Research Article

The $C_{60}(FeCp_2)_2$ -Based Cell Proliferation Accelerator

Andrei Soldatov,¹ Edward Shpilevsky,² Vitaliy Goranov,³
Vladimir Kulchitsky,⁴ and Floriana Tuna⁵

¹ Laboratory for Superconducting Materials Physics, State Scientific and Production Association "Scientific-Practical Materials Research Center of the NAS of Belarus", P. Brovka Street 19, 220072 Minsk, Belarus

² Department of New Materials, Lykov Institute of the Heat and Mass Transfer of the NAS of Belarus, P. Brovka Street 15, 220072 Minsk, Belarus

³ Central Scientific Research Laboratory of the Belarus State Medical University, Dzerzhinski Avenue, 83, 220116 Minsk, Belarus

⁴ Institute of Physiology of the NAS of Belarus, Akademicheskaya Street 28, 220072 Minsk, Belarus

⁵ School of Chemistry, Manchester University, Oxford Road, Manchester M13 9PL, UK

Correspondence should be addressed to Andrei Soldatov; andreisoldatov@mail.ru

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We studied structural and magnetic proprieties of the fulleride $C_{60}(FeCp_2)_2$. The influence of fulleride particles on the cell proliferative activity was also investigated. We found that the proliferative activity of the RINmF5 cells increases (53% versus control) in presence of the $C_{60}(FeCp_2)_2$ nanosized particles. Moreover, it was registered that the cell culture became multilayered and secreted basophile matrix.

1. Introduction

The last two decades studies have showed that fullerene C_{60} derivatives exhibit a great potential in many fields of biology and medicine [1]. These are UV and radioprotection [2], specific DNA cleavage [3], antiviral, antioxidant, and antiamyloid activities [1, 4], allergic response [5] and angiogenesis [6] inhibitions, immune stimulating and antitumor effects [7, 8], enhancing effect on neurite outgrowth [9], and gene delivery [10]. Fiorito and coauthors showed that C -fullerenes, when highly purified, do not stimulate the release of NO by murine macrophage cells in culture, their uptake by human macrophage cells is very low, and they possess a very low toxicity against human macrophage cells [11]. Water-soluble fullerene derivatives protect human keratinocytes from UV-induced cell injuries together with the decreases in intracellular ROS generation and DNA damages [12] and suppress intracellular lipid accumulation [13]. Ferrocene derivatives in particular ferrocenium salts demonstrate antitumor activity [14, 15]. Authors [16] proposed possible mechanisms of action of these compounds on tumors, which can be

assumed as one-electron oxidation of the ferrocene nuclei to the ferrocenium salts Fc^+X^- . These salts have relatively low reduction potentials fit for biochemical process to take place [17].

Many medical applications need that the drug materials possess ferromagnetic properties at room temperature. This allows managing the drug moving by magnetic field. ESR measurements on $C_{60}(FeCp_2)_2$ over a wide temperature range (4–290 K) indicate the formation of a low concentration of C_{60}^- ions and thus the presence of weak charge transfer interactions of C_{60} with ferrocene and the possible occurrence of at least a small amount of ferrocenium cations [18]. The C_{60}^- ESR spectra show appreciable anisotropy at $T < 40$ K, reflecting the freezing of the molecular rotation and the static distortion of the fullerene local symmetry similar to other fulleride salts. Moreover, the temperature variation of the C_{60}^- ESR spectra at low temperatures ($T < 10$ K) suggests the presence of antiferromagnetic spin-spin interactions. A major contribution of iron ions and ferromagnetic particles is identified at $T < 100$ K that may account for the dc magnetic response of the compound. These allow us to

FIGURE 1: As grown $C_{60}(FeCp_2)_2$ single crystal.

believe that ferrocene fullerides will have a good perspective for biomedical applications.

In this work, we studied structural and magnetic properties of the fulleride $C_{60}(FeCp_2)_2$. The influence of fulleride particles on the cell proliferative activity was also investigated.

2. Experimental

Single crystalline specimens of $C_{60}(FeCp_2)_2$ were grown from the solution of a stoichiometric mixture of C_{60} and $FeCp_2$ in benzene by slow evaporation at 300 K as described in [19].

In order to study the influence of the fulleride presence on cell proliferation, we chose the cell line *rin-mf5* (cell derivatives of the human insular tissue). These cells possess the large receptor field and high sensitivity against an attack of no specified cell toxins. These properties make them suitable targets for biotrophic agents. Under the experimental conditions studied, substances were introduced directly into culture medium of the cells *rin-mf5*, grown in the RPMI 1640 medium. The results were examined in comparison with the cell amount grown in the same conditions without any additive. Data simulation was carried out with the "StatOlympus3X" software.

