REVIEW

Nanotransporters for anticancer drugs, modifications, target molecules

Pavel Kopel[1], Dorota Wawrzak[2], Amitava Moulick[1], Vedran Milosavljevic[1] and René Kizek[1]

[1] Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic - European Union;

[2] Institute of Chemistry, Environmental Protection and Biotechnology, Jan Dlugosz University of Czestochowa, Armii Krajowej 13/15, PL-42-201 Czestochowa, Poland - European Union;

* Author to whom correspondence should be addressed; E-Mail: kizek@sci.muni.cz; Tel.: +420-5-4513-3350; Fax: +420-5-4521-2044.

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The nanoparticle based drugs, mostly in liposomes, are already approved for clinical use or under clinical investigation. Many attempts are made to improve nanoparticles sizes, shapes and surface modifications that lead to prolongation of drug circulation in blood stream and targeting to cancer cells. Thus small molecules like polyethylene glycol and targeting ligands like folic acid, peptides, antibodies, aptamers and nucleic acids are bound on the surface of nanoparticles with the aim to increase specific cell uptake. Very promising are multifunctional nanoparticles that combine both diagnostic as well as delivery role together.

Keywords: Liposomes; ferritin; carbon nanomaterials; magnetic nanoparticles

1. Introduction

The concept of using a particle measured in the nanoscale as carriers of drugs and vaccines appeared over three decades ago. Advances in nano medicine has evolved and has raised hopes for the implementation methods of striking antitumor therapy selectively in tumor mass, while reducing the risk of a wide range of side effects, which are encumbering modern pharmacology. Nanoparticles are attractive as drug delivery platforms because it is relatively easy to influence their properties and modify their features, so that they can be useful in creation of effective and precise medicine carriers. Meaningful are not only dimensions of the carrier enabling tissue penetration, but also their shape, and developed different functionalities of surface.

Current progress in the field of nanobiotechnology has led to the development of a new area of nanomedicine, associated with the application of nano biomaterials, both for diagnostic and therapeutic aims creating a new category of nano particles called theranostics. The main expectations and challenges in this regard relate to nano-magnetic properties, received bioengineering methods, with potential used in the transport of drugs, particularly anticancer drugs used in therapy determined using molecular targets. Unique physicochemical properties of magnetic nanoparticles promise hope for the development of modern cancer nanomedicine, acting, inter alia, technological breakthrough in the area of targeted drug delivery and gene therapy of cancer using magnetic hyperthermia, tissue engineering, marking the tumor cells and the molecular magnetic resonance imaging.

Nanotechnology in medicine and health care was initiated over forty years ago with deliverance of the first therapeutic and diagnostic agents in a safer and more efficient manner [1]. Convergention of diagnosis and therapy carried out through exploitation of nanopar-

ticles resulted with increasing number of theradiagnostics went out from research stage and being commercialized or having reached clinical stage.

2. Graphene, graphene oxide and carbon nanotubes

Graphene is a two-dimensional layer of carbon atoms with a thickness of single atom, of a hexagonal arrangement of atoms in a shape of honeycomb, and is often visualized as a homogeneous network of a large size. Graphene has many extraordinary properties such as an extremely high mechanical strength and flexibility, good thermal and electrical conductivity, is nearly transparent. Medical applications of graphene are built around its bacteriostatic and bactericidal properties, which are pledged with selected other features open extremely wide field of possibilities. Graphene oxide (GO) can be prepared by oxidation of graphite and in its structure are oxygen atoms in epoxy, hydroxy and carboxy groups. Biomedical applications relate to the whole family of graphene derivatives. Antibacterial properties of graphene and graphene oxide correspond to two phenomena. The first is a purely mechanical effect of destroying cell membranes by the sharp edges of graphene flakes and GO. The second is destructive to many strains of bacterial interaction of oxygen introduced into the cells on the surface of the GO [2].

