

ANTITUMOR EFFECTS AND HEMATOTOXICITY OF C₆₀-Cis-Pt NANOCOMPLEX IN MICE WITH LEWIS LUNG CARCINOMA

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Background: Cisplatin (Cis-Pt) is a widely used anticancer drug but its therapeutic efficiency is limited by hemato-, cardio-, hepato-, nephro- and neurotoxicity. Complexation of Cis-Pt with C₆₀ fullerene nanoparticle will allow to enhance the antitumor activity of the drug and to reduce its side toxic effects. **Aim:** To estimate the antitumor effects of C₆₀-Cis-Pt nanocomplex in Lewis lung carcinoma (LLC) and analyze hematological toxicity in tumor-bearing mice. **Materials and Methods:** Complexation of C₆₀ fullerene and Cis-Pt molecule was studied by computer simulation. C₆₀-Cis-Pt nanocomplex was i.p. injected to LLC-bearing mice in a total dose of 7.5 mg/kg (C₆₀:Cis-Pt as 3.75:3.75 mg/kg). The survival of tumor-bearing mice and the relative reduction of tumor weight was recorded. Blood indices were determined using the Particle Counter PCE210 automatic hematology analyzer. **Results:** Computer simulation demonstrated the formation of C₆₀-Cis-Pt nanocomplex in physiological medium and its stability due to the hydrophobic interactions. Treatment with C₆₀-Cis-Pt nanocomplex increased survival time of LLC-bearing mice by 32%, normalized hemoglobin content (up to 100 g/l), erythrocyte and platelet count as compared to the untreated LLC-bearing mice. Tumor weight decreased by 35.5%; the mitotic index of tumor cells decreased by 78%, and apoptotic index increased by 75%. The revealed effects of the C₆₀-Cis-Pt nanocomplex were more pronounced than the effects of Cis-Pt or C₆₀ fullerene alone in equivalent dose. **Conclusion:** Treatment with C₆₀-Cis-Pt nanocomplex prolonged the survival of LLC-bearing mice and reduced anemia in LLC-bearing mice. **Key Words:** cisplatin, C₆₀ fullerene, C₆₀-Cis-Pt nanocomplex, Lewis lung carcinoma, tumor-bearing mice survival, hematological indices.

Cisplatin (Cis-[Pt(II)(NH₃)₂Cl₂, Cis-Pt) is a metal-containing alkylating drug that is widely used to treat a number of cancers. Therapeutic efficiency of Cis-Pt is limited by high toxicity, non-selective distribution of the drug between normal and tumor tissues and development of drug resistance [1]. In order to overcome these limitations, nanoparticles have been used as drug delivery and carrier systems in order to promote preferential accumulation in cancer cells and thereby reduce adverse side effects. Recent studies have shown that combination or conjunction of anticancer drug with nanoparticles could enhance its permeability and retention effect, as well as provide selective accumulation in tumor cells [2, 3].

A variety of carriers, organic (biopolymers, polymeric conjugates, micelles and liposomes) and inorganic (carbon, iron oxide, gold and silica) nanoparticles have been used to modify the action of platinum-based drugs [4]. Cis-Pt conjugated with gold-coated iron oxide nanoparticles (Pt@Au@FeNPs) was more toxic towards Cis-Pt-sensitive and resistant human ovarian

carcinoma A2780 cells than the free drug [5]. Cis-Pt (Pt IV) conjugated with PEGylated gold nanorods was more toxic against Cis-Pt-sensitive and resistant lung cancer A549 cells and facilitated drug uptake through endocytosis in comparison with the free drug [6].

The ability of carbon nanoparticles to modulate antitumor activity of Cis-Pt was shown. So, single-walled carbon nanotubes could be promising for targeted drug delivery [7], metallofullerenes with gadolinium [Gd@C₈₂(OH)₂₂]_n overcome tumor resistance to Cis-Pt by reactivating the impaired endocytosis of Cis-Pt-resistant human prostate cancer (CP-r) cells [8], an increased cytotoxic effect of Cis-Pt in combination with fullereneol against HeLa and Lewis lung carcinoma (LLC) cells was demonstrated [9, 10].

