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Induction of Erythroid Differentiation of Human K562 Cells by Cisplatin Analogs

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ABSTRACT. Human leukemic K562 cells can be induced *in vitro* to erythroid differentiation by a variety of chemical compounds, including hemin, butyric acid, 5-azacytidine, and cytosine arabinoside. Differentiation of K562 cells is associated with an increase in the expression of embryo–fetal globin genes, such as the ζ-, ε-, and γ-globin genes. Therefore, the K562 cell line has been proposed as a very useful *in vitro* model system for determining the therapeutic potential of new differentiating compounds as well as for studying the molecular mechanism(s) regulating changes in the expression of embryonic and fetal human globin genes. Inducers of erythroid differentiation that stimulate γ-globin synthesis could be considered for possible use in the experimental therapy of hematological diseases associated with a failure in the expression of adult β-globin genes. In this paper, we analyzed the effects of a series of cisplatin analogs on both cell growth and differentiation of K562 cells. Among seven cisplatin analogs studied, three were found to be potent inducers of erythroid differentiation. Erythroid differentiation was associated with an increase in the accumulation of (a) hemoglobins Gower 1 and Portland and (b) γ-globin mRNA. BIOCHEM PHARMACOL **60**;1:31–40, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. K562 cells; erythroid differentiation; DNA-binding drugs; cisplatin; cisplatin analogs

Pharmacologically mediated regulation of the expression of human γ-globin genes has been proposed as a potential therapeutic strategy in hematological disorders, including β-thalassemia [1–4]. It has been suggested that even modest increases in fetal Hb§ levels can be clinically beneficial in sickle cell disease [5], while a greater increase in HbF is necessary to reduce the severity of β-thalassemia [3]. Therefore, recent studies have been focused on the search for compounds able to stimulate γ-globin gene expression [6–10]. In this respect, the human leukemic K562 cell line has been proposed as a very useful *in vitro* model system for studying the molecular mechanism(s) regulating the expression of embryonic and fetal human globin genes [11–17], as well as for determining the therapeutic potential of new differentiating compounds [3, 11]. This cell line, isolated and characterized by Lozzio and Lozzio [18] from a patient with chronic myelogenous leukemia in blast crisis, exhibits a low proportion of hemoglobin-synthesizing cells under standard cell growth conditions, but is capable of undergoing erythroid differentiation when treated with a variety of compounds, including hemin [11], ara-C [13], butyric acid [13, 17], 5-azacytidine [15], and chromomycin and mithramycin [19]. Following erythroid induction of K562 cells, a sharp increase in cytoplasmic accumulation of Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\varepsilon_2$) is observed, accompanied by an increase in the expression of human ε- and γ-globin genes [14–17]. *In vitro* studies demonstrate that known inducers of K562 erythroid differentiation, such as hydroxyurea, erythropoietin, butyrates, and 5-azacytidine, are also capable of inducing fetal hemoglobin production when administered singularly or in combination to normal erythroid cells [1, 6, 8, 9, 20]. With respect to this point, butyric acid and 5-azacytidine have been the object of recent reports focused on *in vivo* treatment of β-thalassemia.

Among possible biological response modifiers, one of the most interesting classes of compounds are DNA-binding drugs displaying sequence selectivity [21–24]. DNA-binding drugs are capable of interfering with the DNA-binding activity of a variety of transcription factors in a sequence-dependent manner [22–28]. With respect to capacity to induce erythroid differentiation, we have recently demonstrated that the G + C-selective DNA-binding compounds mithramycin and chromomycin are able to stimulate Hb Portland production and γ-globin mRNA accumulation in treated K562 cells [19].

Among DNA-binding compounds, cisplatin (**1**) has been the object of a number of studies. Since the discovery

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§ *Abbreviations:* Hb, hemoglobin; HbF, fetal hemoglobin; DACH, diaminocyclohexane; THMP, trihydroxymethyl phosphine; H_2O_2, hydrogen peroxide; FT-IR, Fourier transformed-infrared; ara-C, 1-β-arabinofuranosylcytosine.

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of its cytostatic activity in 1964 by Rosenberg [29] and its proposed use as anticancer drug since 1979 [30], cisplatin is routinely employed for the treatment of testicular and ovarian cancer and is being increasingly used against cervical, bladder, and head/neck tumors [30]. The mechanism of action of cisplatin is based on the intrastrand cross-linking of the *cis*-$Pt(NH_3)_2$ unit to cellular DNA at two neighboring guanine bases [30]. With respect to a possible use as differentiating agent, cisplatin was shown to induce erythroid differentiation of K562 cells [31]. However, despite being one of the most active chemotherapeutic agents currently available [30–33] and despite having a large clinical use [30], cisplatin exhibits severe side-effects, the most significant of which is acute and chronic nephrotoxicity [34, 35]. In addition, optic neuropathy [36] and ototoxicity [37] of cisplatin have been reported. Therefore, cisplatin analogs are currently being designed, synthesized, and tested in order to find compounds retaining biological activity without exhibiting nephrotoxic effects. In the most successful second generation cisplatin analogs, the chloride ligands have been replaced by carboxylate (e.g. carboplatin, **9**). Despite the fact that the structural variation of carboplatin (**9**) seems to be responsible for a decreased toxicity of this compound [38], to our knowledge no information is available in the literature on the possible capacity of carboplatin to induce erythroid differentiation of K562 cells.

