ONCOLOGY

Effect of a New Antitumor Drug Cycloplatam on DNA Structure and Production

A. G. Tikhomirov, I. S. Sokolova, L. Yu. Dederer, and L. B. Gorbacheva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 125, No. 2, pp. 197-199, February, 1998
Original article submitted December 26, 1996

The new antitumor drug cycloplatam [amine (cyclopentylamine)-S-(-)-malatoplatinum(II)] caused dose-dependent formation of interstrand DNA ligations *in vitro* depending on the duration of incubation. Like cisplatin, cycloplatam caused a deep and long inhibition of DNA production in tumor cells. The effect of cycloplatam in bone marrow and small intestine epithelium cells was weaker and shorter than that of cisplatin.

Key Words: *cycloplatam; interstrand DNA ligations; DNA production*

Cisdichlorodiaminoplatinum derivatives (cisplatin, CP) have been widely used in chemotherapy of testicular and ovarian tumors, small-cell pulmonary carcinoma, tumors of the head and neck. [12]. Some malignant tumors are initially resistant to CP. Tumors sensitive to this drug acquire resistance to it during therapy [14]. Nephro- and neurotoxicity and detrimental effects on hemopoietic and gastrointestinal systems limit clinical application of CP and other first-generation derivatives [10]. This prompts the search for new drugs with better therapeutic properties.

New Russian-manufactured drug amine (cyclopentylamine)-S-(-)-malatoplatinum or cycloplatam (CCP) [3] is a second-generation complex platinum compound. Experiments demonstrated a high antitumor activity of CCP [13]. Recent studies showed complete absence of cross resistance between CP and CCP, which necessitates further investigation of this drug [8]. The second phase of clinical trials of CCP is just over. CP capacity to form intra- and interstrand DNA ligations is believed to be responsible for the cytotoxicity of this compound [9]. Among the possible mechanisms of antitumor effect of CCP, a damaging effect on DNA structure and production appears to be the most probable.

Our purpose was to compare the effects of CCP and CP on DNA structure *in vitro* and on DNA production in tumors and organs of animals with tumors *in vivo*.

MATERIALS AND METHODS

Interstrand DNA ligations were studied on calf thymus DNA (Serva). DNA (0.1 mg/ml in 6.3 mM potassium phosphate buffer with 0.15 mM NaCl, pH 7.2) was incubated at 50°C in the presence of different CP or CCP concentrations for 5-240 min. DNA-DNA ligations were detected by spectrofluorometry with ethidium bromide (EB) [7]. EB forms complexes only with double-stranded DNA; these complexes fluoresce at stimulation wavelength 305 nm and emission wavelength 600 nm. Fluorescence of native DNA complex with EB was taken for 100%, and fluorescence of denatured DNA before incubation with drugs was the zero point. Formation of interstrand DNA-DNA ligations promotes renaturing and increases fluorescence.

Treated or intact DNA (20 μg in 200 μl potassium-phosphate buffer, pH 7.2) was added to 3 ml

Institute of Biochemical Physics, Russian Academy of Sciences

0007-4888/98/0002-0172$20.00

solution containing 5 μg/ml EB, 0.33 mM EDTA, 26 mM K_2HPO_4, pH 12. Some specimens were denatured at 100°C for 5 min and then rapidly cooled to 18-20°C. Fluorescence was measured in 1-cm cuvette in an Elumin spectrofluorimeter (Russian Academy of Medical Sciences).

Experiments *in vivo* were carried out on mice (20-23 g) transplanted lympholeukemia P388 or solid melanoma B16. Leukemic strain P388 was transplanted intraperitoneally to BDF_1 (C57Bl/6×DBA/2) hybrids in a dose of 10^6 cells/mouse. B16 melanoma was transplanted to CDF_1 (C57Bl/6×CBA/2) hybrids by subcutaneous injections of 0.3 ml tumor cell suspension in normal saline (2:1). The drugs were injected intraperitoneally in single doses lower than maximum allowable: CCP in a dose of 55 mg/kg to mice with P388 on day 5 of tumor development, CP in a dose of 8 mg/kg to mice with B16 melanoma on day 12 of tumor development.

DNA production in tumor cells, bone marrow, spleen, and small intestinal epithelium cells was assessed by incorporation of 2-^{14}C-thymidine. Labeled precursor was injected 1 h before sacrifice in a dose of 2 μCi/mouse. Protocols of experiments and treatment and scintillation of radioactive precipitation were described previously [1,4].

RESULTS

CCP causes the formation of interstrand ligations in calf thymus DNA *in vitro*, its effect depending on the concentration and time of incubation. Previously, the possibility of CCP interaction with DNA *in vitro* was demonstrated by the circular dichroism method [5].

