Induction of Polyploid Nuclei in the Plasmodium of Physarum Polycephalum by Platinum Antitumor Compounds

M. Wright, I. Lacorre-Arescaldino, J. P. Macquet, and M. Daffé

Laboratoire de Pharmacie et de Toxicologie Fondamentales, Centre National de la Recherche Scientifique, 205, route de Narbonne, 31400 Toulouse [M. W., J. P. M., M. D.], and Laboratoire de Biologie Cellulaire, Faculté des Sciences de Reims, BP 347, 51062 Reims [I. L-A], France

ABSTRACT

The intranuclear mitosis of the plasmodial nuclei of myxomycetes permits the observation of defects in chromosomal reparation which would probably be lethal in other eukaryotic cells with open mitosis. We found that antitumoral platinum-amine compounds perturbed late mitotic events and induced the formation of giant nuclei which were polyploid in plasmodia of Physarum polycephalum. Using 26 platinum-amine complexes, we have shown that all antitumoral compounds induced the formation of polyploid nuclei for drug concentrations at least three times lower than the amount necessary to block the overall plasmodial growth, whereas platinum compounds without antitumor activity did not behave this way. DNA replication appeared to be quantitatively normal during formation of giant nuclei by antitumoral compounds. These observations suggest that platinum-amine compounds exert their antitumor activity by interfering with mitosis rather than by a gross inhibition of DNA synthesis.

INTRODUCTION

Since the discovery of the antitumor activity of cisplatin (38), numerous platinum-amine compounds have been studied in order to find more active antitumoral agents (28). Various investigations have been performed in order to elucidate the pharmacological action of these compounds in vivo (review in Ref. 37). Cisplatin interacts with a great variety of biological molecules (11, 35, 44), and it is difficult to correlate its antitumoral activity with a specific action on one of these biological molecules, although some facts (2, 17, 22, 27, 33, 35, 36) suggest that DNA could be the pharmacological target. The plasmodium of the myxomycete Physarum polycephalum (8, 23) was used in order to investigate this hypothesis. The plasmodium can be schematically described as a flat giant cell (10 cm in diameter) which contains 10⁶ nuclei (23) showing a synchronous (15) intranuclear division (13, 16) without cytokinesis. In contrast to what occurs in animal cells, chemicals which perturb nuclear division and the equipartition of chromosomes do not lead to immediate death but to the formation of giant nuclei (14, 30, 48). The results of this study suggest that the initial toxicity of antitumoral platinum-amine compounds is due to the perturbation of the mitotic machinery rather than to a gross inhibition of DNA synthesis (17, 22).

MATERIALS AND METHODS

Chemicals. The syntheses of most of the platinum compounds (Table 1) have already been described (28). Compound 8 was prepared from the addition of the isocitrate ion to the platinum daquao species. The trimer Compound 15 was prepared as Compound 14 starting with Compound 10. Compound 22 was obtained by reacting Compound 5 with an excess of ammonia and Compound 26 as described in Ref. 10. Compounds were recrystallized or reprecipitated twice before use. Elemental analyses (C, H, N, Cl) were performed by Parcor Laborotary, Toulouse, France. Platinum content was determined by atomic absorption spectrophotometry (29), and ir spectra were recorded.

Strains. Haploid plasmodia of the colonia strain (Cl) of P. polycephalum (myxomycetes) (3, 45) were directly derived from haploid amebae and grown at 22°C as described previously (49). SDM was used in all cases and was made according to the formula given by Daniel and Rusch (5) and modified by Daniel and Baldwin (4). Synchronous plasmodia were grown on SDM agar obtained by mixing 1 volume of 3% agar with 1 volume of SDM. Microplasmodia grown in SDM were concentrated by decantation, and 1 ml of the pellet was added on the center of a cellulose nitrate membrane or a filter paper (Whatman No. 4 paper). After 2 hr on a metallic grid without medium, the membrane or the filter paper was placed on SDM agar at 22°C. Whatman No. 4 paper was used when Compounds 3 or 16 were included in the agar medium.

