Research Articles

Differential Antigenotoxic and Cytoprotective Effect of Amifostine in Idarubicin-Treated Mice

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In this study we evaluated the antigenotoxic and cytoprotective capabilities of WR-2721 [*S*-2-(3-aminopropylamino)-ethylphosphorothioic acid (amifostine)] in different normal tissues of BALB/c mice treated with idarubicin [4-demethoxydaunorubicin (IDA)]. The aminothiol WR-2721 is a pro-drug that requires dephosphorylation to its active metabolite WR-1065, to produce selectively cytoprotective activity in normal tissues exposed to radio- and chemotherapeutic agents, without protecting malignant tissues. IDA is an effective chemotherapeutic agent against hematological diseases, but produces a broad spectrum of toxicity in nontumoral cells. Animals were injected intravenously with WR-2721 (250 mg/kg) or IDA (6 mg/kg) and WR-2721/IDA. Micronuclei frequency in bone marrow was measured 24 and 48 hr after initiation of the treatments. The IDA-treated group showed increased levels of micronuclei. However, the WR-2721- and WR-2721/IDA-treated groups did not show differences from the controls. Genetic damage was evaluated by alkaline single-cell gel electrophoresis at 24-hr posttreatments. Important DNA damage was observed in liver, spleen, and peripheral blood cells of mice treated with IDA. The presence of WR-2721 diminished that damaging effect only in liver cells. The apoptotic index was measured in liver and spleen tissues by the TUNEL assay 14 and 24 hr after treatment. In liver we observed an increased percentage of apoptotic cells at 24 hr for the IDA-treated group, whereas the WR-2721 and WR-2721/IDA groups remained at low levels. Splenic cells treated with IDA and WR-2721/IDA showed increased DNA fragmentation levels at any time. In conclusion, WR-2721 has a tissue-specific antigenotoxic and cytoprotective effect in IDA-treated mice using these experimental conditions. Environ. Mol. Mutagen. 39:3–9, 2002.   © 2002 Wiley-Liss, Inc.

Key words: idarubicin; amifostine; DNA damage; apoptosis; cytoprotection; DNA topoisomerase II

INTRODUCTION

The use of cytoprotective agents represents an alternative method for reducing radio- and chemotherapeutic toxicity in normal tissues [Hospers et al., 1999], thus preventing the risk of potentially genotoxic effects such as secondary tumor formation [Ferguson and Pearson, 1996].

Amifostine [WR-2721, *S*-2-(3-aminopropylamino)-ethylphosphorothioic acid], an analog of cysteamine, is a phosphorylated aminothiol pro-drug that is dephosphorylated to its active metabolite, the free thiol WR-1065 in normal tissues by membrane-bound alkaline phosphatase [Shaw et al., 1996; Hospers et al., 1999]. This latter compound is selectively incorporated into normal cells, producing the highest antimutagenic and cytoprotective effect of all the metabolites generated [Capizzi, 1996]. The selective uptake of WR-1065 results from both the reduced alkaline phosphatase concentrations in malignant tissue compared with its normal counterpart, and from the lower pH presented in the microenvironment of tumoral tissues, which makes the uptake of WR-1065 by these cells difficult [Korst et al., 1997; Hospers et al., 1999; Romano et al., 1999].

The underlying mechanisms of antigenotoxicity and cytoprotection by WR-1065 include: (1) scavenging free radicals; (2) participating in the chemical repair of DNA through the donation of hydrogen atoms; (3) protecting endogenous antioxidants by forming protein–aminothiol mixed disulfides; (4) inducing an intracellular hypoxic state

that results from auto-oxidation processes; and (5) stabilizing and protecting the DNA by binding to it [Hospers et al., 1999; Romano et al., 1999; Marzatico et al., 2000; Snyder and Grdina, 2000].

In vitro and in vivo experimental studies suggest an effective cytoprotective activity by WR-2721 against the toxicities resulting from radiation therapy or chemical drugs (e.g., cisplatin, cyclophosphamide, carboplatin, doxorubicin, paclitaxel, or 5-fluorouracil) [Taylor et al., 1997; Romano et al., 1999; Santini and Giles, 1999].

