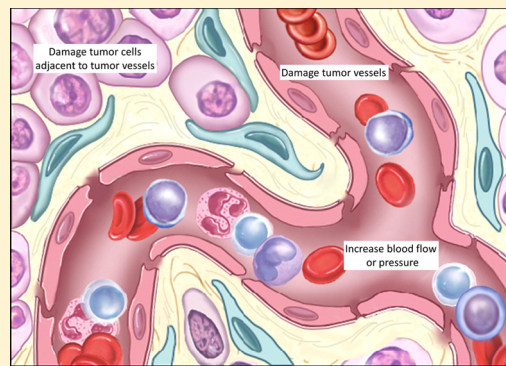


Cancer Drug Delivery: Considerations in the Rational Design of Nanosized Bioconjugates

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ABSTRACT: In order to efficiently deliver anticancer agents to tumors, biocompatible nanoparticles or bioconjugates, including antibody–drug conjugates (ADCs), have recently been designed, synthesized, and tested, some even in clinical trials. Controlled delivery can be enhanced by changing specific design characteristics of the bioconjugate such as its size, the nature of the payload, and the surface features. The delivery of macromolecular drugs to cancers largely relies on the leaky nature of the tumor vasculature compared with healthy vessels in normal organs. When administered intravenously, macromolecular bioconjugates and nanosized agents tend to circulate for prolonged times, unless they are small enough to be excreted by the kidney or stealthy enough to evade the macrophage phagocytic system (MPS), formerly the reticulo-endothelial system (RES). Therefore, macromolecular bioconjugates and nanosized agents with long circulation times leak preferentially into tumor tissue through permeable tumor vessels and are then retained in the tumor bed due to reduced lymphatic drainage. This process is known as the enhanced permeability and retention (EPR) effect. However, success of cancer drug delivery only relying on the EPR effect is still limited. To cure cancer patients, further improvement of drug delivery is required by both designing superior agents and enhancing EPR effects. In this Review, we describe the basis of macromolecular or nanosized bioconjugate delivery into cancer tissue and discuss current diagnostic methods for evaluating leakiness of the tumor vasculature. Then, we discuss methods to augment conventional “permeability and retention” effects for macromolecular or nanosized bioconjugates in cancer tissue.



1. INTRODUCTION

In order to efficiently deliver anticancer agents to tumors, biocompatible nanoparticles or bioconjugates, including antibody–drug conjugates (ADCs), have recently been designed, synthesized, and tested, some even in clinical trials.^{1–4} Macromolecular bioconjugates and nanosized agents have a number of intrinsic advantages over conventional low-molecular-weight agents including a large payload capacity for anticancer agents, the ability to protect the payload from degradation, multivalent targeting moieties, and controlled or sustained release that minimizes side effects while increasing the safety margin of the anticancer agents.^{5–7} Controlled delivery can be enhanced by changing specific design characteristics of the bioconjugate such as its size, the nature of the payload, and the surface features.^{8,9} The delivery of macromolecular drugs to cancers largely relies on the leaky nature of the tumor vasculature compared with healthy vessels in normal organs.¹⁰ When administered intravenously, macromolecular bioconjugates and nanosized agents tend to circulate for prolonged times, unless they are small enough to be excreted by the kidney or stealthy enough to evade the macrophage phagocytic system (MPS), formerly the reticulo-endothelial system (RES).¹¹ Therefore, macromolecular bioconjugates and nanosized agents with long circulation times leak preferentially into tumor tissue through permeable tumor vessels and are then retained in the tumor bed due to

reduced lymphatic drainage. This process is known as the enhanced permeability and retention (EPR) effect.¹² Most macromolecular bioconjugates and nanosized agents tend to accumulate within tumors, due to the EPR effect depending on the vascular characteristics in each tumor, and then release their therapeutic payloads. However, EPR effects provide relatively modest specificity and offer only a 20–30% increase in delivery compared with critical normal organs. Nonetheless, macromolecular bioconjugates and nanosized cancer agents have shown efficacy in animal models of cancer, and several agents are undergoing testing in clinical trials.^{13,14} Clearly, if the EPR effect could be enhanced, potentially great gains could be made in the delivery of macromolecular bioconjugates and nanosized cancer agents, thereby enhancing their anticancer effects.

