EXPERIMENTAL ARTICLES

UDC 615.277.3+615.012]-022.532-092.9 https://doi.org/10.15407/biotech13.03.045

PREVENTION OF CISPLATIN TOXICITY AGAINST NORMAL CELLS BY COMPLEXATION WITH C_{60} FULLERENE

S. V. Prylutska[1]
I. I. Grynyuk[1]
T. D. Skaterna[2]
L. B. Drobot[2]
N. S. Slobodyanik[1]
O. P. Matyshevska[2]

[1]Taras Shevchenko National University of Kyiv, Ukraine

[2]Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv

E-mail: psvit_1977@ukr.net

Received 23.02.2020
Revised 16.05.2020
Accepted 30.06.2020

The aim of this study was to evaluate the toxicity of noncovalent nanocomplex of C_{60} fullerene with cisplatin (C_{60}-Cis-Pt) against normal cells. The toxicity of the C_{60}-Cis-Pt nanocomplex compared to the free Cis-Pt was studied by estimating kidney human embryonic (HEK293) cells viability using MTT assay and rat erythrocytes resistance to acid haemolysis. It was shown that free 40 μM Cis-Pt changed the morphology and reduced the viability of HEK293 cells, as well as increased the number of haemolyzed erythrocytes compared to the control. According to the investigated parameters analysis no cytotoxic effects of C_{60}-Cis-Pt nanocomplex was observed at Cis-Pt equivalent concentration. The prevention of Cis-Pt toxic action against normal cells by its complexation with C_{60} fullerene opens the prospect of nanostructure usage as an effective cytoprotector and a target carrier in tumor cells.

Key words: C_{60} fullerene, cisplatin, nanocomplex, HEK293 cells, cytotoxicity, erythrocytes, haemolysis.

The use of biologically active nanomaterials for targeted drug delivery, enhancement of the traditional anticancer drugs therapeutic efficacy and prevention of its side effects is a significant and complex problem of modern biotechnology. The representative of carbon nanostructures C_{60} fullerene is promising in this direction. It is a chemically stable, nanosized (0.72 nm), almost spherical and hydrophobic molecule that penetrates through biological membranes, localizes within cells [1–3]. As is known, chemical modification affects the physical, chemical and biological properties of C_{60} fullerene. Pristine C_{60} fullerene and its water-soluble derivatives do not cause toxic effects [1, 4, 5]. The accumulation of C_{60} fullerene in tumors of the liver, stomach, intestine, lungs, bones and its selective damaging effect on malignantly transformed cells was detected [6–8]. The surface structure of C_{60} molecule with a system of double π-conjugated electron-deficient bonds is unique and determines the properties of this nanostructure as an antioxidant (free radical scavenger) [9, 10] as well as its ability to generate reactive oxygen species (ROS) after UV-Vis light irradiation [11–14] that can be used in photodynamic therapy of tumors. Besides, C_{60} fullerene can form stable complexes with chemotherapeutic drugs [15–17], that can be used to optimize their action.

The traditional broad-acting anticancer drug is cisplatin (cis-diaminodichloroplatinum, cis-[Pt(II)$(NH_3)_2Cl_2$], Cis-Pt), the cytotoxic effect of which is caused by DNA damage and oxidative stress induction [18–22]. Despite the negative side effects of Cis-Pt, it is widely used in antitumor therapy. Clinical usage of

Cis-Pt might be increased with improving its selectivity, overcoming drug resistance and reducing toxicity.

To increase the effectiveness of Cis-Pt antitumor effect and minimize its side effects, a noncovalent nanocomplex of C_{60} fullerene with Cis-Pt (C_{60}-Cis-Pt) was created [23]. Estimation of C_{60}-Cis-Pt nanocomplex toxicity is an important prerequisite of its usage for biomedical purposes. Thus, the aim of this work was to estimate the toxic effect of free Cis-Pt against normal cells in comparison with C_{60}-Cis-Pt nanocomplex.

Materials and Methods

Creation of C_{60}-Cis-Pt nanocomplex. C_{60} fullerene aqueous colloid solution (C_{60}FAS) (150 μg/ml, $2–10^{-4}$ M, purity 99.95%) was prepared at the Technical University of Ilmenau (Germany) as described in [24, 25]. C_{60} FAS is characterized by a high C_{60} fullerene concentration and stable up to 12 months at +4 °C.

