injected (i.p.) 4 days after tumor inoculation.

The difference between the weight of the lungs of mice treated with doxorubicin (8 mg/kg) and doxorubicin at 4 mg/kg, 2 h before doxorubicin injection were fully protected against its myelosuppressive effects. The effect of AG 1714 on myelosuppression induced by doxorubicin at different doses was investigated. Doxorubicin at 10 and 15 mg/kg induced a 55% and 84% reduction of nucleated bone marrow cells, respectively. In contrast, mice that were pretreated with doxorubicin and AG 1714 fully reconstituted their bone marrow at that time (Fig. 5B).

AG 1714 Prevents Cisplatin-induced Small Intestine Injury. Gastrointestinal injury is a common condition that affects a proportion of cancer patients who receive chemotherapeutic drugs, and it has become the dose-limiting toxicity of a number of chemotherapeutic protocols.

Administration of cisplatin (14 mg/kg) to mice, followed 4 days later by histological analysis, revealed a severe injury to the small intestinal epithelium. The injury was manifested by an extensive disintegration of the brush border small intestinal epithelium (Fig. 6B). Administration of AG 1714 (20 mg/kg), 2 h before cisplatin, prevented the small intestine injury (Fig. 6C).

Effect of Cisplatin and AG 1714 on Established Lung Metastasis of Murine Tumors. After our observation that AG 1714 reduced chemotherapy induced-toxicity in normal tissues, we investigated the effect of AG 1714 on the antitumor effect of chemotherapy using a variety of experimental tumor models. Mice were inoculated (i.v.) with a variety of murine tumors cells, followed 4 days later by a single administration (i.p.) of cisplatin (4 mg/kg). AG 1714 (20 mg/kg) was administered (i.p.) to the appropriate experimental groups 2 h before cisplatin. Mice that were treated with cisplatin alone were injected 2 h before with the vehicle used for AG 1714 solubilization. Twenty-four days after tumor inoculation, the mice were sacrificed, and the extent of tumor load in the lungs was assessed by the lungs' weight (Fig. 7) and by scoring of the lungs' lesions. Tumor lesions in the lungs varied in size. They included discrete lesions of various sizes, conglomerates of multiple lesions, and confluent growth of the tumor throughout the lungs. There was a correlation between the extent of tumor growth in the lungs and their weights.

As seen in Fig. 7A, cisplatin markedly inhibited the growth of MCA-105 fibrosarcoma. AG 1714 by itself had a small antitumor effect. Combined treatment with AG 1714 and cisplatin was as effective as cisplatin alone in suppression of the growth of the established lung metastases of MCA-105. Cisplatin and AG 1714 by themselves had a partial antitumor effect against 3LL. Combined treatment with AG 1714 and cisplatin was more effective than cisplatin also in suppression of the growth of established lung metastases of this tumor (Fig. 7B).

Cisplatin (4 mg/kg) or AG 1714 (20 mg/kg) alone were not effective against established lung metastases of B-16 melanoma (Fig. 7C). Combined treatment with these agents was more effective than each agent alone.

Effect of AG 1714 on Low- and High-Dose Chemotherapy Efficiency and Mortality in Tumor-bearing Mice. We have investigated the effect of AG 1714 on low- and high-dose chemotherapy efficacy and mortality in tumor-bearing mice. As seen in Table 1A, doxorubicin by itself, at a dose of 4 mg/kg, did not cause mortality and at 8 mg/kg induced 62.5% mortality of MCA-105 fibrosarcoma-bearing mice. Administration of AG 1714 (20 mg/kg) 2 h before doxorubicin at 8 mg/kg reduced mortality to 25% and was more effective than doxorubicin at 4 mg/kg.

Cisplatin at 2 × 10 mg/kg induced 71% mortality in MCA-105 fibrosarcoma-bearing mice (Table 1B). Administration of AG 1714 (20 mg/kg) 2 h before cisplatin reduced mortality to 33% and exhibited a more pronounced antitumor effect than cisplatin at 2 × 4 mg/kg. Under similar experimental conditions, AG 1714 allowed doxorubicin dose intensification in B-16 melanoma-bearing mice (Table 2).

Mortality occurred at early development of the tumor and was related to chemotherapy toxicity. The overall mortality (in the experiments depicted in Tables 1 and 2) in mice that were treated with high-dose chemotherapy was 17 of 23 (dead/total) compared with 6 of 22 (dead/total) in mice that were treated with high-dose chemotherapy and AG 1714. The difference in mortality between the two groups was statistically significant (P < 0.001).

