Transvascular Drug Delivery in Solid Tumors

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The microvessel wall is a barrier for the delivery of various therapeutic agents to tumor cells. Tumor microvessels are, in general, more permeable to macromolecules than normal vessels. The hyperpermeability is presumably due to the existence of large pore structures in the vessel wall, induced by various cytokines. The cutoff pore size is tumor dependent, as determined by transport studies of nanoparticles. The vascular permeability is heterogeneous in tumors and dependent on physicochemical properties of molecules as well as the ultrastructure of the vessel wall. The ultrastructure is dynamic and can be modulated by the tumor microenvironment. The microenvironment itself can be altered by the transvascular transport because the transport may facilitate angiogenesis, reduce blood flow, and induce interstitial hypertension in tumors. Future studies of transport need to address mechanisms of the barrier formation and emphasize development of novel strategies for circumventing or exploiting the vascular barrier.

Delivery of therapeutic agents to tumor cells in vivo encounters three major problems: (1) metabolism and clearance of drugs in the body, (2) physiological barriers for transport of therapeutic agents from sites of administration to tumor cells, and (3) drug resistance of tumor cells.[1] The issue of drug delivery to solid tumors is unique for two reasons. First, anticancer drugs are toxic to both tumor and normal cells. Hence, the dose of drugs is limited by normal tissue tolerance. In some cases, tolerance is lower in humans than in experimental animals.[2,3] Therefore, the therapeutic efficacy observed in rodents bearing human tumor xenografts may not be achievable in patients, owing to inadequate drug delivery.[2-5] Second, drug delivery in tumors is nonuniform; there are regions in tumors where the drug exposure is insufficient. The heterogeneous distribution may contribute to the incomplete eradication of tumors by therapeutic agents. In general, heterogeneous distribution of small drugs with short plasma half-life is attributed to the chaotic vasculature and microcirculation in tumors,[4-9] whereas the heterogeneous delivery of macromolecules or nanoparticles (eg, liposomes, viral vectors) is likely due to heterogeneous angiogenesis as well as transvascular and interstitial transport.[4-6,10-12]

Specific problems in tumor interstitial transport include (1) low convective transport because of the interstitial hypertension and the lack of functional lymphatics; (2) outward gradient of the interstitial fluid pressure, which may cause convective transport of extravasated drugs from the interior to the periphery of tumors; (3) large diffusion distance in some regions; and (4) binding of drugs to tumor and stroma cells as well as to the extracellular matrix.[5] Review of the interstitial transport in tumors is beyond the scope of this article (for review, see Jain[5]). Therefore, the following discussion is focused on the transvascular transport of therapeutic agents in solid tumors.

Transvascular Transport in Tumors

Transvascular transport in tumors is heterogeneous. This heterogeneity is exemplified by examining liposomal transport in tumors. When injected into the systemic circulation, fluorescently labeled liposomes accumulate in certain regions in solid tumors but are absent in others (Fig 1).[12] Even along the same vessel, the distribution of liposomes can be nonuniform (Fig 1). Mechanisms of the heterogeneous transport are multifactorial and not well understood.

Transvascular transport is characterized by the hydraulic conductivity to water and the microvascular permeability to other molecules. Both of them are phenomenological quantities for characterizing molecular transport across membranes. The hydraulic conductivity of tumor vessels has not yet been quantified. The capillary filtration coefficient, however, which is the product (L_pS) of the hydraulic conductivity (L_p) and the surface area of vessels (S), has been reported in the literature.[13] The filtration coefficient in an isolated rat mammary adenocarcinoma (R3230AC) perfused ex vivo is much higher than that in normal tissues.[13] It is likely that the hydraulic conductivity in tumors is also higher than normal tissues, as indicated indirectly by the vasogenic cerebral edema in brain tumor patients[14] and the elevated interstitial fluid pressure in most solid tumors.[5]

