Biochemical Pharmacology, Vol. 59, pp. 105–114, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00316-0

COMMENTARY

Biomimetic Transport and Rational Drug Delivery

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ABSTRACT. Medicine and pharmaceutics are encountering critical needs and opportunities for transvascular drug delivery that improves site targeting and tissue permeation by mimicking natural tissue addressing and transport mechanisms. This is driven by the accelerated development of genomic agents requiring targeted controlled release. Although rationally designed for *in vitro* activity, such agents are not highly effective *in vivo*, due to opsonization and degradation by plasma constituents, and failure to transport across the local vascular endothelium and tissue matrix. A growing knowledge of the addresses of the body can be applied to engineer "Bio-Logically" staged delivery systems with sequential bioaddressins complementary to the discontinuous compartments encountered—termed *discontinuum pharmaceutics*. Effective tissue targeting is accomplished by leukocytes, bacteria, and viruses. We are increasingly able to mimic their bioaddressins by genomic means. Approaches described in this commentary include: (a) endothelial-directed adhesion mediated by oligosaccharides and carbohydrates (e.g. dermatan sulfate as a mimic of sulfated CD44) and peptidomimetics interacting with adhesins, selectins, integrins, hyaluronans, and locally induced growth factors (e.g. vascular endothelial growth factor, VEGF) and coagulation factors (e.g. factor VIII antigen); (b) improved tissue permeation conferred by hydrophilically "cloaked" carrier systems; (c) "uncloaking" by matrix dilution or selective triggering near the target cells; and (d) target binding-internalization by terminally exposed hydrophobic moieties, cationic polymers, and receptor-binding lectins, peptides, or carbohydrates. This commentary also describes intermediate technology solutions (e.g. "hybrid drugs"), and highlights the high-resolution, dynamic magnetic resonance imaging and radiopharmaceutical imaging technologies plus the groups and organizations capable of accelerating these important initiatives. BIOCHEM PHARMACOL **59**;2:105–114, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. targeted drug delivery; vascular endothelium; active transport; dermatan sulfate; adenovirus; radiopharmaceuticals in drug development

Transvascular drug delivery is undergoing a revolution in theory and practice, based on the need to target and release genomic agents and biopharmaceuticals in a localized, controlled fashion mimicking that of paracrine hormones [1–4], plus the emerging capacity to produce site-selective bioaddressins by genomic means. An editorial in *Nature Biotechnology* [5] put the problem succinctly: "No payoff without delivery," medically, socially, or economically. Only about one part in ten thousand of an i.v. injected agent reaches its final cellular target when that target is located at a deep tissue site. Drug delivery systems have been categorized by the key method or substance employed in their formulation. This substance typically represents only one component of a multi-component system needed to traverse the sequential barriers and recognize the related bioaddresses encountered *in vivo*. Rational integration of these targeting components is required to achieve pharmacologic concentrations of most genomic, biopharmaceutical, and hydrophobic agents at deep tissue sites. As is frequently the case for complex systems, genomics is beginning now to develop its own drug-delivery solutions by providing rationally designed bioaddressins as well as rationally designed drugs. What remains is, first, to complete the elucidation and cataloguing of the constitutive organ addresses of the body and the induced neoaddresses that are the "gatekeepers" for endothelial transport, tissue-matrix permeation, and target-cell binding-internalization; and, second, to bioengineer "cloaked" and staged vehicles with properly addressed payloads that enable multi-compartment transport.

Our understanding of bioaddressing is currently at the stage where computer networking and telecommunications were 15 years ago, in terms of how to package, sequentially address, transport, receive, and decode bioinformation. Lessons provided by long-trunk telecommunications and local area networking are transferable to bioinformation packaging, addressing, site avoidance, and site targeting. Most of our current drug carriers can now be viewed as entrapment cores comprising the payload capacity required for the "dumb" steps of "long-trunk" blood and tissue transport. The addressing information required for "switching" these "packets" of bioinformation across the interme-