Idarubicin [4-demethoxydaunorubicin (IDA)], an anthracycline analog, is an antineoplastic drug used in the treatment of acute myelogenous leukemia. It produces a wide range of biologic reactions in cells [Hande, 1998]. Its tumor cytotoxicity is attributed to its DNA-intercalating properties [Baguley, 1991] and its poisoning of DNA topoisomerase IIα [Binaschi et al., 1997, 1998]. Its structure is a target for cellular reductases, which generate free radicals and can induce cellular oxidative damage [Hande, 1998]. These effects can produce sufficient DNA [Binaschi et al, 1997] and non-DNA damage to induce cells to activate cell death processes [Binaschi et al., 1998].

The aim of this study was to evaluate the antigenotoxic and cytoprotective effects of WR-2721 against IDA-induced toxicity in normal mice tissues including bone marrow, liver, spleen, and peripheral blood.

MATERIALS AND METHODS

Chemicals

WR-2721 [Actifos®, CAS No. 20537-88-6) was received from Laboratorios Filaxis S.A., Argentina. IDA (Zavedos®, CAS No. 58957-92-9) was donated by Pharmacia and Upjohn, Argentina. Mitomycin C (MMC, CAS No. 50-07-7) was purchased from Sigma (St. Louis, MO).

Animals and Treatment Groups

Male and female BALB/c mice [7–9 weeks of age, 18–22 g average body weight (b.w.)] were used. Animals were kept in rooms controlled for lighting (12-hr cycle) and temperature (25°C). Food (Asociación de Cooperativas Argentinas) and water were available ad libitum. The animals were divided into the following experimental groups: negative control group (received distilled water), a group receiving WR-2721 250 mg/kg b.w. only, a group receiving IDA 6 mg/kg b.w. only, and a group receiving WR-2721 250 mg/kg b.w. 15 min before IDA 6 mg/kg b.w. All chemicals were water soluble and were administered by intravenous treatment in a volume of 0.1 ml/10 g b.w.

Micronucleus Test

Animals (five males and five females per group) were terminated 24 and 48 hr after chemical administration. Bone marrow cells were flushed from the femurs into fetal bovine serum and collected by centrifugation at 1000 rpm for 5 min. Cells were smeared, fixed with methanol, and stained with May–Grünwald Giemsa. Micronuclei (MN) were evaluated in 2000 polychromatic erythrocytes (PCEs) per mouse. MMC was given to mice at a dose of 1 mg/kg b.w. as the positive control.

Cell Dissociation and Viability

Mice were terminated at 24 hr after treatment; their livers and spleens were removed and cells were isolated from each organ using a trypsin dissociation technique. Briefly, the tissues were washed with Ca^{2+}- and Mg^{2+}-free PBS supplemented with 20 mM HEPES and 0.8 mM EDTA. The cell suspensions were incubated for 30 min at 1 g/10 ml in 0.25% trypsin in PBS at 37°C with gentle stirring. The percentage of viable cells, as measured by acridine orange and ethidium bromide assays, was more than 85% in all cases.

Alkaline Single-Cell Gel Electrophoresis (Alkaline Comet Assay)

Murine peripheral blood, liver, and spleen (n = 4) were collected and processed at 24 hr after treatment. Liver and spleen cell suspensions (prepared as above, 1×10^4 cells/15 μl) and blood cells were mixed with 75 μl of low-melting agarose (LMA, 0.75% in Ca^{2+}/Mg^{2+}-free PBS) and spread on a slide precoated with normal melting agarose (0.75% in Ca^{2+}/Mg^{2+}-free PBS). A third layer of LMA was applied, covered with a coverslip, and solidified. The coverslip was removed and the microscope slides were placed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 1% Triton X-100, 10% DMSO) and kept at 4°C for at least 1 hr. Slides were then transferred to an electrophoresis box containing an alkaline solution at pH 13 (300 mM NaOH, 1 mM EDTA). Slides were kept in this solution for a 20-min unwinding time at 4°C. A current of 30 V (250 mA) was then applied for 25 min. The slides were removed, neutralized (0.4 M Tris, pH 7.5), and stained with ethidium bromide (20 μg/ml). Nucleoids were evaluated with a fluorescence microscope. A total of 100 comets on each slide were visually scored as belonging to one of five classes according to tail fluorescence intensity, tail length, and nucleoid integrity. They were then assigned a value of 0 (undamaged), 1, 2, 3, or 4 (maximally damaged). To calculate the mean of the DNA damage grade for each treated group, the following formula was applied:

$$\text{grade of damage} = X_0 \times 0 + X_1 \times 1 + X_2 \times 2 + X_3 \times 3 + X_4 \times 4$$

where X = cell number, 0 = no damage, 1 = low level of damage, 2 = medium level of damage, 3 = high level of damage, and 4 = the highest level of damage.