In this Review, we examine the basis of macromolecular or nanosized bioconjugate delivery into cancer tissue and discuss current diagnostic methods for evaluating leakiness of the tumor vasculature. Then, we discuss methods to augment conventional “permeability and retention” effects for macromolecular or nanosized bioconjugates in cancer tissue.

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2. PHARMACOKINETICS: SMALL VS LARGE MOLECULES

After entry into the systemic circulation, agents are carried via the circulatory system and are distributed into organs. Small molecular weight agents readily leak from the vasculature and distribute within the tissue according to a concentration gradient. For this reason, most small molecular antitumor agents have a large volume of distribution after intravenous administration. While this ensures delivery to the tumor it also exposes normal tissue to toxicity. In addition, rapid clearance from the circulation of such agents can result in challenges in maintaining the drug concentration within the tumor. In contrast, the leakage of macromolecular drugs from vessels is much slower due to their large size in relation to the gaps or fenestrations in normal capillaries. Macromolecular agents have prolonged circulation times, typically measured in hours to days, which provide a larger input function to the tumor. Meanwhile, in combination with leaky tumor vessels, macromolecular agents accumulate within the extravascular space of tumors. This tumor-specific accumulation of large molecular agents affects the injection dose and toxicity resulting in lower doses and reduced toxicity.

When considering the excretion of any agent administered systemically, the renal clearance is the critical determinant of pharmacokinetics. The physiological function of the kidney is to filter the plasma at the glomerular basement membrane. Some molecules that are filtered at the glomerulus may be recovered in the proximal tubules and others that avoid filtration at the glomerulus may be excreted into the urine by proximal tubular epithelium, although this is rare for nanosized agents. The kidney is highly efficient in filtering the plasma, and therefore, glomerular filtration should be taken into account when designing large molecular agents. The glomerular basement membrane is formed by specialized cells and connective tissues, and its surface is negatively charged. The glomerulus has mostly round pores that measure approximately 6 nm in diameter.^{4,11,15} Therefore, the net charge of nanosized agents will highly influence their renal excretion; positively charged or neutral molecules will be filtered into urine more efficiently than negatively charged ones even if they are all approximately 6 nm in diameter.¹⁶ However, strongly positively charged agents could be trapped by the brush border within the proximal tubules. Thus, strongly charged molecules, in general, may have difficulty with renal excretion even if they are at or below the size threshold of the glomerular pore. Additionally, the shape¹⁷ and flexibility (hardness or softness)¹⁸ of the agents will alter filtration; therefore, the hydrodynamic diameter measured by dynamic light scattering (DLS) may be insufficient to predict the degree of renal excretion. "Soft" molecules will more easily be filtered. Another key design factor is that DLS measurements depict the average size of nanoparticles, which may not accurately predict glomerular filtration since nanosized agents typically have a range of sizes from below to above the 6 nm size threshold.

3. EPR EFFECTS FOR MACROMOLECULES AND NANOPARTICLES

Solid tumors often possess a permeable vasculature compared with healthy vessels in normal organs.¹⁰ From the pathophysiological blood/fluid circulation point of view, the endothelial surface is fenestrated with gaps between endothelial cells, and is surrounded by discontinuous or absent basement membranes

and fewer or poorly adherent pericytes.^{19–21} This enables macromolecules to reach tumor cells from the bloodstream in higher concentration than in normal tissues. Furthermore, most solid tumors lack functional intratumoral lymphatic vessels; thus, the clearance of leaked drugs in the tumor extracellular fluid is reduced. Although the lymphatic function is impaired, the tumor has increased intratumoral pressure which leads to convective extravasation from the tumor into the surrounding stroma, somewhat offsetting the deficiency in lymphatics. Thus, although different nanosized agents distribute somehow differently into tumor tissues, the EPR effect results in generally a 20–30% increase in the accumulation of macromolecular agents compared with small molecular weight agents.¹² The liver and MPS recognize and remove foreign bodies from the blood pool including large molecular agents. Therefore, such agents should be designed to evade rapid uptake by the liver or MPS. Hydrophobic smaller nanosized agents frequently bind serum proteins that sometimes accelerate uptake and catabolism of the agent by the liver resulted in short circulation half-life.²² Larger molecules or particles are readily recognized by the MPS. Molecules or particles with highly charged surfaces are also recognized by the MPS and are quickly removed from the circulation (Figure 1). Therefore, useful design parameters