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diate endothelial and tissue barriers, and for "receiving and decoding" the biomolecules at the final target cells, is analogous to the "smart" information-processing steps located at the telecommunications "periphery." In biological systems, this periphery consists of: (a) the bioaddressins that surface their payload cores (and which may contain secondarily addressed, hybrid drugs); and (b) their complementary receptors on the endothelia and final target cells (or microorganisms) located in lesional sites or solid organs. This invokes a new paradigm of discontinuous transport across sequential biological compartments. It accommodates the uniquely different compartment properties and transport addresses of: (a) blood, with its potentially interfering plasma constituents, formed elements, and normal endothelia; (b) the tissue matrix; and (c) the final target cells. By encouraging independent modifications of addressins and payload cores, it allows the physiochemical properties of different active substances to be optimized with core materials for optimal entrapment efficiency and controlled local release. It acknowledges that drug carriers need to "chromatograph" through tissue hydrogels, which also are rich in lipids, and, at pathologic sites, rich in proteolytically cleaved peptides, oligosaccharides, cytokines, and growth factors. Any of these can impede the matrix permeation of drugs and drug carriers with exposed hydrophobic or specific binding moieties. Such impedance results in steep concentration gradients of active agents at incremental (micrometer) distances from porous or actively transporting capillaries [6]. Because the present approach emphasizes differences in transport among sequential biological compartments, it is termed *discontinuum pharmaceutics*. It will be essential for the effective delivery of genomic agents to deep tissue sites in adult humans.

There are three major classes of transvascular targeting with respect to the vascular endothelium: Passive, Indirectly Active, and Active. To achieve site targeting, all agents and delivery systems must encounter and overcome the first-compartment "plasma problem."

THE PLASMA PROBLEM

Targeting begins with cloaking. This is because no intravascular agent has direct access to the endothelium. Except for hydrophilic agents (which include many diagnostic contrast media), up to 95% of agent loss occurs as the result of its initial encounter with plasma. Control of biodistribution and the opportunity for site targeting are enabled only when an agent or delivery system resists opsonization, reformulation, and precipitation by plasma proteins and the aqueous milieu of plasma. Hydrophobic and amphoteric agents bind principally the hydrophobic sites of albumin, and also bind LDL* and VLDL. Electrostatic binding occurs between strongly cationic drugs (e.g. protamine) and endothelial heparan sulfates plus the anionic proteins predominating in plasma. Receptor-specific binding occurs to (a) preformed antibodies; (b) coagulation and complement proteins, such as tissue factor and factor VIII antigen (von Willebrand factor); and (c) serum amyloid protein (SAP), which dissociates histone proteins from chromatin and strongly binds the liberated DNA, leading to its accelerated liver clearance [7]. Amphoteric drugs, such as anthracyclines, taxanes, camptothecins, and polyene antifungal emulsions, also have the propensity to aggregate, reformulate, and precipitate in plasma. Without anticipating the full consequences of doing so, many anti-tumor drugs of today have been screened either *in vitro* or in single-compartment, intraperitoneal leukemia models to predict their *in vivo* efficacies. This has resulted in a predominance of relatively hydrophobic agents, which exhibit good target-cell internalization, but are not as well transported through hydrophilic plasma and tissue environments. For such drugs, intentional hydrophilic cloaking may be of major benefit just to reach the endothelial surface in a transportable form.

PASSIVE SYSTEMS

These include PEG carriers, certain poloxymers, standard liposomes and nanoparticles, PGLA particles, lipid emulsions, and most standard drugs and contrast agents. A subclass, including PEG, sterically stabilized (Stealth) liposomes [8], and other hydrophilic polymers and nanoparticles, generates a "water shell" at its surface. This confers the potential for prolonged circulation times, which is realized if the polymers or particles are of a small size (less than about 150 nm), above which clearance by the liver and spleen increases irrespective of the surface. Prolonged circulation allows more passes through target microvessels and a greater opportunity for passive extravasation across permeable microvessels. This is termed "Enhanced Permeability and Retention" or "EPR effect" [9]. EPR offers moderate and characteristically superficial lesional access and retention of polymeric, small particulate, and lipid emulsion carriers, including albumin-bound small molecules. Extravasation occurs not only in tumors, but also in granulomas, inflammatory lesions, and infectious sites [10]. The passive drug carriers most studied for tumor delivery include albumin, hydroxypropylmethacrylate, dextrans of 10,000–300,000 kDa, and small lipid emulsions, including the arterially administered styrene maleic acid neocarzinostatin (SMANCS) [11]. These have not yet been optimized for deep tumor permeation, and, consequently, the typical therapeutic outcomes have been initial tumor regressions followed by regrowth from non-accessed subregions. The EPR effect is variably enhanced by pre-administering physiologic modulators, including inhibitors of angiotensin-converting enzyme, which elevate bradykinin levels locoregionally, and angiotensin II, which elevates blood pressure and variably increases tumor blood flow [10, 12]. To