TUNEL Assay by Flow Cytometry

The apoptosis measurement was carried out after 14 or 24 hr using an Apoptag® fluorescein direct in situ apoptosis detection kit (Intergen, Purchase, NY). The samples, either splenic or hepatic cell suspensions (2×10^6 cells approximately) were added to 1 ml of PBS. The cells were fixed for 15 min with 1% paraformaldehyde in an ice-cold solution. The fixed cells were then incubated with TdT enzyme and fluorescein–dUTPs for 30 min at 37°C, avoiding exposure to light. After this time, the reaction was stopped by the addition of a stop/wash solution buffer (provided by kit). Finally, cell suspensions were mixed with 1 ml of the counterstain solution, which contained propidium iodide (PI; 0.5 μg/ml in antifade), and were incubated in the dark at room temperature for 15 min. The samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Cell Quest software was run for data acquisition and analysis. Ten thousand cells per tissue sample and per animal (n = 3) were analyzed. The red fluorescence signal of PI was measured at 620 nm using FL2 linear amplification and the green fluorescence corresponding to FITC was assessed at 530 nm by the FL1 detector using logarithmic amplification.

TABLE I. Micronuclei Frequency in Polychromatic Erythrocytes (PCEs) of Mouse Bone Marrow

Treatment group	Sex	Sampling time (hr)	MNPCE/2000 PCEs (Individual values)	MN frequency (‰) (Mean ± SD)
			Frequencies of MNPCE	
Control	M	24	8, 9, 8, 10, 9	4.4 ± 0.4
	F	24	7, 11, 7, 7, 8	4.0 ± 0.9
WR-2721	M	24	10, 11, 8, 11, 10	5.0 ± 0.6
	F	24	10, 15, 6, 8, 10	4.9 ± 1.7
IDA	M	24	36, 28, 26, 25, 22	13.7 ± 2.6*
	F	24	30, 27, 24, 20, 26	12.7 ± 1.9*
WR-2721/IDA	M	24	7, 12, 15, 11, 13	5.8 ± 1.5**
	F	24	8, 14, 17, 8, 12	5.9 ± 2.0**
MMC	M	24	66, 60, 48, 53, 62	28.9 ± 3.6*
	F	24	62, 56, 36, 42, 52	24.7 ± 5.4*
Control	M	48	6, 9, 9, 14, 10	4.8 ± 1.4
	F	48	7, 8, 6, 11, 10	4.2 ± 1.5
WR-2721	M	48	11, 8, 10, 13, 6	4.8 ± 1.4
	F	48	12, 7, 4, 9, 10	4.2 ± 1.5
IDA	M	48	25, 30, 26, 20, 33	13.4 ± 2.5*
	F	48	19, 40, 32, 20, 21	13.2 ± 4.6*
WR-2721/IDA	M	48	15, 8, 12, 16, 10	6.1 ± 1.7**
	F	48	9, 15, 19, 10, 11	6.4 ± 2.1**
MMC	M	48	23, 28, 27, 36, 24	13.8 ± 2.6*
	F	48	30, 28, 25, 30, 25	13.8 ± 1.3*

*Significant differences with respect to control group ($P = 0.0001$).
**Significant differences with respect to IDA-treated group ($P = 0.0001$).

Statistical Analysis

The means of the frequencies of MNPCE were statistically analyzed by the χ^2 test. Differences between the grade values of DNA damage were tested using the Student–Newman–Keuls method. Doubling of the controls' apoptotic index by the treated groups was considered sufficient to indicate a positive result.