Methods. Growth of synchronous plasmodia was estimated by the ratio of the surface covered by the plasmodia at various times after the beginning of the experiment to the surface of the plasmodia at the beginning of the experiment (11 to 12 sq cm). Surfaces were determined using a Hewlett-Packard 9820A calculator equipped with a Hewlett-Packard 9864A digitizer. In the experimental conditions used, there is a linear relationship (0.6 mg of protein per sq cm of plasmodium) between the surface covered by plasmodia and the amount of protein measured as described by Daniel and Baldwin (4). Spreading of 0.5 ml of water on the surface of a plasmodium quarter (12 sq cm) does not perturb either the growth rate or the nuclear size. After 24 hr of growth, the surface reached by the plasmodia, the quantity of protein and the nuclear size were indistinguishable from those of the control. Nuclei were observed under phase-contrast microscopy (15) and/or electron microscopy (6).

Three-dimensional models were built from serial electron microscopic thin sections according to a modification of the procedure of Jordan and Saunders (24). Stereopictures were taken from each 3-dimensional model as described by Wright et al. (47) and observed with a standard stereoviewer (Mattey, Paris, 70-mm eye separation). The amount of DNA per nucleus was determined in isolated nuclei by cytofluorometry. A plasmodium was homogenized in 70 ml of 30% (w/v) glycerol/20% (v/v) dimethyl sulfoxide/0.1 M piperazine-N,N′-bis-(2-ethanesulfonic acid) (pH 6.9)/0.5 mM ethylenediamine(oxyethylene nitrile)tetraacetic acid/5 mM MgCl₂/0.1% (v/v) Triton X-100 with an homogenizer (Sorval Omnimixer, 1 min, 5000 rpm) and centrifuged for 10 min at 4800 × g (5000 rpm; Beckman JS 7.5 rotor with swinging buckets) on a cushion without Triton X-100 but containing 50% (w/v) glycerol. Aliquots of the nuclear suspension put on to slides were fixed by formaldehyde vapors. After a 15-min hydrolysis in 5 N hydrochloric acid at 20°C, the nuclei were stained at 20°C with a Schiff reagent containing p-rosanilin (1 mg/liter; Gurr Co.) (34). Cytofluorometric measurements were carried out with a Leitz cytophotometer (MPV) modified for phase-contrast and epifluorescence.
Thymidine kinase activity was determined as described measuring the fluorescence intensity at wavelengths above 610 nm incident excitation pulse of green light (Filters BG36 and S546) and microscopy (26) with a stabilized 100-watt mercury lamp, using a 1-sec microscope (26).

