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Induction of Apoptosis by the bis-Pt(III) Complex $[Pt_2(2\text{-mercaptopyrimidine})_4Cl_2]$

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ABSTRACT. We analyzed both the cytotoxicity and the type of cell death produced by the novel binuclear Pt(III) compound Pt-Spym ($[Pt_2(2\text{-mercaptopyrimidine})_4Cl_2]$) in kidney human fibroblasts and in human tumor cell lines (HeLa, CH1, CH1*cis*R and HL-60). The data showed that Pt-Spym displayed higher cytotoxicity against these tumor cells than cisplatin. In contrast, Pt-Spym had low toxicity against normal human fibroblasts. Interestingly, Pt-Spym circumvented cisplatin resistance in CH1*cis*R cells. We also observed that Pt-Spym induced the characteristic changes attributed to apoptosis in cells with normal levels of p53 protein (CH1 and CH1*cis*R) and with low levels of p53 protein (HeLa), but not in cells lacking p53 (HL-60). Interestingly, Western blot data indicated that apoptosis induction by Pt-Spym in HeLa, CH1, and CH1*cis*R cells was not associated with drastic changes in p53 levels. However, *cis*-DDP strongly decreased p53 levels in CH1 and CH1*cis*R cells and abolish p53 protein in HeLa cells. Altogether, these results suggest that induction of apoptosis by Pt-Spym requires the presence of p53 protein. BIOCHEM PHARMACOL **60**;3:371–379, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. p53; apoptosis; cytotoxicity; cisplatin; $[Pt_2(2\text{-mercaptopyrimidine})_4Cl_2]$

In recent years, many investigations have been directed toward the synthesis of platinum compounds endowed with higher antitumor activity and lower toxic effects than the clinically used drug *cis*-DDP.§ These studies have led to the design of new platinum complexes in which the drug molecule contains one or several *cis*-Pt(II) centers coordinated to biologically active molecules [1–3]. Moreover, many efforts have been made to design new molecules with metallic centers having an oxidation state different from Pt(II) due to the capacity of many tumor cells to acquire resistance to *cis*-Pt(II)-derived compounds *in vivo*. Thus, a great number of compounds with Pt(IV) centers have been synthesized, with some showing pharmacological advantages relative to *cis*-DDP [4, 5]. However, at present, the number of Pt(III) complexes synthesized that have been biologically tested is limited, due to intrinsic difficulties in achieving the Pt(III) oxidation state [6].

Programmed cell death or apoptosis is an energy-dependent physiological mechanism for the elimination of target cells [7, 8]. The morphological changes associated with apoptosis include chromatin condensation, fragmentation of the nucleus, and packaging of cell remnants into "apoptotic bodies" [9]. One biochemical marker of apoptosis in many, but not all, cell types is the cleavage of nuclear DNA into 180-base pair integer fragments [10]. Many chemical stimuli, including anticancer agents such as cisplatin, are potent inducers of apoptosis in some cell lines [11–15]. Since apoptotic cells are phagocyted and processed by macrophages while necrotic cells lysate and release their constituents to the extracellular medium producing inflammation and other local damage [10], the designing of antitumor drugs that kill cells via apoptosis, thereby avoiding the side effects of necrosis, is of great interest.

On these grounds, we designed several compounds in which biologically relevant ligands were coordinated to Pt(III) atoms and subsequently reported the synthesis, characterization, and DNA-binding properties of the novel Pt(III) complex Pt-Spym [16, 17]. We observed that the DNA modifications induced by this binuclear Pt(III) derivative of 2-mercaptopyrimidine (Fig. 1) are very different from those induced by *cis*-DDP. In fact, the atomic force microscopy technique revealed that Pt-Spym induces compaction of the DNA double helix and interhelical cross-link formation between different DNA molecules [17]. In addition, *in vivo* toxicity tests using BDF1 mice gave an LD_{50} value for the Pt-Spym compound significantly higher than that of *cis*-DDP, and preliminary cytotoxicty tests in HL-60

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§ *Abbreviations*: *cis*-DDP, cisplatin, *cis*-diamminedichloroplatinum(II); FBS, fetal bovine serum; HF, human fibroblasts; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; PI, propidium iodide; Pt-Spym, $[Pt_2(2\text{-mercaptopyrimidine})_4Cl_2]$; Spym, 2-mercaptopyrimidine; and FITC, fluorescein isothiocyanate.

