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REVIEW

THE INFLUENCE OF CONJUGATES ON THEIR FUNCTIONALITY IN THE THERAPY OF SOLID TUMORS

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Abstract: Polymeric carriers may deliver therapeutic substances and biomolecules to the destination place in unchanged form. Their use in the therapy of solid tumors in comparison to unbound drugs brings about several benefits, such as drug dose reduction as well as adverse effects decrease. The primary advantage of the described conjugates is the control release of the pharmacologically or biologically active substance, by means of the use of proper chemical bonds between constituents of drug delivery system (DDS). The system may be transported in an organism in two ways: passively, by using the enhanced permeability and retention (EPR) effect, or actively, by placing on its surface a molecule which selectively creates the way to its destination. To elaborate such a DDS type requires the knowledge of the biological nature of neoplastic tumor and the chemical properties of the carrier and the therapeutic substance or biomolecule.

Keywords: conjugate, polymeric carrier, EPR effect, targeted therapy, amide and ester connector

Abbreviations: 4-ABA - 4-aminobutyric acid, 6-ACA - 6-aminocapronic acid ASGR - asialoglycoprotein receptor, BH3 - homological peptide 3, DAO - D-aminoacid oxidase, DDS - drug delivery system, EPR - enhanced permeability and retention, HPMA - N-(2-hydroxypropyl)metacrylamide, GCSF - granulocyte colony stimulating factor, GDNF - glial cell-derived neurotrophic factor, Gly-Phe-Leu-Gly - glycine-phenylalanine-leucine-glycine, LHRH - luteinizing hormone releasing hormone, MTX - methotrexate, NG - nitroglycerine, PEG - polyethylene glycol, RES - reticuloendothelial system, R_h - small inertia moment, ROS - reactive oxygen species, t1/3β - circulation time, Tf - transferrin, TNF-α - tumor necrosis factor alpha, VEGF - vascular endothelial growth factor, XO - xantine oxidase, ZnPP - zinc protoporphyrine

The rising number of new substances with high therapeutic potential, but of low molecular mass, poorly water-soluble, transported to target tissue in a limited manner, gives the reason for requirement for new drug forms. Nowadays, controlled kinetics release therapeutic system are widely used (1). Expansion of new drug forms requires the use of both biological and chemical knowledge to achieve time and spatial control over a drug molecule *in vivo*, and the primary goal is to achieve maximal clinical benefits. The new systems are: injectable microspheres, nanospheres, amphiphilic polymer carriers like polymeric miceles. The next step to change the kinetics and mechanism of release of active substance from polymeric carriers is construction of conjugates, that is connections of drug molecule with polymeric carriers by chemical bond (2).

It is a challenge for pharmaceutical industry involved in oncology to improve drugs in such a fashion that they would eliminate neoplastic cells with the smallest damage to healthy cells and host cells (3). The conjugates developed to meet this aim consist of two chief components: polymer and pharmacologically active substance, alternatively, with the connector between them (4). The type of the bond connecting polymeric carrier with therapeutic substance influences its release. The development of molecular biology and oncologic etiopathogenesis initiated a new generation of conjugates, which involve a molecule that enables the controlled system of drug release (DDS - drug delivery system) to be delivered to the pathologically changed place of destination – the organ, tissue, or cellular compartment. Moreover, because of quick elimination and

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biotransformation of low molecular pharmacologically active substances, to increase the substances half-life in organism special techniques are used (5). Controlled bio-distribution of conjugates depends then on several factors: the properties of polymeric material, its molecular mass, shape and size of DDS, type of bond between the polymer and therapeutic substance and the quantity of therapeutic substance contained in DDS. The therapeutic effect depends on an optimal selection of conjugate components in relation to specific pathology (6).

Enhanced permeability and retention effect, the gate to passive DDS

As a result of the binding of low molecular therapeutic substances with polymeric carrier of high molecular mass we engender a huge molecular system of drug delivery. Small molecules pass through the cellular membrane into the cell by diffusion, meanwhile the bigger ones by endocytosis, which is a slower than diffusion process. To facilitate the reach of cellular compartment through DDS, the local concentration of DDS is increased in the target location. The accumulation of drug molecules in the place of action is of a special importance in the anti-neoplastic therapy of solid tumors with the use of conjugates polymer, namely therapeutic substance without molecule for targeted therapy (7). This sort of DDS gets to the tumoral tissue by means of passive transport because of the higher permeability and retention of neoplastic tissue. This effect is a consequence of non-connectivity and enhanced permeability of the endothelium of the blood vessels of neoplastic tumor. Moreover, because of rapid tumor growth, its lymphatic system is weakly developed or non-existent, which renders the removal of molecules reaching the tumor difficult and their accumulation (8).