Platinum(1,2-DACH) complexes, including the prototype [$PtCl_2$(1*R*,2*R*-DACH)] (**2**), have also attracted considerable interest. In fact, (**2**) and other 1,2-DACH derivatives showed good *in vitro* activity [32] and *in vivo* efficacy against cisplatin-resistant tumors [33]; low aqueous solubility and molecular instability have been the major impediments to clinical development of Pt(DACH) analogs. Following this perspective, we prepared the new compounds **3** and **4** *cis*-[$Pt(OOCR)_2(NH_3)_2$] (**3**, RCOO = cholate; **4**, RCOO = deoxycholate), bearing bile acids as anionic ligands. In this paper, we report on the synthesis of these cisplatin analogs (see Fig. 1 for chemical structures) and their biological effects on human leukemic K562 cells. The activity of compounds **3** and **4** was compared to that of cisplatin (**1**), carboplatin (**9**), compound **2**, and a parallel group of complexes where the neutral ligand NH_3 is replaced by S-bonded DMSO: *cis*-[$PtCl_2(DMSO)_2$] (**5**), *cis*-[$Pt(OOCR)_2(DMSO)_2$] (**6**, RCOO = cholate; **7**, RCOO = deoxycholate). Finally, we also tested a phosphinic compound (**8**) *cis*-[$PtCl_2(THMP)_2$].

Erythroid differentiation of K562 cells was studied by the benzidine/H_2O_2 reaction [16, 17], hemoglobin production by cellulose acetate gel electrophoresis of postmitochondrial supernatants [39], and γ-globin mRNA accumulation was analyzed by Northern blotting [40]. The results obtained demonstrated that three of the newly synthesized platinum complexes are potent inducers of erythroid differentiation of K562 cells. Erythroid differentiation by these cisplatin analogs was associated with (a) an increase in the content of embryo–fetal hemoglobins and (b) an increase in γ-globin mRNA accumulation.

MATERIALS AND METHODS

Synthesis of Cisplatin and Cisplatin Analogs Containing Chloride Ligands

cis-[$PtCl_2(NH_3)_2$] (cisplatin) (**1**) [41], [$PtCl_2$(1*R*,2*R*-DACH)] (**2**) [42], *cis*-[$PtCl_2(DMSO)_2$] (**5**) [43], and *cis*-[$PtCl_2(THMP)_2$] (**8**) [44] were prepared following reported methods and characterized by elemental analyses and spectroscopic techniques (i.r. and NMR). Carboplatin (**9**) was obtained from Sigma Chemical Co.

Synthesis of cis-[$Pt(OOCR)_2(NH_3)_2$] (3, RCOO = cholate; 4, RCOO = deoxycholate)

Five hundred milligrams of *cis*-[$PtI_2(NH_3)_2$] (1 mmol) was suspended in 2.5 mL of H_2O. With the suspension maintained under stirring, 0.34 g (2 mmol) of $AgNO_3$ dissolved in 2.5 mL of H_2O was added. The reaction mixture was stirred for 20 min at 65° in the dark. The colloidal suspension of AgI was then filtered through a Millipore membrane and a clear solution thus obtained. Sodium cholate (for product **3**) or sodium deoxycholate (for product **4**) (2.2 mmol in 10 mL of water) was added dropwise under stirring. The reaction mixture containing the product as a white precipitate was cooled for 2 hr, then the solid was filtered, washed three times with water, and dried under vacuum.

(3): 70% yield. *FT-IR* (KBr, ν cm^{-1}, selected data): 3300 (OH, NH); 1572 (COO asym); 1377 (COO sym). *^{1}H-NMR* (CD_3OD, δ ppm): 0.7 (6H, s, CH_3); 0.9 (6H, s, CH_3); 1.1 (6 H, d, ^{3}JHH = 6 Hz, CHC*H*$_3$); 1.1–2.4 (48 H, m); 3.4 (2 H, br s, HOC3*H*); 3.9 (2 H, br s, HOC7*H*); 4.0 (2 H, br s, HOC12*H*); in DMSO-d6, 4.3 ppm (6 H, br, NH_3). *Elemental analysis*: calculated values for $C_{48}H_{84}N_2O_{10}Pt$: C% = 55.25; H% = 8.05; N% = 2.68; found: C% = 55.56; H% = 8.06; N% = 2.60.

(4): 68% yield. *FT-IR* (KBr, ν cm^{-1}, selected data): 3300 (OH, NH); 1556 (COO asym); 1381 (COO sym). *^{1}H-NMR* (CD_3OD, δ ppm): 0.7 (6 H, s, CH_3); 0.9 (6 H, s, CH_3); 1.0 (6 H, d, ^{3}JHH = 6 Hz, CHC*H*$_3$); 1.0–2.4 (52 H, m); 3.5 (2 H, br s, HOC3*H*); 3.9 (2 H, br s, HOC12*H*). *Elemental analysis*: calculated value for $C_{48}H_{84}N_2O_8Pt$: C% = 56.97; H% = 8.31; N% = 2.77; found: C% = 56.99; H% = 8.60; N% = 2.65.

Synthesis of cis-[$Pt(OOCR)_2(DMSO)_2$] (6, RCOO = cholate; 7, RCOO = deoxycholate)

Two hundred milligrams ($2.37 \cdot 10^{-4}$ mol) of *cis*-[$PtCl_2(DMSO)_2$] was dissolved in 40 mL of CH_2Cl_2, and $4.74 \cdot 10^{-4}$ mol of silver salt (silver cholate for **6**, silver deoxycholate for **7**) was added. The brown suspension was kept under stirring in the dark for 18 hr and the precipitate