C₆₀ fullerene, being a representative of carbon nanostructures, can be a promising agent for anticancer drug delivery. C₆₀ fullerene is a chemically stable molecule, able to penetrate biological membranes. It localizes in normal cells without toxic effects in a range of 10⁻⁷–10⁻⁵ M and exhibits powerful antioxidant properties [11–17]. A selective accumulation of C₆₀ fullerene nanoparticles in tumor tissue was demonstrated [18–20]. The unique structure of C₆₀ molecule makes it possible to modify its surface with chemotherapeutic agents [21–23]. It was demonstrated that C₆₀ fullerene conjugated with pa-

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Abbreviations used: C₆₀FAS – C₆₀ fullerene aqueous colloid solution; Cis-Pt – cisplatin; HE – hematoxylin and eosin; i.p. – intraperitoneal(l); IR – inhibitory rate; LLC – Lewis lung carcinoma; LS – lifespan.

clitaxel or doxorubicin exhibited increased antitumor activity as compared with free drugs [24–26].

The aim of this study was to estimate the antitumor effects of C₆₀-Cis-Pt nanocomplex in LLC and analyze its hematotoxicity in tumor-bearing mice.

MATERIALS AND METHODS

C₆₀-Cis-Pt nanocomplex. A highly stable pristine C₆₀ fullerene aqueous colloid solution (C₆₀FAS; final concentration 0.15 mg/ml, purity > 99.5%) was obtained at Technical University of Ilmenau (Germany) as described in [27, 28]. In brief, this method is based on transferring C₆₀ fullerene from organic solution into the aqueous phase by ultrasonic treatment. The monitoring of the state of C₆₀ fullerene nanoparticles in aqueous solution using microscopic and spectroscopic measurements showed the presence of both single C₆₀ molecules and their nanoaggregates with the size in the range of 1.2–100 nm. The C₆₀FAS was stable for 6–8 months at room temperature [29]. To obtain C₆₀-Cis-Pt nanocomplex C₆₀FAS (150 µg/ml) and Cis-Pt dissolved in 0.9% NaCl saline solution (150 µg/ml) were mixed in 1:1 volume ratio (C₆₀:Cis-Pt molar ratio 1:2.4). This mixture was treated by ultrasound (22 kHz, 20 min) with further stirring on the magnetic stirrer (400 rpm, 18 h). The final concentrations of C₆₀ fullerene and Cis-Pt in the obtained nanocomplex were 75 and 75 µg/ml, respectively.

In silico experiment. Structures of C₆₀ fullerene and Cis-Pt molecule, as well as the 1:1 C₆₀-Cis-Pt nanocomplex were characterized according to the procedure described in [30]. The energy minimization of the complex in the water environment was accomplished in X-PLOR software (1423 TIP3P water molecules in cubic box) by the method of molecular mechanics with CHARMM27 force field. The resultant 1:1 C₆₀-Cis-Pt structure was further used to populate the surface of C₆₀ fullerene by several Cis-Pt molecules in a way, which creates sterical conditions for the contact between neighbouring Cis-Pt molecules via hydrogen bonds. Further execution of energy minimization procedure allowed testifying the stability of such nanocomplex at each iteration by changing the number of bound Cis-Pt molecules. Such approach enabled to estimate the maximal number of drug molecules, which may bind with C₆₀ fullerene.

In vivo experiments

Animals. Studies were carried out on the male C57Bl/6 mice 2–2.5 months old weighing 22–24 g from the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the NAS of Ukraine, Kyiv, Ukraine. All experiments were conducted in accordance with the international regulations of the European Convention for protection of vertebrate animals under control of the Bio-Ethical Committee of the NAS of Ukraine.

Tumor model. National Bank of Cell Lines and Transplanted Tumors of R.E. Kavetsky IEPOR of the NAS of Ukraine kindly provided LLC cells. LLC cells (5 · 10⁵) were transplanted into the limb by intramuscular injection.

