molecular pharmaceutics

meeting report

Drug Carriers in Medicine and Biology

The Gordon Research Conference on Drug Carriers in Medicine and Biology is an established conference for scientists engaged in research surrounding drug delivery, macromolecular-based therapies including polymers, antibodies, and liposomes, new molecular targets, and preclinical as well as clinical applications of drug carriers. We chaired the most recent meeting, which was held October 5−10, 2004, in Big Sky, Montana. The presentations reflected many of the advancements in such fields as polymers, monoclonal antibodies, and novel small molecules as drug carriers and therapeutic agents. Imaging technologies were described that utilized a variety of carriers. A series of excellent presentations on self-assembling materials with an array of biological applications clearly identified this area as an emerging and promising area of technology. What follows is a summary of the presentations.

The keynote address by Rakesh Jain at Harvard University surrounded the physiology of solid tumors, which consist of such components as tumor and stromal cells, extracellular matrix, and vascular endothelial cells. Blood flow in tumors can be quite different than in normal tissues, due to their tortuosity and leakiness together with impaired lymphatic drainage. These conditions combine to restrict the intratumoral access of a variety of therapeutic agents. A great deal of interest has surrounded the possibility of restricting intratumoral blood flow even further with new classes of drugs known as antiangiogenic agents. One such agent, an antibody against vascular endothelial growth factor known as bevacizumab (Avastin), has just recently been approved for the treatment of colorectal carcinoma. Dr. Jain showed that bevacizumab changes the architecture of tumor blood vessels and decreases tumor hypoxia. The cells then become more susceptible to radiation and drug therapies. This was expanded upon later in the meeting by Herbert Hurwitz at Duke University who presented clinical data showing that the combination of bevacizumab with conventional chemotherapeutic regimens increased the life expectancy of patients with advanced colorectal carcinoma by at least 30%. Results such as these provide convincing validation of the clinical potential of antiangiogenic therapy.

Edward Sausville at the University of Maryland presented the other keynote address on signal transduction inhibitors for cancer therapy. The signal transduction pathways within cancer cells can be quite different from those within normal cells, providing several targets for chemotherapeutic intervention. These include a large array of protein tyrosine and threonine kinases that act in very complex and intertwined pathways. Examples of clinically approved drugs acting at the level of signal transduction inhibition were described, including Gleevec, Iressa, Avastin, Herceptin, Erbitux, geldanamycins, and flavopiridols. Dr. Sausville pointed out that one of the most compelling and as yet unresolved questions around signal transduction inhibitors concerns which combination of kinases need to be inhibited to achieve effective and specific growth inhibition. He described the possibility of generating nontoxic signal transduction inhibitors that become specifically toxic in tumor cells when provided in specific combinations. It is evident that this is an area of research that will lead to several new drugs. Indeed, later in the meeting Dr. Klaus Bosslet at Schering AG described an orally active vascular endothelial growth factor inhibitor that is now in a Phase III clinical trial for the treatment of metastatic colorectal carcinoma. Several other small drugs against this target are also in advanced clinical studies.

The efficiency of macromolecular-based drug targeting for solid tumor therapy needs improvement, since only a small percentage of the administered dose finds its way to the tumor sites and stays there. Therefore, the principals that influence persistence, biodistribution, clearance, and metabolism are of critical importance in developing effective macromolecular based drugs. Sherrie Morrison at UCLA discussed the molecular features of various antibody isotypes, and showed how site-specific mutagenesis has shed light on the critical residues for complement fixation and to binding to Fc receptors. Of particular interest was the description of mAb binding to FcRn receptors, and the role this interaction plays in antibody persistence, penetration, and metabolism. FcRn binding was predicted 40 years ago by Brambell as being central to maintaining antibodies in the circulation and protecting them from catabolism. Dr. Morrison described studies showing that while FcRn binding clearly plays a role in antibody persistence, it is certainly not the only factor involved, since a direct correlation between FcRn binding affinity and half-life was not obtained.