RESULTS

Effects of Cisplatin and trans-Dichlorodiammineplat

Table 1

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Chemical denomination</th>
<th>Pattern of action</th>
<th>nmol of compound</th>
<th>GA/PN ratio</th>
<th>T/C (%)</th>
<th>No. of labile sites</th>
<th>$t_{1/2}$ (hr)</th>
<th>Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potassium tetrachloroplatinate(II)</td>
<td>E</td>
<td>10,000</td>
<td>110</td>
<td>4</td>
<td>8</td>
<td>&lt;2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>2</td>
<td>Potassium trichloroacetylomineplatinate(II)</td>
<td>A</td>
<td>5,000</td>
<td>146</td>
<td>3</td>
<td>6.7</td>
<td>&lt;1</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>cis-Dichlororhodamineplatinate(II)</td>
<td>A</td>
<td>750</td>
<td>205</td>
<td>2</td>
<td>3.9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>cis-Dichloroethylamidineplatinate(II)</td>
<td>A</td>
<td>900</td>
<td>132</td>
<td>2</td>
<td>1.5</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>5</td>
<td>cis-Dichloro-1,2-diaminocyclohexaneplatinate(II)</td>
<td>B</td>
<td>1,000</td>
<td>177</td>
<td>2</td>
<td>2.3</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>6</td>
<td>cis-Oxalato-1,2-diaminocyclohexaneplatinate(II)</td>
<td>B</td>
<td>500</td>
<td>180</td>
<td>2</td>
<td>90</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>7</td>
<td>cis-Leucino-1,2-diaminocyclohexaneplatinate(II)</td>
<td>B</td>
<td>2,000</td>
<td>180</td>
<td>2</td>
<td>110</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>8</td>
<td>cis-Isoxatrizo-1,2-diaminocyclohexaneplatinate(II)</td>
<td>A</td>
<td>2,500</td>
<td>174</td>
<td>2</td>
<td>130</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>9</td>
<td>cis-Sulfomonoacquamineplatinate(II)</td>
<td>B</td>
<td>1,500</td>
<td>119</td>
<td>2</td>
<td>0.1</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>10</td>
<td>cis-Sulfomonoacquamine-1,2-diaminocyclohexaneplatinate(II)</td>
<td>B</td>
<td>500</td>
<td>174</td>
<td>2</td>
<td>&lt;0.1</td>
<td>0</td>
<td>GA&lt;1</td>
</tr>
<tr>
<td>11</td>
<td>cis-Diaquoxidineplatinate(II)</td>
<td>A</td>
<td>2,000</td>
<td>119</td>
<td>2</td>
<td>&lt;0.1</td>
<td>2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>12</td>
<td>cis-Diaquothioplatinate(II)</td>
<td>A</td>
<td>2,500</td>
<td>173</td>
<td>2</td>
<td>&lt;0.1</td>
<td>2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>13</td>
<td>cis-Diaquocyclohexaneplatinate(II)</td>
<td>B</td>
<td>2,500</td>
<td>173</td>
<td>2</td>
<td>&lt;0.1</td>
<td>2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>14</td>
<td>Drp-Dihydroxy-bis(acidamineplatinate(II))</td>
<td>B</td>
<td>2,500</td>
<td>111</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>15</td>
<td>Bis-cyclohexyl-1,2-diaminocyclohexaneplatinate(II)</td>
<td>B</td>
<td>1,000</td>
<td>182</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>16</td>
<td>trans-Dichlorodiammineplatinate(II)</td>
<td>C</td>
<td>&gt;5,000</td>
<td>116</td>
<td>2</td>
<td>2.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>trans-Diaquodiammineplatinate(II)</td>
<td>G</td>
<td>500</td>
<td>113</td>
<td>2</td>
<td>0.5</td>
<td>+2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>18</td>
<td>Monochlororhodamineplatinate(II)</td>
<td>G</td>
<td>5,000</td>
<td>108</td>
<td>2</td>
<td>2.5</td>
<td>+1</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>19</td>
<td>Monocarbldiammineplatinate(II)</td>
<td>F</td>
<td>10,000</td>
<td>106</td>
<td>1</td>
<td>&lt;0.1</td>
<td>+2</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>20</td>
<td>Tetrathiolamineplatinate(II)</td>
<td>C</td>
<td>—</td>
<td>108</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>21</td>
<td>Bis-ethylendiammineplatinate(II)</td>
<td>C</td>
<td>—</td>
<td>98</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>PN&lt;2</td>
</tr>
<tr>
<td>22</td>
<td>cis-Diammine-1,2-diaminocyclohexaneplatinate(II)</td>
<td>C</td>
<td>—</td>
<td>108</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>PN&lt;2</td>
</tr>
</tbody>
</table>

### Note

- GA/PN ratio cannot be calculated since no polyploid nuclei were observed.
- PN is the minimum amount of platinum compound leading to the occurrence of polyploid nuclei (see Chart 4); GA is the minimum amount of platinum compound leading to complete growth arrest (see Chart 4).
- The comparison was performed on female DBA/2 mice which were grafted with 10⁵ L1210 cells on Day 0 and treated only on Day 1. Experiments were terminated on Day 30, and the number of animals still alive at that time was recorded but not included in the calculation of the T/C ratio. Antitumor activity was expressed as (T/C) ± SEAAABBABBBAABGBCGGFCCCEA-BDCnmol.
- No. of labile sites was determined for this study.
- Half-time of the platinum fixation on DNA corresponding to a final $r_0$ of 0.20, $r_0$ being the number of platinum atoms fixed per nucleotide. Experimental conditions are described in Ref. 28.
- The size of nuclei was normal even after 48 hr incubation, in the presence of concentrations of cisplatin as low as 10 μM.
- Antitumor activity is expressed as (T/C) ± SEAAABBABBBAABGBCGGFCCCEA-BDCnmol.
- Not known.
- No surviving animals were obtained.
- No covalent interaction takes place between DNA and platinum compound, since there is no labile site on the platinum compound.
- In vitro assembly of sheep brain tubulin (4 mg/ml) was performed as described in Ref. 31. The amount of platinum in the plasmid was measured in crude extracts (Branson sonifier, 35 watts, 3 times for 10 sec each) by atomic absorption spectrophotometry (Perkin-Elmer 603) with a graphite furnace (Perkin Elmer HGA 76) coupled with an autosampling system (AS-1).
platinum(II) used (0.2 mM in the agar medium). Thus, abnormalities of the nucleus occurred for concentrations of cisplatin which were devoid of effects on plasmodial growth, while abnormal nuclei were not observed for the trans isomer.

