Expert Opinion

The XIIth International Symposium on Recent Advances in Drug Delivery Systems

in honour of Prof. Jan Feisen;

Salt Lake City, UT, USA, 21 – 24 February, 2005

Eric Vivès
INSERM EMI0227 'Immunociblage des Tumeurs et Ingénierie des Anticorps', Centre de Recherche en Cancérologie, CRLC Val d'Aurelle Paul Lamarque, 34298 Montpellier Cedex 05, France

The XIIth International Symposium on Recent Advances in Drug Delivery Systems was held from 21 – 24 February 2005 in Salt Lake City, UT, USA. Approximately 250 people attended this symposium dedicated to a broad variety of topics, ranging from recent advances in drug delivery systems to biomaterials and novel concepts in macromolecular therapeutics. A total of 33 people, all recognised specialists in the aforementioned fields, presented 30-min up-to-date reviews of these topics, as well as discussing recent results. In addition, the symposium included a poster session with ~ 100 displays highlighting various interesting data.

Keywords: cell targeting, cellular uptake, drug delivery, nanotechnology, sustained release

Expert Opin. Drug Deliv. (2005) 2(3):597-601

1. Overview

This year's International Symposium on Recent Advances in Drug Delivery Systems meeting was held in honour of Prof J Feijen from University of Utah celebrating his 60th birthday. This meeting, which is held every 2 years was dedicated especially to a wide variety of drug delivery systems, ranging from recent advances in genes, RNA interference, proteins, vaccines and cell delivery to biomaterials and novel concepts in macromolecular therapeutics. An invitation to the equivalent conference held next year in Europe was extended and can also be found at the conference website [1].

2. Meeting highlights

The symposium began with the plenary lecture provided by R Langer (Massachusetts Institute of Technology). He swiftly introduced a quote from Inder Verma, written in 1999: "There are only three problems in gene therapy: delivery, delivery and delivery". This sentence indeed reminded all the attendants that the main bottleneck for drug delivery systems was the efficient and specific cellular uptake by the targeted cell type.

Langer proceeded by showing an amazing and spectacular property of a polymer made of dioxanone and ε-caprolactone. This polymer adopts different structures depending on the physicochemical environment, such as the temperature or hydrophilicity. Among such polymers, the development of synthetic precursor monolayers were reported that allow for efficient cell adhesion suitable for various plastic surgery applications. Along this line, biopolymers can be designed with the appropriate shape (e.g., a nose or an ear) prior to being seeded with different cell types. The cellular development was shown to be fully effective when employing this polymer, and restored an identical tissue, not only in

terms of appearance, but also in terms of biological function, as was shown in a rat model that had good recovery of motricity in the legs after a spinal cord alteration followed by repair in the rat. Finally, a microarray detection system to determine the more efficient polymer for allowing the adhesion of various cell type was presented.

R Schiffelers (Utrecht University) exposed recent developments with the self-assembling nanoplex-targeted small interfering RNA (siRNA) for inhibiting tumour and ocular angiogenesis *in vivo*. Targeting angiogenesis is certainly a promising strategy in the treatment of these diseases, as $\alpha_v\beta_3$-integrins are one of the proteins overexpressed on angiogenic endothelial cells, and cyclic peptides containing an Arg-Gly-Asp motif have been identified as high-affinity ligands for these integrins. Therefore, complexes of polyethylenimine (PEI), polyethylene glycol (PEG) and Arg-Gly-Asp motifes were designed to deliver specific siRNAs aimed at inhibiting one of the strongest proteinaceous activators of angiogenesis, namely the vascular endothelial growth factor (VEGF) through altering the expression of its receptors VEGFR1 and VEGFR2. These complexes, which are 0.09 nm in size and possess a ζ-potential of 6, show a very good specific interaction with various endothelial cells *in vitro*, but also a noticeable higher tumoural delivery *in vivo*. Furthermore, a disparition of the peritumoural vascularisation was observed, including in the eyes following infection by herpes simplex virus. In addition, it was concluded that the combination of specific ligands with delivery systems could indeed be more efficient for clinical activities.

