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Cytotoxicity, DNA Damage, and Cell Cycle Perturbations Induced by Two Representative Gold(III) Complexes in Human Leukemic Cells With Different Cisplatin Sensitivity

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The gold(III) complexes [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 were recently shown to exert important cytotoxic effects in vitro on human tumor cell lines. To elucidate the biochemical mechanisms leading to cell death, the effects produced by these gold(III) complexes on the leukemic CCRF-CEM cell line—either sensitive (CCRF-CEM) or resistant to cisplatin (CCRF-CEM/CDDP)—were analyzed in detail by various techniques. For comparison purposes the effects produced by equitoxic concentrations of cisplatin were also analyzed. First, the dependence of the IC_{50} values of either complex on the incubation time was investigated. Cytotoxicity experiments confirmed that both gold(III) compounds retain their efficacy against the cisplatin-resistant line: only minimal cross-resistance with cisplatin was detected. Notably, [Au(phen)Cl_2]Cl is more cytotoxic than [Au(dien)Cl]Cl_2, with IC_{50} values of 7.4 and 6.0 M at 24 and 72 h, respectively, on the resistant line. Results of the COMET assay point out that both gold(III) complexes directly damage nuclear DNA. Remarkably, DNA damage inferred by either gold(III) complex in the two cell lines is larger than that produced by equitoxic cisplatin concentrations. Finally, the effects that either gold(III) complex produces on the cell cycle were investigated by flow cytometry. It was found that both complexes cause only moderate and transient cell cycle perturbations. Larger cell cycle perturbations are induced by equitoxic concentrations of cisplatin. The implications of the present results for the mechanism of action of cytotoxic gold(III) complexes are discussed.

Key words: Gold(III) complexes; Cytotoxicity; COMET assay; Flow cytometry

Gold(III) complexes, isoelectronic and isostructural to platinum(II) complexes, hold promise as possible antitumor agents (1–3). Only a few reports (4–10) exist in the literature describing the cytotoxic properties and the in vivo antitumor effects of gold(III) complexes. Preliminary in vitro data suggested that a direct interaction of gold(III) complexes with DNA represents the molecular basis for their cytotoxic effects (7,8). Recently, we showed that the gold(III) center may be largely stabilized, even at neutral pH, through an appropriate choice of the ligands (11,12) while preserving its peculiar biological properties. This led us to prepare and evaluate a certain number of novel gold(III) complexes as possible antitumor drugs. Remarkably, most gold(III) complexes tested in our laboratory turned out to be highly cytotoxic, thus stimulating additional research in the field.

We report here on the effects in vitro of two representative gold(III) complexes—[Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 (13–16) (see Fig. 1)—tested on the leukemic cell lines CCRF-CEM either sensitive (CCRF-CEM) or resistant to cisplatin (CDDP[2]) (CCRF-CEM/CDDP). The aim of the present study was to investigate how tumor cells are modified by [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 in vitro, in comparison with cisplatin, and to uncover the biochemical mechanisms leading to the observed effects. The dependence of cytotoxicity on exposure time was first addressed. To assess directly induced DNA damage the COMET assay was used; the sensitivity of this assay is now clearly demonstrated (17–21). Additional information is provided by detailed flow cytometric analysis (22) of the effects of the gold(III) complexes on cell cycle.

MATERIALS AND METHODS

Chemicals

Common chemical reagents were purchased from Sigma Chemical Co. (Milano, Italy) and Pharmacia; cisplatin was purchased from Teva Pharma Italia (Milano, Italy) and HAuCl4 from Chimet (Arezzo, Italy). Fetal calf serum (FCS), antibiotics, and RPMI-1640 medium were obtained from Gibco Life Technologies Italia (Milano, Italy). [Au(phen)Cl_2]Cl was prepared by addition of phenanthroline-HCl to an $HAuCl_4$ solution at a 1:1 stoichiometry following the procedure reported in Block and Bailar (13). [AuCl(dien)]Cl_2 was prepared according to Nardin et al. (14). The purity of [Au(phen)Cl_2]Cl and [AuCl(dien)]Cl_2 was 99% and 97%, respectively.

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[2]Abbreviations used: CDDP, cisplatin; DPC, DNA–protein cross-links; PI, propidium iodide; TM, tail moment; DTM, decrease of tail moment.

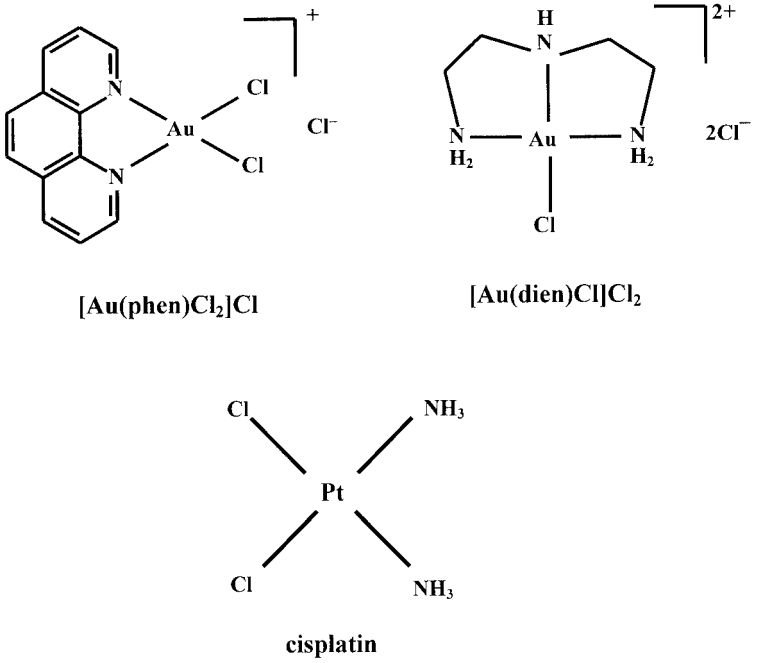

Figure 1. Schematic drawing of the gold(III) complexes [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2; the structure of cisplatin is also reported.

