Pharmaceutical Research, Vol. 20, No. 9, September 2003 (© 2003)

Review

Drug Delivery and Transport to Solid Tumors

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Received March 31, 2003; accepted May 27, 2003

Purpose. The purpose of this review is to provide an overview of the principles of and barriers to drug transport and delivery to solid tumors.

Methods. This review consists of four parts. Part I provides an overview of the differences in the vasculature in normal and tumor tissues, and the relationship between tumor vasculature and drug transport. Part II describes the determinants of transport of drugs and particles across tumor vasculature into surrounding tumor tissues. Part III discusses the determinants and barriers of drug transport, accumulation, and retention in tumors. Part IV summarizes the experimental approaches used to enhance drug delivery and transport in solid tumors.

Results. Drug delivery to solid tumors consists of multiple processes, including transport via blood vessels, transvascular transport, and transport through interstitial spaces. These processes are dynamic and change with time and tumor properties and are affected by multiple physicochemical factors of a drug, multiple tumor biologic factors, and as a consequence of drug treatments. The biologic factors, in turn, have opposing effects on one or more processes in the delivery of drugs to solid tumors.

Conclusion. The effectiveness of cancer therapy depends in part on adequate delivery of the therapeutic agents to tumor cells. A better understanding of the processes and contribution of these factors governing drug delivery may lead to new cancer therapeutic strategies.

KEY WORDS: transport; drug delivery; solid tumors.

INTRODUCTION

Over 85% of human cancers are solid tumors. The effectiveness of cancer therapy in solid tumors depends on adequate delivery of the therapeutic agent to tumor cells. Inadequate delivery would result in residual tumor cells, which in turn would lead to regrowth of tumors and possibly development of resistant cells.

Historically, cancer chemotherapeutic agents are small molecules with molecular weights below several hundred. Recent advances in the molecular targeting approach have led to the discovery of novel therapeutics including monoclonal antibodies, cytokines, sense or antisense oligonucleotides, viral and nonviral gene vectors, and genetically engineered cells (1–8). Delivery of these newer agents to solid tumors, because of the relative large size of these agents, poses new challenges beyond those encountered with traditional small-molecule cytotoxic agents. The processes and factors governing drug delivery to solid tumors are reviewed here.

Cancer chemotherapeutic agents are often administered systemically. Following a systemic administration, drug delivery to cells in solid tumors involves three processes, i.e., transport within a vessel (e.g., blood circulation), transport across vasculature walls into surrounding tissues, and transport through interstitial space within a tumor (9). These processes are determined by the physicochemical properties of a drug or particle (e.g., molecular or particle size, diffusivity, drug binding to cellular macromolecules) and the biologic properties of a tumor [e.g., tumor vasculature, extracellular matrix components, interstitial fluid pressure (IFP), tumor cell density, tissue structure and composition]. As discussed below, several of these properties are unique to tumors and are not found in normal tissues.

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The role of membrane transport and efflux proteins on drug transport and accumulation in tumor cells has been extensively evaluated in the last 15–20 years. Another area of major research focus is the design of drug formulations or

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ABBREVIATIONS: bFGF, basic fibroblast growth factor; EPR, enhanced permeability and retention; GAG, glycosaminoglycan; IFP, interstitial fluid pressure; MVP, microvascular pressure; VEGF, vascular endothelial growth factor.

particles for the purpose of tumor-targeted delivery. These areas have been reviewed elsewhere (10–15) and are not discussed here.

PART I. TUMOR VASCULATURE

Perfusion and drainage of tissues including solid tumors involve blood and lymphatic vessels. As discussed below, the blood and lymphatic vessels in tumors differ from the vessels in normal tissues in several aspects.

Tumor Vasculature

Tumor blood supply plays an important role in the delivery of therapeutic agents to solid tumors (16–19). Small tumors (<2 mm in diameter) are perfused by vasculature originating from surrounding host tissues. Further growth and enlargement of tumors are usually accompanied by newly formed microvessels (20). Tumor vasculature differs from the vasculature in normal tissues both functionally and morphologically; tumor blood vessels are generally more heterogeneous in distribution, larger in size, and more permeable (21–23). There are notable quantitative differences in the vasculature in transplanted animal tumors and spontaneous human tumors, e.g., higher vascular density and better blood circulation in transplanted tumors because of the absence of sinuses (24,25). The vascularization of implanted tumors is also likely to be different from that in spontaneous tumors: neovascularization would be required to support the growth of the relatively large number of implanted tumor cells (typically in the range of several million cells), whereas early-stage spontaneous tumors can be supported by the normal vasculature supplying the adjacent normal tissues until the tumor size exceeds 2 mm in diameter, a process that frequently requires months or years (20). Most of the available data on tumor vasculature were obtained from transplanted tumors. Implantation of colon LS174T tumor cells (2×10^5) resulted in capillary sprouts into the tumor mass after 3 days and establishment of microvasculature in tumors after 10 days (26). A second study using the same tumor model further defined the three stages of tumor neovascularization. In stage I, corresponding to 3–4 days after tumor implantation, the underlying and peripheral normal vessels were dilated while the tumor remained avascular. Vascular sprouts and loops in the periphery and center of the tumor were observed in stage II (day 6–7), and tumor vasculature was fully developed in stage III (days 10–17) (27).

Tumor vasculature is highly heterogeneous in terms of density, length, and diameter; distribution of vessels depends on the location within a tumor and the tumor size (16,23). Four regions are categorized on the basis of tumor vasculature, i.e., (a) avascular necrotic region with no vasculature, (b) seminecrotic region characterized by capillaries, precapillaires, and postcapillaries extended, without branching, toward the avascular necrotic region, (c) stabilized microcirculation region characterized by many venular and venous drainage vessels and few (two to five) arteriolar vessels, and (d) tumor advance front region where flow is similar to percolation in porous medium (28). Generally, peripheral regions of a tumor show higher blood vessel density than central regions (23). The ratio of avascular and seminecrotic regions to well-perfused regions is also a function of tumor size, i.e., larger avascular regions in larger tumors, which partly explains the lower average drug concentration in larger tumors (9,28,29). Heterogeneity in tumor vasculature contributes to uneven drug distribution within solid tumors.