To generate C_{60}-Cis-Pt nanocomplexes, the C_{60} solution (150 μg/ml) and Cis-Pt (Sigma, USA) solution in 0.9% NaCl saline (150 μg/ml) were mixed in 1:1 volume ratio. The mixture was sonicated with ultrasound (22 kHz, 20 min) and stirred (400 rpm, 18 h). Final concentrations of C_{60} fullerene and Cis-Pt were 75 μg/ml (104 μM) and 75 μg/ml (250 μM), respectively. The stability of C_{60}-Cis-Pt nanocomplexes in the aqueous medium was confirmed by the results of the dynamic light scattering technique [26]. The calculated dissociation constant for the obtained noncovalent C_{60}-Cis-Pt nanocomplex is ~ 2 mM [27].

Cell culture. Non-tumor HEK293 (human embryonic kidney 293) cells were kindly supplied by the Bank of Cell Cultures and Transplantable Experimental Tumors of Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine (Kyiv, Ukraine). Cells were maintained in DMEM (Sigma-Aldrich Co, Ltd, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich Co, Ltd, USA), 50 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Cells were incubated for 24 h with or without free Cis-Pt or C_{60}-Cis-Pt nanocomplex in Cis-Pt equivalent concentration.

Cell viability was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma-Aldrich Co, Ltd, USA) reduction assay [28]. At indicated time points of incubation 100 μl aliquots ($10×10^3$ cells) were placed into the 96-well microplates Greiner (Sigma-Aldrich Co, Ltd, USA), 10 μl of MTT solution (4 mg/ml in phosphate-buffered saline (PBS)) was added to each well and the plates were incubated for another 2 h at 37 °C. Precipitates were dissolved with 100 μl of dimethyl sulfoxide (DMSO) (Sigma-Aldrich Co, Ltd, USA). Diformazan formation was determined by measuring absorption at 570 nm with a microplate reader μQuant (BioTEK, USA).

Curve fitting and calculation of the half-maximal inhibitory concentration (IC_{50} value) were done using GraphPad Prism 7 (GraphPad Software Inc., USA). Briefly, individual concentration-effect curves were generated by fitting the logarithm of the tested compound concentration versus corresponding normalized percent of cell viability values using nonlinear regression.

Cells morphology was investigated using phase-contrast microscopy (Olympus CKX41SF, Japan). For light microscopy images an Olympus SP-500UZ (Indonesia) camera was used.

Erythrocytes haemolysis. Erythrocytes isolated from the heparinized rat blood, were incubated at 37 °C with or without C_{60}-Cis-Pt nanocomplex. Erythrocytes haemolysis was induced by addition of hydrochloric acid to the final concentration of 0.001 N [29]. Measurements of the haemolysis dynamics were carried out for 2 min with a 10 s interval on the spectrophotometer (Scinco, Germany) at $\lambda = 630$ nm.

All experiments with animals in this study were performed according to the Bio-Ethics Committee of the abovementioned institution.

Statistical analysis was performed using two-way ANOVA followed by post Bonferroni tests. The IC_{50} value was represented as M ± SD of more than four independent experiments. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Viability and morphology of HEK293 cells. Cytotoxic activity of C_{60}-Cis-Pt nanocomplex against HEK293 cells in Cis-Pt equivalent concentrations of 5–40 μM in comparison with the free drug was studied by MTT test at 24 h of incubation. The viability of cells incubated without additions of C_{60} fullerene, Cis-Pt, or C_{60}-Cis-Pt nanocomplex was taken as 100% (control).

No effect of C_{60} fullerene used alone in the range of 2.8–16.6 μM concentrations,

equivalent to those in C_{60}-Cis-Pt nanocomplex, on HEK293 cells viability during the incubation period was detected (data are not presented). The calculated IC_{50} values for C_{60} fullerene (IC_{50} = 530 μM) (Table 1) action on HEK293 cells showed that it is a low toxic compound. These results are in a good agreement with the literature data. Thus, the toxic effect of pristine C_{60} fullerene against normal baby hamster kidney BHK-21 cells were observed only at high 440 μM concentration [30].

We have detected the cytotoxic effect of Cis-Pt against HEK293 cells at 40 μM concentration. Under the action of the drug at this concentration cell viability at 24 h was reduced by 28% compared to the control (Fig. 1). The decrease of HEK293 cells viability by 43% compared to the control under the action of 50 μM Cis-Pt was also demonstrated in [31]. The calculated IC_{50} value for Cis-Pt was shown to be 75 μM (Table 1).

