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Cellular Delivery of Therapeutic Macromolecules (CDTM) International Symposium 2010: lessons and progress from inter-disciplinary science

Our understanding of disease processes is rapidly increasing and an unprecedented number of macromolecular entities, including biopolymers such as nucleotides, peptides and proteins as well as synthetic polymers, are under investigation as therapeutic agents. The effective delivery of many of these therapeutic macromolecules to their target is constrained by their interaction with biological barriers, be this a feature of the macromolecule absorptive or dispositional processes, or indeed the need for the macromolecule to reach an intracellular target. Challenges faced in the effective delivery of macromolecule therapeutics to tissues, cells and subcellular compartments are considerable. The science underpinning the basic mechanisms of macromolecule interactions with biological barriers, through to the clinical translation of such entities into therapeutic agents serves as the focus of the Cellular Delivery of Therapeutic Macromolecules (CDTM) biennial international symposia series. The CDTM symposia have been held in Cardiff University since 2006 and a major objective for the organizers of this series, Drs Mark Gumbleton and Arwyn Jones, is for the symposia to serve the development of Early Stage Career researchers and to promote the inter-disciplinary collaborations necessary to make real progress in this field. All three symposia in the series CDTM2006, CDTM2008 and CDTM2010 have attracted the highest quality of international speakers and provided unique opportunities for delegates from around the world to interact with others engaged in this research area and to learn from more experienced attendees. For information on the CDTM series go to www.CDTM.cf.ac.uk.

A CDTM symposium has traditionally begun with a perspective on membrane biophysics and biochemistry as it relates to macromolecule cell trafficking. For CDTM2010 Erwin London (Stony Brook University, USA) discussed the domain organisation of proteins and lipids at the plasma membrane prior to Paul Luzio (University of Cambridge, UK) discussing regulators of endocytic pathways that will eventually determine the intracellular fate of macromolecule therapeutics. Endocytic pathways while portals for macromolecule entry into the cell do not afford ready escape from within the endomembrane system. This escape remains a major hurdle to cytoplasmic delivery of macromolecules. Jörgen Wesche (University of Oslo, NO) revealed some of the crucial aspects of the endosomal escape mechanisms for fibroblast growth factor-1. Parallels between natural proteins and synthetic systems were then exposed with presentations on plasma membrane and endosomal membrane interactions of liposomes by Frank Szoka (University of California, San Francisco, USA), of polyplexes by Ernst Wagner (Ludwig Maximilian University Munich, DE) and nanoparticles by Tore-Geir Everson (University of Oslo, NO). Cameron Alexander (Nottingham University, UK) presented a talk on how architecture and bioresponsiveness can influence the cellular delivery of synthetic polymer systems. Viruses are natural delivery vectors and Andrew Baker (University of Glasgow, UK) demonstrated the value of engineered chimeric adenovirus particles as carriers of therapeutic genes in cardiovascular disease.

The design of macromolecule therapeutics able to efficiently cross epithelial and endothelial surfaces will be more efficient once the biological landscape is characterized and an improved perspective is achieved of the mechanisms through which nature overcomes such barriers. The trafficking of IgG by FcRn within, and across, endothelial barriers was the subject of the presentation by E. Sally Ward (Southwestern Medical Centre, University of Texas, USA). Jan Schnitzer (PRISM, San Diego USA) highlighted the use of endothelial proteomics to identify tissue-specific IgGs able to traverse the endothelial barrier to deliver cargo. The blood–brain barrier represents a unique endothelial network and William Banks (VAPSHCS and University of Washington, Seattle, USA) discussed the challenges and opportunities of delivering peptides and proteins to the brain across this restrictive microvasculature.

Cell penetrating peptides (CPPs) can deliver themselves and associated cargo across a wide range of biological membranes and Sandrine Sagan (Université Pierre et Marie Curie-Sciences et Medécine, Fr) discussed technologies for assessing peptide uptake. In a complementary presentation Giles Divita (Centre de Recherches de Biochimie Macromoléculaire, Montpellier, Fr) expanded on CPP-mediated delivery to highlighted successful *in vivo* siRNA delivery strategies using specific CPP sequences.

The unique challenges in the design and development of macromolecules destined for the clinic were demonstrated by presentations from industrial scientists Ted Parton (UCB Celltech, UK) on pegylated antibody fragments, and by David Rozema (Roche-Madison Inc., USA) on polymer conjugates for siRNA delivery.