Further work in antibody structure/function was described by Anna Wu at UCLA. Several novel truncated antibody constructs were described, including single chain Fv-Fc dimers, minibodies which lack the antibody CH1 and CH2 domains, and maxibodies which lack the CH1 domain. These constructs were designed for the delivery of radionuclides to tumors for imaging and therapy. The constructs undergo rapid clearance and provide better images of tumors than whole IgGs, which stay in the circulation for long periods of time. Rapid intratumoral uptake and systemic clearance was necessary to achieve excellent PET images of tumors

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with an ^{124}I-labeled anti CEA antibody. Consistent with these findings were studies showing that radiolabeled minibodies can be administered at much higher doses than corresponding IgGs without toxicity. Novel constructs such as those described have already demonstrated utility for radioimaging, and have significant therapeutic potential in drug delivery, due to their abilities to access tumors with low normal tissue accumulation.

Several applications of mAbs for therapy were described, with an emphasis on issues that affect therapeutic efficacy and toxicity. Hartmut Koeppen at Genentech discussed recent data using mAbs for the delivery of anticancer drugs to solid tumors. The major issues highlighted in the talk included conjugate immunogenicity, tumor penetration, antigen expression on tumors and normal cells, internalization of the antigen, linker stability, biodistribution, and antigen-independent toxicity. Preclinical data were presented with highly potent antiHer2/neu-drug conjugates that were active at well-tolerated doses. Linker instability in some of the conjugates played a significant role in the systemic toxicity observed. The most stable conjugates described included peptide-linked drugs, and drugs that were linked through stable amide bonds. Primate studies showed that the toxicities were manageable at doses considered to be in the efficacious range. These new immunoconjugates, consisting of potent drugs, stable linkers, and well-characterized antigen targets, have significant potential for cancer therapy.

Volker Schellenberger at Genencor International discussed the use of mAb-enzyme fusion proteins for the activation of anticancer prodrugs within solid tumors. The major issues in this field are immunogenicity of foreign enzymes, achieving high tumor-to-blood ratios, and having prodrugs that are unstable, but that release active drugs with well-characterized dose−response effects. A version of β-lactamase was described that was deimmunized according to the results of a T-cell stimulation assay. This enzyme was used to activate a prodrug of melphalan, a drug that is used in high-dose isolated limb perfusion for the treatment of melanoma. The fusion protein was optimized for thermal stability, resistance toward proteolysis, and selective binding under the slightly acidic conditions found within solid tumors. Clinical studies with the fusion protein/prodrug combination are planned.

Dr. David Scheinberg from the Memorial Sloan-Kettering discussed the use of mAbs for the delivery of short-lived α-emitting isotopes to tumors. ^{225}Ac was attached to several different mAbs through a functionalized DOTA chelator, and the resulting conjugates had very high radiochemical purities. This isotope is of interest, since it has a relatively long half-life (10 days) and sequentially decays through three other atoms, resulting in the release of four α particles. The mAb-^{225}Ac constructs effected high levels of cytotoxic activities in hematologic and solid tumors in an antigen-specific manner. Spheroid studies showed that the activities were centered around high concentrations of the conjugates, consistent with the short path length of the α particles. One of the issues with ^{225}Ac is that the daughter ions may not remain in the chelated form. When metal scavengers were used, kidney exposure was significantly reduced. The approach of using ^{225}Ac in the targeted form is of interest, since the half-life may allow for significant levels of intratumoral penetration, and the dosimetry to tumors can be highly favorable. It is possible that one of the recombinant constructs described by Anna Wu would be ideally suited for ^{225}Ac delivery.

Molecules that form supramolecular assemblies were prominently featured in the meeting. Timothy Deming from the University of California at Santa Barbara described self-assembly of block copolymers into hydrogels. New synthetic methods involving catalysts for the preparation of amino acid based block copolymers were described. The use of transition metal complexes as end groups to control the addition of each α-amino acid *N*-carboxyanhydride (NCA) monomer to the growing polymer chain permitted control of the propagation reaction and resulted in copolymers of defined structure and narrow distribution of molecular weights. Diblock copolypeptide amphiphiles self-assembled at low concentrations to form the three-dimensional structure of hydrogels. The unique properties of these hydrogels depended on their structural parameters. Segments that were α-helical formed gels more readily than β-strands, which in turn were better than random coils. The porosity, fast response, and rapid rearrangement after stress of self-assembled hydrogels may prove useful in biomedical applications, particularly for drug delivery. Large molecules such as proteins and DNA could be loaded into the hydrogel structure to be released as the hydrogel responded to a physiological trigger.