Table 1. Effect of AG 1714 on low- and high-dose chemotherapy efficacy and mortality in MCA-105 fibrosarcoma-bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of lungs (mg ± SE)</th>
<th>Mortality (dead/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>908 ± 107</td>
<td>0/8</td>
</tr>
<tr>
<td>AG 1714 (20 mg/kg)</td>
<td>632 ± 74</td>
<td>0/6</td>
</tr>
<tr>
<td>Doxorubicin (4 mg/kg)</td>
<td>477 ± 119</td>
<td>0/5</td>
</tr>
<tr>
<td>AG 1714 + Doxorubicin (20 mg/kg)</td>
<td>494 ± 137</td>
<td>0/5</td>
</tr>
<tr>
<td>Doxorubicin (8 mg/kg)</td>
<td>170 ± 4</td>
<td>58 (62.5%)</td>
</tr>
<tr>
<td>AG 1714 + Doxorubicin (20 mg/kg)</td>
<td>172 ± 6*</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>No tumor</td>
<td>161 ± 12</td>
<td>0/4</td>
</tr>
</tbody>
</table>

Table 2. Effect of AG 1714 on low- and high-dose doxorubicin efficacy and mortality in B-16 melanoma-bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of lungs (mg ± SE)</th>
<th>Mortality (dead/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG 1714 (20 mg/kg)</td>
<td>976 ± 142</td>
<td>0/6</td>
</tr>
<tr>
<td>Doxorubicin (4 mg/kg)</td>
<td>392 ± 87</td>
<td>0/6</td>
</tr>
<tr>
<td>AG 1714 + Doxorubicin (20 mg/kg)</td>
<td>363 ± 28</td>
<td>0/6</td>
</tr>
<tr>
<td>Doxorubicin (8 mg/kg)</td>
<td>246 ± 10</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>AG 1714 + Doxorubicin (20 mg/kg)</td>
<td>232 ± 12</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Mice were sacrificed 25 days after tumor inoculation, and lungs from each animal were weighed.

** Mice died between 4 and 9 days after doxorubicin administration.

† AG 1714 was injected (i.p.) 2 h before doxorubicin.

‡ Doxorubicin was injected (i.p.) 4 days after tumor inoculation.

§ The difference in mortality between mice injected with doxorubicin (8 mg/kg) + AG 1714 (20 mg/kg) and mice injected with doxorubicin (8 mg/kg) alone was statistically significant (P < 0.02).
Effect of Chemotherapy and AG1714 on Human Tumor Xenografts in Nude Mice. The effect of doxorubicin (4 mg/kg) and AG1714 (20 mg/kg) on established lung metastases of human SK-28 melanoma xenograft in nude mice was investigated. As seen in Fig. 7D, doxorubicin markedly suppressed the growth of this tumor, whereas AG1714 by itself had a small antitumor effect. Pretreatment with AG1714 did not impair the effect of doxorubicin alone in suppression of the growth of this tumor.

We investigated the antitumor effect of cisplatin and AG1714 against OVCAR-3, inoculated s.c. in nude mice. Cisplatin had a marked antitumor effect in this model, whereas AG1714 by itself was less effective. AG1714 did not impair the chemotherapeutic effect of cisplatin (Fig. 8).

Effect of AG1714 on Chemotherapeutic Agent-induced Apoptosis of Cell Lines in Vitro. We explored the possibility that the reduction by AG1714 of chemotherapy-induced toxicity of normal cells may result from its inhibition of apoptosis.

A9, a fibroblastic cell line (derived from a normal subject), was incubated in vitro with cisplatin (100 μM) for 20 h in the presence and absence of AG1714 (20 μM). (All cell cultures contained 50 μg/ml cycloheximide.) Apoptosis was assessed by cell-sorting analysis using propidium iodide staining. The extent of apoptosis of untreated A9 cells, cells treated with AG1714 alone, cells treated with cisplatin alone, and cells treated with cisplatin and AG1714 was 16, 11, 43.2, and 12.6%, respectively. In contrast to inhibition by AG1714 of cisplatin-induced apoptosis of A9 cells, AG1714 (20 μM) did not inhibit doxorubicin (1 μM)-induced apoptosis of B-16 melanoma cells after incubation for 14 h. The extent of apoptosis of untreated B-16 melanoma cells, cells treated with AG1714 alone, cells treated with doxorubicin alone, and cells treated with doxorubicin and AG1714 was 7.2, 16.1, 36.2, and 37.6%, respectively. The results are expressed as the means of duplicate experiments, and the deviations from the means did not exceed 6%. The relationship between these findings in vitro and the selective in vivo effects of AG1714 are presently under investigation.

