Challenges of Drug Delivery Systems That Contribute to Cancer Chemotherapy

Alteration of Tumor Microenvironment for Improved Delivery and Intratumor Distribution of Nanocarriers

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Nanocarrier-based cancer chemotherapeutics are thought to increase therapeutic efficiency and reduce the side effects of associated chemotherapeutic agents by altering the agents’ pharmacokinetics and tissue distribution following intravenous administration. In spite of these favorable properties, nanocarrier-based cancer chemotherapeutics are not always effective because of their heterogeneous intratumoral localization. Homogeneous distribution of nanocarriers in a tumor would improve the efficacy of nanocarrier-based cancer chemotherapeutics. In this article, we describe and discuss some trials that attempt to manipulate the barriers in the tumor microenvironment that hinder extravasation through the tumor vasculature and penetration of nanocarriers in solid tumors. Alterations of the tumor microenvironment that relate directly to the intratumoral distribution of nanocarriers may be potential strategies to improve the delivery of nanocarrier-based cancer chemotherapeutics.

Key words nanocarrier; enhanced permeability and retention effect; tumor microenvironment; anticancer therapy

1. INTRODUCTION

Conventional chemotherapy is currently still problematic because of the disproportional relationship between antitumor effects and adverse effects. Anticancer agents are easily distributed in the patient’s body and indiscriminately reach not only tumors, but also normal organs and tissues. Therefore, the development of a suitable tumor-selective drug delivery system is needed to avoid the undesirable systemic side effects of anticancer agents. The most effective strategy is to exploit the anatomical and pathophysiological abnormalities of tumor tissue, particularly the tumor vasculature. Nanoparticles such as polymeric micelles and liposomes, which have a particle size of 50–200 nm, have been found to effectively accumulate in solid tumors due to the abnormal features of the tumor microenvironment. This characteristic is generally known as the “enhanced permeability and retention (EPR)” effect. (Fig. 1) This characteristic is generally known as the “enhanced permeability and retention (EPR)” effect of vascular permeability and/or hypovascularity, appears to become a barrier to extravasation of nanocarriers from tumor vasculature and their diffusion in the interstitium of solid tumors (Fig. 1B). In this article, we describe and discuss some trials that attempt to manipulate the barriers in the tumor microenvironment that hinder extravasation through the tumor vasculature and penetration of nanocarriers in solid tumors.

2. BARRIERS TO ACCUMULATION OF NANOCELLULARIS IN THE TUMOR MICROENVIRONMENT

2.1. Tumor Vasculature and Blood Flow

The vasculature in the solid tumor is structurally and functionally abnormal, leaky, tortuous, dilated and saccular, and has a haphazard pattern of interconnection. These abnormalities contribute to spatial and temporal heterogeneity in tumor blood flow. In addition, solid pressure generated by proliferating tumor cells compresses intratumor blood and lymphatic vessels. Such vascular and lymphatic abnormalities in solid tumors are a major cause of the abnormal tumor microenvironment. With respect to tumor vascular architecture, the vessel wall structure is very abnormal. Large interendothelial junctions, increased numbers of permeable fenestrations, and a lack of normal basement membrane are often found in tumor vessels. In addition, perivascular cells have abnormal morphology and heterogeneous associations with tumor vessels. Consistent with these structural abnormalities in the tumor vessel wall, the vascular permeability of solid tumor vessels relating to the EPR effect is generally higher than that of most normal vessels, resulting in enhanced accumulation of nanocarriers. However, extravasation of nanocarriers is limited by the cut-off size of the “pores” in the walls of tumor ves-
sels, which vary from 100 nm to 2 \( \mu \)m depending on the tumor type, its growth location, and whether it is growing or regressing.\(^{3,13}\) Nanocarriers with a mean diameter of ca. 200 nm are preferred for tumor targeting carrier systems.