Copolymer which first proved the existence of enhanced permeability and retention (EPR) effect was poli(maleic acid styrene-co anhydrate) with neocarcinostatin. Clinical studies of patients suffering from hepatocelullar carcinoma proved its efficacy in the reduction of tumor mass and level of neoplastic markers. Since 1990, this conjugate has been used in Japan in the therapy of hepatocellular carcinoma (8).

Studies on mice with N-(2-hydroxypropyl)-metacrylamide (HPMA)-doxyrubicine conjugate, where Gly-Phe-Leu-Gly (glycine-phenylalanine-leucine-glycine) tetrapeptide was a connector, confirmed a six times higher drug concentration in neoplastic tissue (melanoma), in comparison to healthy tissues, as well as reduction of toxicity and longer half-life in relation to non-conjugated doxorubicin. The copolymer was applied clinically in 1994. The next achievement of this pharmaceutical branch is the HPMS-camptothecin conjugate. The insoluble in blood plasma camptothecin was conjugated with HPMA with the use of metacrylol-glycine connector, as water-soluble substance administered intravenously. However, because of little differences of half-life ($t_{1/2}$) between the conjugated form, and the unbound camptothecin and similar intensification of adverse effects, further studies were dropped. Similarly, because of little solubility of paclitaxel, a HPMA-Gly-Phe-Leu-paclitaxel conjugate was elaborated, which allowed to lengthen the half-life from $t_{1/2}$ = 1.2 h for unbound paclitaxel to $t_{1/2}$ = 6.5 h for the conjugate. However, it is not used because of the risk of neurotoxicity, which is similar for the conjugate and the free drug form (4). At the end of the 20th century, polyethylene glycol (PEG) conjugates with camptothecin and paclitaxel, as well as dextran and doxorubicin, and polymeric conjugates with plasma proteins were created (4, 9).

Maeda – the discoverer of the EPR effect, with collaborators, utilized the possibility of generating stress oxidation which is the feature of reactive oxygen forms (ROS - reactive oxygen species), used as anti-neoplastic factor, and their chemical binding with PEG. With the use of ROS generators: xantine oxidase (XO), D-amino acid oxidase (DAO) and zinc protoporphyrine (ZnPP), they created the following conjugates: PEG-XO, PEG-DAO, PEG-ZnPP. Given to mice, the uptake of these conjugates by neoplastic cells increased due to EPR effect (4). One of the methods enhancing the EPR effect is administration of nitroglycerine (NG), which freeing the amino radical of nitrite ion (NO_2^-) leads to dilatation of blood vessels (9). It has been confirmed that application of nitroglycerine ointment on skin in the neoplastic tumor area causes higher production of NO_2^- in cancerous tissues than in healthy ones (10). In neoplastic tissues, in which oxygen concentration is small, pH low, NO_2^- turns into nitrogen oxide (NO). (9). The interaction of nitrogen oxide (NO) with peroxide anion (O_2^-) produced in the course of leukocyte pass through the endothelium enhances the production of highly reactive and toxic to cancerous cells peroxynitrite, among others peroxynitrite anion $ONOO^-$. An inhibitory influence of NO on the progression of neoplastic disease has been shown by their inhibitory influence on growth stimulating kinase, caspase, and release of cytochrome c in the cells. Other EPR effect enhancing mediators are: bradykinin, prostaglandins, vascular endothelial growth factor (VEGF), L-arginine,

as substrates for nitrogen oxide (10, 11). A contrary effect was observed after administering such substrates as indomethacin, and antagonist of B2 bradykinin receptor, since permeability of tumor vessels decreased by 34 and 46%, respectively (12).