Medical uses of carbon materials are intensively researched especially carbon nanotubes (CNTs). Carbon nanotubes (CNTs) take the form of a hollow cylinder with a rolled-up graphene built. CNTs are used as drug carriers enable the continuous and constant dosing of pathological cells. It may additionally comprise an antibody or specifically targeting the enzyme activity [3]. An example would be the use of MWNTs containing cisplatin, the use of which resulted in inhibition of tumor cell growth [4]. Similar results were obtained by combining doxorubicin with carbon nanotubes in breast cancer [5], or the attachment of carboplatin in the treatment of bladder cancer [6]. Nanomedicine is an extremely important area in which nanotubes can find a variety of uses, both in therapy and diagnosis. Efforts are also made in attempts to combine carbon nanotubes with active particles to create modern target drug transporters, which are particularly important for the pharmaceutical industry [7,8].

The use of carbon nanotubes as a carrier is possible thanks to the progress of research on the chemical modification [9]. Carbon nanotubes can be subjected to functionalization with different active particles responsible for target recognition (targeted therapy), imaging and treatment. In this way, a multifunctional system for transporting a drug can greatly improve the pharmacological profile [9,10]. Carbon nanotubes are also used as nanocontainers. Nanotubes filled with different chemical substances can be used in tumor therapy, diagnostic, and as contrast agents [10].

The first clinical tests are run on coating with nanotubes metal or metal oxide nanoparticles, and at the same time obtaining a surface ligands (folic acid or the corresponding glycoprotein) providing transport of nanoparticles to the tumor cells. Such particles after intravenous administration to achieve the target are subjected to an external magnetic field, which leads to a controlled heating of the metal particles and, consequently, destruction of the transformed cells. The results indicate that this method is more accurate than chemotherapy, carries also less risk of side effects and generates lower costs.

Carbon nanotubes also fulfill a role of gene carrier. Gene therapy is a promising treatment for cancer and genetic disorders. For the transport of viral genes there are special and non-viral carriers (e. g. liposomes, polymers, micro- and nanoparticles). The first ones carry a risk of side effects such as immune response, inflammation and oncogenesis. In contrast, no viral carriers, but more secure, do not always provide the appropriate level of gene expression. Therefore, researchers are making efforts to seek new, more efficient vehicles [7]. High molecular weight and a cationic nature of functionalized carbon nanotubes (f-CNT) allow electrostatic interaction with plasmid DNA. In order to test the ability of f-CNTs to form complexes with nucleic acids and their translocation were combined in various ratios f-CNT and the plasmid

DNA containing the gene of β-galactosidase marker. Obtained images demonstrated the presence of CNT-DNA complexes. F-CNT nanotubes were present in the form of beams, between which there plasmids in the form of annular clusters or super-coiled structures. The study of gene expression level of β-galactosidase showed penetration of these complexes to the cells. Furthermore, it was found that 5-10 times greater levels of gene expression for f-CNT complexes and DNA than for the same helix [11,12].

Carbon nanotubes have also been used as carriers of antigens. Connection of the external walls of the nanotubes with synthetically produced peptides, as for example. Epitopes of antigens create a system which can induce an immune response in a living body [13].

3. Magnetic nanoparticles

Magnetic nanoparticles (MNP) are made of an inorganic core, e.g. iron oxide, cobalt or nickel coated with substances being compatible with respect to the tissue, to which has been implemented [14]. One of the most important features is the MNP to superparamagnetism used in clinical diagnostic techniques. Introduction of MNP to the tested tissue bears effect of disorder of the local magnetic field in the tissue resulting in decrease of the relaxation time, the phenomenon used in magnetic resonance imaging [15,16]. Using MNP significantly improves possibilities of distinction between tumor and healthy tissue. Among the available contrast agents using nanoparticles there are superparamagnetic iron oxide, used for liver imaging called Combidex used in the diagnosis of metastases with a diameter of 5-10 nm in the lymph nodes [17]. In addition to tumor tissue imaging MNP are used to observe the cardiovascular system, especially in the detection of atherosclerotic plaques, and other diseases of the cardiovascular system. MNP can be further combined with organic dyes and fluorescent like rhodamine or fluorescein isothiocyanate (FITC), allowing to define the extent of tumor resection intraoperative study.

Other application of MNP is delivery of medicine to specific pathological tissue by utilizing the affinity of the ligands used in surface and magnetism which allows manipulating with pharmaceuticals through the external magnetic field. Biocompatibility, non-toxicity and high level of accumulation in tumors cause that magnetic nanoparticles are also used in intracellular hyperthermia. This therapy involves the use of MNP and alternating magnetic field to produce a significant amount of heat in tumor cells. Depending on the temperature and time of generated heat it causes death of the tumor cell or at least increase their sensitivity to radiotherapy or chemotherapy [18].