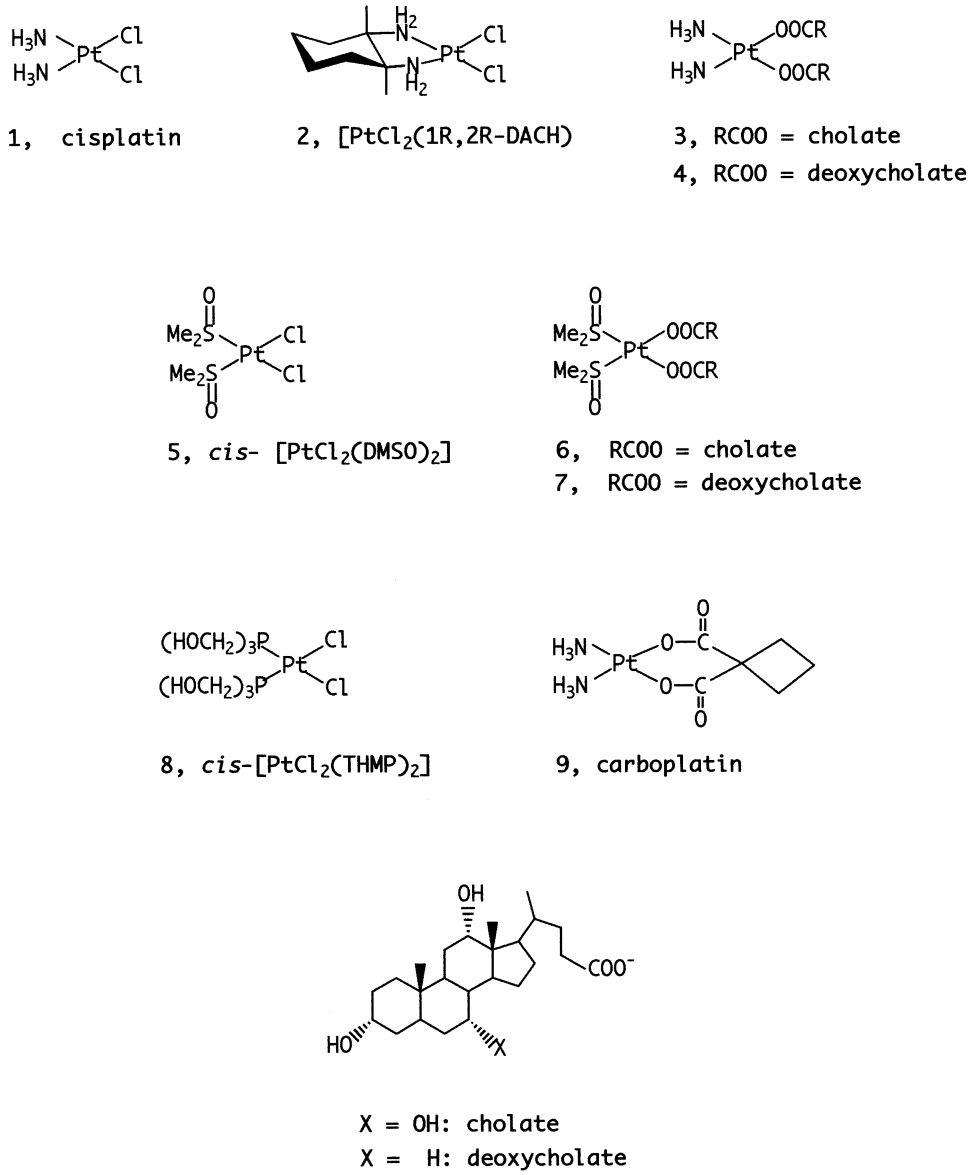

FIG. 1. Chemical structures of cisplatin and the cisplatin analogs employed in this study.

of AgCl then filtered. The clear yellow filtrate was taken to complete dryness, leaving the product as a cream solid.

(6): 80% yield. *IR* (CsI, ν cm^{-1}, selected data): 3422 (OH); 1566 (COO asym), 1381 (COO sym), 1145 (ν SO). *^1H NMR* (CD_3OD, δ ppm): 0.6 (6 H, s, CH_3); 0.9 (6 H, s, CH_3); 1.0 (6 H, d, ^{3}JHH = 6 Hz, CHC*H*$_3$) 1.1–2.4 (48 H, m); 3.4 (2 H, br s, HOC3*H*); 3.45 (12 H, s, ^{3}JHPt = 13 Hz, $OSCH_3$); 3.8 (2H, br s, HOC7*H*); 3.9 (2H, br s, HOC12*H*). *{^1H}^{195}Pt-NMR* (CD_3OD, δ ppm): −452. *Elemental analysis*: calculated values for $C_{52}H_{90}O_{12}PtS_2$: C% = 53.58; H% = 7.72; S% = 5.49; found: C% = 53.8; H% = 7.87; S% = 5.73.

(7): 81% yield. *IR*(KBr, ν cm^{-1}, selected data): 3414 (OH); 1558 (COO asym); 1385 (COO sym), 1033 (SO), 439 (PtS). *^1H NMR* (CD_3OD, δ ppm): 0.7 (6 H, s, CH_3); 0.9 (6 H, s, CH_3); 1.0 (6 H, d, ^{3}JHH = 6 Hz, CHC*H*$_3$); 1.2–2.4 (52 H, m); 3.4 (12 H, s, ^{3}JHPt = 13 Hz, $OSCH_3$); 3.5 (2H, br s, HOC3*H*); 4.0 (2H, br s, HOC12*H*). *{^1H}^{195}Pt-NMR* (CD_3OD, δ ppm): −454.22. *Elemental analysis*: calculated values for $C_{52}H_{90}O_{10}PtS_2$: C% = 55.07; H% = 7.94; S% = 5.65; found: C% = 53.06; H% = 8.74; S% = 5.06.

