Exp Oncol 2019
41, 2, 106–111

Experimental ONCOLOGY

# ANTITUMOR EFFECTS AND HEMATOTOXICITY OF $C_{60}$-Cis-Pt NANOCOMPLEX IN MICE WITH LEWIS LUNG CARCINOMA

*S.V. Prylutska[1,] *, O.V. Lynchak[1], V.V. Kostjukov[2], M.P. Evstigneev[2], O.V. Remeniak[3], V.K. Rybalchenko[1], Yu.I. Prylutskyy[1], U. Ritter[4], P. Scharff[4]*

*[1] Joint Ukrainian-German Center on Nanobiotechnology, Taras Shevchenko National University of Kyiv, Kyiv 01601, Ukraine*

*[2]Belgorod State University, Belgorod 308015, Russian Federation*

*[3]National Pirogov Memorial Medical University, Vinnytsya 21018, Ukraine*

*[4] Joint Ukrainian-German Center on Nanobiotechnology, Technical University of Ilmenau, Ilmenau 98693, Germany*

***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 $C_{60}$ 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 $C_{60}$-Cis-Pt nanocomplex in Lewis lung carcinoma (LLC) and analyze hematological toxicity in tumor-bearing mice. *Materials and Methods*: Complexation of $C_{60}$ fullerene and Cis-Pt molecule was studied by computer simulation. $C_{60}$-Cis-Pt nanocomplex was i.p. injected to LLC-bearing mice in a total dose of 7.5 mg/kg ($C_{60}$: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 $C_{60}$-Cis-Pt nanocomplex in physiological medium and its stability due to the hydrophobic interactions. Treatment with $C_{60}$-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 $C_{60}$-Cis-Pt nanocomplex were more pronounced than the effects of Cis-Pt or $C_{60}$ fullerene alone in equivalent dose. *Conclusion*: Treatment with $C_{60}$-Cis-Pt nanocomplex prolonged the survival of LLC-bearing mice and reduced anemia in LLC-bearing mice.***

***Key Words*: cisplatin, $C_{60}$ fullerene, $C_{60}$-Cis-Pt nanocomplex, Lewis lung carcinoma, tumor-bearing mice survival, hematological indices.**

Cisplatin (Cis-[Pt(II)$(NH_3)_2Cl_2$, 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@$C_{82}(OH)_{22}]_n$ 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 fullerenol against HeLa and Lewis lung carcinoma (LLC) cells was demonstrated [9, 10].

$C_{60}$ fullerene, being a representative of carbon nanostructures, can be a promising agent for anticancer drug delivery. $C_{60}$ 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 $C_{60}$ fullerene nanoparticles in tumor tissue was demonstrated [18–20]. The unique structure of $C_{60}$ molecule makes it possible to modify its surface with chemotherapeutic agents [21–23]. It was demonstrated that $C_{60}$ fullerene conjugated with pa-

Submitted: March 12, 2019.
*Correspondence: E-mail: psvit@bigmir.net
*Abbreviations used*: $C_{60}$FAS – $C_{60}$ fullerene aqueous colloid solution; Cis-Pt – cisplatin; HE – hematoxylin and eosin; i.p. – intraperitoneal(ly); 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 $C_{60}$-Cis-Pt nanocomplex in LLC and analyze its hematotoxicity in tumor-bearing mice.

## MATERIALS AND METHODS

***$C_{60}$-Cis-Pt nanocomplex.*** A highly stable pristine $C_{60}$ fullerene aqueous colloid solution ($C_{60}$FAS; final concentration 0.15 mg/ml, purity > 99.5%) was obtained at Technical University of Ilmenau (Germany) as discribed in [27, 28]. In brief, this method is based on transferring $C_{60}$ fullerene from organic solution into the aqueous phase by ultrasonic treatment. The monitoring of the state of $C_{60}$ fullerene nanoparticles in aqueous solution using microscopic and spectroscopic measurements showed the presence of both single $C_{60}$ molecules and their nanoaggregates with the size in the range of 1.2–100 nm. The $C_{60}$FAS was stable for 6–8 months at room temperature [29]. To obtaine $C_{60}$-Cis-Pt nanocomplex $C_{60}$FAS (150 µg/ml) and Cis-Pt dissolved in 0.9% NaCl saline solution (150 µg/ml) were mixed in 1:1 volume ratio ($C_{60}$: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 $C_{60}$ fullerene and Cis-Pt in the obtained nanocomplex were 75 and 75 µg/ml, respectively.

