aqueous formulation of SMANCS (17). This approach has unfortunately not received much attention.

## Augmentation of the EPR effect and heterogeneity of the effect in cancer: Further enhancement of drug delivery to tumor.

Cancer and inflamed tissues share many vascular mediators that cause gaps between tight endothelial cell-cell junctions to open. As discussed above, BK and NO, among others, play a major role in the EPR effect. Angiotensin-converting enzyme (ACE) can converts angiotensin I (AT-I) to AT-II and also degrade BK because they have the amino acid sequence in common. Thus, ACE degrades BK, and ACE inhibitor blocks BK-degradation (5,6). Consequently, ACE inhibitors such as enalapril cause higher local BK concentrations, with the EPR effect thus being enhanced. Similarly, NO generators such as nitroglycerin (NG) applied topically enhance the EPR effect because NO mediates the effect. NG is quickly absorbed and becomes nitrite *in vivo*, and then nitrite ($NO_2^-$) is reduced to NO in hypoxic tumor tissue (6,8,18). Application of NG ointment to tumor-bearing hosts resulted in a 2- to 3-fold increase in delivery of macromolecular drugs to tumors (18) and an improved clinical effect (19).

By infusing AT-II, which is a physiological vasoconstrictor, one can elevate blood

pressure (eg. from 110 to 150–160 mmHg), and selective delivery of drugs to tumors will increase via passive opening of endothelial gaps (6,16), with enhanced clinical effects (18). NG and ACE inhibitors are safe and inexpensive and will solve the problem of low or heterogeneous EPR effects in tumors (6,19).

The vascular pathology of solid tumors is variable and frequently heterogeneous, with necrotic areas having obstructed blood flow, just as in infarcted cardiac tissue. We found that an augmented EPR effect, as described above, improves tumor blood flow and hence drug delivery. Pretreatment with NG before arterial drug infusion confirmed this result (18).

## Innovations in photodynamic therapy (PDT)

For fluorescent tumor imaging and PDT, rational design of photosensitizers (PSs) and light irradiation are needed. PDT has been known for more than a century, but PDT is not very popular in clinical practice. PDT requires good molecular probes or PSs. Conventional PSs such as Laserphyrin® have low MW and do not demonstrate the typical EPR effect. They were excreted into bile or feces very quickly (>95%), shortly after intravenous injection; only a small fraction, far less than 1%, was found in tumors at 24 h. That is, low-MW PSs are disseminated throughout the body, but more are found

in the liver, kidney, and spleen (20). Polymeric drugs, however, including plasma proteins and other biocompatible polymers, localize more selectively in solid tumors because of the EPR effect (1,6-8).