A comparison of the blood vasculature in a transplanted rat hepatoma tumor and normal subcutaneous tissues showed larger volume (50 vs. 20%), surface area (70 vs. 20 mm^2/mm^3), and length (36 vs. 160 cm/mm^3) of the vessels in tumors with active neovascularization (22). This study also showed the near absence of vessels in the necrotic region of the tumor. Compared to normal colon tissues, the microvessels in rat colon tumors showed larger diameters of capillaries (5–20 vs. 5–8 μm) and venules (15–70 vs. 12–50 μm) (30). Because the diameters of tumor vessels readily exceed the size of most drug molecules (<1–2 μm), the size of tumor microvessels is not likely the limiting factor of drug delivery to solid tumors.

One of the unique features of tumor microvessels is their leakiness as a result of the discontinuity of endothelium (31–33). Several studies using transplanted rodent tumors (34–36) showed that pore size of tumor microvessels varies from 100 to 780 nm in diameter depending on the anatomic location of the tumor (e.g., smaller in cranial tumors compared to subcutaneous tumors) and the tumor growth (e.g., smaller in regressing tumors). In comparison, microvessels in most normal tissues (with the exception of kidney and liver) are less leaky; the tight junctions between endothelial cells are usually less than 2 nm (37), whereas the pore size in postcapillary venules is larger at up to 6 nm (38,39). Fenestrated endothelium of the kidney glomerulus and the sinusoidal endothelium of the liver and spleen show larger pore size of 40–60 nm and 150 nm, respectively (40,41).

Compared to normal tissues, tumors show elevated levels of growth factors, i.e., vascular endothelial growth factor or VEGF (also called vascular permeability factor) (42–45), basic fibroblast growth factor or bFGF (36,46), and other vasoactive factors (bradykinin and nitric oxide). High levels of bradykinin result in vasodilatation and enhance the extravasation of large molecules and their retention in tumors (47,48). Inhibition of bradykinin by a kinin receptor antagonist HOE140 or inhibition of kallikrein, a protease that converts kininogen to bradykinin, by a soybean trypsin inhibitor decreases the extravasation of albumin-bound Evans blue dye and decreases the accumulation of ascitic fluid in mice (48,49). Enhancement of vascular permeability by VEGF and bradykinin is mediated by the generation of nitric oxide; the depletion of nitric oxide with a nitric oxide synthase inhibitor or nitric oxide scavenger (e.g., 2-phenyl-4,4,5,5-teramethylimidazoline-1-oxyl-3-oxide) decreases the extravasation of macromolecules (49); bFGF was also shown to increase vascular permeability, although its effects may be indirectly through other yet unknown mechanisms (46).

Tumor Blood Flow

Tumor blood flow affects drug transport through the vascular space in a tumor. Blood flow is determined by the difference between arterial and venous pressure and flow resistance. The latter, in turn, is affected by viscosity of blood and geometry (e.g., length and diameter) of blood vessels (16). Compared to normal tissues, tumors show a greater blood

viscosity due to the presence of tumor cells and large molecules (e.g., proteins and collagen) drained from the extravascular space, a larger vessel diameter and a longer vessel length. The net result is a greater flow resistance in tumor blood vessels.

Compared to normal tissues, tumor tissues show similar arterial pressure but a lower venous pressure (50). Most of blood vessels in the internal regions of a tumor are veins or venuoles, whereas peripheral regions of tumors have a few of arteries and/or arterioles (33,51). Therefore, the arteriole-venuole pressure difference as a driving force for blood flow is negligible in the central region of a tumor, but is greater in the periphery. This, in part, explains the heterogeneous blood flow within a solid tumor; blood flow is lower in the center but higher in the periphery of the tumor relative to the blood flow in the surrounding normal tissues (28,51–53) On the whole, the average blood flow in tumors is lower than in normal tissues (54).

Tumor Blood Flow Measurement

The major methods used to measure tumor blood flow include magnetic resonance imaging (MRI), positron emission tomography (PET), and Doppler ultrasound imaging. For MRI, the earlier studies measured the clearance or uptake of deuterated water in tumors (55–57), but more recent studies suggest that functional gradient recalled echo MRI (58,59) and perfusion MRI (60) are as effective as the radionuclide-based techniques, both in sensitivity and specificity. Logan *et al.* have used PET scan with $H_2{}^{15}O$ as the tracer to evaluate the tumor blood flow before and after administration of interleukin-1 in patients (61). The applications and limitations of PET have recently been reviewed (62,63). Doppler ultrasound imaging, including color Doppler imaging, pulsed Doppler imaging, and power Doppler imaging, measures the frequency shift or power of Doppler signals to assess tumor blood flow (64,65) and has been used to study blood flow of experimental tumors and patient tumors (66–68). Another less common tumor perfusion measurement method for experimental tumors is the determination of dye concentration per gram of tumor by spectrophotometry after extraction of colored microspheres (69). Jain and colleagues have pioneered the use of intravital microscopy, where a transparent dorsal skin fold chamber is placed in an animal to monitor blood vasculature establishment in tumors (26,27,35,46,70). Some of the above methods require harvesting tumors (e.g., microsphere extraction method) or surgical procedures (e.g., intravital microscopy) that can be conducted only in experimental settings, whereas MRI, PET, and Doppler imaging can also be used in humans.

Lymphatic Drainage in Tumors

Lymphatic vessels are widely distributed throughout the body and are more permeable to fluid and solutes than are blood capillaries. The major function of the lymphatic system is to return the interstitial fluid to the blood circulation. In most normal and inflammatory tissues, macromolecules are cleared from tissues via the lymphatic system (53,71). Large particles such as tumor cells detached from a primary tumor can enter the lymph by passing between the endothelial cells of the lymphatic capillaries (72). An impaired lymphatic system is a characteristic of solid tumors. As discussed below, this property contributes to the retention of macromolecules in tumor interstitium and is used for passive tumor targeting.

PART II. TRANSPORT OF DRUGS ACROSS TUMOR VASCULATURE INTO SURROUNDING TUMOR TISSUES

Transport through Blood Vessels

After being transported to a tumor via the blood circulation, molecules are extravasated from blood vessels. The extravasation of molecules is associated with fluid movement across the vasculature wall. Because the exchange of fluid is dependent on the hydrostatic and osmotic pressure difference between blood vessels and interstitial space, microvascular pressure (MVP) plays an important role as a determinant of transvascular drug transport as well as blood flow in tumor tissues (16).

