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Tumor delivery of macromolecular drugs based on the EPR effect ☆

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ABSTRACT

Enhanced permeability and retention (EPR) effect is the physiology-based principal mechanism of tumor accumulation of large molecules and small particles. This specific issue of *Advanced Drug Delivery Reviews* is summing up multiple data on the EPR effect-based drug design and clinical outcome. In this commentary, the role of the EPR effect in the intratumoral delivery of protein and peptide drugs, macromolecular drugs and drug-loaded long-circulating pharmaceutical nanocarriers is briefly discussed together with some additional opportunities for drug delivery arising from the initial EPR effect-mediated accumulation of drug-containing macromolecular systems in tumors.

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Under the term “macromolecular drugs” we usually understand the whole variety of things. First, those could be macromolecules with useful pharmacological properties, such as proteins and peptides, i.e. protein and peptide drugs. Second, those could be various DNA-based constructs for gene therapy. Third, those could be synthetic or natural biocompatible soluble polymers carrying multiple moieties small drug molecules attached to a carrier protein via biodegradable or non-degradable bonds. In terms of size, this term can also include drug-loaded pharmaceutical nanocarriers, such as liposomes or polymeric micelles. The common feature of all listed preparations is their size in a nanometer region. Many of such drugs are developed for tumor therapy and, as one can expect, the key problem with their use is how to effectively deliver them to and into tumors or even inside individual tumor cells.

It is now a well-established phenomenon that under certain circumstances (inflammation/hypoxia, first of all, which is typical for tumors, infarcts, and some other pathological sites in the body) the endothelial lining of the blood vessel wall becomes more permeable than in the normal state. As a result, in such areas, large molecules and even relatively certain particles ranging from 10 to 500 nm in size, can leave the vascular bed and accumulate inside the interstitial space. This was clearly demonstrated in many tumors [1,2] and in infarcted areas [3,4]. Assuming these large (polymeric) molecules/particles are loaded with a pharmaceutical agent, they can bring this agent into the area with the increased vascular permeability, where the active drug can be eventually released from a carrier. Such spontaneous accumulation or “passive” targeting, which works especially good with tumors because of the lack of lymphatic drainage there, is currently known as enhanced permeability and retention (EPR) effect

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[5–7]. In other words, high permeability of the tumor vasculature allows macromolecules and nanoparticles to enter the tumor interstitial space, while the compromised lymphatic filtration allows them to stay there. Unlike macromolecules, "small" low-molecular-weight pharmaceutical agents are not retained in tumors because of their ability to return to the circulation by diffusion [7]. EPR-mediated drug delivery is currently seen as an effective way to bring drugs to and into tumors, especially macromolecular drugs and drug-loaded pharmaceutical nanocarriers. The mechanisms and approaches underlying EPR-related phenomena are discussed in Greiesh et al. [8].

Interestingly, it was recently shown that EPR effect can be modulated by the blood pressure and substances that can influence it. Thus, elevated blood pressure was suggested as a strategy to increase tumor-targeted delivery of SMANCS [9,10].

1. Some general considerations

Because the cut-off size of the permeabilized vasculature varies from case to case in rather broad limits — from 200 to 800 nm [1,11], the size of a drug-carrying particle may be used to control the efficacy EPR-mediated drug delivery. Clearly, EPR-based type of tumor targeting requires macromolecular drugs and drug delivery systems to be long-circulating (i.e., to stay in the blood for extended periods of time) in order to provide a sufficient level of accumulation in the target. The most usual way to keep drug carriers in the blood long enough is to "mask" them by modifying (grafting) their surface with certain water-soluble polymers with a well-solvated and flexible main chain, such as polyethylene glycol (PEG) [12,13]. The surface-grafted "protective" polymers effectively prevent (slow down) the opsonization of drugs and drug carriers and their clearance by mononuclear phagocytic system (MPS) and also hinder the contact between PEGylated macromolecules and nanoparticles and phagocytic cells. The approach is best developed for liposomes [14,15], although it has a rather broad applicability [16]. Important advantages of prolonged circulation of drugs and drug carriers in the blood flow include the possibility of maintaining a required concentration of an active drug or drug carrier in the blood for a long time after a single administration; the ability to utilize the EPR effect for the accumulation of pharmaceuticals in the areas with leaky vasculature; and the possibility of enhancing ligand-mediated targeting of drugs and drug carriers into the areas with a limited blood supply and/or low concentration of a target antigen, where an extended time is required to allow for a sufficient quantity of a drug in the target zone.

