New delivery systems for macromolecules

Dr Charles Ebert (TheraTech, Salt Lake City, UT, USA) gave the opening address at the AIC conference *Oral & Mucosal Delivery Systems for Macromolecules*, held at St James Court Hotel, London on 28–29 May 1997. He explained that pharmaceutical scientists were no longer seeking the 'holy grail' of drug delivery. Dr Ebert argued that the broad array of therapeutic agents chasing commercial opportunities required scientists to identify the most appropriate means of delivery to maximize the commercial potential. This meeting was designed to review these challenges for the oral and mucosal delivery of macromolecules and to discuss the latest technologies in this field.

Oral and mucosal peptide delivery

In the keynote address, Professor Vince Lee (University of South California, CA, USA) reviewed the various factors that should be considered when selecting an appropriate delivery system, such as the route of administration, pattern of drug release, method of delivery and fabrication of the formulation. Evidence was presented that suggested that the interaction between mucoadhesive agents and cell surfaces may cause changes in cell permeability and stimulate endocytosis.

In considering the various routes of administration, Professor Lee suggested that there were considerable misconceptions regarding the absorption of peptides from the lung and that more work was needed to develop formulations that will provide access to the whole surface area of the lung, in particular the alveolar regions, which account for 95% of the total surface area. With respect to oral delivery, Professor Lee emphasized the importance of drug design over drug formulation in avoiding hepatic clearance. Although drug formulation may be used to overcome problems of drug absorption and gastrointestinal metabolism, the problems of hepatic clearance may only be overcome by developing more metabolically stable analogues of the parent drug.

Dr Karsten Cremer (LTS Lohmann Therapy Systems, Andernach, Germany) described innovative dosage forms for macromolecule delivery to specific absorption sites in the gastrointestinal tract. He discussed the exploitation of intestinal transport proteins, such as vitamin B_{12} carriers, to increase the uptake of peptides and possibly nanoparticles. The potential incorporation of cholera toxin-binding subunits and *Escherichia coli* heat label toxin into vaccine delivery systems to enhance uptake was also discussed along with the use of enzyme inhibitors such as aprotinin, polycarbophil, carbopol and bile salts to reduce peptide degradation. He concluded by suggesting that the oral delivery of biopharmaceutical peptide products of up to 10 amino acids would be feasible in the near future; in the medium term, larger molecules without critical dosing regimes may also be successfully delivered by this route but the oral administration of large peptides with narrow therapeutic tolerances is beyond the capabilities of current technology.

Dr Roger New (Cortecs, London, UK) outlined the principle behind an oil-based carrier system (Macrosol™) for the oral delivery of peptide hormones. The system solubilizes hydrophilic molecules in oils in the absence of an aqueous phase. A wide range of compounds has been incorporated into Macrosol™, including a number of peptides. Dr New described the *in vivo* evaluation of the system for the delivery of calcitonin and showed data clearly illustrating the increased bioavailability of calcitonin on intrajejunal administration to pigs in the carrier system. The use of this carrier system for the delivery of insulin had also been investigated, and encouraging data were presented for the oral delivery of insulin in man.

Evaluating metabolic stability and mucosal uptake

Dr John Woodley (Keele University, UK) discussed the various *in vitro* methods for the evaluation of metabolic stability and mucosal uptake in the gastrointestinal tract, with particular reference to the advantages of using everted gut sacs, developed by Woodley's group, over the Caco-2 and Ussing chamber models for evaluating drug absorption. The use of the everted gut sac to evaluate the uptake of various macromolecules was illustrated by reference to his group's studies illustrating the importance of polymer charge and hydrophobicity on tissue uptake and serosal transfer.

Measuring immune response to oral vaccination

Dr Anu Kantele (University of Helsinki, Finland) discussed the monitoring of circulating antibody-secreting cells as a measure of the immune response to oral vaccination. Traditionally, humoral immune response in humans has been based on the assessment of antibodies in secretions, which may present both practical and ethical problems. Kantele's group have shown that circulating antibody-secreting cells express different homing receptors that bind to specific addressins in various tissues. The circulating antibody-secreting cells induced on oral vaccination in humans all express the $\alpha_4\beta_7$ gut homing receptor, with only a few expressing L-selectin – the homing receptor that leads to cell accumulation in the peripheral lymph nodes. On parenteral administration of the same antigen, the expression of $\alpha_4\beta_7$ receptor was reduced, and there was a concomitant increase in the expression of L-selectin. Oral booster immunization, however, was found to induce the expression of a unique set of homing receptors by antibody-secreting cells, which reflected neither the responses obtained on oral nor parenteral primary immunization. Dr Kantele suggested that

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a more accurate evaluation of hormonal immune response to oral vaccination was assured by assessing both the expression of homing receptor on antibody-secreting cells and the levels of secretory antibodies.