Magnetic measurements of powdered crystals were performed on the magnetometer Quantum Design MPMS XL 7T. X-ray analysis was carried out on the DRON-3 diffractometer.

3. Results and Discussion

As grown single crystals of $C_{60}(FeCp_2)_2$ have pine-like shape (see Figure 1) and a brown color; no impurities have been detected by X-ray analysis. $C_{60}(FeCp_2)_2$ was synthesized first by Crane with coauthors [19]. This matter has triclinic structure with space group of $P\bar{1}$. Our X-ray analysis data is in agreement with results of [19].

Large crystals can be used as a substrate for adhesion and growth of cells (see Figure 2(b)). At the same time, the crystals with size of more the $10\ \mu m$ do not have reliable influence on the proliferative activity of the cells in monolayer culture (Figure 2(c)). After 24 hours of incubation, the cell amount in the experimental samples ($n = 6$) was larger

(but less than 40%) than that observed in control samples. The most intelligible effect of the proliferative activity was found in presence of the $C_{60}(FeCp_2)_2$ nanoparticles. The proliferative activity of the *rin-mf5* cells increases (53% versus control shown at Figure 2(a)) in presence of the $C_{60}(FeCp_2)_2$ nanosized particles ($500\ particles/cm^2$). Moreover, it was registered that at the places where the fulleride particles are accumulated, the cell culture became multilayered and secreted basophile matrix (see Figure 2(d)).

To our opinion, it can be related to the particle size of the fulleride nanocrystals $C_{60}(FeCp_2)_2$, which probably contain the $Fe^+C_{60}^-$ salt. The possible mechanism of the proliferation acceleration consists in nonspecific endocytosis of fulleride nanoparticles by *rin-mf5* cells. Due to relatively low reduction potentials Fe^+ cation that is suitable for intracellular biochemical process to take place, the $C_{60}(FeCp_2)_2$ nanoparticle can be a catalyst of the intracellular regulatory systems and accelerate the *rin-mf5* proliferation.

Magnetic measurements were performed by heating from 2 K up to room temperature under the magnetic field of 1 T. The results do not confirm the existence any ferromagnetic or antiferromagnetic ordering of $C_{60}(FeCp_2)_2$ as described earlier in the investigated temperature interval. As shown in Figure 3, $C_{60}(FeCp_2)_2$ is paramagnetic at liquid helium temperature. During the heating, it was transforming to the diamagnetic state near 60 K and was not changing the magnetic state up to room temperature.

4. Conclusion

$C_{60}(FeCp_2)_2$ does not possess any ferromagnetic or antiferromagnetic ordering from 2 K up to room temperature.

The proliferative activity of the RINmF5 cells in presence of the $C_{60}(FeCp_2)_2$ nanoparticles increases (53% versus control). It is proposed that the proliferation rate depends on the fulleride particle size. The possible mechanism of the proliferation acceleration consists in nonspecific endocytosis of fulleride nanoparticles by *rin-mf5* cells. Due to relatively low reduction potentials Fe^+ cation that is suitable for intracellular biochemical process to take place, the $C_{60}(FeCp_2)_2$ nanoparticle can be a catalyst of the intracellular regulatory systems and accelerate the *rin-mf5* proliferation.

FIGURE 2: The *rin-mf5* cell proliferation in presence of the $C_{60}(FeCp_2)_2$ particles. (a) Cell proliferation (standard condition in monolayer). (b) Adhesion and growth of cells on the big crystal. (c) Influence of the crystals with size of nearly $5\ \mu m$ on the cell proliferation. (d) Cell proliferation in presence of the $C_{60}(FeCp_2)_2$ nanoparticles.

FIGURE 3: Temperature dependence of the $C_{60}(FeCp_2)_2$ magnetization.