**Cytological Characterization of Giant Nuclei Formed by Cisplatin.** When a plasmodium was treated in the presence of 0.1 mM cisplatin in the agar medium abnormally large nuclei were observed. A similar result was obtained also when a single application of 100 nmol of cisplatin was spread on a plasmodium (Fig. 1). Under phase-contrast microscopy, one or several enlarged nucleoli (Fig. 1) were observed in these giant interphase nuclei. Under electron microscopy, the main ultrastructural characteristic of the giant nuclei was the frequent presence of a mass of macrotubules with inner and outer diameters of 24 and 39 nm, respectively. These macrotubules were reminiscent of those observed in a cell cycle-thermosensitive mutant of Physarum (6) and were not detected in control nuclei. Determination of the amount of DNA per nucleus was estimated by fluorescence photometry (26) on isolated nuclei stained by the procedure of Feulgen (34). Interphase nuclei isolated in late G2 (20 min before metaphase) were used as internal control. Since the strain used in this work is haploid (3), control late G2 nuclei contained twice the haploid DNA content. In contrast, after 48 hr of treatment with cisplatin, the content of DNA per nucleus was 4 times higher than the DNA content of G2 control nuclei (Chart 1). However, the histogram of DNA content per nucleus of these polyploid nuclei was broader than the histogram which could be expected from control G2 nuclei (Chart 1). Such an effect could be due to a perturbation of the equipartition of chromosomes during mitosis occurring in the presence of the drug and/or to a loss of synchrony between nuclei during the polyploidization process.

**Effect of Cisplatin on Mitosis and DNA Synthesis.** In the presence of 0.1 mM cisplatin, a concentration which does not interfere with the overall growth, the timing of metaphase remained undisturbed when the treatment was applied less than one cell cycle before the control mitosis, i.e., after the preceding S phase (there is no G1 phase in Physarum). When the treatment was begun before the preceding S phase, mitosis was delayed for 4 hr (Chart 2) in contrast with the observations of Mohrová and Drobník (32), who reported no disturbance of the mitotic periodicity of Physarum by cisplatin. A doubling of the length of prophase stages of mitosis was occasionally observed, but all other stages of mitosis appeared normal under phase-contrast microscopy. In the presence of 0.2 mM cisplatin, a concentration known to interfere with the overall growth, similar results were obtained except that prophase stages were more consistently lengthened (Chart 2). After a single treatment with 250 nmol of cisplatin, the second mitosis following the treatment was delayed and showed a lengthening both of the prophase stages (8.5 hr) and of the metaphase stages (1.25 hr) in contrast with their usual length in control plasmodia (30 and 4 min, respectively) and in agreement with the increased length of mitosis observed by Mohrová and Drobník (32) after a 1-hr pulse with 0.1 mM cisplatin. Although the prophase and metaphase figures observed by electron microscopy were all typical, unusual posttelophase nuclei (corresponding to the so-called “reconstruction” stage) were frequently observed (55% of posttelophase nuclei reconstructed from serial thin sections). Three-dimensional models (Fig. 2a) obtained from electron microscopic serial thin sections showed that these nuclei, devoid of microtubules and exhibiting a nucleolus in the “reconstruction” process (13, 16, 20, 21, 25, 39, 41, 46), were linked by a nuclear bridge limited by the nuclear
envelope (Fig. 2, a and b). These abortive division stages may account for the presence of interphase nuclei with 2 nucleoli and with or without a central constriction of the nuclear envelope (Fig. 2c) and for the occurrence of polyploid nuclei due to the impairment of the final stage of nuclear division.

