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A Novel Nanoconjugate of Landomycin A with C_{60} Fullerene for Cancer Targeted Therapy: *In Vitro* Studies

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Abstract

Introduction—Landomycins are a subgroup of angucycline antibiotics that are produced by *Streptomyces* bacteria and possess strong antineoplastic potential. Literature data suggest that enhancement of the therapeutic activity of this drug may be achieved by means of creating specific drug delivery systems. Here we propose to adopt C_{60} fullerene as flexible and stable nanocarrier for landomycin delivery into tumor cells.

Methods—The methods of molecular modelling, dynamic light scattering and Fourier transform infrared spectroscopy were used to study the assembly of C_{60} fullerene and the anticancer drug Landomycin A (LA) in aqueous solution. Cytotoxic activity of this nanocomplex was studied *in vitro* towards two cancer cell lines in comparison to human mesenchymal stem cells (hMSCs) using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT) test and a live/dead assay. The morphology of the cells incubated with fullerene–drug nanoparticles and their uptake into target cells were studied by scanning electron microscopy and fluorescence light microscopy.

Results—The viability of primary cells (hMSCs, as a model for healthy cells) and cancer cell lines (human osteosarcoma cells, MG-63, and mouse mammary cells, 4T1, as models for cancer cells) was studied after incubation with water-soluble C_{60} fullerenes, LA and the mixture C_{60} + LA. The C_{60} + LA nanocomplex in contrast to LA alone showed higher toxicity towards cancer cells and lower toxicity towards normal cells, whereas the water-soluble C_{60} fullerenes at the same concentration were not toxic for the cells.

Conclusions—The obtained physico-chemical data indicate a complexation between the two compounds, leading to the formation of a C_{60} + LA nanocomposite. It was concluded that immobilization of LA on C_{60} fullerene enhances selectivity of action of this anticancer drug *in vitro*, indicating on possibility of further preclinical studies of novel C_{60} + LA nanocomposites on animal tumor models.

Keywords—C_{60} fullerene, Landomycin A, Complexation, Cytotoxicity, Membranotropic effect, Molecular modelling, Dynamic light scattering, Fourier transform infrared spectroscopy, Scanning electron microscopy, Fluorescence microscopy.

ABBREVIATIONS

4T1	Mouse mammary cells
C_{60}FAS	C_{60} fullerene aqueous solution
Cis	Cisplatin
DAPI	4′, 6 Diamidino 2 phenylindole
DLS	Dynamic light scattering
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
Dox	Doxorubicin
EPR	Enhanced permeability and retention
FCS	Fetal calf serum
FTIR	Fourier transform infrared spectroscopy
hMSCs	Human mesenchymal stem cells
LA	Landomycin A
MG-63	Human osteosarcoma cells
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide
PBS	Phosphate-buffered saline
PDI	Polydispersity index
SEM	Scanning electron microscopy

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INTRODUCTION

Landomycins (LAs) are a subgroup of angucycline antibiotics that are produced by *Streptomyces* bacteria and possess strong antineoplastic potential.[12] All natural LAs share the same aglycon (landomycinone) and vary only in the number of sugar residues in their linear glycosidic chain containing only di- and trideoxysugars (β-D-olivose and α-L-rhodinose).[4,12] It has been shown that LAs with longer saccharide chains have a higher activity towards tumor cell lines *in vitro*,[19,45] and that LA with six sugar residues (LA-A) constitutes the most potent family member. Although the molecular mechanisms of the action of LAs are still poorly understood, recent studies on another family member, i.e., LA with three sugar residues (LA-E), have indicated the crucial role of specific extramitochondrial hydrogen peroxide production and early caspase-7 activation upstream of mitochondria.[28]

Enhancement of the therapeutic activity of anticancer drugs by means of specific drug delivery systems is considered as one of the most promising approaches in current pharmacology and medicine.[15,26,47,48] Here we propose to adopt C_{60} fullerene as flexible and stable nanocarrier for drug delivery into tumor cells.[2,10,14,27,43] This idea is based on our previous extensive studies on the bioactivity of water-soluble pristine C_{60} fullerenes which demonstrated a preferable targeting of tumor cells.[20,32] This may be explained by their strong membranotropic properties[8,29,44] as well as by a low toxicity towards normal cells and tissues of the organism.[30,31,50] C_{60} fullerene easily forms complexes with various compounds, including the well-known anticancer drugs doxorubicin (Dox) and cisplatin (Cis) as we demonstrated earlier.[37–39] Moreover, the conjugation of Dox and Cis to C_{60} fullerene led to a significant increase of their cytotoxic activity *in vitro* and a therapeutic activity towards Lewis lung carcinoma *in vivo*,[33–35] thus confirming the role of C_{60} fullerene as a versatile drug delivery platform that can be widely applied in clinical oncology.

Previously, by means of such powerful physico-chemical methods as the small-angle neutron scattering and atomic force microscopy, we accomplished a pilot study of intermolecular complexation and reported the effect of binding between C_{60} fullerene and a novel anticancer drug candidate, i.e., LA (LA-A).[40] In the present work this result was further investigated in more detail using the dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectroscopy and molecular simulation techniques. The physico-chemical data were supplemented by *in vitro* studies of toxic effect of C_{60} + LA nanocomplex towards normal and tumor cells and its penetration into target cells.

