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molecular pharmaceutics

Meeting Report: Drug Carriers in Medicine and Biology

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Received December 12, 2008; Accepted December 15, 2008

The Gordon Research Conference on Drug Carriers in Medicine and Biology celebrated its 30th anniversary with a highly attended meeting held August 24−29, 2008 in Big Sky, Montana. The meeting was cochaired by David Scheinberg from the Memorial Sloan Kettering Cancer Center and Kyung-Dall Lee from the University of Michigan. This interdisciplinary meeting brings together scientists involved in many aspects of drug development, including drug delivery, molecular targets, drug and prodrug design, macromolecular-based drug carriers and delivery devices, and technologies to measure drug uptake and effectiveness. The presentations in the 2008 meeting illustrated not only the recent advancements made in these areas but also the challenges that lie ahead. Readers of *Molecular Pharmaceutics* could benefit from knowing what transpired, since the talks fall within the scope of the journal.

The meeting was opened with a keynote address by Gregory Verdine at Harvard University in a session entitled "Drugging the Undruggable". Current biological and small molecule therapeutics apply to only a small percentage of all possible molecular targets on or within cells involved in pathologic conditions. Technologies are available to derive peptides that bind to almost any target of interest, but peptides can be unstable in the body, have low affinities for their targets, and have poor permeability through cell membranes. Dr. Verdine described a technology to create highly stable α-helical peptides through total synthesis, in which amino acids with olefin containing sidechains are incorporated in spatial configurations that allow them to be cross-linked through olefin methathesis in organic solvents. The resulting "stapled peptides", some of which contained only 1 or 2 cross-linked residues, had very high T_m values and low susceptibility toward proteolytic cleavage and were capable of entering cells. Examples were provided of novel constructs that bound to p53 and Notch, proteins that are involved in cancer and stem cell differentiation, respectively. This approach to drug design may allow for the generation of novel pharmaceuticals directed against a wide array of targets of interest.

Another example of the use of engineered peptide drugs was described by Yana Reshetnyak (University of Rhode Island), who developed a low pH sensitive peptide that inserts into and crosses membranes. The pH low-insertion peptides (pHLIP) were nontoxic, did not cause fusion or leakage, and were unidirectional in delivery. The peptides were capable of delivering cargo such as imaging agents.

Throughout the meeting, there were examples of how an understanding of the molecular basis of a disease of interest can provide a framework for new drug development. One example of this was presented by Adrian Krainer from the Cold Spring Harbor Laboratory, who described how point mutations in exons can lead to genetic diseases such as spinal muscular atrophy through altered RNA splicing in a process known as exon skipping. Knowing the nucleotide sequences involved in this disease has allowed for the design and application of antisense and siRNA-based molecules that suppressed exon skipping through changes in splicing patterns. Kathleen Giacomini from the University of California at San Francisco described how genetic variations in the membrane transporters can have a significant impact on susceptibility to drug therapy, for example in diabetes. Transporters recognized by the metformin, an antihyperglycemic drug used for the treatment of type II diabetes mellitus, were studied. The drug binds to the OCT1 transporter, and individuals with reduced transporter function have altered drug pharmacokinetic parameters which may lead to drug resistance.

There were several presentations on synthetic and biologic carriers that allowed for conditional drug release in predetermined manners. Solid tumors are often associated with hypoxia, and this was exploited by Martin Brown at Stanford University in a presentation on clostridia-directed enzyme prodrug therapy. This approach exploits a nonpathogenic

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⊥ D.A.S. has no financial conflict disclosures related to this work.