* *Abbreviations*: LDL, low density lipoprotein; VLDL, very low density lipoprotein; PEG, polyethylene glycol; PGLA, polyglycolic-polylactic acid; EPR, enhanced permeability and retention; MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography; and PET, positron emission tomography.

date, passive delivery has remained an incomplete solution for treating most solid tumors and abscesses due to anatomic and physiologic inhomogeneities of endothelial maturation, porosity, and responsiveness to EPR modulators, plus general matrix impedance of deep carrier diffusion [13]. Also, the prolonged circulation times (ranging from 10 to 100 hr) result in uptake at non-target sites and organs of clearance. Resulting toxicities have limited the potentially achievable improvements in therapeutic index. An additional disadvantage of passive delivery is that anti-tumor and anti-infective agents benefit from very quickly achieving high lesional concentrations. The slow tissue pharmacokinetics of passive systems are not well suited to this objective. In the author's view, physiologic up-modulators of EPR may provide a modest adjunctive benefit to lesional site uptake. To further optimize tissue pharmacokinetics, it may be of benefit to co-administer a rapidly localizing bolus of free drug together with the more slowly localizing drug-delivery formulation. There are reports of successful passive tumor imaging with radiolabeled short-chain antisense oligonucleotides [14]. However, for systemic genomic therapy, the tissue levels required are so much higher that we remain orders of magnitude away from efficacious passive delivery.

CYTOTOXIC PRIMING AND ADJUNCTIVE APPROACHES

What appears to hold greater promise for deep tumor delivery is priming with an intravenous cytotoxic agent, followed, after an interval of 24–40 hr, by administration of a second cytotoxic drug in polymeric form, ideally as a hydrophilic formulation. To determine the tumor priming effect on second-agent uptake and localization, the author has primed human BRO melanoma xenografts with i.v. diphtheria toxin, and 36 hr later injected (i.v.) the polymeric MRI contrast agent gadolinium diethylenetriaminepentaacetate conjugated to dextran 70 (Gd:DTPA–dextran with a modal molecular mass of 36 kDa) [15]. Comparing each primed tumor with its own pre-primed control, the uptake of the MRI agent was doubled, as assessed by increments in T1-weighted MRI image intensities and equivalent increments in ^{153}Gd radiolabel uptake. By region-of-interest analysis, the matrix diffusion of Gd:DTPA–dextran was increased several-fold in rate and distance from the peripherally well-perfused capillaries, into centrally non-perfused subregions. On special histologic staining of the tumor explants for endothelial integrity, these image changes correlated with marked microvascular permeabilization and diffuse extravasation of PAS-positive plasma proteins. Analogous MRI studies are now routinely performed in clinical settings at submillimeter resolution. Polymeric MRI and radionuclide imaging agents are experimentally available now and are currently under development for the noninvasive clinical monitoring of tumor blood flow and endothelial permeability effects. These and other imaging techniques hold great promise for studying the tumor increments and distributions of macromolecular drugs and genomics, such as nucleotides, cytokines, and antibodies.

A novel adjunctive means to improve tissue delivery is retrograde venous perfusion of target tissues and organs at elevated pressures. This induces endothelial gaps in dermal microvasculature [16], and also has been studied in the portal system of the liver [17] and in the brain [18]. Although drug-delivery studies are just beginning, this approach may be of future benefit for enhancing extravasation and parenchymal-cell delivery in solid organs. However, to be effective, retrograde venous perfusion must combine adequate exposure times with delivery systems capable of deep tissue permeation, and this combination remains to be optimized.

INDIRECTLY ACTIVE SYSTEMS

Indirectly active targeting takes place when agents, such as porphyrins, bind plasma LDL–VLDL and adopt the biodistribution and localization properties of the endogenous plasma recipients. Photoporphyrins localize selectively in tumors [19] and atheromatous plaques [20] due to local up-regulation of endothelial LDL–VLDL transport. The principal uses of these agents are for specialized laser ablations and diagnostic procedures performed in tertiary care centers. The drawback is that standard porphyrins are transported nonselectively into a variety of activated sites, as well as deposited in the liver and skin. It remains to be determined what advantages the new, more water-soluble porphyrins may offer. The major challenge with all indirectly active systems is that the administered formulations become altered rapidly *in vivo*, the kinetics of transition are not under control, and the plasma concentrations of adoptive carriers vary from individual to individual.