The microvascular permeability in tumors has

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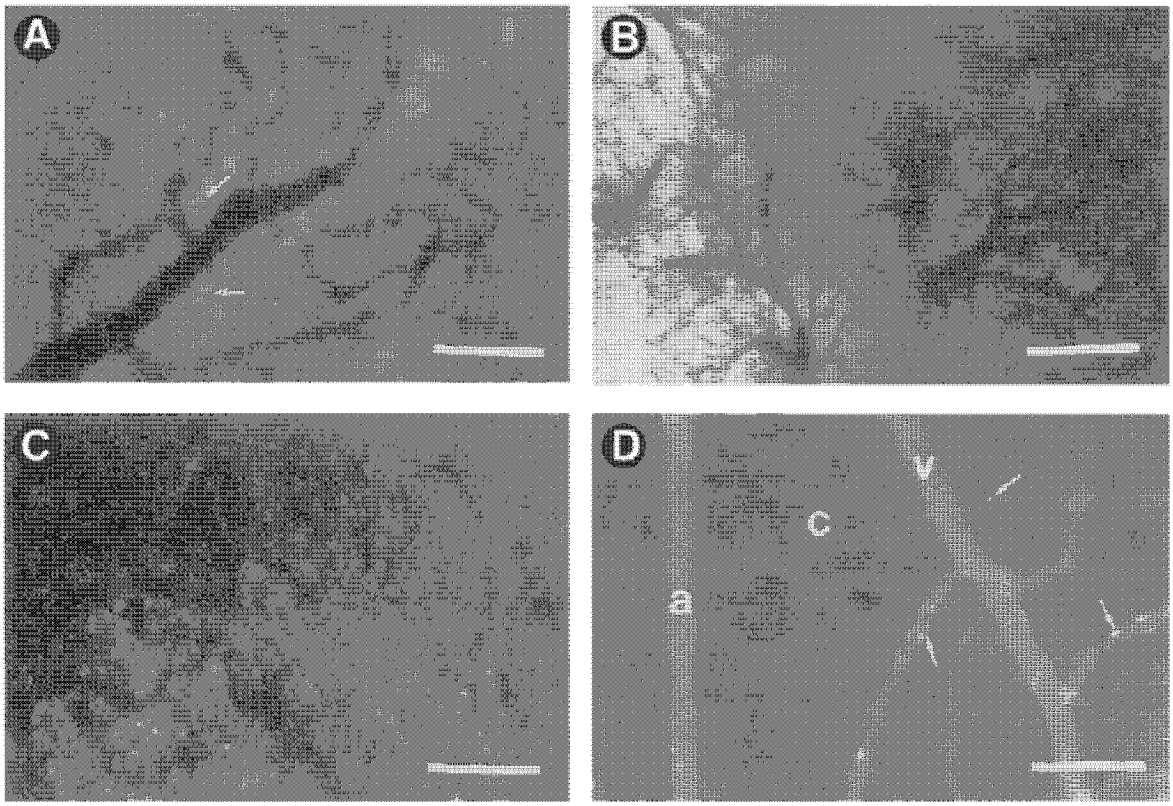

Figure 1. Heterogeneous distribution of liposomes in tumor and normal tissues. Human colon adenocarcinoma cells (LS174T) were transplanted in dorsal skinfold chambers in severe combined immunodeficient mice. Fifteen to 32 days after tumor cell transplantation, fluorescently labeled liposomes were injected intravenously. The photos were taken 2 days after injections. (A) Local heterogeneity: Liposomes accumulated only in perivascular regions in solid tumors. Bar, 100 μm. (B) Regional heterogeneity: There was a significant extravasation of liposomes on the left and nearly no extravasation on the right. Both sides of the tumor were vascularized and well perfused. Bar, 400 μm. (C) Most extravasated liposomes accumulated near the roots of capillary sprouts, whereas the sprouts per se showed minimal leakiness. Bar, 200 μm. (D) Liposomes accumulated only in the wall of small postcapillary venules (6 to 25 μm in diameter) in normal subcutaneous tissues as indicated by arrows. Neither parallel capillaries (c) nor arterioles (a) and large collecting venules (v, >25 μm) were labeled by liposomes. Bar, 100 μm. (Reprinted with permission.[12])

been studied extensively. The data from the literature, as summarized in Table 1, demonstrate that tumor microvascular permeability is in general elevated (Fig 1).[5,10-12,15-23] This is presumably due to the exposure of tumor vascular endothelial cells to cytokines, such as vascular endothelial growth factor/vascular permeability factor (VEGF/VPF).[5,20,24] Primary brain tumors, however, may represent a case different from other tumors.