Table 1. **IC_{50} values for C_{60} fullerene, Cis-Pt and C_{60}-Cis-Pt nanocomplex in HEK293 cells ($M \pm m$, $n = 6$)**

Compounds	IC_{50} (μM), 24 h
C_{60} fullerene	530 ± 43
Cis-Pt	75 ± 5.6
C_{60}-Cis-Pt nanocomplex	90 ± 6.8*

Note: * — $P < 0.05$ in comparison with Cis-Pt.

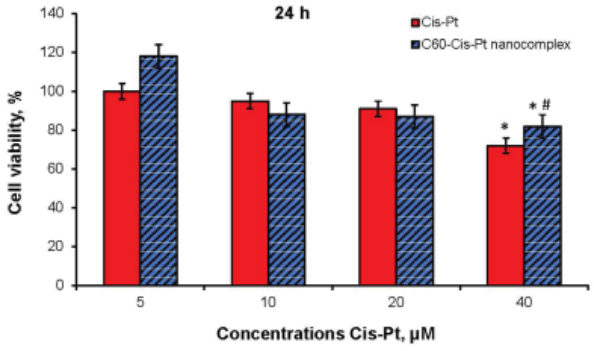

Fig. 1. **Viability of HEK294 cells treated with free Cis-Pt or C_{60}-Cis-Pt nanocomplex in Cis-Pt equivalent concentrations at 24 h of incubation ($M \pm m$, $n = 6$):**
* $P < 0.05$ in comparison with control (untreated cells); # $P < 0.05$ in comparison with Cis-Pt

With the action of 5 μM C_{60}-Cis-Pt nanocomplex the viability of HEK293 cells was increased by 20% compared to the control (Fig. 1), probably due to the initial adaptive response to the compound. C_{60}-Cis-Pt nanocomplex at 40 μM Cis-Pt equivalent concentration inhibited HEK293 viability, but the toxic effect appeared to be only 18% as compared to control (Fig. 1). The calculated value of IC_{50} for C_{60}-Cis-Pt nanocomplex was higher (90 μM) than that for free Cis-Pt (Table 1) confirming the decreased cytotoxicity of Cis-Pt against non-tumor cells at complexation with C_{60} fullerene.

Morphological studies showed that untreated (control) HEK293 cells formed elongated epithelioid structures and dense monolayer in some areas, a large number of intercellular contacts were observed. The cytotoxic effect of 40 μM Cis-Pt on HEK293 cells was evidenced by morphological changes of cells (Fig. 2, Table 2).

Most of HEK293 cells treated with 40 μM Cis-Pt were characterized by atypical morphology and smaller size (Table 2). No evident effect of C_{60}-Cis-Pt nanocomplex in equivalent 40 μM Cis-Pt concentration on cells morphology was detected.

Therefore, the toxic effect of Cis-Pt on normal cells at complexation with C_{60} fullerene was reduced. The protective effects of C_{60} fullerene against the toxic effects of Cis-Pt may be due to the antioxidant properties of the carbon nanostructure [32, 33, 34]. We have previously shown that C_{60} fullerene prevented ROS production in thymocytes and prevented the decrease of thymocytes viability induced by Cis-Pt [35, 36].

Erythrocytes haemolysis. The use of platinum-based drugs in chemotherapy is limited due to its high haematotoxicity, that is the cause of haemolytic anemia and bone marrow disease [37]. So the search for erythrocyte protection pathways against drug damage is of current interest.

The effect of Cis-Pt and C_{60}-Cis-Pt nanocomplex at the level of cells plasma membrane was estimated by the dynamics of erythrocytes haemolysis, which reflects the dynamics of erythrocyte plasma membrane destruction and the release of haemoglobin into the environment. Erythrocytes haemolysis of the control (untreated red blood cells) was accelerated at 40 s after the treatment with haemolytic and reached the maximum at 60 s, the number of haemolyzed cells was 30 ± 2% (Fig. 3).

