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Pharmaceuticals for Binary Radiotherapy

V. N. Kulakov[a,b], A. A. Lipengol'ts[b,c], and N. L. Shimanovskii[d]

[a] *Lomonosov Moscow State University, Moscow, 119991 Russia*
e-mail: vikov@ rambler.ru

[b] *Burnazyan Federal Medical Biophysical Center, Federal Medical Biological Agency, ul. Zhivopisnaya 46, Moscow, 123186 Russia*
e-mail: lipengolz@mail.ru

[c] *Blokhin Cancer Research Center, Kashirskoe shosse 24, Moscow, 115478 Russia*

[d] *Pirogov Russian National Research Medical University, ul. Ostrovityanova 1, Moscow, 117997 Russia*
e-mail: shiman@rsmu.ru

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Abstract—Improvement of the efficiency of external-beam cancer radiotherapy still remains an urgent medical problem. One of the approaches to this problem is provided by binary radiotherapy which ensures a minimum radiation load on normal body tissues surrounding the pathological area.

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The aim of this paper is to introduce binary radiotherapy (BRT) and give a brief analysis of the main lines of research and development of drugs for binary radiotherapy.

Binary radiotherapy is based on the localization of physical interaction of ionizing radiation with chemical elements (absorption of heat neutrons or X-ray photoeffect) directly in a target being irradiated. Therewith, the radiation dose of the target is lower that the radiotolerance limit of normal cells, due to which selective destruction of tumor cells takes place, provided the latter are saturated with a special preparation (Fig. 1). In other words, high energy is delivered to tumor due to specific properties of the preparation rather than due to perfect characteristics of the radiation source.

Unlike external-beam radiotherapy which employs nothing but an ionizing radiation source (neutrons, photons, electrons, or ions of certain elements, such as H, C, etc.), BRT requires two components: a radiation source and a special preparation containing a chemical element capable of interacting with external radiation to produce secondary energy. There are two types of BRT, which are differentiated by the physical process: neutron capture therapy (NCT) and photon capture therapy (PCT) (see table).

At present the BRT technology is under development. First of all, preparations with a high differential accumulation factor (tumor-to-normal tissue concentration ratio) should be developed. The differential accumulation factors of known pharmaceuticals:

Principal physical characteristics of binary radiotherapy technologies[a]

Type of binary radiotherapy	External radiation source	Principal physical process
Neutron-capture therapy	Thermal and epithermal neutrons (up to 10 keV)	$^{10}B + n \rightarrow \alpha(1.47\ MeV) + {}^{7}Li\ (0.84\ MeV) + \gamma\ (0.48\ MeV)$; $^{157}Gd + n \rightarrow [{}^{158}Gd] \rightarrow \gamma\ (7.88\ MeV) + \bar{e}$ (Auger and Coster–Kronig electrons, 5−9 keV) + $\bar{e}$ conversions (45−66 keV)
Photon-capture therapy	X-ray emission (up to 300 keV)	$\gamma + M \rightarrow [M^*] \rightarrow M + \gamma\ (\sim E_{shell}) + \bar{e}\ (E_\gamma - E_{shell}$, Auger electrons, etc.)

[a] (E_{shell}) Binding energy of K-shell electrons and (E_γ) incidence photon energy.

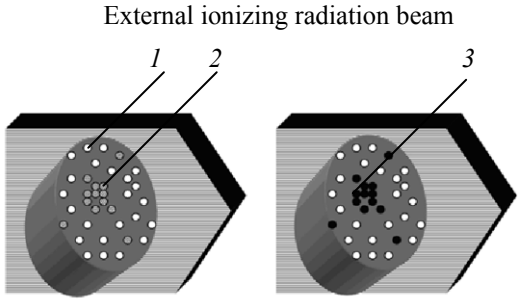

Fig. 1. Schematics of binary radiotherapy: (*1*) normal cells, (*2*) tumor cells, and (*3*) damaged tumor cells.

[^{10}B]-*p*-dihydroxy-L-phenylalanine and sodium mercapto-*closo*-dodecaborate $Na_2[B_{12}H_{11}SH]$ are about 3.