Cell Lines and Culture Conditions

Human erythroleukemia K562(S) cells [11] were cultured in a humidified atmosphere at 5% CO_2 in RPMI-1640 (Flow Laboratories) supplemented with 10% fetal bovine serum (FBS, Celbio), 50 units/mL of penicillin, and 50 μg/mL of streptomycin [16]. Cell growth was studied by determining the cell number/mL after different days of *in vitro* cell culture [14, 15, 17]. Stock solutions of cisplatin

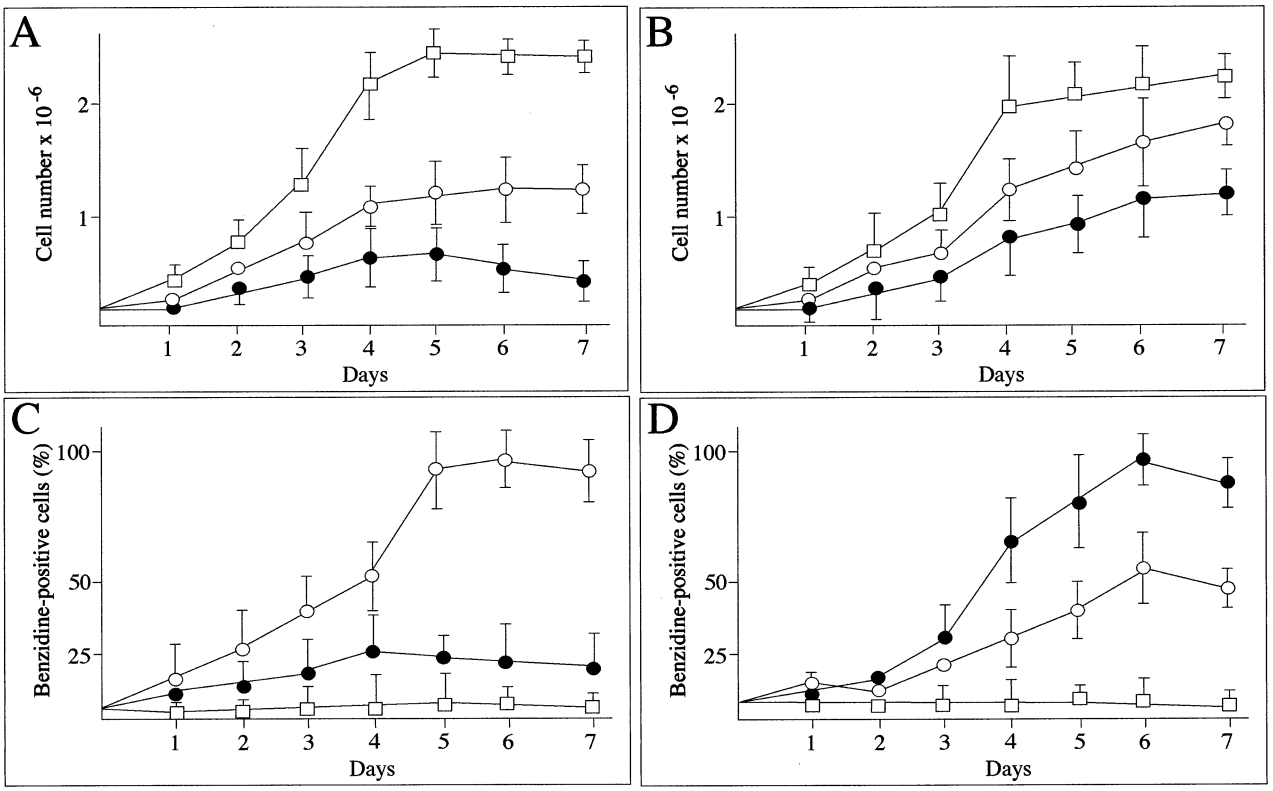

FIG. 2. Effects of cisplatin (1) (A, C) and carboplatin (9) (B, D) on *in vitro* growth (A, B) and differentiation (B, D) of K562 cells. Cells were cultured in the absence (open squares) or the presence of 6 μM cisplatin (A, C, open circles), 25 μM cisplatin (A, C, closed circles), 12 μM carboplatin (B, D, open circles), and 25 μM carboplatin (B, D, closed circles). After different days, the cell number/mL and the proportion of benzidine-positive cells were determined in each K562 cell population. Results represent the average of 6 (A, C) and 4 (B, D) independent experiments ± SD. At day 0, the concentration of uninduced cells was 7.5 × 10^4 cells/mL; at day 0, the percentage of benzidine-positive cells was found to be 5.6 ± 2.8 (C) and 6.5 ± 3.1 (D).

(10 mM) and ara-C (10 μM) were stored at −20° in the dark and diluted immediately before use. Treatment with the indicated concentrations of cisplatin and cisplatin analogs was carried out by adding the appropriate drug concentrations at the beginning of the experiment. The medium was not changed during the induction period. The chemical structures of cisplatin and cisplatin analogs are shown in Fig. 1.

Hemoglobin Determination

K562 cells containing heme or hemoglobin were detected by specific reaction with a benzidine/hydrogen peroxide solution as reported elsewhere [15, 16]. The final concentration of benzidine was 0.2% in 5 M glacial acetic acid, 10% H_2O_2 [15, 16]. In order to analyze hemoglobin production by erythroid-induced K562 cells, 2 μL of total fresh postmitochondrial cell lysates was electrophoresed on cellulose acetate strips (Poliphorm) in Tris–EDTA–borate buffer [17, 39]. After an electrophoresis of 30 min at 5 mA, the gels were stained with benzidine/hydrogen peroxide (1% benzidine in 4.3 M acetic acid, 3% H_2O_2) and photographed [39].