***In silico experiment.*** Structures of $C_{60}$ fullerene and Cis-Pt molecule, as well as the 1:1 $C_{60}$-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 $C_{60}$-Cis-Pt structure was further used to populate the surface of $C_{60}$ 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 $C_{60}$ 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 ($5 \cdot 10^5$) 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); $C_{60}$ fullerene group ($C_{60}$FAS); Cis-Pt group (Cis-Pt in 0.9% NaCl saline solution); $C_{60}$-Cis-Pt nanocomplex group ($C_{60}$-Cis-Pt nanocomplex solution). The intact group (0.9% NaCl saline solution) consisted of healthy mice without transplanted tumor. $C_{60}$ fullerene (in a total dose of 3.75 mg/kg), Cis-Pt (in a total dose of 3.75 mg/kg), $C_{60}$-Cis-Pt nanocomplex (in a total dose of 7.5 mg/kg ($C_{60}$: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 $CO_2$ 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

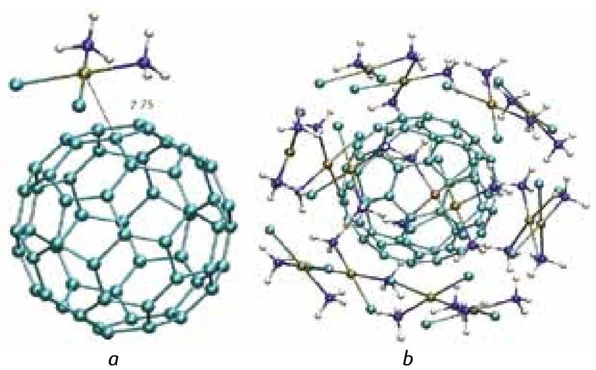
***Computer simulation***. Results of the structural modeling of $C_{60}$-Cis-Pt nanocomplex in the 1:1 ratio of $C_{60}$ fullerene and Cis-Pt molecule (Fig. 1, *a*) evidenced the stability of this structure in aqueous media. The minimal distance between $C_{60}$ fullerene and Cis-Pt molecule was found to be 2.75 Å indicating the importance of dispersive van der Waals attractive interactions between Pt atom and aromatic $C_{60}$ fullerene surface in stabilization of the nanocomplex.

The energy-minimized structure with the theoretically maximal filling of the $C_{60}$ 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 $C_{60}$ fullerene. The planes of cruciform Cis-Pt molecules were parallel to the planes tangent to the surface of $C_{60}$ fullerene. No preferential arrangement of Cis-Pt molecules over the five- or six-membered $C_{60}$ 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 $C_{60}$ fullerene. Numerical calculations showed that the total binding energy of Cis-Pt molecules with $C_{60}$ 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 $C_{60}$ fullerene.

The *in silico* results point out on interaction of $C_{60}$ fullerene and Cis-Pt molecules and stability of $C_{60}$-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 $C_{60}$ fullerene or its nanocluster resulting in formation of noncovalent $C_{60}$-Cis-Pt nanocomplex stabilized by hydrophobic interactions [23].

***The survival of LLC-bearing mice treated with Cis-Pt and $C_{60}$ fullerene separately or with $C_{60}$-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 $C_{60}$-Cis-Pt nanocomplex in a total dose of 7.5 mg/kg ($C_{60}$:Cis-Pt as 3.75:3.75 mg/kg).



*a* *b*

**Fig. 1.** The calculated structure of 1:1 (*a*) and 1:15 (*b*) $C_{60}$-Cis-Pt nanocomplexes

Recent studies have shown that metalofullerene $Gd@C_{82}(OH)_{22}$ under i.p. administration of 3.8 mg/kg (once a day for 7 days) did not cause pronounced side effects in mice with transplantated 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, $C_{60}$ fullerene, or $C_{60}$-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 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].