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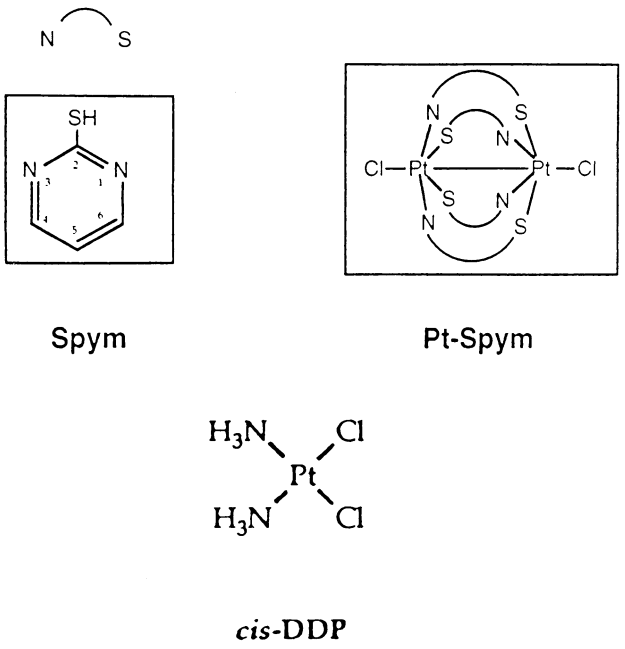

FIG. 1. Structures of Spym (2-mercaptopyrimidine), Pt(III)-Spym, and *cis*-DDP.

and HeLa cells indicated that the compound was biologically active [16].

In view of the interesting biochemical and biological properties of Pt-Spym, we further analyzed the cytotoxic activity of the compound against several human tumor cells and the type of cell death induced by the compound. The results showed that Pt-Spym had high cytotoxic activity in HeLa and CH1 cells as well as in cisplatin-resistant CH1*cis*R cells. Interestingly, Pt-Spym induced characteristic features of apoptosis in all these cancer cells endowed with functional p53 protein in spite of the fact that CH1 and CH1*cis*R cells have normal levels of p53, while HeLa cells have low levels of p53. In contrast, Pt-Spym did not induce apoptotic features in HL-60 leukemic cells, although this compound was also active against this p53-negative tumor cell line. Therefore, induction of apoptosis by Pt-Spym might be related to the presence of p53 in cancer cells.

MATERIALS AND METHODS

Reagents and Drugs

One hundred-millimeter culture and microwell plates were obtained from Nunclon. MTT and PI were purchased from Sigma Chemical Co. Fluorescein-conjugated annexin V-FITC was supplied by PharMingen International. FBS was supplied by GIBCO BRL. *cis*-DDP was a gift from Bristol-Myers Squibb. Spym was obtained from Aldrich. Pt-Spym was synthesized as previously described [16]. *cis*-DDP, Spym, and Pt-Spym were dissolved in PBS as 1 mg/mL stock solutions. Stock solutions were freshly prepared before use.

Cell Lines and Culture Conditions

HL-60, a human promyelocytic leukemia cell line lacking the *p53* gene [18, 19], was cultured in RPMI-1640 (Rosental Park Memorial Institute Medium) supplemented with 10% FBS, 2 mM glutamine, 100 units/mL of penicillin, and 100 mg/mL of streptomycin at 37° in an atmosphere of 95% air and 5% CO_2. HeLa (low levels of wild-type p53) cervix carcinoma cells and CH1 (normal levels of p53 wild-type) and CH1*cis*R (normal levels of p53 wild-type) ovarian cancer cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS, 2 mM glutamine, 100 units/mL of penicillin, and 100 mg/mL of streptomycin at 37° in an atmosphere of 5% CO_2. CH1*cis*R cells are endowed with acquired resistance to cisplatin and derive from CH1 cells. Resistance to cisplatin is primarily due to enhanced DNA repair [2, 20]. Primary cultures of kidney HF were maintained in MEM (modified Eagle's medium) supplemented with 10% FBS, 2 mM glutamine, 100 units/mL of penicillin, and 100 mg/mL of streptomycin under the above-mentioned conditions. The tumor cells (HeLa, CH1, CH1*cis*R, and HL-60) and the human kidney fibroblasts were passaged twice weekly, showing a doubling time of approximately 24 hr.

Drug Cytotoxicity

HL-60, HeLa, CH1, CH1*cis*R, and HF cellular survival was evaluated by using the MTT method [21]. Cells were plated onto 96-well sterile plates, at a density of 10^4 cells/well in 100 μL of medium, and incubated for 3–4 hr. Drugs were added to final concentrations from 0 to 150 μM in a volume of 100 μL/well. Sixteen, twenty-four, and forty-eight hours later, 50 μL of freshly diluted MTT solution (1/5 in culture medium) was added to a concentration of 1 mg/mL into each well and the plate was incubated for 5 hr at 37° in a humidified 5% CO_2 atmosphere. After the specified periods, cell viability was determined by measuring the absorbance at 520 nm, using a Whittaker Microplate Reader 2001. All experiments were done in quadruplicate.

Agarose Gel Electrophoresis

In order to study if the drugs produce internucleosomal DNA degradation characteristic of apoptosis in the different cell lines, "DNA laddering" was analyzed after 24 hr of drug treatment by conventional agarose gel electrophoresis as previously reported [22].