RESULTS

In Vivo Micronucleus Test

The results of the micronucleus induction studies in mice bone marrow (n = 5) at 24 and 48 hr after treatment are presented in Table I. No sex differences were observed in the frequencies of MNPCEs in the control and treated groups. Administration of IDA 6 mg/kg b.w. produced a significant increase ($P = 0.0001$) in the frequencies of MNPCE compared with that of controls at 24 and 48 hr posttreatment. These values were threefold higher than those of the control groups. MMC-treated mice showed a significant increase in MNPCE frequencies compared with those of untreated mice ($P = 0.0001$), which were the highest at 24 h. WR-2721 250 mg/kg b.w. caused no change in the MNPCE values with respect to the control. There were no differences between the MNPCE frequencies in mice treated with the combination of WR-2721/IDA and the

control group, except for the females exposed to both chemicals for 48 hr ($P = 0.0322$). However, the values of MNPCE in these mice were significantly diminished with respect to those produced in the IDA-treated group ($P = 0.0001$).

Alkaline Single-Cell Gel Electrophoresis

Table II shows the grade of DNA damage in mice liver, spleen, and peripheral blood cells treated with IDA, WR-2721, or combined treatments at 24 hr (n = 4). An important DNA damage was observed in liver ($P < 0.05$), in peripheral blood ($P < 0.05$), and in spleen cells of mice treated with IDA compared with the control groups. WR-2721 given alone had no effect on DNA damage induction in any of the tissues assayed.

The grade of damage in liver cells treated with WR-2721 before IDA did not show differences from the control values. The number of extremely damaged cells (grade 4) was 11.7 ± 7.5 and 20.5 ± 10.0% in WR-2721/IDA and control groups, respectively. On the other hand, when the combination of WR-2721/IDA was administrated, the grades of damage in spleen ($P < 0.05$) and peripheral blood cells ($P < 0.05$) were significantly higher than the control values. In these cases, the number of extremely damaged cells was 58.6 ± 10.4% in spleen and 15.7 ± 11.1% in peripheral blood cells, and 21.4 ± 11.1 and 1.5 ± 0.8% in control cells, respectively.

Induced-Apoptosis Detection

The percentages of TUNEL$^+$-cells or cells presenting highly fragmented DNA in liver and spleen tissues are shown in Figure 1. Such fragmentation represents a late stage in the process of apoptotic cell death.

At 14 hr after treatment, the percentage of TUNEL$^+$-cells found in liver tissue was similar in all treated groups, whereas a significant increase in this percentage was found in the IDA-treated group at 24 hr after dosing.

In spleen tissues of mice exposed to IDA, an increase of highly fragmented DNA levels was observed at 14 hr posttreatment. IDA and WR-2721/IDA produced a strong induction of cell death as measured after 24 hr. Histological sections were stained with hematoxylin and eosin (Fig. 2). In IDA-treated mice, the presence of greatly depleted cell regions without cellular residues was observed in spleens, although a low number of apoptotic cells were found in liver tissues.

DISCUSSION

The micronucleus test in murine bone marrow cells, an actively proliferating type of tissue, is based on the observation of MNPCEs [Schmid, 1975]. The present studies indicate that IDA increases the frequency of MNPCE at 24

TABLE II. Grade of DNA Damage in Mice Liver, Spleen, and Peripheral Blood Cells at 24 Hr