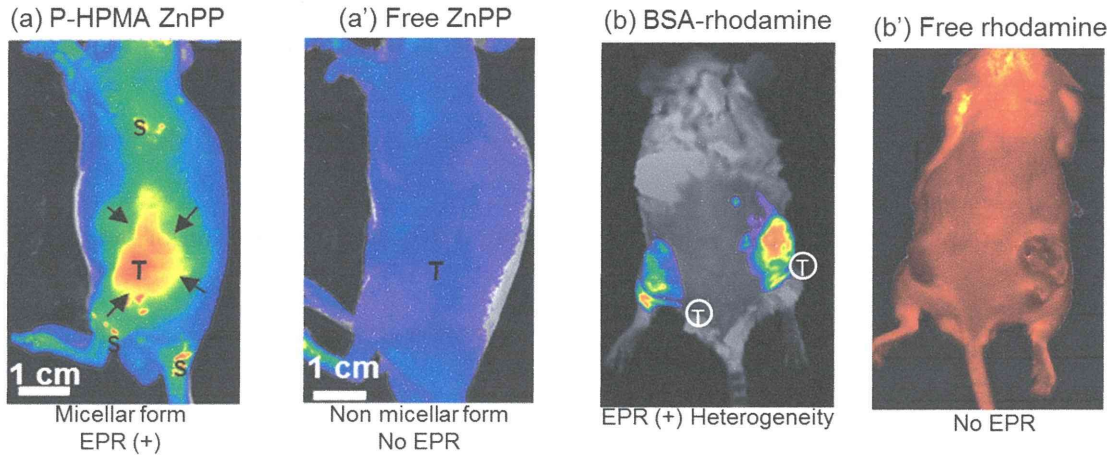


Figure 1. The EPR effect of polymer (HPMA)-conjugated zinc protoporphyrin (ZnPP) (a), free ZnPP (a'). It is also seen with bovine serum albumin (BSA)-conjugated rhodamine (b), and free tetramethyl rhodamine (b') in tumor-bearing mice. Macromolecules, both HPMA Zn-PP and BSA-rhodamine with apparent MWs about 500 kDa and 70 kDa, respectively, selectively accumulated in tumors, because of the EPR effect, as shown by the *in vivo* fluorescent imaging system; on the contrary both free ZnPP and free rhodamine, with MWs less than 1000 Da, showed little tumor uptake. Adapted from (6) with permission.

In addition to the spectroscopic system used in traditional PDT, He/Ne and YAG lasers are utilized, which do not emit light at the protoporphyrin wavelength (about 430 nm) but instead emit light with a wavelength of 633 or 532 nm. Excitation of many of current PSs thus produces a very low quantum yield. Our zinc protoporphyrin (the PS)-conjugated polymer caused impressive regression of chemically induced

autochthonous breast cancer in rats, and this regression could be seen, via either blue fluorescent light (emission maximum 410–440 nm) or xenon-based endoscopic light, from the body surface.

## Conclusion

Macromolecular drugs, or nanomedicines, to treat cancer are now being developed. Many advantages exist for these drugs, not only for tumor targeting based on the EPR effect but also for the potential to control metastasis via lymphotropism, and elucidation of these clinical treatments is warranted (10,11). The unique tumor environment, such as low pH, that can lead to release of drugs from carrier particles or polymer chains can be exploited for such nanomedicines, but not simple low-MW compounds. The EPR effect will be enhanced by using non-toxic, inexpensive vascular mediators, which will be of great clinical benefit. PDT should also be investigated with this concept in mind, to stimulate future innovations.

### Executive Summary

- EPR is the first most critical step at vascular level to achieve tumor selective drug delivery.
- Advantage of nanomedicine is a capacity to remain high in plasma concentration and thus utilization of the EPR effect.

- EPR-based tumor selective delivery can be increased further by common vascular mediator like nitroglycerin and others.
- Polymer-conjugated drug can be easily designed to respond to the tumor environment and release active drug in tumor.
- Many common vascular mediators operate in cancer and inflammation that affect vascular leakiness.
- Lymphtropic, and thus antimetastatic property, and oral formulation, may be possible by lipid formulation of the polymer conjugated low MW drugs.

(1,718 words)


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*Review article for* ***Expert Opinion on Drug Delivery***

# Development of next-generation macromolecular drugs based on the EPR effect: challenges and pitfalls

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***Areas covered***

Based on the EPR effect, the mechanism for more universal tumor delivery using macromolecular drugs, to cover wider tumor-types than single molecular target, is discussed. Unique properties of solid tumor vasculature in the tumor tissue are discussed, especially leakiness of the blood vessels and factors involved and impaired clearance of macromolecular drugs from the tumor interstitium via the lymphatic system. Criteria of such macromolecular drugs or nanomedicines for effective tumor accumulation is commented. Importance of long plasma retention time of such drugs, and a need of release of active principles from nanoparticles at target site, are also commented. Methods to augment the EPR effect and tumor delivery (2-3 times), and its application to the photodynamic therapy (PDT) are also commented.