10.1021/mp800258g CCC: $40.75
Published on Web 01/06/2009

form of Clostridia that grows in hypoxic and necrotic sites that are relatively specific to solid tumors. The bacteria were engineered to express an enzyme that could convert a relatively nontoxic prodrug into an active drug. Dr. Brown showed data with Clostridia genetically engineered to produce the *Escherichia coli* nitroreductase enzyme together with the novel dinitrobenzamide mustard prodrug, PR-104, an anticancer drug currently in clinical testing. He demonstrated greater efficacy of the combination in various rodent tumor models than could be achieved with the use of the chemotherapeutic agent alone. Neil Forbes also presented a strategy in the use of bacterial targeting using a strain of *Salmonella typhimurium* that grows more rapidly within tumors than in normal tissues. The agent has cytotoxic activity, and can also be engineered to deliver therapeutic agents and diagnostic markers to tumors.

The acidic environment within solid tumors, as well as that within the endosomes and lysosomes of target cells, provided the basis for a number of interesting approaches for drug delivery. Mark Grinstaff from Boston University described a new class of dendrimeric-based nanoparticles that expand under mildly acidic conditions through the hydrolysis of labile acetal groups that are incorporated into the structure. Nanoparticles were developed that encapsulate such drugs as paclitaxel, and release the drug at pH 5.0. Jean Fréchet from the University of California at Berkeley described a number of novel cross-linked acid-sensitive polymeric constructs that form emulsions capable of encapsulating macromolecules for antigen presentation. Several acid sensitive acetals were described, some of which were exquisitely sensitive to lysosomal pH. Upon hydrolysis, the encapsulated payload is released in the target cell where it can be degraded and presented via MHC 1 on the cell surface. Considerable progress has been made in the general area of liposomal-mediated delivery, as illustrated in several talks during the meeting. David Thompson from Purdue University described pH sensitive liposomes for drug delivery. To achieve the requisite level of stability in the blood and lability within tumor tissues, a number of vinyl ether derivatives of varying pH sensitivity were designed computationally and then used to form steroid-PEG derivatives that get incorporated within the lipid bilayer. The resulting liposomes are labile within acidic vesicles within the target cells. Folate targeting was used to target the liposomes to folate-receptor positive tumor cells in tissue culture. Similar vinyl ether constructs were used in preparing pH-sensitive polyrotaxanes for gene delivery. Stavroula Sofou (Polytechnic Institute of New York University) discussed pH sensitive liposomes actuated by lateral phase separation of DPPC:DOPE:PEG-lipid mixtures. At the slightly acidic pH of the tumor interstitium, lipid phase separation results in membrane areas enriched in and other areas depleted of PEG-modified lipids. In the depleted areas, functional groups conjugated directly on the membrane surface become exposed, resulting in pronounced vesicle binding. Anu Puri (National Cancer Institute) provided data on a novel class of tunable liposomes that are activated to release their contents upon radiosensitized polymerization of diyne lipids. The design principle relies on partitioning of diacetylenic lipids as segregated patches in the liposome membrane and included an appropriate tunable photosensitizer in the aqueous compartment of liposomes to promote electromagnetic radiation-triggered chemical modification in $DC_{8,9}PC$ for localized drug delivery. Theresa Allen (University of Alberta) discussed the antitumor activities of targeted stealth liposomes. These differ from early generation liposomes in their ability to evade mechanisms for rapid clearance to home to tumors by virtue of having appended antibody fragments that recognize tumor associated antigens. The role of the EPR effect for initial tumor uptake followed by more selective ligand targeting to cells was discussed.