2.2. Tumor Substance and Extracellular Matrix

To deliver the associated-anticancer agents to tumor cells, nanocarriers require their transport from the bloodstream to the tumor interstitium, and then diffuse through the tumor interstitium. The diffusion of nanocarriers is strongly affected by the extracellular matrix (ECM) composition and by its geometry.\(^{14–16}\) Compared with normal tissues, the tumor interstitium is characterized by an altered ECM and an increased number of fibroblasts that extensively synthesize adhesion molecules.\(^{17}\) The ECM components vary greatly among tumors in both amount and composition.\(^{18}\) Tumor-associated ECM is composed mainly of type 1 collagen, glycosaminoglycans such as hyaluronan, and proteoglycans such as decorin and glycol protein.\(^{19,20}\) and these ECM components form a complex structured gel. The resistance to interstitial flow is strongly linked to glycosaminoglycans, and especially hyaluronan, in the interstitial space of tumors.\(^{15,21}\) Although the diffusion rate of nanocarriers depends, of course, on the tumor type and the size and charge of the nanoparticles,\(^{16,22,23}\) the ECM is one of the major barriers to the diffusion of nanocarriers in the tumor interstitium.

3. BREAKTHROUGH TO ENHANCE EXTRAVASATION AND SUBSEQUENT DIFFUSION OF NANOCARRIERS IN TUMOR

3.1. Increased Blood Flow in Tumors

Diphtheria Toxin Treatment

Treatment with diphtheria toxin, which is much less cytotoxic to mouse cells than to human ones, caused cellular death (apoptosis) in human-tumor xenografts grown in mice,\(^{24}\) while the treatment showed no effect on murine tumors. As a consequence, diphtheria toxin treatment led to a greater fraction of blood and lymphatic vessels with an open lumen in human tumors.\(^{25}\) This study confirms that proliferating tumor cells cause intratumor vessels, particularly those without supportive stromal structures, to collapse, thus leading to impaired blood flow. Tumor-selective cytotoxic therapy, which affects growing vascular endothelial cells, growing smooth muscle cells, and growing tumor cells, may result in more homogeneous blood flow in tumors and thereby enhance the efficient delivery of nanocarrier-based drugs to tumor tissue.

Angiotensin-II (AT-II)-Induced Hypertension

It has been reported that the smooth muscle layer, which plays a vital role in regulating blood pressure and flow, is lost in vasculature in some parts of tumor tissue. In normal blood vessels, angiotensin-II (AT-II), a vascular mediator, causes hypertension (increasing blood pressure and flow rate) via AT-II receptors...
on vascular smooth muscle cells. However, under these conditions the blood flow volume remains constant in normal tissue because of the existence of smooth muscle actin. \(^{26–28}\) On the other hand, the tumor blood vessels cannot regulate the blood flow volume because of the absence of the smooth muscle layer. Consequently, blood flow volume in tumor tissue increases 2–6 times in proportion to elevated blood pressure. \(^{26}\)

The induction of the hypertensive state by AT-II is, therefore, expected to augment the EPR effect and, thereby, the delivery of macromolecular drugs and nanocarriers. \(^{6,7,29}\)

**3.2. Enhanced Extravasation of Nanoparticles from Tumor Blood Circulation**

**Inhibition of Transforming Growth Factor-β (TGF-β) Signaling**

TGF-β is known as a multifunctional cytokine, which regulates the growth, differentiation, migration, adhesion, and apoptosis of various types of cells. TGF-β inhibits the growth and migration of blood vascular endothelial cells in vitro, whereas it induces angiogenesis in vivo. \(^{30}\) Mice lacking certain components related to TGF-β signaling (e.g., TGF-β1, TGF-β2, or TGF-β3) exhibited abnormalities in blood vessels. \(^{31–33}\) Although the dextran (2 MDa, 50 nm) remained mostly in the intravascular space in the control tumor, the use of low dose TGF-β1 inhibitor resulted in extravasation and a far broader distribution of the dextran around the tumor neovascularity. \(^{34,35}\) These findings suggest that low-dose TGF-β1 inhibitor can maintain blood flow in the tumor vasculature and simultaneously induce extravasation of macromolecules. TGF-β1 inhibitor co-administered with DXR-containing PEGylated liposome and polymeric micelles significantly enhanced intratumoral accumulation of DXR in a xenograft murine model of human BxPc3 cells. \(^{6,7}\) Consequently, a strategy in combination with TGF-β signaling could be a breakthrough in chemotherapy delivered by nanocarriers in solid tumors.

