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Perspectives of Fullerene Derivatives in PDT and Radiotherapy of Cancers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Nanoparticles of fullerenes and their water-soluble derivatives have been firmly introduced into solution of medical problems. Although there are still debates about their toxicity and long-term consequences of their application in the clinic, the success of fullerenes application in some sections is undeniable, in particular, in photodynamic therapy (PDT) of cancer tumors. Besides there are interesting data on radiotherapy where fullerenes appear to be more transporters than drugs, but due to own cytoprotective properties, the fullerene adducts can also participate in the combined treatment. This review evaluates the status of these sections of fullerene chemistry in terms of development and recent trends.

Keywords: Fullerenes; PDT; ionizing radiation; proliferation; cancer chemotherapies.

1. INTRODUCTION

Due to its unique spherical structure, C_{60} has a possibility to accept up to 6 electrons [1] and possesses extended π-system. When C_{60} is subjected to action of light, then an electron

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moves to higher energetic level, thereby producing an excited singlet C_{60} that reacts with O_2 to form a singlet oxygen ($^1O_2$) [2,3]. Fullerenes are exclusively effective generators of the singlet oxygen with quantum yield of $^1O_2$ close to one [4]. They absorb in UV- and in visible spectrum [5] that allows their usage in photodynamic therapy (PDT). Originally formed the singlet excited state ($^SC_{60}$*) undergoes intercombination transition into a triplet state ($^TC_{60}$*). The triplet excited state is an excellent acceptor of electrons and potential producer of the superoxide anion radical $O_2^{-\cdot}$ (Fig.1). A latter way is more pronounced in organic solvents, predominantly, in polar ones, especially, in the presence of reducers such as NADH [6].

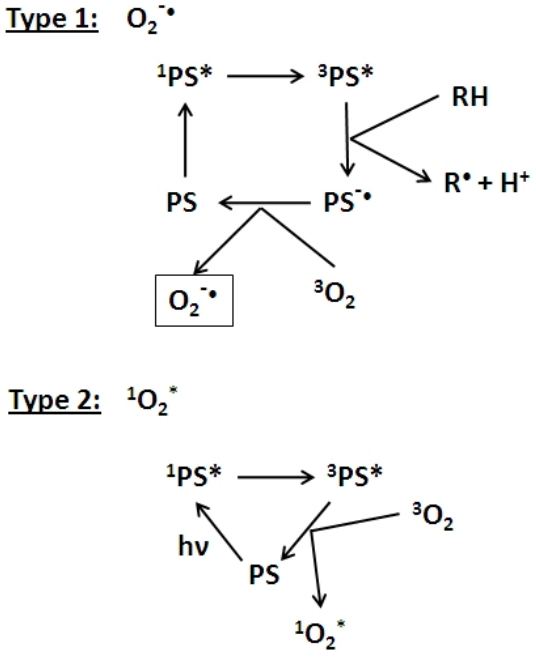

Fig. 1. Mechanisms of generation of singlet oxygen and superoxide radical by fullerenes

Increasing number of functional groups added to fullerene, leads to decreasing of quantum yield of the singlet oxygen. Toxicity is significantly decreased or even is fully absent in hydrophilic fullerenes [7]. Under UV- irradiation the fullerenes demonstrate the ability to generate reactive oxygen species (ROS) with rate of 10 nmol mL^{-1} min^{-1} as confirmed by ESR method [8]. The efficiency of fullerene photocytotoxicity depends on the degree of modification as well as on the size of the fullerene cage [9]. More extended π-system generates a higher phototoxicity. That is why the photodynamic activity of C_{70} is greater than C_{60}-fullerene.

Recently the development of nanotechnology caused a careful investigation of medical capabilities of carbon nanoparticles, in particular, fullerene derivatives (adducts). Nanoparticles of 1-100 nm in size have principally others physical and chemical parameters, mechanisms and biomedical application. This is due to location of majority of their atoms at interphase of outer surface of the particles that, in turn, leads to new quantum and mechanical mechanisms of their action. To imagine the size of nanoparticles let's take a height of a child of 4 years old to be equal to 1, then a diameter of the red blood cell will be

in the range of 10^{-5}-10^{-6}, diameter of hepatitis C virus is ~10^{-7}, of carbon nanotube - ~10^{-8}-10^{-9}, and of water molecule – 10^{-10} [10]. In the scale of nanoparticles the fullerenes are included to the levels from individual molecules up supramolecules and assemblies.

Now there is understanding of general parameters that are of fundamental importance to medical applications of fullerenes. Among these parameters there are a size of nanoparticles and an ability of self-aggregation due to the structure of the side chains and the formation of hydrogen bonds. The size and shape of the nanoparticles define the morphology of the aggregates which depends on the method of fullerene preparation. As a rule smaller nanoparticles demonstrate higher cytotoxicity (although there are exceptions) which have caused by various factors, in particular, by the ability of small nanoparticles to integrate into proteins, membranes and DNA helix [11-14] affecting signaling pathways of apoptosis [15] that represented in Fig. 2.