Because of leakiness and high permeability of tumor vasculature, the major pathway of drug transport across tumor microvascular wall is by extravasation via diffusion and/or convection through the discontinuous endothelial junctions. In comparison, transcytosis plays a relatively minor role (73,74). The pore size of tumor microvessels (i.e., 100–780 nm) (34–36) limits the distribution of molecules/particles larger than 1 μm across the tumor vasculature.

As discussed below, the difference in vascular permeability between tumor and normal tissues partly explains the passive tumor targeting, i.e., the tumor-selective delivery of macromolecules such as liposomes and drug-conjugated high-molecular-weight polymers (40,74).

Transport through Lymphatic System

The lack of lymphatic drainage in solid tumors has two effects on drug delivery and retention in solid tumors. First, defective lymphatic flow in solid tumors decreases the clearance of high-molecular-weight compounds from tumor interstitium (75–87). This, together with leaky tumor blood vessels, results in enhanced accumulation and retention of high-molecular-weight compounds in solid tumors, a phenomenon recognized as the enhanced permeability and retention (EPR) effect (3,75–80). EPR is predominant for compounds with molecular weights larger than 40 kDa but negligible for smaller molecules that readily redistribute to blood circulation via diffusion and/or convection (71,78). One study reported that EPR is affected by the tumor size, with a greater EPR in smaller tumors, probably because of the greater vessel density as compared to larger tumors containing an avascular region (88).

A comparison of the accumulation of radioiodinated (2-hydroxypropyl) methacrylamide copolymers with molecular weights ranging from 4.5 to 800 kDa administered intravenously to mice bearing sarcomas showed equal tumor accumulation/retention for all copolymers at early time points (within 10 min), whereas the accumulation/retention after 6 h was significantly greater for the larger copolymers with molecular weights exceeding 50 kDa. In comparison, smaller co-

polymers with molecular weights less than 40 kDa were cleared more rapidly from tumor interstitium. Hence, enhanced retention as a result of impaired lymphatic drainage is considered more important than enhanced extravasation from greater blood vessel permeability for the accumulation of high-molecular-weight compounds in tumors (77).

Second, the lack of lymphatic system in solid tumors increases IFP. This may be a major reason for the limited extravasation of macromolecules in spite of leaky microvasculature in tumors. Enhanced IFP induces outward convective flow, inhibiting the transvascular transport of molecules as well as transport in tumor interstitial space, as discussed below (73,85–87).

Drug Transport in Tumor Interstitial Space

Transport of small molecules in interstitial space is mainly by diffusion, whereas transport of large molecules is mainly by convection (89). Drug diffusion depends on diffusivity and concentration gradient, and convection depends on hydraulic conductivity and pressure difference. Because of the higher IFP in tumors compared to normal tissues, the net convection flow in tumor interstitium is outward from the core of a tumor.

After extravasation, drugs move through interstitial space to reach tumor cells located distal to blood vessels (1,9,85). Distance between capillaries is dependent on the status of vascularization (e.g., vascular region vs. avascular region). Vascularization is a function of tumor size; the ratio of avascular and poorly vascularized regions to well-vascularized regions increases with tumor stage, which is indicative of size (28,29). For example, the intercapillary distance is 49 μm in well-vascularized regions of mammary adenocarcinoma R3230CA tumor grown in the rat ovarian tissue-isolated tumor preparation (90), and 304 μm in Stage IIb and III carcinoma of the cervix uteri in human patients (91). Intercapillary distance in solid tumors also increases with tumor size in mouse mammary tumor (31) and rat tumors (92).

PART III. DETERMINANTS OF AND BARRIERS TO DRUG TRANSPORT, ACCUMULATION, AND RETENTION IN TUMORS

In addition to the abovementioned physicochemical, physiologic, and biologic factors, other factors such as tissue composition, tissue architecture, and drug binding to cellular components also affect drug transport, accumulation, and retention in tumors. Furthermore, solid tumors represent a dynamic system because of the time-dependent development of new vasculature as well as the time-dependent changes in tumor cell density as a result of drug-induced cell death. Accordingly, drug delivery in tumors should be viewed as a dynamic process that changes with time and is dependent on the drug effect on tumor cells.

Binding of Drug to Cellular Macromolecules

Most anticancer drugs target macromolecules such as proteins and nucleic acids. Some of these drugs are extensively bound to intracellular and/or extracellular macromolecules. The relationship between cellular drug binding and drug penetration into solid tumors has been studied using three-dimensional spheroids. Spheroids consist of tumor cells aggregated with irradiated HeLa cells, with the latter serving as the extracellular matrix (93). Spheroids have many characteristics of a solid tumor including multicellular structures, intratumoral heterogeneity including necrotic regions and oxygen gradients, and extracellular matrix. Hence, compared to monolayer or suspension cultures, spheroids are more similar to *in vivo* tumors and have been used to evaluate drug delivery and effectiveness of radiotherapy and chemotherapy (94–98).

Studies using tumor cell spheroids show that drug binding affects drug penetration and spatial distribution within spheroids. Drugs that do not bind to cellular macromolecules or cannot cross cell membranes readily penetrate spheroids. For example, 5-fluorouracil, cisplatin, thymidine-5′-triphosphate, sucrose, inulin, and monoclonal antibody against anticarcinoembryonic antigen are evenly distributed in thyroid cancer cell spheroids within 15 min (99–101). In contrast, drugs such as doxorubicin, daunomycin, actinomycin D, methotrexate, vinblastine, and paclitaxel, which bind to cellular macromolecules, remain localized in the periphery of spheroids (99,102–105). In spite of uneven intratumoral distribution, these high-binding drugs, because of their extensive binding and retention in cells, show higher average concentrations per spheroid as compared to low-binding drugs. This indicates that average tumor concentration is not a good indicator of drug distribution within a tumor and highlights the need for studies of spatial drug distribution within tumors.

Extracellular Matrix Composition

Extracellular matrix of solid tumors is composed of macromolecules such as fibrous proteins (e.g., collagen and elastin) and polysaccharides (e.g., hyaluronan and proteoglycan). These macromolecules are produced by host cells, but their production is regulated by tumor cells (85,106). The physiologic functions of extracellular matrix in normal tissue are to maintain homeostasis, stabilize the spatial and functional relations between cells (e.g., generating tissue cohesiveness), pose as a barrier to bacterial invasion, and regulate macromolecule transport through interstitium (107). In tumors, the extracellular matrix proteins are a source of physical resistance to drug transport (85,108).