Cell Culture and Cytotoxicity Assay

The CCRF-CEM human leukemic cell line was used in the present study. The resistant subline, CCRF-CEM/CDDP, was selected by stepwise exposing the parental cells to increasing cisplatin concentrations up to 10 μM. Then the cells were grown in the constant presence of 10 μM cisplatin. Cells were cultured in RPMI-1640 medium containing 10% FCS and antibiotics (streptomycin 100 μg/ml and penicillin 100 U/ml). Drugs were dissolved in sterile bidistilled water and IC_{50} values (drug concentration inhibiting cell growth by 50%) were determined after 72-h exposure or at 72 h following 1-, 2-, 4-, and 24-h exposure to drugs, by cell counting (Model D Coulter counter, Coulter electronics, Ltd., Luton, Bedfordshire, England). Cell viability was also evaluated by the trypan blue exclusion test.

COMET Assay

For the detection of cross-links, a modification of the alkaline version of the COMET assay was performed (16). Drug-treated cells (i.e., exposed for 24-h IC_{50}) and untreated controls were washed twice and about 1.2×10^5 cells were suspended in 90 μl of prewarmed low melting point agarose [0.7% in phosphate-buffered saline (PBS)]; 80 μl of the suspension was rapidly spread on frosted microscope slides, precoated with two layers of normal melting point agarose (0.5% in PBS, Ca^{2+} and Mg^{2+} free) and covered with a coverslip. After gelling for 10 min at 0°C, the coverslip was gently removed and a fourth layer of 75 μl low melting point agarose was added. Two slides of each sample treated with drug and two slides of the untreated control were treated for 5 min with 30 μM H_2O_2 (CCRF-CEM) or 100 μM H_2O_2 (CCRF-CEM/CDDP) at 0–1°C to produce random DNA breakage. Slides were then put in a tank filled with lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 1% *N*-lauroyl-sarcosine, pH 10; 1% Triton X-100 and 10% DMSO were added fresh) for 1 h at 4°C. The slides were then removed from lysis solution and incubated in a fresh electrophoresis buffer (300 mM NaOH and 1 mM Na_2EDTA, pH 12.7) for 20 min at room temperature, to allow unwinding of DNA. Electrophoresis was performed for 20 min at 25 V and 300 mA. After electrophoresis, slides were washed three times for 5 min in fresh neutralization buffer (0.4 M Tris-HCl, pH 7.5). Slides were stained with 100 μl ethidium bromide solution (20 μg/ml), covered with a coverslip, and stored at 4°C.

Fifty cells per slide were examined with a fluorescence microscope and analyzed by computerized image analysis system, determining the mean tail moment (i.e., percentage of DNA in the tail times tail length; tail length measured from center of head) (19). In the presence of cross-links, H_2O_2-induced DNA migration was reduced. The relative reduction of H_2O_2-induced DNA migration was used as an indicator of cross-links. Cross-linking was expressed as the percentage decrease in tail moment (DTM) calculated by the formula (20):

$$\% \text{ decrease in tail moment} = \left[1 - \left(\frac{\text{TMdp} - \text{T Mcu}}{\text{TMcp} - \text{TMcu}}\right)\right] \times 100$$

where TMdp = tail moment drug-treated and peroxide-treated sample; TMcu = tail moment of drug-untreated control; and TMcp = tail moment of drug-untreated and peroxide-treated control.

To discriminate between DNA–protein cross-links

(DPC) and DNA–DNA cross-links, we performed a proteinase K treatment. After lysis, slides were washed three times in TE buffer (5 μM Na_2EDTA, 100 μM Tris-HCl, pH 10), then covered with 100 μl proteinase K (1 mg/ml TE buffer) and incubated for 2 h at 37°C in a humidified incubator. Controls were incubated with 100 μl TE buffer only. After removing the coverslip, slides were processed as usual.

Cell Cycle Analysis

Cell cultures were synchronized by double thymidine block (21). Briefly, cells at 3×10^5 cells/ml were incubated at 37°C in 5% CO_2 in the presence of 0.5 mM of thymidine for 24 h, then for 24 h in thymidine-free medium. Synchronization of the cells at the G_1/S transition was then achieved by a further 24-h incubation in medium containing 0.5 mM thymidine. Immediately after release from the second block, cells were exposed to drugs at equitoxic concentrations (IC_{50}) for 4 h, then washed and allowed to progress through the cell cycle in drug-free medium. At different times, cells were fixed in 70% ice-cold ethanol and stored at 4°C. Cells were then rehydrated in PBS and stained in propidium iodide (50 g/ml) solution containing RNase A (5 U/ml) for 30 min (22,23). Propidium iodide (PI)-stained cells were analyzed for DNA content with a Coulter Elite equipped with a Spectra-physic argon-ion laser with an output of 200 mW at 488 nm. Data from 20,000 cells were analyzed with forward and scattered light, and red fluorescence was recorded. Cell cycle distribution of the cells was determined on a linear scale; a logarithmic scale of PI fluorescence intensity was alternatively used to evidence DNA fragmentation at values lower than those of G_0/G_1. The percentages of cells in cycle phases were determined by a graphic curve-fitting method of DNA distribution using Multicycle software (Phoenix, Sorrento Valley, San Diego, CA).