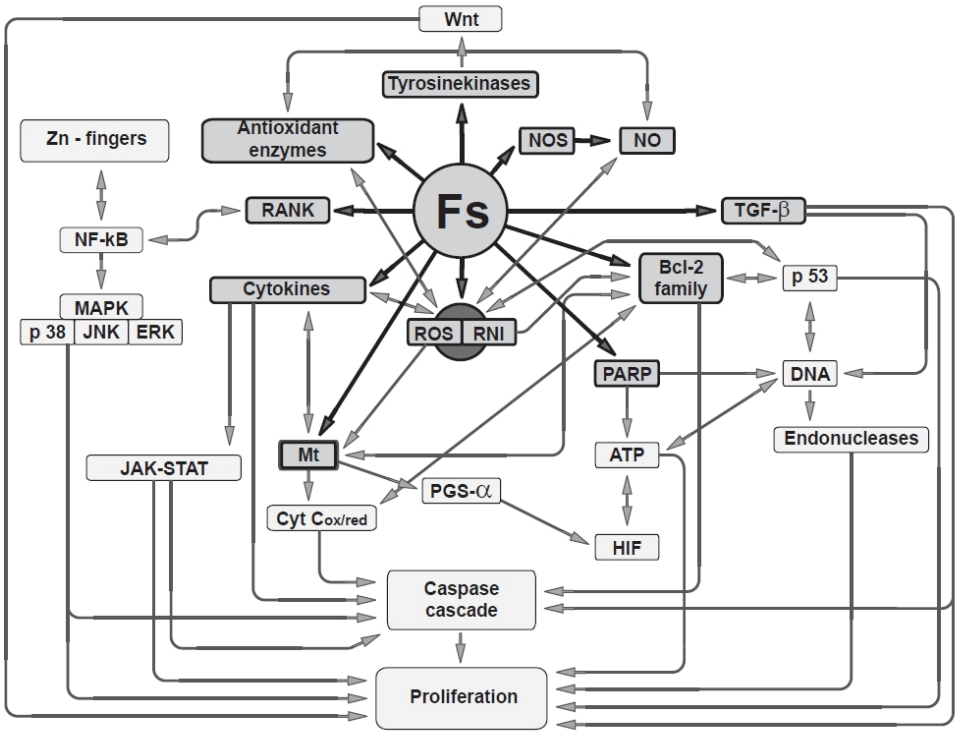

Fig. 2. Effects of fullerenes (Fs) on some signaling pathways of apoptosis

Fullerenes are able to activate two contrary actions in the body, namely, a generation of apoptotic or necrotic cell death due to the appearance of ROS or a protective of cells acting as antioxidant agents (called sponges of radicals), in some cases, at the level of SOD-mimetics. In the first case, the fullerenes can be considered as potential anti-tumor agents [16-18] or preparations for PDT. As PDT agents fullerene derivatives may be a part of the conjugates, aggregates and supramolecules. Development of strategy of fullerene based nanoplatforms with drugs of different actions represents promising possibilities for carbon nanomedicine [19], especially, in cancer therapy.

A number of books that comprehensively describe an organic chemistry of fullerenes [2,20] and their application in biochemistry and medicine [2,21,22] have been published. Many reviews appeared [23-28] to describe the investigation of synthesis of new compounds based fullerene, and their possible medical usage [29-35], in particularly, in PDT [27,36]. However, the trends in PDT as well as in the emergence of new fullerene derivatives are changing very rapidly and this requires analysis of their medical options as effectors of the apoptosis of normal and cancer cells.

Two aspects of development of fullerene chemistry are described in this work: new trends in PDT with features of biological usage and possibilities of applying of fullerenes in radiotherapy and radiodiagnostics.

2. PHOTOTOXICITY OF FULLERENES. PHOTODYNAMIC THERAPY

Photodynamic therapy as a treatment method involves the application of a photosensitive substance or its metabolic precursor, followed by irradiation with a focused light of a certain wavelength [28]. This results in the photochemical generation of singlet oxygen and/or ROS in targeted tissues. The active species initiate oxidation of sensitive biomolecules, resulting in the damage of cells, start of signaling pathways of apoptosis, autophagy, anoikis or necrosis. An ability to significant accumulation in the tumor, to stability and rapid elimination (clearance) as well as to the absence of general toxicity, synergistic effects with other drugs and to the availability for the action of a specific wavelength light irradiation are necessary properties of photosensitizers [29].

The method of PDT is widely used in cancer treatment. Two types of effects are distinguished [31], where reacting agents are superoxide radicals or singlet oxygen (Fig. 1). For fullerenes the method is based on the selective accumulation of photosensitizer in tumor cells and its ability to generate both agents (singlet oxygen as well as superoxide radical) under irradiation with light beam of definite wavelength. Thus, cellular uptake of a preparation and selectivity of its action, namely, on cancer cells play a certain role in damaging of a tumor.

Such selective necrosis of cancer cells was observed in the presence of the fullerene adduct with some polyester chains and irradiation by light (λ=400-505 nm) [32].

Till now photosensitizers on the basis of carbon nanostructures did not pass clinical trials and are not allowed for their practical use [30]. Nevertheless, investigations are widely carried out with hope for their further usage. When PDT is fullerene mediated it can be used to damage multiple types of cancer cells including head and neck, breast and esophageal cancers which poorly respond to other kinds of cancer therapy.

Fullerene adducts that exhibited the phototoxic effect against cancer cells are shown in Table. 1.

Table 1. Effect of light irradiation in the presence of fullerenes

I. Fullerene adducts as a protectors against the photoirradiation

Fullerene adduct	Cells	Conditions	Effect	Ref.
$C_{60}(OH)_{44}$ $8H_2O$ (I) $C_{60}(OH)_{6\text{-}12}$ (II) $C_{60}(OH)_{32\text{-}34}$ $7H_2O$ (III)	Skin keratinocytes HaCaT	UVA, UVB	ROS acceptors: (I)>(III)>(II). (I) shows higher protective effect in UVB than in UVA	70
Carboxyl-fullerene[C_{60}]	HaCaT	UVB	Reduction of blocking cell proliferation. Without the participation of Bcl-2 family	71
PVP/C_{60}	HaCaT	UVA	Blocks the signaling pathways of apoptosis	73
C_{60} incorporated into the phospholipid membrane	HaCaT	UVA, 10 J sm^{-2} 74.5 nm, C=150ppm	restoration of the cell viability	74
		Chronic UVA to 4 J sm^{-2}. In total 76 J sm^{-2}.	Inhibition of cell damages	75
		250 ppm, C=0.75 ppm C_{60}		
C_{60} integrated in liposomes	HaCaT	UVA (12 J sm^{-2}), UVB (500 mJ sm^{-2}), 75.6 nm	Prevention of cell morphological degeneration from OH radicals	77
Tris-malonyl-C_{60}	A431	UV	The maintaining a network of cytoskeleton components is carried out only in the presence of the substance during irradiation	72