Cell Morphology and Staining with PI

For examination of altered morphology associated with contrasting modes of cell death (apoptosis versus necrosis), control and treated cancer cells were photographed in a phase-contrast microscope and stained with PI. For phase-contrast microscopy, 10 mL of cell culture (10^5 cells/mL)

was plated in P100 plates and incubated for 4–5 hr, after which Pt-Spym was added at twice the IC_{50} final concentrations and the cells incubated for 24 hr. Phase-contrast photographs were made using a Zeiss microscope at different treatment times. For fluorescence microscopy, 3 mL of cell cultures (10^5 cells/mL) was plated in P60 plates containing glass coverslips. After drug treatment for 24 hr, the coverslips with the cells were washed twice with PBS and incubated with 100 μL of PI solution (100 mM in PBS) for 30 min. The cells were fixed over the coverslips with a drop of Mowiol and immediately examined with a Zeiss microscope with fluorescence attachment. Orange fluorescence was detected. Cells exhibiting bright orange fluorescent fragmented nuclei were interpreted as apoptotic cells. Viable cells were interpreted as cells exhibiting a orange, diffusely stained and easily distinguished from apoptotic cells, nuclei [5].

Quantitative Evaluation of Apoptosis by Annexin V-FITC

HeLa cells (10^5 cells/plate) were plated in 100-mm sterile dishes at 37° until plate attachment. The cells were treated with Pt-Spym at 2 × IC_{50} for different periods of time between 3 and 48 hr. Then, cells were collected using a cell scraper and pelleted by centrifugation. The cells were washed twice with cold PBS and resuspended in 1× binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$) at a concentration of 1×10^6 cells/mL. Then, 100 μL of the solution was transferred to a 5-mL culture tube and 3 μL of annexin V-FITC and 3 μL of PI were added. The cells were vortexed and incubated for 15 min at room temperature in the dark. After this incubation, cells were washed with 400 μL of 1 × binding buffer and their fluorescence measured using a FACSCalibur Becton Dickinson flow cytometer. The percentage of apoptosis in treated cells was normalized by subtraction of the percentage of apoptosis found in control undamaged cells (3.6% ± 0.5).

Protein Extraction and Western Blot Analysis

Expression of p53 protein was detected by Western blot analysis as described previously [5]. Briefly, aliquots of lysates of HL-60, HeLa, CH1, and CH1*cis*R drug-treated cultures containing 10 μg of total protein were subjected to SDS-12% polyacrylamide gel electrophoresis. Gels were transferred into nitrocellulose filters using an electroblotting apparatus (Millipore). The filters were blocked with a PBS solution containing 3% (w/v) milk powder, 2% (w/v) glycine, and 0.1% (w/w) $Tween_{20}$ and incubated first with anti-p53 antibody (DO-1), a mouse monoclonal antibody that recognizes both wild-type and mutant p53 protein of human origin under both denaturing and non-denaturing conditions (Santa Cruz Biotechnology). After washing with PBS–0.1% $Tween_{20}$, the filters were incubated with a secondary antibody conjugated with peroxidase (goat anti-mouse IgG, immunoglobulin G, Fluka). Chemiluminiscence was performed using ECL (Amersham) according to the manufacturer's instructions. The filters were then exposed to X-ray film and the protein bands of the autoradiographs analyzed by laser densitometry using a Molecular Dynamics densitometer.

RESULTS

Cytotoxicity of the Drugs

Figure 2 shows that treatment with Pt-Spym or *cis*-DDP in a concentration range between 0 and 150 μM resulted in a dose- and time-dependent inhibition of cell survival in HL-60 and HeLa cells. In all cases, the highest effect was obtained after 24 hr of treatment. A plateau was reached after this time. In addition, Table 1 shows that Pt-Spym displayed IC_{50} values against HeLa, CH1, and CH1*cis*R cells 10-fold, 30-fold, and 210-fold lower, respectively, than those of *cis*-DDP after 24 hr of treatment. Moreover, Pt-Spym was able to circumvent cisplatin resistance in CH1*cis*R cells, showing a resistance factor 7-fold lower than that of cisplatin. In contrast, we observed that the cytotoxic activity of Pt-Spym against human fibroblasts was 2.6 times lower than that of *cis*-DDP (IC_{50} values of 135 and 52 μM, respectively). Interestingly, the Spym ligand did not have any effect on cell survival in any of the cell lines, even at the highest concentration of drug tested (150 μM).

Cell Morphology of Pt-Spym-Treated Human Tumor Cells

We used phase-contrast microscopy to visualize the types of morphological changes occurring in the control and treated Pt-Spym cells. It was observed that Pt-Spym induced cell detachment from the plate surface and the appearance of extensive morphological changes in a large percentage of the HeLa cells even after 16 hr of incubation (Fig. 3, C and D). Similar results were obtained in CH1 and CH1*cis*R cell cultures (data not shown). In contrast, when the same study was carried out in the HL-60 cell line, it was observed that the number of cells decreased due to rapid cellular lysis, suggesting that Pt-Spym produces cell death in HL-60 cultures by necrosis (data not shown).

"DNA Laddering" Detection by Agarose Gel Electrophoresis

In order to study the type of cell death (apoptosis versus necrosis) produced by Pt-Spym in the different cell lines, 1.5% agarose gel electrophoresis of extracted genomic DNA from control and drug-treated cells was performed. As expected, it was observed that the DNA in human fibroblasts appeared as a band of high molecular size corresponding to genomic DNA (Fig. 4A, lanes 2 and 3). On the other hand, a classical 180-base pair integer oligonucleosome "ladder" was obtained when HeLa cells were treated with the drug (Fig. 4B, lane 3), but not in untreated cells (Fig.

