Expert Opinion

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Innovative therapeutics: from molecules to medicines

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The Socrates Intensive Programme offers annual courses focusing on the specific aspects of innovative therapeutics. The 2004 meeting was coordinated by the University of Parma and covered various subjects in the field of advanced drug delivery and pharmaceutical technology, including sessions on biopharmaceutics, pharmacokinetics, polymers, oral delivery, colloidal vectors, peptide and protein delivery, vaccines, oligonucleotide delivery, gene delivery, non-conventional routes of administration, and a graduate student symposium. The meeting had a highly interactive character and provided a unique opportunity for young scientists to present and discuss their work in an international setting.

Keywords: colloidal carriers, drug delivery, drug targeting, gene delivery, peptide and protein delivery, pharmaceutical technology, route of administration, vaccine delivery

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1. Introduction to the Socrates network

The Socrates Intensive Programme offers annual courses focusing on the specific aspects of innovative therapeutics, including new molecules, new technologies and alternative administration routes. The Socrates Network is based on two programmes: the first was originally proposed in 1995 by Professor F Puisieux on novel drug delivery systems; the second was initiated in 2000 by Professor E Fattal on the delivery of fragile molecules (proteins, vaccines and nucleic acids). The programme is currently coordinated by P Santi (University of Parma) and involves a network of 11 European universities. The programme is primarily intended for graduate students and other researchers from the participating universities. The lecturers come from the same universities, supplemented by well-known invited speakers. The official site of the programme can be found at [101].

2. The 2004 meeting on innovative therapeutics

The 2004 meeting was coordinated by the University of Parma, Department of Pharmacy, through the Inter-University Consortium on Innovative Pharmaceutical Technologies 'TEFARCO Innova', which comprises 10 Italian universities. The 2004 meeting was located at the University of Parma and focused on the delivery of innovative molecules, through approaches based on modern pharmaceutical technologies. The meeting included sessions on biopharmaceutics, pharmacokinetics, polymers, oral delivery, colloidal vectors, peptide and protein delivery, vaccines, oligonucleotide delivery, gene delivery, non-conventional routes of administration, and a PhD student symposium.

Various lectures had a highly tutorial character; for example, fundamentals were explained about the diffusion or mass transport applied to the delivery and release of drugs from drug delivery systems; the gastrointestinal (GI) tract and oral delivery; the lung and pulmonary delivery; the nose and nasal delivery; the skin and transdermal

delivery; the blood–brain barrier and delivery to the CNS; polymer and liposome technology; and the structure and characterisation of macromolecules. In addition, recent research achievements were also presented by the participating scientists. The increasingly important role of macromolecules, such as proteins and DNA, in our arsenal of therapeutic agents was reflected by the large number of presentations in the fields of (targeted) delivery of proteins, genetic materials and vaccines. Because of their complex structures and hydrophilic nature, they require special approaches with respect to their stabilisation, characterisation and delivery [1,2]. In the following sections some of the topics presented during the meeting will be summarised.

3. Meeting highlights

3.1 Polymers, lipids and colloidal carriers

Following a general introduction by P Caliceti (University of Padua, Italy) about natural and synthetic polymers currently used for controlled drug delivery and their synthesis, F Alhaique (University La Sapienza, Rome, Italy) focused the attention on polysaccharides that can be used for the preparation of 'modified-release' dosage forms. Both chemical and physical cross-link technologies were illustrated. Particular attention was devoted to the design of hydrogels based on scleroglucan, a fungal polysaccharide [3,4].

R Bettini (University of Parma, Italy) dealt with swellable matrices for oral drug delivery. Issues relevant to the manufacturing of swellable matrices (i.e., methodology and type of polymers) were discussed. Most of the talk was devoted to the mechanism(s) of drug release from such delivery systems and in particular the rate-controlling processes.