Presence of glycosaminoglycan (GAG) is associated with a lower hydraulic conductivity and lower convective flow in interstitium (85,107,109). However, several studies showed that GAG content alone does not fully explain the high resistance for water and solute transport in many soft tissues (107,110,111). For example, one study showed that treatment of cornea with hyaluronidase reduces the GAG contents by 75% and increases the corneal stroma conductivity by 6.5-fold (111). However, the same study showed that the residual resistivity (i.e., inverse of hydraulic conductivity) was about 30 times higher than the resistivity calculated for 25% GAG, suggesting the presence of other more important determinants of the resistance to the convective flow. Although higher levels of GAG are found in tumors than in normal tissues (112–114), the role of GAG in drug transport in solid tumors has not been studied.

The presence of collagen in tumor extracellular matrix contributes to drug transport resistance in interstitium (115). The diffusion coefficient of IgG, measured *in situ* by fluores-

cence redistribution after photobleaching, is inversely related to the collagen content in a tumor. Treatment with collagenase increased the diffusion coefficient of IgG in tumors with high collagen contents by about twofold. In several transplanted tumors that contained high and variable collagen levels but comparable GAG levels, treatment with collagenase and/or relaxin, a human hormone that up-regulates the expression of collagenase and inhibits collagen synthesis by fibroblasts, nearly doubled the diffusion coefficient of IgG and dextran without affecting the GAG contents (115,116), indicating that GAG content is not an important determinant of drug transport in tumors with high collagen contents. Likewise, treatment of tumors with high contents of collagen or hyaluronan with hyaluronidase did not increase the diffusion coefficient of IgG and dextran in either tumor, indicating that hyaluronan is not an important determinant in drug transport (115).

Collectively, these earlier studies point to collagen as a major determinant of resistance of drug transport in solid tumors and suggest reduction of collagen content in tumors as a method for enhancing drug delivery to solid tumors (115,116). It is noted that these studies focused on diffusivity of macromolecules and did not evaluate spatial drug distribution. Hence, it is not known whether reduction in collagen (e.g., by relaxin treatment) is sufficient to ascertain even distribution of macromolecules throughout a tumor (116). Further, because collagen is a widespread constituent in tissues, it is uncertain whether the collagen-targeting approach can selectively and effectively reduce the collagen content in tumors without causing significant side effects.

Tumor Structure and Composition

Importance of Tumor Cell Density

As discussed above, diffusion through tumor interstitial space is a major mode of drug transport in solid tumors. In general, drug diffusion in a gel structure is a function of porosity and tortuosity (117,118). A larger fraction of interstitial space and/or a decrease in tortuosity would result in more rapid drug diffusion.

Our laboratory has used tumor histocultures to study the spatial relationships among interstitial space, tumor cell density, and drug penetration in solid tumors (119–121). Histocultures are cultures of fragments of tumors (approximately 1 mm^3 in size) (122,123). Histocultures are maintained on a collagen matrix. Similar to spheroids, histocultures provide a model system that retains three-dimensional multicellular structures and heterogeneity in oxygenation status. The histoculture system offers several additional advantages, as follows. In contrast to spheroids that are derived from tumor cells maintained under *in vitro* conditions, histocultures are derived from tumors maintained *in vivo* and therefore reflect the tissue composition in a tumor (i.e., presence of both epithelial tumor cells and stromal tissues) and the tissue architecture (i.e., presence of interstitial space). Histocultures derived from patient tumors further offer the advantage of their multiclonal nature and potential clinical relevance. As discussed below, stromal tissues and interstitial space in tumors are important determinants of drug delivery and transport.

We used histocultures of animal xenograft tumors and patient tumors to evaluate the role of tumor cell density and presence of stromal tissues on the transport of two drugs that are highly bound to cellular macromolecules (i.e., paclitaxel and doxorubicin). The results for paclitaxel are presented in Fig. 1. Xenograft tumors, because they are established by implanting a large number of tumor cells that grow relatively rapidly, typically consist of a small fraction of stroma tissues and high tumor cell density. In comparison, human tumors are developed from expansion of a single or few malignant cells, a process that is much slower than the growth of transplanted tumors, and therefore have a larger fraction of stromal tissues, a larger fraction of interstitial space, and a lower tumor cell density. For example, xenograft tumors showed a 60% higher tumor cell density and 2.5-fold lower stroma tissues than do patient tumors (119,121). Our results show a slower drug penetration in xenograft tumors; even distribution of paclitaxel and doxorubicin within histocultures was achieved in < 24 h for human tumors and >48 h in xenograft tumors. However, xenograft tumors showed about two- and 1.5-fold higher accumulation of paclitaxel and doxorubicin, respectively, compared to human tumors. These differences were not accounted for by the differences in the expression of *mdr1* p-glycoprotein in xenograft and patient tumors (119). Further examination of the rate of drug penetration in solid tumors and the spatial relationship among drug penetration, tumor architectures, and tumor cell distribution showed the following: (a) more rapid drug penetration in tumors with a lower tumor cell density and a greater fraction of interstitial space and/or stromal tissue, (b) preferential drug distribution to the areas with a low epithelial cell density compared to areas with a high cell density, and (c) higher drug accumulation in xenograft tumors as a result of drug binding in tumor cells, which exists in greater abundance in xenograft tumors than in patient tumors. These results indicate the important role of tissue composition and architecture, and tumor cell density in determining the rate and extent of drug penetration and the spatial distribution in solid tumors.

In summary, drug transport through interstitial space, similar to drug transport via blood circulation, is a major mode of drug distribution or delivery throughout a solid tumor. Hence, the relative importance of the transport through the interstitial space is likely to be greater in poorly vascularized tumors with reduced drug transport via blood circulation, as compared to highly vascularized tumors.