4. Liposomes and their modificationsce

Liposomes have been the most successful drug delivery carriers. Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Because of their biocompatibility, biodegradability, low toxicity and possibility to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased rate of both as an investigational system and commercially as a drug delivery system. The drugs inside the liposomes are protected from oxidation and degradation during circulation in bloodstream. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ, or system where the contents will be utilized [19]. Since the introduction of Doxil (a PEGylated liposomal doxorubicin), several products have been approved worldwide [20]. Liposomes are mostly used for the passive targeting having blood lifetime one or two days. It is required that the size of liposomes is less than 200 nm to facilitate fenestration through the leaky blood vessels around the tumour site. In general, the release of drug from liposome has to be slow enough not to let free drug to be removed quickly from the bloodstream.

Most of the liposomes for cancer treatment were approved on the basis of reduced side effects due to their passive targeting capabilities. Other liposomal anticancer products, such as DaunoXome and Myocet were primarily used to reduce toxicity in comparison to free

doxorubicin and not to sustained release of encapsulated drug [21].

Except of conventional liposomes there are modified ones called stealth liposomes [22]. Coating liposomes with PEG reduces the percentage of uptake by macrophages and leads to a prolonged circulation and, therefore, make available abundant time for these liposomes to leak from the circulation. Stealth liposomes are transporters with a membrane composed of phospholipid bilayer used to deliver drugs into a cell. A liposome can be made of naturally phospholipids with mixed lipid chains coated by polymers of PEG and colloidal in nature. Stealth liposomes are a new generation of compounds used for controlled drug release [23]. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil (Janssen Biotech, Inc., Horsham, USA) or Caelyx (Schering-Plough Corporation, Kenilworth, USA) for the treatment of solid tumors [24]. There are plenty of anticancer liposomes under clinical trials. To the group belong PEGylated lipoplatin, S-CKD602 [25] and NL CPT-11 containing cisplatin, CKD-602 (a camptothecin analogue) and irinotecan (CPT-11), respectively. From the trials it follows that lipoplatin is less toxic and of the same activity like cisplatin applicable for various cancers [26] [27] [28].

To improve efficacy of cancer treatment by liposomes it is necessary to modify either liposomes or their surface by peptides, RNA or antibodies which also serve for targeted delivery to specific cancer tissue. For example, dual-ligand liposomal delivery system for targeting the delivery of paclitaxel to lung cancer was prepared. The specific ligand peptide HAIYPRH (T7) and the cationic cell-penetrating peptide TAT were connected with phospholipid via a polyethylene glycol (PEG) spacer to prepare the dual-ligand liposomes (T7/TAT-LP-PTX) [29]. Active targeting molecules displayed better cell selectivity but were shadowed by the poor tumor penetration effect. Cell penetrating peptides could increase the uptake of the carriers but were limited by their non-specificity. Dual-ligand system may possess a synergistic effect and create a more ideal drug delivery effect. Thus, liposome system modified with RGD, TAT and cleavable PEG was designed [30]. Liposomal drug delivery system conjugated with cyclic arginine-glycine-aspartic acid-tyrosine-lysine peptide (cRGDyk) was developed to improve therapeutic efficacy in a mice model of bone metastasis from prostate cancer [31].

5. Natural nanotransporters – ferritin

Ferritin belongs to proteins with cage like structure, which can be used to bind molecules in its cavity. Maxiferritins are formed from 24 subunits 12 nm in diameter with 8 nm cavities with MW = 480 kDa and miniferritins formed from 12 subunits 8 nm in diameter with 5 nm diameter cavities of MW = 240 kDa [32]. Mostly maxiferritins are used, especially horse spleen ferritin for its commercial availability. Ferritin wide occurrence as well as its ability to reversibly store and release iron ions to the living cells has attracted the interest of researchers worldwide.

It was found that the cavity can be utilized for storage of other ions and molecules and can be utilized for synthesis of nanoparticles with defined size. Apart from interior cavity, the surface of apoferritin can be modified. This offers further possibility of delivering encapsulated drug to a target cell in more effective way and minimalizing thus side effects particularly toxicity to nontarget organs by drugs.

