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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Antitumor Effect of Arabinogalactan and Platinum Complex

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Abstract—The article presents the results of investigation of antitumor properties of platinum–arabinogalactan complex. We showed the ability of the complex to inhibit the growth of Ehrlich ascites tumor cells. It is found that the distribution of the platinum–arabinogalactan complex is not specific only for tumor cells in mice. The complex was found in all tissues and organs examined (ascites cells, embryonic cells, kidney, and liver). The mechanism of action of the arabinogalactan–platinum complex may be similar to cisplatin as the complex is able to accumulate in tumor cells.

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The prospects for obtaining new drugs based on arabinogalactan, a polysaccharide that can form conjugates with different compounds in the form of spherical particles, are currently opening [1, 2]. Arabinogalactans contain numerous arabinose and galactose residues through which they interact with asialoglycoprotein receptors [3]. Due to this property, these polysaccharides can be used to deliver into cells the compounds that are unable to penetrate through the outer cell membrane. Galactose-containing polysaccharides exhibit a high biological activity and have immunomodulating effects [4]. Arabinogalactan-based conjugates exhibit the properties of this polysaccharide and the functional groups introduced into it.

In this study, we investigated the possibility of using arabinogalactan for targeted delivery to tumor cells of platinum, which exhibits antitumor activity and is a component of cisplatin, a pharmaceutical that is widely used in oncological clinical practice [5, 6], as well as the efficiency of the anticancer effect of the complex of arabinogalactan with platinum.

The complex of arabinogalactan with platinum (CAP) was synthesized as described in [7]. The efficiency of the anticancer effect of CAP in vivo was studied using male outbred ICR mice obtained from the animal breeding facility of the Vector State Research Center (Koltsovo, Novosibirsk oblast). All mice that were transplanted with Ehrlich ascites carcinoma cells were divided into three groups. Mice of the first group were intraperitoneally injected with a CAP solution at a dose of 20 mg/kg and an equal volume of 5% glucose solution. Animals of the second group were injected with a cisplatin solution at a dose of 20 mg/kg and an equal volume of 5% glucose solution. Mice of the control group received sterile saline and 5% glucose solution. Drugs were administered on days 3, 5, 7, 9, and 11 after the tumor inoculation.

The penetration of CAP into tumor cells in vitro was estimated by fluorescence microscopy. For this purpose, cells were incubated at 37°C for 2 h in Hanks solution (Chumakov Institute of Poliomyelitis and Viral Encephalites, Russia) containing 0.9 mg/mL CAP and a fluorescent label. After incubation, the cells were washed three times with Hanks solution. The fluorescence of the drug inside the cell was evaluated at an excitation wavelength of 493 nm and an emission wavelength of 520 nm under an AxioImager D1 microscope (Carl Zeiss, Germany).

To study the distribution of CAP in the body of mice bearing Ehrlich ascites carcinoma, the animals on days 5, 6, and 7 of tumor development were injected with the preparation containing the fluorescent label at a dose of 20 mg/kg body weight. In these experiments, five male and two pregnant female ICR

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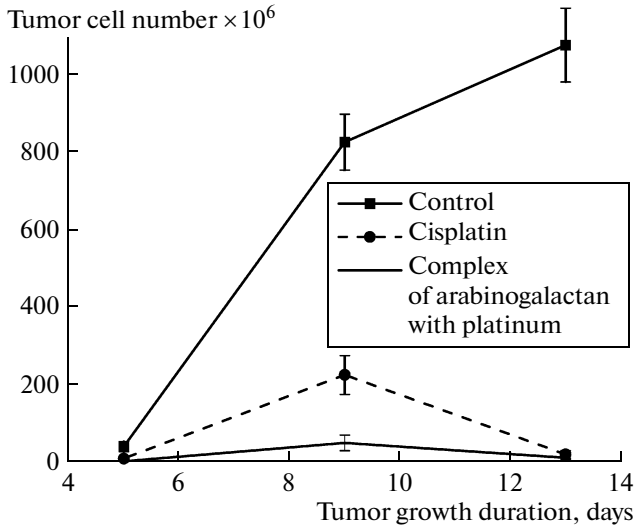

Fig. 1. Effect of cisplatin and CAP on the growth of Ehrlich ascites carcinoma. Here and in Fig. 3, the mean values and 95% confidence intervals are shown ($n = 15$ for each group).

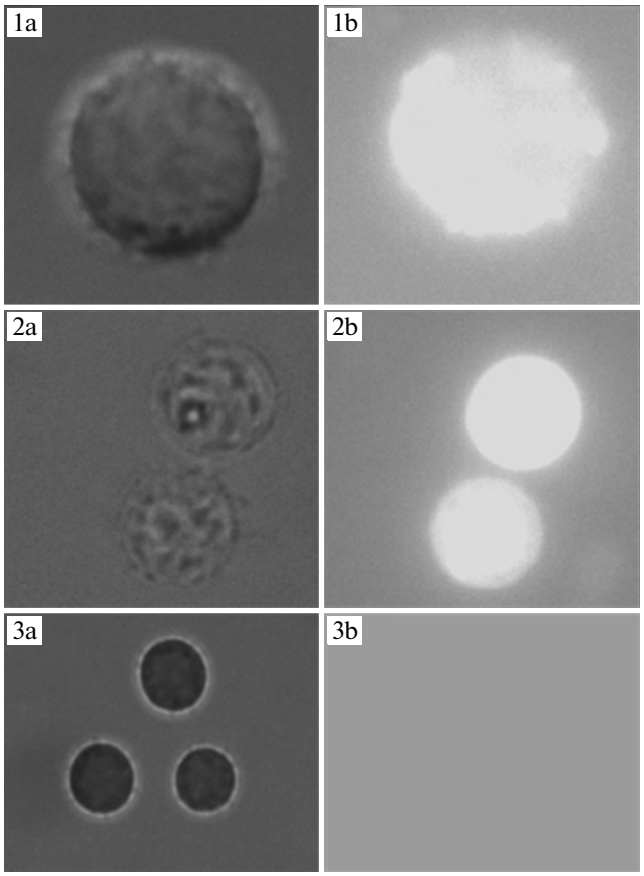

Fig. 2. CAP fluorescence in (1) ascites cells, (2) lymphocytes, and (3) erythrocytes. (a) Light microscopy, (b) fluorescent microscopy.