Design of the experiment. Treatment (5 consecutive days with 1-day interval) started on the 2nd day after tumor transplantation. LLC-bearing mice were randomized by weight and divided into 4 groups with 20 animals per group (12 mice for survival and 8 mice for haematological and histological parameters study): untreated mice (0.9% NaCl saline solution); C₆₀ fullerene group (C₆₀FAS); Cis-Pt group (Cis-Pt in 0.9% NaCl saline solution); C₆₀-Cis-Pt nanocomplex group (C₆₀-Cis-Pt nanocomplex solution). The intact group (0.9% NaCl saline solution) consisted of healthy mice without transplanted tumor. C₆₀ fullerene (in a total dose of 3.75 mg/kg), Cis-Pt (in a total dose of 3.75 mg/kg), C₆₀-Cis-Pt nanocomplex (in a total dose of 7.5 mg/kg (C₆₀:Cis-Pt as 3.75:3.75 mg/kg)) were injected intraperitoneally (i.p.).

An average lifespan (LS, days) of mice in each group, a coefficient of animal life prolongation (*cp*, % relative to the untreated tumor-bearing mice) and survival of tumor-bearing mice (%) in each group during the experiment were calculated.

Hematological analysis. The blood was collected from the tail vein into the tube with K3EDTA anticoagulant (C-Sanguis Counting Kotrollblutherstellungs-und Vertriebs GmbH, Germany) on the 22nd day after transplantation of LLC. Determination of hematological indices in blood was performed using automatic hematology analyzer Particle Counter PCE-210 (ERMA Inc., Japan).

Antitumor activity. Autopsy was carried out immediately after euthanasia with CO₂ on the 22nd day after LLC transplantation. The inhibitory rate (IR, %) of the tumor was calculated as follows

$$IR = \frac{tw_c - tw_t}{tw_c} \times 100\%,$$

where *tw_c* and *tw_t* is average tumor weight (g) in untreated tumor-bearing animals (*c*) and treatment (*t*) groups of animals, respectively.

Histological study. Tumors were excised and fixed in 10% neutral buffered formalin, and then subjected to standard histological processing and staining with hematoxylin and eosin (HE). Slides were analyzed using a light microscope Olympus BX-41 (Olympus Europe GmbH, Japan) and camera Olympus C-5050 Zoom (Olympus Europe GmbH, Japan). Slides were screened for apoptosis and mitosis. Apoptotic index was calculated as the number of apoptotic cells and apoptotic bodies. Mitotic index was calculated by counting mitosis among 1000 cells. Apoptotic and mitotic indices were expressed as percentage of total cell number.

Statistical analysis. All statistical analyses were performed with the Origin 8.0 software (OriginLab Corporation, USA). Student's test was used to compare groups with *p* < 0.05 being considered significant.

RESULTS AND DISCUSSION

Computer simulation. Results of the structural modeling of C₆₀-Cis-Pt nanocomplex in the 1:1 ratio of C₆₀ fullerene and Cis-Pt molecule (Fig. 1, a) evidenced the stability of this structure in aqueous media. The minimal distance between C₆₀ fullerene and Cis-Pt molecule was found to be 2.75 Å indicating the

importance of dispersive van der Waals attractive interactions between Pt atom and aromatic C₆₀ fullerene surface in stabilization of the nanocomplex.

The energy-minimized structure with the theoretically maximal filling of the C₆₀ fullerene surface with Cis-Pt molecules is shown in Fig. 1, b. The data obtained evidenced the possibility of the binding of fifteen Cis-Pt molecules with C₆₀ fullerene. The planes of cruciform Cis-Pt molecules were parallel to the planes tangent to the surface of C₆₀ fullerene. No preferential arrangement of Cis-Pt molecules over the five- or six-membered C₆₀ fullerene rings was shown. Apparently, this is determined by the lack of aromatic ring in Cis-Pt molecule and the absence of a classical π -stacking with the C₆₀ fullerene. Numerical calculations showed that the total binding energy of Cis-Pt molecules with C₆₀ fullerene is about -8.6 kcal/mol and the main contribution to the stabilization of the nanocomplex is made by the hydrophobic interactions. Electrostatic interactions both between molecules in the nanocomplex and with water molecules were very weak due to the electroneutrality of both molecules and the non-polarity of C₆₀ fullerene.

The *in silico* results point out on interaction of C₆₀ fullerene and Cis-Pt molecules and stability of C₆₀-Cis-Pt nanocomplex in physiological media under the conditions of *in vivo* experiment. This fact should be taken into consideration when interpreting biological data with these compounds used in combination.