Dynamic Changes in Tumors

Angiogenesis

Tumor angiogenesis, or development of new microvessels, is a dynamic process (20). During the initial growth phase (up to 1–2 mm in diameter), tumor cells can obtain oxygen and nutrients from the existing blood supply to the surrounding normal tissues. Angiogenesis is required to support the further growth of tumors beyond the microscopic stage. During angiogenesis, new blood vessels sprouting from mature blood vessels in the surrounding normal tissues grow toward tumor cells. As discussed in Part I, blood vessels in tumors are morphologically different from blood vessels in normal tissues and are altered as a function of tumor size. The maintenance of these new vessels requires the presence of growth factors. bFGF was the first growth factor found to induce

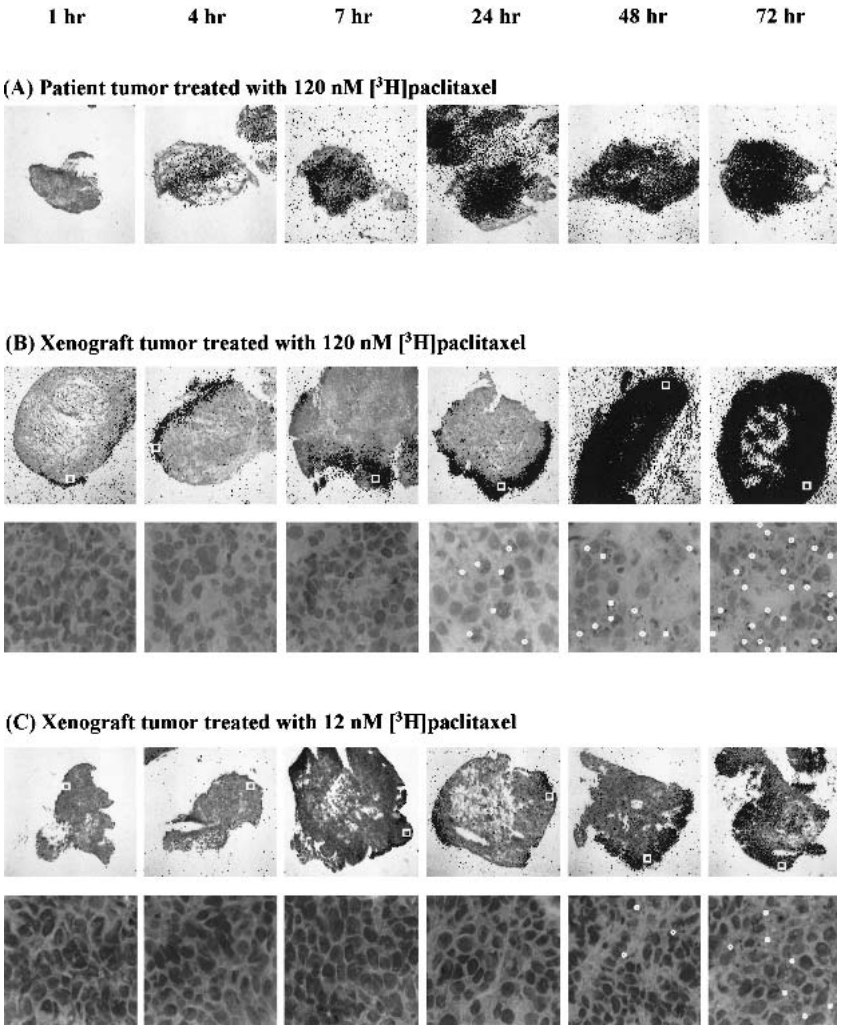

Fig. 1. Effect of tumor composition and importance of interstitial space on drug penetration. The penetration of [^{3}H]paclitaxel in patient and xenograft tumor histocultures was compared. A, Histocultures of a head and neck patient tumor were treated with 120 nM [^{3}H]paclitaxel. This dose was sufficient to induce apoptosis. Images of autoradiographic film were overlaid on histologic images. 25× magnification. B, Histocultures of FaDu tumor were treated with 120 nM [^{3}H]paclitaxel. This dose was sufficient to induce apoptosis. Upper panel: Images of autoradiographic film overlaid on histologic images, 25× magnification. Lower panel: Enlargement of the indicated boxed region of the slide in the upper panel, to demonstrate the presence of apoptotic cells (indicated by white dots), 400× magnification. The fractions of apoptotic cells were ~30% and ~50% at 24 and 72 h, respectively. C, Histocultures of FaDu tumor were treated with 12 nM [^{3}H]paclitaxel. This dose was not sufficient to induce apoptosis. Upper and lower panels: Same as in panel B. Very few apoptotic cells (<7% of total cells) were detected throughout 72 h. Reproduced from an earlier publication (119) with permission.

angiogenesis in normal tissues. However, there is uncertainty whether bFGF is a major inducer of tumor angiogenesis. VEGF is now recognized as the key inducer of tumor angiogenesis (124). Because tumor vasculature is a key determinant of drug delivery, the dynamic nature of formation and maintenance of new blood vessels in tumors indicates that drug supply to tumors is a dynamic process that changes with time and the microenvironment within a tumor.

Drug-Induced Cell Death

Many anticancer drugs act by inducing apoptosis. Apoptosis is a controlled physiologic process that occurs in a morphologically and biochemically distinct manner and ultimately leads to cell death. The apoptosis process involves a sequence of events including cell shrinkage, increased cytoplasmic density, chromatic condensation and segregation into sharply circumscribed masses, and the formation of membrane-bound surface apoptotic bodies (125). Apoptotic cells are phagocytosed from the midst of living tissues by neighboring cells or macrophages without eliciting an inflammatory reaction.

We examined the role of apoptosis in drug delivery to tumors. Using the histocultures system, we have shown that drug-induced apoptosis led to decreased tumor cell density and expanded interstitial space, which in turn resulted in an enhanced rate of drug penetration to the inner layers of a solid tumor (119–121). The results further showed that a 30% apoptotic cell fraction was sufficient to enhance drug transport, whereas a smaller apoptotic cell fraction (<7%) did not enhance drug transport (Fig. 1). The typical 16- to 24-h delay in the completion of apoptosis in epithelial tumor cells results in changes of drug transport in tumors after a 16- to 24-h lag time (126–132). Furthermore, because drug effect is a func-

tion of drug concentration and treatment duration, drug-induced apoptosis and drug-induced enhancement of drug transport in solid tumors also depend on the drug treatment schedule. Accordingly, the transport of a highly protein-bound drug in a solid tumor is a dynamic process and is determined by the drug effect. As discussed below, we have used the apoptosis-induction approach to enhance the delivery of highly protein-bound drugs that normally do not readily penetrate into solid tumors (120).