Apoferritin was employed to encapsulate anticancer drugs cisplatin and carboplatin [33] [34]. It is well known, that clinical application of platinum-based anticancer drugs is largely limited by severe general toxicity and drug resistance. Drug delivery systems with tumor-targeting potential are highly desired for improving the efficacy and applicability of these drugs. The delivery of platinum drugs cisplatin, carboplatin and oxaliplatin by encapsulating each of them in the cavity of apoferritin was studied. The encapsulation was achieved through reassembly route at pH 2.0 and 7.4, respectively, in saturated drug solution. Carboplatin and oxaliplatin complexes in apoferritin exhibit a marginal cytotoxicity towards this cell line under similar concentrations [34]. A novel antibody-drug

conjugate was synthesized incorporating ferritin cisplatin nanoparticles [35]. An average of three molecules of monoclonal antibody (mAb) Ep1 to the human melanoma-specific antigen CSPG4 were conjugated to a single ferritin cage encapsulating about 50 cisplatin molecules. The flow cytometry demonstrated specific binding to a CSPG4(+) melanoma cell line, but not to a CSPG4(-) breast carcinoma cell line. As compared to the cisplatin-containing ferritin nanoparticle alone, which inhibited thymidine incorporation more efficiently in breast carcinoma than melanoma cells, the mAb-derivatized nanoparticle had a 25-fold preference for the latter. Anticancer activity was also studied on a methylene blue-encapsulated apoferritin complex. The complex shows cytotoxic effects on MCF-7 human breast adenocarcinoma cells when irradiated at the appropriate wavelength [36].

Ferritin can be genetically modified to present a peptide sequence on the surface [37]. Thus Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys (RGD4C)-modified ferritin can efficiently target tumors through RGD-integrin interaction. It was shown that after being precomplexed with Cu(II), doxorubicin can be loaded onto RGD modified apoferritin with high efficiency. These doxorubicin-loaded ferritin nanocages showed on U87MG subcutaneous tumor models a longer circulation half-life, higher tumor uptake, better tumor growth inhibition, and less cardiotoxicity than free doxorubicin [37]. Ma-Ham et al. [38] studied daunomycin, an anthracycline antibiotic drug, that is used for specific types of cancer treatment such as acute myeloid leukemia and acute lymphocytic leukemia, encapsulated within apoferritin cage.

The apoferritin-doxorubicin complex has been formed by reassembly of the apoferritin sphere in the presence of doxorubicin [39]. The doxorubicin encapsulation was carried out using direct and step-wise change of pH of the solution from 2.5 to 7.4. It was found that up to 28 molecules of doxorubicin can be capsulated per apoferritin protein and no significant drug leakage occurs during the first two days.

Magnetic particle mediated transport in combination with nanomaterial based drug carrier has a great potential for targeted cancer therapy. Doxorubicin encapsulated into the apoferritin was conjugated with magnetic particles and investigated by capillary electrophoresis with laser-induced fluorescence detection (CE--LIF). This combination of magnetic particles and drug encapsulated in apoferritin can be potentially used for magnetic resonance imaging, thermotherapy and chemotherapy [40]. Apoferritin and liposome encapsulated forms of doxorubicin were prepared and their toxicity were compared with doxorubicin alone and Myocet on prostate cell lines [41]. Three different prostatic cell lines PNT1A, 22Rv1, and LNCaP were chosen. The toxicity was compared using the MTT assay, real-time cell impedance-based cell growth method (RTCA), and flow cytometry. The efficiency of doxorubicin entrapment was 56% in apoferritin cages and 42% in the liposome carrier. Apodox IC50 was determined as follows: 603.1, 1344.2, and 931.2 nM for PNT1A, 22Rv1, and LNCaP, respectively.

6. Conclusions

The main objective of research in recent years is to provide a multifunctional nanoparticles and nanomaterials whose properties can be controlled in the body through the local environment and external factors, e. g. external magnetic field. Many pharmaceutical companies have their own research programs aimed at the introduction of new products based on nanoparticles and nanomaterials and improve current pharmaceuticals. Nanosubstances appeared to be commercially and started to be used widely in the diagnosis or treatment of cancer, among others. Intensive nanotechnology research in the future will lead to extend the functions of nanoparticles in nanodiagnostics, in nanopharmacology and in many new medical applications.

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