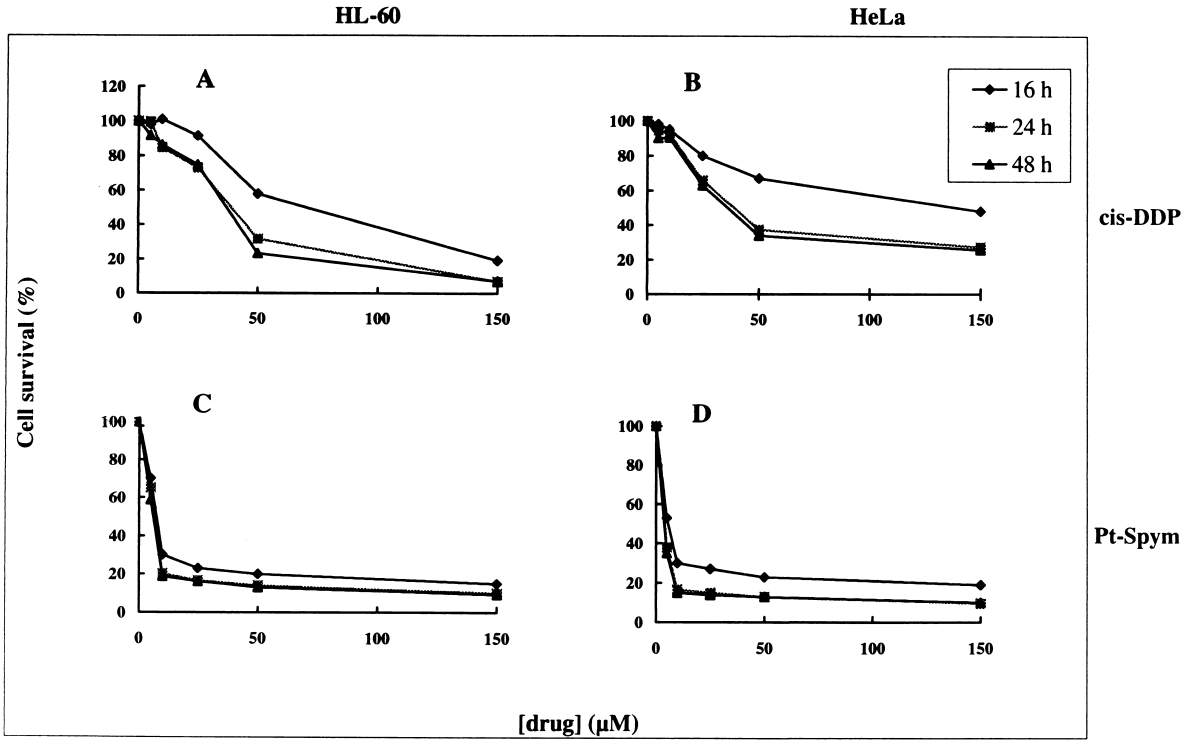

FIG. 2. Time– and dose–response effect of Pt-Spym and *cis*-DDP on the survival of HeLa and HL-60 cells. Cells were cultured with different drug concentrations for 16, 24, and 48 hr, and cell survival was determined by the MTT method. (A) HL-60 cells and (B) HeLa cells incubated with *cis*-DDP; (C) HL-60 cells and (D) HeLa cells incubated with Pt-Spym.

4B, lane 2). Similar results were obtained in CH1 and CH1*cis*R cells (data not shown). However, such an "oligonucleosome ladder" was not observed in the Pt-Spym-treated HL-60 cells (Fig. 4C, lane 3). In these cells, the DNA appeared unspecifically degraded as a smudge, and it was not possible to find the classical 180-base pair "DNA ladder" in any of the samples. Because the lack of internucleosomal DNA cleavage in HL-60 cells is a good marker of cell death through necrosis [23], it is likely that cell killing induced by Pt-Spym in HeLa cells occurs via apoptosis and in HL-60 cells and fibroblasts via necrosis.

Nuclei Morphology of Pt-Spym-Treated HeLa Cells

We confirmed the induction of apoptosis by Pt-Spym by studying the nuclear morphological features characteristic of apoptotic cell death via PI staining (Fig. 5, A and B). Exposure of the cells to twice the IC_{50} of Pt-Spym for 24 hr resulted in the appearance of apoptotic features in the majority of the HeLa cells, including cell shrinkage, nuclear condensation, and membrane-bound apoptotic bodies. Similar results were obtained in CH1 and CH1*cis*R cells (data not shown).