II. Fullerenes in PDT

Fullerene adduct	Cells	Conditions	Effect	Ref.
C_{60}	T-leukemic lymphocytes, Jurcat	UV, visible 12-50 nm, 50 μmole/L	Cytotoxic effect	35
C_{60} and C_{70} incorporated into lipid membranes	HeLa	λ>400 nm	Phototoxicity of C_{70} is higher than C_{60}.	60
C_{70}, incorporated into surface-cross-linked liposome (cerasomes)	HeLa	UV	High photoreactivity	80
C_{60}-aminoacid: -Phe -L-Arg C_{60}-folic acid	HeLa,	5 μg/ml, visible light	The decrease of mitochondrial membrane potential, viability, activity of antioxidant enzymes: SOD, CAT, Gpx. An increase of MDA level and magnification of caspase-3.	32
Copolymer C_{60}-*N*-vinylpyrrolidone	HeLa,	100 μg/ml	30% of the cells are killed. Damage of membrane cells.	42
	osteogenic sarcoma cells	5 μg/ml		
Bis-methano-phosphonate-C_{60} (BMPF)	HeLa,	4 μmol/L, green light	An increase in MDA. The enhancement of lipid peroxidation.	30
Carboxy-C_{60} in present of Ca^{2+}	Superhelical DNA pBR 322	Visible light	Cleavage at guanine bases. Mono-substituted adducts are higher phototoxicity	30
C_{60}-pyropheophorbide	Jurcat		Phototoxicity	34
C_{60}/PEG with different terminal chains			PEG with methyl terminal structure produces the maximum accumulation in the tumor	38
Gd^{3+}@C_{60}/PEG	PDT+MRT		Theranostics	37
multimeric (crosslinked) PEG/C_{60}	KB-tumor grafted into mice	λ=670 nm (10 min)	A raising the temperature up to 44^0 and tumor regression	39
N-methyl pyrrolidine – C_{60} in Cremophor EL-micelles	Irradiation through the abdominal wall	White light	Predominance of necrosis	44

Cationic micelles of poly(*N*-vinyl-caprolactam)-C_{60}			Photosensitizer, antiviral drug	40
Cationic adduct C_{60} (Fig. 4) integrated into liposome	HeLa		Highly effective phototoxic agent	76
Carbohydrate substituted fullerene-C_{60}	Cancer cells	UV and visible light λ=355 nm, laser irradiation	Selective degradation of cells and HIV-protease	62
	Normal fibroblasts		no cytotoxicity	64
Glycol chitosan-C_{60}	KB-tumor of human carcinoma of the cervix	λ=670 nm	Selective accumulation in the tumor and high generation of singlet oxygen	66
Malonic acid-C_{70}		400-700 nm	Necrosis, blebbing	9
FHP1 FHP6 FHP12			Compound II is more phototoxic due to higher intracellular uptake	61
$C_{60}(OH)_x$	human retinal pigment epithelial cells	Visible light, 83 J sm^{-2}, >5 µmole/L	Early apoptosis	54
$C_{60}(OH)_{24}$	Human lens and retina cells		penetrate eye barriers	51,53
$C_{60}(OH)_{19}(ONa)_{17}$ $18H_2O$	Different cells		High phototoxicity	55,56
Deca cationic $C_{84}O_2$-Malonate Quaternary Ammonium iodide/triiodide Salts		The relation-ship of the wavelength to the cell photo-toxicity in the present of a donor in the side chain (a tertiary amine)	cyclic process enhancing the generation of OH radicals	50

$(\gamma CD)_2/C_{60}$	HLE B3	UVA, visible, 2 μmole/L	1) high toxicity in the absence of aggregation 2) transport through eye barriers	57
α,β,γ-CD/C_{60}	DNA	400 nm	DNA cleavage: α<β<γ-CD	58
Conjugates C_{60} with lipophilic meso-aryl- porphyrins with long chain substituents				49
C_{60}-porphyrin incorporated into liposomal vesicles	Hep-2	1 μmole/L, D=54 J/sm^2	death of cancer cells (80%)	48
C_{60}-porphyrin	Hep-2		Caspase-dependent pathway of apoptosis	46
5,10,15,20-tetra-kis(4-phenyl)porphyropolyvinyl-pyrrolidone-C_{60} (TPP/PVP/C_{60})	1) K562	436 nm, 1000 mJ/sm^2, 20-200 mW/sm^2, 0.05 μmole/L	Apoptosis without the participation of caspase-3	2
	2)rat with Walker 256 carcinoma inoculated subcutaneously	685 nm, 50 J/sm^2, t=15 min, 10 mg/kg intraperitoneally	Oxidative stress leads to the destruction of cancer cells	26
Naphthalene-diimide-C_{60}			Photosensitizers with light-harvesting antenna	82
C_{60}- cycloveratrilen with glucose or lactose residues			Photoreactive supramoleculs	86

Colloid solutions of C_{60} and C_{70}, water-soluble fullerenes functionalized by amino acids, folic or malonic acids, some copolymers, inclusion compounds, conjugates, dyads and triads, some fullerols etc. are used for this purpose. The mechanism of action may be various. Incubation of tumor cells with fullerenes with subsequent irradiation by light causes apoptosis in different types of cancer through 4-6 hours after irradiation [34]. Most of fullerene derivatives induce oxidative stress, which, depending on the cellular uptake of a particular compound, as well as on the type of cells, leads to a selective or non-selective damage of cells or tumor tissue by apoptosis or necrosis mechanisms. Typically, these events are accompanied by the development of mitochondrial dysfunction as well as by the decline of activity of antioxidant enzymes and the growth of MDA level. Antioxidant functions of fullerenes in the conditions of a strong oxidative stress may acquire greater significance and to contradict the anti-tumor activity of the compound. Mono-adducts of C_{60} (in comparison with bis- or tris-adducts) demonstrated the greatest activity in relation to cancer cells due to their high cell uptake and enhanced localization in mitochondria.

2.1 Phototoxicity of fullerene adducts to HeLa cells

The aminoacid-C_{60} derivatives (Fig. 3): phenylalanine-C_{60}, L-arginine-C_{60} (Fig. 3.3) and folic acid-C_{60} (Fig. 3.4) - in concentration of 5 μg/mL did not cause cytotoxicity in the dark during long time and possessed selectivity for cancer HeLa cells [33]. The decrease of mitochondrial membrane potential, of cell viability and of SOD, CAT, Gpx activity and the increase of MDA level were observed after irradiation by visible light. Finally, the apoptosis proceeded through enhancement of caspase-3 activity.