Several examples of novel delivery systems being developed as drug carriers were presented. Paula Hammond from MIT described the preparation and properties of linear dendritic block polymers, known as "patchy particles", that allowed for the organized presentation of cell binding molecules. One example included folic acid attached to the surface of polyester dendrons for the delivery of the dendrimers to folic acid receptor positive tumor cells. Ligand valency and patterning was related to pharmacokinetics, tumor retention, and the rate of intracellular uptake. These novel constructs were capable of homing to tumors in antigen dependent manners. Shiladitya Sengupta from MIT utilized poly(lactide-*co*-glycolide) (PLGA) microspheres that were coated with anticancer drug combinations, such as doxorubicin and combretastatin 4A. This particular combination was of interest, since the agents act synergistically on tumor cells through complementary mechanisms of activity. Several other drug combinations were explored, including Pt-based alkylating agents and BCNU. Kimberly Hamad-Schifferli from MIT described the formation, properties and utilities of gold nanorods which were synthesized with a cationic surfactant, cetyltrimethylammocium bromide (CTAB). The surfactant could be then exchanged with thiolated ligands, such as DNA with an appended thiol group, leading to well-characterized molecules that are stable and relatively uniform in size. Upon ultrafast laser irradiation, the nanorods melt and release the bound drugs, allowing for both temporal and spatial control of molecules of interest. The wavelength of light required to induce cargo release was dependent upon the shape and size of the nanorods. Preparations that resulted in capsule shaped nanorods utilized short wavelengths for release, while the dog bone shaped nanorods required longer wavelengths. This approach to drug delivery demonstrated how the shape and composition of the nanoparticle could influence the factors leading to selective control over drug release of multiple species.

Particle based delivery systems were also described that allow for the control of drug pharmacokinetics and pharmacodynamics. One such presentation was given by Joseph DeSimone, University of North Carolina, Chapel Hill, who presented research on a technology called "Particle Replication in Non-Wetting Templates" or PRINT. Using the PRINT method, drugs are embedded in a perfluoropolyether matrix that can then be fabricated to form monodisperse particles

with well-defined shapes and sizes. The particles can be generated in large quantities with nanometer to micrometer size ranges, depending on the particular conditions used in preparation. Drugs and proteins can be embedded throughout the particles or on just the exposed surfaces. Some of the biological properties of particles with the same shape and size as red blood cells were presented, and current investigations include particle trafficking in the body as a function of both shape and size. The role of size shape in nanoparticle kinetics and function was also discussed by Omolola Adefeso (University of Michigan), with a particular emphasis on the impact that shear forces have on vascular flow rates.

Another microfabricated device for drug release was described by Kristy Ainslie developed in the laboratory of Tejal Desai at the University of California at San Francisco. The microdevice protects drugs from enzymatic degradation, and releases them in an asymmetric manner toward the epithelial barrier of the intestine. A PEG hydrogel has been incorporated in these devices, and this hydrogel has been shown to release up to three therapeutics simultaneously. The results indicate that, with this device, drug is concentrated at the in vitro epithelial cell barrier and greater than 6 times the amount of drug permeates the barrier compared to conventional particles that release drug non-asymmetrically. Jinming Gao from the University of Texas Southwestern Medical School provided model simulation studies of cell targeting of nanoparticles. The results provided predictions about how shape and size affect targeting efficiency to receptors under physiologically relevant flow conditions. Experimental validation is in progress to verify the model data.

Many drugs fail due to unfavorable ADMET (adsorption, distribution, metabolism, elimination and toxicity) characteristics that often reveal themselves late in clinical development. Michael Shuler (Cornell University) overviewed the development of microfabricated chips ("A Body on a Chip") with several chambers connected by series of microchannels to model ex vivo pharmacokinetics. The chambers each contain tissue mimics for organs in the body that are often affected by the drugs used to treat diseases of interest. For example, chambers containing liver cells, bone marrow, kidney cells, tumor and many other tissues are used for predicting the ADMET properties of approved and experimental anticancer drugs and various nanoparticles of interest. The goal of this work is to more rapidly and economically model kinetics and metabolism before the initiation of phase 1 human trials.