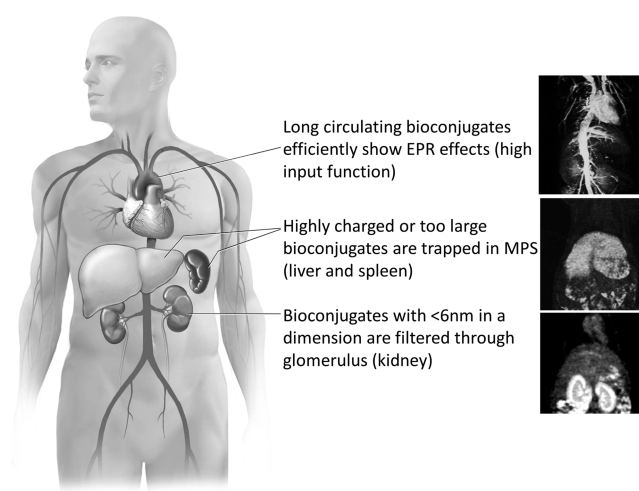


Figure 1. General pharmacokinetics of macromolecular and nanosized bioconjugates when injected intravenously. EPR effects operate only with sufficiently long circulation of bioconjugates.

for a large molecular agent include limiting the size (probably to <300 nm in diameter) and maintaining a net charge as close to neutral as possible while providing a predominantly hydrophilic surface.²³ To achieve this design, hydrophilic and neutral polymers including polyethylene glycol and polysaccharides are commonly used on the surface of nanosized agents to make them "stealthy", thereby avoiding recognition by the liver and MPS.^{24–26}

4. DRUG DELIVERY TO TUMOR: IS THERE A VALUE TO KNOWING THE VASCULAR KINETICS?

The slow clearance of anticancer bioconjugates results in a favorable input function to the tumor. The leakiness of the tumor vessels further contributes to drug delivery. Information regarding the permeability and perfusion of tumors can be obtained with a variety of imaging methods that measure the