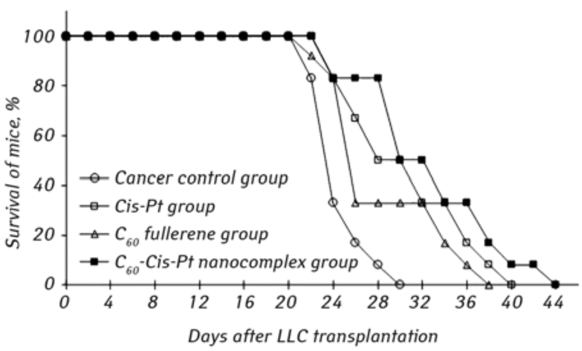



**Fig. 2**. Survival (%) of LLC-bearing mice treated with either Cis-Pt and $C_{60}$ fullerene separately or with $C_{60}$-Cis-Pt nanocomplex

**Table 1.** Average LS and coefficient of life prolongation (*cp*) of LLC-bearing mice treated with either Cis-Pt and $C_{60}$ fullerene separately or with $C_{60}$-Cis-Pt nanocomplex

| Experimental groups | *LS*, days | *cp*, % |
|---|---|---|
| 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 $C_{60}$ fullerene (Table 1). $C_{60}$-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 $C_{60}$ 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.

***Hematological toxicity in LLC-bearing mice treated with Cis-Pt, $C_{60}$ fullerene or with $C_{60}$-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 $C_{60}$ fullerene were confirmed *in vitro* and *in vivo* experiments [40–43]. We have previously shown that $C_{60}$ 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 $C_{60}$ fullerene the increased reactive oxygen species level induced by $H_2O_2$ 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 $C_{60}$ fullerene prevented Dox-dependent oxidative insults in LLC-bearing mice liver and heart [42]. In present study, $C_{60}$ fullerene reduced anemia in LLC-bearing mice (Table 2) with hematologic indices approaching those in intact animals. $C_{60}$-Cis-Pt nanocomplex reduced the hematotoxicity in tumor-bearing mice caused by tumor growth and drug treatment. These finding 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, $C_{60}$ fullerene or $C_{60}$-Cis-Pt nanocomplex.*** The inhibitory effect on tumor growth was evident in all treated groups. Treatment of LLC-bearing mice with Cis-Pt or $C_{60}$ 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 $C_{60}$-Cis-Pt nanocomplex was accompanied by 1.55-fold decrease of tumor weight with 35.5% IR (Table 3). Earlier, we have shown that antimetaststic effects of $C_{60}$-Cis-Pt nanocomplex exceed those of Cis-Pt (75% *vs* 57% of total metastases volume reduction) [46]. The data on antitumor effects of $C_{60}$-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 $C_{60}$ fullerene separately or with $C_{60}$-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.

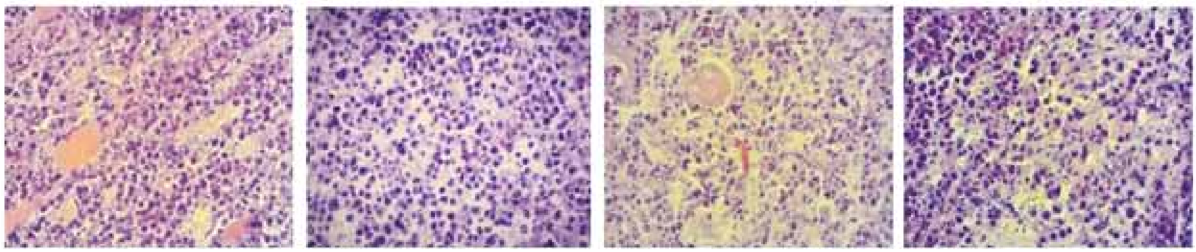
***Tumor morphology after treatment with Cis-Pt, $C_{60}$ fullerene or with $C_{60}$-Cis-Pt nanocomplex***. Histological examination of the tumor nodules in untreated mice group showed that tissue consisted of a cluster of highly polymorphic, atypical cancer cells (Fig. 3, *a*). A large number of cells 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 $C_{60}$ 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 $C_{60}$-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 $C_{60}$ fullerene separately or with $C_{60}$-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.



