Early Effects of Cis-Dichlorodiammine Platinum (II) on Tumor Progression and Programmed Death of Ehrlich Ascites Carcinoma Cells

B. V. Razin, E. V. Inzhevatkin, V. V. Nefedova, A. B. Egorova, V. I. Kazbanov*, and V. P. Nefedov

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We studied the effects of cis-dichlorodiammine platinum (II) on different pathways of cell death in cultured Ehrlich ascites carcinoma *in vitro* and on subsequent growth of transplanted tumor *in vivo*. One-hour incubation with the cytostatic modulated apoptosis in cell culture. However, exposure of cell culture to a minimal effective concentration of cis-platinum(II)diammine dichloride promoted the growth of transplanted tumor.

Key Words: *Ehrlich ascites carcinoma; cis-dichlorodiammine platinum (II); apoptosis; necrosis*

Cis-dichlorodiammine platinum (II) (CDDP) is a potent cytostatic used for antitumor therapy [1,3]. The mechanisms underlying cytostatic effects of this substance are now extensively studied. It was hypothesized that cytostatic effects of CDDP are related to initiation of tumor cell apoptosis.

Recent studies showed that cytostatics (*e.g.*, CDDP) in low concentrations stimulate proliferation of some cells [1,6]. It can be hypothesized that cytostatics can stimulate proliferation of tumor cells. However, we found no data on the direct effects of CDDP in low concentrations on tumor cells.

Here we studied the effects of low CDDP concentrations on apoptotic and necrotic death of cultured Ehrlich ascites carcinoma (EAC) cells *in vitro* and on *in vivo* growth of transplanted tumor.

MATERIALS AND METHODS

CDDP was synthesized and purified by the method developed at the Institute of Chemistry and Chemical Technology.

International Research Center for Extreme Body States, Presidium of the Krasnoyarsk Research Center, Siberian Division of the Russian Academy of Sciences; *Institute of Chemistry and Chemical Technology, Siberian Division of the Russian Academy of Sciences, Krasnoyarsk. ***Address for correspondence:*** inscience@mail.ru. Razin B. V.

Asynchronous EAC culture was incubated in medium 199 containing CDDP in concentrations of 10^{-12}, 10^{-9}, and 5×10^{-4} M at 37°C for 1 h. Control cells were incubated under the same conditions without CDDP. EAC cell concentration in medium 199 during incubation was 1.5×10^{10} cells/ml.

Dead cells were counted in a Goryaev chamber immediately after incubation (large squares). EAC cells were stained with methylene blue: 10 μl cell suspension and 40 μl 0.5% methylene blue in 0.85% NaCl were added to 800 μl medium 199. Stained cells were considered as dead cells. Enlarged cells with relatively weakly stained cytoplasm and organelles were markers of necrotic death. Small intensively stained cells or weakly stained cells of normal size and shape with signs of blebbing and heterogeneous staining of intracellular organelles were characteristic of apoptosis.

After 1-h incubation with CDDP, morphological analysis of EAC cell suspensions was carried out, the cells were washed 3 times with 10-fold volume of medium 199 and were intraperitoneally inoculated (2.4×10^6 cells/ml) to intact mice. The total number of tumor cells was estimated in a Goryaev chamber 9 days after *in vivo* EAC growth.

The results were analyzed by Student's *t* test [2].

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TABLE 1. Effect of CDDP on *In Vitro* Cell Death and *in Vivo* EAC Growth ($M \pm m$)

Parameter	Control	CDDP, M		
		10^{-12}	10^{-9}	5×10^{-4}
Cell death				
apoptotic cells, %	1.21±0.54	1.79±0.73	8.92±2.65**	28.33±4.40*
necrotic cells, %	0.52±0.19	0.41±0.11	0.68±0.28	1.42±0.62***
EAC cell count on day 9 of *in vivo* tumor growth	$6.01\times10^8\pm2.13\times10^8$	$6.53\times10^8\pm1.34\times10^8$	$9.54\times10^8\pm2.60\times10^{8}$***	$2.44\times10^7\pm3.21\times10^6$*

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control.

RESULTS

CDDP in a concentration of 10^{-12} M had no effect on the death of cultured EAC cell (Table 1). CDDP in a concentration of 10^{-9} M 5-fold increased the count of apoptotic cells, but had no effect on necrotic death. Increasing the concentration of CDDP to 5×10^{-4} M promoted cell apoptosis and 3-fold increased the count of necrotic cells (compared to the control and other experimental groups). However, the count of necrotic cells was 20 times lower than that of apoptotic cells. Therefore, the contribution of necrosis into cultured EAC cell death was insignificant.

Preincubation of cells with 10^{-12} M CDDP had no effect on *in vivo* growth of EAC. Incubation of EAC cells with CDDP in a concentration of 5×10^{-4} M markedly decelerated the growth of transplanted tumor. It should be emphasized that preincubation of EAC cells with 10^{-9} M CDDP increased the count of tumor cells.

It was reported that CDDP inhibits proliferation of some cells, but accelerates division of others. Cell responsiveness to apoptotic signals is determined by activity of mitosis-regulating enzymes. In CDDP-sensitive cells, the preparation stimulates expression of the universal inhibitor $p21^{waf1/cip1}$ and arrests the culture in S and G_2 phases of the cell cycle [8]. By contrast, in cisplatin-resistant cells CDDP activates cyclin-dependent kinase $p34^{cdc2/cdk1}$ and cyclin B1 through dephosphorylation of $p34^{cdc2/cdk1}$ by protein phosphatase cdc25C [7], stimulates expression of this cyclin-kinase complex [10], and inactivates kinase inhibitor $p27^{Kip1}$ [6]. Cisplatin up-regulates cyclin D1 and cyclin-dependent kinase cdk4 activities, decreases the content of $p16^{INK4a}$ (endogenous inhibitor of the D1-cdk4 complex) and, therefore, stimulates pRb phosphorylation, which leads to inactivation of this negative regulator of cell division and promotes cell transition from the late G_1 into S phase [5].

Our findings can be explained by heterogeneity of the tumor cell culture and the presence of cisplatin-sensitive cells. Minimal effective concentration of the cytostatic induced rapid apoptotic death of these cells during 1-h *in vitro* incubation with CDDP. At the same time, CDDP accelerates division of other tumor cells, thus modulating subsequent tumor growth *in vivo*.

Our results indicate that the cytopathogenic effect of CDDP is associated with induction of tumor cell apoptosis. However, this preparation in the minimal effective concentration can stimulate tumor growth.

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