Tissue/Treatment group[a]	Distribution of grade of DNA damage (%) (Mean ± SD)					Grade of damage
	0	1	2	3	4	
Liver						
Control	13.0 ± 4.8	22.8 ± 7.2	25.2 ± 12.9	18.5 ± 5.3	20.5 ± 10.0	197.8 ± 57.2
WR-2721	13.0 ± 3.6	22.7 ± 8.5	27.3 ± 4.1	20.6 ± 5.6	16.4 ± 5.0	204.4 ± 17.4
IDA	3.5 ± 1.5	9.4 ± 3.7	21.9 ± 6.0	30.5 ± 11.4	34.7 ± 5.5	283.8 ± 13.6*
WR-2721/IDA	16.7 ± 6.6	28.6 ± 9.7	29.5 ± 4.1	13.5 ± 6.7	11.7 ± 7.5	174.8 ± 32.9
Spleen						
Control	19.4 ± 4.3	22.4 ± 9.4	23.0 ± 3.3	13.8 ± 4.0	21.4 ± 11.1	195.2 ± 42.7
WR-2721	20.0 ± 11.1	17.1 ± 10.4	14.0 ± 1.5	16.2 ± 3.1	32.7 ± 19.5	225.9 ± 72.3
IDA	10.0 ± 6.5	18.2 ± 7.4	13.6 ± 5.3	13.9 ± 4.4	44.3 ± 14.4	276.3 ± 39.5
WR-2721/IDA	7.6 ± 7.3	9.2 ± 4.8	13.1 ± 2.6	11.5 ± 5.4	58.6 ± 10.4	304.4 ± 42.5*
Peripheral blood						
Control	73.7 ± 16.4	18.0 ± 15.1	4.6 ± 2.3	2.2 ± 1.0	1.5 ± 0.8	39.7 ± 19.6
WR-2721	66.0 ± 13.3	22.8 ± 11.2	7.1 ± 2.1	1.3 ± 1.1	2.8 ± 2.1	56.8 ± 12.5
IDA	17.0 ± 18.2	19.3 ± 13.4	22.7 ± 6.9	28.2 ± 21.7	12.8 ± 7.4	200.0 ± 79.3*
WR-2721/IDA	9.4 ± 5.8	29.8 ± 11.8	25.3 ± 4.2	19.8 ± 5.6	15.7 ± 11.1	202.5 ± 46.7*

[a]Both male and female mice were used (4 animals per treatment group).
*Significant differences with respect to control group ($P < 0.05$).

and 48 hr posttreatment. Similarly, IDA has been shown to elevate the MN frequency in human tumor cell lines [Stopper et al., 1999]. The MN formation attributed to the action of clastogenic agents presumably arises from the inhibition of the DNA topoisomerase II enzyme. During this process, the enzyme–drug–DNA, a stable ternary complex, produces double-strand breaks, which become targets for mutagenic events [Froelich-Ammon and Osheroff, 1995]. The positive control group treated with MMC showed a significant increase in MNPCE and the magnitude of this response decreased with increasing sample time. Similar results were found by Crebelli et al. [1999]. The administration of WR-2721 reduced the induction of MNPCE in mice treated with IDA, demonstrating its antigenotoxic effect in the mouse erythropoietic system. This finding is in accordance with previous reports in which treatment with WR-2721 prior to cyclophosphamide [Czyzewska and Mazur, 1995] or to X-irradiation [Mazur, 2000] decreased the MNPCE frequencies in mice. Snyder and Grdina [2000] proposed that the active thiol form WR-1065 protected against MN formation by catalytic inactivation of topoisomerase II. Murley et al. [1997] demonstrated that WR-1065 seems to affect topoisomerase IIα activity by altering its phosphorylation status. This could lead to the accumulation of cells in the G2 phase [Murley et al., 1997; Snyder and Grdina, 2000] and a prolongation of the cell cycle, which could provide more time for DNA repair to occur. In addition, its antigenotoxicity involves effects on endogenous enzymatic systems required in processing and removing DNA damage [Capizzi, 1996; Marzatico et al., 2000].

The comet assay is a sensitive technique for detecting the presence of DNA strand-breaks and for revealing alkali-labile sites such as AP sites in individual cells. In this study, the comet assay was applied to detect DNA damage in liver, spleen, and peripheral blood cells at 24 hr after dosing. The

differences among the control values for DNA damage in different tissues analyzed could be the result of the enzyme dissociation technique applied to obtain the cellular suspen-
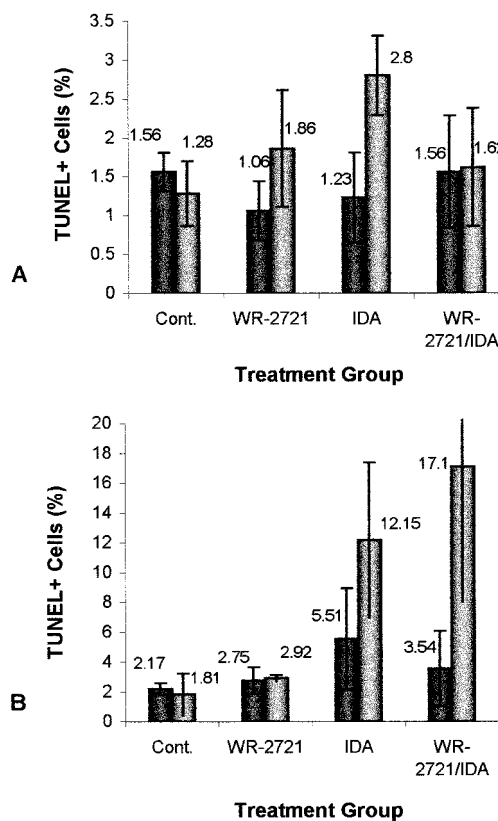