Although typical bipolar metaphases with normal microtubules were observed, a direct effect of cisplatin on microtubules could account for the disturbance of the timing of mitosis, the lengthening of mitosis, and the presence of macrotubules in interphase amebae. Thus, we investigated the action of cis- and trans-dichlorodiammineplatinum(II) on the in vitro assembly of sheep brain tubulin at 37° (31). The extent of the inhibition of microtubule assembly was dependent on the concentration of the platinum isomer and the preincubation time of tubulin with platinum at 4°. The trans isomer was more potent than the cis isomer (Chart 3) (half-inhibition of microtubules assembly, 0.3 and 1.2 mM, respectively). Thus, the in vitro action of cis- and trans-dichlorodiammineplatinum(II) on tubulin assembly cannot account for their different in vivo effects.

Since the observed perturbations of mitosis could be associated with some earlier perturbation of the cell cycle, we investigated the action of 0.1 mM cisplatin into the agar medium on the occurrence of 2 significant cell cycle events, DNA synthesis (8, 23) and the periodic increase of thymidine kinase synthesis (9, 23, 40, 50, 51). In agreement with the results of Mohrová and Drobník (32), we failed to observe any significant differences in the incorporation of radioactive thymidine into an acid-insoluble form during S phase even after a pretreatment for 8 hr in the presence of 0.1 mM cisplatin (100 and 110% in the absence and in the presence, respectively, of 0.1 mM cisplatin). However, in the presence of 0.2 mM cisplatin, the incorporation was 58% of the incorporation observed in the control. Likewise, we found that the intensity of the peak of thymidine kinase synthesis was normal (80 and 86 pmol of dTMP synthesized per min and per mg of protein in the absence and in the presence, respectively, of 0.1 mM cisplatin) although its timing was delayed of 3.5 hr with mitosis in the presence of the drug.

Effects of Various Platinum-Amine Compounds on the Occurrence of Polyploid Nuclei and on Growth Inhibition. Since the antitumoral compound cisplatin induced the formation of polyploid nuclei at concentrations which had no inhibitory action on growth, we hypothesized that the antitumoral activity might be linked to the molecular events leading to the induction of polyploid nuclei in Physarum synchronous plasmodia rather than to the general effect of growth inhibition. In order to check this possibility, the action of 26 platinum(II)- and (IV)-amine compounds on Physarum synchronous plasmodia was studied. Defined quantities of compounds were directly applied in 0.5 ml of distilled water onto the surface of quarters of 10-cm-diameter synchronous plasmodia which were subsequently incubated for 48 hr at 22°. Then plasmodial growth and occurrence of polyploid nuclei were recorded (Table 1). Several types of dose responses were observed (Chart 4). As can be observed in the case of the cis-isocitratoto-1,2-diaminocyclohexaneplatinum(II) complex, there was good reproducibility between the shape of the curves in 2 different sets of experiments (Chart 4B), although the whole curve could be displaced. A small variation of the thickness of synchronous plasmodia from one experiment to another may explain these differences.

No effect on growth or on nuclear size was observed with less than 50 nmol of cisplatin. Polyploid nuclei were detected in the presence of higher amounts of the drug without any concomitant

![Chart 3](image-url)

Chart 3. Inhibitory activity of cisplatin and trans-dichlorodiammineplatinum(II) on the in vitro assembly of sheep brain tubulin. Abscissa, concentration of platinum-amine compounds; ordinate, inhibition of tubulin assembly (percentage of control). Purified sheep brain tubulin was preincubated at 4° for 5 hr in the presence of various concentrations of platinum-amine compounds, and the temperature was shifted to 37° to induce microtubule assembly. The increase of optical density was recorded for various drug concentrations, and the plateau levels were used to calculate the percentage of inhibition. ◊, cisplatin; □, trans-dichlorodiammineplatinum(II); •, cisplatin.