The knowledge of the endothelium of neoplasmic tumor blood vessels and stoppage of tumor vascularization became a new aim of the pharmacotherapy of solid tumors. A conjugate HPMA with calpastatin, which is a substance of anti-angiogenic action, was obtained, where Gly-Phe-Leu-Gly-ethylenediamine was used as connector. Studies on animals demonstrated prolongation of circulation time and activity of caplastatin, predominantly, accumulation in tumor blood vessels and inhibition of their growth (4).

Conjugates actively transported to neoplasmic tissue

Lots of cancerous tumors are poorly vascularized. The therapy with the conjugates, whose intracellular transport is based on EPR effect, in such cases is ineffective. Therefore, conjugates with a molecule actively directing the DDS were formed (4, 10). Active transport of DDS is based on the polymer carrier bond with a pharmacologically or biologically active molecule component, which enables selective binding with receptors or antigens on the surface of targeted cells (13-15). This mechanism ensures selectivity, and the molecules recognized by membrane receptors are carried into neoplastic cells faster, than in the case of passive transport. DDS transport does not require big accumula-

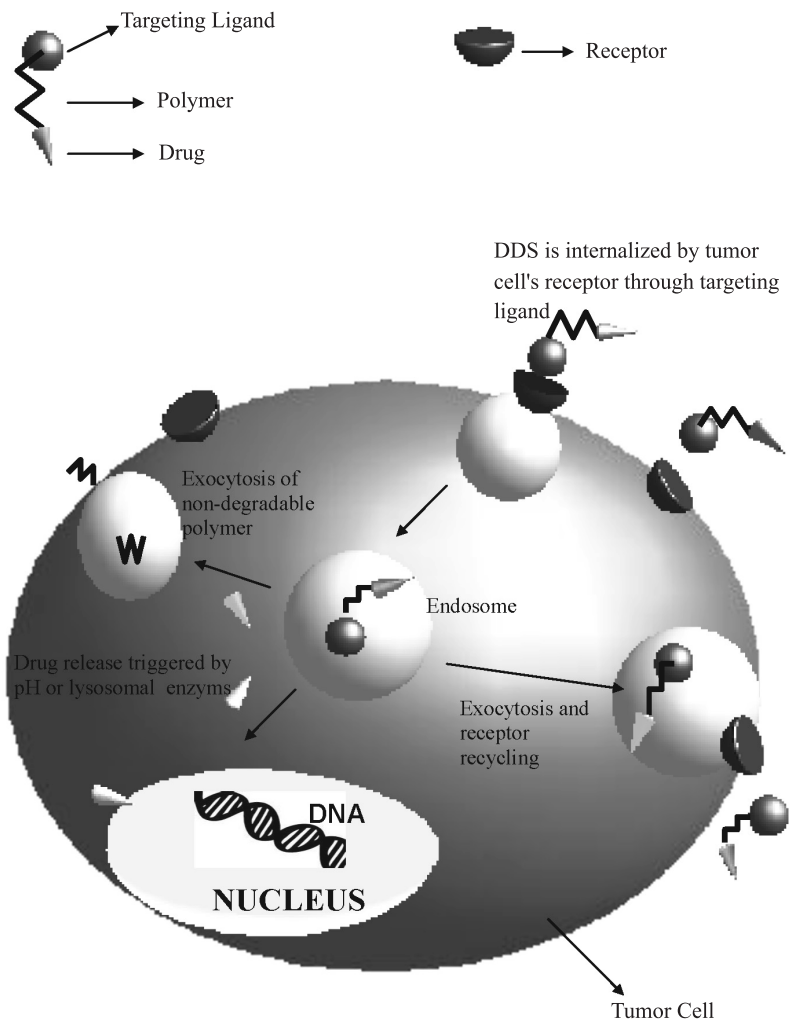

Figure 1. Phases of mechanism occurs between DDS and tumor cell's compartments

tion of conjugate outside the cell, nor a difference between the external and the internal environment of cell. This process consists of a few stages (Fig. 1):

1) molecule interaction with the membrane receptor;

2) DDS transport in the endosome into the cell;

3) fusion with the lizosome, degradation of the connector and freeing the therapeutic substance in the target space;

4) degradation of the polymer carrier in the cell or its transport outside the cell (16).