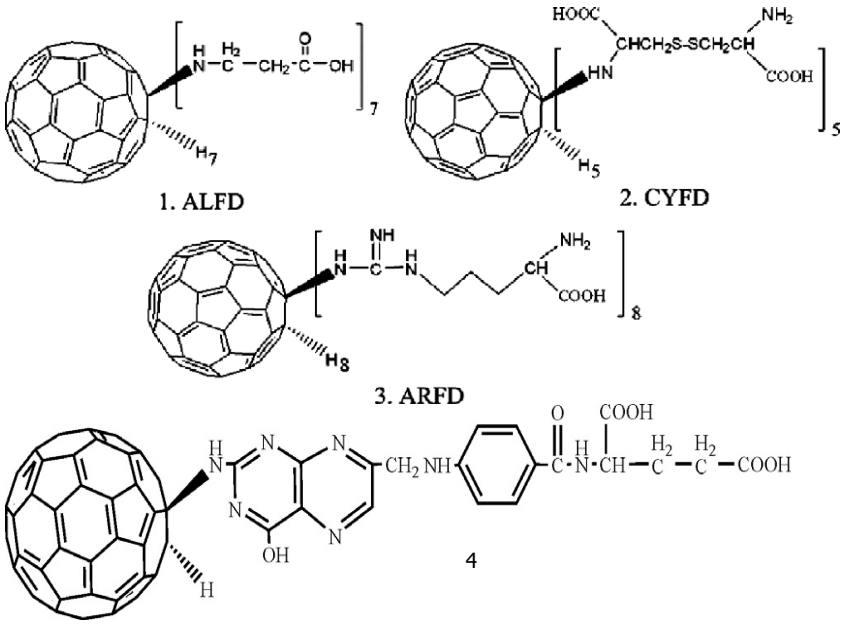

Fig. 3. Aminoacid-C_{60}: Ala-C_{60}(1), Cystine-C_{60}, Arg-C_{60}(3). Folic-C_{60} (4)

Cationic adduct C_{60} (Fig. 4) incorporated into the liposome, was demonstrating a high efficiency of cell destruction in the PDT [37].

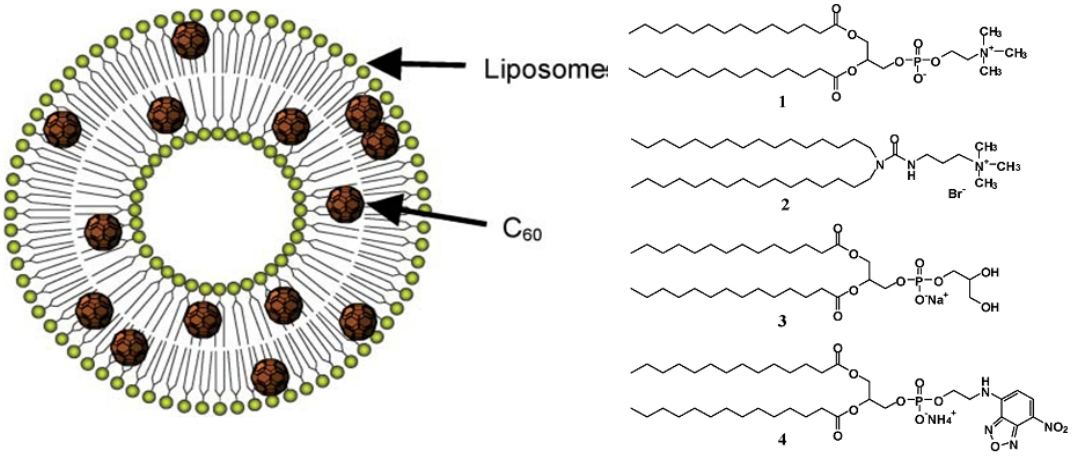

Fig. 4. Complex C_{60}-liposome having different surface charges due to compounds 1-4 [76]

By using BMPF (Table 1.II) it was shown [38] that ROS together with calcium ions are involved in the process of sensibilization of apoptosis and necrosis. The presence of extracellular calcium promotes activity of fullerene derivatives, but its removal does not interrupt their ability of membrane damaging. That is indicating to the existence of both calcium-dependent and calcium-independent manners in the process. Simultaneously, BMPF enhances lipid peroxidation in dependence on dose and duration of irradiation [31].

Photodynamic activity of C_{70}-γ-cyclodextrine (C_{70}/γ-CD) inclusion compound was sufficiently greater than those for C_{60} in relation to HeLa cells [39]. Similar picture was observed at comparison of photodynamic activity (λ>400 nm) of C_{60} and C_{70} incorporated into lipid membrane [40]. Authors consider that this is linked with a simple difference in their ability to generate singlet oxygen, due to asymmetry of C_{70} [9]. Similar highly photoreactive properties were showed by C_{70} included in surface-cross-linked liposomes (cerasomes). The photosensitizer was obtained by inclusion C_{70}/CD into a lipid membrane using the reaction of exchange [41] and C_{70} is capable of acting without the release from the cerasomes of membranes. Dynamics of spontaneous penetration of pristine C_{60} and N-substituted fullero[C_{60}]pyrrolidines into phospholipid membranes and its distribution there have been described [42]. The nature of fullerenes as well as a method of their introduction into liposomes plays a significant role [43]. Fullerene-containing liposomes with the highest potential for photo cleavage of DNA were obtained in photo triggered the exchange reaction, but not in case of heat or microwave irradiation.

Fullerene-C_{70}, modified by malonic acid, caused a much more active cell death by necrosis than the C_{60} derivative. This process accompanied by blebbing that is rare in PDT [9].

Co-polymers of C_{60}-N-vinylpyrrolidon showed the best phototoxicity in relation to HeLa and murine osteogenic sarcoma cells (Tabl.) in PDT [44]. Cationic micelles of block-copolymers of poly (N-vinylcaprolactam)-C_{60} demonstrated themselves as a potential antivirus drug [45] and, simultaneously, as strong photosensitizers under UV-irradiation [46].

2.2 Phototoxicity of Fullerene Adducts to Leukemic Cells

Action of C_{60} colloid solution and irradiation (in UV- and visible ranges) produced cytotoxic effects on leukemic Jurkat cell line (but not on thymocytes) [47]. The process was accompanied by elevated activation of caspase-3. C_{60}-pyrofeoforbids with different amount of substitutes showed varying phototoxicity in relation to Jurkat cells [35]. Effects were determined by difference in their physical and chemical properties and, as consequence, by different intracellular uptake.

2.3 Photoinduced DNA-cleavage

Photoinduced cleavage of super-helix DNA in pBR322 (a plasmid used *E. coli* cloning vectors) predominantly at guanine bases [31] was observed after incubation with carboxy-fullerenes-C_{60} and irradiation by visible light (but no in the darkness). CD/C_{60} is also effective photosensitizers of DNA cleavage in PDT [48]. Effectiveness are increased in order of α-<, β-<, γ-CD/C_{60}. The difference is due to varying aggregation behavior of the inclusion complexes in aqueous solution, and correlates with the size of the cavity of host molecules - cyclodextrin (174 <262 <474 A^3, respectively). Moreover, photodynamic activity of γ-CD/C_{70} complex was much higher than in the case of C_{60} [39].