Haemolysis of erythrocytes treated with 40 μM Cis-Pt slightly slowed, however, the

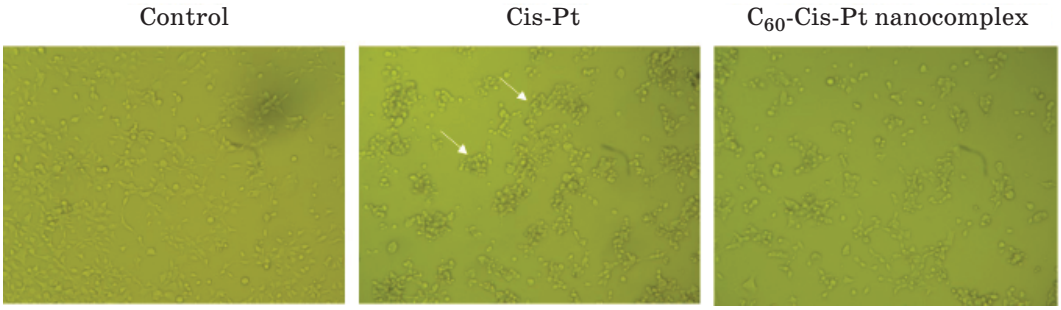

Fig. 2. **Microphotographs of HEK293 cells incubated for 24 h in the presence of free 40 μM Cis-Pt or C_{60}-Cis-Pt nanocomplex** (phase-contrast microscopy, × 400)

Table 2. **Morphological features in HEK293 cells at 24 h after action 40 μM Cis-Pt or C_{60}-Cis-Pt nanocomplex** ($M \pm m$, $n = 6$)

Compounds	Changes		
	Atypical cell morphology	Smaller cell size	Roud shape and nonadherent pattern
Cis-Pt	++++	++	++++
C_{60}-Cis-Pt nanocomplex	+	–	+

Note: + few, ++ moderate, +++ severe, ++++ many.

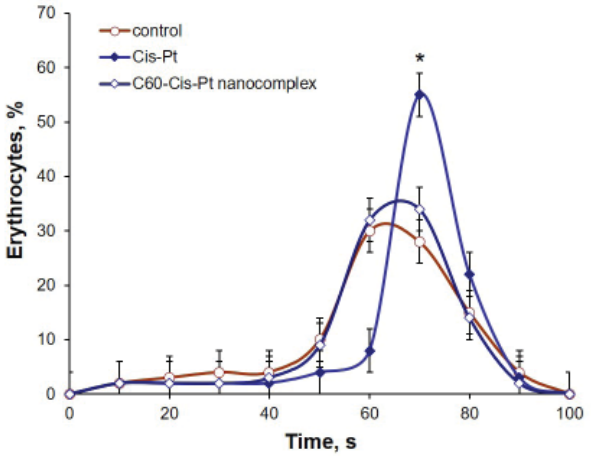

Fig. 3. **Erythrocytes haemolysis after action 40 μM Cis-Pt or C_{60}-Cis-Pt nanocomplex** ($M \pm m$, $n = 6$): * $P < 0.05$ in comparison with control (untreated cells)

number of haemolyzed cells reached 55 ± 2% (Fig. 3), indicating on Cis-Pt-induced decrease of cells resistance to haemolysis. As it was shown in [38], Cis-Pt at 35 μM concentration caused morphological changes of human erythrocyte membrane by embedding into the inner layer of cells. The extranuclear mechanism of Cis-Pt cytotoxic action is associated with its high affinity to phosphatidylserine the inner monolayer of the erythrocyte plasma membrane [39].

C_{60} fullerene at 16.6 μM concentration equivalent to that in C_{60}-Cis-Pt nanocomplex did not change parameters of erythrogram, indicating on the absence of haemolytic effect of carbon nanostructure (data are not presented). As it was shown by us previously [40, 41], the slight haemolytic activity of C_{60} fullerene was detected at concentrations > 50 μM.

No haemolytic effect was detected when erythrocytes were treated with C_{60}-Cis-Pt nanocomplex at 40 μM Cis-Pt equivalent concentration. The parameters of erythrogramm were the same as in control (Fig. 3) indicating that C_{60} fullerene weakened Cis-Pt interaction with erythrocyte membrane and was able to increase the resistance of erythrocyte membrane to Cis-Pt induced haemolysis.

The data obtained showed that the cytotoxic effect of Cis-Pt against normal cells was prevented at complexation with C_{60} fullerene and that C_{60}-Cis-Pt nanocomplex has a potential to be used for minimization of anticancer drug side effects.

This work was partially supported by the budget themes of Taras Shevchenko National University of Kyiv (State Registration Numbers 0119U100316 and 0119U100331).

The authors declare that they have no conflicts of interest.