**Keywords**

enhanced permeability and retention (EPR) effect of solid tumor, tumor selective drug delivery, macromolecular drug, barriers to tumor delivery, application of EPR effect to PDT, nanomedicine, augmentation of EPR effect

## *Introduction*

The door to the antitumor chemotherapy opened with the first publication of the report that nitrogen mustard gas, a classic chemical warfare agent, markedly suppressed lymphoid tumors. Since 1948, various anticancer therapeutic agents have been developed [1, 2]. Most such drugs were cytotoxic and exerted a therapeutic effect by

inhibiting DNA synthesis or various enzyme functions, followed by apoptosis or necrosis. However, these cytotoxic mechanisms were not selective for cancer cells. They also affected normal cells, and therefore whole body distribution of such cytotoxic agents caused many serious adverse effects. Such non-selective toxicity to normal tissues and vital organs such as bone marrow led to dose-limiting toxicity, so maximal drug dosages were restricted, which resulted in inadequate therapeutic outcomes. Developing tumor-selective therapies has therefore been a goal of cancer researchers. Surgical excision and radiation therapy have provided more direct elimination of, or damage to, tumor tissues. However, the efficacy of these therapeutic modalities was mainly limited to primary tumors or to clearly visible or detectable tumors.

Malignant tumors possess the inherent property of metastasis; that is, primary tumor cells spread to distant sites in the body and form metastatic tumor nodules. The hallmark event in this regard is lymph node metastasis. After tumor cells metastasize to distant sites, they become extremely difficult to find, and thus eradicating cancer completely by surgical removal or radiation therapy alone is a formidable task. Under such circumstances, chemotherapy offers hope for treating metastatic tumors, because drugs can be delivered to the entire body via the systemic circulation. However, indiscriminate distribution of most, if not all, low-molecular-weight (low-MW) drugs causes serious systemic adverse effects, as just mentioned. To achieve the desired tumor-selective delivery of chemotherapeutic agents, drugs must distinguish tumor tissues from normal tissues. This concept of selective toxicity was proposed at the end of 19th century by Paul Ehrlich. Although selective toxicity in bacterial chemotherapy was successful with the use of sulfonamide and penicillin [3], no drug successfully achieved such universal selectivity for solid tumors.

Before 1979, when we developed the first polymeric conjugate drug SMANCS (poly(styrene-co-maleic acid)-conjugated neocarzinostatin) [4], no anticancer macromolecular drugs, which can utilize the pathophysiological characteristics of tumor blood vessels for tumor-selective toxicity, existed. Classic cytotoxic anticancer drugs affect rapidly dividing cancer cells more than non-dividing normal cells, which occur in most normal tissues, by inhibiting DNA synthesis [1].

Furthermore, classic drug-screening models most often utilized ascitic leukemia models such as murine L1210 or P388, in which tumor cells propagated in the peritoneal cavity (i.e. intraperitoneally [i.p.]), and candidate drugs were therefore administered i.p. This system involved little pharmacokinetic consideration or utilization of the vascular physiology of solid tumors.

The more recent trend in anticancer drug development, with so-called

molecular target drugs and antibody drugs, will in theory produce a greater tumor-selective effect by utilizing unique enzymes in tumors such as protein kinase inhibitors, by inhibiting growth factors (EGF [endothelial growth factor] or VEGF [vascular endothelial growth factor]), or by utilizing receptor antagonists of these factors [5]. According to this concept, molecular target drugs should be safer and highly selective for tumors and will not affect normal tissues and organs. Imatinib, the most successful example, is used to treat chronic myelogenous leukemia, in which the abnormal fusion protein (BCR-ABL) is the key protein and imatinib inhibits the function of the fusion protein kinase [6, 7]. Imatinib became a first-line drug and improved the 10-year survival rate in patients with chronic myelogenous leukemia, from 20% to 85% [8].