Dynamics of spontaneous penetration of pristine C_{60} and N-substituted fullero[C_{60}]pyrrolidines into phospholipid membranes and its distribution there have been described [42]. The nature of fullerenes as well as a method of introducing them into liposomes is playing a significant role in DNA damaging [43]. Fullerene-containing liposomes with the highest potential for photo cleavage of DNA were obtained in photo-triggered exchange reaction, but not by heat or microwave irradiation. The liposomes were much higher photoreactive due to the lack of self-quenching of photoexcited fullerenes. That is resulted in the introduction of only isolated fullerenes because of controlling their self-aggregation.

2.4 Effect of Fullerene Adducts against Different Tumors *In vivo*

The dominant necrotic effects over apoptosis were observed under treatment by N-methyl-pyrrolidine-C_{60} placed in Cremophor-EL-micelles with simultaneous irradiation with white light through the abdominal wall in case of intraperitoneal carcinomatosis [49].

Multimeric (cross-linked) PEG-C_{60} - nanoparticles [50] are used in photothermal/photodynamic therapy of mice with inoculated KB-tumor. At the laser irradiation (λ = 670 nm, 10 min) the temperature on the surface of tumor was increased to 44ºC and caused high level of singlet oxygen leading to tumor regression.

Conjugates of fullerene with glycol chitosan (30 nm) were photoreactive in relation to cells of KB-tumor human cervical carcinoma. The selective accumulation of photosensitizer in the tumor and high generation of singlet oxygen were observed [51].

A system of 5,10,15,20-tetrakis (4-phenyl) porphyrin-polyvinylpyrrolidon-C_{60} (TPP/PVP/C_{60}) was tested on rats with subcutaneously inoculated Walker 256 carcinoma. Under the irradiation with red light (λ = 685 nm) after intraperitoneal injection of 10 mg of preparation per kg of body weight the oxidative stress, lipid peroxidation and changes in antioxidant

systems were observed in the tumor tissue that resulted in the destruction of cancer cells [19].

2.5 A Role of Substitutes

An investigation of phototoxicity of three fullerene hexa-cis-adducts in combination with different amount of introduced photosensitizers: bis (3^1,3^2-didehydrophytochlorine) fullerene [5:1]-hexa-adduct (FHP1), fullerene [5:1]-hexa-adduct with six 3^1,3^2- didehydrophytochlorine groups (FHP6) and fullerene[6:0]-hexa-adduct with 12-3^1,3^2- didehydrophytochlorine units (FHP12) was performed and FHP6 was recognized as the most prospective one [52]. The degree of intracellular uptake, which depended on the size and asymmetry of the fullerene complex through a changing of quantum yield of the singlet oxygen, had the greatest meaning.

2.6 A Role of Aggregation

Dendro [C_{60}] fullerene [53] inhibited growth of cells in the darkness and was slightly phototoxic under UV-irradiation due to independent formation of aggregates. A method of preparation of fullerene nanoparticles played a great role in its phototoxic activity.

2.7 Photoactivity of Inclusion Compounds

It is considered that PEG-fullerenes C_{60} possess good potential to be used in PDT with very small side effects for normal cells [54]. PEG-fullerene-C_{60} demonstrated phototoxicity (λ=400-600 nm, 140 J/cm^2) in relation to human fibrosarcoma cells HT1080, significantly decreasing their viability. Normal fibroblasts in the same conditions kept viability of 85-93%. PEG-conjugated fullerenes, containing Gd^{3+} ion, were used for photodynamic therapy in combination with magnetic resonance tomography (theranostics) [55]. Samples of PEG with different terminal structures and molecular weights were conjugated with C_{60} by covalent binding. All studied conjugates demonstrated *in vitro* equal ability to generate the superoxide anion and anti-tumor activity in PDT. However, C_{60}-PEG conjugates *in vivo* possess a tumor suppression activity, the longest half-life in blood circulation and the highest accumulation in the tumor if they have the methyl-terminal PEG only [56].

Water-soluble derivative $(\gamma\text{-CD})_2/C_{60}$ is used in two ways: as drug transporter through eye barriers and photosensitizer in PDT for treatment of tumors [57]. Starting with 2 μmol L^{-1} $(\gamma\text{-CD})_2/C_{60}$ was highly toxic to the HLE B-3 cells under UVA irradiation. In the meantime, aggregated nanoparticles did not provide such an effect even at 30 μmol/L, and the effect was not observed under irradiation by a visible light. Obviously the singlet oxygen is an important intermediate product of phototoxicity of monomer $(\gamma\text{-CD})_2/C_{60}$ and ^sO production decreases with an aggregation of the particles.

2.8 Fullerols as Photosensitizers

Fullerol ($C_{60}(OH)_{19}(ONA)_{17}18H_2O$) also exhibited a high phototoxicity in water solutions in relation to different cancer cells and cellular compounds [58,59] that was explained by authors [279] as a result of possible accumulation of ROS products. Fullerol $C_{60}(OH)_{24}$ is a powerful photosensitizer [60] at the expense of superoxide radicals and singlet oxygen generation through effective resonance transfer of energy [61]. $C_{60}(OH)_{24}$ is used in PDT in ophthalmology, because it is able to penetrate eye barriers, and some correlation between

intracellular distribution of $C_{60}(OH)_{24}$ and progression in damage of human lens and retina *in vivo* is observed [62]. Fullerols demonstrated phototoxicity against human retina pigment epithelial cells causing the appearance of early apoptosis [63].

2.9 Conjugates and Supramoleculs with Fullerene Adducts in PDT

Fullerene-carbohydrate hybrids (Fig. 5) can produce the selective degradation of HIV-1 protease [64]. However, these compounds did not demonstrate cytotoxicity against normal fibroblasts [65], besides; these hybrids are able to inhibit a lipid peroxidation in blood plasma [66].