REFERENCES

1. *Prylutska S. V., Grebinyk A. G., Lynchak O. V., Byelinska I. V., Cherepanov V. V., Tauscher E., Matyshevska O. P., Prylutskyy Yu. I., Rybalchenko V. K., Ritter U., Frohme M. In vitro* and *in vivo* toxicity of pristine C_{60} fullerene aqueous colloid solution. *Fullerenes, Nanotubes and Carbon Nanostructures.* 2019, 27 (9), 715–728. https://doi.org/10.1080/1536383X.2019.1634055
2. *Franskevych D., Palyvoda K., Petukhov D., Prylutska S., Grynyuk I., Schuetze C., Drobot L., Matyshevska O., Ritter U.* Fullerene C_{60} penetration into leukemic cells and its photoinduced cytotoxic effects. *Nanoscale Research Letters.* 2017, V. 12, P. 40–49. https://doi.org/10.1186/s11671-016-1819-5
3. *Grebinyk A., Grebinyk S., Prylutska S., Ritter U., Matyshevska O., Dandekar T., Frohme M.* C_{60} fullerene accumulation in human leukemic cells and perspectives of LED-mediated photodynamic therapy. *Free Radical Biology and Medicine.* 2018, V. 124, P. 319–327. https://doi.org/10.1016/j.freeradbiomed.2018.06.022
4. *Kolosnjaj J., Szwarc H., Moussa F.* Toxicity studies of fullerenes and derivatives. *Adv. Exp. Med. Biol.* 2007, V. 620, P. 168–180. https://doi.org/10.1007/978-0-387-76713-0_13
5. *Prylutska S. V., Matyshevska O. P., Golub A. A., Prylutskyy Yu. I., Potebnya G. P., Ritter U., Scharff P.* Study of C_{60} fullerenes and C_{60}-containing composites cytotoxicity in vitro. *Mater. Sci. Engineer. C.* 2007, V. 27, P. 1121–1124. doi: 10.1016/j.msec.2006.07.009
6. *Tabata Y., Murakami Y., Ikada Y.* Photodynamic effect of polyethylene glycolmodified fullerene on tumor. *Jpn. J. Cancer Res.* 1997, 88 (11), 1108–1116. https://doi.org/10.1111/j.1349-7006.1997.tb00336.x
7. *Ji Z. Q., Sun H., Wang H., Xie Q., Liu Y., Wang Z.* Biodistribution and tumor uptake of $C_{60}(OH)_X$ in mice. *J. Nanopart. Res.* 2006, V. 8, P. 53–63. http://dx.doi.org/10.1007/s11051-005-9001-5
8. *Zhu J., Ji Zh., Wang J., Sun R., Zhang X., Gao Y., Sun H., Liu Y., Wang Zh., Li A., Ma J., Wang T., Jia G., Gu Y.* Tumor-inhibitory effect and immunomodulatory activity of fullerol $C_{60}(OH)_x$. *Small.* 2008, 4 (8), 1168–1175. https://doi.org/10.1002/smll.200701219
9. *Yin J.-J., Lao F., Fu P. P., Wamer W. G., Zhao Y., Wang P.C., Qiu Y., Sun B., Xing G., Dong J., Liang X.-J., Chen C.* The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials. *Biomaterials.* 2009, 30 (4), 611–621. https://doi.org/10.1016/j.biomaterials.2008.09.061