Northern Blotting

Total RNA was phenol-chloroform-extracted from cytoplasms of uninduced K562 cells or K562 cells induced to erythroid differentiation with cisplatin and cisplatin analogs [40]. Fifteen micrograms of total RNA was loaded onto 1% agarose gel, electrophoresed, transferred to a nylon membrane (GeneScreen plus), and hybridized with the ^{32}P-labeled γ-pUCA probe. This probe is a 3.3-kb *Hind*III fragment of the human A-γ-globin gene cloned in the *Hind*III site of pUC18 [45].

RESULTS

Cell Growth and Differentiation of K562 Cells Cultured in the Presence of Newly Synthesized Cisplatin Analogs

Figure 2 shows the effects of cisplatin and carboplatin on K562 cell growth (Fig. 2, A and B) and differentiation (Fig. 2, C and D). In these experiments, K562 cells were seeded in the absence or presence of 6 and 25 μM cisplatin (A and C) and 12 and 25 μM carboplatin (B and D). Both compounds clearly induced (a) a dose-dependent inhibition of *in vitro* proliferation of K562 cells and (b) an increase in the proportion of benzidine-positive (hemoglobin-contain-

ing) cells. It should be noted that high concentrations of cisplatin (25–50 μM, see also Fig. 3A) were highly toxic, leading to a strong inhibition of cell growth, associated with a blocking of erythroid differentiation of treated K562 cells. Trypan blue exclusion tests demonstrated that a high number of K562 cells treated with 25 μM cisplatin (60.8 ± 3.8%; N = 6) efficiently incorporated the dye, thus confirming the high cytotoxicity of treatment with cisplatin. By contrast, carboplatin was found to be less cytotoxic at the same concentrations, the cells being positive to the trypan blue exclusion test 7.5 ± 2.5% (N = 4). Figure 3 shows a comparison of the effects of cisplatin and cisplatin analogs on K562 cell growth and differentiation. For all the compounds, experiments similar to those reported in Fig. 2 were conducted and the results obtained after 7 days of cell culture were compared. Figure 3 (open symbols) shows that cisplatin and cisplatin analogs **2**, **3**, **4**, and **8** caused a dose-dependent decrease in the proliferation efficiency of K562 cells. Fifty percent inhibition of cell growth (IC_{50}) occurred when K562 cells were cultured for 7 days in the presence of 4 μM cisplatin (**1**), 20 μM (**2**), 35 μM (**8**), 8 μM (**3**), and 20 μM (**4**), respectively. On the contrary, compounds **5**, **6**, and **7** exhibited much lower inhibitory activity on K562 *in vitro* cell growth. Trypan blue exclusion tests, analysis of caspase activity, and the dUTP nick end labeling (TUNEL) assay demonstrated that the antiproliferative activity of compounds **2**, **3**, **4**, and **8** was not associated with cytotoxicity, but rather with activation of apoptosis.* Figure 3 (closed symbols) also demonstrates that cisplatin and cisplatin analogs displayed a clearly different ability to induce a significant increase in the proportion of benzidine-positive (hemoglobin-containing) K562 cells after 7 days of induction. Cisplatin and compounds **2**, **3**, and **4** were found to be effective inducers of K562 erythroid differentiation. By contrast, compounds **8**, **5**, **6**, and **7** did not induce erythroid differentiation. This lack of induction ability was particularly significant for compound **8**, which, unlike **5**, **6**, and **7**, exhibited antiproliferative activity.

The kinetics of induction of erythroid differentiation by compounds **2**, **3**, and **4** in comparison with cisplatin and ara-C, one of the most powerful inducers of K562 cell differentiation, are shown in Fig. 4 [13]. Therein, the kinetics of induction of compounds **2** and **3** are also compared to the effects of cholate and deoxycholate (see panel C). It should be noted that the induction ability of **2** (Fig. 4A), **3** (Fig. 4B), and **4** (Fig. 4B) was similar to that of ara-C (Fig. 4A) and other inducers of erythroid differentiation of K562 cells, such as mithramycin [19] and hydroxyurea ([8, 19] and data not shown). Control experiments performed by treating K562 cells with cholate and deoxycholate demonstrated that these compounds were not active in inducing erythroid differentiation of K562 cells (Fig. 4C) and inhibiting cell growth (data not shown).

* Mischiati C, Bianchi N and Gambari R, manuscript in preparation.

Hemoglobin Accumulation in K562 Cells Cultured with Cisplatin and Compounds 2, 3, and 4

Cellogel acetate gel electrophoresis of postmitochondrial cell lysates is reported in Fig. 5. Low levels of accumulation of both Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\epsilon_2$) were found in uninduced K562 cells, in agreement with already published observations from our and other laboratories [12, 19, 46, 47]. The results obtained (Fig. 5) demonstrate that, following 7 days of treatment of K562 cells with ara-C, cisplatin, and compounds **2**, **3**, and **4**, both Hb Portland and Hb Gower 1 were abundantly expressed, suggesting that erythroid differentiation induced by these cisplatin analogs is accompanied by an increase in the expression of the embryo–fetal globin genes (the β-like ε and γ and the α-like ζ). The percentage values of benzidine-positive cells in this experiment were 6.5% (uninduced cells), 85.2% (cisplatin-treated cells), 75.4% (cells treated with compound **2**), 72.3% (cells treated with compound **3**), and 76.5% (cells treated with compound **4**).

Northern Blotting Analysis

Total RNA was isolated from both uninduced and erythroid-induced K562 cells treated for 7 days in the presence of cisplatin, compounds **2**, **3**, and **4**, and 250 nM ara-C. In order to analyze the expression of γ-globin genes, Northern blotting was performed and hybridization carried out with a ^{32}P-labeled plasmid specifically recognizing human γ-globin mRNA sequences. The results are shown in Fig. 6 and clearly demonstrate that treatment of K562 cells with compounds **2**, **3**, and **4** induced a sharp increase in accumulation of γ-globin mRNA.