EXPERIMENTAL

Material Preparation

A stable pristine C_{60} fullerene aqueous colloidal dispersion (C_{60}FAS) at the maximum concentration of 150 μg mL^{-1} was prepared as described earlier,[36,42] based on the original methodology which includes the transfer of C_{60} molecules from toluene into an aqueous phase under ultrasonication.

In all experiments, we used LA powder (99.5% purity according to the high-performance liquid chromatography data) which was extracted from culture of *Streptomyces globisporus S136* strain. LA was dissolved in methanol at the maximum concentration 200 μg mL^{-1}. Immobilization of LA on C_{60} fullerene (i.e., the C_{60} + LA mixture) was accomplished as follows[40]: the initial solution of C_{60}FAS and LA (with maximum concentration) were mixed in the volume ratio 1:1. The resulting mixture was ultrasonicated for 30 min, followed by magnetic stirring for 12 h by at room temperature.

DLS and ζ-Potential Determination

The size distribution and the ζ-Potential for C_{60}FAS and C_{60} + LA mixture were determined by DLS on a Zetasizer Nano-ZS90 (Malvern, Worcestershire, UK) at room temperature. The instrument was equipped with a He–Ne laser (5 mW) operating at 633 nm. The results were analyzed under the Smoluchowski approximation.

FTIR Study

FTIR spectroscopy was used to study the structural organization of pristine C_{60} fullerene with LA in aqueous solution. The FTIR spectra were recorded with a Perkin-Elmer BX-II spectrophotometer with a spectral resolution of 1 cm^{-1} in the range of 400–4000 cm^{-1}. The samples were examined as thin film between two NaBr plates. For optimum signal-to-noise ratio, 64 scans were recorded per spectrum.

Structure Calculations

The procedure for structural analysis of the C_{60} + LA nanocomplex corresponded to those used previously to study of the same nanocomplexes,[40] except that in the present work we modelled the structure with maximum coverage of the C_{60} fullerene surface by the ligand molecules. Briefly, the calculations of the spatial structure of C_{60} + LA nanocomplex were performed by molecular mechanics using the X-PLOR software with the CHARMM27 force field. Quantum-

mechanical calculations of partial atomic charges on C_{60} fullerene and ligand atoms were performed with the Gaussian03 software within the framework of DFT (B3LYP) using a 6-31G* basis set by the Merz–Kollman method.

Cell Culture

The cytotoxic activity of the C_{60} + LA nanocomplex was studied *in vitro* on human osteosarcoma cells (MG-63), mouse mammary cells (4T1), and primary human mesenchymal stem cells (hMSCs). The cell lines were obtained from the Collection of Microorganisms and Cell Cultures of the University of Duisburg-Essen. MG-63 and 4T1 cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal calf serum (FCS), 100 U mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin at 37 °C in humidified atmosphere at 5% CO_2. hMSC were cultured in mesenchymal stem cell growth medium (MSCGM BulletKit™; Lonza). 12 h prior to uptake experiments, the cells were trypsinized and seeded in 48-well plates at a density of 25×10^3 cells per well in 0.25 mL DMEM with FCS for MG-63 and 4T1 cells and with MSCGM for hMSCs.

Scanning Electron Microscopy (SEM)

The attachment of free and LA-immobilized C_{60} fullerene to the membrane of MG-63 cells, 4T1 cells, and hMSCs was visualized by SEM. Cells were cultured for 1 h either with or without C_{60}FAS, and rinsed twice with phosphate-buffered saline (PBS). Then the cells were fixed with 3.7% glutaraldehyde (Sigma-Aldrich, Taufkirchen, Germany) in PBS for 15 min. After a 2-fold washing with PBS, the fixed cells were dehydrated with an ascending sequence of ethanol (20, 40, 60, 80 and 96–98%). Subsequently, after evaporation of ethanol, the samples were left for 2 h at 37 °C and then analyzed by SEM. SEM was performed with an ESEM FEI Quanta 400 instrument with gold/palladium-sputtered samples.

Fluorescence Microscopy

The uptake studies of C_{60}FAS (75 μg mL^{-1}), of LA (100 μg mL^{-1}), and of the C_{60} + LA mixture (75 + 100 μg mL^{-1}) were carried out as follows. The cells were incubated with 5 μL dispersion/solution of the indicated compounds for 3 and 24 h, respectively. Immediately after the indicated time, the cells were washed three times with PBS to remove all dissolved compounds that were not attached to the cells. The cellular uptake was measured by transmission light microscopy and fluorescence microscopy with a Keyence Biorevo BZ-9000 instrument (Osaka, Japan), equipped with filters for 4′, 6 diamidino 2 phenylindole (DAPI, EX 360/40, DM 400, BA 460/50) at ×20 magnification. C_{60} + LA nanocomplexes were visible as blue fluorescing dots.