A presentation by K Kataoka (University of Tokyo) followed in which different preparations of micelles containing a drug reservoir for drug delivery systems were discussed. It was shown that reticulation through the formation of disulfide (SS) bonds could improve the stability of such formulations by increasing the extracellular stability and allowing further release following intracellular reduction of the SS bridges. Again, drug-mediated delivery by micelles containing Arg-Gly-Asp ligands were shown to improve the recovery following synovium space injection. In addition, triblock copolymers consisting of PEG, poly-3-morpholinopropylaspartamide and poly-L-lysine were used for the preparation of three-layered polyplex micelles for pDNA delivery. The role of poly-L-lysine is to condense DNA; the poly-3-morpholinopropylaspartamide shows a good buffering capacity for enhanced cytoplasmic delivery; and the biocompatible segments of PEG were exposed at the surface of the micelles. After intravenous injection, luciferase activity was found to be 100-fold greater in tumour than in the rest of the body, making these triblock polymers very promising candidates as nanocarriers for *in vivo* gene delivery systems.

L Chiarantini (University of Urbino) then provided a comparison of novel delivery systems for antisense peptide nucleic acids (PNAs). Three different PNA delivery strategies have been evaluated:

- modified PNA was extended at the C-terminus with a hydrophobic tetrapeptide (Phe-Leu-Phe-Leu)
- modified PNA was extended at the N-terminus with three lysine residues and then electrostatically bound to the surface of polymeric core-shell microspheres (MSs);
- antisense PNA was loaded into autologous red blood cells (RBCs) by hypotonic dialysis

The delivery efficacy was evaluated following the inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. RBCs and core-shell MS delivery systems were shown to be the most effective methods for transfecting murine macrophages *in vivo* with a PNA concentration of 40 nM.

RJ Levy (Children's Hospital of Philadelphia) presented the macroscopic coating of metal stents, which are used for dilating clinically obstructed arteries, with viral gene vectors via high-affinity proteins, such as antibodies or receptor-peptides, for promoting a local delivery process. This approach demonstrated the successful gene transfer *in vivo* for several cardiovascular pathologies.

M Akashi (Osaka University) introduced the development of polymeric nanospheres (NSs) for intranasal immunisation using inactivated HIV-1. These core–corona NSs are composed of a hydrophobic polystyrene and hydrophilic macromonomers made of easily controllable chemical structures. Lectine-immobilised NSs can efficiently capture HIV-1 particles. Akashi and colleagues intranasally immunised rhesus macaques with these HIV-1-loaded NSs. They then measured HIV-1 gp120-specific IgA and IgG antibody levels using an enzyme-linked immunosorbent assay method in plasma, vaginal washes and fecal extracts. The protective effect after challenge with a pathogenic virus was determined by quantitative polymerase chain reaction of the viral RNA. After a series of six immunisations, vaginal anti-HIV gp120 IgA and IgG were detected in all HIV-NS-immunised macaques. After intravaginal challenge, the plasma load of viral RNA in infected macaque was substantially less than in non-immunised control macaque and reached below the detectable level. Moreover, in intravenous challenge experiment, HIV-NS-immunised macaques showed lower viral RNA loads and higher $CD4^+$ T-cell counts than control animals. Akashi further mentioned that a more degradable polymer (i.e., γ-polyglutamic acid) was used to replace the not degradable polystyrene moiety. It was concluded that these HIV-NS may have a great potential as a prophylactic vaccine against HIV infection.