M Fresta (University of Catanzaro, Italy) and C Vauthier (University of Paris South, France) presented fundamentals on liposomes, polymeric micelles and nanoparticles, including their structure, preparation, characterisation and possible applications [5,6]. Innovative applications for the delivery of biopharmaceuticals (peptides, proteins and genetic materials) were presented by C Vauthier, A Bochot (University of Paris South, France) and T Kissel (University of Marburg, Germany). For example, the association of oligonucleotides with nanoparticles or liposomes has been shown to improve their therapeutic use through enhanced stability *in vivo* and improved intracellular penetration [7,8]; nanoparticles composed of biodegradable graft polyesters have been successfully applied for protein encapsulation [9,10]. V Préat (Catholic University of Louvain, Belgium) discussed the application of polymeric micelles consisting of self-assembling amphiphilic polymers for the oral delivery of poorly water-soluble drugs [11].

3.2 Peptide and protein delivery

Recent advances in recombinant DNA technology have made the production of large numbers of peptides and proteins possible. Many of these peptides/proteins have been shown to be highly potent and effective therapeutic agents. However, major problems exist for the successful delivery of peptide/protein drugs, including protein instability during formulation, storage and handling, degradation of the drug after administration by various proteolytic enzymes and poor permeability through lipid membranes due to the large size and non-lipophilic nature of these compounds [2].

M Lane (Trinity College Dublin, Ireland) discussed the present status of the various approaches described in the literature to enhance oral peptide delivery, including absorption enhancers, enzyme inhibitors and site-specific delivery [12]. M Alonso (University of Santiago de Compostela, Spain) explained how nanoparticles can be employed as carriers for oral peptide delivery. The nanosystems described comprised a lipid core surrounded by a hydrophilic coating, consisting of either a non-charged polymer, such as polyethyleneglycol (PEG), or the positively-charged polysaccharide chitosan [13,14]. The ability of these systems to enhance absorption of salmon calcitonin was described in detail [15].

W Jiskoot (Utrecht University, Netherlands) reviewed analytical methods used to characterise proteins [16]. These techniques are vital to detect small changes in structure, which can result in a loss of therapeutic activity and/or immunogenicity [2,17]. The suitability of several techniques, including fluorescence, circular dichroism, infrared spectroscopy and mass spectrometry, were discussed and the advantages and limitations of each method were illustrated by examples from Jiskoot's laboratory.

F Veronese (University of Padua, Italy) presented covalent polymer conjugation as a means of achieving improved delivery and reduced immunogenicity of protein therapeutics. Common problems encountered in the preparation of reproducible polymer conjugation were identified and potential solutions were presented [18]. So far, the most successful polymer in use is PEG and the strategy of drug modification known as PEGylation was discussed. Examples of PEG–drug conjugates, including insulin and IFNs, were used to illustrate the success of this approach [19].

C O'Driscoll (University College Cork, Ireland) gave an update about lymphatic delivery of peptides. Advantages of lymphatic delivery include the avoidance of hepatic first pass metabolism and targeting to the lymph nodes for vaccination purposes or for treatment of lymphatic diseases. Delivery systems employed to target peptides to the lymphatic system were discussed, in particular lipid-based vehicles used to enhance intestinal lymphatic transport [20].

G Martin (King's College London, UK) talked about the use of particulate systems for inhaled protein delivery and *in vitro* models employed for studying pulmonary delivery. Such systems, apart from the powdered protein, should contain excipients for stabilising the protein [21,22]. Excipients may also enable efficient dispersion of the drug, and, perhaps, controlled delivery of the therapeutic agent.