mice were used. Tissues and cells were extracted on day 9 of tumor development. The liver, kidneys, and embryos were perfused with sterile saline. Then, the organs were placed in deionized water (wt/vol ratio, 1 : 3 (g/mL)), homogenized for 10 min, centrifuged at 1800 *g*. The resulting supernatant was used for further study. To isolate the ascites cells and erythrocytes, the contents of the abdominal cavity and the whole blood were centrifuged to obtain ascites fluid and blood plasma. Erythrocytes and ascites cells were washed twice with saline and then diluted with deionized water at a ratio of 1 : 3 (cells/water). The fluorescence of CAP containing the fluorescent label was measured with an Aminco Bowman Series 2 spectrofluorometer (Thermo Spectronic, United States) for 30 s at an excitation wavelength of 493 nm and an emission wavelength of 520 nm. For each measurement, a 100-μL aliquot of the study sample was added to the measuring cell.

The experimental data were statistically processed using STATISTICA 7.0 software. The differences between the groups were evaluated using Student's *t* test, because the number of cells and the fluorescence intensity had a normal distribution (according to the Shapiro–Wilk test) and the same variance (according to Levene's test). Differences were considered significant at $p \le 0.05$.

The results of studies in vivo have shown that cisplatin and CAP effectively suppressed the growth of Ehrlich ascites carcinoma (Fig. 1). At the end of the experiment, the number of ascites cells in the tumors of the control animals increased in 358 times; in the tumors of the animal that were treated with cisplatin, in 7 times; and in the tumors of mice that were treated with CAP, only in 4 times. Furthermore, in the group of mice treated with cisplatin, 11% of animals died. In the control group, the animals that died by the end of the experiment accounted for 8%, whereas all animals from the group treated with CAP survived.

It is known that the mechanism of the anticancer effect of cisplatin is determined by DNA alkylation, which leads to a prolonged inhibition of the biosynthesis of nucleic acids and, ultimately, to cell death [5]. To investigate the mechanism of action of CAP, we studied its ability to penetrate into tumor cells using a fluorescent label. Experiments (Fig. 2) showed that CAP accumulated in the tumor, as is the case with cisplatin [5], but the mechanism of action of this drug requires a special study.

It is known that cisplatin has no specificity of action on tumor cells only and is distributed in all biological fluids and tissues. For this reason, cisplatin therapy may cause renal dysfunction, anaphylactic reactions, leukopenia, thrombocytopenia, anemia, and neuropathy [5]. The toxic effect of cisplatin on the functional state of organs and tissues is primarily determined by its antiproliferative effect on rapidly dividing cells. The study of the interstitial and intracellular distribution CAP showed that, similarly to cis-

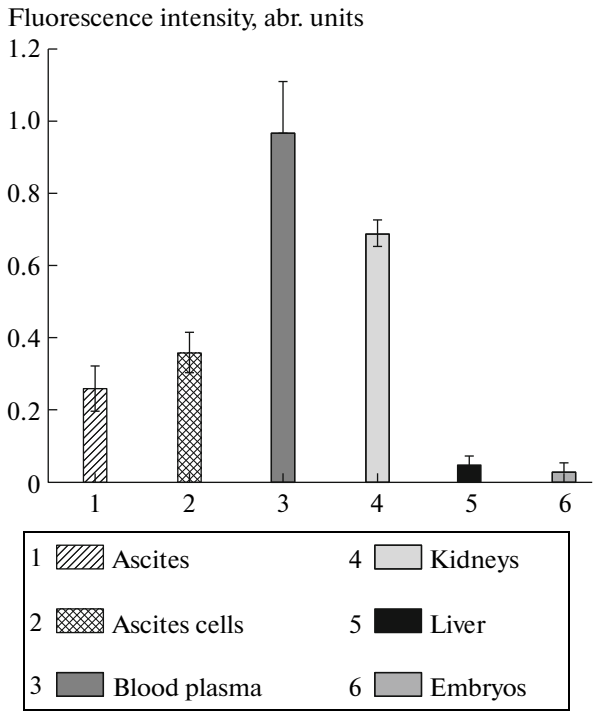

Fig. 3. CAP distribution in tissues of tumor-bearing mice ($n = 5$ for groups 1–5, and $n = 3$ for group 6).

platin [6], it does not selectively accumulate only in tumor cells (Fig. 3). We have found that this drug accumulated in ascites cells and embryos but was absent in erythrocytes. The highest quantities of CAP were detected in the blood plasma and kidneys, which are apparently the main route of CAP excretion.

Thus, the main results obtained in our study are as follows: CAP can effectively suppress tumor growth without causing significant toxic effects; the mechanism of action of CAP is based on its ability to penetrate into the cell; and the distribution of CAP in the body is not specific only for tumor cells.

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