Along the same line, B Gander (Institute of Pharmaceutical Sciences) presented the use of biodegradable MSs made of polylactide-co-glycosides (PLGA) and chitosan for vaccination and immunotherapy with subunit antigens and related nucleic acids. This strategy has been found to induce strong and long-lasting immune responses of both T helper cell type 1 and 2 following unspecific phagocytosis by macrophages *in vivo*. It was also shown that loading antigen-presenting cells with such antigen-containing MS markedly

prolonged the antigen presentation to T cells. Several criteria, such as the production of TNF-α, reactive oxygen intermediates, iNOS or COX-2, and the viability of cells, were recorded for following the cellular responses. All results focused on the ready ingestion of the MSs by the different macrophages, meaning that particle macrophagocytosis does not change essential markers of metabolic activity. The *in vitro* release and immune response induced by PLGA-MSs containing an antimalarial antigen were also examined. Interestingly, antibody titres were still at a level of 1/410,100 after 25 weeks. Therefore, these PLGA-MS, for example, could be safely used for *ex vivo* antigen loading and reinjection for immunotherapy purposes.

J-H Kim (University of Utah) proposed an approach to improve the stability of biopharmaceutical proteins following temporal and reversible complexation with synthetic weak basic polyelectrolytes, such as poly-L-histidin. Kim and colleagues evaluated the physical characteristics of complexes with various model acidic proteins, such as bovine serum albumine, insulin or glucagon-like peptide-1, and the long-term storage (≤ 60 days) of PLGA-MSs loaded with these complexes was characterised. It was reported that the addition of a multifunctional PEG-polycation can be a beneficial strategy.

Listeriolysin-O (LLO), a 58-kDa *Listeria monocytogenes* protein, has been evaluated by K-D Lee (University of Michigan) and colleagues for its ability to promote the endosomial escape of a pay-load molecule, such as cytotoxic protein, antisense oligonucleotides or plasmid DNA. A single cystein residue of LLO allowed the covalent attachment of the drug to be carried into cells. The chimaera was then loaded into liposomes or polymeric particles. Once inside the lysosome, the endosomolytic properties of LLO following pore formation induced an increased cytosolic uptake of the active molecule. This property has been assessed both *in vitro* and in mice.

Reactive oxygen species including hydrogen peroxide (H_2O_2), are powerful oxidants that, at high concentrations, are toxic to cells and cause tissue damage. However, at sublethal doses, H_2O_2 activates the expression of several activators of the metastatic tumour growth. Therefore, it has been suggested that the targeted delivery of catalase, an enzyme detoxifying H_2O_2, could prevent tumour metastasis. For instance, M Nishikawa (Kyoto University) showed that liver and lung delivery could be achieved by using a galactose- and a PEG-catalase chimaera, respectively. A reduction of pulmonary tumour proliferation was observed after the injection of PEG-catalase. Thus, the targeted or sustained delivery of catalase to sites where tumour cells metastasise could be a promising approach for inhibiting or preventing the tumour from spreading to various sites of the body.

The second day of the symposium commenced with a session about biohybrid systems, scaffolds and biomaterials.

An interesting way to improve the cellular delivery of plasmid DNA was provided by H Ghandehari (University of Baltimore). Silk-elastin protein polymers (SELPs) were used for the delivery of naked DNA and adenovirus particles to solid tumours. Composed of silk (Gly-Ala-Gly-Ala-Gly-Ser) and elastin (Gly-Val-Gly-Val-Pro) amino acid motifs, SELPs can be prepared with the appropriate sequence and composition to undergo an irreversible solid-to-gel transition. The released plasmids were thus viable for ≤ 28 days *in vitro* and, in a murine model of human breast cancer, the intratumoural transfection efficiency using DNA-containing hydrogels was enhanced by one- to threefold compared with direct injection of nude plasmid DNA. SELPs were also shown to be suitable for the localised delivery of adenovirus particles as they can be mixed with polymers without using organic solvents that may reduce the virus viability. It was thus seen that adenoviral particles embedded in SELP hydrogels retain bioactivity for ≤ 22 days. A recombinant polymer containing lysine residues for condensing DNA, a fibroblast growth factor-2 (FGF-2) motif for promoting a cancer cell targeting and nuclear localisation and histidine residues to allow escape from endosomes was obtained. In addition, folate was also attached at the surface of particles to increase the specific tumoural delivery of paclitaxel.