In addition to apoptosis, anticancer drugs can also induce necrosis. Whether a drug induces apoptosis or necrosis appears to be dependent on the intensity of the initial drug-induced insult, with necrosis occurring at higher intensity (125,133,134). Although both apoptosis and necrosis produce cell death and thereby reduce tumor cell density, there are significant differences in the nature of cell death by these two processes. Apoptosis occurs in an orderly fashion and does not elicit inflammation, whereas necrotic cell death is accompanied by extensive inflammation. Whether inflammation and the resulting pathologic changes (e.g., accumulation of cells and fluid) alter drug transport in tumors is unknown. Furthermore, apoptosis, because it occurs in cells scattered throughout a tumor, would result in expansion of interstitial space throughout a tumor. This is more desirable than space expansion in isolated areas of a tumor, as would be expected in the case of necrosis, where cell death occurs in large groups of contiguous cells (125,134). Finally, apoptosis induction typically requires lower drug concentrations and is therefore more readily attainable as a result of clinically relevant doses (125,133). Further studies to define the role of drug-induced necrosis in tumor drug delivery are warranted.

PART IV. EXPERIMENTAL APPROACHES TO IMPROVE DRUG DELIVERY TO TUMORS

Enhancement of Drug Delivery by Altering Tumor Blood Flow

Several strategies to enhance tumor blood flow, including physical and pharmacologic methods, have been examined. It is noted that these approaches, because they depend on the existing vasculature, may improve the drug delivery to vascular regions of tumors but will not improve the delivery to avascular regions.

Local hyperthermia enhances the delivery of radioimmunoconjugate and monoclonal antibody in animals (135–139) and human patients (140), presumably through an initial increase in tumor blood flow. However, later studies show that hyperthermia leads to dilatation of precapillary arterioles and results in a decrease in the arteriolar–venular pressure gradient and thereby in a decrease of tumor blood flow (16,141). Hence, enhanced drug delivery by local hyperthermia results from factors other than increased blood flow.

The ability of vasopressors to increase tumor blood flow has been tested; angiotensin II was effective, whereas adrenergic vasopressors (e.g., epinephrine and methoxamine) were not effective (142–145). At a systemic blood pressure between 100 and 150 mm Hg, angiotensin II enhances tumor blood flow without changing the blood flow of normal organs such as liver, brain, and bone marrow (142,143). The selective increase in tumor blood flow results from the loss of autoregulation of blood flow and homeostasis in tumor blood vessels (142), presumably because tumor blood vessels lack both smooth muscle cells surrounding the endothelial cells and angiotensin II receptors (71). In constrast, other vasopressors such as epinephrine and methoxamine reduce rather than enhance tumor blood flow. This is because these molecules act on different sites of the arteriole network (145). Angiotensin II increases the vascular resistance of terminal arterioles but not the upstream and larger vessels, and increases the perfusion pressure of the larger vessels. These effects of angiotensin II result in increased blood flow into tumor vessels that originate at or near the junction of the terminal arterioles and larger vessels, whereas epinephrine and methoxamine increase the vascular resistance of the larger vessels and, hence, reduce the blood flow.

The concept of using angiotensin II to improve the delivery and/or efficacy of chemotherapeutic agents to solid tumors has been verified experimentally. One study showed that the antitumor effects of mitomycin C against a subcutaneously implanted hepatoma tumor in rats, including reduction of tumor size, reduction of lymph node metastases, and prolongation of survival time, were significantly improved by angiotensin II–induced hypertension (142). In another study using tumor-bearing rats, angiotensin II–induced hypertension (~145 mm Hg vs. ~100 mm Hg in untreated controls) resulted in an approximately twofold increase in the intratumoral concentration of FITC-labeled neocarzinostatin (146). It is noted that the angiotensin II–induced hypertension requires the presence of angiotensin II and, because of its short half-life of <1 min (147,148), is terminated at the end of infusion. Infusion of angiotensin II is usually limited to 10 min, probably to avoid side effects associated with sustained hypertension. Clinical studies evaluating the use of angiotensin II were initiated in Japan in the early 1990s (149–151). Its clinical utility remains to be demonstrated.

Enhancement of Drug Retention in Tumors

The EPR effect is being evaluated as a passive tumor-targeting approach to deliver macromolecules. Tumor-selective accumulation of soluble macromolecules, such as polymeric drug conjugates {e.g., poly(styrene-*co*-maleic acid-half-*n*-butylate)-conjugated neocarzinostatin (75,81) and PK1[N-(2-hydroxypropyl)-methacrylamide copolymer doxorubicin]} (3), proteins (75,82), and liposomes (83,84), have been demonstrated. Some of these compounds are currently in clinical evaluation (3,81,84).

Theoretically, increasing the levels of vasoactive factors such as VEGF, bFGF, bradykinin, and nitric oxide may enhance vessel permeability. However, this approach may have limited practicality in part because of the instability of these molecules. Furthermore, these molecules have biologic activity that may counteract the advantage of increased drug delivery. For example, VEGF is associated with enhancing tumor growth and metastasis (152,153), and bFGF is associated with tumor resistance to chemotherapy (154–156). The use of an angiotensin-converting enzyme inhibitor such as enalapril or tempocapril, which also inhibit the degradation of bradykinin, to increase the EPR effect has been suggested (71). However, to date, this principle has not been verified experimentally or clinically.

Enhanced Drug Delivery by Modulating Vascular and Interstitial Pressure

A greater MVP results in an increase in transvascular fluid filtration, i.e., convection flow across the vascular wall, and, in turn, enhances transvascular drug transport to tumors (73). A lower IFP results in the same effects. Hence, a larger difference between MVP and IFP may result in a greater convective flow and fluid extravasation and thereby enhance the delivery of macromolecules (9,73,157,158). In theory, either decreasing IFP or increasing MVP may enhance drug delivery to solid tumors. This approach has been evaluated in experiment model systems (9,157–159) but has not been tested in patients.

A study showed that paclitaxel and doxetaxel reduced IFP in solid tumors and reduced compression of blood vessel (159). It has been proposed that the reduced IFP may reflect a reduction in cell density secondary to drug-induced apoptosis (9). However, this hypothesis is not in agreement with the data on the kinetics of drug-induced apoptosis and reduction in cell density or the data on the kinetics of tumor IFP reduction. If reduced tumor cell density were the cause of the decreased IFP, then the changes in IFP over time should mirror the kinetics of reduction in tumor cell density. This was not the case; paclitaxel-induced apoptosis and reduction of tumor cell density increased with time up to 24 h and then decreased to the control level at 96 h, whereas IFP decreased continuously until 96 h (159). In addition, because this study did not evaluate the effect of drug-induced apoptosis on spatial drug distribution in solid tumors, it is unclear if the reduced IFP secondary to drug-induced apoptosis is low enough to enhance drug delivery to solid tumors. As we have shown (discussed below), expansion of interstitial space by apoptosis significantly enhances drug delivery. Hence, increased interstitial space rather than reduced IFP as the major cause of enhanced drug delivery cannot be ruled out.