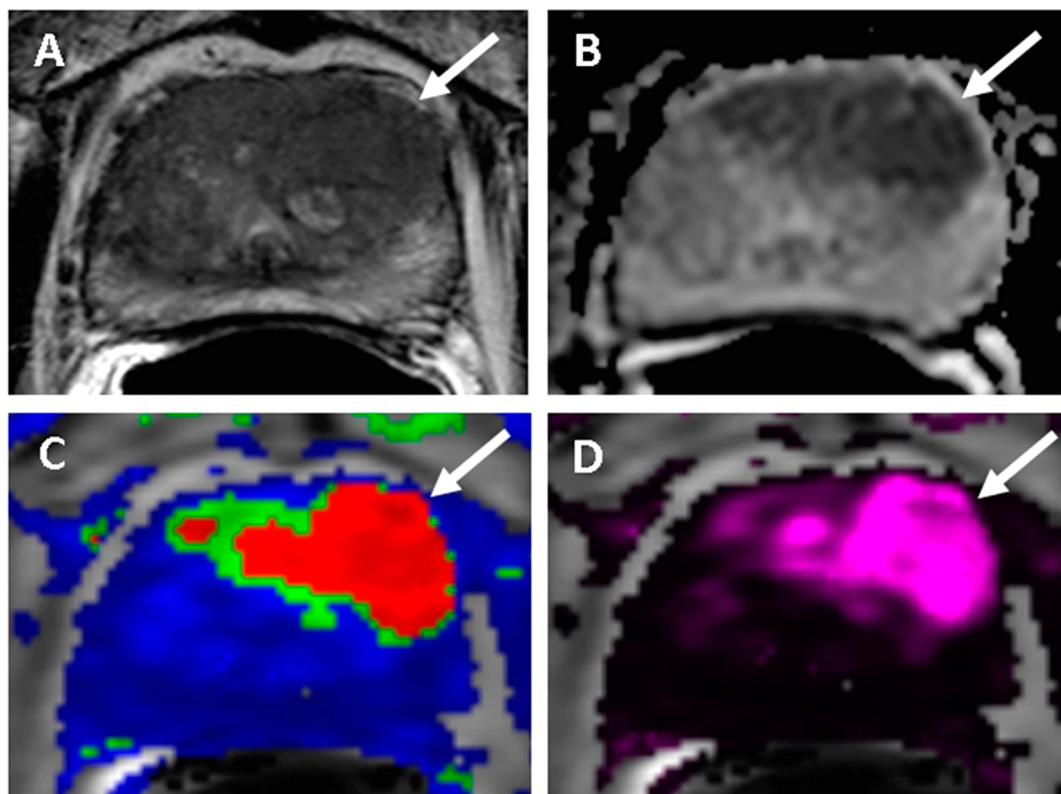


Figure 2. Images of a patient with high serum PSA (17.8 ng/mL) are shown. Axial T2W MRI (A) and ADC maps of diffusion weighted MRI (B) show a midline to the left anterior transition zone lesion in the mid prostate level (arrows). K_{trans} (C) and k_{ep} (D) maps generated from the DCE MRI data using a two-compartment model quantitative technique show an area of leaky vasculature that highly suggests cancer (arrows). Targeted biopsy revealed that highly malignant prostate cancer grew within the suggested lesion.

kinetics of contrast media enhancement, including dynamic imaging using MRI, CT, and ultrasound.

Dynamic contrast enhanced MRI (DCE-MRI) is a functional MRI technique that provides insight into tumor vascular kinetics. DCE-MRI relies on the acquisition of gradient recalled echo (GRE) MRI sequences which are obtained before, during, and after a bolus of intravenous administration, typically a Gadolinium (Gd)-chelate contrast material. These are mainly low molecular weight contrast media [e.g., Gd-DTPA, molecular weight = 567 Da], although newer agents such as gadofosveset dimeglumine can bind albumin and behave as a large agent. During DCE-MRI, tumors are characterized by their rapid, intense enhancement followed by a relatively rapid washout compared to normal background tissue.²⁷

DCE-MRI can be evaluated using one or more of three different approaches:

Qualitative analysis. This is the easiest and the most popular approach. This involves the visual detection of focal early, strong enhancement with early washout—compared with that of normal tissue.

Semiquantitative Analysis. This involves evaluation of the shape of the signal intensity (SI) vs time curve, its onset time, gradient of the upslope of enhancement, peak SI, and the washout rate. Semiquantitative methods have the advantage of being simple to perform and enable the straightforward calculation of SI changes. However, this method depends on a linear relationship between SI and concentration of the Gd chelate, an assumption that is usually not correct. In order to avoid these issues SI should be converted into Gd

concentration by applying a T1 map to the precontrast images and calculating Gd concentration.

Quantitative Analysis. This depends on curves depicting Gd concentration varying over time and uses multicompartiment pharmacokinetic models to calculate permeability constants. The dynamic data obtained via DCE-MRI is used to generate curves which are mathematically fit to two or more compartment pharmacokinetic models. This approach enables the calculation of quantitative parameters such as K_{trans} (forward flow rate constant [wash in]), k_{ep} (reverse flow rate constant [wash out]), fpV (plasma volume fraction compared to whole tissue volume), and V_e (extravascular, extracellular volume fraction of the tumor), using the modified Toft's model (Figure 2).²⁸ The kinetic parameters are usually higher in tumors than in surrounding healthy background tissue and these values can decrease after oncologic treatments such as chemotherapy, antiangiogenic therapy, radiotherapy, and embolotherapy for various cancer types.

Thus, DCE-MRI is a potential biomarker for clinical oncology applications; however, it has several limitations. There is currently no standardization and consensus on which imaging protocol and analysis method should be used. Its intra- and interpatient repeatability and reproducibility are variable. Finally, DCE-MRI may not be feasible for some patient groups, especially for individuals with renal failure (due to the risk of nephrogenic systemic fibrosis after Gd-chelate administration), those with implanted MRI incompatible metallic devices, and those with severe claustrophobia.²⁹