Underpinning much of the innovative science are cutting edge technological

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.10.010

advancements and a unique feature of the CDTM series is the integration of 'Technical Application' sessions. For CDTM2010 Olav Scheimann (St Andrews University, UK) presented a session on EPR spectroscopy and its value in resolving conformations and dynamics of membrane associated proteins. Werner Witke (Leica Microsystems, DE) presented a session on Total Internal Reflection Microscopy (TIRF) and its capacity to study crucial events that occur on and very close to the plasma membrane. Dries Vercauteren (Ghent University, BE) presented a session comparing pharmacological and molecular approaches to inhibit endocytic pathways and the requirements to integrate both to gain a more accurate picture of uptake pathways.

CDTM2010 welcomed over 180 registered delegates from 27 different countries and from five continents. Over 90 delegate posters were presented, with six of these also selected to highlight their work via short talks. The publication of the CDTM2010 abstracts in the journal *Drug Discovery Today* is a major landmark for the symposia series reflecting its international standing in the scientific community, its maturation into an important event in the Drug Delivery calendar and its ability to consistently deliver high quality science both on the podium and through the delegate contributions. It is hoped that all who attended CDTM2010 departed with renewed energy for the scientific challenges they face.

We look forward to CDTM2012.

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DELEGATE ABSTRACTS

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Design and development of polymeric nanoparticles for targeted delivery of nucleic acid-based therapeutics to tumor sites

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Nucleic acids are widely used as potent therapeutics in cancer research. They can either promote gene expression by bringing a gene either not expressed or under-expressed into tumor cells (cDNA), or alternatively silence expression of genes such as oncogenes (RNAi mediators). However, before they can be efficiently translated to the clinic, this technology requires some optimization: nucleic acids and their vehicles need for instance to be protected from rapid elimination from the bloodstream (opsonization, clearance, and nuclease-mediated degradation) and the specificity of tumor addressing has to be validated. Hence a polymeric nanoparticular carrier encapsulating nucleic acids, either plasmid DNA or siRNA, was developed. Nanoparticles are composed of (1) PLGA, a well tolerated and biodegradable polymer, (2) PEG groups to avoid opsonization, (3) PEI moieties to complex nucleic acids and to enhance cytosolic delivery and (4) RGD sequence for active tumor targeting. Nanoparticles were formulated by double emulsion or water-in-oil-in-water method. Physical properties of such nanoparticles were assessed by dynamic light scattering (size and polydispersity index) and laser doppler electrophoresis (zeta potential). The efficiency of nucleic acid encapsulation into the carrier was determined by the Picogreen assay. Cytotoxicity and transfection capacity were assessed in an *in vitro* model of B16F10 melanoma cells. To date, various designs of nanoparticles were successfully formulated with appropriate size, surface charge and encapsulation efficiency. The PLGA nanoparticles did not show cytotoxic effects on cells and, although less efficient than PEI alone, allowed DNA delivery into tumor cells.

doi:10.1016/j.drudis.2010.09.355

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Pulmonary delivery of mRNA: *in vitro* and *in vivo* evaluation

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Gene therapy is a very promising field of research in medicine. The success of gene based therapeutics will depend on a well thought-out and well-designed delivery system, which should guide the nucleic acids into the desired compartment of the selected cells. However, humans and other organisms have developed natural barriers that protect their body against different kinds of pathogens or intruders. During the evolution of the human being, these barriers have become almost perfect and difficult to overcome. The nuclear membrane, one of the final barriers that protect our genes, appears to be the most important and the crucial one to overcome in non-viral gene delivery. In this work we try to avoid the need to overcome this barrier by intracellular delivery of mRNA instead of pDNA. mRNA delivery has many advantages. First, mRNA does not have to overcome the nuclear barrier and therefore mRNA can transfect also non-dividing cells or dividing cells independent of their cell cycle. Second, mRNA cannot integrate in the genome. Consequently, mRNA mediated gene expression is transient and the risk of insertional mutagenesis can be excluded. Third, there is no need to select a promoter [1]. In this work we evaluate whether mRNA complexed with cationic liposomes (composed of e.g. the cationic lipid GL67) are able to transfect the respiratory tissue of mice. The efficacy of the mRNA:liposome complexes and the gene expression kinetics will be studied and compared with pDNA:liposome complexes. In this study we focus in particularly on GL67-based liposomes. GL67 is an amphiphile consisting of a cholesterol anchor lined to a spermine headgroup in a 'T-shape' configuration. It was proven that GL67 based liposomes are the most effective non-viral pulmonary gene delivery systems [2]. Evaluation of the