Fig. 1. Comparison between percentages of cells with highly fragmented DNA induced by different treatments, 14 hr (dark-shaded) and 24 hr (light-shaded) after dosing in (**A**) hepatic and (**B**) splenic cells from both male and female mice. The measurement was carried out by the fluorescent TUNEL assay.
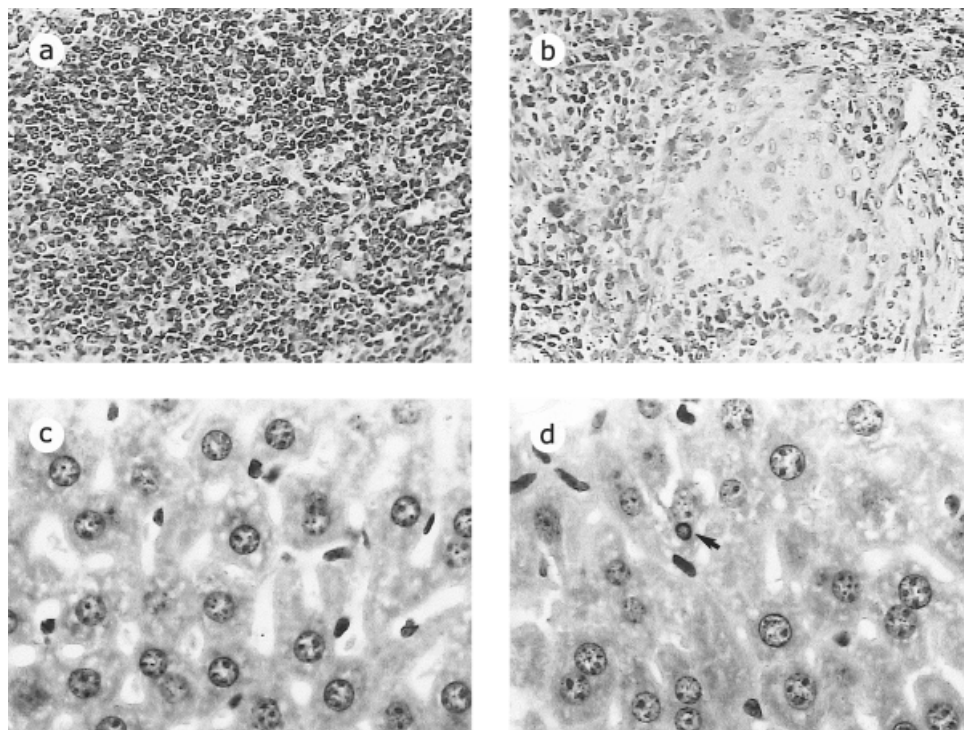
Fig. 2. Histological examination (hematoxylin and eosin–stained) of spleen and liver tissues from mice at 24 hr after treatment. **a:** Section of a lymphatic nodule from a control mouse (100 × magnification); **b:** section of a lymphatic nodule from an IDA-treated mouse (100 ×); **c:** section of hepatic tissue from a control mouse (400 ×); **d:** section of hepatic tissue from an IDA-treated mouse (400 ×). arrow: apoptotic nucleus.

sions of liver and spleen compared to those used for peripheral blood cells. Our results show that IDA was able to damage DNA in liver, spleen, and peripheral blood cells of the treated mice. This could be because IDA is an anthraquinone that can undergo electron reduction, producing reactive compounds that damage macromolecules and lipid membranes [Peddie et al., 1997; Hande, 1998; Liu et al., 1999]. We have also observed that WR-2721 protected liver cells against IDA-induced DNA damage, but failed to prevent this effect in spleen and peripheral blood cells.

