Rapamycin 'glue strategy'

Rapamycin is a highly specialized molecular glue. That is the essence of a recently published paper [*Science* (1996) 273, 239–242] on the structure of this immunosuppressive agent associated with two of its binding proteins: the FK506-binding protein (FKBP12) and the FKBP-rapamycin-associated protein (FRAP). The structure was determined in the laboratory of Dr Jon Clardy at Cornell University (Ithaca, NY, USA) in collaboration with Dr Stuart Schreiber and coworkers at Harvard University (Boston, MA, USA).

A striking aspect of the complex is the almost complete absence of physical interaction between the two protein molecules. The complex is held together by the simultaneous insertion of two distinct regions of rapamycin into hydrophobic clefts on the surface of FKBP12 and FRAP. The binding occurs sequentially; first, rapamycin and FKBP12 form a complex and then FRAP binds to its complementary region of rapamycin. The role of rapamycin is to serve as a tether, holding two disparate proteins in close proximity.

Cyclosporin is another immunosuppressive agent that acts by forming a ternary complex with two proteins: cyclophylin and calcineurin, a protein serine/threonine phosphatase. Again, the binding is sequential; cyclosporin and cyclophylin interact first, and then a ternary complex is formed with calcineurin, which inhibits its phosphatase activity. In contrast to rapamycin binding, the binding site for calcineurin is spread over the surface of both cyclosporin and cyclophylin.

Both rapamycin and cyclosporin are naturally occurring cyclic peptides that were originally discovered from soil microbes as antifungal agents. They both possess potent immunosuppresive activity, but have distinct mechanisms of action. Cyclosporin inhibits the signaling pathway leading from the T-cell receptor to the activation of the IL-1 gene, presumably through inhibition of the phosphatase activity of calcineurin. Rapamycin blocks the signaling pathway leading from the IL-1 receptor to the nuclear factors that trigger cell proliferation, perhaps through inhibition of FRAP. FRAP is a member of a class of proteins that are known to be important in cell-cycle progression and regulates the p70 ribosomal protein, S6 kinase. Entrapping FRAP in a complex with rapamycin and FKBP12 probably blocks its ability to regulate the p70 kinase.

The work from Clardy's laboratory may also lead to the development of new tools for genetic engineering. By splicing the rapamycin-binding domains from FKBP12 and FRAP onto other proteins, it will be possible to use the rapamycin glue to join proteins that would otherwise never associate. This may have practical value for gene therapy. According to Clardy, "It's not hard to introduce new genes. What's hard is getting them to turn on." Already, scientists from Ariad Pharmaceuticals (Cambridge, MA, USA) have used the rapamycin glue strategy to join a DNA-binding protein with a regulatory protein to trigger a foreign gene in mice to produce human growth hormone. The ability to tether proteins by this mechanism may be an important key to making the promise of genetic therapies a reality.

Robert W. Wallace

Strategic use of drug delivery systems

The pharmaceutical industry is indeed 'living in interesting times', but not with the doom and gloom forecast by that Chinese curse. Behind the recent media attention focusing on multi-million-dollar mergers and acquisitions surrounding the big players, there is much growth and dynamism within the industry, centring around small organizations that focus on niche drug delivery technology. Such technology can provide benefits both commercially to the pharmaceutical company and clinically to the patient. The pharmaceutical industry is changing in attitude and culture towards the needs of the 'customer', and drug delivery systems (DDS) are playing an increasing role in meeting these needs. This was a recurring theme at a two-day IBC conference held in June, entitled *The Strategic Use of Drug Delivery Systems.*

DDS in strategic marketing

Dr Lisa Nolan (Elan Corp, Athlone, Ireland) set the scene for the first day's proceedings by providing a strategic marketing perspective. She highlighted the clinical benefits of DDS, which often translate commercially. Specialized delivery technology can prove lifesaving to important new chemical entities that would otherwise drop out of development owing to inappropriate pharmacokinetics. From a commercial perspective, a novel therapeutic may make it to the market, but if it has a suboptimal dosage regime or delivery system, it is susceptible to market share erosion; this point is particularly important in the current climate in which the period of pioneer exclusivity is decreasing. Life-cycle management of a product through the strategic use of DDS in line extension can prove highly important. This is exemplified by the success story of Cardizem™ (diltiazem) in the US angina market: Marion Merrell Dow used an internal cannibalization strategy to increase the market share of their once-daily formulation after the initial diltiazem formulation patent expiry. The lack of availability of delivery technology in-house may, however, be the stumbling block to product line extension. In that case, the