Vessels in some but not all primary brain tumors are nearly impermeable to therapeutic drugs or diagnostic agents. The tight blood-tumor barrier (BTB) has been observed both clinically and experimentally. For example, vessels in a human glioblastoma xenograft (HGL21) transplanted in mice have been shown to be similar to the blood-brain barrier (BBB) that is impermeable to sodium fluorescein (MW = 376) and Lissamine green (MW = 577).[18] In the clinic, computed tomography and magnetic resonance imaging studies demonstrate that some human glioblastomas and cerebral lymphomas do not show contrast enhancement after infusion of contrast agents,[25-28] and the percentage of contrast enhancement may depend on the type, stage, and location of tumors as well as the age of patients.[26,28] Ultrastructural studies of human brain tumors also reveal heterogeneous results. Fenestrated vessels are observed in some glial tumors[29] but not in others.[30] For nonglial tumors and brain metastasis, the results are more consistent; vessels in these tumors are fenestrated.[30-32] The existence of fenestrated vessels in solid tumors, however, has been challenged by animal studies,[24,33,34] as discussed later.

In addition to tumor vessel wall, the BTB includes

Table 1. The Microvascular Permeability of Tumor Vessels

Tumor Tissue	*Host Tissue*	*Tracer*	*Size of Tracer**	*Permeability (10^{-7} cm/s)*	*Reference*
Human colon aca (LS174T)	SCID† sc tissue	Fc fragment	25,000	4.3 ± 1.6	19
		Fab′ fragment	25,000	4.6 ± 1.0	19
		Ovalbumin	45,000	5.9 ± 1.2	19
		Albumin	66,000	1.4 ± 0.5	19
		Concanavalin A	104,000	1.9 ± 0.7	19
		$F(ab')_2$ fragment	110,000	1.4 ± 0.3	19
		IgG	160,000	2.6 ± 1.1	19
		Stabilized liposome	90 nm	0.20 ± 0.16	12
	SCID liver	Albumin	66,000	4.8 ± 3.5	23
	SCID pia mater	Albumin	66,000	4.5 ± 0.9	20
Human glioblastoma (HGL21)	SCID pia mater	Albumin	66,000	0.11 ± 0.05	18
Human glioblastoma (U87)	SCID pia mater	Albumin	66,000	3.8 ± 1.2	18
Human melanoma (P-MEL)	SCID pia mater	Albumin	66,000	1.0 ± 0.1	20
Mouse mammary aca (MCaIV)	C3H sc tissue	Albumin	66,000	2.1 ± 0.7	22
	C3H pia mater	Albumin	66,000	2.9 ± 1.5	18
	SCID pia mater	Albumin	66,000	3.1 ± 0.5	20
Rat mammary aca (R3230AC)	Rat granulation tissue	Sulforhodamine B	558	340 ± 70	16
		Albumin	66,000	7.8 ± 1.2	16
		Stabilized liposome	82 nm	3.4 ± 0.8	10
		Conventional liposome	91 nm	1.8 ± 0.4	10
	Rat pia mater	Albumin	66,000	1.7 ± 0.6	18
Rabbit mammary aca (VX2)	Rabbit granulation tissue	Dextran	150,000	5.7 ± 3.9	15

*The size of tracers is indicated by either the molecular weight (without unit) or the diameter (nm).
†SCID (severe combined immunodeficient) and C3H are two strains of mice.
Abbreviations: aca, adenocarcinoma; sc, subcutaneous.

the wall of normal vessels in surrounding tissues. This is because tumor cells often invade normal tissues, and the permeability of normal vessels is low, especially in the brain.[35,36] Therefore, these tumor cells can be protected from systemic drug treatment and are responsible partially for the local tumor recurrence.

The vascular permeability in both tumor and normal tissues depends on physicochemical properties of drugs and the ultrastructure of the vessel wall,[5,37,38] which, in turn, can be modulated by tissue microenvironment.[5] Mechanisms of heterogeneous transvascular transport and the formation of the tight BTB remain the subject of ongoing investigations. Several key issues regarding the transvascular transport are discussed.

Pathways of Transvascular Transport

Several potential pathways for the transvascular transport of molecules and nanoparticles have been identified (for review, see Renkin[39]). Mechanistic explanations for transvascular transport of macromolecules and nanoparticles in tumors, however, are still controversial. The main point in the argument is not which pathways are available but which ones are the dominant channels for the transvascular transport. There are two competing hypotheses. One of them, proposed by Dvorak et al,[24,33,34] suggests that the major pathway is the interconnected vesiculovacuolar organelles (VVOs). The number of VVOs can be up-regulated by VEGF/VPF and other vasoactive agents; the size of VVOs ranges from 50 to 415 nm in diameter.[40] VVOs are separated by diaphragms; the opening and closing of the diaphragms regulate the rate of transport.[40] The second hypothesis suggests that the major pathway is the open endothelial junction/fenestra.[30-32,41-43] These structures can be induced by VEGF/VPF and other cytokines and are up to 700 nm in width.[41,42] Despite the differences discussed, both hypotheses agree that the transvascular pathways for macromolecules and nanoparticles are channel-like structures. The discrepancy between the VVO and the open junction/fenestra hypotheses is unlikely caused by differences in tumors used in the studies. Other issues regarding the experimental design and data interpretation have to be addressed in future studies.