ACTIVE SYSTEMS

These are transported by active endothelial mechanisms based on conjugated or physically associated binding moieties complementary to either constitutive or induced microvascular surface receptors. Historically, their efficacies have varied depending on: (a) how well cloaked the vehicle was *en route*, (b) how physically available the envisioned endothelial receptors actually were to the binding moiety (i.e. how well the binding substance recognized or penetrated the camouflaging mucous and coagulant substances that overcoat endothelial protein targets in many subregions of pathologic microcirculations), and (c) how well designed the vehicle was to induce active extravasation, which typically is optimized by a multivalent binding surface with individual epitopes of only moderate binding affinity (see below). Early attempts at active tumor targeting utilized antibodies and antibody fragments addressed to the final tumor cells and not to the local endothelium. In doing so, they actually became passive carriers dependent on the EPR effect. They also suffered from being: (a) too

specific, leading to failed recognition of mutated cells; (b) too small for optimal pharmacokinetics at the sizes ideal for EPR uptake (*ca.*15–20 kDa); and (c) too exposed to avoid disproportionate adsorption by proximal antigens, resulting in dramatic tissue gradients at micrometer distances from the porous capillaries of active tumor subregions [21, 22]. Just now being elucidated are the endothelial integrins and adhesins that correspond to different normal organs [23] and pathologic lesions [24–26]. Completion of this cataloguing effort is of the greatest importance for our future-generation targeting systems. Additional needs include: (a) better protection of core payloads against premature release; (b) "triggerable" systems that "burst release" their payloads upon interaction with target-cell receptors [27] or upon exposure to local pH changes, temperature increments (e.g. from room to body), or resonant ultrasound energy; (c) improvements in tissue-retention times and target-cell internalization [28–31]; and (d) solutions to the very low permeation across the tight junctions of normal parenchymal organs, in order to reach the tissue concentrations of genomic agents in normal adult organs that currently are achieved in lesional sites.

NEXT-GENERATION ACTIVE SYSTEMS

Currently under development are a number of rational delivery systems that enhance tissue access by addressing the discontinuities of sequential compartments. According to this paradigm, the vascular endothelium is viewed as the "state address," the tissue matrix as traversable "city streets," and the final cellular target as the "home address" [2]. State-addressing substances are directed at neovascular or organ-selective endothelial determinants. Binding substances of special interest include endothelial-targeted: (a) antibodies [32]; (b) native and modified tissue pathway factor inhibitors [33, 34]; (c) RGD phage-display peptides, for example, CDCRGDCFC-phage [23]; (c) sulfated glycosaminoglycans [35–38]; and (d) the tetrasaccharides, sialyl Lewis A and X, Lewis Y, and their synthetic analogues, which are related to E-selectin (ELAM-1) binding [39], and their novel 12-mer peptidomimetics [40]. The latter have not yet been tested *in vivo*, but the oligosaccharide analogues of sialyl Lewis X have been shown to inhibit angiogenesis both *in vitro* and *in vivo* [41]. Their problem as drug candidates is that their very low binding affinity and short chain length require that they be infused continuously at high doses to achieve anti-inflammatory efficacy. Biochemical synthesis of longer-chain saccharides, although feasible, remains prohibitively expensive for therapeutic uses. Combined biosynthetic–biochemical solutions involve chemically derivatizing the biosynthesized oligosaccharides (or oligopeptides) with heterobifunctional poly(ethylene)glycols [42], and assembling these hybrid molecules into the surfaces of nanoparticles and liposomes [27, 42] to give multivalent binding surfaces of moderate avidity. The presently available phage-display peptides (above) exhibit serious binding and transport deficiencies, including uneven addressing of tumor endothelial subregions, and minimal, if any, deep tumor permeation. In experimental mice, this results in tumors that stop growing, but remain viable and dormant [43].