Drug targeting was featured and emphasized throughout the meeting, and several approaches and applications were discussed. Defining the biology and the molecular receptors for targeted delivery is a critical aspect to this work as illustrated by David Lyden from the Weill Cornell Medical College in a lecture the premetastatic microenvironment. Over 100 hundred years ago, Steven Paget's "Seed and Soil" pivotal hypothesis of metastasis introduced the concept that a receptive microenvironment is required for disseminating malignant cells to engraft in distant tissues. Dr. Lyden demonstrated that there are molecular and cellular changes required at these future sites of metastasis known as the premetastatic niche. Several significant alterations occurring in the parenchyma at destination sites of future metastasis were shown to encourage the homing and engraftment of circulating tumor cells. The initial proliferation of tumor cells in the metastatic niche leads to development of micrometastases. Assembly of a functional vasculature is required to enable progression to macrometastases, and bone marrow derived endothelial progenitor cells are critical regulators of this process. In addition to primary tumor treatment, systemic therapies targeting each stage of metastatic progression (premetastatic, micrometastatic and macrometastatic) may be beneficial. The presentation discussed specific targets that might be critical determinants in each of these stages.

Bronek Pytowski from Imclone Systems provided an overview of the lymphatic structure of solid tumors together with a rationale for utilizing antibodies against vascular endothelial growth factor receptor-3 (VEGFR-3) for targeting tumor lymphatic endothelium. VEGF-R3 is overexpressed in tumor lymphatics, and is also involved in the generation of angiogenic sprouts and formation of new vascular networks. One application of anti-VEGF-R3 mAbs is for the treatment of inflammatory breast carcinoma, which invades and grows in local lymph nodes. A mAb against VEGFR-3 was described that had antitumor activity in vivo, and acted through the intended mechanism of inhibiting the development of the tumor lymphatic system. This antibody, which displays a novel mechanism of activity, is being developed for human clinical trials.

Recombinant fusion proteins were featured in presentations illustrating their activities for treating cancer, autoimmunity and inflammatory diseases, and for treating blood clots. Vladimir Muzykantov from the University of Pennsylvania provided an overview of the physiology of vascular endothelium. Cell adhesion molecules expressed on the luminal surface of include stably expressed PECAM-1 and pathologically up-regulated ICAM-1. These represent good target determinants for delivery of therapeutics. Interestingly, endothelial cells internalize multivalent conjugates cross-linking multiple copies of ICAM-1 or PECAM-1, but not monoclonal antibodies to these antigens. A series of anti-PECAM recombinant fusion proteins was reported. One such molecule was the anti-PECAM single chain Fv (scFv) fused to pro-urokinase mutant, which is activated by thrombin. This fusion protein specifically augments fibrinolytic capacity of the vasculature to dissolve pathological blood clots. Another molecule described was the anti-PECAM scFv-thrombomodulin fusion protein, which had very similar targeting capacities, but has anticoagulant and anti-inflammatory activities. These recombinant targeted therapeutics may allow for safer, more effective, specific and durable antithrombotic and anti-inflammatory activities.

There were several other presentations regarding the use of mAbs and mAb-conjugates for cancer therapy. Ezio Bonvini from Macrogenics, Inc., discussed how bispecific fusion proteins can be applied for the treatment of autoim-

mune diseases. The Fc receptors CD16A, CD16B, CD32A and CD32B mediate a variety of immunoregulatory functions, acting as either activators (CD16A and B, CD32A) or inhibitors (CD32B). A bispecific fusion protein consisting of VH and VL domains that bind the inhibitory receptor CD32B and CD79B, a component of the B-cell receptor, inhibited B-cell activation and was active in a collagen-induced arthritis model in mice. The possibility of exploiting both activating and inhibitory receptors for inducing immune responses was discussed.

Ira Pastan (National Cancer Institute) described antibody-pseudomonas exotoxin 40 (PE40) fusion proteins for cancer therapy. Results of a phase I clinical trial in patients with hairy cell leukemia treated with an anti-CD22-PE40 fusion protein were presented. There were 19 complete regressions, and 6 partial regressions out of 46 patients treated. The phase II dose was 40 μg/kg, and strong efficacy was obtained with few signs of immunogenicity. A new antibody with much higher affinity was obtained by mutating three of the amino acids in the variable region, and phase I trials of this novel construct are underway. These studies were extended to include targeting the mesothelin receptor on solid tumors. Mutations in PE40 were reported that were less immunogenic in mice compared to the wild type protein.