In some countries (USA, Japan, Netherlands, Sweden, Finland, Argentina, etc.), thermal and epithermal neutron beams for clinical applications have already been developed. In Russia, experimental NCT research is being conducted [1–4], a medical beam system is being developed at the Moscow Engineering Physics Institute (MEPhI), a prototype photon radiation source has been fabricated, and active radiobiology research in the field of PCT is in progress [5, 6].

Specialized preparations for both types of BRT are virtually lacking. A series of research was carried out on NCT with compounds enriched with the stable isotope ^{10}B. There are two ^{10}B-containing pharmaceuticals used in clinical research (vide supra). A series of tests on dogs with spontaneous melanoma of oral cavity, performed at the MEPhI, gave evidence for a high therapeutic efficiency of inoperable melanoma: Complete tumor resorption was observed in 80% of cases [7]. The technology of NCT with replantation was tested to success in therapy of spontaneous osteosarcoma [8]. It was shown that disodium mercapto-*closo*-dodecaborate is biologically active and can find applications in new NCT technologies [9].

The iodine-containing X-ray contrast agents and gadolinium-containing magnetic resonance (MR) contrast agents most commonly used in PCT research are not tumor-specific. Their differential accumulation occurs due to breaking through the blood–brain barrier (for brain tumors); in some cases, intratumor injections are used. The PCT experiments with contrast agents on mice [10] and rabbits [11] with c implanted tumors, as well as on dogs with spontaneous brain tumors [12] demonstrated a high efficiency of this technology. Clinical trials (Phase I) were performed in the USA in mid-1990s [13]. Functionalized gold nanoparticles, too, have found wide application in PCT research. Both *in vitro* and *in vivo* results show that they are more efficient than contrast agents [14]. However, whether gold nanoparticles can be introduced in clinical practice is still unclear because of the insufficiency of knowledge on their delayed effects. Thus, development of new BRT agents with accumulation factors higher than 3 is quite an urgent task.

Known MR contrast agents like magnevist [15] are used in MR diagnostics of malignant neoplasms. It should be noted that no one of such clinically used contrast agents does not show tendency for specific accumulation in tumors of different etiologies. Final diagnosis is based on the results of complex investigation. At the same time, the visualization of malignant neoplasms by means of tumorotropic agents (chemical elements which exhibit affinity to specific organs and tissues) allow therapeutic activities, including external-beam radiotherapy, to be initiated as early as possible, and this favors better treatment results. Moreover, 3D visualization of the target and information on tumor pharmacokinetics of the drug are necessary for planning the BRT procedure. An optimal approach which would ensure timely treatment is to use chemically identical drugs in computer and MR tomography and BRT.

What are fields where we could expect success in the development of such drugs? To answer this question, let us analyze the potential of various agents tried previously in an attempt to solve this problem. It should be emphasized that this is a problem common in many respects both for BRT and chemotherapy. At the same time, in radiotherapy one should care about nothing but how to increase the concentration of required elements in tumor, which makes the problem much easier to solve.

PORPHYRINS COMPLEXES WITH GAGOLINIUM AND OTHER PARAMAGNETIC LANTHANIDES

There agents have never tested in BRT. The methods of synthesis of porphyrin derivatives are difficult to scale up, which prevents commercialization of porphyrin drugs.

Antibody-Based Drugs

Complexes of MR contrast agents with monoclonal antibodies may prove highly specific contrast agents