Future developments are likely to be based on substances that bind induced endothelial (a) hyaluronan, the apparent endothelial target for activated leukocyte CD44 [24–26], which binds with even greater avidity after natural sulfation of its glycan residues [44]; (b) selectins, especially of the E type, which is unique to the endothelium [45, 46]; (c) integrins, in particular $\alpha_v\beta_3$, due to its selectivity for angiogenesis and tumor targeting [43, 47]; (d) the α/β subunits of surface ATP synthase, which is a target for angiostatin [48]; (e) locally induced coagulation factors, including tissue pathway factor, factor VIIa, factor VIII antigen, thrombospondin, fibrin split products, platelet factors IIB/IIIA, PF-4 [35], and others that may overcoat underlying protein receptors [34, 49–54]; and (f) tissue-matrix substances exposed by induced proteinases, including plasminogen fragments, fibronectin peptides [55, 56], laminin chains [57], fragments of collagen types IV [35, 36, 58, 59], XIV [60], and XVIII, glycosaminoglycan oligosaccharides [35, 45, 46], and others. Importantly, the peptide fragments of plasminogen and collagen XVIII comprise the antiangiogenic peptides angiostatin and endostatin, respectively [61, 62]; and a 300-kDa polysaccharide, CM101, derived from group B *Streptococcus*, when administered i.v., also exhibits strong antiangiogenic activity, as well as adjunctive neuroprotection in murine acute spinal cord injuries [63, 64].

NOVEL GLYCOSAMINOGLYCAN MIMICS OF LEUKOCYTE TARGETING

A unique class of next-generation carriers is based on glycosaminoglycan polymers (of *ca.* 18 kDa) [36, 38] and glycosaminoglycan-surfaced nanoparticles (50–110 nm in diameter) [35, 37, 65, 66], which appear to mimic the local bioadhesion, rolling, and stopping of activated leukocytes. Leukocyte rolling is mediated by bioadhesion of their induced and sulfated CD44 glycans to locally up-regulated vascular adhesins, initially hyaluronan [24–26]. A preferred biomimetic polymer is the naturally derived, non-anticoagulant glycosaminoglycan dermatan sulfate, of 18.5 kDa, which is chemically fractionated to further enrich it for 2,4-disulfated disaccharides, occurring as short runs of 2 to 4 such disaccharides separated by less sulfated sequences [67]. Agent-delivery vehicles have been prepared by: (a) covalently conjugating the active substances to dermatan, and (b) noncovalently formulating very basic active substances (e.g. with an amine group of $pK_a > 9$) by strong paired-ion binding to the highly acidic sulfate groups of dermatan. Formulations are titrated to a slight excess of the dermatan carrier, to confer a negative Zeta potential of -25 to -45 mV, which repels the heparan sulfates of normal endothelia. Covalent formulations have a typically low agent-to-carrier payload of 5–12% agent by weight; how-

ever, the physically formulated systems have high payloads, ranging from > 40% agent (by weight) for the soluble polymers, to > 95% agent (by weight) for the nanoparticles, which assemble in a stable fashion around amphoteric drug cores. The multivalent binding surface of dermatan selectively bioadheres to tumor neovascular endothelium and rapidly induces high-capacity, active transport of the carrier and active substance into a variety of experimental animal tumors. Importantly, following extravasation, these systems permeate from perfused subregions into and throughout poorly perfused and necrotic subregions [35–38]. Summarizing from several independent reports, these dermatan systems putatively bind both basic neovascular growth factors and adhesins, including: (a) vascular endothelial growth factor (VEGF); (b) hyaluronan [24–26, 44, 68] and chondroitin sulfate-like molecules [35–38, 68]; (c) selectins; and (d) one or more of the induced coagulation factors, including factor VIII antigen (von Willebrand) [35–38]. Dermatan carriers are highly unique in avoiding normal endothelia, due in part to their noninteraction with antithrombin III; however, they bind redundantly and quite homogeneously to the multiply induced receptors on neovascular endothelium.

In the author's studies [37] where special iron stains were performed to determine the tumor microdistribution of a ferrioxamine–dermatan agent, the tumor endothelium stained strongly positive at 7 min post-injection. After 60 min, the endothelium stained weakly and the tumor cells were strongly positive. Subcellular localization was detected on the tumor-cell surface, intracytoplasmically, and in perinuclear and nuclear locations. Based on the additionally unique property of glycosaminoglycans to self-associate up a concentration gradient, and the recent reports of chondroitin-like molecules present on the surfaces of many different tumor cells [69], it is possible that cross-association of the dermatan and chondroitin chains facilitates the observed tumor-cell binding and internalization. Until these mechanisms are further elucidated, the unique functional observation regarding dermatan systems is that they transport themselves and their associated agents, apparently at selectin–carbohydrate selectivities, across all three of the key compartment barriers: tumor endothelium, the tissue matrix, and tumor-cell membranes. From these studies, the following general pattern has emerged: exposed carriers with high affinity epitopes (e.g. K_d values $\geq 10^7$), such as antibodies, lectins, and phage display peptides, are internalized by endothelium but not well released from the abluminal surface, whereas carbohydrate carriers with multiple, more widely spaced epitopes of lower affinity (e.g. each with a K_d of *ca.* 10^4) are internalized, transported, well released, and favorably diffused into deep lesional sites.