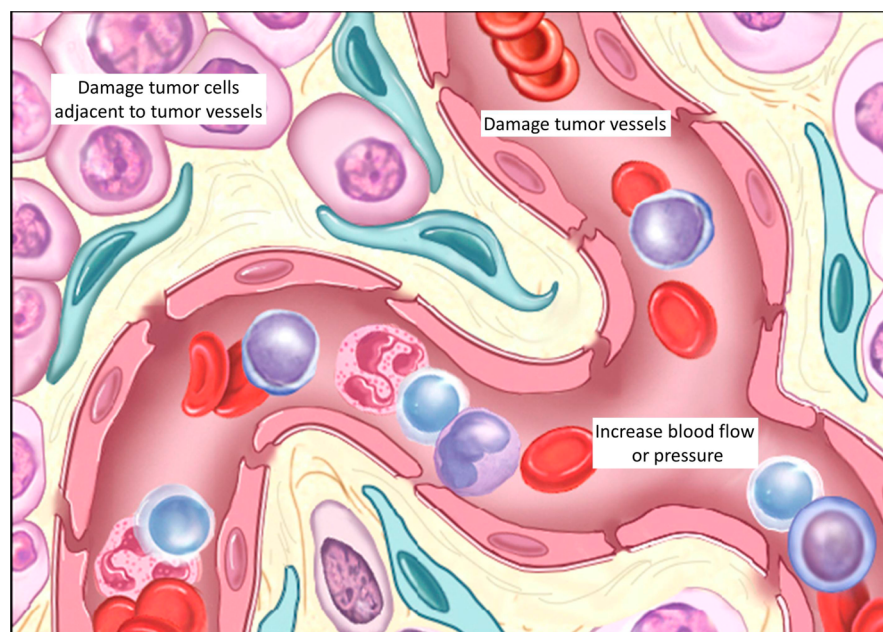


Figure 3. Strategies for further improving cancer delivery of macromolecular and nanosized bioconjugates over conventional EPR effects.

5. HOW TO IMPROVE CANCER DRUG DELIVERY

Macromolecular agents generally tend to accumulate within tumors at higher concentrations than small molecular agent due to the EPR effect. However, as mentioned, EPR effects provide relatively modest increases of approximately 20–30% in delivery compared with normal organs.

The extent of the EPR effect is dependent on several factors.^{30,31} By manipulating either local tumor stroma or systemic conditions, EPR effects can be altered leading to superior macromolecular drug delivery. The three most important modifiable parameters that improve nanosized agent delivery include (1) altering normal physiology without modifying the tumor environment; (2) altering the tumor vasculature or stroma; (3) killing the cancer cells to reduce their barrier function (Figure 3).³²

In this section, we will discuss nonselective and selective molecular targeting methods for further improving the “permeability and retention” effect for large molecular agents in cancer tissue based on modification of these three conditions.

5.1. Physiologic Conditions. In order to improve drug delivery within cancers while avoiding normal tissue, one can increase the input function of the agent. Normal vessels retain their ability to respond to extrinsic vasoconstrictors whereas tumor vessels lose their responsiveness to such agents. In normal vessels, muscular fibers in the vessel wall will contract, limiting blood flow. Therefore, when vasoconstrictive drugs are administered, normal vessels are constricted and blood pressure is increased. In contrast, tumor vessels do not respond to vasoconstrictors because of insufficient muscular structure. This leads to a relative increase in the input function in vessels supplying tumors.³³ This phenomenon was recognized in the 1970s during diagnostic angiography for tumor localization and was termed “pharmacangiography”.³⁴ During diagnostic angiography, vasoconstricting agents including alpha receptor agonists were injected via a catheter to constrict normal vessels while accentuating tumor vessels.^{35,36} Later, pharmacangiography was used to constrict vessels after the delivery of

nanodrug therapy to reduce washout and increase exposure of the tumor to the therapy.³⁷ Diagnostic pharmacangiography is no longer needed for conventional diagnostic scanning because CT and MRI have become so proficient at detecting cancers, but the effect can still be put to use to selectively increase drug delivery.