MTT and Live/Dead Assay

The cell viability was analyzed by an 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT) assay 72 h after the incubation of MG-63 cells, 4T1 cells and hMSCs with C_{60}FAS (75 μg mL^{-1}), LA (100 μg mL^{-1}) or the mixture of C_{60} + LA (75 + 100 μg mL^{-1}). The following volumes of dispersion were added to the cells (total volume of 250 μL per well): 0.5, 2.5, 5 or 10 μL, which corresponds to the following concentrations: 0.15, 0.75, 1.5, 3.0 μg mL^{-1} of C_{60}FAS, 0.2, 1.0, 2.0 and 4.0 μg mL^{-1} of LA, 0.15 + 0.2, 0.75 + 1.0, 1.5 + 2.0 and 3.0 + 4.0 μg mL^{-1} of C_{60} + LA mixture. The used ratio of concentrations in the studied mixtures, presumably, corresponds to the optimum binding efficiency of LA to C_{60} fullerene clusters as evidenced by structural analysis (see below).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; Sigma, Taufkirchen, Germany) was dissolved in PBS (5 mg mL^{-1}) and then diluted to 1 mg mL^{-1} in the cell culture medium. The culture medium of the incubated cells was replaced by 300 μL of the MTT solution. The cells were incubated for 1 h at 37 °C under 5% CO_2 in humidified atmosphere. 300 μL of dimethyl sulfoxide (DMSO) was added to the cells. After 30 min, a 100 μL aliquot was taken for spectrophotometric analysis with a Multiscan FC instrument (Thermo Fisher Scientific, Vantaa, Finland) at λ = 570 nm. The absorption of incubated cells was normalized to that of *control* (untreated) cells, thereby indicating the relative level of cell viability. Each concentration of the studied compounds was run in triplicate and normalized to blank controls, containing the equivalent volume of culture medium.

A live/dead assay was carried out according to the following protocol. 72 h after the incubation of MG-63 cells, 4T1 cells, and hMSCs with 5 μL of C_{60}FAS (75 μg mL^{-1}), LA (100 μg mL^{-1}) and C_{60} + LA mixture (75 + 100 μg mL^{-1}), respectively, the cells were washed with PBS and stained with a live/dead viability/cytotoxicity assay for mammalian cells (L3224, Invitrogen Co.) to evaluate the cell viability. 150 μL of the calcein AM and ethidium homodimer-1 working solution were directly added to the cells. Afterwards, the cells were subsequently incubated for 30 min at 37 °C. After incubation, the stained cells were imaged by fluorescence microscopy (Keyence). The live/dead kit determines the cell viability based on

the cell membrane integrity. Living cells are stained by calcein AM which emits a green fluorescence (517 nm) when excited by blue light (494 nm), whereas dead cells are stained by EthD-1 which emits a red fluorescence (617 nm) when excited by green light (528 nm). All experiments were carried out in triplicate.

Statistics

Statistical analysis was performed by conventional methods of variation statistics. The significance of the differences between the control and experimental measurements was estimated by Student's t test using the Origin 8.0 software (OriginLab Corporation, USA). The difference between the compared values was considered to be significant at $p < 0.05$.

TABLE 1. The hydrodynamic particle diameter, the *PDI* value, and the ζ-Potential for C_{60}FAS and C_{60} + LA aggregates at room temperature in aqueous dispersion, measured by DLS method.

Samples	Diameter (nm)	*PDI*	ζ-Potential (mV)
C_{60}FAS	184 ± 18	0.209	− 17.0 ± 2.0
C_{60} + LA mixture	190 ± 20	0.219	− 1.0 ± 0.1

RESULTS AND DISCUSSION

Material Characterization

It has been established that the size of C_{60} fullerene particles directly correlates with their biodistribution and toxicity.[21,30,31,50] Depending on the size, C_{60} fullerene particles can penetrate through the plasma membrane into the cell or be adsorbed on the surface of the membrane.[8,9,29,44] Therefore, DLS measurements of C_{60}FAS and C_{60} + LA dispersions were performed (Table 1).

C_{60} fullerenes and C_{60} fullerenes with LA form particles (aggregates) in water with an average hydrodynamic size 184 and 190 nm, respectively, in good agreement with previous results.[40] The obtained result