DISCUSSION

AG1714 reduced toxicity in different normal tissues induced by doxorubicin and cisplatin. The observation that AG1714 did not impair the antitumor activity induced by some of these agents strongly suggests that AG1714 did not elicit its protective activity by neutralizing the chemotherapeutic agents themselves. It is plausible that intestinal injury and infection are major causes of death that were prevented by AG1714. In fact, AG1714 effectively reduced cisplatin-induced intestinal injury (Fig. 6B) and doxorubicin-induced myelosuppression (Figs. 4 and 5).

AG1714 was more effective in reducing cisplatin-induced nephrotoxicity upon its administration 2 h before cisplatin as compared with its administration 2 h after chemotherapy. The pharmacokinetic data of AG1714 are not yet available. We have found previously that other benzylidene malononitriles were rapidly cleared (serum half-life of less than 10 minutes) from circulation. This raises the possibility that AG1714 may exert its protective effect by an inductive process leading to tolerance. The possibility that the protective effects of AG1714 resulted from its effect on the pharmacokinetics of the chemotherapeutic agents should also be considered. However, the findings that AG1714 reduced toxicity of structurally different agents make this possibility less plausible.

The myelosuppressive effect of doxorubicin at a high dose was only partially protected by AG1714. However, pretreatment with AG1714 under these conditions facilitated the recovery of the myelosuppressed bone marrow. This effect of AG1714 was most probably due to its protective effect against doxorubicin-induced depletion of bone marrow stem cells.

In striking contrast to the protective effect of AG1714 against chemotherapy-induced toxicity in a variety of normal tissues, this agent did not impair the antitumor effect of doxorubicin and cisplatin. This was demonstrated in experiments using various murine tumors and human tumor xenografts in athymic mice.

In some experiments in which low doses of chemotherapeutic agents were used, a cooperative effect between AG1714 and chemotherapy was noted (Fig. 7 and Table 1). Experiments in which low and high doses of chemotherapy were used (Tables 1 and 2) demonstrated that AG1714 reduced mortality of high-dose chemotherapy and increased its therapeutic index. These findings suggest that AG1714 may have a potential clinical use in chemotherapy by allowing dose intensification. AG1714 by itself was reported previously to inhibit tumor growth in mice (27).
The mechanism for the selective inhibiting effect of AG1714 against chemotherapy toxicity is not known. Recent studies indicated that some chemotherapeutic agents elicit their cytotoxic activity by inducing programmed cell death (apoptosis). It is, therefore, tempting to assume that AG1714 could selectively modulate apoptosis in normal and cancer cells. Our findings that AG1714 inhibited cisplatin-induced apoptosis of A9 cells in vitro and did not affect doxorubicin-induced apoptosis of B-16 melanoma cells in vitro may support this possibility. We recently observed\(^3\) that AG1714 reduced by 60% the acute liver damage that was induced by the in vivo administration to mice of anti-fas antibody. Activation of the apoptotic pathway, culminating in severe liver damage, is induced under these experimental conditions (28). Of interest are recent studies that indicated that doxorubicin-induced apoptosis of cell lines in vitro was inhibited by blocking of the fas-mediated pathway (29).

The molecular targets of AG1714 are unknown. AG1714 is structurally related to the tyrphostin family, agents that selectively inhibit protein tyrosine kinases (24). Inhibitors of tyrosine kinases were shown to modulate apoptosis induced by a variety of agents in different cell types (20–23). It should be noted, however, that AG1714 is not a derivative of hydroxybenzylidene malononitrile like most of the reported tyrphostins.

AG1714 may enable intensification of the presently used chemotherapy and also may enable the therapeutic use of novel, anticancer drugs that have unacceptable toxic effects. AG1714 by itself seems to be nontoxic. Multiple administration of AG1714 to mice at a cumulative dose exceeding the maximal single therapeutic dose used in this study by 15-fold did not result in toxicity. Toxicity was assessed by mortality, growth appearance, body and organ weight, blood count and chemistry, and histological analysis. Regardless of its mechanism of action, AG1714 may provide a novel, useful tool to improve chemotherapy of human cancer.

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REFERENCES


\(^3\) A. Novogrodsky, M. Patya, and A. Vanichkin, unpublished data.

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