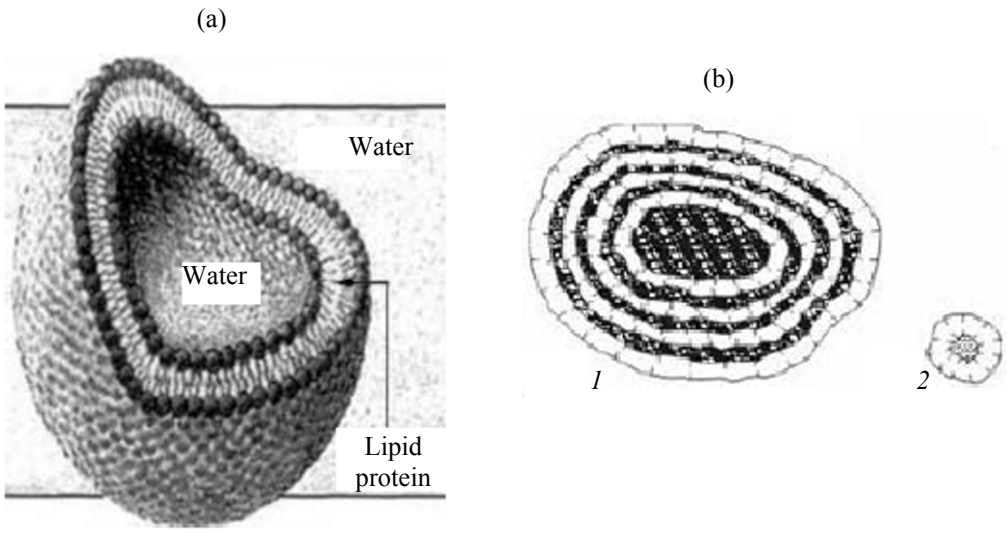

Fig. 2. (a) Structure of bilayer liposome and (b) types of liposomes: (*1*) multilayer and (*2*) monolayer: (dashed fields) water and (light fields) bilayer lipid layer with the hydrophobic parts of molecules pointing inward.

not only for normal but also for pathologically changed tissues. Such complexes have already been prepared, for example, immune complexes specific to the carcinoembryonic antigen of colon cancer [16]. Unfortunately, researchers revealed a lot of obstacles to the development of tumor-specific contrast agents. The most important of them are that the surface of malignized cells contains too little available binding sites and that the synthesized antibodies exhibit quite low specificity. The biotransformation of a contrast agent–antigen complex in the body starts with cleavage of immunoglobulin and, therefore, a loss of specificity.

Polymers

Binding gadolinium complexes to polymers allows one to combine functional diagnostics with high-resolution MR imaging [17]. For visualization of blood vessels, a Gd–diethylenetriaminepentaacetic acid (DTPA) are covalently bound to macromolecular substances, such as albumin, dextrane, polylisine, etc. In animal experiments, such MR contrast agents allowed improvement of the quality of tomograms of arteries and veins less than 1 mm in diameter. They can also be used in direct and indirect MR lymphography. Such complexes were found to break through the blood–brain barrier not only in the case of severe damage of the vasculature, but also in the case of increased capillary permeability (for example, inflammation in the active phase, tumors, or ischemic disease) [18]. However, macromolecular contrast agents may cause body sensitization. The enzyme-linked immunosorbent assay (ELISA) was used to show that the sensitization of rats with Gd–DTPA–albumin, Gd–DTPA–dimeglumine, Gd–DTPA–dextran, and Gd–DTPA–polylysine induces production of IgG antibodies specific to Gd–DTPA [18]. Undeniably, further research is required to determine the potential of immune response to macromolecular MR contrast agents in clinical practice.

Liposomal Formulations

Liposomes are spheres with a thick hydrophobic shell and aqueous solution inside and outside [19]. Unilamellar liposomes are from 20 nm to a few hundred nanometers in diameter. The residence time of liposomes in the circulatory system is short (from a few minutes to a few hours). Liposomes readily adsorb blood plasma proteins, after which the liposomes are entrapped by macrophages and then destroyed and removed from the body. The problem for liposomes to break through natural body barriers was solved in an unexpected and a fairly facile manner. As known, liposomes include various lipids. It was found that cells which tend to remove liposomes from blood circulation can be “deceived” by imparting strong hydrophilicity to liposome surface, thus making it inaccessible or even invisible, by modifying it with lipids conjugated with hydrophilic polymers (MW 1000–6000 Da); as a result, the circulation time of liposomes becomes much longer.

Lipid molecules can form two different types of structures in water: liposomes (Fig. 2) and micelles.

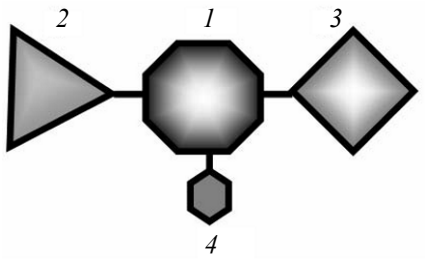

Fig. 3. Drug design: (*1*) fragment responsible for physicochemical characteristics (solubility, sign and value of the total charge, dimensions, etc.); (*2*) fragment responsible for targeting to a specific site or tissue; (*3*) fragment responsible for the diagnostic or therapeutic effect, in binary radiology inclusive; and (*4*) fragment which makes possible quantitation of the drug.