Quantitative Evaluation of Apoptosis by Annexin V-FITC

In order to analyze the kinetics of apoptosis induction in HeLa cells treated with Pt-Spym, we used the Annexin V method by quantifying the percentage of apoptotic cells versus the period of drug treatment in a flow cytometer. The percentage of apoptotic cells in drug-treated cultures was normalized relative to control untreated drug cultures (see Materials and Methods). Figure 6 shows that the

TABLE 1. IC_{50} mean values obtained for *cis*-DDP, Pt-Spym, and Spym against HeLa, CH1, CH1*cis*R, and HL-60 tumor cell lines and against human fibroblasts (HF) after 24 hr of incubation at 37° with the drugs

	HeLa		CH1		CH1*cis*R		HL-60		HF	
Compound	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL
cis-DDP	37.0 ± 1.3	12.3 ± 0.4	3.0 ± 0.2	1.0 ± 0.1	42.0 ± 3.0 (14)	14.0 ± 1.0	35.0	10.6 ± 0.2	52.0 ± 4.0	17.0 ± 2.0
Spym	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pt-Spym	3.5 ± 0.1	3.3 ± 0.1	0.10 ± 0.02	0.09 ± 0.01	0.20 ± 0.01 (2)	0.19 ± 0.01	3.5 ± 0.3	3.3 ± 0.1	135 ± 6.0	124.0 ± 4.0

Numbers in parentheses = resistance factors: IC_{50} resistant line/IC_{50} parent line. ND, not determined. These values were higher than 150 μM. Data represent the means of four experiments ± standard deviation.

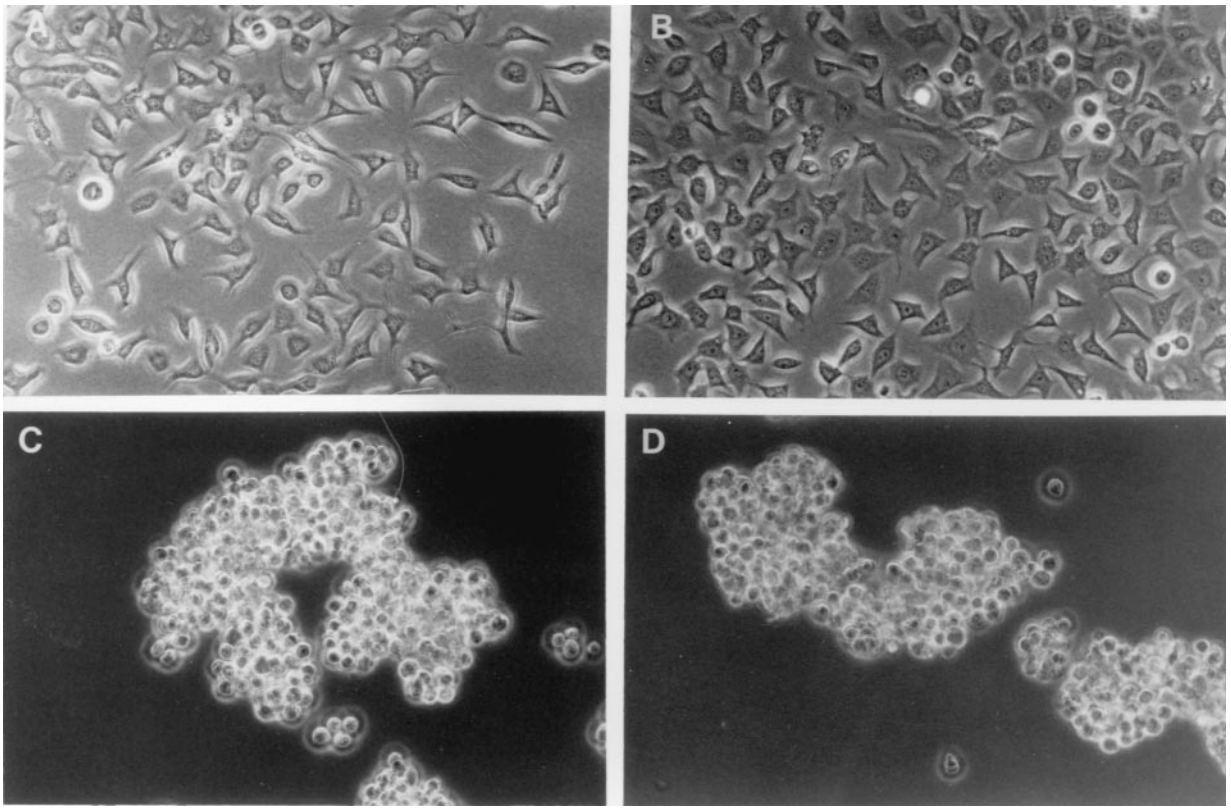

FIG. 3. Morphological changes in control untreated HeLa cells after 16 hr (A) or 24 hr (B) or in cells treated with 2 × IC_{50} of Pt-Spym for 16 (C) or 24 hr (D) and photographed under a phase-contrast microscopy at a magnification of ×200.