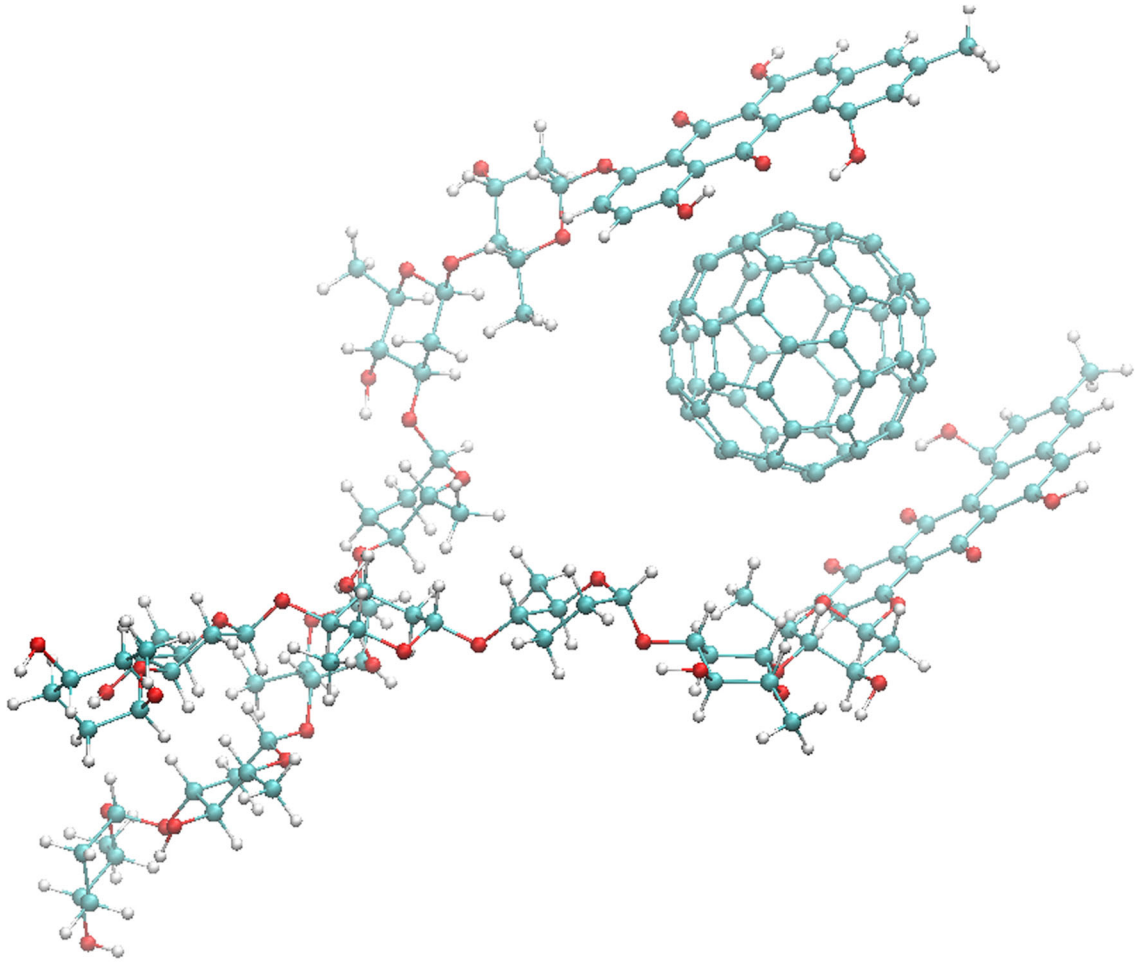

FIGURE 1. Calculated structure of 1:2 C_{60} + LA nanocomplex.

means that presence of LA solution only slightly affects the distribution of C_{60} fullerene aggregates in aqueous solution. The value of polydispersity index (*PDI*, about 0.21) indicates a low polydispersity of these systems. The magnitude of ζ-Potential measured for C_{60} + LA mixture shifted to -1 mV from the initial value of − 17 mV measured for C_{60}FAS, indicating an interaction between C_{60} fullerene and LA.

The calculated structure of the C_{60} + LA nanocomplex (Fig. 1) indicates the possibility of binding of up to two LA molecules to the surface of one C_{60} fullerene molecule, suggesting a favorable hydrophobic complexation effect. Both LA ligands are stabilized by π-stacking with the C_{60} molecule surface which is known as the dominant force in C_{60} fullerene–aromatic interactions.[33–35,37–40] Importantly, the maximal binding ratio 1:2 provides rough estimate of the ratio of concentrations (1:1…1:2) to be further used in *in vitro* experiments and corresponding to optimal binding efficacy of LA to fullerene clusters in solution.

Further characterization of the C_{60} + LA interaction was carried out by FTIR spectroscopy. The FTIR spectrum of C_{60} fullerene with LA mixture is shown in Fig. 2 together with the reference spectra of C_{60}FAS and LA. The spectrum of C_{60}FAS exhibits four intense peaks at 1423, 1180, 576, and 526 cm^{-1} attributed to the C–C vibrational modes of the C_{60} molecule and weak bands at 3372 and 1666 cm^{-1}, corresponding to water hydrogen-bonded O–H stretching and bending,

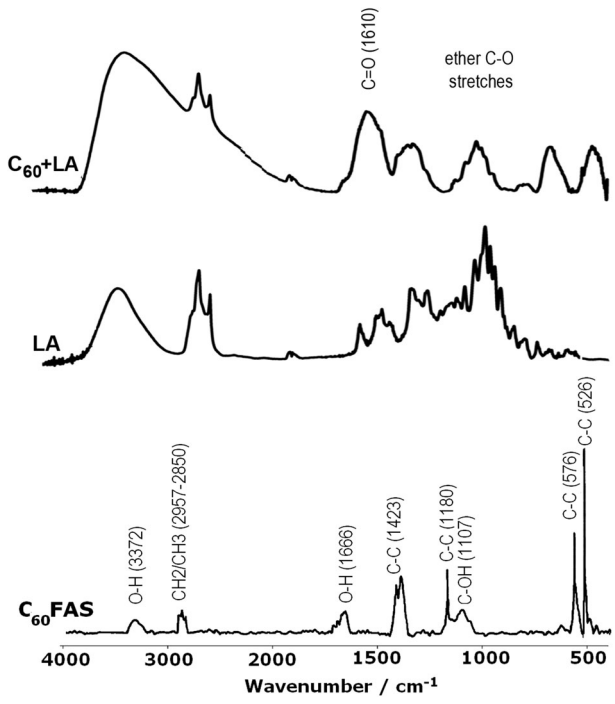

FIGURE 2. FTIR spectra of C_{60}FAS, LA and C_{60} + LA mixture.