JW Singer (Cell Therapeutics) provided an overview of various poly-L-glutamic acid (PG) drug conjugates, such as Xyotax™ (MD Anderson Cancer Centre; paclitaxel polyglumex [PPX] with CT-2106 [PG-glycin-camptothecin]). PPX was conjugated covalently through an ester bond to PG with an average molecular weight of 48 kDa containing 37% paclitaxel (weight/weight). *Ex vivo* human plasma stability studies showed that ~ 12% of the PPX was hydrolysed to paclitaxel over a period of 24 h. PPX demonstrated enhanced efficacy compared with paclitaxel in animal tumour models when administered as a single agent or in conjunction with radiation. PPX was also shown to be well-tolerated even in patients with extensive prior therapy.

Results from a Phase I study demonstrate that CT-2106 persists in plasma as a stable conjugate with a half-life of ~ 33 h. Again, it was shown from clinical trials of PPX and CT-2106 that polymer conjugation could enhance the therapeutic effectiveness and tolerability of anticancer drugs.

TA Desai (Boston University) described the successful construct of bioadhesive microdevices from polymethylmethacrylate. These microdevices have been designed to provide an internal reservoir via which drug can be delivered and a modified face to promote various cell-specific interactions through interaction with recognition motifs, mainly within the gastrointestinal tract. Interactions between microdevices and Caco-2 cell monolayers were investigated.

Along the same line, JL West (Rice University) presented biomimetic hydrogels as scaffolds in tissue engineering. More precisely, biofunctional derivatives of PEG were designed with inserted peptide sequences as substrates for targeted proteolytic enzymes involved in tissue formation, cell migration and extracellular matrix remodelling. It was shown that such polymers achieved biodegradation in response to the targeted proteases, whereas the materials are stable in the absence of proteolytic enzymes or when exposed to nontargeted tissues. Furthermore, as PEG is non-adhesive in cells, hydrogels were modified with the common

Arg-Gly-Asp-Ser cell adhesion peptide. Following such an addition, cell adhesion and spreading occurred specifically in a dose-dependent fashion. That was also the case when elastin-derived adhesion peptide Val-Ala-Gly-Pro, an adhesive for smooth muscle cells, was grafted into PEG-based hydrogels. These hydrogels can also be modified with various biomolecules, including the growth factors (transforming growth factor-β, epidermal growth factor, FGF-β and nerve growth factor). In each case, bioactivity of growth factors immobilised to the hydrogel was higher than that of their soluble counterparts.

DL Kaplan (Tufts University) exposed the use of emulsans as tools for controlled release. Emulsans are complex anionic polysaccharides produced from a variety of Gram-negative bacterium. They form an extracellular cell-associated capsule, which is subsequently released into the fermentation broth. This family of bioemulsifiers was investigated for biomedical applications. As an example, emulsan-alginate MSs were prepared. A two- to threefold improvement in adsorption for the emulsan-alginate MSs was recorded compared with alginate alone for the cellular delivery of bovine serum albumine. When subtilisin was bound to emulsan/alginate MSs, treatment with lipase, which cleaves the fatty esters from the emulsan structure, allowed the release of the bound protein. In conclusion, emulsan MS systems can be bioengineered to specifically release absorbed proteins by treatment with lipase. Combined with the structural versatility and biological activation features for these emulsan/alginate MSs, the presented results suggest some interesting future options in areas of controlled release.

As an alternative way of the phage display techniques to identify peptides interacting specifically with various cell types, KS Lam (University of California, Davis Cancer Center) and colleagues developed a 'one bead–one compound' strategy to identify cell-binding peptides. Basically, a single short peptide sequence was grown on one support bead. The beads were screened for various cell types. It is then possible to detect cell growth on beads harbouring the interacting peptide. Sequencing the corresponding bead allowed for the characterisation of the responsible attachment peptide. Following this strategy, the researchers identified new peptide ligands with high affinity and high specificity against prostate cancer, non-small-cell lung cancer, ovarian cancer and non-Hodgkin's lymphoma. From these peptide sequences, it was possible to design new types of specific ligand made with non-natural amino acids. The *in vivo* evaluation of some of the presented peptides by near infrared imaging was quite impressive, with specific tumour accumulation still detected 46 h after injection. Indeed, it is expected that such 'homing peptides' will be used in the near future for improving the delivery of different biologically active compounds.