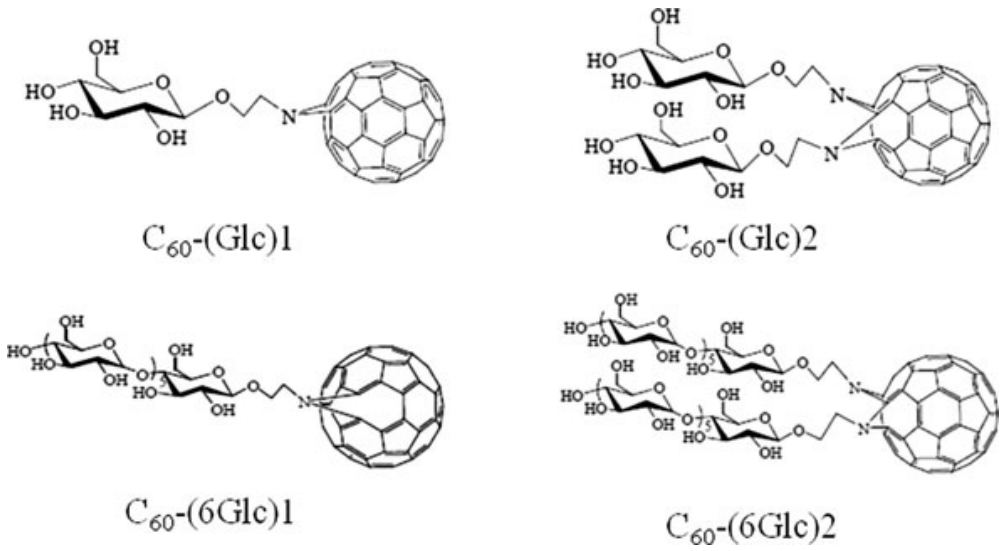

Fig. 5. Carbohydrate substituted [C_{60}] fullerenes [62,64]

Recently, the conjugates of C_{60} and lipophilic meso-aryl-porphyrins with long chain substituents were obtained for using in PDT [67].

Among fullerene nanomaterials a porphyrin-fullerenes play an important role due to high bioavailability and relative non-toxicity [68]. Therefore, photosensitizers in the form of dyads and triads, which include porphyrin and fullerene derivatives, are produced. Dyad C_{60}-porphyrin (C_{60}-P) was used in PDT against human cells Hep-2 cancer of the larynx, causing apoptosis in the caspase-dependence manner [69]. These dyads, being included (C = 1 μmol L^{-1}) into liposomal vesicles, have caused under light irradiation the death of 80% of the cells [70].

Apoptosis without participation of caspase-3 was observed when the human lymphoblast cell line (K562) was treated by TPP/PVP/C_{60} [2]. The usage of TPP is linked with its ability to generate singlet oxygen with high quantum yield (0.63) [71]. Three types of interactions were registered (Fig. 3) in this dyad: electrostatic, hydrogen bonds in TPP-PVP and the donor-acceptor bonds between fullerene and other components [27]. Here the high ability of these compounds to the formation of photo-induced state with divided (isolated) charges was first noted. Cell survival was dependent on the level of illumination and high phototoxic effect persisted even in an atmosphere of argon. Depending on the microenvironment of the

sensitizer site localization, the tissue is damaged either through the mechanism of $^1O_2$ - mediated photoreaction process or through ROS attack at a low concentration of oxygen. Apoptosis by caspase-3-dependent pathway (58% of apoptotic cells) has been replaced by predominant necrotic phenomena in anaerobic conditions.

2.10 Complex Drugs Containing Fullerene Derivatives

By using deca cationic $C_{84}O_2$-malonate quaternary ammonium iodide/triiodide salts, it has been shown [72] that the generation of hydroxyl radicals can be enhanced by application of short excitation wavelength in the presence of a combination of electron donors of amine and low concentrations of ascorbic acid (AA). AA acts as an effective reducer of tertiary amine cation radicals formed during photoinduced intramolecular electron transfer from the hexa-bis (aminoethyl) amidated malonic donor fragment to the fullerene cage. AA electron reduction can regenerate neutral deca-tertiary aminoethyl fragments and, therefore, continue photo-induced oxidation-reduction cycles that ultimately leads to an increase of hydroxyl radicals generation. $C_{84}O_2$-fullerene derivatives exhibit the properties of effective photosensitizers.

The photoelectric effect can be further amplified by using the light-harvesting liposomal system with introduced light-harvesting antenna molecule [32]. To improve the photodynamic activity of liposomal photosensitizer against cancer cells, the construct as "paddle" with the light-harvesting molecules 1,10 - dioctadecyl - 3,3,30,30-tetra-methyl-indodicarbocyanine (DiD) and C_{60} in bilayer lipid membranes was developed [73]. The light energy (λ = 610-740 nm) is absorbed by the antenna and transmitted to the C_{60} molecules for the ROS generation as shown in Fig. 6.

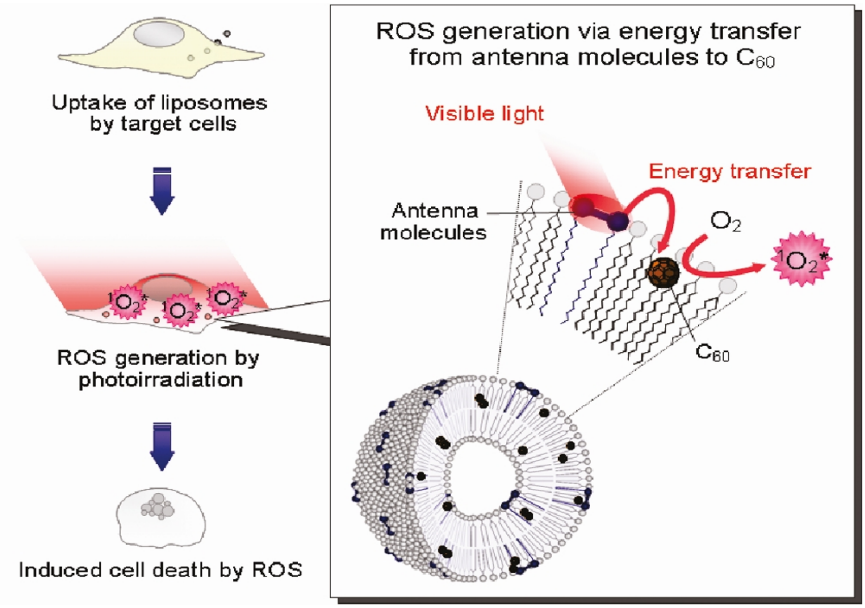

Fig. 6. Light-harvesting liposomal photosensitizer, which is a lipid membrane containing C_{60} and antenna molecule (1,10-dioctadecyl-3,3,30,30-tetramethylindodicarbocyanine - DiD). Copied from [81]

Two naphthalene-diimide-C_{60} (NDI-C_{60}) dyads in which alkyl amino substituents acted as light-harvesting antennas, and C_{60} served as a spin converter for intercombination transition from the singlet excited to the triplet excited state were proposed for PDT [74].

The idea of association of fullerene and porphyrin structures is considered to create new photosensitizers with enhanced generation of singlet oxygen and more simple entry into tumors. Some polyads have been synthesized [75], but they have not been tested in medicine yet [76].