NONINVASIVE IMAGING OF SITE TARGETING

By MRI of dermatan–ferrioxamine employed as an 18.5-kDa polymeric MRI contrast agent in syngeneic rat AT-1 prostate tumors, the leading edge of agent diffusion was visualized noninvasively at submillimeter resolution to move through the tumor matrix at 0.5 to 0.75 cm/hr [70–72]. Also, radioimaging studies have been performed in rats bearing 1- to 3-cm AT-1 prostate tumors and syngeneic NIH 13672 NF breast adenocarcinomas.* Polymeric dermatan–deferoxamine was radiolabeled with ^{67}Ga for single-photon (SPECT) [73–75] and with ^{68}Ga for positron (PET) [76] gamma scintigraphy. As seen by image analysis over the tumor region, tumor uptake was very rapid, peaking at 5 min after i.v. injection and washing out with a $T_{1/2}$ of 54 min. Blood clearance (analyzed over the heart region) had a $T_{1/2}$ of 18 min and occurred almost exclusively by the renal route, as was typical for all of these soluble polymeric formulations. Uniquely for this dermatan class of glycosaminoglycan carriers, tumor uptake was linearly related to the administered dose over a 1600-fold range of 1.55 to 2500 μmol/kg, while the blood clearance times remained virtually unchanged. The nanoparticulate formulations differed from the soluble polymers in requiring 1–3 hr for site targeting, and they also cleared more slowly by the hepatic route. However, they favorably targeted high payloads of agent for subsequently controlled release [37, 65, 66, 77].

TARGETED THERAPY

Tests of amphotericin B–heparin nanoparticles (95:5, w/w; 110-nm diameter) in murine lung coccidoides infections gave *ca.* 1/2 log improvement in therapeutic index and a shift from renal to liver clearance, plus avoidance of amphotericin B-induced renal tubular necrosis, and no new hepatotoxicity in 14-day murine toxicity studies [65, 66, 78]. Tests of doxorubicin–dermatan nanoparticles (40:60, w/w; 50-nm diameter) in human MX1 breast cancer xenografts gave a 2.5-fold improvement in therapeutic index, with the bone marrow remaining the limiting site of toxicity [37; and Ranney D, unpublished studies]. As further evidence of deep lesional access, 50–70% long-term survivors were observed for both of these glycosaminoglycan formulations versus zero to low percentages for their respective standard agents.

VIRAL AND BACTERIAL DELIVERY WITH ACTIVE MATRIX PROPAGATION

A number of replication-competent cytolytic viruses have been investigated as selective cancer therapeutics, including herpes simplex, autonomous parvoviruses, Newcastle disease virus, and various adenoviruses [79]. Modified adenoviruses have been produced with genomic deletions allowing them to replicate selectively in p53-deficient tumor cells [80], which are present in about half of human tumors. Early clinical trials involving their direct injection into head and neck tumors have shown promising initial results, including a number of apparently stable remissions. These adenoviruses replicate and lyse their initial tumor

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hosts and are successively released, generating a cascade of matrix propagation that continues up to and stops at the tumor boundary with normal tissues. This represents a very unique paradigm and a major step forward in biomimetically permeating the lesional matrix. However, major challenges remain in developing successful loco-regional and systemic delivery formulations. This is because an extremely high fraction (>95%) of i.v. administered adenovirus is cleared extremely rapidly by the liver and spleen, and to a lesser extent by the lungs [81]. This is based on mechanisms as yet to be elucidated, but which appear only minimally dependent on the binding of viral penton base to hepatic α_v integrins [82]. When liver uptake occurs above a certain limiting dose, which is currently below that of systemic efficacy, acute hepatocellular necrosis results as a limiting (and preclinically lethal) side-effect [81]. Liver site avoidance, reductions in other side-effects, and increased genomic payload capacity are the subjects of intensive efforts [83]. Predictably, major challenges still will remain in camouflaging the viral surface and affording adequate site avoidance. This is because complete surface coatings are nearly impossible to achieve by genomic engineering, and because specific receptor retargeting from low-efficiency, CAR-dependent uptake to high-affinity tumor-cell tropism (e.g. by using the basic fibroblast growth factor ligand, FGF2) [84], although effective *in vitro*, has not yet proven useful *in vivo*. This is because the major delivery barrier remains endothelial extravasation and not tumor-cell internalization. Development of effective systemic delivery will probably proceed by: (a) receptor-engineered viruses that are noncovalently overcoated with synthetic polymers under conditions adjusted to retain viral infectivity; and (b) loco-regional perfusion of organs containing identified tumor masses, perhaps utilizing adjunctive venous retroperfusion methods. These intermediate solutions eventually may progress to fully systemic administration, with progressively advanced implementation of viral cloaking, endothelial transport, and liver avoidance [35, 37].