![Chart 4](image-url)

Chart 4. Induction of polyploid nuclei and effect on growth of several platinum-amine compounds. Abscissa, amount of platinum-amine compound; ordinate, relative increase in the surface area of the plasmodium after 24 hr in the presence of drug. Points surrounded with a circle, presence of polyploid nuclei after 48 hr in the presence of the drug. The horizontal dashed line corresponded to the absence of growth. In B, the vertical dashed lines show the minimum amount of complex (PR) which gives rise to polyploid nuclei; arrows, minimum amount of complex (GA) which leads to absence of growth in 2 independent sets of experiments (9, 10). Pattern A, Compound 11; Pattern B, Compound 8; Pattern C, Compound 20; Pattern D, Compound 25; Pattern E, Compound 23; Pattern F, Compound 19; Pattern G, Compound 18. In each experiment, 2 controls were run: absence of treatment; and spreading of 0.5 ml of water without platinum-amine compounds. In all cases, growth was not inhibited, nor were polyploid nuclei formed.
Induction of Polyploid Nuclei in Physarum by Platinum Compounds

There is no correlation between the extent of antitumoral antitumoral activity towards L1210 leukemia in mice, although amine compounds with GA/PN ratios higher than 3 show an antitumoral activity on L1210 leukemia in vivo when T/C (see Table 1) is equal or greater than 125%. Thus, except for Compounds 9 and 11 which are chemically closely related and probably form similar aminated species in water, all platinum-amine compounds showing a T/C lower than 125% gave a GA/PN ratio inferior to 2.5. Alternatively, all antitumor platinum-amine compounds gave a GA/PN ratio larger than 3 (Table 1). However, there are no quantitative relationships between the values of the GA/PN ratio and the T/C ratio, the charge, or the half-life of the labile groups of the platinum complexes (Table 1).

**DISCUSSION**

Structural requirements of the platinum-amine compounds for the formation of giant nuclei (Table 1) are in close agreement with those necessary for antitumor activity against leukemia L1210 (28). In both cases, the biological activity is correlated with a pair of cis-labile ligands and is independent of their rate of hydrolysis and of the charge of the compounds. Only platinum-amine compounds with GA/PN ratios higher than 3 show an antitumoral activity towards L1210 leukemia in mice, although there is no correlation between the extent of the antitumoral activity and the value of the GA/PN ratio. This necessary condition implies that the formation of polyploid nuclei in Physarum plasmodia in the absence of a strong inhibition of growth could be related to the mode of action of these substances on tumoral L1210 cells. Complexes 9 and 11 (Table 1), the aquation products of which are probably similar and exhibit a high GA/PN ratio but are devoid of antitumoral activity, demonstrate that this condition is not sufficient. The GA/PN ratio and the 50% inhibition dose for growth of L1210 cells in culture (28) both predict that Compounds 9 and 11 are sufficiently toxic towards tumor cells to be active antitumor compounds. However, these compounds are quite lethal toward mice, and it seems that sufficiently high doses for antitumor activity cannot be administered.

Some antitumoral alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea and thiotepe (Triethylene thiophosphoramide) were devoid of toxicity on Physarum plasmodia. However, caryolysin [methylis[chloroethyl]amine hydrochloride], a dinitrogen mustard, induced giant nuclei in Physarum plasmodia (30) and exhibited a GA/PN ratio of 6.25. This observation suggests that some alkylating agents could share this mechanism of toxicity with the platinum antitumor compounds.