Furthermore, antibody drugs, most of them being monoclonal antibodies (mAbs) targeting VEGF and growth factor receptors on the tumor cell surface, have been used to treat colon, lung, and breast cancers [5, 9, 10]. Contrary to initial expectations, the adverse effects of molecular target drugs, although occurring with low frequency, are not negligible and may lead to fetalities [11]. In a number of cases, mAb-based molecular target drugs, such as mAbs targeting CTLA-4 (cytotoxic T-lymphocyte antigen 4) or PD-1 (programmed cell death 1), had a significant antitumor effect [12, 13]. In most cases, however, the therapeutic effect was not as marked as that of imatinib and the cost of the drugs did not merit their use [8]; this cost issue is a frequent concern of many specialists and agencies. These discrepancies between the theoretical expectation and clinical results may be due to genetic diversity or heterogeneity and polyclonal properties of tumors in clinical settings, as seen via genomic analyses of many solid tumors observed in clinics [14-16].

## 1. The enhanced permeability and retention (EPR) effect—the first step in tumor-selective drug delivery

The EPR effect is observed in most solid tumors, primarily with biocompatible macromolecules when they are administered intravenously (i.v.). An EPR effect-mediated drug accumulation was observed with various macromolecules such as lipid particles, proteins, synthetic polymers, liposomes, and micelles, with molecular sizes of more than 40 kDa, or 7-8 nm [17]. Therefore, tumor-selective delivery or selective toxicity of antitumor drugs can be achieved by conjugating or encapsulating low-MW antitumor drugs to macromolecular vehicles (proteins, liposomes, polymers, and micelles) (Figure 1). The EPR phenomenon occurs at physiological or tissue levels, not at cellular or subcellular levels. Thus, we can utilize this phenomenon for a

universal method to deliver drugs to various solid tumors and obtain selective toxicity [18]. This unique property of the EPR effect, namely, an elevated vascular permeability and retention of drugs in tumor tissues, derives primarily from three components: (i) defective vascular architecture (large gaps between endothelial cell linings and other defect in vascular architecture, (ii) excessive production of vascular mediators (e.g. bradykinin and nitric oxide [NO]), and (iii) impaired lymphatic recovery from the tumor interstitium.

### 1.1 Factors enhancing vascular permeability

A phenomenon similar to the EPR effect occurs in infected and inflamed tissues; various inflammatory cytokines and chemokines are secreted, thereby increasing vascular permeability and leading to edema, fever, and/or pain. The leaky nature of the tumor vasculature becomes more apparent with most biocompatible plasma proteins, such as albumin (67 kDa), transferrin (90 kDa), and immunoglobulin G (IgG) (170 kDa). The increased vascular permeability in tumor tissue is caused by architectural defects and overproduction of vascular mediators, most of which are proinflammatory effectors. Vascular permeability factors overproduced at or near tumor tissues include bradykinin, NO, prostaglandins, VEGF, tumor necrosis factor-α (TNF-α), carbon monoxide (CO) by heme oxygenase (HO)-1, and others [19, 20]. Many reports on this issue have been published [16, 19, 21, 22].

These vascular mediators facilitate the opening of vascular endothelial cell-cell gaps in normal blood vessels as well, which allows extravasation of macromolecules or nanoparticles of more than 10 nm, including viruses and even bacteria with sizes in the micrometer range (Fang et al., unpublished). Intercellular gap openings in the tumor vasculature may range from 20 nm to 2 μm or larger [23, 24]. This situation is great contrast to endothelial gaps seen in normal vasculature (<5 nm).

### 1.2 Cause of longer retention of macromolecules in tumor tissue and impaired lymphatic clearance

The lymphatic network exists throughout the body and acts as a drainage system for components of interstitial tissue fluid, particularly lipid particles and plasma proteins that extravasate from the blood circulation. However, we observed impaired lymphatic function in tumor tissues, which contributes to retention of such macromolecules in tumors [21, 25]. The issue of lymphatic clearance in tumor tissues differs somewhat from that in inflamed tissues. That is, extravasated proteins and lipid particles in inflamed tissues are cleared by the lymphatic system within about a week. Clearance