10. *Saitoh Y., Ohta H., Hyodo S.* Protective effects of polyvinylpyrrolidone-wrapped fullerene against intermittent ultraviolet-A irradiation-induced cell injury in HaCaT cells. *J. Photochem. Photobiol. B.* 2016, V. 163, P. 22–29. https://doi.org/10.1016/j.jphotobiol.2016.08.001
11. *Yamakoshi Y., Umezava N., Ryu A., Arakane K., Miyata N., Goda Y., Masumizu T., Nagano T.* Active oxygen species generated from photoexited fullerene (C_{60}) as potential medicines: $O_2^{\bullet-}$ versus $^1O2$. *J. Chem. Soc.* 2003, V. 125, P. 12803–12809. https://doi.org/10.1021/ja0355574
12. *Huang Y.-Y., Sharma S. K., Yin R., Agrawal T., Chiang L. Y., Hamblin M. R.* Functionalized fullerenes in photodynamic therapy. *J. Biomed. Nanotechnol.* 2014, 10 (9), 1918–1936. https://doi.org/10.1166/jbn.2014.1963
13. *Moor K. J., Snow S. D., Kim J. H.* Differential photoactivity of aqueous [C_{60}] and [C_{70}] fullerene aggregates. *Environ. Sci Technol.* 2015, V. 49, P. 5990–5998. https://doi.org/10.1021/acs.est.5b00100
14. *Mroz P., Pawlak A., Satti M., Lee H., Wharton T., Gali H. Sarna T., Hamblin M. R.* Functionalized fullerenes mediate photodynamic killing of cancer cells: Type I versus Type II photochemical mechanism. *Free Radic. Biol. Med.* 2007, V. 43, P. 711–719. https://doi.org/10.1016/j.freeradbiomed.2007.05.005
15. *Zakharian T. Y., Seryshev A., Sitharaman B., Gilbert B. E., Knight V., Wilson L. J. A* Fullerene-paclitaxel chemotherapeutic: Synthesis, characterization, and study of biological activity in tissue culture. *J. Am. Chem. Soc.* 2005, V. 127, P. 12508–12509. http://dx.doi.org/10.1021/ja0546525
16. *Chaudhuri P., Paraskar A., Soni S., Mashelkar R. A., Sengupta S.* Fullerenol cytotoxic conjugates for cancer chemotherapy. *ASC Nano.* 2009, 3 (9), 2505–2514. http://dx.doi.org/10.1021/nn900318y
17. *Lu F., Haque S. A., Yang S. T., Luo P. G., Gu L., Kitaygorodskiy A., Li H., Lacher S., Sun Y.-P.* Aqueous compatible fullerene-doxorubicin conjugates. *J. Phys. Chem. C.* 2009, 113 (41), 17768–17773. http://dx.doi.org/10.1021/jp906750z
18. *Berndtsson M., Heagg M., Panaretakis T., Havelka A. M., Shoshan M. C., Linder S.* Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. *Int. J. Cancer.* 2007, 120 (1), 175–180. https://doi.org/10.1002/ijc.22132
19. *Cepeda V., Fuertes M. A., Castilla J., Alonso C., Quevedo C., Pérez J. M.* Biochemical Mechanisms of Cisplatin Cytotoxicity. *Anticancer. Agents Med. Chem.* 2007, 7 (1), 3–18. http://dx.doi.org/10.2174/187152007779314044