Whether one or the other form is prevailing depends on the structure and properties of lipids and special additives.

Many drugs of new generation are combined with delivery systems to allow slow drug release to specific organs and target cells and to improve the pharmacologic characteristic of the drug [20]. Even though liposomes are fairly unstable, their size together with the targeting fragment make this dosage form a promising candidate not only for antitumor drug, but also for MR tomography and BRT applications.

Dendrimer-Based Drug Formulations

Dendrimers are branched, tree-like structures [21]. They can be assembled (synthesized) from monomer units of various chemical structures. Dendrimers are highly stable in aqueous media, which is quite an important property for drug formulations. Dendrimers can be conjugated with fragments with various functional groups, and, therefore, impart desired characteristics to the resulting structure. Such fragments can include therapeutic or diagnostic drugs conjugated with dendrimer molecules by spacers or antibodies, as well as other targeting molecules; the role of modifying fragments can also be played by compounds which impart desired physicochemical characteristics to the final construction [21].

Of great interest are the works of the research groups from the USA [22, 23] and Netherlands [24] on dendrimer formulations with the third-to-fifth generation polyamidoamine dendrimers as fragment providing required physicochemical properties (Fig. 3). In [22, 23], matrix metalloproteinases were used to activate an antitumor cationic peptide bearing a gadolinium complex as the fragment responsible for the diagnostic or therapeutic effect and/or fluorescent label as the fragment responsible for visualization of tumor margins during surgery.

The Dutch researchers published their results soon after the USA group. We would like to emphasize that the Dutch group work mostly with a polyimine dendrimer (PPI) synthesized by their developed scheme. The third-generation dendrimer PPI has 18 reactive groups, which allows strong covalent bonding with chemically diverse antitumor and diagnostic agents. The main goal of the Dutch researchers, specifically, to activate the cationic peptide for facilitating its transport through the tumor cell membrane, was successfully achieved. Quite apparently, new antitumor drug formulations on the basis of such construction will be developed in the near future.

The fact that liposomes and dendrimers both lack affinity to tumor cells and have similar mechanisms of

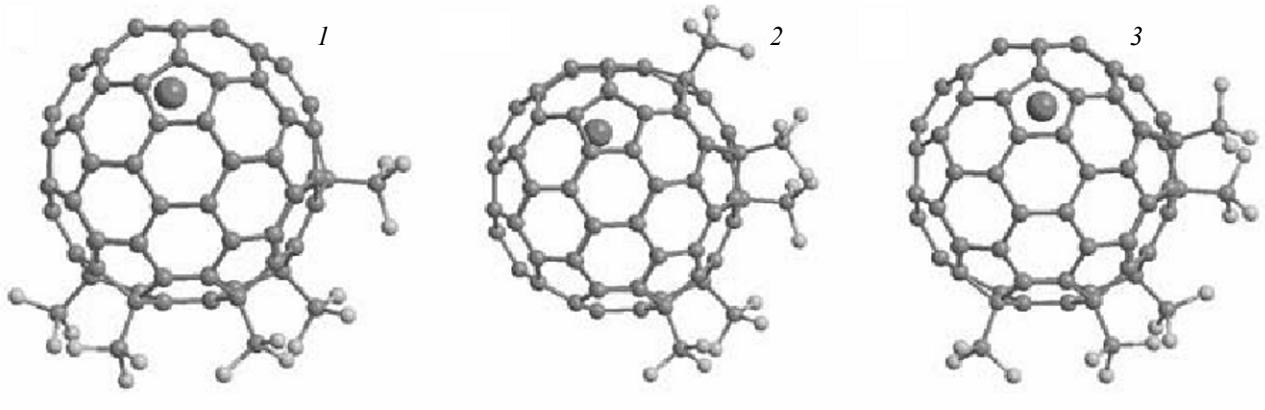

Fig. 4. Structures of trifluoromethyl-substituted gadolinium fullerenes C_{82}. Thermodynamic stability order: *1* > *2* > *3*.

permeation into normal and malignant cells makes it necessary to combine these structures with fragments which would target their encapsulated drug to tumor cells. The transport containers for targeted drug delivery are liposomes, dendrimers, and nanocapsules from biodegradable polymers. They protect drugs from degradation in the body, can deliver water-insoluble substances to cells, and prolong the half-life time. The most suitable targeting structures are peptides containing fragments complementary to tumor receptors. The drug formulations to be developed will feature reduced immune reactivity and high specificity to different tumor cells.