Tumor Necrosis Factor (TNF-α) TNF-α is one of the most thoroughly investigated cytokines and affects tumor-associated vasculature, not the vascularization of normal tissue. \(^{37}\) Seynhaeve et al. \(^{38}\) indicated that the alteration of tumor vasculature caused by TNF-α led to further abnormalities—in particular, vascular permeability in the tumor—and that treatment of low-dose TNF-α in combination with i.v. administration of PEGylated liposome facilitated extravasation of the liposome from blood vessels and more homogeneous distribution in solid tumors. This revealed that treatment with TNF-α not only increases the leakiness of some tumor vessels, but also renders more vessels permeable to nanocarriers such as liposomes 100–200 nm in mean diameter, while leaving the vascular function (e.g., flow) intact. They finally demonstrated an improved tumor response due to a more homogeneous distribution of anticancer drug-containing nanocarriers in the tumor.

**Vascular Endothelial Growth Factor (VEGF)**

VEGF is considered the central factor in both physiological and pathological angiogenesis. \(^{39,40}\) Its receptors are predominantly expressed on neovascularization in tumors. VEGF has vasoactive properties and is 50000 times more potent than histamine in increasing permeability. \(^{41,42}\) Together with angiopoietins, VEGF regulates the interaction of endothelial cells with other endothelial cells, pericytes and basal membranes. Hence, VEGF triggers dissociation of the endothelial cells, resulting in leakage and the generation of edema. \(^{43–45}\) So, the exogenous addition of VEGF to hypopermeable vessels could be used to increase transvascular transport of macromolecules, including nanocarriers, in these regions. \(^{31}\)

Nitric Oxide (NO) NO, synthesized from l-arginine by NO synthase (NOS), is a well-known vasoactive agent that increases vascular permeability in tumors. \(^{46–48}\) Because of such vasoactivity, NO may increase the EPR effect against nanocarriers by widening the endothelial gaps of tumor-feeding arteries. In one such case, in humans, treatment with the NO-releasing agent isosorbide dinitrate enhanced the opening of the tumor-feeding artery, and more drug entered the tumor. \(^{49}\) This technique, plus co-treatment with AT-II, further enhanced the site-specific delivery of SMANCS–Lipiodol to the tumor. \(^{49}\)

**3.3. Augmentation of Distribution of Nanocarriers in Solid Tumor**

**Alteration of Extracellular Matrix (ECM)**

Composition Nanocarriers must extravasate from blood and penetrate through the ECM in the tumor interstitial space in a tumor. The movement of nanocarriers through the ECM relies on passive diffusive transport, \(^{50}\) but the movement via passive diffusion becomes less efficient for nanocarriers. Collagenase treatment efficiently digests collagen in tumor ECM, and was found to increase macromolecule diffusion in tumors. \(^{21,51,52}\) Digestion of type I collagen by collagenase produced a 2-fold increase in the diffusion of 10 kDa dextran at all tumor depths and similar increase in the diffusion of 500 kDa dextran and albumin, but only in superficial tumors. These indicate that collagen in the interstitial space of tumors plays an important role in regulating the initial distribution of macromolecules in tumors.

Digestion of decorin, a major ECM, by cathepsin C produced a more substantial increase in the diffusion of 500 kDa dextran deeper in tumor tissue. \(^{53}\) Decorin plays a role as an important determinant of macromolecule diffusion in tumor tissue ECM. Decorin digestion may produce a widening of collagen interfibrillar spaces. \(^{54}\) This strongly indicates that decorin is a target to enhance macromolecule diffusion in tumors.