The identification of transport pathways has di-

rect implication in drug delivery to solid tumors because the development of novel strategies for modifying tumor and normal vascular permeabilities relies on mechanisms of transvascular transport. If the vascular permeability can be modulated differentially between tumor and normal tissues, the specificity of drug delivery to tumors will be improved significantly.

Cutoff Pore Size

Both structural and functional analyses indicate that large pores exist in tumor vessels that are permeable to macromolecules and nanoparticles,[5,20,34,42,45] and the cutoff size of pores is dependent on tumor and organ environment.[10,11,18,19,43] For example, vessels in a human glioblastoma (HGL21) transplanted in mouse cranial windows have been shown to be impermeable to small molecules (molecular weight <600), indicating that the cutoff pore size in this model is smaller than 1 nm.[18] The cutoff pore size, however, in a mouse mammary adenocarcinoma (MCaIV) transplanted in dorsal skinfold chambers is between 1200 and 2000 nm, as determined by extravasation studies of liposomes with different sizes.[43] The cutoff pore size in other tumors has been reported to be between 100 nm and 800 nm.[10-12,19,43] Large pores also exist in discontinuous endothelia of normal sinusoids in the reticuloendothelial system (RES).[39] The cutoff size of pores in liver sinusoids is approximately 100 nm.[46]

The size and the number of pores in the vascular endothelium can be altered via local application of various endothelial growth factors or vasoactive agents.[41,42,47] Alternatively, neutralization or elimination of endothelial growth factors can significantly reduce tumor vascular permeability and pore cutoff size.[20,43] Therefore, it is likely that large pores in tumor vessels are induced and maintained by growth factors and other cytokines released by tumor and stroma cells.

Large pores in tumor vessels have provided a therapeutic window for specific drug delivery to solid tumors. For example, the heart microvessels are permeable only to molecules smaller than or similar to horseradish peroxidase (approximately 5 nm in diameter), although gaps spaced 10 to 20 nm apart can also be found occasionally in these vessels.[48] Therefore, one would expect that the therapeutic agents, larger than 20 nm and smaller than the cutoff size of pores in tumor vessels, will accumulate preferentially in tumors and a few normal organs (eg, the RES). This is, indeed, the case in the delivery of doxorubicin to solid tumors in patients.[49-52] When doxorubicin is encapsulated in sterically stabilized liposomes of approximately 100 nm in diameter, the severe cardiotoxicity caused by the treatment with free doxorubicin can be completely eliminated.[49,51,52] In addition, these stabilized liposomes enhance drug delivery to solid tumors, in comparison with conventional liposomes. The enhancement is attributed to prolonged plasma half-life, reduced uptake of these particles in the RES, and enhanced vascular permeability.[10,50,53] The increase in drug delivery, however, may not necessarily enhance the efficacy of drugs. Therefore, the clinical outcome of the liposome-mediated cancer chemotherapy remains to be determined.

Effect of Physicochemical Properties of Drugs on the Microvascular Permeability

Physicochemical properties of drugs, such as charge, size, configuration, and polarity, may affect the transport across the vessel wall.[5,37,38] In general, the permeability of both normal and tumor microvessels is inversely correlated with the size of molecules (Table 1),[19,54-56] presumably because of the size exclusion effect of pores in the endothelium and the extracellular matrix surrounding endothelial cells. The vascular permeability in tumors,[19,57,58] however, is less sensitive to the molecular weight, compared with that in normal tissues.[54-56,59] The reduced sensitivity in tumors is probably due to large pores in tumor vessels as discussed earlier[19,43,48] because the permeability is susceptible to the molecular size only if the size is comparable to the dimension of pores in the vessel wall.