Our results indicate that the antitumoral activity of cisplatin seems to be caused by an impairment of the final stage of nuclear division rather than by a gross inhibition of DNA synthesis. Polyploid nuclei have been observed previously in animal (1, 18, 19, 42, 43) and plant (7) cells after a treatment with cisplatin. In Physarum plasmodia, which have an intranuclear mitosis, the perturbation of chromosome distribution leads to polyploid giant nuclei without immediately killing the cell. In eukaryotes with open mitosis, such effects would be toxic more rapidly. Hence, we postulate that the Physarum plasmodium model has permitted us to observe the toxicity which occurs in mammalian cells at the lowest concentration of cisplatin. These effects are observed at doses which do not sensibly inhibit gross DNA synthesis. The occurrence of polyploid nuclei after a treatment with cisplatin seems to be due to the impairment of the separation of the 2 daughter nuclei in telophase. This observation is in agreement with the results of Sodhi (42), who observed in Sarcoma 180 cells treated with cisplatin that the nuclei of a single giant cell were in communication with another through thin nuclear strands and were enclosed in a common nuclear envelope. Polyploid nuclei can also be induced in Physarum plasmodia with aphidicolin, an inhibitor of DNA polymerase α (references in Ref. 9), and with griseofulvin (14) or methylbenzimidazole carbamate (48), 2 mutant poisons. Based upon experiments with the assembly of sheep brain tubulin and in vitro observation of typical bipolar metaphase figures in treated plasmodia, it seems unlikely that a direct perturbation of microtubule assembly could account for the different effects of cisplatin and trans-dichlorodiammineplatinum(II) on Physarum plasmodia. Treatment of plasmodia with either aphidicolin or microtubular poisons gave abnormal metaphase figures. However, no attached daughter nuclei were observed. Thus, the impairment of the final stage of nuclear division in Physarum plasmodia seems to be a specific action of platinum-amine compounds. The molecular basis of this effect is still unknown. We cannot reject the possibility that the cytotoxic action of antitumoral platinum-amine compounds on mitosis could be mediated by a qualitative effect of these compounds on DNA.
replication. Such a hypothesis would explain why mitotic delays occurred only when cisplatin was applied before S phase. However, inhibitors of DNA synthesis such as aphidicolin (9), fluorodeoxyuridine (52) and hydroxyurea do not delay the peak of thymidine kinase with mitosis, as has been observed with cisplatin.

ACKNOWLEDGMENTS

The skillful determination of the antitumoral activity of several platinum-amine compounds has been performed by Dr. S. C. Cross, G. Francois, C. Galy, and A. M. Mazard. Dr. J. L. Butour is acknowledged for the determination of the t<sub>50</sub>. The action of platinum-amine compounds on the in vitro assembly of tubulin was performed by B. Allal during a pregraduate research project. Electron microscopic observations were kindly made by A. Moisand. The numerous discussions, comments, and corrections of Dr. N. P. Johnson have been highly stimulating and have been greatly appreciated. 1,3-bis(2-chloroethyl)-1-nitrosourea, thiotepa, and caryopline were generous gifts of the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

REFERENCES

cell cycle of Physarum polycephalum by the heat sensitive system which 

52. Wright, M., Tollon, Y., and Eon-Gerhardt, R. Regulation of thymidine kinase 
synthesis and mitosis during the cell cycle of the Myxomycete Physarum 

53. Wright, M., Tollon, Y., and Lundström, H. Inhibitory action of Nephroma water 
extracts on the metabolism of Physarum polycephalum. Planta Med. 35: 323- 

Fig. 1. Increase of the nuclear size in the presence of cisplatin. Nuclei were 
observed on smears under phase contrast microscopy after 72 hr of incubation 
without (a) or with (b, c) 100 nmol cisplatin. The dense masses inside the nuclei 
are the nucleoli.

Fig. 2. Abnormal nuclei observed after the second mitosis following treatment with cisplatin. Cisplatin (250 nmol) was applied directly in 0.5 ml of distilled water onto 
the surface of a quarter of 10-cm-diameter synchronous plasmodium. The different phases of the second mitosis following the treatment with cisplatin were observed 
under both phase-contrast and electron microscopy. ne, nuclear envelope; and nu, nucleolus. Arrows, bridge between the 2 daughter nuclei. a, stereoscopic view of an 
electron microscopic 3-dimensional model of 2 attached reconstruction nuclei; b, electron microscopic picture of a serial thin section of the 3-dimensional model of a; c, 
electron microscopic picture of a serial thin section in 3 binucleolate interphase nuclei with or without a central constriction of the nuclear envelope showing the 
disappearance of the bridge.
Induction of Polyploid Nuclei in the Plasmodium of *Physarum Polycephalum* by Platinum Antitumor Compounds


*Cancer Res* 1984;44:777-783.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/2/777

**E-mail alerts** Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions** To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.