In our opinion, an optimal design for tumorotropic agents for application in MR tomography diagnostics and BRT is presented in Fig. 3 [25].

Nanocapsules of biodegradable polymers [26] and development on their basis of antitumor drug formulations is one of the ways to practical realization of the task to construct new-generation drugs.

At first glance, the encapsulation of the paramagnetic gadolinium atom in fullerenes (Fig. 4) [27, 28] opens up the way to new drugs via introduction of various substituents to the fullerene core, the more so as proton-containing substituents in, for example, fullerene C_{60}, is known to enhance the T_1 signal [29].

At the same time, the contradictory results of toxicological testing of nanosized carbon carriers (nanotubes and fullerenes) [29] does not allow us to consider them as safe drug carriers. For this reason, gadolinium fullerene derivatives (Gd@C[60]) are not good candidates for application in MR tomography diagnostics and BRT.

Gold nanoparticles (Fig. 5), which are able to adsorb antibodies, are suitable for BRT. Such constructions reach 45 nm in size and contain up to 10 pmol IgG per 1 mL (see, for example, [30]). The size of such constructions can be easily measured by UV spectrophotometry.

Gold particles, like carbon nanotubes and fullerenes, undergo no biodegradation in the body. By this reason, strong evidence for the safety of gold nanoparticles for humans is required before Au-based drug formulations will be introduced in the BRT practice.

The method of molecular recognition which is used in the development of highly specific catalysts [31] has scarcely been used in pharmaceutics. Compared to enzyme–substrate, antigen–antibody, and receptor interactions, the chemical approach to molecular recognition or imprinting is more versatile. Imprinting allows much more selective recognition with subsequent binding of virtually any chemical compound, provided the latter is capable of forming inclusion compounds with the chosen organic matrix. New drug formulations whose mechanism of accumulation in the target is based on imprinting open up the way to minimize cross-binding of the drug construction beyond the target site. Note that at present no such formulations are under development.

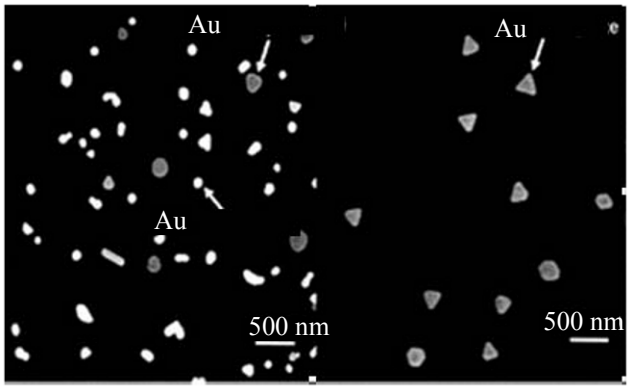

Fig. 5. Gold nanoparticles (left) before and (right) after purification.

In summary we would like to formulate the principal conclusions concerning development of preparations for BRT.

(1) Nanoscale size and biodegradability of the fragment responsible for the physicochemical characteristics of the drug and creating optimal conditions for its permeation into tumor cells.

(2) Third-to-fifth generation dendrimers hold the greatest promise as a basis for development of a universal transport platform.

(3) Most promising seem to be PPI dendrimers with a high content of end functional groups.

(4) The fragment responsible for targeting the drug construction to specific cells and organs is presently technically available.

(5) Effort should be taken to search for short peptides assisting drug permeation into tumor cells.

Thus, more efficient tumorotropic preparations are

need for large-scale introduction of BRT in clinical practice. Successful solution of this task will not only increase efficiency of the therapy of malignant neoplasms in itself, but also will allow computer and MRT diagnostics for their visualization, and will also provide a way to quantitative assessment of accumulation and elimination of such preparations, which is quite necessary to improve the BRT planning process.

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