Digestion of hyaluronic acid (HA) by hyaluronidase administration was found to enhance the therapeutic efficacy of antitumor agents associated with nanocarriers. \(^{55,56}\) Hyaluronidase treatment increased the tumor uptake and improved the distribution of liposomal DXR. \(^{57}\) On the other hand, Magzoub et al. \(^{58}\) reported that digestion of hyaluronan slowed macromolecule diffusion in tumor tissue. This discrepancy with respect to the consequences of hyaluronidase treatment is not well understood. Further research is needed.

**Creation of Void Space in Extravascular-Interstitial compartments in Tumors**

The existence of ECM is not the only barrier to the diffusion of nanocarriers through tumor interstitium. The narrow spacing of ca. 20 nm between tumor cells also hinders the penetration of most nanoparticles. Nagano et al. \(^{59}\) tested whether the void spaces, enlarged due to tumor cell apoptosis, enhance the initial penetration of microspheres (100 nm) in tumors. Tumor cell apoptosis was induced by treatment with doxycycline-regulated expression of CD8/caspase-8, paclitaxel, or paclitaxel plus tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). In the control tumor, the microspheres were restricted to small areas in the tumor center. By contrast, in the treated tumor, the microspheres were distributed in larger areas and found in the periphery of the tumor. Their results suggest that induction of apoptosis and the resulting void space enlargement in the tumor facili-
tate penetration of nanoparticles into the tumor tissue.

Treatment with Chemotherapeutic Agents Paclitaxel tumor priming promoted the interstitial transport of nanoparticles, because such priming reduced the tumor cell density as a result of cellular death, expanded the microvessel diameter, and increased tumor perfusion.60)

We recently reported that metronomic oral cyclophosphamide (CPA) dosing promoted enhanced accumulation of PEGylated liposomes in solid tumor tissue and increased the therapeutic efficacy of DXR associated with PEGylated liposomes.61,62) We assume that this enhancing effect on the EPR effect reflects the transient increase in density of microvessels in the tumor tissues. Anti-angiogenesis induced by metronomic CPA dosing might cause tumor tissue hypoxia by diminishing blood flow, and the resulting hypoxia and acidification of the surrounding tissue might induce a transient increase in the density of microvessels, consequently creating higher permeability for the PEGylated liposome in the tumor.

Similar tumor augmentation to CPA was observed with metronomic S-1 dosing.63) S-1 is a novel oral fluoropyrimidine derivative and consists of the three pharmacological agents Tegafur (TF), 5-chloro-2,4-dihydroxypyrimidine (cdHP) and potassium oxonate (Oxo) in a molar ratio of 1:0.4:1.64) Its antitumor activity is achieved by an increased and prolonged retention of 5-FU in the blood derived from prodrug TF.65,66) S-1 metronomic dosing also enhanced the accumulation and distribution of both oxaliplatin-containing PEG-coated liposomes65) and PEG-coated siRNA-lipoplexes67) in solid tumor, resulting in a potent tumor growth suppressive effect. The superior antitumor activity in the combined treatment might be due to the following tentative mechanism: pretreatment with metronomic S-1 dosing results in a preferential intratumoral accumulation of nanocarriers, along with cytotoxic action on tumor cells and endothelial cells, and permits efficient delivery and homogeneous distribution of nanocarriers in the tumor tissue, thereby potentiating the cytotoxic effects of the payloads in the nanocarriers.

The approach using clinically approved chemotherapeutic agents, such as Paclitaxel, CPA and S-1, to augment the tumor microenvironment may be considered a breakthrough in drug delivery strategies with nanocarriers and may be hopefully translated into clinical settings.

4. CONCLUSION

The therapeutic efficiency of cancer treatment by means of nanocarriers is clearly dependent on effective delivery of the encapsulated anticancer agents into tumors. However, the effectiveness of delivery is often impaired because of barriers in the tumor microenvironment, as described in this chapter. We here described the importance of alteration of the tumor microenvironment which augments the nanocarriers’ extravasation and diffusion process to promote enhanced therapeutic effects of anticancer agents associated with nanocarriers. Innovations in the strategies to control the tumor microenvironment may lead to a breakthrough in nanocarrier-based chemotherapy.

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