2. Tumor delivery of proteins and peptides

Many peptides and proteins possess biological activity that makes them potent and therapeutic, in particular, anticancer agents. Advances in solid-phase peptide synthesis and recombinant DNA and hybridoma technology allows for the production of unlimited quantities of clinical grade peptides and proteins. Their use as therapeutic agents is hampered, however, by their fast elimination from circulation mostly because of renal filtration, fast enzymatic degradation, uptake by the mononuclear phagocyte (MPS), and accumulation in non-targeted organs and tissues. Fast elimination and distribution into non-targeted organs and tissues causes the need to administer a drug in large quantities, which is often not economical and sometimes impossible due to non-specific toxicity. Conjugation of polypeptides with water-soluble polymers slows down the renal filtration [17] thus increasing their longevity in circulation and allowing for their better accumulation in tumors via the EPR effect.

Currently, polyethylene glycol (PEG) is the most popular polymer for the modification of peptides and proteins with therapeutic potential [18]. Thus, PEGylated L-asparaginase has a circulation life-time of 5.7 days in humans compared to 1.2 days for the original enzyme [19]. Several polypeptide-PEG conjugates are already used as anticancer drugs. PEG–asparaginase used for the treatment of lymphoma and leukemia [19,20] and PEGylated interferon (a non-specific stimulator of the immune system), the activity of which against various tumors is well documented [21], can serve as good examples. PEGylated G-CSF, some other PEGylated hematopoietic growth factors (GM-CSF, MGDF), PEGylated lymphokines, and PEGylated cytokine inhibitors are also subjects of preclinical and clinical trials as EPR-based anticancer agents, see Eliason [22] for review.

3. Tumor delivery of macromolecules

In some cases, the conjugation of drugs with polymers that are not large enough to prevent the renal clearance, but attach themselves and conjugated drug to natural long-circulating blood plasma components, like serum albumin or lipoproteins, can also be used to increase the circulation time. An example of such polymer is poly (styrene-co-maleic acid anhydride) (SMA). It has been shown that the conjugation of peptides and proteins with a polymer of this type as short as 1.5 kDa increases circulation time of anticancer proteins and peptides via the binding of the conjugates to plasma albumin [7]. It has also been shown that, similar to conjugation with high-molecular-weight polymers, the conjugation with SMA protects proteins from enzymatic degradation, and decreases immunogenicity of modified proteins [23]. Neocarzinostatin/SMA conjugate (SMANCS) used for the treatment of hepatoma [7] was clearly shown to accumulate in solid tumors via the EPR effect [5,23] and has served as a template for constructing other anticancer macromolecular drugs acting via the same mechanism [24].

Another family of macromolecular therapeutics delivered into tumors via the EPR effect is based on hydroxypropylmethacrylate (HPMA), to which a broad variety of anticancer drugs was attached [25,26]. Moreover, HPMA-based macromolecular drugs accumulated in tumors via the EPR effect could be eventually delivered inside tumor cells and even specifically to cell nuclei by the attachment of certain additional functions to HPMA allowing for an enhanced intracellular uptake and organelle-specific targeting. Some additional data on macromolecular therapeutics for tumor treatment including those bypassing the multidrug resistance could be found in Refs. [27–33].

4. Tumor delivery of nanoparticles

Long-circulating pharmaceutical nanocarriers, such as liposomes, micells, or polymeric nanoparticles, are capable of accumulating in various pathological areas with affected vasculature via the EPR effect, and have been repeatedly used for drug delivery into tumors via passive accumulation. Long-circulating liposomes and other nanocarriers demonstrate dose-independent, nonsaturable, log-linear kinetics, and increased bioavailability [34]. The anticancer drug doxorubicin incorporated into long-circulating PEG-coated liposomes, which is currently used in clinical conditions, demonstrates high efficacy in EPR-based tumor therapy and strongly diminishes the side-effects [35,36] characteristic of free doxorubicin. Doxorubicin in PEG-liposomes (Doxil® and Caelyx®) is successfully used for the treatment of solid tumors in patients with breast carcinoma metastases, with subsequent survival improvement [37–39]. The same set of indications was targeted by the combination therapy involving liposomal doxorubicin and paclitaxel [40] or Doxil/Caelyx and carboplatin [41]. Caelyx is currently also in Phase II studies for patients with squamous cell cancer of the head and neck [42] and ovarian cancer [43]. Clinical data showed the impressive effect of doxorubicin in PEG-liposomes against unresectable hepatocellular carcinoma [44], cutaneous T-cell lymphoma [45], and sarcoma [46]. The recent review on the successful use of Caelyx in the treatment of ovarian cancer can be found in Perez-Lopez et al. [47]. PEG was also attached to the liposome surface in a removable fashion to facilitate liposome capture by the cell after PEG-liposomes accumulate in target

site via the EPR effect, and the PEG coating is detached under the action of local pathological conditions (decreased pH in tumors). Detachable PEG conjugates are described in [48], where the detachment process is based on the mild thiolysis of the dithiobenzylurethane linkage between PEG and an amino-containing substrate (such as PE). Low pH-degradable PEG-lipid conjugates based on the hydrazone linkage between PEG and lipid have also been described [49; 50]. It should, however, be noted here that recent evidence showed that PEG-liposomes, previously considered biologically inert, could still induce certain side-reactions via activation of the complement system [51,52].