Calculations carried out by DFT-method, have shown [77] that some of the metal-containing fullerenes (especially, bis-fullerenes) have even more powerful potential as photosensitizers.

Two sugar-functionalized (glucose- and lactose) water-soluble cyclotriveratrylene derivatives that are capable to form photoreactive supramolecular compounds with C_{60} are described [78].

To enhance the photoactivity of drugs, fullerene derivatives are covalently linked with well-known photosensitizers, for example, with zinc phthalocyanines. Compounds of C_{60}-pyrrolidine tris-acid ethyl ester (PyC_{60}) are obtained with a series of photosensitizers-zinc phthalocyanines (ZnPc) among them unsubstituted ZnPc, zinc - 1,4,8,11,15,18,22,25 – octa butoxy-29H, 31H - phthalocyanine and zinc - 2,3,9,10,16,17,23,24 - octakis - (octyloxy) - 29H, 31H-phthalocyanine [79]. Also there are similar compounds for C_{70} [80]. Theoretical calculations of the photoexcitation mechanism showed that interaction between ZnPc and PyC_{60} is regulated by electrostatic mechanism, but not dispersive forces associated with π-π interaction.

Photodynamic activity of fullerenes can be used not only in the treatment of cancer, but also for the sensitization of antimicrobial action [81]. The inactivation rate of MS2 bacteriophage and the rate of generation of singlet oxygen increased in the following order of four suspensions of fullerene photosensitizers [82]: aqu-nC_{60} <C_{60} $(OH)_6$ ≈ $C_{60}(OH)_{24}$ <C_{60} $(NH_2)_6$. In all cases the inactivation mechanism has involved a destruction of capsid by $C_{60}(NH_2)_6$, an infringement of the secondary structure and oxidation of the capsid proteins.

Some modern aspects of fullerene application in PDT, in particular, therapeutic perspectives of their using, are considered in the review [83]. It is necessary to account that fullerenes may be more effective in PDT of hypoxia tumors (where the oxygen level is low), because they are able to change a mechanism of cell damage.

Thus, photo-sensitizing effect of fullerene derivatives may be performed at the expense of $^1O_2$ (C_{60}) and ROS enhancing ($C_{60}(OH)_{18}$). It is necessary to take into account the result of differential interaction with various structures, for example, binding to molecules of the lipid membrane, increasing of OH-generation etc. The effect may be additionally enhanced by using of light gathering antenna molecule and conjugates involving components reinforcing each other effect. The greatest phototoxicity is characteristic for mono-substituted C_{60} adducts that have no ability to self-aggregation, especially in the presence of calcium ions. The process is a dose- and concentration-dependent and the method of preparation of fullerene nanoparticles is of a great importance.

3. PHOTOPROTECTIVE ROLE OF FULLERENES

Some works are devoted to dual role of fullerene as pro-oxidant as well as antioxidant in UV-induced damages of different tissues, i.e. synergic or cumulative effects in PDT [84]. This problem is very important in production of cosmetic anti-sunburn means and also in some skin diseases. It is known that under UV-irradiation a set of events, leading to pro-apoptotic alterations (generation of ROS, cell rounding, bubbling of its surface etc.) and anoikis, is observed at the cellular level [85]. In mice, sebaceous glands fulfill a role of a main site for ROS generation in UVB-irradiation [86]. Use of C_{60} did not lead to emergence of toxicity, but index of ROS and index of apoptosis were decreased. More significant decrease was detected at simultaneous use of ascorbic acid and fullerene that, possibly, is provided by binding of fullerene with AA and decreasing of the Fenton reaction yield due to intercalation of AA to the heme pocket. Thus, use of a sum of fullerene+AA in combination with UV-irradiation is an effective remedy against oxidative damage of a skin.

C_{60}, incorporated into phospholipid membrane (74.5 nm), in the case of its injection before or after UVA irradiation, restores viability of cells by decreasing of 30% the level of ROS [87]. Liposome-fullerene under full absence of own toxicity significantly inhibited damage caused by chronic UVA irradiation of skin (Table 1.I) [88]. Water-soluble nanoparticles containing fullerene-C_{60} incorporated into liposomes (75.6 nm) showed dose-dependent protective effect HaCaT cells against OH-radicals, emerging under UVA- and UVB- irradiation. Prevention of cell degeneration was observed and any protective effect exists in the presence of C_{60} only [89].

Carboxyl-fullerene-C_{60} sufficiently reduced a blocking of proliferation of human keratinocytes induced by UVB-irradiation [90] and simultaneously decreasing an amount of cells with depolarized mitochondria. The mechanism of action had included interference of the preparation to the process of ROS generation by depolarized mitochondria, however, without participation of Bcl-2.

Tris-malonyl-C_{60} (C3) is able to protect, selectively, cells against intracellular and/or membrane changes in UV-irradiation [91]. It suggested that protection of epithelial cells A431 is linked with ability of namely this compound to maintain a network of cytoskeleton components and the integrity of coordination linkage, but only in the case, when fullerene derivative presents during irradiation. It is not excluded that the ability of a preparation to capture the superoxide radical before its conversion into OH- radical is a substantial component of the antioxidant action. C3 actually have been localized in the cell membrane.

Skin keratinocytes (HaCaT) were used for approbating of different fullerols: $C_{60}(OH)_{44}$ $8H_2O$ (SHH-F), $C_{60}(OH)_{6\text{-}12}$ (LH-F) and $C_{60}(OH)_{32\text{-}34}$ $7H_2O$ (HH-F) [92] in conditions of UVA and UVB-irradiation. Acceptor activity in relation to ROS was higher for HH-F and SHH-F, than for LH-F that determined a degree of their cytoprotective effect, which was greater in the case of SHH-F. Protective effect of SHH-F in relation to UVB- induced damages was higher than those in relation to UVA. Thus, SHH-F is a highly effective cytoprotector under UV-irradiation.

PVP/C_{60} [93] repressed the changes caused by UVA in HaCaT cells in the form of translocations of the transcription factor NF-κB into cytoplasm to the nucleus of keratinocytes. Protective effect and a blockage of abnormal signaling pathways were observed.