*a* *b* *c* *d*

**Fig. 3.** HE staining of histological sections of tumor tissue (magnification ×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 efector 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 $C_{60}(OH)_n$ 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]. $^{125}I$-$C_{60}(OH)_x$ after intravenously injection accumulated

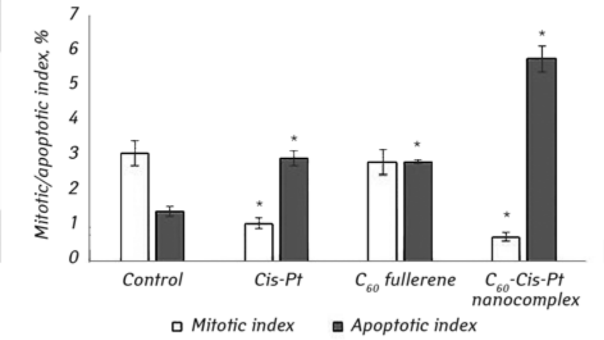



**Fig. 4.** Tumor mitotic and apoptotic indexes in LLC-bearing mice treated with either Cis-Pt and $C_{60}$ fullerene separately or with $C_{60}$-Cis-Pt nanocomplex. $^*p < 0.05$ as compared to untreated group

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.

## ACKNOWLEDGMENTS

Dr. S.V. Prylutska is grateful to Dr. G.V. Didenko (R.E. Kavetsky IEPOR, NAS of Ukraine) for supporting *in vivo* experiments. This study was partially supported by the STCU project N 6256. State support to leading research groups (5889.2018.3) is acknowledged (MPE).

## REFERENCES

1. **Florea AM, Büsselberg D.** Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers (Basel) 2011; **3**: 1351–71.

2. **Maeda H.** The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 2001; **41**: 189–207.

3. **Greish K.** Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. Methods Mol Biol 2010; **624**: 25–37.

4. **Duan X, He C, Kron SJ, Lin W.** Nanoparticle formulations of cisplatin for cancer therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2016; **8**: 776–91.

5. **Wagstaff AJ, Brown SD, Holden MR, *et al.*** Cisplatin drug delivery using gold-coated iron oxide nanoparticles for enhanced tumour targeting with external magnetic fields. Inorg Chim Acta 2012; **393**: 328–33.

6. **Min Y, Mao C-Q, Chen S, *et al.*** Combating the drug resistance of cisplatin using a platinum prodrug based delivery system. Angew Chem Int Edit 2012; **51**: 6742–47.

7. **Mejri A, Vardanega D, Tangour B, *et al.*** Encapsulation into carbon nanotubes and release of anticancer cisplatin drug molecule. J Phys Chem B 2015; **119**: 604–11.

8. **Liang X-J, Meng H, Wang Y, *et al.*** Metallofullerene nanoparticles circumvent tumor resistance to cisplatin by reactivating endocytosis. Proc Natl Acad Sci USA 2010; **107**: 7449–54.

9. **Chaudhuri P, Paraskar A, Soni S, *et al.*** Fullerenol cytotoxic conjugates for cancer chemotherapy. ACS Nano 2009; **3**: 2505–14.

10. **Niu Y, Yan C.** The effect of fullerenol combined with cisplatin on the proliferation of cervical cancer Hela cells. J Cancer Ther 2016; **7**: 232–8.

11. **Foley S, Crowley C, Smaihi M, *et al.*** Cellular localisation of a water-soluble fullerene derivative. Biochem Biophys Res Commun 2002; **294**: 116–9.

**12. Prylutska S, Grynyuk I, Grebinyk S, *et al.*** Comparative study of biological action of fullerenes $C_{60}$ and carbon nanotubes in thymus cells. Mat-wiss u Werkstofftech 2009; **40**: 238–41.

**13. Johnston HJ, Hutchison GR, Christensen FM, *et al.*** The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. Toxicol Sci 2010; **114**: 162–82.

**14. Russ KA, Elvati P, Parsonage TL, *et al.*** $C_{60}$ fullerene localization and membrane interactions in RAW 264.7 immortalized mouse macrophages. Nanoscale 2016; **8**: 4134–44.

**15. Larner SF, Wang J, Goodman J, *et al.*** *In vitro* neurotoxicity resulting from exposure of cultured neural cells to several types of nanoparticles. J Cell Death 2017; **10**: 1–7.