DISCUSSION

We describe in this paper three platinum complexes (compounds **2**, **3**, and **4**) able to induce K562 cell erythroid differentiation at a level similar to cisplatin (**1**) and carboplatin (**9**). Erythroid differentiation of K562 cells with compounds **2**, **3**, and **4** is associated with production of both Hb Gower 1 and Hb Portland. Control experiments suggest that these related compounds induce differentiation of K562 cells at levels (a) similar to those of the most effective inducers ara-C and mithramycin [12, 18] and (b) higher than those of other studied K562 cell inducers, such as butyric acid, 5-azacytidine, and hemin [11, 12, 15].

In order to show that the induction of erythroid differentiation observed for cisplatin and cisplatin analogs **2**, **3**, and **4** is not related to a mere administration to cells of whatever platinum-containing compounds, we tested as comparison a parallel group of complexes where the neutral ligand NH_3 is replaced by S-bonded DMSO: *cis*-$[PtCl_2(DMSO)_2]$ (**5**), *cis*-$[Pt(OOCR)_2(DMSO)_2]$ (**6**, RCOO = cholate; **7**, RCOO = deoxycholate). Despite the fact that we found that the absence of antiproliferative activity for **5**, **6**, and **7** is consistent with no activity as

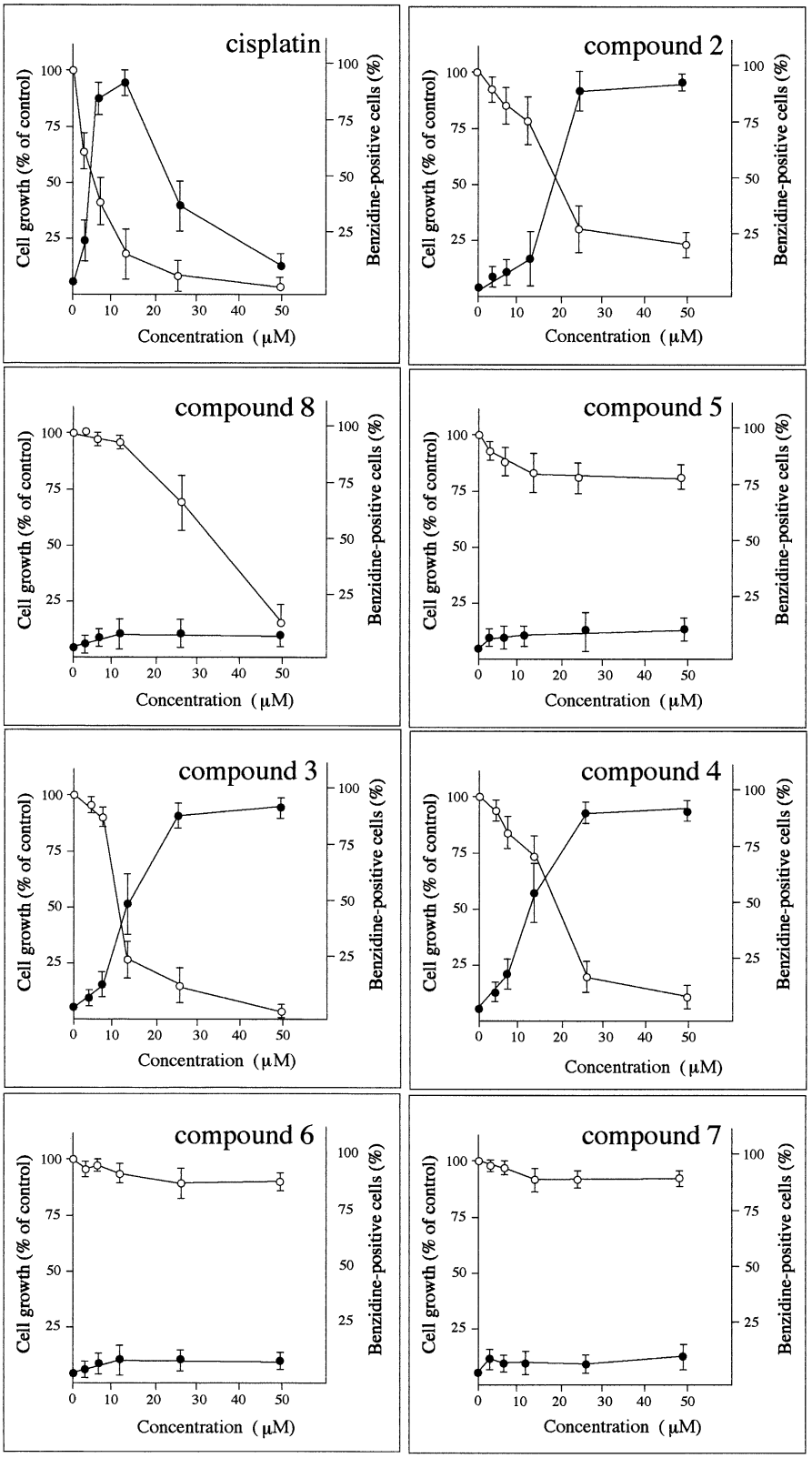

FIG. 3. Effects of cisplatin and cisplatin analogs on *in vitro* growth (open symbols) and differentiation (closed symbols) of K562 cells. Cells were cultured in the absence (0) or the presence of different concentrations of cisplatin and cisplatin analogs. After 7 days, the cell number/mL and the proportion of benzidine-positive (hemoglobin-containing) cells were determined in each K562 cell population. The data values of cell number/mL in treated cells were compared to those of untreated cultures and expressed as % of control. Results represent the average ± SD of four independent experiments. After 7 days' culture of untreated K562 cells, the percentage of benzidine-positive cells was 7.9 ± 2.7.