Finally, it is important to note that using various physico-chemical methods, including the small-angle neutron scattering, scanning and atomic force microscopies, dynamic light scattering and UV-Vis spectroscopy as well as the isothermal titration calorimetry, it was shown that Cis-Pt molecules could be adsorbed on the surface of single C₆₀ fullerene or its nanocluster resulting in formation of noncovalent C₆₀-Cis-Pt nanocomplex stabilized by hydrophobic interactions [23].

The survival of LLC-bearing mice treated with Cis-Pt and C₆₀ fullerene separately or with C₆₀-Cis-Pt nanocomplex. Antitumor activity of Cis-Pt was investigated in the range of high concentrations, mainly more than 10 mg/kg body weight of the animal [31], but significant toxicity of the drug and high lethal effect complicate the long-term evaluation of its antitumor activity. We used the model of multiple i.p.

injections of Cis-Pt in a total dose of 3.75 mg/kg and C₆₀-Cis-Pt nanocomplex in a total dose of 7.5 mg/kg (C₆₀:Cis-Pt as 3.75:3.75 mg/kg).

Recent studies have shown that metalofullerene Gd@C₈₂(OH)₂₂ under i.p. administration of 3.8 mg/kg (once a day for 7 days) did not cause pronounced side effects in mice with transplanted human breast cancer MCF-7 cells, none of animals died, the body weight of animals corresponded to normal values, while animal lethality was observed in the untreated group and in the group that received paclitaxel in a dose of 10 mg/kg (4 times each 3 days) [32].

In our study, the average survival of untreated tumor-bearing mice was 25 days. Survival and average LS of LLC-bearing mice treated with Cis-Pt, C₆₀ fullerene, or C₆₀-Cis-Pt nanocomplex increased as compared to control, with the average survival of 31, 29, and 33 days, respectively (Fig. 2, Table 1). It is worth

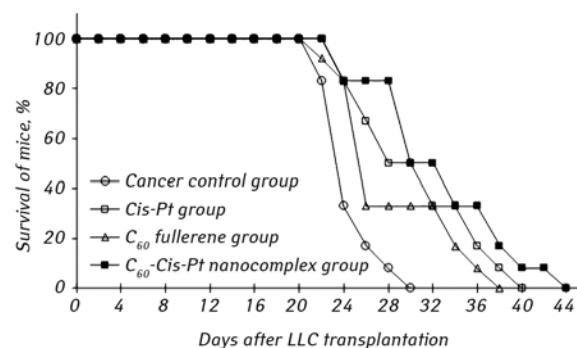


Fig. 2. Survival (%) of LLC-bearing mice treated with either Cis-Pt and C₆₀ fullerene separately or with C₆₀-Cis-Pt nanocomplex

noting that the survival prolongation of tumor-transplanted mice by 25% and more in groups under the effect of drugs compared to untreated group is considered to be an effective antitumor response [33].

Table 1. Average LS and coefficient of life prolongation (*cp*) of LLC-bearing mice treated with either Cis-Pt and C₆₀ fullerene separately or with C₆₀-Cis-Pt nanocomplex

Experimental groups	LS, days	<i>cp</i> , %
Untreated	25 ± 2	—
+ Cis-Pt	31 ± 3	24 ± 4*
+ C ₆₀ fullerene	29 ± 2	16 ± 4
+ C ₆₀ -Cis-Pt nanocomplex	33 ± 3*	32 ± 5*

Note: **p* < 0.05 as compared to tumor-bearing mice treated with saline.

The prolongation of the survival in LLC-bearing mice in experimental groups evidences the effective antitumor action of Cis-Pt both separately and at complexation with C₆₀ fullerene (Table 1). C₆₀-Cis-Pt nanocomplex was observed to perform the most potent antitumor effect, as coefficient of animal life prolongation in this group of tumor-bearing animals was higher compared to the one exposed to separate administration of Cis-Pt and C₆₀ fullerene by 1.3 and 2 times, respectively.

Our results are in a good agreement with the data presented in [34], where a 2-fold prolongation of Dalton's ascites lymphoma-transplanted mice survival time under the action of Cis-Pt was shown as compared with untreated group.