The folate-mediated targeting of NSs made of biodegradable methoxypolyethylene glycol/poly-ε-caprolactone was presented by YM Lee (Hanyang University). The confocal microscopy study revealed that folate-conjugated NSs loaded with paclitaxel exhibited a greater extent of cellular uptake than folate-unconjugated NSs against MCF-7 breast carcinoma cells. Moreover, higher toxicity was also measured, providing evidence that folate-mediated endocytosis played an important role in transporting drugs within the tumour cells.

J-C Leroux (University of Montreal) then discussed the preparation, characterisation and application of block copolymer micelles. This group recently synthesised diblock, triblock and star-shaped polymers composed of either poly-*N*-vinylpyrrolidone or poly-*N*-(2-hydroxyprolyl)methacrylamide as the hydrophilic segment exposed at the surface of the micelle, and poly-D,L-lactide as the internal core-forming block. These polymers were found to have low critical association concentrations inversely proportional to the degree of hydrophobicity. Association of poorly water-soluble drugs, such as paclitaxel, yields stable micelles with sizes in the range of 20 – 180 nm. Recently, micelles were prepared following a drug incorporation technique whereby both the hydrophobic paclitaxel and the polymer are dissolved in a water/tert-butanol mixture prior to freeze-drying and rehydratation. It was shown that paclitaxel-loaded micelles exerted reduced toxicity, thus allowing for doses threefold higher than the maximum tolerated dose of Taxol® (Bristol Myers Squibb) to be administrated. In addition, different pH-sensitive diblock copolymers were also synthesised for the oral administration of hydrophobic drugs. The release kinetics was found to be dependent on the hydrophobicity of the copolymer as well as on the pH level. This could indeed be very valuable once the complex reaches the intestinal tract. Therefore, the relevance of these developments in the field of biomedical research was evaluated.

KL Wooley (Washington University) focused her presentation on shell crosslinked knedel-like polymer micelles. Various moieties, such as the Tat peptide or saccharide, were attached to these nanoparticles to evaluate the efficiency of packaging of the drug and efficient cellular delivery.

Y Fu (Akina, Inc.) followed this presentation by highlighting the recent developments in the formulation of fast melting tablets. Various parameters were investigated, which yielded to the definition of a tablet as possessing good hardness and fast disintegration time following a production method that is easy to scale-up. Along the same lines, YR Byun (Gwangju Institute of Science and Technology) presented oral heparin derivatives in aqueous formulations. Three kinds of low molecular weight heparin (LMWH)-deoxycholic acid (DOCA) were prepared according to various DOCA conjugation ratios. It was shown that the aqueous formulation containing 10% dimethyl sulfoxide could solubilise a LMWH–DOCA conjugate and were more effectively absorbed into the gastrointestinal tract.

K Fowers (MacroMed) then presented various preclinical and clinical developments of a physically targeted controlled-released paclitaxel product, OncoGel® (MacroMed), for solid tumour management. The aim of the study was to examine the safety and tolerability of OncoGel to support its clinical development. Data showed that the maximum tolerated dose

was not reached when OncoGel was administered as a single intralesional injection. Plasma measurement of the paclitaxel released from the injection area showed negligible concentrations, meaning that paclitaxel was scavenged efficiently within the tumour and, therefore, offers the possibility of reducing the side effects usually observed when using paclitaxel.

DF Ranney (Global Biomedical Solutions) described the use of carbohydrate polymers for promoting angiogenic targeting. Heparin-surfaced amphotericin B and dermatan sulfate-surfaced doxorubicin were prepared and evaluated for their antifungal efficacy and tumour retention, respectively. Both polymers, and other structurally similar carbohydrates, were shown to undergo homogeneous endothelial binding, rapid, active and high capacity extravation, deep matrix penetration and cellular internalisation at numerous focal disease sites.