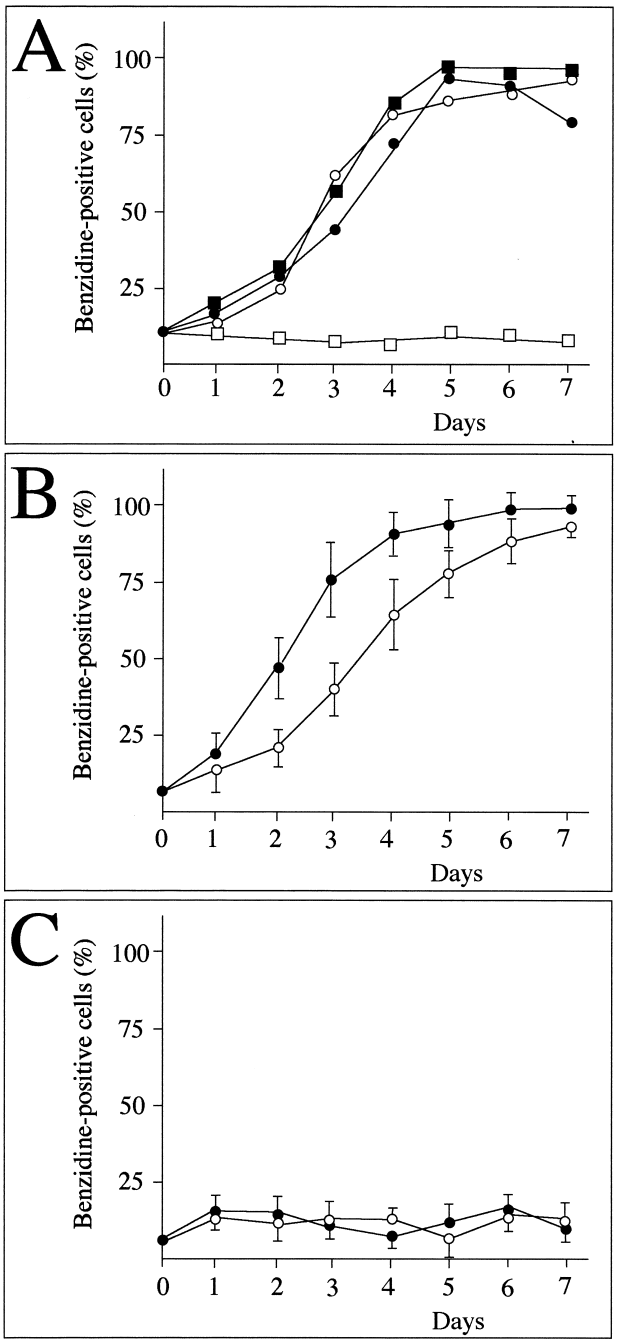

FIG. 4. Kinetics of erythroid induction of K562 cells by ara-C, cisplatin, compounds 2, 3, and 4, cholate, and deoxycholate. (A) Closed squares, cells treated with 500 nM ara-C; closed circles, cells treated with 12 μM cisplatin; open circles, cells treated with 25 μM compound 2. (B) Open circles, cells treated with 25 μM compound 3; closed circles, cells treated with 25 μM compound 4. (C) Open circles, cells treated with 50 μM cholate; closed circles, cells treated with 50 μM deoxycholate. K562 cells were cultured as indicated and after different lengths of time, the proportion of benzidine-positive (hemoglobin-containing) cells was determined. Results reported in panel A are from a single control experiment, while those reported in panels B and C are from three independent experiments and represent the average ± SD. At day 0, the percentage of benzidine-positive cells was 6.7 ± 2.2.

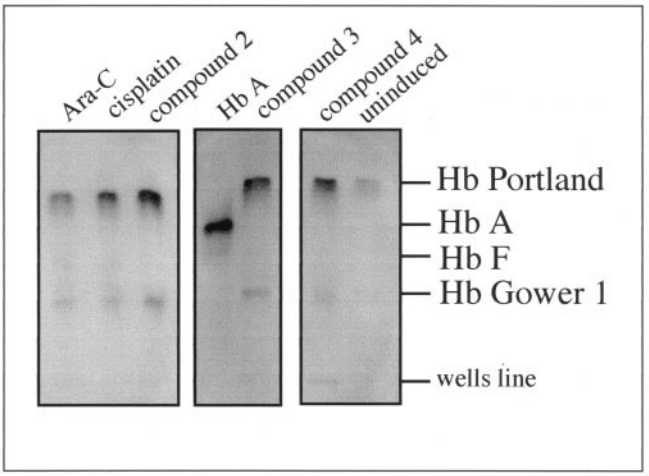

FIG. 5. A. Cellulose acetate gel electrophoresis of hemoglobin produced by K562 cells. Postmitochondrial cell lysates from uninduced K562 cells or from cells induced for 7 days with 250 nM ara-C, 12 μM cisplatin, and 25 μM compounds 2, 3, and 4 were layered on cellulose acetate strips, electrophoresed, and stained with benzidine. Electrophoretic migration of Hb Portland, Hb Gower 1, and HbF is indicated. Electrophoretic migration of HbA is also shown.

erythroid differentiation inducers, our study demonstrates that inhibition of cell proliferation by platinum complexes does not necessarily lead to induction of erythroid differentiation. In fact, when we tested the phosphinic compound (**8**) *cis*-$[PtCl_2(THMP)_2]$, we found that this platinum complex exhibits antiproliferative activity without inducing an increase in the proportion of benzidine-positive cells.