At present, various mechanisms and bond types between DDS and chemical structure on the surface of neoplasmic cell: ligand-receptor, antibody-antigen, lectin-bicarbonate, avidin-biotin (7) are being studied. A majority of experiments refers to the mechanism based on binding a ligand with a receptor (17). One of the studies resulted in a conjugate containing camptothecin and a homological peptide 3 (BH3). Its task was to activate the apoptosis of cancerous cells. As the factor enabling a targeted therapy luteinotropin was used (LHRH - luteinizing hormone releasing hormone). Since some types of neoplasm have numerous LHRH receptors on its surface, an enhanced uptake and transport of conjugate into cancerous cells was observed (7).

Neoplastic cells possess many transferring receptors. This fact was reflected in the project of transferrin-polyethylene glycol-tumor necrosis factor alpha (Tf-PEG-TNF-α), in which transferrin plays the role of ligand conditioning the specificity of bond, and the factor of neoplasm necrosis α plays the role of anti-neoplastic cytokinine. As neoplastic cells have a receptor also for TNF-α, the system was bound to cellular membrane in two places. PEG, however, as a long connector between Tf and TNF enabled optimal accommodation of these molecules to receptors. As a result, enhanced selectivity and binding precision were achieved. Nevertheless, there is an issue on action duration of such a complex, since the necessity of connecting two ligands to cellular surface may be prolonged (18, 19).

Also was analyzed galactosamine, which, as a ligand for asialoglycoprotein receptors (ASGR) present on hepatocytes, enables selective transport of the HPMA-Gly-Phe-Leu-Gly-doxorubicin-galactosamine conjugate to the cells of metastatic liver cancer. Eighty per cent of the intravenously administered copolymer was localized in the hepatic cells. Studies proved that the therapy was effective, and the asiaoglycoprotein receptors require polyvalent ligands, hence a pivotal requirement is high content of galactosamine in the polymeric chain and ASGR saturation (4, 20).

Many scientific papers were devoted to therapeutic monoclonal antibodies, as ligands specific exclusively for one protein/antigen epitope, being neutral to all other antigens. A few conjugates containing antibodies, e.g., gemtuzumab (Mylotarg), trositumomab and ibritumomab (Zevalin), have been currently obtained. Many conjugates containing monoclonal antibodies are being presently in laboratories and pre-clinically studied (20-23).

It is thought to be of importance to develop the knowledge of conjugates which employ binding with neoplastic cell on the ligand-receptor basis, where non-covalent interaction occurs, and there is no valent electrons exchange. Interaction refers to the energy, structure and functioning of molecules, as well as to the complex surroundings. Studies of the described DDS are in progress (15, 24).

Size, mass, shape and ramifications of the polymeric carrier

Low molecular anti-neoplastic substances undergo quick biotransformation and renal filtration in organism, and because of large distributional volume they cause many adverse effects. Connecting chemical substance to polymer which functions as a carrier may solve these problems (25).

As molecular mass increases, the mean circulation time (t1/3β) of the system transporting therapeutic substance increases as well. It is explained by decreased glomerular filtration of DDS in kidneys. It should be noticed, however, that the reticuloendothelial system (RES) recognizes and eliminates DDS the faster the bigger its size. Generally, as for polymers which have identical chemical properties, architecture and molecular conformation, (t1/3β) increases along with the rise of molecular mass up to the point, where polymers are still unrecognized by RES (8).

Prolongation of DDS circulation time in organism is inseparably connected with the increase of its uptake by neoplastic cells. In the case of DDS, using the EPR effect, the initial concentration of the system around the tumor is high, due to little elimination of the DDS by the kidneys. What happens later with therapeutic substance depends on the vascularization of tumor, permeability of its blood vessels, and the properties of the polymer. The design of DDS using the EPR effect depends on the difference between the size of fissures in the endothelium of capillaries in healthy tissue and neoplastic tissue (Tab. 1), and the size of the drug carrier (Tab. 2). It is of no significance, however, for the DDS actively