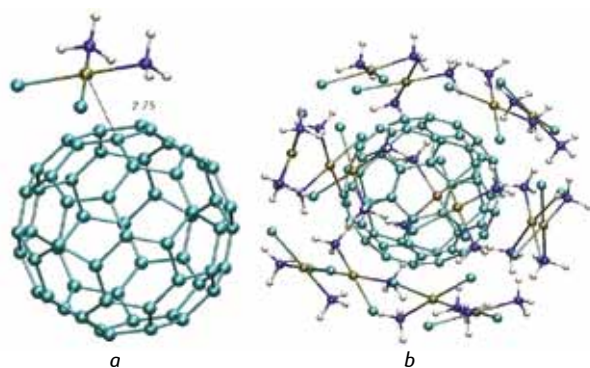


Fig. 1. The calculated structure of 1:1 (a) and 1:15 (b) C₆₀-Cis-Pt nanocomplexes

Hematological toxicity in LLC-bearing mice treated with Cis-Pt, C₆₀ fullerene or with C₆₀-Cis-Pt nanocomplex. Results of blood analysis on the 22nd day after LLC transplantation to mice are presented in Table 2. The decrease in hemoglobin content (by 11%) and hematocrit (by 21%) as well as a decrease in the count of erythrocytes by 38%, lymphocytes by 30%, and thrombocytes by 30% on the 22th day after tumor transplantation as compared to the corresponding indices in intact animals evidenced anemia development in tumor-bearing mice. 1.4-fold increase in leukocyte count indicated leukocytosis as tumor-associated inflammatory process (Table 2). Earlier, the leukemoid reaction with a significant increase of monocyte count was demonstrated in animals with highly angiogenic LLC [35].

Antitumor drugs are known to suppress hematopoiesis. Cis-Pt caused hematotoxicity at both low (10⁻⁸–10⁻¹² g/ml per rat) [36] and high (4–16 mg/kg) concentrations [34, 37–39], with the development of anemic conditions both in intact and tumor-bearing animals. In our study, Cis-Pt in a total dose of 3.75 mg/kg aggravated anemia in LLC-bearing mice as evidenced by the reduction of hemoglobin and erythrocyte levels by 21% and 20%, respectively, and lymphocytopenia (Table 2).

In order to prevent anemic conditions during and after chemotherapy, antioxidants have become widely used in a medical practice. The side effects of chemotherapy, in particular, Cis-Pt-induced anemia in tumor-bearing animals, were prevented by combined treatment with antioxidants, such as selenium Se and ascorbic acid [34, 39]. The powerful antioxidant properties of pristine water-soluble C₆₀ fullerene were confirmed *in vitro* and *in vivo* experiments [40–43]. We have previously shown that C₆₀ fullerene protected thymocytes from the damage induced by 100 μM hydrogen peroxide or Cis-Pt (3.3 and 16.7 μM). After the treatment of cells with 10⁻⁵ M C₆₀ fullerene the increased reactive oxygen species level induced by H₂O₂ or Cis-Pt in thymocytes was normalized and erythrocytes hemolysis induced by Cis-Pt was prevented [12, 41, 43]. In addition, it was demonstrated that C₆₀ fullerene prevented Dox-dependent oxidative insults in LLC-bearing mice liver and heart [42]. In present study, C₆₀ fullerene reduced anemia in LLC-bearing mice (Table 2) with hematologic indices approaching those in intact animals. C₆₀-Cis-Pt nanocomplex reduced the hematotoxicity in tumor-bearing mice caused by tumor growth and drug treatment. These findings are of some interest taking into account the systemic effects of the

tumor on the host and the development of paraneoplastic syndrome [45].

Antitumor effects of Cis-Pt, C₆₀ fullerene or C₆₀-Cis-Pt nanocomplex. The inhibitory effect on tumor growth was evident in all treated groups. Treatment of LLC-bearing mice with Cis-Pt or C₆₀ fullerene was accompanied by 1.36 and 1.23-fold decrease of tumor weight with 26.3 and 18.4% IR, respectively, in comparison with untreated group, while treatment with C₆₀-Cis-Pt nanocomplex was accompanied by 1.55-fold decrease of tumor weight with 35.5% IR (Table 3). Earlier, we have shown that antimetastatic effects of C₆₀-Cis-Pt nanocomplex exceed those of Cis-Pt (75% vs 57% of total metastases volume reduction) [46]. The data on antitumor effects of C₆₀-Cis-Pt nanocomplex and decreasing hematological toxicity as compared to the free drug are consistent with antimetastatic effects of this complex.