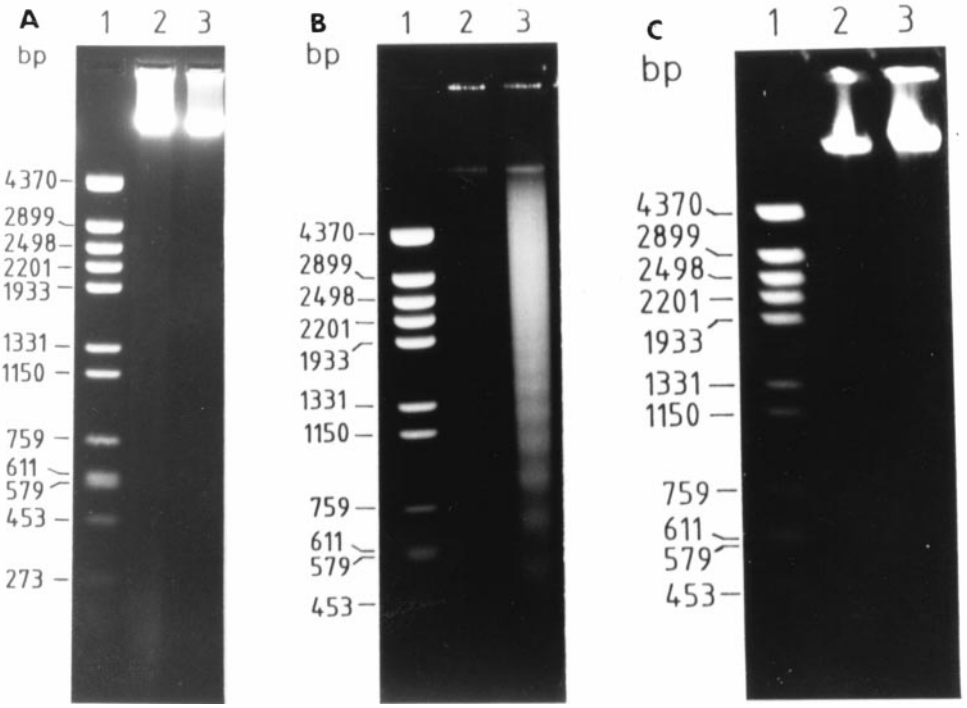

FIG. 4. Agarose gel electrophoresis (1.5%) of genomic DNA extracted from control and Pt-Spym treated for 24 hr with 2 × IC_{50} of drug. Human fibroblasts (A), control and Pt-Spym-treated (2 × IC_{50}) HeLa ovarian tumor cells (B), and control and Pt-Spym-treated (2 × IC_{50}) leukemic HL-60 cells (C).

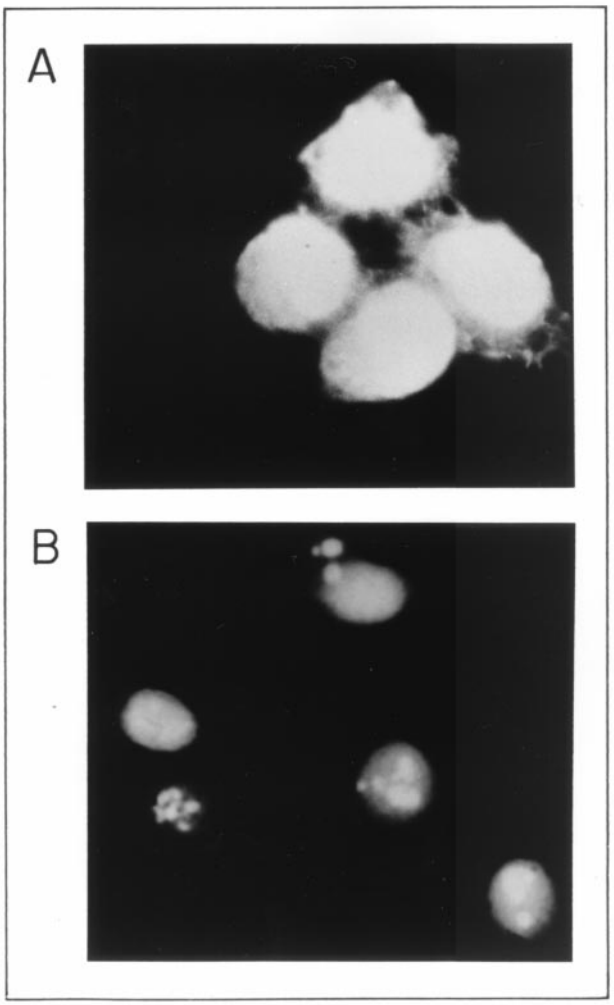

FIG. 5. Morphological changes in nuclei of HeLa cells after 24 hr of incubation with Pt-Spym. Cultures of HeLa cells were treated with 2 × IC_{50} concentrations of Pt-Spym and photographed under a Zeiss microscope with fluorescence attachment at a magnification of ×660. (A) Control cells; (B) Pt-Spym-treated cells.

percentage of apoptosis increased with the period of drug treatment. Thus, after 3 hr of incubation with 2 × IC_{50} of Pt-Spym, 5% of apoptotic cells was produced with 83% of the cells becoming apoptotic after 24 hr. After 24 hr of Pt-Spym treatment, apoptosis reached a plateau.