RESULTS

Cytotoxicity

The cytotoxic properties of the individual complexes were investigated by cell counting. Table 1 reports the drug concentration values required to inhibit cell growth by 50% compared with the control (IC_{50}); data were measured at 72 h following drug treatments of 1, 2, 4, and 24 h. Additional experiments were carried out with continuous exposure to the drugs for 72 h. Notably, the observed cytotoxic effects drastically increase as the incubation times are increased.

A cisplatin IC_{50} value of 25.9 ± 4.2 μM was measured on the CCRF-CEM cell line after 1-h exposure; by increasing the exposure time up to 72 h the cisplatin IC_{50} value fell to 0.70.1 μM. $[Au(phen)Cl_2]Cl$, tested on the same cell line, showed higher IC_{50} values at short exposure times (169.2 ± 7.1 and 98.6 μM at 1 h and 4 h); IC_{50} values <5 μM were obtained at 24 and 72 h. Compared with $[Au(phen)Cl_2]Cl$, $[Au(dien)Cl]Cl_2$ turned out to be virtually noncytotoxic at short exposure times (>600 and 259 ± 36.7 μM at 1 h and 4 h, respectively); cytotoxicity increased at 24 and 72 h with IC_{50} values of 58.2 and 12.6 μM, respectively.

The CCRF-CEM/CDDP cell line, compared with CCRF-CEM, exhibited a resistance index to cisplatin equal to 34, following 24-h incubation. $[Au(phen)Cl_2]Cl$ showed lower cytotoxic properties than cisplatin after short exposure times (1–4 h), but was able to overcome resistance to cisplatin (resistance index 2.6 at 72 h): IC_{50} values of 7.4 μM at 24 h and 6 μM at 72 h. Like $[Au(phen)Cl_2]Cl$, $[Au(dien)Cl]Cl_2$ overcame resistance to cisplatin, but showed higher IC_{50} values for all tested exposure times.

COMET Assay

Figure 2 shows typical comet images (representative of both cell lines) obtained on the parental CCRF-CEM cell line either in the presence or in the absence of the metal complexes. After H_2O_2 treatment of control cells, distinct comets were observed (Fig. 2b). In H_2O_2-treated cells, preincubated with the metal complexes (Fig. 2c–e), comet tails are still visible but are of different shape and smaller length. Thus, reduction in tail size is a direct consequence of the interaction of metal complexes with DNA. H_2O_2-untreated cells, exposed to drugs under the same experimental conditions, exhibited comet images similar to those of the control (data not shown) characterized by 10 tail moment (TM) value.

Table 1. IC_{50} (μM) Values of Cisplatin, $[Au(phen)Cl_2]Cl$, and $[Au(dien)Cl]Cl_2$ in Human Leukemic Cells CCRF-CEM, Sensitive or Resistant to Cisplatin

	CCRF-CEM					CCRF-CEM/CDDP				
Drugs	1 h	2 h	4 h	24 h	72 h*	1 h	2 h	4 h	24 h	72 h*
Cisplatin	25.9 ± 4.2	9.1	5.3 ± 1.2	0.9 ± 0.4	0.7 ± 0.1	>186 (>7)	119.8 (13.2)	66.2 (12.5)	29.4 ± 1.1 (34)	19.8 (28.3)
$[Au(phen)Cl_2]Cl$	169.2 ± 7.1	128.7	98.6	4.4	2.3	214 (1.3)	111.9 (0.9)	90 (0.9)	7.4 (1.7)	6 (2.6)
$[Au(dien)Cl]Cl_2$	>600	500	259 ± 36.7	58.2	12.6	>600	590 (1.2)	324 (1.3)	153.3 ± 39 (2.8)	32.7 ± 6.6 (2.6)

Data were collected 72 h after removing the drugs. Values are IC_{50} (μM) expressed as mean ± SE of at least three determinations or mean of two determinations. Ratio between IC_{50} values on CCRF-CEM/CDDP cells and on the parental cell line is shown in parentheses.
*Data were collected immediately after 72-h exposure to drugs.