respectively; the broad intense band near 1107 cm^{-1} is attributed to C–OH stretching; a weak band at 2957–2850 cm^{-1}, corresponds to C–H stretching.[18] Two strong broad bands centered at 3410 and 3373 cm^{-1} corresponding to hydrogen-bonded O–H stretching [*v*(OH)] of the methanol and a weak band at 1734 cm^{-1} [δ(OH)] were observed in the FTIR spectra of the LA and C_{60} + LA mixture, respectively, because the measurement was performed on alcohol substance. The O–H stretching and CH_3 stretch bands of the LA in the FTIR spectra of the methanol solution and C_{60} + LA mixture had low intensities and overlapped with bands of the solvent. Aromatic C=C stretches of LA at 1368 and 1450 cm^{-1} had low intensities and overlapped with bands of the C–C vibrational modes and C–O–H bending of the C_{60}FAS system.[18] The group of peaks from 1111 to 975 cm^{-1} centered at 1055 cm^{-1}, corresponding to dialkyl- and alkyl–aryl ether C–O stretches of LA. Also, the characteristic strong band at 1610 cm^{-1} assigned to a C=O stretch conjugated with the aromatic system is found both for the LA and C_{60} + LA.

It is known that the stacking of aromatic surfaces results in a high-frequency shift of C=O functional groups by few cm^{-1}.[7] Such shifts were initially expected for the C_{60} + LA interaction, which, however, they were not observable on the measured FTIR spectrum of the mixture due to strong line broadening (Fig. 2). However, the presence of C–OH vibrations in FTIR spectrum of C_{60}FAS provides another important source of C_{60} + LA nanocomplex stabilization due to intermolecular hydrogen bonding to proton acceptor groups of the ligand. Note, such additional stabilization is well known for aromatic–aromatic π-stacked complexes in solution.[6]

In Vitro Experiments

Pure LA and the C_{60} + LA mixture showed a clear concentration-dependent cytotoxic effect towards MG-63 cells, 4T1 cells, and hMSCs after 72 h incubation (Fig. 3). No toxic effects of C_{60}FAS towards these cell lines were observed even at the highest concentration. The same result was observed for C_{60}FAS *in vitro* against to normal cells (thymocytes) and cancer cells (Ehrlich ascitic carcinoma, leukemia L1210 and HeLa).[30,31,50] LA alone was toxic for all three cell types, and its cytotoxicity increased with increasing LA concentration. In particular, IC_{50} values for LA were 1.81, 0.55 and 0.88 μg mL^{-1} for MG-63, 4T1 cells and hMSCs, respectively. Interestingly, at the increasing concentration of the C_{60} + LA mixture, we observed a higher toxicity for cancer cells and a weakly pronounced toxicity (up to 40%) for hMSCs. These data were also confirmed by analysis of IC_{50} value of

