Cellular Delivery of Therapeutic Macromolecules


Challenges to macromolecular drug delivery

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Abstract
The use of macromolecules, particularly monoclonal antibodies, as therapeutic agents has come to the forefront in recent years. The biodistribution and delivery issues for protein drugs are shared to a substantial degree with other emerging therapeutic approaches including pharmacologically active nucleic acids and nanoparticles. A generalized approach to these issues involves consideration of the multiple biological barriers that stand between the macromolecular drug or nanoparticle at its site of administration and its ultimate biological target. Considerations of size, stability, non-specific versus specific associations and potency versus toxicity all play a role. The creation of delivery approaches that combine high specificity for the target cell or tissue, high therapeutic payload and modest toxicity remains a challenge, although some very promising examples have emerged recently. A variety of sophisticated targeting strategies, based primarily on combinatorial library methods, when used in combination with new technologies to identify cell-surface receptor ‘signatures’ of specific tissues, will facilitate advances in targeted delivery of macromolecules and nanoparticles. The challenges to contemporary macromolecule drug delivery are complex, thus new research paradigms are emerging that combine the talents of physical and biological scientists to address this key issue for modern pharmacology and therapeutics.

Introduction
The era of macromolecular therapeutics is already upon us. Over the last decade, numerous non-traditional agents have entered the clinic or are in advanced clinical trials. This includes approx. 15 monoclonal antibodies and six liposomal drug formulations already in clinical use, as well as two FDA-approved oligonucleotides with more than 50 oligonucleotide-based drugs in clinical trials [1,2]. Despite this impressive accomplishment, many issues and challenges still remain for macromolecular-based therapies, particularly those involving intracellular targets. Many of the key issues are the traditional ones concerning the stability and biodistribution of macromolecular agents. Rapid degradation, clearance by the mononuclear phagocytes of the reticuloendothelial system, inability to penetrate into target tissues and failure to permeate cell membranes are old challenges [3] that are still with us. A newer issue is the increasing appreciation that even the most precisely designed macromolecular agent will have undesired or ‘off-target’ effects, a fact revealed by contemporary analytical tools such as DNA chips.

Biodistribution and targeting of macromolecular drugs
Fortunately, a great deal of progress has been made over the last decade in understanding and controlling the biodistribution of macromolecules. Two very important thrusts have been (i) techniques for the identification of unique cellular targets, and (ii) novel methods for delivering macromolecules to cells. Combinatorial library approaches, notably phage display, have been extremely important in the detection of cell-surface markers that are unique (or at least highly enriched) in specific tissues. An important variant

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of this approach has been ‘in vivo phage display’ [4]; this has allowed the identification of endothelial-specific markers in several tissues. Coupled with the identification of new markers, there have been vast improvements in our ability to generate reagents that can selectively target these markers. Once again, combinatorial library technologies have been a key part of this thrust [5,6]. Another vital element has been the evolution of better understanding of the pathways by which cells take up macromolecules and viruses [7–9]. This has led to efforts to create peptides and proteins that mimic the ability of viruses and some plant and bacterial toxins to breach membrane barriers and enter the cytosol. Thus a number of investigators have explored the use of the so-called ‘cell-penetrating peptides’ (CPPs) for the intracellular delivery of proteins, oligonucleotides and other macromolecules [10,11]. CPPs seemingly bind to cell surfaces, enter endosomes and then escape from the endosomes by mechanisms that remain poorly defined. In doing so, they can also carry ‘cargo’ molecules including other peptides, some proteins and oligonucleotides, thus allowing these macromolecules access to the cytosol. Further refinements of existing CPP types and the anticipated discovery of novel classes of CPPs may play an important role in the delivery of therapeutic macromolecules that need to act within cells [such as antisense and siRNA (small interfering RNA) for example].

The role of nanotechnology
Advances in the delivery of macromolecular drugs will no doubt be heavily influenced by the development of modern nanotechnology [12,13]. In particular, ‘smart’ nanoparticles having the ability to target specific cells or tissues will probably emerge as important drug carriers. Traditional methods of preparing nanoparticles have involved ‘bottom-up’ or self-assembly approaches that rely on emulsification or controlled aggregation; this can lead to substantial heterodispersity of particle characteristics. However, new ‘top-down’ nanotechnology approaches are being developed that will allow the fabrication of smart nanoparticles with the precise control of size and shape that is associated with computer chip manufacturing [14]. This and other nanotechnology advances, including sophisticated biosensors and imaging approaches, will facilitate the intelligent use of therapeutic macromolecules.

Selectivity of macromolecular drugs
A particularly important issue concerns the specificity of macromolecular drugs since even the most precisely designed moiety is likely to have off-target effects. To illustrate this, I will discuss some work that we have done evaluating the specificity of antisense oligonucleotides using DNA arrays. We are interested in the pharmacology of oligonucleotides coupled to CPPs [15]. In one study, we utilized both ‘free’ and peptide-coupled antisense oligos that were targeted to MDR1, a gene involved in cancer cell drug resistance [16]. We used DNA arrays to evaluate the effect of these agents on mRNA levels both for the target (MDR1) and for other genes. Interestingly, in addition to MDR1, approx. 2% of the genes tested showed significant changes in response to the supposedly MDR1-specific antisense. Both down-regulation and up-regulation were observed, and, in many cases, the mRNAs affected had little or no sequence homology with MDR1. Thus antisense oligonucleotides, although being somewhat specific, can give rise to multiple off-target effects. The same considerations prevail for siRNAs. Initially it was claimed that siRNAs were highly specific and could even discriminate sequences differing by a single base. However, the preponderance of recent studies indicates that siRNAs can affect many off-target genes in addition to that targeted, with multiple mechanisms contributing to the off-target effects [17,18]. This type of intensive gene expression analysis has been used primarily with nucleic-acid-based therapeutics and has been applied to only a limited degree to other types of macromolecular or nanoparticulate drugs, which is possibly a serious omission in the characterization of these agents. In addition to measuring effects on mRNA and protein levels, it will also be important to use functional approaches to examine potential off-target effects of macromolecular drugs. An interesting approach of this type is the multiplexed analysis of signalling cascades using phospho-specific antibodies and flow cytometry [19].

Summary
Great strides are being made in the evolution of macromolecular therapeutics. In the future, we can expect further advances based on several intersecting approaches. First, sophisticated biology including use of combinatorial library approaches to discover and target cell-type-specific markers will make major contributions to the selectivity of macromolecular drugs. Secondly, advances in nanotechnology will provide new carrier systems, as well as new diagnostic approaches to allow informed use of macromolecular therapeutic agents. Finally, increasingly sophisticated analysis of specificity versus off-target effects will aid in the design of better macromolecular drugs and their more intelligent use in therapy.

References

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