It should be underlined that differential chemical stability under tissue culture conditions as well as the hydrophilicity/lipophilicity balance of the chemical functionalities in each complex could play a relevant role in determining the observed difference in activity between the cisplatin analogs studied. With respect to the stability of platinum complexes, it is known that cisplatin undergoes hydrolysis of the chloride ligands [rate constant $k = 2.5 \cdot 10^{-5}$ sec^{-1} at 25°] [48], and this process has been recognized as a step of the mechanism producing the anticancer activity. Carboxylate analogs, such as carboplatin, have greater kinetic inertness to anionic ligand substitution than does cisplatin [49–51]. On the other hand, the integrity of the $(NH_3)_2Pt$ or (DACH)Pt groups, which have been found to be bonded to DNA, has been proved; indeed, the N donor ligands are called "non-leaving groups" [52]. Finally, regarding the inactive compounds **5–7**, it is known that coordinated DMSO can be replaced by a number of nucleophiles [46, 47, 53]. Further experiments will be necessary to clarify this specific point. With respect to the molecular basis of the induction effects of cisplatin and cisplatin analogs, Northern blotting experiments (Fig. 6) demonstrate that erythroid differentiation of K562 cells treated with compounds **2**, **3**, and **4** is associated with a sharp increase in γ-globin mRNA content (Fig. 6). The study of molecular mecha-

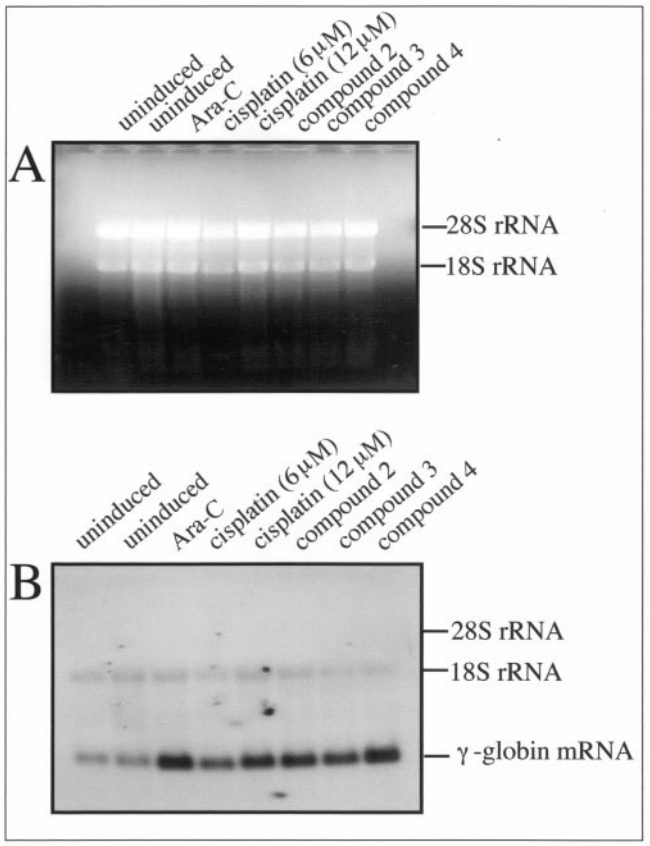

FIG. 6. Northern blotting analysis. Total RNA was isolated from uninduced and erythroid-induced K562 cells. Induction was carried out for 7 days in the presence of 250 nM ara-C, 6 μM or 12 μM cisplatin, and 25 μM compounds 2, 3, and 4, as indicated. Fifteen micrograms of total RNA was electrophoresed, Northern blotting was performed, and hybridization was carried out with a ^{32}P-labeled γ-globin probe. Ethidium-bromide staining of the gel is shown in panel A; autoradiography is shown in panel B.

nisms underlying the switch between ε- and γ-globin genes is crucial for experiments aimed at the induction of γ-globin gene expression in adults [2]. Pharmacologically mediated regulation of the expression of human γ-globin genes could be of interest in the search for potential therapeutic agents in hematological disorders, including β-thalassemia [3, 6, 8, 9, 54–57], as recently published observations demonstrate that hormones, cytotoxic agents, hemopoietic cytokines, and short fatty acids are agents capable of augmenting fetal hemoglobin levels in humans [2]. In particular, butyric acid and 5-azacytidine have been the object of recent reports focusing on *in vivo* treatment of β-thalassemia patients [1, 2, 9]. This is a major issue in this field, since it is well established that an increase in HbF production as low as 30% leads to a significant improvement in the clinical status [3]. Accordingly, our data should encourage studies on possible effects of the cisplatin analogs used on γ-globin gene expression on normal erythroid precursor cells from peripheral blood as well as from bone marrow.

Despite the fact that our data do not provide information on the mechanism of action of the studied platinum complexes, in agreement with the proposed mechanism of action of cisplatin [58–60], *in vitro* DNase I footprinting experiments demonstrate that these DNA-binding drugs are able to interact with the γ-globin promoter of human genomic DNA (data not shown); *in vivo* footprinting experiments could clarify this specific point. Molecular analyses are in this case very important, as it is known that structurally related compounds could induce hemoglobin synthesis in K562 cells by distinct mechanisms [61, 62].

Whatever the mechanism of action of compounds **2**, **3**, and **4** may be, our results demonstrate that these compounds are potent new inducers of K562 erythroid differentiation, leading to accumulation of γ-globin mRNA and production of embryo–fetal hemoglobins.

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