20. *Pratibha R., Sameer R., Rataboli P. V., Bhiwgade D. A., Dhume C. Y.* Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. *Eur. J. Pharmacol.* 2006, V. 532, P. 290–293. https://doi.org/10.1016/j.ejphar.2006.01.007
21. *Florea A.-M., Buesselberg D.* Cisplatin as an Anti-Tumor Drug: Cellular Mechanisms of Activity, Drug Resistance and Induced Side Effects. *Cancers (Basel)*. 2011, 3 (1), 1351–1371. https://doi.org/10.3390/cancers3011351
22. *Galluzzi L., Vitale I., Michels J., Brenner C., Szabadkai G., Harel-Bellan A., Castedo M., Kroemer G.* Systems biology of cisplatin resistance: past, present and future. *Cell Death Dis.* 2014, 5 (5), 1–18. https://doi.org/10.1038/cddis.2013.428
23. *Prylutska S. V., Lynchak O. V., Kostjukov V. V., Evstigneev M. P., Remeniak O. V., Rybalchenko V. K., Prylutskyy Yu. I., Ritter U., Scharff P.* Antitumor effects and hematotoxicity of C_{60}-Cis-Pt nanocomplex in mice with Lewis lung carcinoma. *Exp. Oncol.* 2019, 41 (2), 106–111.
24. *Golub O., Matyshevska S., Prylutska V., Sysoyev L., Ped V., Kudrenko E., Radchenko Yu., Prylutskyy P., Scharff T.* Braun. Fullerenes immobilized at silica surface: topology, structure and bioactivity. *J. Mol. Liq.* 2003, 105 (2–3), 141–147. http://dx.doi.org/10.1016/S0167-7322(03)00044-8
25. *Schuetze C., Ritter U., Scharff P., Bychko A., Prylutska S., Rybalchenko V., Prylutskyy Yu.* Interaction of N-fluorescein-5-isothiocyanate pyrrolidine-C_{60} compound with a model bimolecular lipid membrane. *Mater. Sci. Engineer. C.* 2011, 31 (5), 1148–1150. https://doi.org/10.1016/j.msec.2011.02.026
26. *Prylutska S. V., Grynyuk I. I., Skaterna T. D., Horak I. R., Grebinyk A. G., Drobot L. B., Matyshevska O. P., Senenko A. I., Prylutskyy Yu. I., Naumovets A. G., Ritter U., Frohme M.* Toxicity of C_{60} fullerene-cisplatin nanocomplex against Lewis lung carcinoma cells. *Arch. Toxicol.* 2019, 93 (5), 1213–1226. https://doi.org/10.1007/s00204-019-02441-6
27. *Mosunov A., Evstigneev V., Buchelnikov A., Salo V., Prylutskyy Y., Evstigneev M.* General up-scaled model of ligand binding with C_{60} fullerene clusters in aqueous solution. *Chemical Physics Letters*. 2019, V. 721, P. 22–26. https://doi.org/10.1016/j.cplett.2019.01.051
28. *Carmichael J., Degraff W. G., Gazdar A. F., Minna J. D., Mitchell J. B.* Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* 1987, V. 47, P. 936–942.
29. *Terskov I. A., Gitelzon I. I.* Method of chemical (acid) erythrograms. *Biofizika*. 1957, 2 (2), 259–266 (In Russian).
30. *Liu S., Liu H., Yin Z., Guo K., Gao X.* Cytotoxicity of pristine C_{60} fullerene on baby hamster kidney cells in solution. *J. Biomater. Nanobiotechnol*. 2012, 3 (3), 385–390. https://doi.org/10.4236/jbnb.2012.33037
31. *Atilano-Roque A., Wen X., Aleksunes L. M., Joy M. S.* Nrf2 activators as potential modulators of injury in human kidney cells. *Toxicol Rep.* 2016, V. 3, P. 153–159. https://doi.org/10.1016/j.toxrep.2016.01.006
32. *Gharbi N., Pressac M., Hadchouel M., Szwarc H., Wilson S. R., Moussa F.* C_{60} fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett*. 2005, V. 5, P. 2578–2585. https://doi.org/10.1021/nl051866b
33. *Ferreira C. A., Ni D., Rosenkrans Z. T., Cai W.* Scavenging of reactive oxygen and nitrogen species with nanomaterials. *Nano Res.* 2018, V. 11, P. 4955–4984. https://doi.org/10.1007/s12274-018-2092-y
34. *Grynyuk I., Grebinyk S., Prylutska S., Mykhailova A., Franskevich D., Matyshevska O., Schütze C., Ritter U.* Photoexcited fullerene C_{60} disturbs prooxidant-antioxidant balance in leukemic L1210 cells. *Mat.-wiss. und Werkstofftech.* 2013, 44 (2–3), 139–143. https://doi.org/10.1002/mawe.201300105
35. *Prylutska S. V., Grynyuk I. I., Grebinyk S. M., Matyshevska O. P., Prylutskyy Y. I., Ritter U., Siegmund C., Scharff P.* Comparative study of biological action of fullerenes C_{60} and carbon nanotubes in thymus cells. *Mat.-wiss. und Werkstofftech.* 2009, V. 40, P. 238–241. https://doi.org/10.1002/mawe.200900433
36. *Franskevych D. V., Grynyuk I. I., Prylutska S. V., Matyshevska O. P.* Modulation of cisplatin-induced reactive oxygen species production by fullerene C_{60} in normal and transformed lymphoid cells. *Ukr. Biochem. J.* 2016, V. 88, P. 44–50. https://doi.org/10.15407/ubj88.01.044
37. *Kutwin M., Sawosz E., Jaworski S.* Structural damage of chicken red blood cells exposed to platinum nanoparticles and cisplatin. *Nanoscale Res. Lett.* 2014, 9 (1), 257–283. https://doi.org/10.1186/1556-276X-9-257
38. *Suwalsky M., Hernández P., Villena F., Sotomayor C. P.* The anticancer drug cisplatin interacts with the human erythrocyte membrane. *Z. Naturforsch. C J. Biosci.* 2000, 55 (5–6), 461–466. https://doi.org/10.1515/znc-2000-5-624
39. *Rebillard A., Lagadic-Gossmann D., Dimanche-Boitrel M. T.* Cisplatin cytotoxicity: DNA and plasma membrane targets. *Curr. Med. Chem.* 2008, 15 (26), 2656–2663. https://doi.org/10.2174/092986708786242903