3.3 Vaccine delivery

Over the last 10 years, advances in the vaccine delivery field have confirmed the utility of biodegradable particles as delivery

systems for antigens via non-invasive mucosal routes [23]. Alonso gave a detailed presentation on the topic of micro/nanoparticles as vaccine delivery vehicles. As a result of the preferential uptake of nanoparticles by mucosal surfaces when compared to microparticles, there has been increasing focus on nanoparticles as transmucosal vaccine carriers. The design of new nanoparticle formulations intended for intranasal immunisation was clearly illustrated [24,25]. The development of these nanoparticles is based on two critical criteria: high antigen loadings and protection of the antigen from degradation as it traverses mucosal barriers. The presence of a PEG coating around polylactide nanoparticles enhances their stability following contact with mucosal fluids, as well as the transport of the associated antigen across the nasal and intestinal mucosae [14,26]. These observations correlate well with improved immune responses elicited by the encapsulated antigens. Following nasal administration of tetanus toxoid encapsulated into polylactic-co-glycolic acid (PLG)–PEG nanoparticles to mice, high and long-lasting mucosal and humoral immune responses were observed [25]. A further strategy has been the design of nanoparticles based on chitosan, a mucoadhesive polymer. Chitosan nanoparticles can be generated spontaneously under exceptionally mild conditions and demonstrate high affinity for associated antigen [13]. Nasal administration of chitosan nanoparticles containing tetanus toxoid resulted in high and long-lasting systemic and local immune responses [24].

Jiskoot gave an overview of liposomal and other lipid-based antigen carriers [27]. Such lipidic structures will closely mimic the outside of pathogens and, when used to deliver a suitable antigen, would be expected to result in vaccine formulations with stronger and longer-lasting immune responses than would result from administration of the antigen alone. The two main classes of lipid nanostructures, which show potential for vaccine development for administration in humans, are liposomal systems and immune stimulating complexes (ISCOMs). ISCOMs are composed of cholesterol, phospholipid, saponin (QuilA) and antigen. ISCOMs are typically 40 nm in diameter and are suitable as carriers for antigens such as amphiphilic proteins. Liposomes are highly versatile with regard to composition, size, physicochemical and immunological characteristics. This allows a flexible design in order to maximise functionality, and has led to the emergence of a whole group of liposome-like structures, many of which are being tested as antigen carriers. Examples of such liposomal systems are liposomes containing the outer membrane protein PorA of *Neisseria meningitidis* type B (PorA-liposomes). The PorA-liposomes are as immunogenic as bacterial vesicles [28]. Efficient *in vitro* and *in vivo* targeting of PorA-liposomes to dendritic cells may be achieved through manipulation of the phospholipid membrane [29,30]. However, this does not necessarily lead to higher protective immune responses [29].

3.4 Non-viral gene delivery and oligonucleotide delivery

Gene therapy has attracted considerable interest because of the possibility to interfere with life-threatening diseases on a molecular level. The delivery of the genes to the nuclei of the target cells has proven to be a critical factor for the success of this approach. Various gene therapeutics based on viral vectors have reached clinical testing, but tolerability and safety issues remain obstacles. Non-viral vectors based on cationic polymers (polyplexes) and/or cationic lipids (lipoplexes), with plasmid DNA, seem to be less problematic with respect to safety, but have not yet reached the transfection efficiency of viral vectors [31].

T Kissel explained the use of polyethylene imine (PEI) based polycations for gene delivery. Modification of PEI with PEG was shown to offer considerable potential for gene delivery *in vivo*. By systematically modifying the structure of PEI–PEG, insight into DNA complexation behaviour and its influence on transfection, cytotoxicity and biodistribution in mice was obtained [32-35]. Moreover, the conjugation of PEI–PEG with targeting moieties was shown to allow for the targeted delivery of plasmid DNA to tumour cells *in vivo* [36].

The use of cationic liposomes for non-viral gene delivery was discussed by S Simões (University of Coimbra, Portugal). In particular, strategies were presented to overcome barriers for successful (targeted) gene therapy, such as passage of cytoplasmic, endosomal and nuclear membranes [37-39].

E Fattal (University of Paris South, France) explained how colloidal vectors can be utilised to increase the efficiency of antisense oligonucleotide (ODN) therapeutics [40]. Adsorption to biodegradable polymeric nanoparticles via electrostatic interactions or encapsulation of ODN/polymer complexes in biodegradable microspheres was shown to protect the ODNs against nucleases and improve their cellular entry.

