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Review article

Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect

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ABSTRACT

For over half a century extensive research has been undertaken for the control of cancer. However, success has been limited to certain malignancies, and surgical intervention is potentially curative for early stage patients. For the majority of patients with advanced stage of cancer, the treatment is limited to chemotherapy or radiation. Chemotherapy in particular has limitations due to the lack of selectivity with severe toxicity. Under these circumstances tumor-targeted delivery of anticancer drugs is perhaps one of the most important steps for cancer chemotherapy. We reported such a drug for the first time, styrene-maleic acid copolymer-conjugated neocarzinostatin (SMANCS) in 1979, and it eventually led to formulate the concept of the enhanced permeability and retention (EPR) effect of solid tumors in 1986. Monoclonal antibody conjugates are another direction, of which interest is increasing recently though with limited success. The EPR-effect appears as a universal phenomenon in solid tumors which warrants the development of other polymeric drugs or nanomedicine.

EPR-effect is applicable for any biocompatible macromolecular compounds above 40 kDa, even larger than 800 kDa, or of the size of bacteria; thus complexed molecules like micelles and liposomes containing anticancer drugs are hallmark examples. The drug concentration in tumor compared to that of the blood (T/B ratio) can be usually as high as 10–30 times. In case of SMANCS/Lipiodol given via tumor feeding artery, the T/B ratio can be as high as 2000, a real pin-point targeting. EPR-effect is not just passive targeting for momentary tumor delivery, but it means prolonged drug retention for more than several weeks or longer.

This review describes the pathophysiological mechanisms of the EPR-effect, architectural difference of tumor blood vessel, various factors involved and artificial augmentation of EPR-effect with respect to tumor-selective delivery, and then advantages and problems of macromolecular drugs.

1. Introduction

The field of drug delivery systems (DDS) utilizing synthetic polymers either by covalent conjugation or by composite of micellar drugs has become a new domain for new drug development for numerous diseases. Synthetic polymers become an indispensable component for micellar or stealth liposome drugs and protein–polymer conjugates [1–3]. These polymer-based new drug entities are called "polymer therapeutics" [2,3] or macromolecular drugs, and they overlap with nanomedicine that becomes popular in recent years [4]. The polymer therapeutics or nanomedicines are designed to improve drug performance by utilizing pathophysiological uniqueness of solid tumor, of which conventional low molecular weight drugs are incapable. Macromolecular drugs or nanomedicines show improved tumor-selective targeting; the improved therapeutic efficacy and fewer side effects are their primary benefits, in which prolonged circulation time plays a crucial role [4–9].

Most conventional low molecular weight anticancer drugs have inherent character to traverse in and out of blood vessels freely, unless the drug is linked with a tumor-specific molecular ligand having high binding constant. For instance, low molecular weight

Abbreviations: AT-II, angiotensin-II; AUC, area under the concentration curve (vs time); CML, chronic myeloid leukemia; EPR, enhanced permeability and retention effect (of macromolecular drugs in solid tumor); HPMA, poly(hydroxypropyl methacrylic acid); HCC, hepatocellular carcinoma (hepatoma); i.v., intravenously; i.a., intra-arterially; MDR, multidrug resistance; NCS, neocarzinostatin; NO, nitric oxide; NOS, nitric oxide synthase; $ONOO^-$, peroxynitrite; PEG, polyethylene glycol (also called polyoxyethylene); PGs, prostaglandins; PEG-poly(Asp), block copolymer (polyethylene glycol) linked to poly (aspartic acid-benzyl ester); SMA, copolymer of styrene-maleic acid; SMANCS, copoly (styrene-maleic acid) conjugated neocarzinostatin; SOD, superoxide dismutase; T/B, tumor to blood ratio of drug (delivered concentration); VPF, vascular permeability factor; VEGF, vascular endothelial growth factor.

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drugs injected intramuscularly reach a distant site of the body in 10 min. Consequently, their undesirable indiscriminatory distribution in normal tissues causes severe systemic side effects in case of anticancer agents. Namely, free diffusion of toxic drugs in a non-selective manner in the body, and their inability to accumulate selectively in tumor tissues make them disastrous to patients.

Experiences in antibiotic research tell us that selective toxicity is possible in prokaryotic kingdom, where different types of biochemical machinery are used from the animal kingdom. For instance, machinery for protein-synthesis (ribosomes) in prokaryotes is different from eukaryotic cells. Similarly, the cell-wall synthesis of peptidoglycan in bacteria (a target for penicillin) does not exist in eukaryotes. On the contrary, it is difficult to get tumor-selective toxicity because the biological events taking place in cancer cells are essentially the same as that of the host cells. Namely, there is little difference in biochemical or molecular machinery between cancer and normal cells at a cellular or molecular level. Therefore, to target tumor cells more selectively, active targeting based on antibodies or the receptor-mediated targeting with cancer-specific ligands are developed. However, recent clinical results of molecular target-based drugs were somewhat disappointing, if not completely.

Tumor cells have inherent heterogeneity and epitopic diversification as a result of great magnitude of mutation frequency even amongst the same cancer patient [10,12]. The recent results of cancer genomics showed that most human solid tumors were not only single gene-based events, but also multiple genomic alterations. Namely, there were no specific alterations or gene mutations common among individual patients if not name p53, a cancer suppressor gene. Extensive genomic studies of 11 colon cancer and 11 breast cancer patients revealed numerous genetic variants arising from a single solid malignancy. On average about 90 or so such variants were found in a patient. This makes the task of specific antibodies for each of these diverse epitopic targets inefficient if not unrealistic from antibody therapy [10,11].