40. *Prylutska S. V., Grynyuk I. I., Golub A. A., Matyshevska O. P.* Evaluation of cytotoxicity parameters of C_{60} and C_{60}-containing composites *in vitro*. *Dopov. Nats. akad. nauk Ukr.* 2006, N 1, P. 163–167 (In Ukrainian).

41. *Rozhkov S. P., Goryunov A. S., Sukhanova G. A., Borisova A. G., Rozhkova N. N., Andrievsky G. V.* Protein interaction with hydrated C_{60} fullerene in aqueous solutions. *Biochem. Biophys. Res. Commun.* 2003, V. 303, P. 562–566. https://doi.org/10.1016/s0006-291x(03)00392-9

ЗАПОБІГАННЯ ТОКСИЧНІЙ ДІЇ ЦИСПЛАТИНУ ЩОДО НОРМАЛЬНИХ КЛІТИН ЗА КОМПЛЕКСОУТВОРЕННЯ ІЗ C_{60} ФУЛЕРЕНОМ

С. В. Прилуцька[1], *І. І. Гринюк*[1], *Т. Д. Скатерна*[2], *Л. Б. Дробот*[2], *М. С. Слободяник*[1], *О. П. Матишевська*[2]

[1]Київський національний університет імені Тараса Шевченка, Україна
[2]Інститут біохімії ім. О. В. Палладіна НАН України, Київ

E-mail: psvit_1977@ukr.net

Метою роботи було оцінити токсичність нековалентного нанокомплексу C_{60} фулерену з цисплатином (C_{60}-Cis-Pt) щодо нормальних клітин. Токсичність C_{60}-Cis-Pt нанокомплексу, порівняно з вільним Cis-Pt, вивчали на клітинах ембріональної нирки людини (HEK293), оцінюючи їх життєздатність за допомогою МТТ-тесту та на еритроцитах щура за їхньою стійкістю до кислотного гемолізу. Було показано, що вільний 40 мкМ Cis-Pt змінював морфологію та знижував життєздатність клітин HEK293 на 28%, а також збільшував кількість гемолізованих еритроцитів на 25% порівняно з контролем. C_{60}-Cis-Pt нанокомплекс за еквівалентної концентрації Cis-Pt не впливав на досліджувані показники і не спричиняв цитотоксичних ефектів. Запобігання токсичній дії Cis-Pt на нормальні клітини за його комплексоутворення із C_{60} фулереном відкриває перспективу використання наноструктури як ефективного цитопротектора і таргетного носія у пухлинні клітини.

Ключові слова: C_{60} фулерен, цисплатин, нанокомплекс, HEK293 клітини, цитотоксичність, еритроцити, гемоліз.

ПРЕДОТВРАЩЕНИЕ ТОКСИЧЕСКОГО ДЕЙСТВИЯ ЦИСПЛАТИНА НА НОРМАЛЬНЫЕ КЛЕТКИ ПУТЕМ КОМПЛЕКСООБРАЗОВАНИЯ С C_{60} ФУЛЛЕРЕНОМ

С. В. Прилуцкая[1], *И. И. Гринюк*[1], *Т. Д. Скатерна*[2], *Л. Б. Дробот*[2], *Н. С. Слободяник*[1], *О. П. Матышевская*[2]

[1]Киевский национальный университет имени Тараса Шевченко, Украина
[2]Институт биохимии им. А. В. Палладина НАН Украины, Киев

E-mail: psvit_1977@ukr.net

Целью работы было оценить токсичность нековалентного нанокомплекса C_{60} фуллерена с цисплатином (C_{60}-Cis-Pt) относительно нормальных клеток. Токсичность C_{60}-Cis-Pt нанокомплекса, в сравнении со свободным Cis-Pt, изучали путем оценки жизнеспособности клеток эмбриональной почки человека (HEK293) с помощью МТТ-теста и на эритроцитах крысы по их устойчивости к кислотному гемолизу. Было показано, что свободный 40 мкМ Cis-Pt вызывал морфологические изменения и снижение жизнеспособности клеток HEK293 на 28%, а также увеличение количества гемолизированных эритроцитов на 25% по сравнению с контролем. C_{60}-Cis-Pt нанокомплекс в эквивалентной концентрации Cis-Pt не влиял на исследуемые показатели и не вызывал цитотоксических эффектов. Предотвращение токсического действия Cis-Pt на нормальные клетки при комплексообразовании с C_{60} фуллереном открывает перспективу применения наноструктуры как эффективного цитопротектора и таргетного носителя в опухолевые клетки.

Ключевые слова: C_{60} фуллерен, цисплатин, нанокомплекс, HEK293 клетки, цитотоксичность, эритроциты, гемолиз.