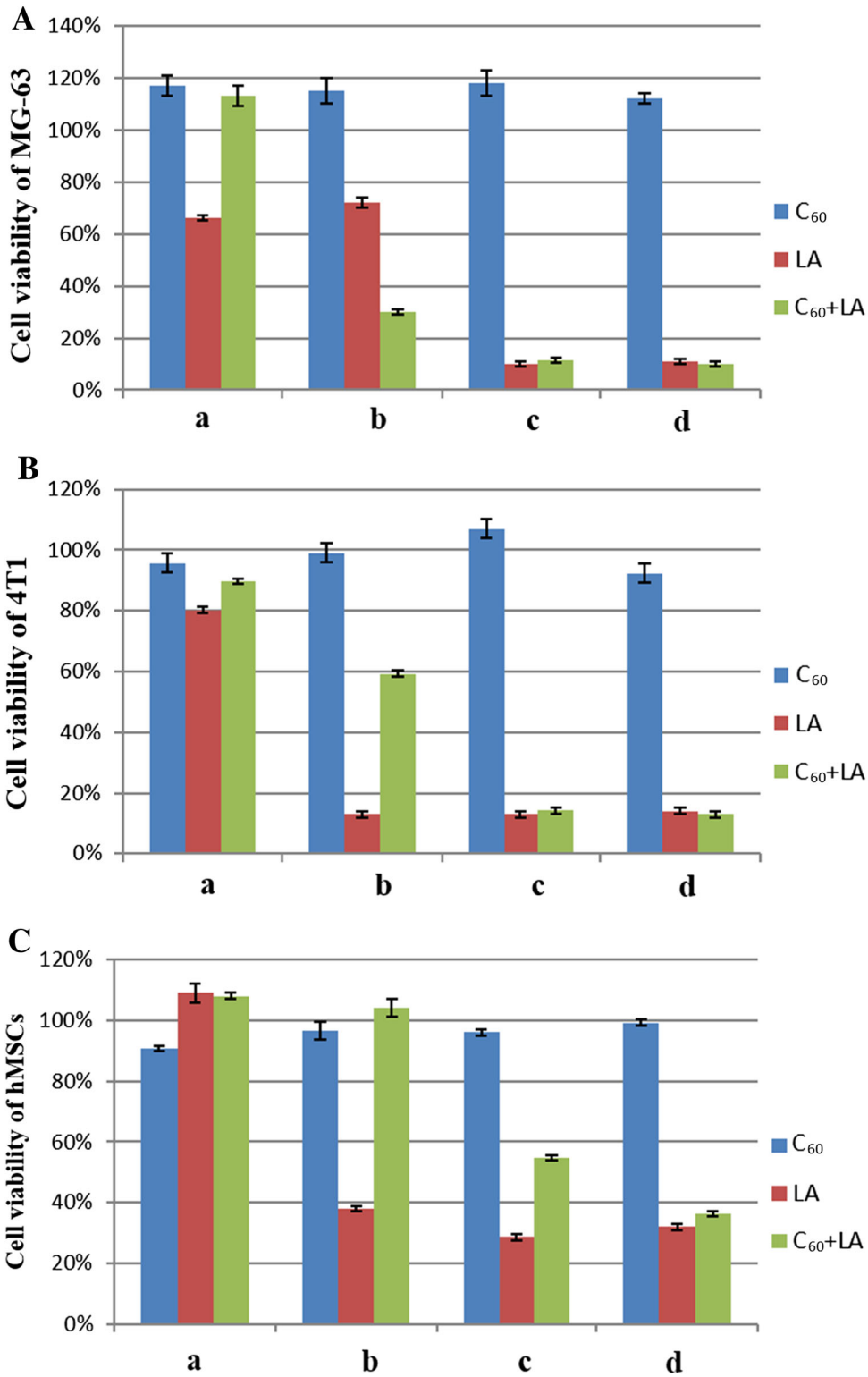

FIGURE 3. MTT assay for MG-63 cells (A), 4T1 cells (B) and hMSCs (C) after 72 h of incubation with C_{60}FAS, LA and C_{60} + LA mixture at different concentrations: 0.15, 0.2 and 0.15 + 0.2 μg mL^{-1} (*a*), 0.75, 1.0 and 0.75 + 1.0 μg mL^{-1} (*b*), 1.5, 2.0 and 1.5 + 2.0 μg mL^{-1} (*c*), 3.0, 4.0 and 3.0 + 4.0 μg mL^{-1} (*d*). Data are given relative to the untreated control samples ($p < 0.05$) and represent the mean ± SD of three independent experiments.

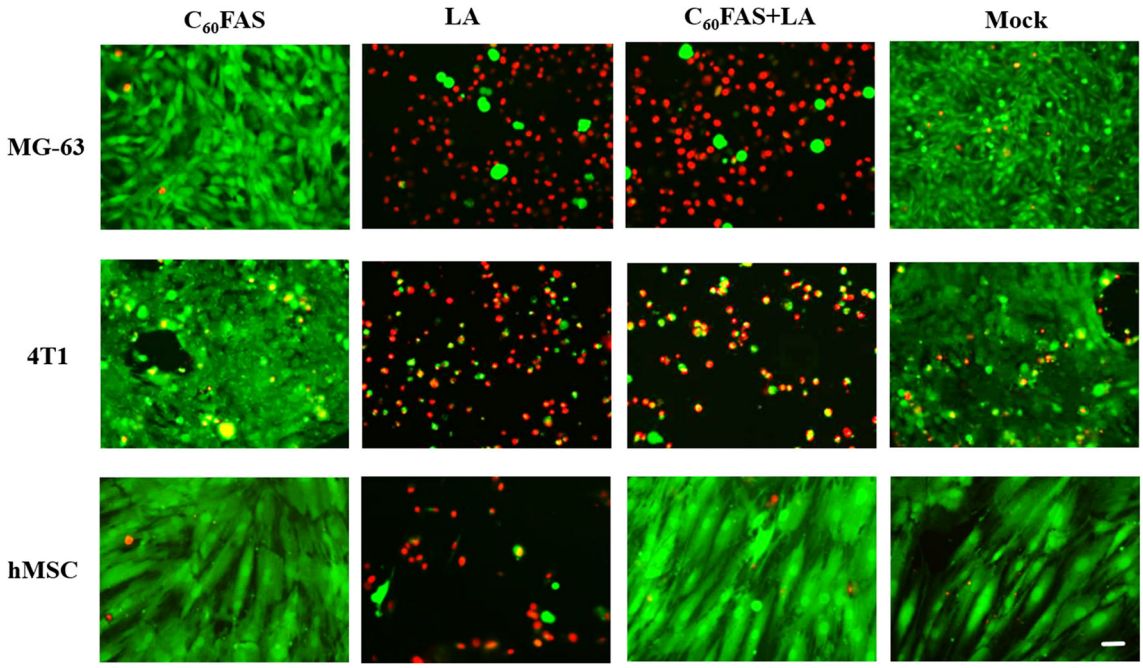

FIGURE 4. Representative live/dead staining of MG-63 cells, 4T1 cells and hMSCs after 72 h of incubation with C_{60}FAS (1.5 μg mL^{-1}), LA (2.0 μg mL^{-1}) or C_{60} + LA mixture (1.5 + 2.0 μg mL^{-1}) and mock (untreated cells). Scale bar 20 μm.

C_{60} + LA nanocomplex: it decreased 2.5-fold for MG-63 cells (from 1.81 down to 0.74 μg mL^{-1}), but gradually increased 2-fold for hMSCs (from 0.88 up to 1.58 μg mL^{-1}). The live/dead assay gave the same results: C_{60}FAS was not toxic for the cells, LA killed almost all cells and the C_{60} + LA mixture, in contrast to LA alone, showed high toxicity for cancer cells and low toxicity for hMSCs (Fig. 4).