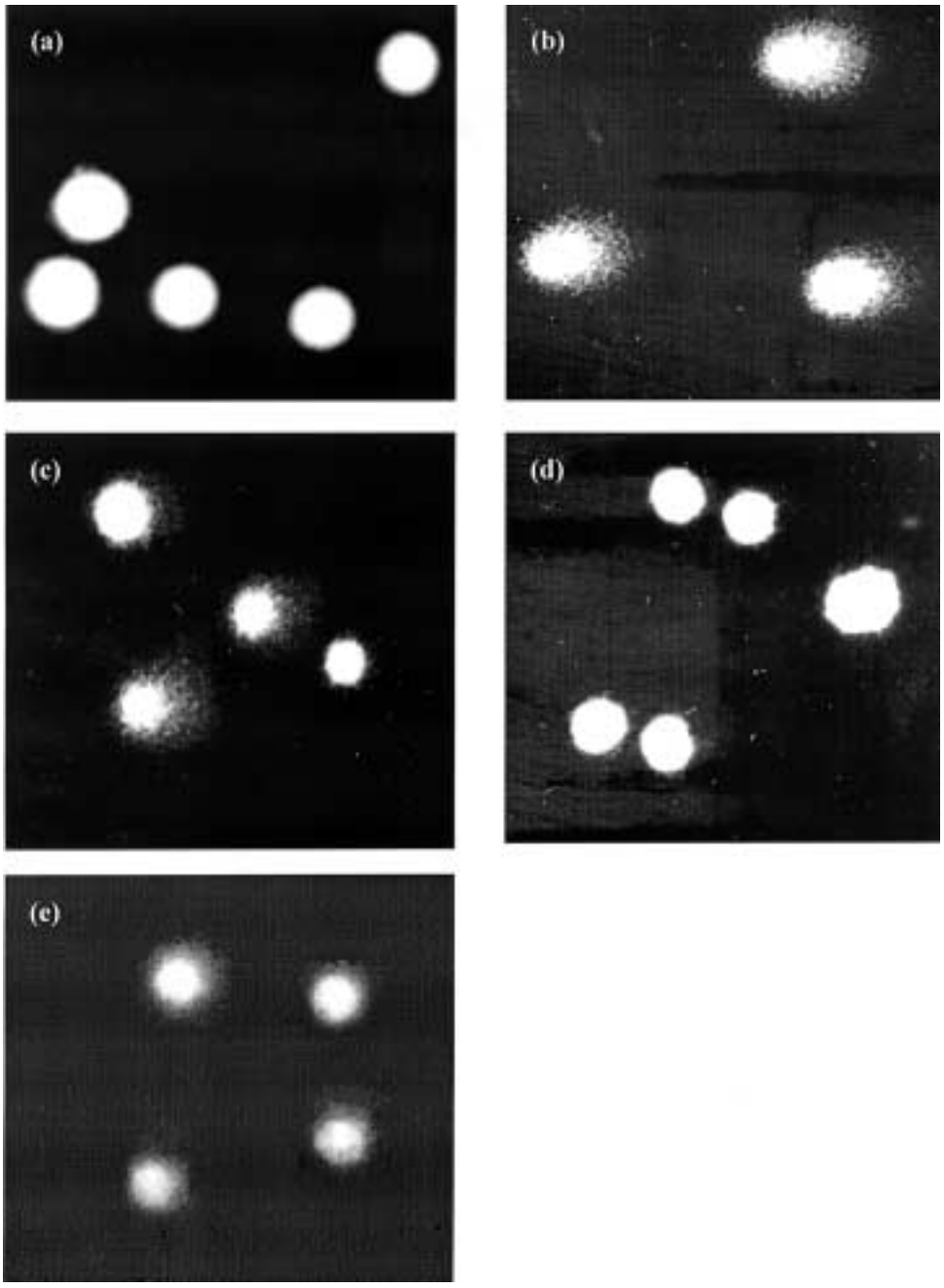

Figure 2. Typical comet images from CCRF-CEM cell line: (a) untreated cells; (b) drug-untreated and peroxide-treated cells; (c) cisplatin- and peroxide-treated cells; (d) $[Au(phen)Cl_2]Cl$- and peroxide-treated cells; (e) $[Au(dien)Cl]Cl_2$- and peroxide-treated cells. Similar comet images were obtained on the CCRF-CEM/CDDP cell line. Cells were exposed to 24-h IC_{50} drugs.

To compare the effects observed in the two cell lines, data are expressed as percentage of decrease in tail moment compared with H_2O_2-treated controls. In Figure 3 the DTM values determined after 24 h IC_{50} treatment are reported. In CCRF-CEM cell line large DTM values were observed for $[Au(phen)Cl_2]Cl$ (88.8%); lower values were observed for cisplatin (39.2%) and $[Au(dien)Cl]Cl_2$ (60.3%). Under the same experimental conditions the CCRF-CEM/CDDP cells exhibited similar DTM values for $[Au(phen)Cl_2]Cl$; in contrast, DTM values were higher for cisplatin (64.8%) and $[Au(dien)Cl]Cl_2$ (76.5%).

To establish whether DPC might be involved in the reduction of H_2O_2-induced DNA migration, slides were treated with proteinase K after lysis. Posttreatment incubation with proteinase K clearly abolished $[Au(dien)Cl]Cl_2$-induced reduction in DNA migration, but had no effect on the sample treated with $[Au(phen)Cl_2]Cl$ (Fig. 3).

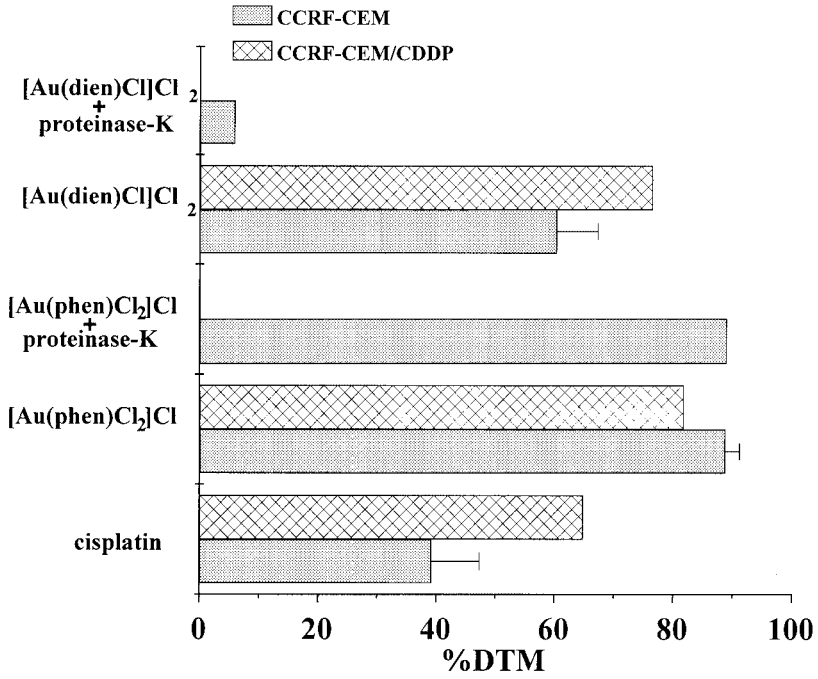

Figure 3. The influence of cisplatin and gold(III) complexes on the reduction of peroxide-induced DNA migration (% decrease tail moment) in the comet assay in CCRF-CEM and CCRF-CEM/CDDP cell lines (see Fig. 2). The effects of treatment with proteinase K on the DNA migration of peroxide-treated CCRF-CEM pretreated with the gold(III) complexes is also reported. Each column represents the mean of least two determinations or the mean ± SE of three determinations.