Several other physical, chemical, and pharmacologic approaches including heat, radiation, photodynamic therapy, mannitol (osmotic agents), nicotinamide, dexamethasone (corticosteroids), pentoxifylline, and tumor necrosis factor-α (TNF-α) were used to lower IFP (9,157). It remains to be shown that these approaches can reduce IFP sufficiently to enhance drug delivery and transport in solid tumors and can be applied clinically.

To date, there are no practical methods to successfully increase MVP for the purpose of increasing drug delivery to solid tumors. One study showed that enhancement of MVP by angiotensin II resulted in a slight increase in transvascular pressure (5 mm Hg increase for a 50 mm Hg increase in mean arterial blood pressure) and increased fluid extravasation for a short duration (i.e., 5 s). These effects were not sufficient to result in a greater delivery of IgG, a nonspecific antibody that does not bind to tumor cells, presumably because of the rapid exchange of the antibody across the vascular wall. When CC49, a high-affinity antibody that binds to tumor cells, was used instead of IgG, angiotensin II infusion resulted in a 40% greater accumulation in tumors (158). Hence, enhanced fluid filtration can potentially increase the intratumoral delivery of macromolecules, but this effect is limited to macromolecules that show high-affinity binding to tumor cells. Note that because angiotensin II enhances tumor blood flow (145,160), it is unclear whether the enhanced drug delivery is caused by increased MVP or increased tumor blood flow. In addition, because of the impaired lymphatic drainage in solid tumors, the enhanced fluid filtration from increased MVP also results in increased tumor IFP (160–162), which may counteract the advantage of increasing MVP.

Enhancement of Drug Delivery Using Apoptosis-Inducing Pretreatment

Drug transport in tumor interstitium increases with expansion of interstitial space and reduction in tumor cell density. Our laboratory has investigated the use of apoptosis-inducing pretreatment (referred to as tissue priming) to increase the tumor transport of highly protein-bound drugs (i.e., paclitaxel and doxorubicin) (119–121). Note that these drugs are also efficient in inducing apoptosis. *In vitro* studies using histocultures of xenograft and human patient tumors as well as *in vivo* studies in tumor-bearing animals have shown that tissue priming with these drugs enhances the rate and extent of drug delivery and eliminates the steep drug concentration gradient between the periphery and the core of solid tumors. The *in vitro* tissue-priming studies were performed in the absence of blood flow or vasculature and therefore not subject to the effects of MVP or IFP. Hence, the finding that tissue priming improves drug delivery and distribution suggests that interstitial space plays a more important role in drug delivery than MVP or IFP.

Because apoptosis is a pharmacologic action of paclitaxel, we hypothesized that its tumor delivery is indirectly determined by its pharmacodynamics, which, in turn, is determined by the treatment schedule. This hypothesis was tested under *in vitro* and *in vivo* conditions using histocultures and tumor-bearing animals (120). Figure 2 and Table 1 summarize the results of *in vitro* and *in vivo* studies, respectively. The results show that a treatment schedule that includes an apoptosis-inducing dose followed by the remainder of the dose given 24 h later results in higher fraction of apoptotic cells, lower tumor cell density, a more rapid drug penetration, a higher drug accumulation, and a more even drug distribution throughout the tumors, as compared to continuous infusion of the same total dose over 24 h. These studies further established the requirement of a 24-h interval, which was the time needed for apoptosis in epithelial tumor cells, between the apoptosis-inducing pretreatment and the subsequent dose in order to enhance drug delivery (120). Schedule-dependent antitumor activity of paclitaxel in patients has been reported; ovarian cancer patients who are refractory to the every-3-week schedule are found to respond to the weekly schedule (163). Additional studies are needed to determine whether the tissue-priming approach can enhance the delivery of large molecules and particles to tumors and whether the schedule-dependent antitumor activity of paclitaxel results from the schedule- and time-dependent changes in drug delivery.

The ability of tissue priming to enhance drug delivery to solid tumors may explain the finding that pretreatment with intravenous diphtheria toxin enhanced the delivery of a 36-kDa polymeric contrast agent (gadolinium diethylenetriaminepentaacetate conjugated to dextran 70), administered 44 h later, to human BRO melanoma xenograft in mice; the pretreatment resulted in a more even distribution and enhanced delivery to vascular and avascular regions of the tumor by about twofold (164,165). Although they did not elu-

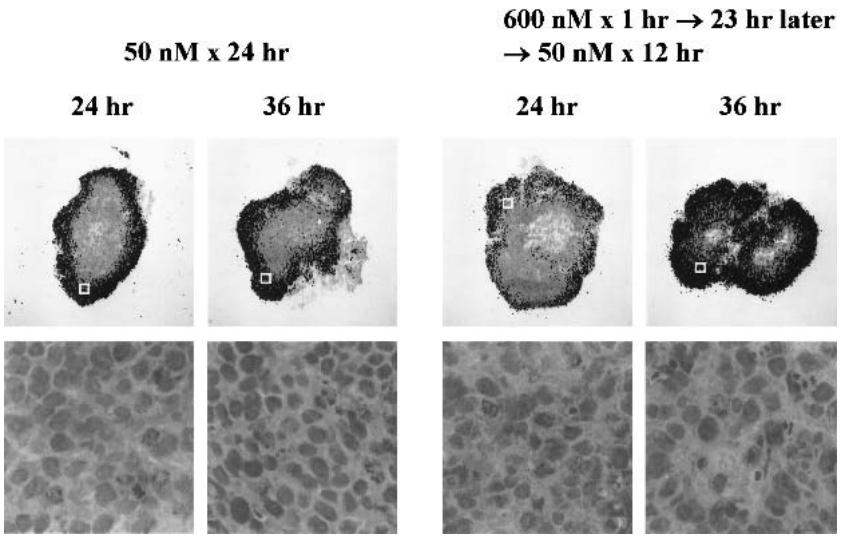

Fig. 2. Importance of apoptosis induction on drug penetration in tumors: Effect of treatment schedule. We examined the effect of treatment schedule on the rate of paclitaxel penetration and tissue morphology in tumor histocultures. Two groups of FaDu tumor histocultures were treated with the same drug exposure, i.e., 1200 nM•h, but by different schedules. One group was treated with 600 nM for 1 h and, 23 h later, 50 nM for 12 h. This treatment schedule, because it delivered the pulse of a high drug concentration sufficient to induce apoptosis, enhanced drug penetration in tumors. The second group was treated continuously with 50 nM for 24 h. This schedule did not induce apoptosis. Top panels show autoradiographic images overlaid on histologic images, 25× magnification. Bottom panels show histologic images of the indicated boxed region in the autoradiographic images, at 400× magnification. The indicated times refer to the times after initiation of treatment. Reproduced from an earlier publication (120) with permission.