p53 Levels in Drug-Treated Cells

Since p53 protein is broadly implicated in apoptosis induction, we analyzed whether the induction of apoptosis by the Pt-Spym compound in CH1, CH1*cis*R, and HeLa cells was associated with changes in the levels of p53 protein. CH1 and CH1*cis*R cells have normal levels of wild-type p53 [2], HeLa cells have low levels of wild-type p53 [24], and HL-60 cells lack p53 protein [25]. Figure 7 (upper panel) shows the levels of p53 protein in CH1, CH1*cis*R, and HeLa cells treated with 2 × IC_{50} of Pt-Spym or *cis*-DDP for 24 hr. It may be observed that, after Pt-Spym treatment, the levels of p53 protein in CH1, CH1*cis*R, and HeLa cells were slightly lower than those of the control untreated cells (Fig. 7: lanes, 2, 5, and 8 vs lanes 1, 4, and 7; see caption to Fig. 7 for protein quantification). In contrast, treatment with *cis*-DDP produced a 100-fold decrease in the levels of p53 protein in CH1 and CH1*cis*R cells (Fig. 7: lanes 3 and 6 vs lanes 1 and 4) and complete annihilation of p53 protein in HeLa cells (Fig. 7: lane 9 vs lane 7). On the other hand, in control experiments it was observed that neither Pt-Spym nor *cis*-DDP induced changes in the levels of α-actin. (Fig. 7: bottom panel).

DISCUSSION

It is well known that one of the handicaps of cancer chemotherapy is the local toxicity produced by the currently used drugs, due to their accumulation in several organs and tissues at their therapeutic doses [26]. Apoptosis, on the other hand, is considered to be a cleaner system of cell death than necrosis, because the lysis of the necrotic cells leads to the production of local side effects due to release of toxic substances from inside the cell to the extracellular matrix [9]. Thus, efforts are being directed toward the synthesis of novel compounds with antineoplastic activity resulting from apoptosis induction.

Cell survival assays indicate that Pt-Spym shows remarkable cytotoxic activity against tumor cells in a dose- and time-dependent way. Interestingly, Pt-Spym is capable of circumventing cisplatin resistance in CH1*cis*R cells. Because the primary mechanism of resistance to cisplatin of CH1*cis*R cells is the result of enhanced DNA repair [20], it is most likely that the DNA adducts formed by Pt-Spym are more difficult to remove by the repair machinery of CH1*cis*R cells than those of cisplatin. In fact, atomic force microscopy experiments have revealed that Pt-Spym produces cross-links between several DNA double helices (interhelical cross-links) [17]. Because the ability of a DNA-binding drug to kill tumor cells might be a consequence of the genetic instability associated with malignant transformation, which lowers the threshold at which DNA injury triggers apoptosis [13], we further analyzed the type of cell death induced by Pt-Spym in the different tumor cell lines. A first indication that the cytotoxic activity of the Pt-Spym compound in HeLa, CH1, and CH1*cis*R cells is due to apoptosis was shown by the analysis, through phase contrast microscopy, of the morphological changes originating in drug-treated cells. The appearance of both a classical 180-base pair integer oligonucleosome "ladder" and of fragmented nuclei in Pt-Spym-treated HeLa, CH1, and CH1 ovarian cancer cells confirmed the induction of apoptosis. Interestingly, *cis*-DDP is also able to induce cell death through apoptosis in HeLa and CH1 cells, but at drug concentrations about 10 times higher than those of Pt-Spym [27]. However, cisplatin is unable to induce apoptosis in CH1*cis*R cells [20]. On the other hand, in spite of the remarkable cytotoxic activity of Pt-Spym against HL-60, the drug does not seem to induce apoptosis in this leukemia

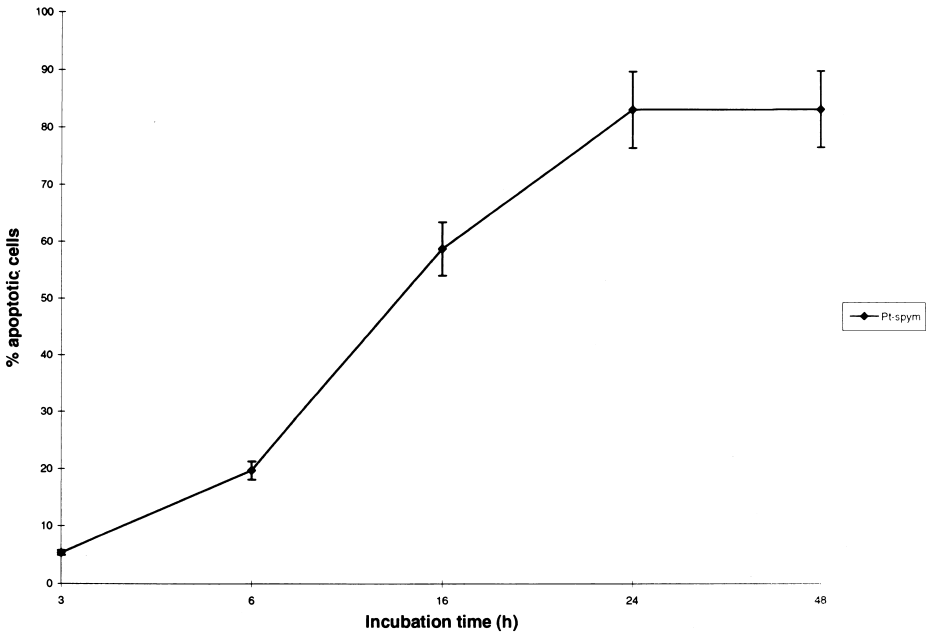

FIG. 6. Percentage of apoptotic HeLa cells after Pt-Spym treatment (2 × IC_{50}) as calculated from the amount of fragmented and integer DNA versus the incubation time. Error bars indicate standard deviations.