transported to neoplastic tissue (8). Studies of actively transported DDS demonstrated that the EPR effect and the fissure size in the endothelium have minor influence on the degree of accumulation of these systems within neoplasmatic tissue. The chief role in the improvement of pharmacokinetics through lengthening the half-life of DDS in organism plays its molecular mass (26). The chemotherapeutic activity of L-asparaginase in the treatment of acute lymphoblastic leukemia was proved already in the 1970s. But low t1/3β and hypersensitivity reaction in several patients restricted its use. Studies carried out from 1970 to 1980 led to the development of PEG-L-asparaginase conjugate, which bearing the name Oncaspar was registered in Japan in 1994, as a drug for acute lymphoblastic leukemia. The conjugated form is characterized by a longer t1/3 (8-30 h for unbound L-asparaginase to 14 days for the conjugate), which significantly lowers the frequency of doses. It was also observed that the hypersensitivity of organism to the used conjugate is considerably lower (8% of patients) in comparison to an unbound L-asparaginase (47% of patients). L-asparaginase was the first protein, which connection with PEG increased the half-life in organism and decreased immunogenicity in relation to the native form. The process described above, universally called pegylation, was applied to other pharmacologically and biologically active molecules. Low molecular anti-neoplastic substances usually unselectively penetrate all tissues, as they easily pass the cellular membrane by diffusion, and are rapidly filtered in renal glomeruli. For that reason, connecting a molecule to a polymeric carrier, e.g., PEG is the solution. Another example is a conjugate of granulocyte colony stimulating factor (GCSF) with PEG. Connection of GCSF of 19,000 Da mass with PEG chain of about 20,000 Da mass resulted in a macromolecular conjugate, which half-life in organism was prolonged from 3.7 h (free GCSF) to 42 h (conjugate). The PEG-GCSF conjugate was introduced to clinical setting as Neulasta and is today used in the therapy on neutropenia, as a supporting agent in chemotherapy (4). Conjugation of PEG with adenosine deaminase – an enzyme reducing the amount of arginine, one of the principal nutritional substances utilized by neoplastic tumors to grow, lengthened the half-life and minimized the immunogenicity of enzyme. Pegylation modifies biodistribution of pharmacologically and biologically active substances, ensuring their stability, protecting them against chemical or proteolytic degradation. It reduces toxicity and hypersensitivity as well as immunogenicity in the case of proteins and antibodies (5).

Table 1. Pore size relationship between normal and malignant tissue [6].

Tissue type	Pore size [nm]
Renal pores	5
Large pores in healthy, normal tissue	8
Pores of typical tumors	40-80
Large pores of malignant tissue	500

Table 2. Relationship between diameter of a polymer carrier and a carrier bound to a bioactive substance [6].

Polymer/ DDS	Diameter [nm]
Polyvinyl alcohol (PVA)	5
PVA with macromolecule	100

Because of the shape and size of fissures in the endothelium of capillary vessels, the shape of polymer from which a drug carrier has been obtained is important. Experiments carried out on mice demonstrate that the longest half-life in organisms belongs to globular polymers or ramificated polymers, slightly shorter half-life characterizes those globular polymers which are easily soluble. Line polymers are eliminated the fastest (8). Great interest is roused by ramificated polymers, whose one of the branches functions as a carrier selectively transporting towards a tumor. Polymers of high molecular mass possess reduced capacity for passing through the fissures of the blood vessels of tumor – high molecular mass limits the penetration of DDS into tumor. It is linked also with a higher pressure inside the tumor in comparison to its periphery. Furthermore, glycoproteins such as hyaluronate, and collagen fibers in the extra-cellular matrix decrease the diffusion of macromolecular DDS to the tumoral cells (8).

A particularly difficult access refers to brain tumors. It is estimated that about 98% of molecules with low molecular mass and almost 100% of macrocellular peptides and proteins do not cross the blood-brain barrier. Nevertheless, there has been progress in recent years in the field of DDS and materials based on nanotechnology, including conjugates. The advantages of these carriers have been noticed in non-invasive cases, where surgery is not needed. The greatest benefits have been recorded in reference to nanomolecules whose surface is covered with molecules facilitating the passage through

the blood-brain barrier and with trophic factors, such as glial cell-derived neurotrophic factor (GDNF), proteins potentially promoting growth and survivability of neurons or neurotransmitters, such as dopamine or noradrenaline, which are employed to treat the Parkinson's disease. Nanomolecules may also contain yttrium and cerium oxides, which work as antioxidants. An example are nanomolecules made from PLGA with addition of nerve growth factor administered in rats affected by Huntington's chorea (16, 20, 25, 27, 28).