3.5 Routes of administration

3.5.1 Oral delivery

C Reppas (University of Athens, Greece) highlighted the exploitation of dissolution testing *in vitro* for predicting the performance of formulations in the intralumenal GI tract and, in certain cases, the drug plasma levels. The key factors affecting dissolution and absorption processes were discussed and selection criteria were proposed for suitable media to be utilised for a biorelevant dissolution test [41-43]. Specific aspects of GI physiology relevant to oral absorption of xenobiotics were also discussed. The composition of intralumenal contents, together with the motility of the GI tract and its effects on transit rates was presented. Moreover, canine data on the responses of the GI tract to glucose solutions were considered. In particular, the effects of nutrient concentration, osmolarity and viscosity on upper GI transit and net water flux were discussed in view of blood glucose levels. Moreover, the relevance of canine to human data was discussed [44-46].

A Gazzaniga (University of Milan, Italy) covered aspects of colonic drug delivery, including methods proposed so far for targeting the colon with orally-administered delivery systems. Special emphasis was given to time-dependent approaches. Most of the approaches proposed over the last three decades lack reproducibility due to high inter- and

intra-subject variability on physicochemical and/or physiological characteristics in the colonic region, such as microflora distribution, pH, residence time and intralumenal pressure. Recently, hydroxypropyl methylcellulose matrices were developed to overcome some of these problems [47].

3.5.2 *Pulmonary and nasal delivery*

A Chiesi (Chiesi Farmaceutici, Parma, Italy) gave a general introduction to drug delivery via the lungs, including the physiological aspects and the rationale for pulmonary delivery. Attention was focused on the devices used at present, such as pressurised metered-dose inhalers, dry powder inhalers and nebulisers. Bettini illustrated the advantages of nasal administration, such as the low proteolytic activity, the avoidance of the hepatic first-pass metabolism and the possibility to achieve a direct brain targeting. Following on, some examples of nasal delivery were illustrated, such as the case of the peptide calcitonin. Problems linked to the choice of the device as well as with the formulation were examined. In particular, the use of powder formulations proved to superior to liquid formulations.

3.5.3 *Transdermal delivery*

R Guy (University of Geneva, Switzerland) gave an overview on the applications and future trends of transdermal drug delivery and non-invasive monitoring [48]. Particular attention was focused on the methodologies that increase the permeability of the skin, either by the application of an additional force (over and above the concentration gradient) to the drug of interest, or by lowering the diffusional resistance of the barrier. In the former category, iontophoresis has been well studied and can claim some success; in the latter case, various approaches have been examined, ranging from the use of chemical enhancers and the formulation of complex vehicles, to electroporation, sonophoresis and a spectrum of minimally invasive techniques (e.g., microneedles, microperforation techniques).

3.6 Graduate student symposium

The graduate student symposium comprised ~ 15 podium presentations and 15 posters by graduate students from the participating universities. The symposium encompassed excellent presentations about both mechanistic studies and applications of polymers and colloidal carrier systems for the oral, transdermal, pulmonary and parenteral delivery of low-molecular-mass compounds and biopharmaceuticals.

4. Expert opinion and conclusions

The meeting largely covered the broad area of advanced drug delivery and focused on modern approaches to improve the delivery of drugs, genes and vaccines via various routes of administration. The meeting was a fruitful combination of tutorial lectures and presentations about ongoing research in several pharmaceutical laboratories in the European Union. Moreover, owing to the informal atmosphere, the meeting had a highly interactive character and offered a unique opportunity for young scientists to learn about the state-of-the-art in the field of advanced drug delivery and pharmaceutical technology, and discuss their own projects with internationally recognised scientists in this research area. Finally and importantly, the excellent quality of the contributions by the participating graduate students holds great promise for the near future of research into advanced drug delivery in the European Union.

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