Furthermore, according to recent reports in the annual meeting of *American Society of Clinical Oncology* (*ASCO*), efficacy of molecular target drugs exhibited only 4–5% of response rate despite very high expectation and very high cost of manufacturing. It is generally thought this much efficacy is only useful as adjuvant or supplementary. Although it is beyond this review, the costs of antibody drugs are so expensive that public and national insurance systems may be at risk aside from the low response rate achieved with these therapies. In this regard, for example, Avastin was not recommended in the UK for reimbursement of national insurance [13]. Thus, 'the cost-benefit' will be considered more than ever for drug approval. Recently, the editorial in the Lancet criticized these issues one step further for drug appraisal [14].

Under these circumstances, a more universal and efficient strategy for anticancer drug design having high selectivity to tumor tissues must be developed. To solve this problem, the phenomenon of "enhanced permeability and retention (EPR)-effect" discovered by Maeda and Matsumura is now becoming the gold-standard in cancer-targeting drug designing that is based on macromolecular, micellar and lipidic particles [5–9], all utilizing EPR-effect as a guiding principle, and the EPR-effect is applicable for almost all rapidly growing solid tumors [7–9,15–18].

Most importantly, EPR-effect can be observed in almost all human cancers with the exception of hypovascular tumors such as prostate cancer or pancreatic cancer. As clinical examples for this, we have experienced that SMANCS/Lipiodol given via the hepatic artery accumulated selectively in hepatocellular carcinoma distinctively [7,17,19–22]. A similar result in clinical setting was also reported for Doxil, a liposomal type of doxorubicin. Namely, Doxil mimic was prepared for radio scintigraphy, and clear tumor accumulation was seen in the whole body scintigram [23]. Another clinical example of EPR-effect can be demonstrated in the traditional tumor imaging in the clinic that utilizes (γ)-emitting gallium scintigraphy based on the selective accumulation of radioactive gallium (used as citrate) in the tumor. ^{65}Gallium ion as injected i.v. will bind to plasma protein transferrin (90 kDa) in the blood, thus radioactive transferrin will accumulate in the tumor by EPR-effect, which will take more than 24 h. Usually radio-scintigram is obtained 2–3 days after intravenous injection of ^{65}Ga when signal/noise ratio is improved; while its clearance from the normal tissues will take place in a day or so via the lymphatic system. The tumor, however, retains this ^{65}Ga-transferrin for several days at high levels by EPR-effect.

Another case of EPR-effect observed in human tumor is the selective accumulation of Lipiodol in the tumor after intra-arterial infusion of Lipiodol which is visualized by X-ray CT-scan [19–21]. This tumor detection method by use of Lipiodol staining after our report [19–22] is now becoming a routine examination before hepatic tumor resection.

Conventional angiography for tumor detection uses water-soluble low molecular weight radio contrast agent, and its high electron density yields staining of tumor as it is infused intra-arterially. This means increased uptake (staining) of this contrast agent by the tumor mass, which is a part of EPR-effect though it is washed out rapidly by diffusion due to the small molecular size (i.e. no retention). Thus, the tumor staining in this angiography is only transitory, less than a few minutes or so. This is a passive delivery of drug, but not EPR-effect which requires long time retention.

Based on EPR-effect, many polymeric drugs are being developed as a new class of antitumor agents [1,24–28], including nanoparticles [16], polymer micelles [27,28] and liposomes [28–31]. Further, EPR-effect is not only limited to these nanoparticles, but it is also valid for tumor-imaging contrast agent Lipiodol as described above, where Lipiodol shows virtually pin-point targeted delivery to the tumor, i.e. tumor/blood (T/B) ratio of more than 2000 can be obtained [19–22,32]. Further, bacterial cells as well as quantum dots (QDs) as ultra-sensitive imaging probe showed more selective accumulation into tumor tissue, which can be explained by EPR-effect [33–35].

In this review, we will describe current problems in cancer chemotherapy, the mechanism of EPR-effect and factors involved, artificial augmentation of EPR-effect for polymeric or macromolecular drugs under the angiotensin-II (AT-II) induced hypertension, and advantages of macromolecular drugs are also discussed.

2. How good is cancer chemotherapy; status quo

In the past 40–50 years, low molecular weight anticancer drugs have been the main treatment modality for many cancers of advanced stage, but have offered no improvement in the cure rate [36,37]. The biggest limitation of these therapeutic agents is overwhelming toxicity due to lack of selectivity. Scientists realized this fact finally towards the end of 20th century and thus cancer-selective targeting became one of the most important goals.

Theoretically, in drug development, molecular target drug is considered more ideal not only in cancer but also in other diseases such as inflammation, primarily to avoid side effects. In this context, *Meares*, editor in chief of *Bioconjugate Chemistry*, a journal of *American Chemical Society*, commented that drug targeting was the most popular subject recently in this journal [38]. In any event, cancer still remains a major cause of death in most developed countries, and the lack of effective control of many cancers is becoming increasingly burdensome on the health care system [36].

As discussed in above references [36,37], the death rate of major cancers such as lung, breast, colon, prostate, and pancreas at advanced stage, has not changed much in the last half century. Further, the clinical benefit of chemotherapy in the most common cancers, for instance the breast cancer and the prostate cancer, is