Avirulent strains of *Salmonella typhimurium* have been engineered as enzyme vectors in which cytosine deaminase has been cloned as a secreted fusion protein for cancer prodrug therapy [85]. Following i.v. administration, these bacteria proliferate preferentially in tumors versus normal tissues and propagate throughout the tumor matrix. With further optimization, this system may become a useful vector for selective, deep tumor delivery.

HYBRID DRUGS

Until the preceding systems are developed sufficiently to garner favorable regulatory review, we are likely to see the nearer-term solutions of "hybrid drugs," which are conjugates of active agents with receptor-binding moieties. Estramustine is one such approved agent, which combines a nitrogen-mustard active moiety with an estrogen binding moiety. Examples currently in clinical trials are thalidomide and its various analogues. Experimental agents include: (a) doxorubicin conjugated to internalizing antibodies [28] and RGD peptides [43]; (b) genomics conjugated to internalizing 16-mer homeodomain peptides [29–31]; and (c) a novel class of genetically engineered silk elastin-like block copolymers, which have specific insertion sequences that can confer stimulus-induced drug release as a function of pH or temperature [86, 87]. The challenges with these early systems include solubility issues, pleiotropic targeting, and unanticipated toxicities. In certain cases, these challenges may be overcome by formulating hybrid drugs as polymeric and particulate agents (above) [35, 37].

RADIOIMAGING AND RADIOTHERAPY OF NEW AGENTS

Radiolabeled agents are the "original" hybrid drugs and diagnostic agents. Because targeting and clearance can be screened accurately only under *in vivo* conditions, gamma scintigraphy is being used increasingly at the earliest research stages to guide synthesis, select preferred molecules and formulations, provide an early snapshot of efficacy and toxicity, and accelerate development timelines. Small-molecular, biomimetic imaging agents include ^{18}F-deoxyglucose, ^{111}In-labeled octreoscan and folate radioconjugates, ^{11}C-labeled acetate and amino acids, and radiolabeled thymidine analogues. Pilot screening of ^{123}I-labeled peptides and other agents, performed in as few as three animals over as little as 3 hr, often gives sufficient information for development decisions. Radiolabeling by electrophilic or nucleophilic substitutions of ^{18}F and methylation reactions using ^{11}C can be performed rapidly, using standard automated equipment modules, to give positron-labeled small molecules, proteins, and delivery vehicles. With access to nearby production facilities and small-animal imagers (needed to accommodate the very short half-lives of PET isotopes), invaluable information on biotargeting and tissue responses can be acquired rapidly. Existing small-animal "microPET" imagers now achieve 2-mm intrinsic detector resolution and 8-mm^3 tissue-volume resolution [88]. New small-animal PET–SPECT imagers currently under development will give submillimeter resolution plus computed tomography of the co-registered anatomy.* Human imagers of this sort should become available in the near future, and these are projected to markedly accelerate the development of biomimetic transport agents. Radiotherapeutic ^{111}In and ^{153}Sm chelates of protein-peptide agents also are beginning to show promise. For an extensive recent review of nuclear bioimaging in drug development, the reader is referred to Ref. 89.