Cell Cycle Analysis: Effects of Gold(III) Compounds on Synchronized Cells Compared With Cisplatin

The effects produced by cisplatin, [Au(phen)Cl_2]Cl, and [Au(dien)Cl]Cl_2 (i.e., exposed to 4-h IC_{50}) on the progression through the cell cycle were analyzed by flow cytometry on synchronized CCRF-CEM and CCRF-CEM/CDDP cell lines. The untreated sensitive and resistant control cells progressed similarly and synchronously through the cell cycle and recovered, 24 h after release of the thymidine (G_1/S) block, a DNA content distribution typical of the respective asynchronous cell population. In the CCRF-CEM cell line, distribution in the cycle phases was about 30.7% of G_0/G_1, 56.8% of S, and 12.5% of G_2M, while in the CCRF-CEM/CDDP cells we found 23.7% of G_0/G_1, 51% of S, and 25.3% of G_2M.

Drug Effects on the CCRF-CEM Cell Line

In the sensitive line, exposure to 5 μM cisplatin (IC_{50}) (Fig. 4b) for 4 h induced a clear G_2M block after 24 h from drug release that persisted up to 48 h (about 45.6% and 12.8% of total cells for treated and control cells, respectively). Exposure to 100 μM [Au(phen)Cl_2]Cl (Fig. 4c) produced a slower progression from G_1 to S phase compared with synchronized control (Fig. 4a): notably, 6 h after drug release, the percentage of control cells in S phase was consistently above that of treated cells (71.1% and 57.8%, respectively). The effects of 300 μM [Au(dien)Cl]Cl_2 (Fig. 4d) were minor but qualitatively similar to those produced by [Au(phen)Cl_2]Cl. The only difference was observed during the first 6 h, when an enlargement in G_2M population with decrease in S phase cells was evident, suggesting rapid cell progression through the cycle. As reported in Figure 5a, appreciable DNA fragmentation is caused by [Au(phen)Cl_2]Cl (12% and 32% at 6 and 72 h, respectively) and by [Au(dien)-Cl]Cl_2 (13% and 22% at 3 and 72 h, respectively). DNA fragmentation caused by cisplatin was instead observed at 24 h (16%) and 168 h (32%). In the control sample, DNA fragmentation was always below 10%.

Drug Effects on the CCRF-CEM/CDDP Cell Line

In the resistant cell line (Fig. 6b) following exposure to 60 μM cisplatin (IC_{50}) for 4 h, a rapid progression from G_1 to early S phase (95% at 6 h) and increased transit time through S phase were observed at 24 h after drug exposure (78% S phase cells for treated cells and 44% for control cells). Exposure to 100 μM [Au(phen)-Cl_2]Cl (Fig. 6c) produced a slower progression from G_1 to S phase compared with the synchronized control. At 6 h the percentage of treated cells in S phase (40%) was consistently below that of control cells (94%). A marked increase of S phase cells was observed only at 24 h (64%), with a simultaneous increase in the G_2 phase compartment. By 96 h cells recovered and displayed a cell cycle distribution pattern similar to that of the con-