In addition to the steric effect, the vascular barrier is selectively permeable to charged molecules.[5,38,60-62] In general, the barrier is more permeable to cationic or neutral molecules than anionic ones,[5,38,60-62] presumably owing to the negative charge of the basement membrane and the extracellular matrix layer (glycocalyx) on the luminal surface of the vessel wall.[38,63-65] For instance, the vascular permeability to ribonuclease (net charge, +4; MW, 13,683) in the frog mesentery is approximately twice as high as that to α-lactalbumin, a molecule with similar size (MW, 14,176) but negative charge (net charge, −10).[61] The same trend has been observed in tumors.[62] Mechanisms of the charge selectivity are still controversial. Electron microscopy studies have demonstrated that cationized ferritin binds to glycocalyx and basement membrane in normal vessels,[60,64,66] suggesting that the charge effect on the vascular permeability is

mediated through electrostatic binding or repulsion between tracer molecules and the vessel wall. Smit and Comper,[63] however, proposed that electrostatic interactions between albumin and other polyions were negligible under physiological conditions. The charge selectivity of the vessel wall is an important issue in gene delivery. Although the cationic charge of delivery vehicles (eg, polycationic liposomes[67] and amino polymers[68]) may improve the efficiency of gene transfer into cells, the electrostatic binding of the vehicles to the vessel wall may significantly influence the pharmacokinetics of gene delivery.

Convection Versus Diffusion

Transport of drugs across vessel wall involves both diffusion and convection. *Diffusion* is the random motion of molecules or small particles. The mass flux is from high-concentration to low-concentration regions and is proportional to the concentration difference between these two regions. *Convection* is mediated by the movement of fluid. The fluid flux is determined by the balance between the hydrostatic and osmotic pressures. In normal tissues, convection is the dominant mode of transport for macromolecules, whereas diffusion is more important for small molecules.[69] The situation in tumors can be significantly different, however, because of the vascular leakiness and the interstitial hypertension. To understand mechanisms that govern the transvascular transport in tumors, Lichtenbeld et al[21] quantified the effective microvascular permeability in a human colon adenocarcinoma (LS174T) transplanted in the mouse dorsal skinfold chamber, using the single-vessel-perfusion technique. They found that the vascular permeability to albumin was independent of the perfusion pressure in the range of 20 to 35 mm Hg, indicating that convection was not the dominant mode of transport across the vessel wall. The study also implies that diffusion is the dominant mode of transport in nonperipheral regions in solid tumors, where the microvascular pressure is nearly equal to the interstitial fluid pressure.[70] Convection may play a role in drug delivery to peripheral tumor tissues, however, where a significant drop of the interstitial fluid pressure occurs.[71] This pressure gradient facilitates extravasation of macromolecules in the periphery and causes extravasated macromolecules oozing from the tumor.[71] Consequently, higher accumulation of macromolecules (eg, monoclonal antibodies) may be observed at the interface between tumor and normal tissues.[72]

The previously mentioned study of convection versus diffusion provides only qualitative results because the single-vessel-perfusion technique itself can cause an increase in tumor vascular permeability to macromolecules.[21] The same problem has also been encountered in the study of tissue-isolated tumors,[73,74] in which the fluid loss from the periphery of some tumors perfused ex vivo was approximately one order of magnitude higher than that from nonperfused tumors in vivo. The perfusion-induced vascular leakiness may be caused by chemical ingredients of the perfusate because it has been shown that the permeability of vessels perfused with albumin solution is higher than that perfused with serum.[54] Although the exact mechanism remains to be determined,[54,56] the perfusion-induced vascular leakiness can be exploited for improving drug delivery to solid tumors during the isolated perfusion of the limb,[75,76] the kidney,[77] the lung,[78] and the liver.[79]

Effect of the Organ Microenvironment on the Vascular Permeability

The vascular permeability of tumors may depend on the tissue microenvironment. Our previous studies demonstrated that the vascular permeability of a human colon adenocarcinoma transplanted in cranial windows or in the liver was higher than the permeability of the same tumor transplanted in dorsal skinfold chambers.[19,20,23] Similarly, growth factor–induced vessels in collagen gels were more leaky to macromolecules when gels were transplanted in mouse cranial windows, in comparison with the same gel transplanted in mouse dorsal skinfold chambers.[80] Therefore, how is the microvascular permeability determined in vivo?

Stewart and Wiley[81] demonstrated, based on the study of quail-chick transplantation chimeras, that newly formed vessels in brain grafts transplanted in the abdominal tissues were similar to the BBB. In contrast, mesodermal grafts transplanted in the brain did not possess the BBB.[81] In another study, Vajkoczy et al[82] found that vessels in rat pancreatic islets transplanted in hamster dorsal skinfold chambers were structurally similar to those in normal rat pancreatic islets, containing diaphragmed fenestrae, although they were originated from nonfenestrated hamster subcutaneous vessels. Both studies described above suggest that endothelial microenvironment instead of the origin of vessels determines the vascular structure. Our preliminary study of brain tumors, however, suggested that host environment

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