5.2. Tumor Vasculature or Stroma. Another approach for improving nanodrug delivery to cancers is to physiologically modify the tumor vasculature. Several anti-angiogenic drugs have been approved and are in common use. Among them, the anti-vascular endothelial growth factor (VEGF) monoclonal antibody, bevacizumab, is used for blocking the effect of VEGF, thus inhibiting tumor angiogenesis, decreasing vascular permeability, and suppressing tumor growth.³⁸ On the other hand, the administration of VEGF itself may temporally increase leakiness and perfusion in tumor tissue and thus is a potential way to physiologically augment the EPR effect.³⁹ It has also been argued that anti-angiogenic treatment initially results in vascular normalization which temporarily improves the distribution of blood in the center of the tumor by reducing interstitial pressure and, thus, improves delivery of drugs, although this phenomenon is generally believed to be short-lived.⁴⁰

In recent work, the endothelial cells of the tumor vasculature have been targeted using the $\alpha v \beta 3$ integrin which is highly expressed in growing neovessels. The RGD-peptide sequence has high affinity for $\alpha v \beta 3$ integrin. To prolong its clearance, the RGD peptide can be conjugated to a gold nanoparticle. When light is applied, the gold nanoparticle induces photothermal damage leading to enhanced EPR effects and cell death.⁴¹ Similar effects have been seen with targeted ultrasound microbubbles.⁴² While it might be tempting to damage endothelial cells in an attempt to increase permeability, this can only be achieved at the risk of decreasing or even eliminating blood flow to the tumor due to thrombosis. Paradoxically, this could further reduce the amount of circulating drug available to the tumor.

There are several other approaches to targeting the vasculature or stroma to promote more vascular supply and

vascular permeability in tumors. These approaches include hyperthermia^{43,44} radiotherapy,⁴⁵ high intensity focused ultrasound,⁴⁶ and various mediators including bradykinin,^{47–50} nitric oxide-releasing agent,^{51,52} angiotensin-converting enzyme inhibitors,^{47–50} tumor necrosis factor α ,^{51,53} heme oxygenase-1,^{48,54} and proteases including collagenase⁵⁵ or hyaluronidase.⁵⁶ Cancer cells under hypoxic condition intrinsically produce some of these humeral factors. Most of these mediators are low molecular weight compounds and thus, when additionally injected into systemic circulation, will affect normal blood vessels in the vicinity of tumor, thus facilitating extravasation not only within tumors but around them as well. A theoretical concern is that compromising the integrity of cancer stroma may promote metastasis; however, this has not been extensively investigated.³²

As an alternative method to manipulating vascular endothelial cell function by physical or chemical or biological stimulation, the use of active transport across the endothelial cells via caveoli or ICAM-1 has been investigated.^{57–59} However, although active transport into cancer tissue has been investigated for more than a decade by a few groups and these methods might be promising, active transport technology has not been widely applied or accepted for designing superior bioconjugate agents or nanosized particles for cancer treatment.

5.3. Killing Tumor Cells. Nanodrug delivery reportedly increases after many cancer therapies. The likely explanation for this is that tumor cells themselves act as a barrier to deeper penetration of nanodrugs. The heterogeneity of the blood supply within the tumor microenvironment leads to marked heterogeneity in the rate of cell proliferation; cancer cells near the vessels proliferate rapidly, while cancer cells further away from the vessels suffer nutrient deprivation and proliferate more slowly.^{60,61} Microscopy reveals that tumor cells grow as sleeves or sheaths concentric with tumor vessels.⁶² Such highly cellular layers may interfere with drug penetration.⁶³

For instance, a single application of X-ray therapy damages cancer cells but leaves the vasculature intact. Thus, an increase in the delivery of nanosized molecules up to 2.2-fold at a peak of 8–12 h after radiation has been observed.⁶⁴ In this case the radiation killed well oxygenated cancer cells near tumor vessels, therefore temporarily increasing vascular permeability by reducing the barrier function of the cancer cells. The greatest cell damage occurred in perivascular cancer cells which subsequently underwent apoptosis. Interestingly, excessive radiation damaged the vessels sufficiently to shut down blood flow which negatively affected nanodrug delivery. Similar vascular shutdown was reported during photodynamic therapy (PDT).⁶⁵ Since PDT damages both cancer cells and normal cells, PDT often reduces tumor blood flow.⁶⁶ Similar effects were observed with some chemotherapy including paclitaxel or docetaxel,⁶⁷ which preferably killed tumor cells close to blood vessels.