4. IONIZING RADIATION AND FULLERENES

It is known that impact of radiation in great degree is determined by generation of large amounts of ROS. Cytotoxicity or, on the contrary, antioxidant effects of fullerenes may play a substantial role in these conditions, increasing or compensating impact of ionizing radiation on the organism. Moreover, properties of the fullerenes may also be changed under action of ionizing radiation. For example, nanoC_{60} in certain conditions inhibits tumor cells growth, expressing properties of a sensitizer under radiotherapy, enhancing apoptosis [94]. However, γ-irradiation is able to influence on cytotoxicity of n-C_{60} (THF) [95]. Irradiated fullerenes not only did not cause oxidative stress and induce ERK-dependent death of different mammalian cells, but, on the contrary, they are protecting cells against oxidative stress, induced by THF-nC_{60} or hydrogen peroxide. Thus, γ-irradiation is able to alter physical and chemical properties of n-C_{60}, leading to full loss of its cytotoxicity and transforming it into a cytoprotective agent. In this case, it is not possible to exclude a possibility of functionalization of fullerene surface by products of water radiolysis that, in addition to a possible role of the residual THF in cytotoxicity, may cause the loss of cytotoxicity and acquisition of new properties by the fullerene.

Radioprotective (anti-radical) activity of hydrated fullerene C_{60}HyFn and its labile nanosize clusters at concentrations of 10^{-11}-10^{-6} mole/L was observed under X-ray irradiation of DNA (1-7 Gy *in vitro*) [96]. C_{60}HyFn at concentrations of 10^{-7}-10^{-6} mole/L protects nucleic acids against radical-induced damage. An optimal radioprotective concentration of C_{60}HyFn was equal to 1 mg/kg i*n vivo* (mice, intraperitoneally, 1 hour before or 15 min. after irradiation) at the lethal dose of 7 Gy. Lysine-C_{60} was used for preliminary treatment of human lymphoblastic cells AHH-1 before γ-irradiation (>400 mg/L) and did not show visible toxicity. This sufficiently increased the cell survival after irradiation by decreasing the apoptosis level in a dose-dependent manner [97].

Dendrite adduct of [C_{60}] fullerene, containing 18 carboxyl groups (C_{60}DF) [98], is able to protect human lymphocytes and intestine cells from consequences of impact of high doses of γ-irradiation. The process includes decrease of ROS level, inhibition of radiation-induced apoptosis and cells necrosis, DNA damages, oxidative stress. However, the process is not selective, because no difference between modest protective effect of the dendro-fullerene on normal fibroblasts (dose modifying factor=1.1 [99]) and on tumor cells was observed *in vivo*. $LD_{50/30}$ for mice, received 300 mg/kg of the dendro-fullerene before irradiation, was equal to 10.09 Gy in comparison with 8.29 Gy for control group. No protective effect was described for a dose of the drug of 200 mg/kg. Linked with antioxidant effects of C_{60}DF protective effect also was observed under irradiation of *zebrafish* embryos in a dose of 20 Gy and 40 Gy [100].

Observed protective effect of polyhydroxylated derivatives of fullerene attracts great attention, however, the mechanism of action is not clarified up to now, excluding its antioxidant action. It is known [101,102] that effects of fullerene derivatives are predominantly linked with mitochondria. It is supposed that nanosize particles may be captured by reticule-endothelial cells [103]. Namely, the specific localization of fullerene derivatives, may serve as decisive factor of their effector activity. The using of $C_{60}(OH)_{24}$ [104] in a dose of 40 mg/kg for 2 weeks before irradiation of the mice with a lethal dose of γ-irradiation (^{60}Co) has led to a decrease in mortality due to increase of immunity, reduction of oxidative damages and improvement of mitochondria functioning (by restoring of mitochondrial membrane potential) in comparison with control group of animals.

Fullerols demonstrated radioprotective effect during X-ray irradiation of animals (8 Gy) [105] exhibiting protective effect in relation to heart and liver against chronic toxicity induced by doxorubicin [106].

C_{60}/PVP and γ-CD/$C_{60,}$ as antioxidants, are able to stabilize radioprotective properties of β-carotene by preventing its oxidation [107]. This fact increases the interest to inclusion complexes as compensating drugs during cancer treatment by using radiotherapy.

The combined effect of sulfa-containing radioprotectors and C_{60}-derivatives resulted in reduction of side effects of the original drug (for example, amifostine) [108].

Fullerenes, serving as drugs, additionally can be transporters of radiomarkers and radioagents and as such intended to be used in radiotherapy and radiodiagnostics. For example, complex $C_{60}(OH)_{20}$ with $^{99m}Tc(CO)_3$ [109] rapidly reaches all tissues of the body except the brain and is retained there for 3 h without losing activity. Clearance is 24 h.

Complex $^{212}Pb@C_{60}$-malic acid [110] was stable under the beta decay of ^{212}Pb to ^{212}Bi and caused a weakening of ^{212}Pb myelotoxicity. $^{177}Lu_xLu_{(3-x)}N@C_{80}$, conjugated with IL-13 [111], was interesting for radioimmunotherapy (RIT).

To prove the efficiency of $C_{60}(OH)_x$ for RTD, ^{125}I-$C_{60}(OH)_x$ was used, which demonstrated more rapid and steady accumulation in cancer tumors in mice [112] compared to normal tissues.

The combined use of fullerene derivatives and radiotherapy is still insufficiently explored, but in future it could be a far more efficient procedure than even the PDT.

The synergistic effects in radiotherapy described earlier [113] becomes all more urgent now due to the development of nanomedicine.

5. CONCLUSION

Recent trends in the use of fullerene derivatives in medicine are related to development of nanoplatforms that contain drugs of different composition and are capable to carry out selective delivery of them to specific organs. The main medicinal targets are cancer cells of different types. It is believed that in this aspect the fullerenes are of great interest because of their opportunity to participate in the composition of such nanoplatforms in several roles: cytotoxic agent as well as, conversely, an antioxidant (these roles may change depending on accumulation in different organs and tissues) ones; as transporter of drugs; as photo- or radiosensitizer (or protector).

However, the complexity of the problem is that until now there is no predictive model of action of fullerene derivatives under concrete conditions for a specific cell type. Moreover, the set of possible mechanisms of the effect of fullerenes on the signaling pathways of apoptosis varied (Fig. 2) and depends on many factors that are difficult to administrate. All this hinders the active using of fullerene nanoparticles in medical practice. However, the individual success of some fullerene derivatives in a particular application, such as action against HIV, the selectivity to certain lines of cancer cells without damaging normal tissue, the possibility of using in theranostics [114] suggest good perspectives of fullerenes in the field of nanomedicine.

Apparently, porphyrin fullerenes of different composition that act well in transportation (drug delivery), PDT and targeting are highly successful.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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