Types of connectors used to form polymeric conjugates with a drug

To introduce a connector between the therapeutic substance and the carrier means to decrease the spatial obstacle between them and to control the release of pharmacologically active agent (7). The choice of connector depends on the mechanism and place of action of the therapeutic substance transported by a polymer. Basically, two types are distinguished: a connector containing an amide bond, and a connector with ester bond. The ester bond undergoes hydrolysis in the plasma, whilst the amide bond is broken down by intra-cellular enzymes (20). Due to the specificity of neoplastic disease, it is better to use amide connectors which will release the therapeutic substance after reaching the intended intra-cellular space in tumor tissue. Substances connected by amide bond may be selectively released from the carrier thanks to the action of proteolytic enzymes. Many neoplastic cells demonstrate high activity of these enzymes, including cysteine proteinase, aspartyl proteinase, serine proteinase, lisosomal thiol-dependent catepsin protease B (3, 20, 29). Most commonly used connectors are amino acids which have amine and carboxyl group, connected to tetrasubstituted chiral carbon, which facilitates conjugation with hydroxyl, thiol, or amide group of polymers or biomolecules. Amino acids are bio-compatible with human organism; they have short chains and allow the active therapeutic substance to be released from conjugate. It has been demonstrated that conjugates with the glycine end undergo hydrolysis faster than hydrophobic amino acids. The HPMA-Gly-Phe-Ala-Leu-mitomycin C conjugate quickly releases the anti-neoplastic substance at the presence of colagenase IV, which makes such conjugates pro-drugs of mitomycin C. The HPMA conjugate with adriamycin, with peptide Gly-Phe-Leu-Gly used as a connecting branch, cumulated in neoplastic tumor well and showed lower toxicity in relation to therapy based on free adriamycin (7). Gly-Phe-Leu-Gly- is a degradable connector and undergoes hydrolysis by lysosomal protease (catepsin B) (20). The peptide Pro-Val-Gly-Leu-Ile-Gly, in turn, is degraded by metalloproteinase II and IX, highly concentrated in neoplastic tumors (7). Of all amino acids, frequently used arginine, has the highest proton affinity, as it contains the guanidine group which renders it alkaline. Unbound and in proton form it possesses 6 sites (hydrogen) which are proton donors, which makes it well water-soluble. It commonly creates electrostatic bonds. It is a positively charged molecule for it holds three nitrogen atoms. Its derivates containing more amine groups, generated in the way of post translation modification influence greatly the capacity for biomolecular binding (24).

Studies confirmed a favorable influence of adding a connector to DDS to protect the therapeutic substance against hydrolysis and biotransformation in plasma. Connection of PEG with anti-neoplastic substance showed weak reactivity of conjugate. When the conjugate was expanded with amino alcohol e.g., H_2N-$[CH_2$-CH_2-$O]_2$-H, the therapy turned out more effective due to higher concentration of the anti-neoplastic substance in tumor tissue. It has been often proved that the use of proper connector influences the separation on therapeutic substance from carrier in the targeted compartment, which might be plasma or cell. Three trials were carried out with the use of unbound methotrexate (MTX), MTX-amide-dextran, MTX-ester-dextran. Those trials showed that conjugated MTX more effectively increases the survivability of rats with gliosarcoma. Unbound MTX is eliminated faster. The use of macromolecular carriers reduces the possibility of biotransformation of therapeutic substance and reduces the possibility of drug elimination. MTX dissociation from dextran is different when the connector is an amide and ester bond. The latter is more labile in water environment and breaks down in blood plasma, thus the substance does not get to the neoplastic cell (7). However, both the amide bond and the ester bond guarantee better control of DDS pharmacokinetics and its repeatability during the release of therapeutic substance (7, 30). Amide bonds show particular lability in low pH (6.5-4) present in neoplastic cells. Therefore, pH-sensitive cis-aconityl, hydrazines, acetyl or aminoester connections are used as connectors, which were applied in the PEG-LHRH conjugate (5, 29, 30). Other connectors employed in anti-neoplastic therapy are: glycine, alanine, 6-aminocapronic acid (6-ACA), 4-aminobutyric acid (4 ABA), succinimidyl (7).