Previous reports on studies in mice demonstrated that there was a higher accumulation of the active labeled metabolite WR-1065 in liver than in spleen tissues [Shaw et al., 1994; Dorr, 1996]. Other studies using mouse tissue homogenates indicated a major dephosphorylation activity in liver compared to that in spleen, whereas there was no activity in serum [Mori et al., 1984]. In addition, the administration of WR-2721 resulted in an increase in glutathione levels in liver cells, increasing the endogenous antioxidant concentrations and thereby counteracting free-radical–associated damage [Grdina et al., 1995; Hospers et al, 1999]. In these noncycling cells, the free-radical scavenging activity of WR-2721 could be its main mechanism of antigenotoxicity [Capizzi, 1996; Marzatico et al., 2000]. In contrast with these results, Kataoka et al. [1996] reported that WR-2721 reduced cyclophosphamide-induced hypox-

anthine–guanine phosphoribosyl transferase mutation frequency in mice splenocytes. Buschini et al. [2000] also found that WR-2721 protected normal human white blood cells against the in vitro genotoxic effect of melphalan.

The DNA fragmentation results in one of the most common biochemical features of apoptotic cells [Li et al., 1995; Ferlini et al., 1996]. The TUNEL assay by flow cytometry was used to elucidate apoptotic cell death in liver and spleen tissues. The mechanism of action of the enzyme terminal deoxynucleotidyl transferase (TdT) allows the addition of fluorescein–dUTPs to the free 3′-OH termini generated by DNA strand breaks [Negoescu et al., 1997]. Bursch et al. [1990] previously reported that a rate of tissue regression as rapid as 25% per day can result from apparent apoptosis in only 2–3% of the cells. Thus, the criterion used to consider differences between treatments was the duplication of control apoptotic levels.

IDA induced an increase in apoptotic cells in liver after 24 hr and in spleen after 14 hr following injection. This observation suggests a major and faster activity of IDA in spleen than in liver cells. IDA has also been shown to induce apoptosis in HL-60 cells after treatment by high doses [Marekova et al., 2000].

Interestingly, we observed that in both liver and spleen the percentage of apoptotic cells was related to the accumulation of cells with a G0–G1 DNA content (unpublished

data), suggesting non-S-phase specificity. It was previously demonstrated that IDA-induced apoptosis occurs in the G1 and/or early S phases of the U 937 cell line [Vial et al., 1997]. Moreover, the anthracyclines daunorubicin and doxorubicin triggered apoptosis of nonactivated peripheral lymphocytes in the G0–G1 phases of the cell cycle [Ferraro et al., 2000].

Our findings show that IDA and the combined treatment promote cell death events in spleen measured at 14 and 24 hr after dosing. The spleen cells of these groups analyzed by flow cytometry 24 hr after treatment showed an increase in the uptake of the fluorochrome PI (unpublished data). This effect could be caused by a relaxed chromatin structure. The presence of more binding sites to interact with PI would indicate necrotic cell death more than an apoptotic pattern. The necrotic cells can be labeled by TdT with less specificity because they present an irregular DNA fragmentation form. Histological sections were studied to confirm the necrotic nature of this cell death through the presence of massive cell death [Darzynkiewicz and Traganos, 1998]. In spleen tissues, IDA induced an important cell depletion 24 hr after treatment. In agreement with our results, Ferraro et al. [2000] reported a cellular depletion in spleen, lymph nodes, and thymus in daunorubicin- and doxorubicin-treated mice.

In our experience, such depletion was not observed in liver tissues of mice treated with IDA, where a low number of apoptotic cells could be found.

WR-2721 reduced the apoptosis induced by IDA only in liver tissue. A previous report on the apoptotic preventive activity of WR-2721 supported this observation [Provinciali et al., 1999].

The present data establish evidence for a differential in vivo antigenotoxic and cytoprotective activity of WR-2721 in IDA-treated mice. Taking into consideration that the side effects of IDA treatment include tissue toxicity and secondary malignancies, the knowledge of target sites of action for WR-2721 will permit the design of new strategies for cytoprotection, depending on the tissues and drugs involved.

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