cell line, as suggested by the fact that the 180-base pair integer oligonucleosome "ladder" was not observed. Although DNA "laddering" in HL-60 cells might occur this rapidly, prior to secondary necrosis, we observed that Pt-Spym-treated HL-60 cells did not show evidence of fragmented nuclei or apoptotic bodies in time–course experiments. Thus, it is likely that Pt-Spym kills HL-60 cells via necrosis.

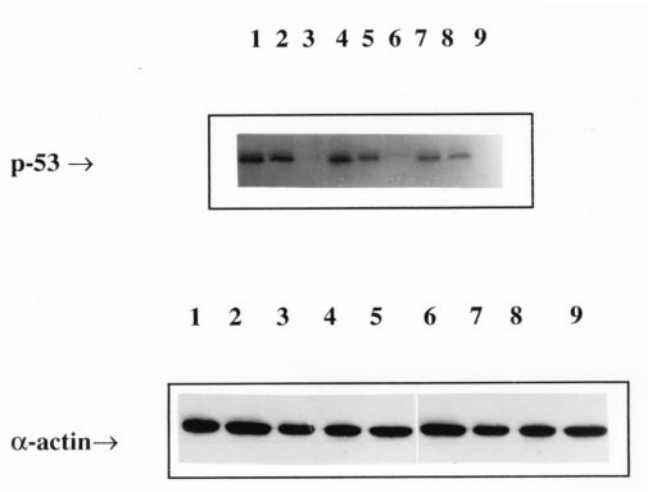

FIG. 7. (Upper panel). Levels of expression of p53 protein in CH1, CH1*cis*R, and HeLa cells after treatment with 2 × IC_{50} of the Pt-Spym complex and *cis*-DDP. Lane 1, control untreated CH1 cells (1.0 μg of p53); lane 2, CH1 cells treated with Pt-Spym (0.8 μg of p53); lane 3, CH1 cells treated with *cis*-DDP (0.01 μg of p53); lane 4, control CH1*cis*R cells (1 μg of p53); lane 5, CH1*cis*R cells treated with Pt-Spym (0.7 μg of p53); lane 6, CH1*cis*R cells treated with *cis*-DDP (0.01 μg of p53); lane 7, control HeLa (0.5 μg of p53); lane 8, HeLa cells treated with Pt-Spym (0.4 μg of p53); and lane 9, HeLa cells treated with *cis*-DDP (absence of p53). (Bottom panel). Control levels of α-actin in treated and non-treated cells as in (A).

The results suggest that the different behavior of Pt-Spym regarding HeLa, CH1, and CH1*cis*R cells on the one hand and HL-60 cells on the other might be related to the presence or absence of p53 protein. In fact, the Western blot data reported here show that treatment with Pt-Spym does not substantially modify the levels of p53 protein in HeLa, CH1, and CH1*cis*R cells. In contrast, *cis*-DDP induces a drastic decrease in the levels of p53 protein in CH1 cisplatin-sensitive and CH1*cis*R cisplatin-resistant cells and abolishes p53 protein in HeLa cells.

Accumulation of p53 in response to cisplatin has been observed in a majority of tumor cells, supporting the view that the wild-type p53 protein may inhibit cell growth to allow DNA repair, and in the event of irreparable damage, initiate apoptotic cell death [15]. However, after treatment with cisplatin, no p53 protein has been detected in tumor cells such as 41M, 41McisR, HX155, and HX155cisR [28]. Thus, p53 response to a DNA-damaging agent may vary depending on the cell line type. Because no increase in p53 mRNA has been observed in several cell lines after cisplatin treatment, it is likely that accumulated p53 protein may require ongoing translation together with a high metabolic stability of p53 mRNA [29]. It is likely, then, that cisplatin may affect the stability of p53 mRNA in some cell lines targeting p53 for degradation. In fact, according to previously reported data, either depletion or complete removal of p53 protein by *cis*-DDP would explain the lower cytotoxic potency of cisplatin in comparison with Pt-Spym in the panel of tumor cell lines tested [30, 31]. We think that the

apoptotic activity of Pt-Spym in tumor cells with functional p53 suggests that the drug might achieve better results against malignancies expressing this tumor suppressor gene. However, because more than 50% of neoplasias have non-functional p53, due to p53 mutations or even the absence of p53 [19], it is possible that Pt-Spym could induce non-specific local toxicity when used against p53-negative cancer types. However, it is interesting to note that Pt-Spym induces cell killing through apoptosis in HeLa cells that have a markedly defective p53 pathway (low levels of wild-type p53) due to expression of the *HPV E6* gene that targets p53 for degradation [24]. To our knowledge, Pt-Spym is the first reported Pt(III) compound that provokes tumor cell killing through apoptosis.

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