In the case of micellar preparations [53] of anticancer drugs, passive EPR-based micelle targeting to pathological organs or tissues can further increase the pharmaceutical efficiency of a micelle-encapsulated drug. Among drugs delivered by passively targeted micelles, one can name paclitaxel, which was shown to accumulate in tumors much better than its commercial formulation Taxol®, when loaded into micelles made of PEG-b-poly(4-phenyl-1-butanoate)-l-aspartamide conjugates [54]. With this preparation, an almost 100-fold increase in the AUC, a 15-fold decrease in the volume of distribution and a significant decrease of drug clearance was achieved, which resulted in a 25-fold improved drug accumulation in C-26 tumors in mice and a corresponding increase in antitumor activity. Some other micellar preparations for passive targeting of paclitaxel have also been tested with variable degrees of success [54,55]. One of polymeric micelle-based preparation of paclitxel, Genexol-PM has already found its way to the clinic [55]. PEG-b-poly(amino acid)-based micelles loaded with cisplatin (CDDP) were designed for passive drug targeting into tumors and are undergoing clinical trials [56]. Among other micellar preparations for passive drug targeting in clinical trials, one can also mention doxorubicin in micelles made of PEG-block-poly(l-aspartate)–doxorubicin conjugate (these micelles contain both free and hydrophobic block-conjugated drug) [57] as well as doxorubicin in micelles made of Pluronic [58]. The problems with drug delivery using polymeric micelles for EPR-mediated passive targeting are usually associated with too fast drug release from the micelles and with the difficulties of intracellular drug delivery [59]. In order to minimize drug release from the micelles, the drug can be chemically conjugated with the hydrophobic blocks of micelle-forming components or drug-loaded micelles can be additionally chemically cross-linked [60–63].

5. Tumor delivery of DNA and related products

DNA and related products can also be delivered into tumors via the EPR effect as a part of long-circulating macromolecular or nanoparticular systems, in particular combining the passive EPR-mediated targeting with an additional ligand-mediating targeting facilitating the intracellular delivery [64,65]. Similar approaches are currently used for the tumor-specific delivery of siRNA [66,67]. In all cases, a sufficient longevity of siRNA-loaded carriers is required to provide an initial EPR-mediated accumulation in tumors [68,69].

5.1. Then what?

Many current tumor drug delivery systems are trying to go further than just providing tumor accumulation. Attempts are under way to combine tumor cell-specific targeting with longevity-sponsored EPR accumulation. Thus, various antitumor antibodies are used to modify PEGylated drug-containing liposomes and further enhance their therapeutic effect via better delivery inside cells, for example, by receptor-mediated endocytosis [70–73]. Intracellular delivery of drug-loaded macromolecular conjugates and pharmaceutical nanocarriers accumulated in tumors via the EPR effect can be facilitated by various means. Thus, when the liposome is made of pH-sensitive components then, after being endocytosed, it fuses with the endovacuolar membrane under the action of lowered pH inside the endosome and destabilizes it, releasing its content into the cytoplasm, i.e. endosomes become the gates from the outside into the cell cytoplasm [74]. This approach has been reviewed many times in various publications (in 2004, endosomal escape by pH-sensitive drug delivery systems was specifically discussed in a special issue of Advanced Drug Delivery Reviews #56, J.C. Leroux, ed.).

Passively targeted polymeric micelles can also demonstrate pH sensitivity and ability to escape from endosomes. Thus, micelles prepared from PEG-poly(aspartate hydrazone adriamycin) easily release an active drug at lowered pH values typical for endosomes and facilitate its cytoplasmic delivery and toxicity against cancer cells [75]. Alternatively, micelles for intracellular delivery of antisense ODN were prepared from ODN–PEG conjugates complexed with a cationic fusogenic peptide, KALA, and provided much higher intracellular delivery of the ODN than could be achieved with free ODN [76].

Endosomal escape could be also facilitated by using pH-sensitive fusogenic peptides attached to drug/gene-loaded nanocarriers [77] or endosomolytic polymers introduced as additional components to delivery systems [78]. Intracellular delivery of various systems could be enhanced by using cell-penetrating proteins and peptides [79], sometimes built into complex systems with other functions in such a way that each function (longevity, targeting, and cell penetration) works only when required [49,50,80].

The recently suggested role of NO in facilitating the EPR effect and the possibility of using nitroglycerine in enhancing the EPR-mediated penetration of macromolecular drugs in tumors and infarcts [81] together with the generally favorable clinical effect of nitroglycerine in combination anticancer therapy [82,83], open a new direction in exploring the EPR effect.

So, the opportunities for the intratumoral drug delivery look just great. But whatever complex schemes are being developed to effectively bring anticancer drugs and genes into tumor cells, the EPR effect-mediated tumor accumulation remains the first crucial step of any scheme.

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