opportunities presented by codevelopment can prove attractive. Collaboration is likely to involve some kind of licensing agreement, a subject dealt with in some detail by Mr David Scott (Independent Consultant, Loughborough, UK). Once the potential DDS technology has been sourced, it is vital to pose questions regarding the type and scope of the desired agreement and 'ideal product profile' requirements to ensure mutual benefit. Above all, the importance of a good working relationship cannot be overstressed, a sentiment that was echoed by Dr Ralph Ecclestone (Scientific Generics, Cambridge, UK), as he reviewed the rationale and benefits of alliances and partnerships. Although not widespread throughout the industry, appropriate strategic partnerships have reduced product development time and costs, provided necessary innovation and achieved extended product life through intellectual property. Dr Peter Kolker (Intellectual Property Consultant, Crewe, UK) addressed this last issue in his presentation, concentrating on DDS covered by downstream or composition patents. Such patents may be held by a potential DDS partner. Alternatively, the ownership of a patent, for example in polymer synthesis, might prove attractive to a DDS company that uses such a class of material.

Design and development

Dr Peter Brand (GlaxoWellcome, Ware, UK) stressed the importance of considering the design and development of DDS as early as possible in the product life cycle. From an experienced engineering perspective, he described the challenges and issues involved in medical device and DDS development through to successful product manufacture and marketing. In a more light-hearted vein, Mr Tom Blackett (Interbrand, London, UK) provided a look at the use of DDS to increase product brand image. 'Brands belong in the mind', he reminded his audience; 'They are unique and timeless.' It is this uniqueness that can impart product differentiation through intellectual property rights, and that leads the big players to recognize the impact of their name, logo, pack design and 'personality' on the end-user.

The strategic issues outlined above underpin much of the product development that is currently in the pharmaceutical industry pipeline, and specific examples of this development in the areas of respiratory, transdermal, transmucosal and peptide delivery were presented on the second day.

Respiratory drug delivery

Tony Wilkinson (3M Healthcare, Loughborough, UK) presented a case study on the development of the Airomir™ inhaler (salbutamol), the first metered dose inhaler to be marketed (March 1995) containing a hydrofluoroalkane propellant. The product demonstrates equivalence, in the laboratory and more importantly in the clinic, to conventional salbutamol CFC-containing metered dose inhalers. The immediate future of the Airomir technology lies in the development of a beclomethasone-containing version. Using a solution instead of the conventional drug suspension, a finer particle size distribution of beclomethasone is achieved, with improved *in vitro* delivery characteristics. The ongoing clinical programme will assess whether the desired therapeutic effect can be achieved with a significantly reduced daily dose.

Transdermal delivery

Two contributions addressed the challenge facing the transdermal delivery of drugs. Dr Phillip Green (Becton Dickinson, Franklin Lakes, NJ, USA) presented an update on recent developments in delivery systems, particularly iontophoresis – 'the delivery of therapeutic compounds through the skin using low levels of electric current'. Iontophoretic systems are controllable and programmable, and are able to provide continuous or spiked drug plasma levels as desired. A particularly interesting development employed electro-osmosis or reverse iontophoresis to monitor serum glucose levels continuously by noninvasive transdermal extraction – a system elegantly packaged in a 'watch' format. We may even see the 'portable pancreas', a noninvasive system linking insulin delivery with continuous glucose monitoring. Dr Gordon Saul (Oxford Biosciences, Oxford, UK) introduced Powderject™ technology. This system uses compressed gas to propel powdered therapeutic compounds across the skin at supersonic velocity. Despite prior art appearing to be held by 'Star Trek', Dr Saul assured his audience that there was nothing fantastical about Powderject or Oraljject™ (the transmucosal version), and this assurance was supported by the *in vivo* studies he presented.

Transmucosal delivery

An update on recent advances in transmucosal delivery was provided by Prof. Bob Davis (DanBioSyst, Nottingham, UK). 'Challenging molecules' such as peptides, proteins and the products of biotechnology are the target compounds for delivery systems designed 'to make an improvement to the patient'. The most promising route emerging from Phase 2 studies is the intranasal route, where bioadhesive formulations are exploited to increase residence times in the nasal cavity. The challenges facing pulmonary delivery still lie in the delivery of the product to the lung itself and in providing adequate bioavailability comparable to that provided intranasally.

Gene and protein delivery

Dr Colin Pouton (University of Bath, UK) closed the proceedings with a concise presentation on gene and protein delivery systems. Protein delivery at the genetic level (gene therapy) is researched as part of the current developments in the treatment of chronic lung diseases such as cystic fibrosis. Transient gene therapy, employing plasmid DNA or adenoviral vectors perhaps linked with cationic lipids to enhance endothelial penetration, seems likely to emerge from development first. Gene therapy involving retroviral DNA may hold the greatest possible benefit to patients, by ensuring that genetic modifications are permanent, but the associated genetic risks are at present unknown.

This was a conference with plenty to ponder on for the marketing and research professional alike. It demonstrated the potential of drug delivery systems to provide strategic solutions for the pharmaceutical industry, increase commercial opportunities and, most importantly, provide improved healthcare for the patient.

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