Buccal drug delivery

Dr Charles Ebert (TheraTech) opened the second day with a discussion of the advantages of the buccal route for the administration of peptide drugs. The TheraTech delivery system for buccal/gingival administration is based on a bilayer muco-adhesive tablet, comprising an adhesive layer and an outer drug layer, which dissolves with time. Dr Ebert described initial randomized double-blind clinical trials that demonstrated the tolerability of this system, based on measurement of local erythema, pain and discomfort. A market research study demonstrated consumer acceptance of the tablets, and showed that consumer acceptance increased with increased tablet usage. Tablet performance was also assessed in human clinical trials using glucagon-like insulinotropic peptide (GLP-1), a 30-amino-acid endogenous peptide with potential use in Type II non-insulin-dependent diabetes mellitus. GLP-1 delivered in the TheraTech transmucosal system caused a rise in serum insulin levels, and a decrease in serum glucagon and glucose levels, in comparison with a placebo, demonstrating the feasibility of oral transmucosal delivery for small molecular weight peptides.

Dr Gary DeGrande (3M Pharmaceuticals, St Paul, MN, USA) described the 3M transmucosal/transbuccal drug delivery system Cydot™. The Cydot system comprises a non-dissolving patch, which can be formulated as a matrix or reservoir system. In contrast to the TheraTech system, in which the drug moves outwards from the tablet into the mouth, the Cydot system contains an impermeable backing layer, so that drug movement is unidirectional towards the mucosal membrane. Dr DeGrande described the evaluation of a Cydot matrix patch for the buccal delivery of melatonin, which demonstrated favourable pharmacokinetics (e.g. rapid onset and sustained delivery), as well as good tolerability and adhesion. A comparison of transmucosal delivery with oral controlled release and transdermal drug delivery demonstrated that transmucosal delivery is the best dosage form to mimic endogenous melatonin secretion. He further described the use of the Cydot system for the transmucosal delivery of buprenorphine and low molecular weight heparin.

Microparticles for vaccine delivery

Dr Derek O'Hagan (Chiron Vaccines, Emeryville, CA, USA) described the advantages, use and potential of poly-(lactide-co-glycolide) (PLG) microparticles for vaccine development. PLG microparticles containing entrapped ovalbumin were capable of inducing a disseminated mucosal immune response after oral administration. A single immunization with diphtheria toxin (DT) in PLG microparticles induced a greater immune response than three doses of DT administered with alum, demonstrating the potential of the microparticles as a single-dose controlled-release vaccine.

He also provided an overview of various adjuvants currently under study, including MF59 (an oil-in-water emulsion), ISCOM (cage-like particulates composed of glycosides, Quill A cholesterol and phospholipids), Iscomatrix and LTK 63 (a genetically detoxified mutant). A comparative study, which measured the IgA titres in nasal, salivary and vaginal washes following the intranasal immunization with gD2 vaccine and the various adjuvants, demonstrated the superiority of PLG microparticles in the induction of a disseminated mucosal response, in comparison with the other adjuvants. The LTK 63 system (soluble adjuvant) was also capable of inducing high antibody responses. For the particulate systems, the need for a physical association of the antigen with the carrier was demonstrated. Dose–response studies with PLG microparticles and LTK 63 with gD2 were also described.

Dr Oya Alpar (Aston University, Birmingham, UK) also described the potential of PLG microparticles as vaccine adjuvants, using cholera toxin B subunit and tetanus toxoid as model antigens for oral and intranasal administration. She continued with a discussion of the influence of the physicochemical properties of microspheres on the resulting immune response, concluding that:

- particles with the highest hydrophobicity induced the highest serum IgG and gut-wash IgA antibodies (although this effect was critically dependent on the antigen used), and
- micro-sized particles provided the most promising mucosal responses.