Samuel Stupp from Northwestern University described amphiphiles that form three-dimensional fibrous networks upon addition to aqueous solutions. Specific peptide sequences were incorporated into the amphiphiles to impart features into the resulting nanofibers such as promotion of neuronal, pancreatic islet, and bone growth. Other amphiphiles were described that generate chemokine binding networks that inhibit angiogenesis or that support cell differentiation. The observation that rapid selective differentiation was linked to the amplification of bioactive epitope presentation to cells by nanofiber scaffolds will have a great impact on the design of numerous self-assembling systems. This is an emerging area of research with potentially a large number of medical applications.

M. Reza Ghadiri from the Scripps Institute described cyclic peptide sequences that spontaneously form nanotubes capable of penetrating through cell walls and membranes. The resulting nanotubes are able to kill bacterial cells within 5 min, whether they are growing or static. Depending on the peptide sequence employed, the resulting nanotubes can be specific for Gram-positive and Gram-negative bacteria, virally infected cells, and subsets of mammalian cells.

A number of talks concerned the use of polymers and polymer conjugates for therapy and imaging. The potential of vesicular carriers for drug delivery were described by Karen Wooley from Washington University, Kazunori Kataoka from the University of Tokyo, and Alexander Kabanov from the University of Nebraska. Dr. Wooley

described supramolecular assemblies formed from dilute solutions of block copolymers. The resulting micelles assume a variety of shapes, depending on the hydrophilicities and amphiphilic characteristics of the building blocks. Such nanoparticles have been shell cross-linked and further modified with peptide sequences to promote binding to cell surfaces and/or transduction. Dr. Kataoka described nanocarriers consisting of block copolymers of poly(ethylene glycol) and poly(aspartic acid) and a dendrimer with a photosensitizer in its core. Enhanced photodynamic effects of these constructs, over cationic dendrimer photosensitizer and polyionic complex micelles, were clearly demonstrated. This approach is suitable for the delivery of photosensitizers of various structures.

Dr. Kabanov used Pluronic block copolymers as an example to demonstrate the effect of macromolecules and macromolecular therapeutics on gene expression profiles in treated cells. In particular the mechanism through which the Pluronic block copolymers sensitize multidrug resistant tumors to anthracycline antibiotics was presented. The mechanism of sensitization may involve disruption of mitochondrial activity and target cell ATP depletion. A relationship has been established between the extent of the cytotoxic activity of doxorubicin and the extent of ATP depletion by Pluronic block copolymer. Data from clinical trials of doxorubicin-containing Pluronic-based micelles were presented.

One of the most advanced polymer−drug conjugates in the clinic, polyglutamic acid−paclitaxel (Xyotax), was described by Jack Singer at Cell Therapeutics. This water soluble conjugate releases the anticancer drug paclitaxel through polymer degradation and ester bond hydrolysis. The advantages of Xyotax over paclitaxel appear to be higher intratumoral drug concentrations, lower systemic toxicity, and activity in settings where paclitaxel is inactive. Phase III clinical trials are in advanced stages, and the outcome should be available next year. If approved, this would represent the first covalent polymer−drug conjugate for cancer therapy and would provide validation for the whole concept.

The meeting included talks surrounding novel targeted constructs for drug delivery. Charles Wilson from the Archemix Corporation described aptamers, which are molecules composed of small oligonucleotides selected for binding through a reiterative PCR-based process. Aptamers to such proteins as thrombin and platelet derived growth factor, together with their pharmacokinetic parameters in unmodified or poly(ethylene glycol) modified forms, were described. Michael Flanagan from Sunesis Pharmaceuticals demonstrated that new small molecular weight drugs could be developed through a process known as tethering. Proteins were mutated at several residues near their binding sites with cysteines, and were then allowed to react with chemical cocktails containing agents with reactive disulfides. Molecules that bound were then tethered to other molecules that bound to nearby cysteines on the proteins, leading to new entities with high binding affinities. This resulted in the identification of new agents that inhibited such proteins as thymidylate synthase and interleukin 2. Peter Dervan from California Institute of Technology described minor groove binding distamycin derivatives that bound in a base-specific manner. Small molecular weight agents were generated to bind to HIFα, a promoter for vascular endothelial growth factor, whose expression was down-regulated.