The SEM micrographs of MG-63 cells, 4T1 cells, and hMSCs, incubated with C_{60} fullerene, LA and C_{60} + LA mixture, as well as without any additions (as a control) are shown in Fig. 5. The SEM images of single cells were chosen to represent the whole population in each group as all cells in the group had the same morphology. The SEM images underline the results obtained from live/dead assay, i.e., at a LA concentration of 2 μg mL^{-1} both types of cancer cells as well as the primary cells were dead in contrast to the C_{60} + LA mixture, where we observed a high toxic effect for cancer cells, but only a low toxicity for primary cells.

The membranotropic effect of LA and C_{60} + LA nanoparticles on MG-63 cells and hMSCs (as a representative model for cancer and primary cells) was studied by fluorescence microscopy. In the Fig. 6, the uptake of LA and C_{60} + LA nanocomplex into cells after 2 h of incubation is shown. It was revealed that LA weakly fluoresces at DAPI channel (blue) of fluorescence microscope in the hMSC and MG-63 cells. In the control (untreated cells) no blue fluorescence was detected (data not shown). Immobilization of LA on C_{60} fullerene leads to enhancement of LA fluorescence inside the cells in a concentration-dependent manner. Thus, C_{60} fullerene enhances LA's entry inside tumor cells due to its membranotropic activity, thus improving cytotoxic activity of these nanocomplexes. Taking into consideration that similar effects of C_{60} fullerene were already observed by us for C_{60} + Dox and C_{60} + Cis nanocomplexes, one may conclude that enhancement of drug uptake into tumor cells is the unique feature of C_{60} fullerene, which may be of high importance for further pre-clinical trials of the C_{60} fullerene–drug nanocomplexes.[25,41]

The peculiarity of the biological effect of the water-soluble C_{60} fullerenes is their ability to selectively damage of the tumor cells, whereas they do not affect the normal cells.[23,46] On the other hand, C_{60} fullerene, as a carrier of antitumor drug, thanks to its powerful antioxidant properties,[5,51] effectively reduces the side (toxic) effects of drug relative to the normal cells.[1,35]

It is known that the blood vessels in most solid tumors are characterized by intense angiogenesis, high density and increased vascular permeability, defective vascular networks and defective or depressed lymphatic drainage in the intratissue space of the tumor,[22] which allows nanocompounds to penetrate, accumulate and hold in tumors. In addition, due to impaired endocytosis, the tumor cells are able to accumulate more and maintain longer, than the normal cells,

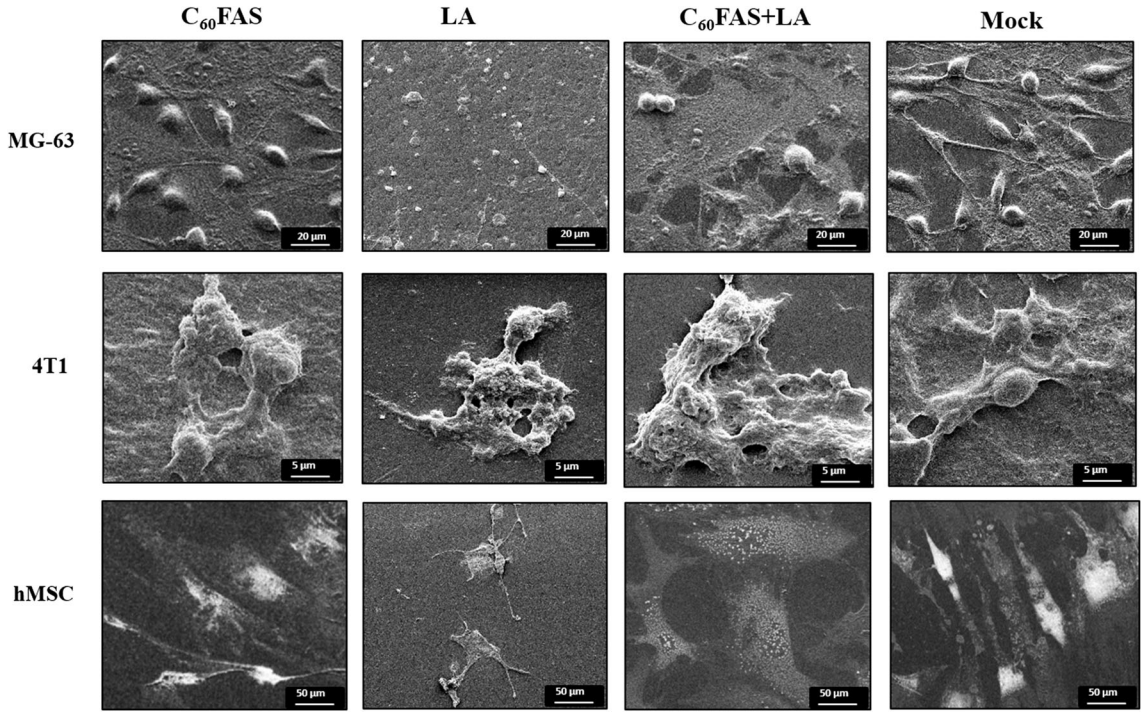

FIGURE 5. SEM micrographs of MG-63 cells, 4T1 cells and hMSCs after 72 h of incubation with C_{60}FAS (1.5 μg mL^{-1}), LA (2.0 μg mL^{-1}) or C_{60} + LA mixture (1.5 + 2.0 μg mL^{-1}) and mock (untreated cells).