She then discussed the potential of the nasal route for vaccine delivery, and described the enhanced immunological responses obtained following the intranasal delivery of PLG co-encapsulated *Yersinia pestis* subunit antigens.

Oral DNA vaccines

Dr 'Taff' Jones (CAMR, Porton Down, UK) described the work of his group on the potential use of PLG particles for the oral delivery of DNA vaccines. Studies have shown that DNA plasmids can be encapsulated without any significant loss of biological function. A single intraperitoneal or oral dose of the encapsulated DNA was shown to elicit a substantial humoral response, and oral dosing also led to a vigorous mucosal humoral response. Studies also suggested that this system elicits the production of serum antibodies and offers significant protection in a measles model.

Bacterial vectors

A major advantage of bacterial delivery systems is their capacity to deliver large sequences to the gastrointestinal-associated lymphoid tissue. Dr David Hone (University of Maryland, Baltimore, MD, USA) described a combination HIV-1 vaccine for oral/intranasal administration capable of inducing both humoral and cell-mediated responses, comprising:

- an HIV-1 envelope subunit vaccine (to induce mucosal and serum HIV-1 neutralizing antibodies), and
- live oral *Salmonella* bearing multiple HIV antigens (to induce a T-cell response).

Oral vaccination with *Salmonella*-HIV vaccine vectors demonstrated HIV-specific T-cell responses. The evidence suggested that the response was driven by T_{H1} cells, and also that secreted HIV antigens are more immunogenic for T-cell responses than their cytoplasmic counterparts. He further described the potential of a live oral *Salmonella* vaccine vector for protection against enterotoxigenic *E. coli* (ETEC). A *Salmonella* vector expressing colonization factor antigens, which mediate attachment of ETEC to enterocytes in the small intestine, elicited mucosal and serum antibodies against the antigens, whereas the free antigens induced no immune response. Interestingly, the studies indicated a biphasic cytokine response, with an initial T_{H2} response progressing with time to a T_{H1} response, which may have important implications for future rational mucosal vaccine design. He concluded with a description of some studies to develop and evaluate non-pyrogenic *Shigella* vectors for mucosal DNA vaccination strategies.

Dr Patricia Londono (Imperial College, London, UK) further discussed the use of *Salmonella* strains as vaccine vectors, with particular reference to the stability of foreign antigen expression *in vivo* and the importance of establishing the optimal expression system for each particular heterologous antigen. She discussed the value of the *nirB* promoter as a suitable promoter for stable foreign antigen expression in *Salmonella*. Foreign antigens can be expressed directly under the control of *nirB*, or can be fused to an immunogenic carrier protein (e.g. in multiple tandem copies attached to the C-terminal end of tetanus toxic fragment C, or on the surface of *Salmonella* delivered hepatitis B core particles). Various expression strategies were compared, with particular emphasis on the systemic and mucosal immune responses elicited by the resulting *Salmonella* strains in murine models.

Intranasal delivery

Continuing with the theme of mucosal immunity, Dr Linda Klavinskis (Guy's & St Thomas' Hospital, London, UK) discussed the concept of combining mucosal vaccination with DNA vaccination, with particular reference to the treatment of HIV infection. After giving a brief review of the common mucosal immunization system and the advantages of DNA vaccination, she proceeded to describe the use of cytofectins (neutral and cationic lipids that incorporate polyanionic DNA into stable multilamellar vesicles) for DNA vaccination. Intranasal administration to mice of cationic DNA 'liposomes' (the delivery system is actually comprised of lamellar structures composed of cationic and neutral lipid with DNA sandwiched in between the lipid layers, measuring approximately 100 nm across) encoding the reporter gene firefly luciferase, resulted in luciferase activity approximately 30-fold higher than when naked DNA was administered, with a peak in activity observed at day 3 and sustained at a lower level for at least 28 days. A serum IgG response, a mucosal IgA response in vaginal and rectal fluids, and T-cell proliferative and cytotoxic T-lymphocyte (CTL) responses were all detected following intranasal immunization. The CTL response was induced in the spleen, cervical, mesenteric and iliac lymph nodes. In rhesus macaques, DNA encoding simian immune deficiency virus (SIV) proteins given rectally or subcutaneously near the iliac lymph nodes ('targeting' to the iliac lymph nodes) induced rectal sIgA responses and also a cell-mediated immune response.

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