Drug carriers have had pronounced effects on imaging technologies. Jonathan Lindner from the University of Virginia described the diagnostic imaging and therapeutic applications of encapsulated microbubbles and other acoustically active microparticles. Application of these agents relies on surface modification with mAbs, peptides, or other ligands that facilitate binding to target cells involved in inflammation, angiogenesis, and atherogenesis. Ultrasonic irradiation of free or targeted microbubbles produces cavitation and other high-energy events such as microstreaming and ballistic shell dispersion that proved useful for imaging. Specific examples were discussed in which ultrasound destruction of payload-bearing microbubbles was used to amplify and site-localize gene and drug delivery. Other imaging technologies described in the meeting included quantum dots that were modified with biomolecules for cell recognition, described by Marcel Bruchez from the Quantum Dot Corporation. Zheng-Rong Lu from the University of Utah presented the design and synthesis of new macromolecular gadolinium complexes as MRI contrast agents. These new macromolecular structures, containing degradable disulfide bonds, permit control of the signal-to-background ratio, resulting in MRI blood pool contrast enhancement and increased sensitivity of tumor detection. Dr. Alexei Bogdanov from Massachusetts General Hospital described imaging technologies that allowed for the detection of differentially expressed enzymes such as cathepsin B, and matrix metalloproteinases 2 and 9 in tumors.

Throughout the meeting, there were presentations surrounding technologies for the identification of promising therapeutic targets and the generation of interesting new molecules. Dieter Brömme from the University of British Columbia discussed the importance of cathepsin K activities in osteoporosis and rheumatoid arthritis. The design of novel cathepsin K inhibitors and the possibility to incorporate them into polymeric drug delivery systems presents a new paradigm for macromolecular therapeutics in the treatment of bone and cartilage related diseases. Paul Herrmann from the National Cancer Institute discussed the potential of proteomic technologies for individualization and improvement of cancer diagnostics, therapy, and prognosis. This technology, in combination with gene expression profiling, may enhance our understanding of the impact of the different subcellular fate and subcellular pharmacokinetics of polymer-bound drugs on their mechanism of action when compared to free drugs. Such data are a prerequisite for the identification of specific targets unique for macromolecular therapeutics. Bernard Lebleu from the University of Montpelier reviewed the area of drug delivery systems containing cell-penetrating peptides. The relationships between the structures

of cell-penetrating peptide-containing drug delivery systems and their internalization and subcellular fates were discussed.

Carston Wagner from the University of Minnesota described novel dihydrofolate reductase fusion proteins that could undergo self-assembly when treated with cross-linked dimers of methotrexate. This technology may have application in forming complex ligand structures capable of binding receptors with high affinities. If such chemical conjugation and recombinant technologies are insufficient for generating bioconjugates of interest, David Tirrell from California Institute of Technology discussed methods for expanding the genetic code to make proteins that would otherwise be exceedingly difficult to make. Dr. Tirrell described how tRNAs can accept unnatural amino acids, which are then incorporated into proteins in both residue and site-specific manners. Such proteins have unusual biological properties and may find use as therapeutics and novel drug carriers, and they should also provide insight into protein structure−activity relationships.

In summary, the Gordon Research Conference on Drug Carriers in Medicine and Biology focused on cutting edge research that likely will lead to valuable new therapeutics. Since the meeting as a whole falls within the scope of *Molecular Pharmaceutics*, we felt that a timely comprehensive overview was of importance. Areas of particular interest in cancer medicine were identified as targeted drug delivery and new molecules that interfere with signaling pathways vital to tumor cell growth. Several new drug carriers and conjugation technologies were described that have medical applications in cancer, autoimmunity, and infectious disease therapy. Self-assembling molecules that can be used as matrices for cell growth, cell recruitment, tissue engineering, and elimination of selected cell populations are of great interest. The next Gordon Research Conference in Drug Carriers in Medicine and Biology will be held in the summer of 2006.

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