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References

- [1] H. L. Ma and X. J. Liang, “Fullerenes as unique nanopharmaceuticals for disease treatment,” *Science China Chemistry*, vol. 53, no. 11, pp. 2233–2340, 2010.
- [2] C. A. Theriot, R. C. Casey, V. C. Moore et al., “Dendro[C_{60}]fullerene DF-1 provides radioprotection to radiosensitive mammalian cells,” *Radiation and Environmental Biophysics*, vol. 49, no. 3, pp. 437–445, 2010.
- [3] R. D. Bolskar, A. F. Benedetto, L. O. Husebo et al., “First soluble $M@C_{60}$ derivatives provide enhanced access to metallofullerenes and permit *In vivo* evaluation of $Gd@C_{60}[C(COOH)_2]_{10}$ as a MRI contrast agent,” *Journal of the American Chemical Society*, vol. 125, no. 18, pp. 5471–5478, 2003.
- [4] S. S. Ali, J. I. Hardt, K. L. Quick et al., “A biologically effective fullerene (C_{60}) derivative with superoxide dismutase mimetic properties,” *Free Radical Biology and Medicine*, vol. 37, no. 8, pp. 1191–1202, 2004.
- [5] J. J. Ryan, H. R. Bateman, A. Stover et al., “Fullerene nanomaterials inhibit the allergic response,” *Journal of Immunology*, vol. 179, no. 1, pp. 665–672, 2007.
- [6] H. Meng, G. Xing, B. Sun et al., “Potent angiogenesis inhibition by the particulate form of fullerene derivatives,” *ACS Nano*, vol. 4, no. 5, pp. 2773–2783, 2010.
- [7] J. Zhu, Z. Ji, J. Wang et al., “Tumor-inhibitory effect and immunomodulatory activity of fullerol $C_{60}(OH)_x$,” *Small*, vol. 4, no. 8, pp. 1168–1175, 2008.

- [8] Y. Y. Xu, J. D. Zhu, K. Xiang, Y. K. Li, R. H. Sun, and J. Ma, "Synthesis and immunomodulatory activity of [60]fullerene-tuftsin conjugates," *Biomaterials*, vol. 32, no. 36, pp. 9940–9949, 2011.
- [9] H. Tsumoto, S. Kawahara, Y. Fujisawa et al., "Syntheses of water-soluble [60]fullerene derivatives and their enhancing effect on neurite outgrowth in NGF-treated PC12 cells," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 6, pp. 1948–1952, 2010.
- [10] R. Maeda-Mamiya, E. Noiri, H. Isobe et al., "In vivo gene delivery by cationic tetraamino fullerene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 12, pp. 5339–5344, 2010.
- [11] S. Fiorito, A. Serafino, F. Andreola, and P. Bernier, "Effects of fullerenes and single-wall carbon nanotubes on murine and human macrophages," *Carbon*, vol. 44, no. 6, pp. 1100–1105, 2006.
- [12] Y. Saitoh, A. Miyanishi, H. Mizuno et al., "Super-highly hydroxylated fullerene derivative protects human keratinocytes from UV-induced cell injuries together with the decreases in intracellular ROS generation and DNA damages," *Journal of Photochemistry and Photobiology B*, vol. 102, no. 1, pp. 69–76, 2011.
- [13] S. Yasukazu, M. Hiromi, X. Li, H. Sayuri, K. Ken, and M. Nobuhiko, "Polyhydroxylated fullerene $C_{60}(OH)_{44}$ suppresses intracellular lipid accumulation together with repression of intracellular superoxide anion radicals and subsequent $PPAR\gamma_2$ expression during spontaneous differentiation of OP_9 preadipocytes into adipocytes," *Molecular and Cellular Biochemistry*, vol. 366, no. 1–2, pp. 191–200, 2012.
- [14] P. Kopf-Maier, H. Kopf, and E. W. Neuse, "Ferrocenium salts—the first antitumor iron compounds," *Angewandte Chemie*, vol. 96, no. 6, pp. 446–447, 1984.
- [15] P. Kopf-Maier, H. Kopf, and E. W. Neuse, "Ferrocenium complexes: a new type of water-soluble antitumor agent," *Journal of Cancer Research and Clinical Oncology*, vol. 108, no. 3, pp. 336–340, 1984.
- [16] V. N. Babin, P. M. Raevskii, K. G. Shitkov, L. V. Snegur, and S. Y. Nekrasov, "Antitumor activity of metallocenes," *Mendeleev Chemistry Journal*, vol. 39, no. 2, pp. 17–23, 1995.
- [17] A. J. Deeming, "Mononuclear Iron Compounds with $\eta^2 - \eta^6$ Hydrocarbon Ligands," in *Comprehensive organometallic chemistry*, G. Wilkinson, F. G. A. Stone, and E. W. Abel, Eds., p. 481, Pergamon Press, 1982.
- [18] N. Guskos, A. G. Soldatov, G. Zolnierkiewicz, V. Likodimos, and S. Glenis, "Spin dynamics and charge transfer in C_{60} -2ferrocene studied by electron spin resonance," *Journal of Non-Crystalline Solids*, vol. 354, no. 35–39, pp. 4334–4337, 2008.
- [19] J. D. Crane, P. B. Hitchcock, H. W. Kroto, R. Taylor, and D. R. M. Walton, "Preparation and characterisation of $C_{60}(\text{ferrocene})_2$," *Journal of the Chemical Society, Chemical Communications*, no. 24, pp. 1764–1765, 1992.