Some therapeutic applications for antibody drug conjugates were presented by Allen Ebens at Genentech. Several mAbs recognizing antigens on hematologic malignancies were used to form conjugates with highly potent drugs such as auristatins and maytansines. The drugs were attached using linkers that were either cleavable through lysosomal proteolysis or disulfide reduction, or were noncleavable, and required complete degradation of the mAb to release active drug. Cleavable linkers were successfully applied to mAbs that recognized both internalizing (CD19, CD22) and non-internalizing (CD20, CD21) antigens, in contrast to the noncleavable linkers that were active mainly on mAbs against internalizing antigens. mAbs with cysteines that were introduced using site directed mutagenesis provided well tolerated ADCs with well defined positions for drug substitution.

The therapeutic utility of radioimmunoconjugates is exemplified by such drugs as Bexxar and Zevalin, which are clinically approved for the treatment of non-Hodgkin's lymphoma. Oliver Press from the Fred Hutchinson Cancer Research Center demonstrated that Bexxar/drug combinations are therapeutically superior and less toxic to patients than total body irradiation/drug combinations. The rationale is that targeted therapy provides an advantageous tumor to normal tissue exposure ratio compared to cytotoxic radiation. The ratio can be increased yet further through pretargeting, which in this case involves the administration of a mAb−streptavidin conjugate which localized to tumors and clears from the systemic circulation. In a second step, radiolabeled biotin is given. The small molecule freely penetrates into tissues, binds to localized streptavidin, and rapidly clears from the blood. Significant therapeutic efficacy was obtained without excessive toxicity. The advantages of pretargeting and several variants of this technology were discussed.

New approaches to molecular imaging were presented in the final session of the meeting. Brian Ross from the University of Michigan described imaging applications of biomarkers to monitor the effects of cancer chemotherapy. One example was the use of functional diffusion maps as an imaging biomarker to monitor the effectiveness of therapy. Quantitative MRI imaging of the diffusion of water molecules in tumors was found to correlate with and predict therapeutic outcomes and survival. Dwight Seferos from Northwestern University (Chad Mirkin laboratory) described the application of "nano-flares" for intracellular imaging. These consist of oligonucleotide-modified gold particles that are hybridized to fluorophores. The nano-flares are small enough to internalize and can detect mRNA in living cells. The particles are quite stable, can be taken up without transfection agents, and provide a novel class of intracellular probes. David Piwnica-Worms from Washington University, St. Louis, closed the meeting with a presentation on dynamic imaging of cell signal transduction pathways and target-specific drug pharmacodynamics. New approaches to molecular imaging involving bioluminescent reporters were described that facilitate studies of cell migration, signaling pathways, drug action and interacting protein partners in vivo within the context of living animals. Applications of these technologies for analyzing β-catenin protein levels in living cells in response to Wnt, and for the characterization of protein−protein interactions in vivo, were presented.

To summarize, a number of novel approaches and advanced technologies for new drug development were presented at the Gordon Research Conference on Drug Carriers in Medicine and Biology. These included devices for specific drug release, new delivery vehicles and biological targets for therapeutic intervention, and cutting edge technologies to monitor effectiveness and off-target toxicities. Many of the advancements, as detailed in this summary, have the potential of leading to novel therapies for treating cancer, neurodegenerative disorders, autoimmunity, diabetes and cardiovascular diseases. In addition, the delivery systems have applications for improving efficacy of existing drugs and diminishing their undesired side effects. Some of the presentations showed successful strategies that are now in trials or FDA approved for sale. The next meeting will be held in the summer of 2010.

Acknowledgment. D.A.S. is supported by NIH RO1 CA 55349, PO1 CA 33049, and the Experimental Therapeutics Center of MSKCC.

MP800258G