**16. Franskevych D, Palyvoda K, Petukhov D, *et al.*** Fullerene $C_{60}$ penetration into leukemic cells and its photoinduced cytotoxic effects. Nanoscale Res Lett 2017; **12**: 40.

**17. Gonchar OO, Maznychenko AV, Bulgakova NV, *et al.*** $C_{60}$ fullerene prevents restraint stress-induced oxidative disorders in rat tissues: possible involvement of the Nrf2/ARE-antioxidant pathway. Oxidative Med Cell Longevity 2018; **2018**: 2518676.

**18. Tabata Y, Murakami Y, Ikada Y.** Photodynamic effect of polyethylene glycolmodified fullerene on tumor. Jpn J Cancer Res 1997; **88**: 108–16.

**19. Ji ZQ, Sun H, Wang H, *et al.*** Biodistribution and tumor uptake of $C_{60}(OH)_x$ in mice. J Nanopart Res 2006; **8**: 53–63.

**20. Zhu J, Ji Z, Wang J, *et al.*** Tumor-inhibitory effect and immunomodulatory activity of fullerol $C_{60}(OH)_x$. Small 2008; **4**: 1168–75.

**21. Skamrova GB, Laponogov IV, Buchelnikov AS, *et al.*** Interceptor effect of $C_{60}$ fullerene on the *in vitro* action of aromatic drug molecules. Eur Biophys J 2014; **43**: 265–76.

**22. Prylutskyy YuI, Evstigneev MP, Cherepanov VV, *et al.*** Structural organization of $C_{60}$ fullerene, doxorubicin and their complex in physiological solution as promising antitumor agents. J Nanopart Res 2015; **17**: 45.

**23. Prylutskyy YuI, Cherepanov VV, Evstigneev MP, *et al.*** Structural self-organization of $C_{60}$ and cisplatin in physiological solution. Phys Chem Chem Phys 2015; **17**: 26084–92.

**24. Zakharian TY, Seryshev A, Sitharaman B, *et al.*** A fullerene-paclitaxel chemotherapeutic: synthesis, characterization, and study of biological activity in tissue culture. J Am Chem Soc 2005; **127**: 12508–9.

**25. Lu F, Haque SA, Yang ST, *et al.*** Aqueous compatible fullerene-doxorubicin conjugates. J Phys Chem C 2009; **113**: 17768–73.

**26. Prylutska SV, Skivka LM, Didenko GV, *et al.*** Complex of $C_{60}$ fullerene with doxorubicin as a promising agent in antitumor therapy. Nanoscale Res Lett 2015; **10**: 499.

**27. Scharff P, Carta-Abelmann L, Siegmund C, *et al.*** Effect of X-ray and UV irradiation of the $C_{60}$ fullerene aqueous solution on biological samples. Carbon 2004; **42**: 1199–1201.

**28. Turov VV, Chekhun VF, Krupskaya TV, *et al.*** Effect of small addition of $C_{60}$ fullerenes on the hydrated properties of nanocomposites based on highly dispersed silica and DNA. Chem Phys Lett 2010; **496**: 152–6.

**29. Grynyuk I, Prylutska S, Slobodyanik N, *et al.*** The aggregate state of $C_{60}$ fullerene in various media. Biotechnol Acta 2013; **6**: 71–6.

**30. Prylutska S, Politenkova S, Afanasieva K, *et al.*** A nanocomplex of $C_{60}$ fullerene with cisplatin: design, characterization and toxicity. Beilstein J Nanotechnol 2017; **8**: 1494–1501.

**31. Kondo A, Maeta M, Oka A, *et al.*** Hypotonic intraperitoneal cisplatin chemotherapy for peritoneal carcinomatosis in mice. British J Cancer 1996; **73**: 1166–70.

**32. Meng H, Xing GM, Sun BY, *et al.*** Potent angiogenesis inhibition by the particulate form of fullerene derivatives. ACS Nano 2010; **4**: 2773–83.

**33. Chaklader M, Das P, Pereira JA, *et al.*** 17-AAG mediated targeting of HSP90 limits tert activity in peritoneal sarcoma related malignant ascites by downregulating cyclin D1 during cell cycle entry. Exp Oncol 2012; **34**: 90–6.