Table 3. Tumor weights and tumor IR in LLC-bearing mice treated with either Cis-Pt and C₆₀ fullerene separately or with C₆₀-Cis-Pt nanocomplex

Experimental groups	Tumor weight, g	IR, %
Untreated	7.6 ± 0.9	
+ Cis-Pt	5.6 ± 0.5*	26.3
+ C ₆₀ fullerene	6.2 ± 0.5*	18.4
+ C ₆₀ -Cis-Pt nanocomplex	4.9 ± 0.4*	35.5

Note: *p < 0.05 as compared to tumor-bearing mice treated with saline.

Tumor morphology after treatment with Cis-Pt, C₆₀ fullerene or with C₆₀-Cis-Pt nanocomplex. Histological examination of the tumor nodules in untreated mice group showed that tissue consisted of a cluster of highly polymorphic, atypical cancer cells (Fig. 3, a). A large number of cells with large nuclei and nucleoli, condensed chromatin grains and light areas of karyoplasm were encountered (Fig. 3, b). The giant polymorphic cells were also found among carcinoma cells. The necrosis foci with perifocal inflammatory infiltrates were detected in the stroma of the tumor tissue. Tumors in C₆₀ fullerene treated group showed a decrease in signs of polymorphism of carcinoma cells that were predominantly round-oval in shape, small in size; also cells with segmented nuclei were found (Fig. 3, c). The loose arrangement of cells was noted; in some areas, the tumor tissue had a cellular structure (Fig. 3, c). In the stroma of the tumor tissue in C₆₀-Cis-Pt nanocomplex group, the extensive foci of necrosis with the presence of a moderately pronounced perifocal inflammatory cell response were found (Fig. 3, d).

In separate tumor cells, mitosis was observed, which reflects their high proliferative activity (Fig. 4). In the Cis-Pt treated group, the microscopic examination of the tumors showed a decrease in number

Table 2. Hemogram of LLC-bearing mice treated with either Cis-Pt and C₆₀ fullerene separately or with C₆₀-Cis-Pt nanocomplex (8 mice per group, M ± m, n = 5)

Indices	Intact mice	Tumor-bearing untreated	Treatment		
			C ₆₀ fullerene	Cis-Pt	C ₆₀ -Cis-Pt nanocomplex
Hemoglobin, g/l	107 ± 11	95 ± 7	108 ± 10	75 ± 8 ^{§*}	100 ± 6 [‡]
Hematocrit, %	38 ± 4	30 ± 3 [§]	31 ± 8	25 ± 4 [§]	31 ± 8
Erythrocytes, 10 ¹² /l	9.23 ± 0.90	5.7 ± 0.48 [§]	7.5 ± 1.9*	4.6 ± 1.2 ^{§*}	6.8 ± 0.8**
Leukocytes, 10 ⁹ /l	7.0 ± 1.2	9.9 ± 0.6 [§]	8.9 ± 1.4 [§]	13.0 ± 2.1 ^{§*}	9.4 ± 1.3*
Lymphocytes, 10 ⁹ /l	4.0 ± 0.3	2.8 ± 0.1 [§]	3.8 ± 0.2*	1.8 ± 0.1 ^{§*}	3.0 ± 0.2*
Platelets, 10 ⁹ /l	400 ± 21	282 ± 21 [§]	360 ± 39*	320 ± 22 [§]	356 ± 33*

Note: [§]p < 0.05 as compared to intact mice; *p < 0.05 as compared to tumor-bearing mice treated with saline; [‡]p < 0.05 as compared to tumor-bearing mice treated with Cis-Pt.