cidate the mechanism, the similar results with the above two studies, including the interval between pretreatment and the second dose, are consistent with the tissue-priming concept.

CONCLUSIONS

Multiple factors affect the delivery and transport of a drug or macromolecule in a tumor. Factors such as binding to extracellular and intracellular components and diffusivity are related to the physicochemical properties of the drug/macromolecule. Factors such as tumor blood flow, lymph flow, MVP, IFP, angiogenesis, regional vessel distribution, tumor cell density, and the extent of stromal tissues and interstitial space are related to the biologic properties of a solid tumor. Some of the tumor biologic properties such as microvessel density, tumor cell density, IFP, and interstitial space are dynamic properties that change with time, and/or are affected by drug-induced apoptosis or necrosis. Hence, transport of drugs in solid tumors should be viewed as a dynamic process that changes with time and drug treatment. This is especially true for drugs that show high binding to macromolecules, because their transport is mainly by convection that is affected more by the above dynamic biologic properties as compared to small molecule drugs that are transported by simple diffusion. For example, the transport of an apoptosis-inducing drug in a tumor with high tumor cell density is expected to be slower in

Table I. Effect of Pretreatment and Treatment Schedule on Drug Accumulation in Tumors under *in Vivo* Conditions, and the Relationship between Drug Accumulation and Apoptosis Cell Density

Group (n)	Infusion rate (mg/kg/h) duration initiated at the indicated time	Total dose (mg/kg)	Time for harvesting tumor (h)	Tumor concentration (μg/g)	Plasma concentration at the time of tumor harvesting (μg/mL)	Tumor-to-plasma concentration ratio	Apoptotic fraction (%)	Cell density (cells/field)
1 (5)	5 × 1 h at 0 h + 0.83 × 6 h at 24 h	10	30	3.94 ± 0.35[a]	1.42 ± 0.11	2.80 ± 0.28[a]	14.5 ± 2.5[b]	83 ± 4.69[b]
2 (4)	5 × 1 h at 0 h	5	24	<0.35[c]	Not detected	Not applicable	11.2 ± 2.3[d]	85 ± 4.52[d]
3 (4)	0.83 × 6 h at 0 h	5	6	2.26 ± 0.13	1.31 ± 0.12	1.73 ± 0.18	3.5 ± 1.5	112 ± 6.08
4 (4)	5 × 1 h at 0 h + 0.83 × 6 h at 1.2 h	10	7.2	3.24 ± 0.31	2.98 ± 0.60	1.20 ± 0.30	5.8 ± 2.2	108 ± 5.78
5 (5)	0.83 × 12 h at 0 h	10	12	2.95 ± 0.34	1.50 ± 0.06	1.98 ± 0.23	6.5 ± 1.6	115 ± 5.18

Note: Animals received the indicated treatment. Concentrations of paclitaxel in tumors and plasma were determined by HPLC. Fraction of apoptotic cells and cell density were determined by counting the number of total and apoptotic cells in 400× microscopic fields (five fields per tumor). Mean ± SD. Reproduced from an earlier publication (120) with permission.

[a] $p < 0.05$, compared to all other groups.

[b] $p < 0.01$, compared to all other groups except group 2.

[c] Drug concentrations in the four rats were 0.35 and 0.25 μg/g in two rats, and below the detection limit (0.2 μg/g) in the remaining two rats.

[d] $p < 0.01$, compared to all other groups except group 1.

the first 24 h or before substantial apoptosis occurs as compared to the transport at later time points after apoptosis has occurred.

It is noteworthy that some of the tumor biologic factors have opposite effects on drug delivery and transport in solid tumors and that these factors are intertwined and interdependent. For example, increased blood pressure can increase drug delivery to solid tumors via blood perfusion and across vessel wall, whereas increased blood pressure also leads to higher IFP and thereby reduces drug transport through interstitial space. With this in mind, it is important that studies on drug delivery and transport in tumors take into account the multiple variables that govern drug distribution.

Significant efforts have been expended on establishing the effects of diffusion coefficients and IFP on drug delivery in tumors. An important area that has received relatively little attention is the spatial drug distribution within a tumor. The effectiveness of cancer treatment depends on the delivery of the therapeutic agent to all tumor cells located in different regions of a tumor because clonal expansion of the residual tumor cells may result in tumor regrowth and development of resistant cells. Accordingly, future efforts should focus on elucidating the barriers to drug transport within a tumor and on evaluating methods to overcome these barriers in order to achieve even drug distribution to vascular and avascular regions of a tumor. This requires better imaging capability to visualize drug distribution in solid tumors under *in vitro* and *in vivo* conditions. A recent report highlights the importance of high-resolution *in vivo* imaging (166).

Our laboratory is particularly interested in using a pharmacodynamically based approach to enhance drug delivery and transport in solid tumors. As we have shown, the delivery and spatial distribution of macromolecule-bound drugs are altered by using the tissue-priming approach. This may be used to design formulations with appropriate release profiles to take advantage of the apoptosis-mediated enhancement of drug penetration.

In summary, drug delivery, transport, and spatial distribution in solid tumors are affected by multiple physicochemical and biologic factors, some of which are dynamic properties that change with time and drug treatment. A better understanding of the contributions of these various factors may lead to therapeutic strategies that permit passive and/or active tumor targeting. The arrival of novel therapeutic agents such as drug-conjugated macromolecules, gene carriers, proteins, antibodies, and genetically engineered cells, which are usually relatively large in size and/or show high binding to macromolecules, highlights the need for additional research in this area.

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