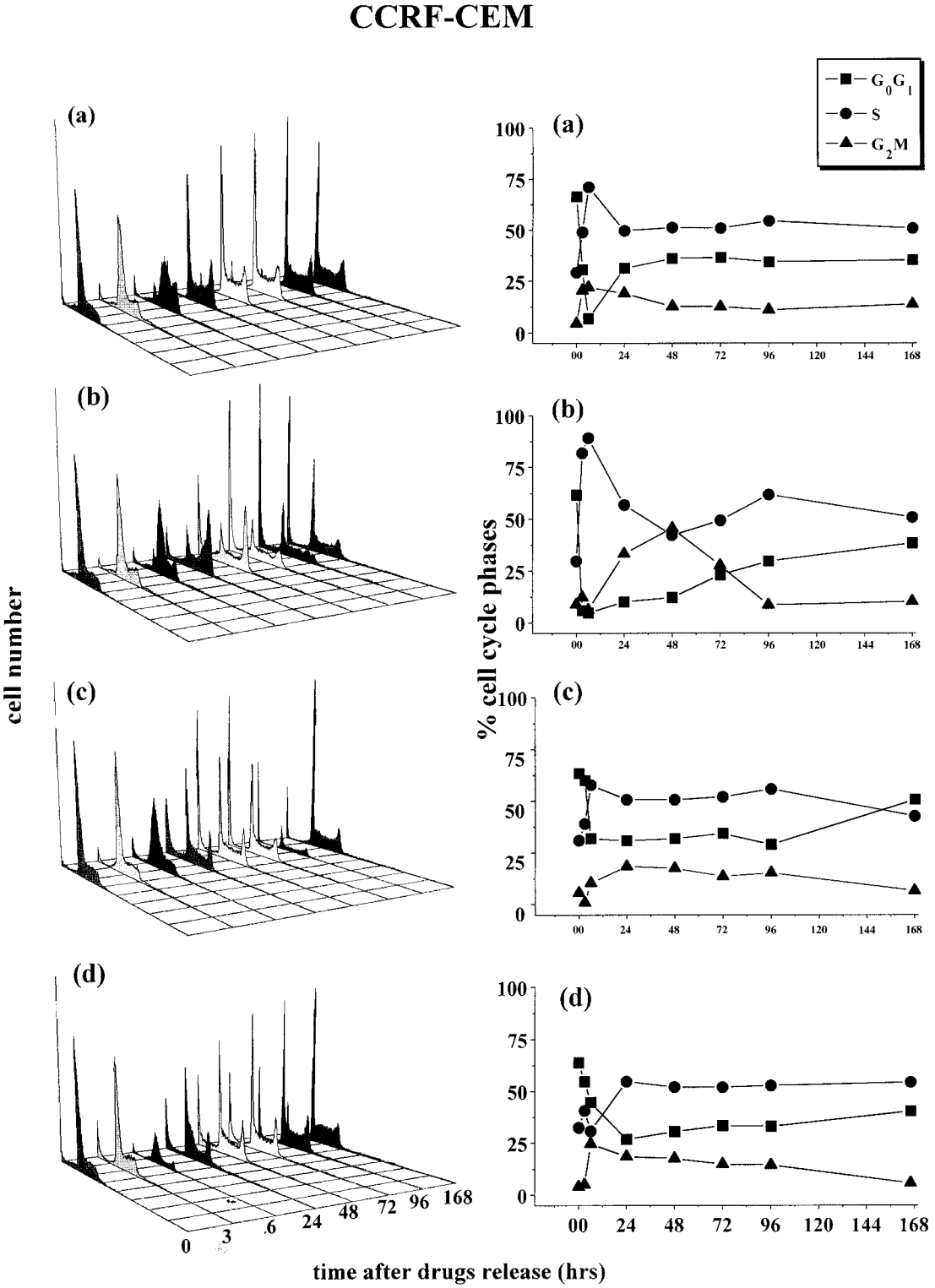

Figure 4. Cell cycle profiles and respective distributions of synchronized CCRF-CEM cell line exposed to 4-h IC_{50} different drugs, then processed at different time intervals for flow cytometric analysis. (a) Untreated cells; (b) cells + 5 μM of cisplatin; (c) cells + 100 μM of $[Au(phen)Cl_2]Cl$; (d) cells + 300 μM of $[Au(dien)Cl]Cl_2$. Data refer to a representative experiment, repeated at least twice.

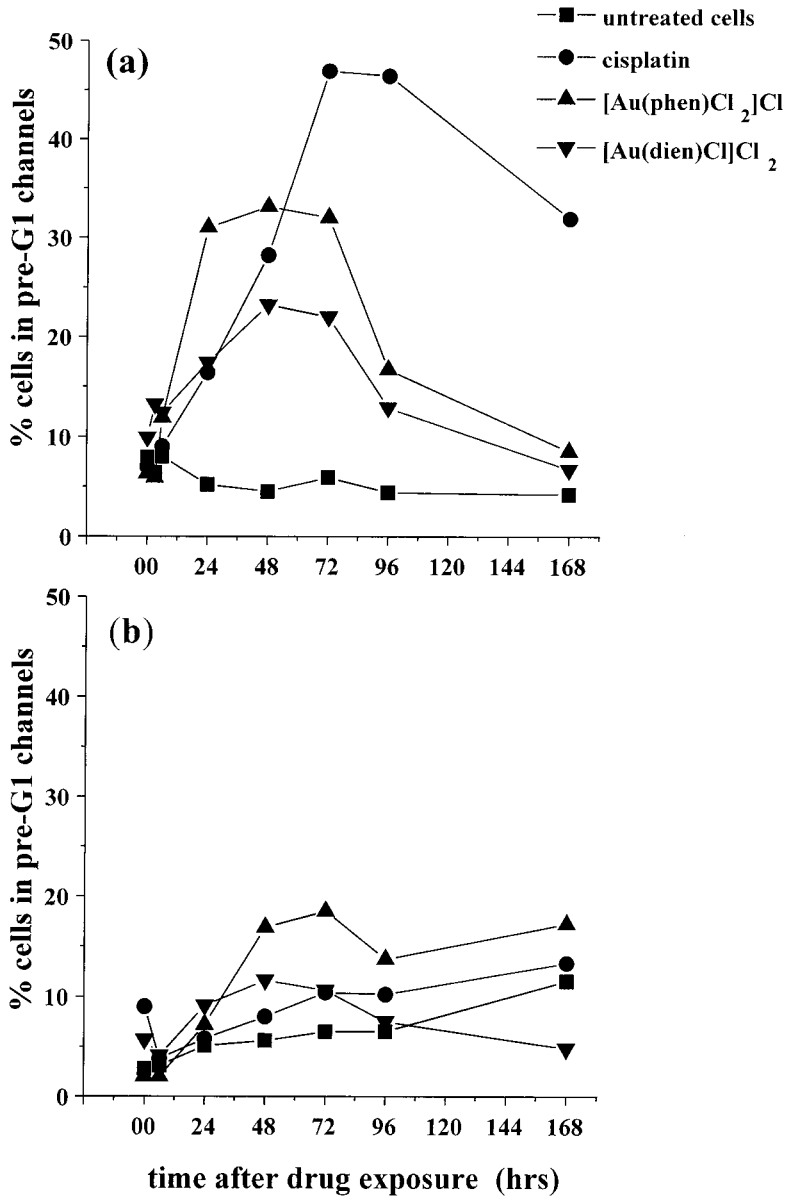

Figure 5. Percentage of CCRF-CEM (a) and CCRF-CEM/CDDP (b) cells with DNA fragmentation determined by PI staining and log-scale acquisition of PI fluorescence (data not shown). Data were obtained from histograms reported in Figures 4 and 6, respectively.

trol (Fig. 6a). [Au(dien)Cl]Cl_2 (300 μM) (Fig. 6d) at 6 h produced an increase in the S phase compartment (78%), similarly to cisplatin, but the percentage was slightly lower; 72 h after drug release the percentage of the S phase was similar to that of the control (48.1% and 47.9%, respectively). All the metal complexes investigated produced a modest increase in DNA fragmentation in this cell line (Fig. 5b); in comparison to the control, only [Au(phen)Cl_2]Cl was able to induce about 18.5% DNA fragmentation after 72 h from drug release.