After 1-h incubation of DNA with CCP in concentrations of up to 1 mM, the drug induced a lesser number of ligations than CP (Fig. 1). The kinetics of DNA-CCP and DNA-CP interactions with the drugs used in equimolar concentrations differed: CP induced the formation of maximum number of ligations after 2 h, whereas CCP only after at least 4 h (Fig. 2, *1, 3*). Equitoxic (therapeutic) doses of CP and CCP are different (about 7-fold): a single dose of CP is 8 mg/kg *vs.* 60 mg/kg for CCP. Comparison of equitoxic concentrations of the drugs shows that CCP induces a greater number of ligations than CP: 26 and 12% after 1 h and 66 and 47% after 3 h, respectively (Fig. 2, *1, 2*).

The formation of DNA—DNA sutures by CCP probably impairs DNA matrix activity and production *in vivo*. Figure 3 shows that a single injection of CCP or CP to mice with tumors causes a deep and prolonged inhibition of DNA production in the tumors and spleen. The pattern of DNA production

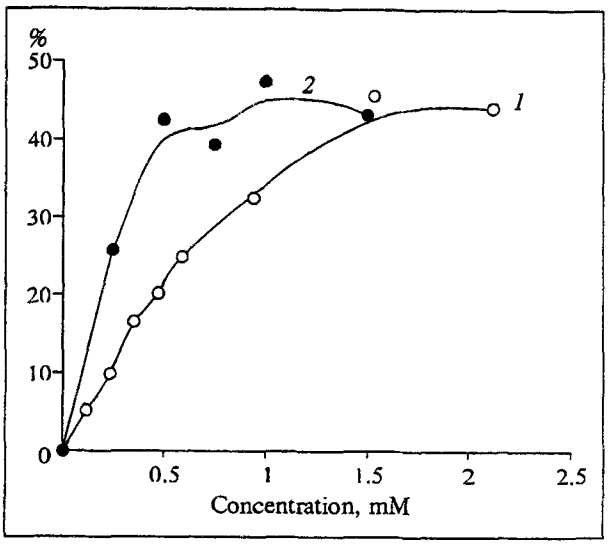

Fig. 1. Relationship between formation of interstrand ligations in calf thymus DNA and cycloplatam (*1*) and cisplatin (*2*) concentrations after 1-h incubation.

in tumor cells under the action of both drugs is virtually the same, which is in good correlation with their antitumor activity in these experimental models [2]. Both drugs impair DNA production in bone marrow and small intestinal epithelium cells, but the effect of CCP is weaker and shorter than that of CP, which indicates a lower damaging effect of CCP on these cells.

These effects can result from impairment of the enzymatic system of replication and repair or from specific disorders in DNA structure. The role of DNA modifications in realization of cytotoxicity of CP derivatives is unknown. It is noteworthy that CP and its inactive transisomer form different interstrand ligations.

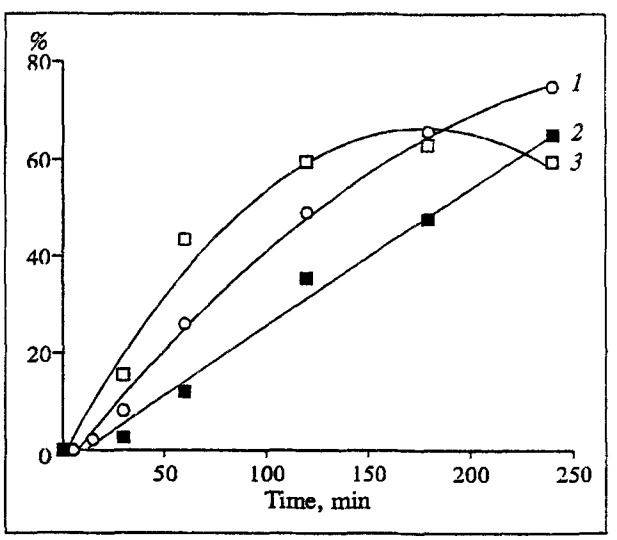

Fig. 2. Kinetics of formation of interstrand ligations in calf thymus DNA after treatment with cycloplatam in concentration of 0.35 mM (*1*) and cisplatin in concentrations of 0.05 mM (*2*) and 0.35 mM (*3*).