When a connecter is added between polymer and therapeutic substance or biomolecule, each of reagents has to contain reactive groups, e.g., –COOH, –OH, –SH, $–NH_2$. An ideal connector should be stable in physiological pH and release the therapeutic substance or biomolecules in destination place (7). Thus, the greatest challenge for modern DDS projecting with the use of connector is the aspect of influence of environmental changes in organism on connectors stability. It is of special importance in the treatment of cerebral tumors when the therapeutic substance should be protected by polymeric carrier until it passes the blood-brain barrier and is released in a non-metabolized form in tumoral tissue (30, 33).

Active formations capable of conjugation

To form a conjugate directly linking polymer to bioactive substance, it is necessary the presence of reactive functional groups both in the polymer chain and in the therapeutic substance or biomolecule. Most commonly carboxyl –COOH, hydroxyl –OH, thiol –SH, and amine $–NH_2$ groups are used. Many molecules, e.g., peptides, proteins, hydrocarbons, fats, polymers, nucleic acids, oligonucleotides, contain in their molecule such functional groups. However, their structure is often modified to enhance affinity of two molecules to each other or to increase the amount of bioactive substance in polymeric carrier (7). PEG is a frequently used carrier for conjugation of therapeutic substances. However, because of only two active groups its ability to conjugate is limited. Therefore, the structure of PEG is often modified by addition of methoxyl or hydroxyl group (7).

Dextran is also employed as a carrier – it is a polymer made of glucose monomers, which has characteristic α-1,6 bonds and primary and secondary hydroxyl groups which are the conjugation site for anti-neoplastic substances. This is how several conjugates were developed: dextran-tacrolimus, dextran-camptothecin, dextran-doxorubicin. Dextran-petide-methotrexate conjugate was also obtained, where the peptide is the connecting branch (7).

A noteworthy group of polymeric carriers are dendrimers which have several functional groups capable of conjugating with biomolecules and therapeutic substances. They may simultaneously carry anti-neoplastic substances and molecules which selectively connect with cells. The number of added to the dendrimer molecules is the result of spatial obstacle created by biomolecules, their low reactivity, small inertia moment (R_h), and accumulation of terminal functional groups (7).

Conjugation takes place as long as there are reactive groups in the therapeutic substance or biomolecule. Usually, these are groups containing donors or acceptors of electrons. Doxorubicin possesses in its side chain an active hydrazone bond, while camptothecin conjugates are made with the use of favored site in the lactone ring C20-OH (7, 20).

Possibilities of increasing the amount of therapeutic substance connected with carrier

The amount of bioactive substance which we can add using line polymers as carriers is small. In the case of HPMA, the optimal amount of anti-neoplastic substance is 10 weight per cent of the whole system; in the case of PGA the amount is bigger and equals about 37 weight per cent. The more ramificated the carrier, the more are molecules of therapeutic substance or biomolecules, which may be connected to it. The most ramificated polymeric carriers are dendrimers. Their branches may stem from one or many cores, where to each branch a bioactive substance can be attached. A great amount of therapeutic substance can be added to poloxamer block co-polymers (Pluronics) used in regeneration and repair of the brain. In addition, their surface can be so modified that their passage through the blood-brain barrier is easier (16, 34, 35).

CONCLUSION

Several decades ago Paul Ehrlich defined the term “magic sphere,” describing perfectly directed therapy, in which the entire dose of pharmacologically or biologically active substance reaches the target organ, tissue, or cellular compartment. The studies by Maeda et al. referring to the EPR effect brought about numerous polymer-therapeutic substance conjugates. Improvement of drug delivery systems through ligands which enable selective transport to diseased tissue allowed a substantial progress in the battle against neoplasms. New methods of modification of the structure of conjugates used in anti-neoplastic therapies and optimization of synthesis facilitate the control of pharmacokinetics of the described DDS, their bio-accessibility, and reduced immunogenicity, which renders anti-neoplastic therapy more effective and its side-effects lesser. The pegylation technique improved anti-neoplastic therapies, reduced the frequency of drug administration, additionally, enabled to provide short half-life substances to the organism. The synthesis of new carriers for therapeutic substances, as well as the development of their technology and pro-

cessing methods, augments the chances of success in the fight against neoplastic diseases. Research into the biology of cancerous cells also delivers greatly valuable information in this area. The news from several months ago regarding the phases of neoplastic cell life, fluidity of cellular membranes, and possibility of changed localization of membranous receptors may improve conjugates and make the conception of "perfectly directed therapy" a reality (36, 38).

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