Parallel experiments carried out on nonsynchronized cells, treated with equitoxic concentrations of the same drugs, yielded similar results (data not shown).

DISCUSSION

Recent studies, carried out in different laboratories, demonstrate that gold(III) complexes may be conveniently developed and evaluated as possible cytotoxic and antitumor drugs. In particular, some gold(III) complexes have shown important cell-killing effects in vitro associated with their ability to overcome resistance to cisplatin. The main objective of the present investigation

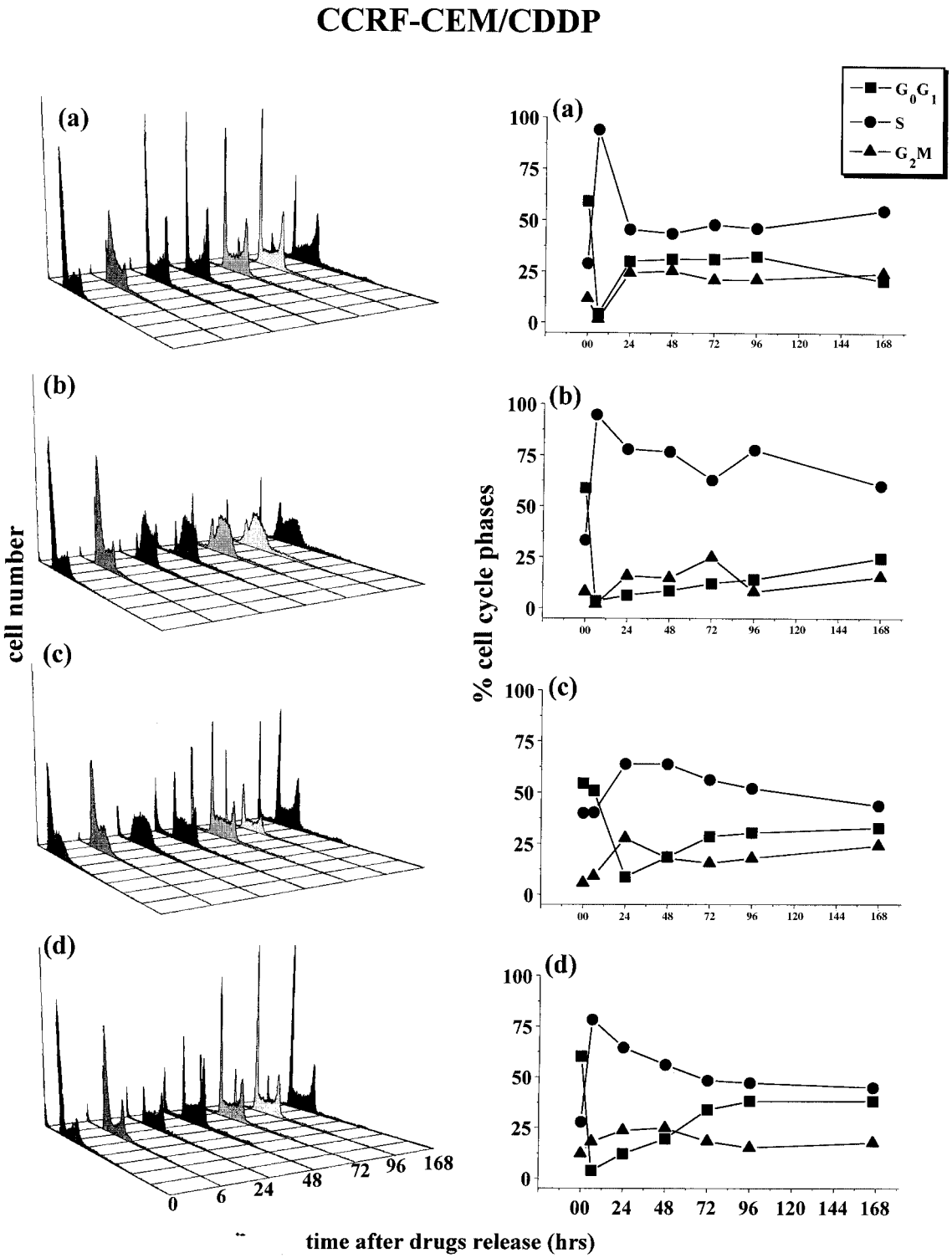

Figure 6. Cell cycle profiles and respective distributions of synchronized CCRF-CEM/CDDP cell line exposed to 4-h IC_{50} to different drugs, then processed at different time intervals for flow cytometric analysis. (a) Untreated cells; (b) cells + 60 μM of cisplatin; (c) cells + 100 μM of $[Au(phen)Cl_2]Cl$; (d) cells + 300 μM of $[Au(dien)Cl]Cl_2$. Data refer to a representative experiment, repeated at least twice.

was to shed light on the biochemical mechanisms through which cytotoxic gold(III) complexes produce their effects, a research area yet almost unexplored. This goal was pursued by a series of experiments, carried out on the leukemic cell line CCRF-CEM, either sensitive or resistant to cisplatin, following treatment with two representative gold(III) complexes.

Specifically, the effects of [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 on the above cell lines were monitored by analysis of the time dependence of cytotoxicity, by the COMET assay, and by flow cytometry studies. This approach has resulted in valuable insight on the response of a tumor cell population to treatment with gold(III) complexes.