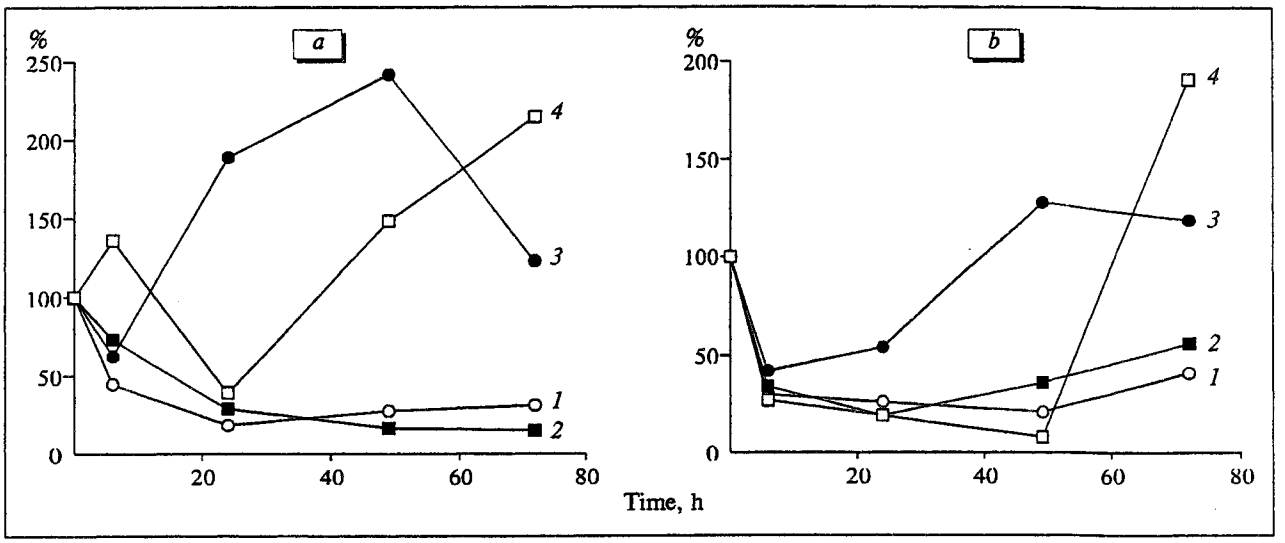

Fig. 3. Relationship between 2-^{14}C-thymidine incorporation in DNA and duration of exposure to cycloplatam and cisplatin. *a*) mice with P388 leukemia treated with cycloplatam; *b*) mice with B16 melanoma treated with cisplatin; *1*) tumor cells; *2*) spleen; *3*) bone marrow; *4*) small intestinal epithelium.

CP binds guanine residues and transplatine guanine and cytosine (complementary to guanine) residues [11]. Adducts are formed at different rates and induce different conformation deformations of DNA [6].

The lack of complete cross resistance between CP and CCP [8] suggests differences in the site-specific CP- and CCP-adducts and DNA—DNA ligations.

REFERENCES

1. L. Yu. Dederer, V. Z. Lankin, A. L. Konovalova, and L. B. Gorbacheva, *Biokhimiya,* **60,** No. 4, 602-609 (1995).
2. A. L. Konovalova, in: *Cycloplatam* [in Russian], Moscow (1993), pp. 3-15.
3. A. L. Konovalova, P. A. Chel'tsov, A. N. Kravchenko, and R. N. Shchelokov, *Dokl. Akad. Nauk SSSR,* **294,** 726-730 (1987).
4. I. S. Sokolova, G. V. Kukushkina, and L. B. Gorbacheva, *Ibid.,* **245,** 260-264 (1979).
5. V. A. Struchkov, in: *Cycloplatam* [in Russian], Moscow (1993), pp. 90-99.
6. V. Brabec and M. Leng, *Proc. Natl. Acad. Sci. USA,* **90,** 5345-5349 (1993).
7. T. R. Brent, *Cancer Res.,* **44,** 1887-1892 (1984).
8. M. Drees, W. M. Dengler, H. R. Hendriks, *et al., Eur. J. Cancer,* **31A,** 356-361 (1995).
9. A. Eastman, *Pharmacol. Ther.,* **34,** 155-166 (1987).
10. K. K. Harrap, M. Jones, C. R. Wilkinson, *et al.,* in: *Cisplatin: Current Status and New Developments,* New York (1980), pp. 193-212.
11. M. A. Lemaire, A. Schwartz, A. R. Rahmouni, and M. Leng, *Proc. Natl. Acad. Sci. USA,* **88,** 1982-1985 (1991).
12. P. J. Loehrer and L. H. Einhorn, *Ann. Intern. Med.,* **100,** 704-713 (1984).
13. M. A. Presnov and A. L. Konovalova, *Arch. Geschwulstforsch.,* **1,** 43-49 (1988).
14. B. Rosenberg, *Cancer,* **55,** 1271-1277 (1985).