JT Santini (MicroCHIPS, Inc.) presented the design of electrothermally activated microchips for implantable drug delivery and biosensing, in order to allow the 'on-demand' release of a drug entrapped in the sealed reservoir of a microchip. Amazingly, the drug was simply released following resistive heating from an applied current to rupture a metal cap covering the reservoir.

AG Mikos (Rice University) then described the use of injectable scaffold for bone and cartilage tissue engineering. These scaffolds are based on fumarate polymers. The main advantage of these polymers is the possibility to allow the solution to be injected to a defect site and to be crosslinked *in situ* following either thermal or photoinitiation procedures. It was shown that, depending on the crosslinking degree, the scaffold could be degraded within 14 weeks, thus allowing the sustained release of the included drug.

Another kind of biodegradable polymer for the delivery of genes, polyphosphazenes, was presented by WE Hennink (Utrecht University). Such polymers were cationised with 2-dimethylaminoethylamine (DMAEA) to promote efficient DNA binding. Good *in vitro* transfection was observed in COS-7 cells, even in the presence of serum. Higher tumour transfection efficiency data compared with the transfection with PEI were also provided. Toxicity of DMAEA polymers was shown to be 20-times lower than observed with PEI, and work with this approach is in progress, such as attaching specific targeting ligand to promo.te cell specificity or PEG to prolong the circulation time of the complex.

Along the same line, K Anwer (Expression Genetics described the use of a biocompatible gene-delivery system made of PEG-PEI-cholesterol (PPC) with an average size of 100 – 150 nm. Transfection efficacy was shown to be higher than with PEI alone. *IL-12* gene transfection was studied for local delivery to human cancer cells. Significant dose-dependent inhibition of tumour growth and improved survival of mice with solid mammary, ovarian or head and neck tumours were recorded. Moreover, the inhibitory effect of the IL-12/PPC was enhanced when it was applied in combination with specific chemotherapeutic agents. The first Phase I human clinical trial of the *IL-12* gene therapeutic is expected to commence this year.

Eventually, T Kissel (Phillips University) also developed the use of PEI associated with the Tat peptide to promote the efficient delivery of DNA and drugs to the lung. Two kinds of complexes were synthesised: one with a ratio of negative/positive charges of 8, and another with a ratio of 10. The cellular delivery of a Tat–PEI–DNA complex with a charge ration of 10 was shown to be the most effective. Following a MTT toxicity test, it was also shown that Tat–PEI complexes were less toxic than the corresponding PEI complex.

3. Expert opinion and conclusion

This was a very interesting symposium; the development of new drug delivery systems covers a very wide range of different strategies from the formulation of original synthetic polymers to the design of various, more sophisticated microdevices aimed to induce the *in situ* sustained release of the drug. However, a recurrent problem was often highlighted: improving the specific drug delivery at the affected tissue remains very challenging. Indeed, promoting the efficient *in vitro* delivery of any drug into cells is usually easy to perform and was well documented during this meeting. However, the remaining efforts for most of these strategies are the requirement of a very efficient 'addressing-signal' directing the drug to the targeted tissue. Therefore, these efficient delivery systems will be associated with various cell-specific ligands in the near future. Next year's meeting, to be held on the 5 – 7 April 2006 at Twente University in The Netherlands, will offer an excellent new opportunity to discuss these various aspects of drug delivery systems.

Websites

1. http://www.bmti.utwente.nl/cdd/
Ninth European Symposium on Controlled Drug Delivery, 5 – 7 April 2006, Twente University, the Netherlands.

Affiliation

Eric Vivès
INSERM EMI0227 'Immunociblage des Tumeurs et Ingénierie des Anticorps', Centre de Recherche en Cancérologie, CRLC Val d'Aurelle Paul Lamarque, 34298 Montpellier Cedex 05, France
Tel: +33 467 61 25 18; Fax: +33 467 61 37 87;
E-mail: evives@univ-montp2.fr