**34. Longchar A, Prasad SB.** Ascorbic acid (vitamin C) ameliorates cisplatin-induced henatotoxicity in tumor-bearing mice. World J Pharmacy Pharmaceutical Sci 2016; **5**: 1870–91.

**35. Fedorchuk OG, Pyaskovskaya OM, Skivka LM, *et al.*** Paraneoplastic syndrome in mice bearing high-angiogenic variant of Lewis lung carcinoma: relations with tumor derived VEGF. Cytokine 2012; **57**: 81–8.

**36. Malarczyk E, Kandefer-Szerszeń M, Jarosz-Wilkołazka A.** Influence of very low doses of cisplatin on tumor cell proliferation *in vitro* and some hematological and enzymatic parameters of healthy rats. Nonlinearity Biol Toxicol Med 2003; **1**: 123–37.

**37. Ohno S, Strebel FR, Stephens LC, *et al.*** Haematological toxicity of carboplatin and cisplatin combined with whole body hyperthermia in rats. Br J Cancer 1993; **68**: 469–74.

**38. Wood PA, Hrushesky WJ.** Cisplatin-associated anemia: an erythropoietin deficiency syndrome. J Clinical Invest 1995; **95**: 1650–9.

**39. Markovic SD, Zizk JB, Djavic DS, *et al.*** Alteration of oxidative stress parameters in red blood cells of rats after chronic *in vivo* treatment with cisplatin and selenium. Arch Biol Sci Belgrade 2011; **63**: 991–9.

**40. Gharbi N, Pressac M, Hadchouel M, *et al.*** $C_{60}$ fullerene is a powerful antioxidant *in vivo* with no acute or subacute toxicity. Nano Lett 2005; **5**: 2578–85.

**41. Grynyuk I, Grebinyk S, Prylutska S, *et al.*** Photoexcited fullerene $C_{60}$ disturbs prooxidant-antioxidant balance in leukemic L1210 cells. Mat-wiss u Werkstofftech 2013; **44**: 139–43.

**42. Prylutska S, Grynyuk I, Matyshevska O, *et al.*** $C_{60}$ Fullerene as synergistic agent in tumor-inhibitory doxorubicin treatment. Drugs R D 2014; **14**: 333–40.

**43. Franskevych DV, Grynyuk II, Prylutska SV, Matyshevska OP.** Modulation of cisplatin-induced reactive oxygen species production by fullerene $C_{60}$ in normal and transformed lymphoid cells. Ukr Biochem J 2016; **88**: 44–50.

**44. Vereshchaka IV, Bulgakova NV, Maznychenko AV, *et al.*** $C_{60}$ fullerenes diminish the muscle fatigue in rats comparable to N-acetylcysteine or β-alanine. Front Physiol 2018; **9**: 517.

**45. Mathew DG, Rooban T, Janani V, *et al.*** Review of paraneoplastic syndromes associated with oropharyngeal squamous cell carcinoma. J Oral Maxillofac Pathol 2010; **14**: 41–7.

**46. Prylutska S, Panchuk R, Gołuński G, *et al.*** $C_{60}$ fullerene enhances cisplatin anticancer activity and overcomes tumor cells drug resistance. Nano Res 2017; **10**: 652–71.

**47. Didenko G, Prylutska S, Kichmarenko Y, *et al.*** Evaluation of the antitumor immune response to $C_{60}$ fullerene. Mat-wiss u Werkstofftech 2013; **44**: 124–8.

**48. Skivka LM, Prylutska SV, Rudyk MP, *et al.*** $C_{60}$ fullerene and its nanocomplexes with anticancer drugs modulate circulating phagocyte functions and dramatically increase ROS generation in transformed monocytes. Cancer Nanotechnol 2018; **9**: 8–31.

**49. Asada R, Liao F, Saitoh Y, Miwa N.** Photodynamic anticancer effects of fullerene [C60]-PEG complex on fibrosarcomas preferentially over normal fibroblasts in terms of fullerene uptake and cytotoxicity. Mol Cell Biochem 2014; **390**: 175–84.

**50. Raoof M, Mackeyev Y, Cheney MA, *et al.*** The internalization of $C_{60}$ fullerenes into cancer cells with accumulation in the nucleus through the nuclear pore complex. Biomater 2012; **33**: 2952–60.