SUMMARY

It is by local "site saturation" that the body achieves efficacious lesional therapy. Our own tissue defenses will not infrequently sacrifice entire sites or regions for the

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survival of the body. By analogy, tissue-biomimetic systems will come to more closely resemble "practical shotgun pellets" than "magic bullets." If we simply achieve saturation of lesional sites, many of our currently resistant diseases will yield to the small molecules of today, and site-targeted genomic therapies will be on their way to realization. For transvascular targeting of pathologic foci with co-induced endothelia, the new paradigm is *discontinuum pharmaceutics*, and the endothelium is the gatekeeper carrying the bioaddresses for tissue access. By comparison, the recently described approach of *continuum pharmacokinetics* is more suitable for delivery in the reverse direction, namely from local tissue implants into blood, and for indications where sustained blood levels are acceptable at 100-fold lower concentrations than those generated around the topical implants [90, 91]. Within tissues, it is not antibodies and high specificities that rule the kingdom, but leukocytes, bacteria, viruses, and proteases, with their moderately selective but redundant adhesins, selectins, integrins, growth factors, chemokines, and matrix plus cell-surface carbohydrates. A new paradigm for delivery of cytotoxic agents is relatively shorter circulation and clearance times, coupled with high-capacity active endothelial transport, and sufficiently frequent administration to maintain site saturation commensurate with plasma and body clearance. For noncytotoxic agents (e.g. "dormancy" therapeutics) the new paradigms are currently evolving. Hydrophilic polymers with a multivalent binding surface (e.g. dermatan sulfates and other natural and synthetic anionic saccharides) currently are the optimal ones for active, homogeneous endothelial transport and release. Hydrophilically cloaked agents (e.g. with surface anionic carbohydrates or PEG) are essential for traversing the aqueous plasma and tissue compartments. Near the target-cell surface, a complete transition is required to maximally expose the hydrophobic sites and receptor-binding moieties that mediate target-cell binding and internalization. Promising cell-internalization vectors are the 16-mer homeodomain peptides, which, after conjugation to active substances, can co-internalize even hydrophilic peptides and small proteins via membrane vesicles with caveolae-like properties [28–30]. Replication-competent lytic adenoviruses and proenzymatic *Salmonella* bacteria provide novel and important paradigms for actively permeating tumor matrices. In targeting solid organs without induced endothelia, the biomimetic paradigm shifts even more dramatically to one of replacing (or ablating and replacing) entire zones of epithelia without unduly damaging the basement membranes, by administering combinations of cytotoxic or apoptotic agents, growth factors, and replication-competent, growth-restricted, genetically prepared cells. Early genomic therapies are already underway in humans, including myocardial and peripheral revascularization, a variety of antiangiogenic therapies, and genetic reversal of cystic fibrosis by the inhalation route. It is natural that successes should occur first where the initial barrier is the target. These early successes need to be extended from lesional sites to the deep epithelia of solid organs. Noninvasive radiopharmaceutical imaging (SPECT/PET) and submillimeter MRI are essential for investigating the kinetics, subregion localization, and clearance of site-targeted agents; and they are becoming increasingly important commercially for identifying new agents, accelerating their development, and lowering costs. Valuable radiotherapies are also beginning to emerge by substituting therapeutic isotopes on optimized diagnostic carriers.

A number of key scientific and industrial groups and organizations are capable of catalyzing these important initiatives. They include the Controlled Release Society, the American Association of Pharmaceutical Scientists, the Society of Nuclear Imaging in Drug Development, the Society of Magnetic Resonance Imaging in Medicine, plus integrative scientific groups, business managers and licensing teams in academia, biotechnology companies, and large pharmaceutical firms. US Oncology, which has resulted from the merger of Physician Reliance Network and American Oncology Resources, represents a major national resource of nearly 1000 oncologists, for integrating the multi-center clinical trials capable of assisting in the development and PET imaging of novel, tumor-localizing therapeutics and monitoring diagnostics. Within the Food and Drug Administration, there are interactive groups in the Biologics and Devices Divisions, which have the expertise to evaluate these novel, biologically and genomically addressed agents. Our major national funding agencies, the National Science Foundation and especially the National Institutes of Health, have a critical role to play in catalyzing the genomic delivery initiatives essential to achieving the latent but large medical, social, and economic benefits. Indeed, these initiatives are so pivotal to enabling our twenty-first century genomic therapies and national biomedical and economic developments that strong consideration should be given to establishing a "National Institute of Drug Delivery." In this regard, the NIH is to be congratulated on its Planning Grants for "Cellular and Molecular Imaging Centers," whose priority is to develop the capabilities for localized, noninvasive, high-resolution imaging in oncology. Reader feedback is welcome and invited by mail or at globemed@flash.net.

This work was performed at, and sponsored by, the University of Texas Southwestern Medical Center at Dallas and ACCESS Pharmaceuticals, Inc.

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