Time Dependence of the Cytotoxic Effects

In principle, analysis of the dependence of the observed cytotoxic effects on the exposure time may provide indirect information on drug uptake mechanisms. In fact, one can assume that the amount of gold that enters cells is roughly proportional to the exposure time, at least during the first hours. Thus, at least qualitatively, the longer the exposure time, the lower the drug concentration that is required to kill cells. Such a trend was obtained with all tested complexes; in some cases, however, the relationship between cytotoxicity and the exposure time is not linear. [Au(phen)Cl_2]Cl exhibits a profile of cytotoxicity similar to that of cisplatin on the sensitive line, although the actual concentrations that are needed to achieve the same effects are at least three times higher. Remarkably, [Au(phen)Cl_2]Cl largely overcomes resistance to cisplatin, so that it is at least three times more effective than cisplatin itself on the resistant line. Generally [Au(dien)Cl]Cl_2 is much less effective than [Au(phen)Cl_2]Cl, on both cell lines, although their time-dependent profiles are qualitatively similar.

The COMET Test

The single-cell gel electrophoresis assay (COMET assay) is an established method to measure DNA strand breaks (24); more generally, the COMET method is now a recognized tool to reveal DNA damage produced by drugs. To allow detection and quantitation of DNA cross-linking at the single-cell level, the standard method was appropriately modified so that a fixed level of random DNA strand breakage was produced by postdrug H_2O_2 treatment (18). The electrophoretic mobility of alkaline denatured cellular DNA is retarded by cross-links; cross-links are thus quantitated as the decrease in the tail moment, compared with H_2O_2-treated controls.

We observed that the tail moment decreases (DTM) induced by [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 on both cell lines are high and largely independent of drug concentration; notably, a concentration of cisplatin 30 times higher in the CCRF-CEM/CDDP cell line induces a DTM only 25% higher than in the sensitive line. It may be hypothesized that repair mechanisms involving DNA cutting begin immediately in cisplatin-treated cells; possibly, in the case of gold(III) complexes, DNA damage is recognized later. Although the concentration of each drug used in the test corresponds to 24-h IC_{50}, the delay in DNA migration (after treatment with H_2O_2) caused by [Au(phen)Cl_2]Cl and [Au(dien)Cl]Cl_2 is larger than for cisplatin, possibly because of a different mode of binding. This hypothesis is confirmed by posttreatment incubation with proteinase K. The latter experiment suggests that [Au(dien)Cl]Cl_2 reduces DNA migration by binding to DNA histones (other mechanisms cannot be excluded), while direct binding of [Au(phen)Cl_2]Cl to DNA–protein is excluded. Moreover, the modifications produced by cisplatin on the double helix are far more persistent than those produced by gold(III) complexes.

No clear relationship can be established between the DNA damage and the cytotoxic activity. A possible explanation is that the cytotoxicity largely depends on the activity and the efficiency of DNA repair (excision and polymerization phenomena, for example).

However, apart from difficulties in explaining some features of our experimental results, it is clear that comet profiles are drastically modified by both gold(III) complexes, thus providing unambiguous evidence for direct interaction of these gold(III) complexes with DNA.

The Effects on the Cell Cycle

One might expect that gold-induced DNA damage will result in significant cell cycle modifications. This prompted us to perform careful cell cycle studies by flow cytometry. It is shown that cisplatin induces a transient G_2M block on the sensitive cell line that is recovered after 48 h. A G_2M block appears to be the major change induced by platinum complexes on several tumor cell lines (25–27). At variance, in the resistant line, the major cell cycle effect appears to be an S phase slowdown at all the observation times; no significant accumulation of cells in the G_2M compartment was detected. It is likely that higher cisplatin concentrations might result in G_2 arrest.

Flow cytometry results show that both gold(III) complexes modify the cell cycle far less than cisplatin. The effects induced by gold(III) complexes on the sensitive line are modest, while transient but significant modifications are observed in the resistant line. Most [Au(phen)Cl_2]Cl-treated CCRF-CEM/CDDP cells (80%), after initial delayed progression from G_1 to S phase, progress in the cycle, while the DNA of others (20% at 48 h) is fragmented (for cisplatin it is only 7%). This finding can explain the ability of [Au(phen)Cl_2]Cl to overcome cisplatin resistance. [Au(dien)Cl]Cl_2 is also able to overcome cisplatin resistance, but the required concentrations are too high to be clinically meaningful. Further studies will be needed to elucidate the exact meaning of DNA fragmentation. Moreover, it is interesting to verify whether apoptosis is involved in the mechanism of gold (III) complexes.

CONCLUSIONS

The mechanisms by which cytotoxic gold(III) complexes exert their effects are yet not understood. Cytotoxicity studies have shown that [Au(phen)Cl_2]Cl is about six times more effective than [Au(dien)Cl]Cl_2 on

both leukemic cell lines. Notably, [Au(phen)Cl_2]Cl is less effective than cisplatin on the cisplatin-sensitive phenotype, but more effective on the cisplatin-resistant one. Analysis of the dependence of the cytotoxic effects on the exposure times reveals similar patterns for all complexes, suggesting that drug entry into cells is relatively slow. The most relevant result of this study is that both gold(III) complexes target cellular DNA; as revealed by the COMET assay, the modifications of the comet profiles produced by gold(III) complexes are significantly larger than those brought about by equitoxic concentrations of cisplatin. Surprisingly, the observed DNA damage does not reflect into large modifications of the cell cycle. In spite of that, both gold(III) complexes, but especially [Au(phen)Cl_2]Cl, were shown to induce significant DNA fragmentation.

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