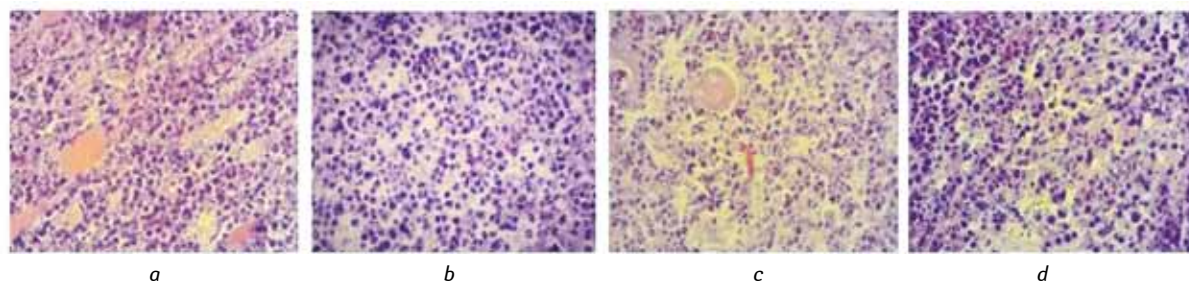


Fig. 3. HE staining of histological sections of tumor tissue (magnification $\times 400$): (a) untreated group, (b) Cis-Pt group, (c) C_{60} fullerene group, (d) C_{60} -Cis-Pt nanocomplex group

of mitotic cells by 65% and an increase in number of apoptotic cells by 50% (Fig. 4). In the C_{60} fullerene group, the mitotic index of tumor cells was the same as in control group, but the number of apoptotic cells was higher than in untreated LLC-bearing mice by 50% (Fig. 4). Compared to control, treatment with C_{60} -Cis-Pt nanocomplex was found to decrease the mitotic index of tumor cells by 78% and increase apoptotic index by 75% (Fig. 4).

The action of the C_{60} -Cis-Pt nanocomplex in tumor tissue reduced the number of mitotic cells, but increased the number of apoptotic tumor cells, which may be one of the mechanisms of its antitumor activity. It is believed that antitumor efficacy of C_{60} fullerene at complexation with Cis-Pt is not due to the direct killing of tumor cells but is the result of activation of the immune response [47]. Thus, C_{60} fullerene at complexation with Cis-Pt suppressed Cis-Pt-induced intracellular reactive oxygen and reactive nitrogen species production in human peripheral blood phagocytes [48]. Also, antineoplastic effect of C_{60} fullerene has been observed by direct toxic effect against human myeloid U937 cells, as well as through the modulation of the functions of the effector cells of antitumor immunity.

According to literary data, C_{60} fullerene shows the selectivity of accumulation in the body of animals. So, C_{60} -PEG and $C_{60}(\text{OH})_n$ accumulated more and retained for a longer time in tumor tissues than in normal ones [18–20, 49]. C_{60} -ser-PromoFluor-633 conjugate after intravenously injection to mouse with liver cancer was permeated through the altered vasculature of the tumor, evading the reticulo-endothelial system [50]. ^{125}I - $C_{60}(\text{OH})_x$ after intravenously injection accumulated

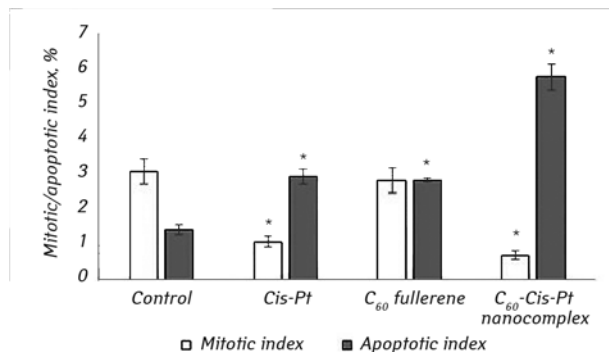


Fig. 4. Tumor mitotic and apoptotic indexes in LLC-bearing mice treated with either Cis-Pt and C_{60} fullerene separately or with C_{60} -Cis-Pt nanocomplex. * $p < 0.05$ as compared to untreated group

in the murine H22 hepatocarcinoma due to enhanced permeability and retention effects, and carbon nanoparticles were also taken up by the mononuclear phagocyte system [19].

Our results revealed C_{60} -Cis-Pt nanocomplex ability to prolong a survival of LLC-bearing mice and to prevent tumor- and Cis-Pt-induced hematotoxicity. These results need to be investigated further in a number of tumor models.

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