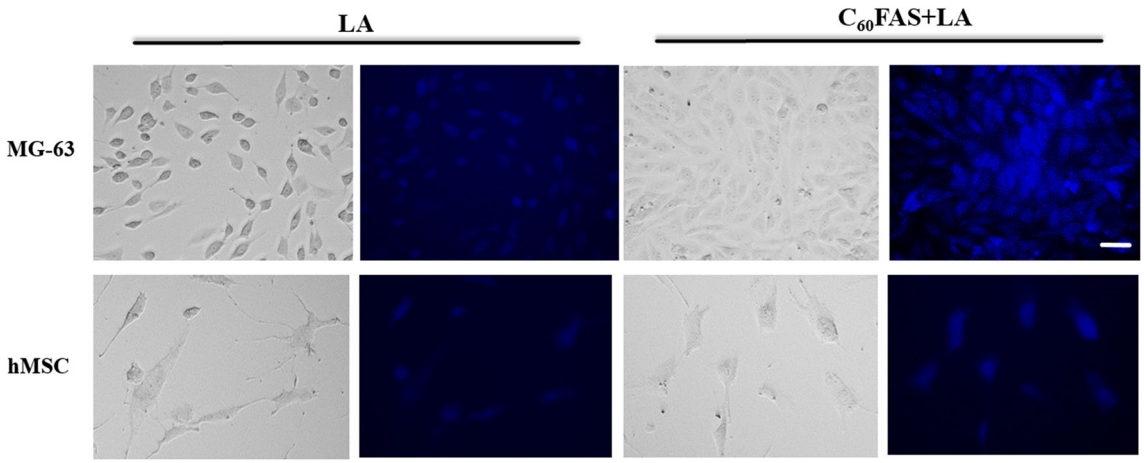

FIGURE 6. Transmission light microscopy and fluorescence microscopy of MG-63 and hMSC cells after 2 h of incubation with LA (2.0 μg mL^{-1}) and C_{60} + LA mixture (1.5 + 2.0 μg mL^{-1}). The blue fluorescence indicates the co-localization of LA and C_{60} + LA nanocomplex on the cells. Note that C_{60} fullerene alone is not fluorescent. Scale bar 20 μm.

exogenous compounds, especially with high molecular weight.[49] Therefore, it can be assumed that with an increase in the size/mass of C_{60} molecule due to its complexation with the chemotherapeutic drug and simultaneous association with the serum proteins, there is a passive accumulation of these nanoparticles in the tumor through the enhanced permeability and retention (EPR) effect in the tumor vasculature.[3,11,13,24] In this regard, the recent study[16] clearly demonstrated the feasibility of tracking and quantifying the delivery kinetics and intratumoral biodistribution of C_{60} fullerene-based drug delivery platform, consistent with the EPR effect on short timescales and passive transport to tumors. On the other hand, it was found that C_{60} fullerenes increase the sensitivity of tumor cells to the action of cytostatics due to their interaction and non-recognition by ABC-transporters.[17,34]

CONCLUSION

A complexation between pristine C_{60} fullerene and LA molecule in aqueous solution was characterized by molecular modelling, DLS and FTIR techniques. Cytotoxic activity of this nanocomplex was studied *in vitro* towards two cancer cell lines in comparison to hMSCs using MTT test and a live/dead assay. It was shown that C_{60} fullerene nanoparticles were non-toxic for the cells, LA at the concentration of 2 μg mL^{-1} killed all tested cell lines, while its nanocomplex with C_{60} fullerene at the same concentration of LA demonstrated high toxicity for cancer cells and much lower toxicity for mesenchymal stem cells. The same results were obtained by live/dead assay: C_{60} + LA mixture had shown high toxicity for cancer cells, but not for the mesenchymal stem cells. These data were supported by fluorescence microscopy studies, which have shown higher uptake of C_{60} + LA nanocomplex by tumor cells, and lower one by mesenchymal stem cells. The obtained results indicate on importance of using C_{60} fullerene as a nanoplatform for drug delivery, and, in particular, huge potential of C_{60} + LA nanocomplex as a novel anticancer agent.

AUTHORS' CONTRIBUTIONS

The work presented here was carried out in collaboration between all the authors. RP, JR, VO and YP created and characterized nanomaterials. VB and VS performed *in vitro* and fluorescence microscopy studies. OL and SP characterized nanomaterials using FTIR analysis. ME performed the computer simulations. UR synthesized and characterized C_{60}FAS. M. Epple and YP coordinated the experimental work, analyzed the data, performed the statistical analysis, and wrote the manuscript. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

V. Bilobrov, V. Sokolova, S. Prylutska, R. Panchuk, O. Litsis, V. Osetskyi, M. Evstigneev, Yu. Prylutskyy, M. Epple, U. Ritter, J. Rohr declare that they have no conflicts of interest.

ETHICAL APPROVAL

Neither human studies, nor animal studies were carried out by the authors for this article.

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