Photothermal damage is another method of selectively increasing EPR effects. By using a GRP78-targeting peptide conjugated to a PEGylated gold nanorod, photothermal damage could be induced after the application of light.⁶⁸ Furthermore, systemic radioimmunoconjugates preferably killed perivascular tumor cells resulting in improved drug delivery.^{69,70} However, these methods could also damage tumor vasculatures resulting in thrombotic occlusion from the bystander effect. More recently, another more selective method of killing tumor cells to augment drug delivery, named photoimmunotherapy (PIT), has been described.⁷¹ PIT can

specifically kill cancer cells exposed to near-infrared light by inducing immediate necrosis without damaging normal cells, including vascular endothelial cells. Since most of the initial cell killing occurs in the perivascular tumor sheaths, increases in nanodrug delivery of up to 24-fold compared with untreated control tumors can be observed (Figure 4).⁷² This increased

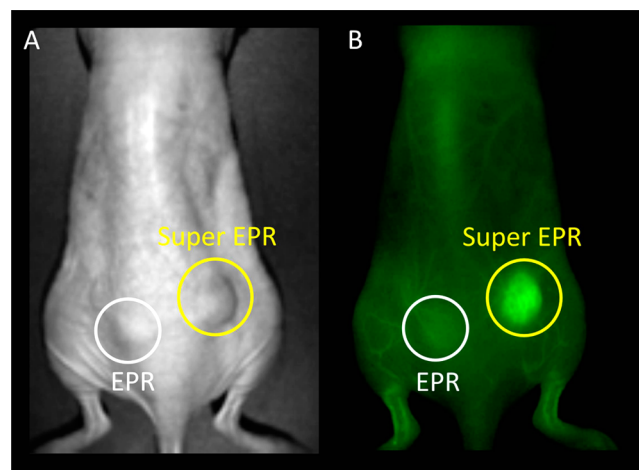


Figure 4. Photoimmunotherapy induced super-enhanced permeability and retention (SUPR) effects delivered PEGylated quantum dots (800 nm emission; 50 nm in diameter) into PIT-treated tumor 24-fold higher concentration than in nontreated tumor with conventional EPR effects at 1 h after injection. (A: white light image, B: 800 nm fluorescence image.)

permeability was induced immediately after exposure to near-infrared light. In order to analyze tumor vascular permeability and delivery of nanosized agents, static and dynamic fluorescence imaging with fluorescent proteins and nanoparticles was commonly used.^{73–75} Dynamic fluorescence imaging showed that intravenously injected, nontargeted polyethylene glycol coated quantum dots (PEG-QD) quickly accumulated in the PIT-treated tumor bed compared with nontreated controls. Histology after PIT showed a markedly dilated tumor vasculature in the widened tumor interstitium along with cancer cell debris. Additionally, intravenously injected PEG-QD leaked throughout the cancer tissue following PIT. Thus, PIT induces an immediate necrosis especially in the layers of cancer cells surrounding the tumor vasculature without damaging vascular cells themselves. This initially leads to decreased interstitial pressure and a commensurate rise in perfusion and leakage into the tumor bed. Therefore, PIT induces selective damage to perivascular cancer tissues markedly augmenting the EPR effect and dramatically increasing drug delivery. This super-enhanced EPR has also been referred to as SUPR to distinguish it from conventional EPR.

6. CONCLUSION

Macromolecular bioconjugates and nanosized cancer agents are promising for improving cancer chemotherapy because they can achieve target-specific or controlled delivery of large payloads of anticancer agents, resulting in improved tumor delivery based on the unmodified EPR effect. There are several clinically available diagnostic imaging methods to evaluate leakiness of tumor vasculature. Furthermore, a few methods to further improve nanodrug delivery by augmenting the conven-

tional EPR effects have been discovered. Among them, super-enhanced EPR effects which occur after PIT induce damage in the layers of cancer cells immediately adjacent to the tumor vasculature, and this can have dramatic effects on perfusion with improvements in the delivery of nanoparticles of up to 24-fold compared with untreated tumors. The magnitude of the nanodelivery improvement could have a direct impact on the therapeutic effects of nanosized cancer drugs possibly resulting in dose reductions when used sequentially after PIT.

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Notes

The authors declare no competing financial interest.

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