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Formulation of therapeutic synthetic polymers for drug and gene delivery ▼

In their paper in a recent issue of *Drug Discovery Today* on the use of synthetic polymers in therapeutic applications [1], Hunter and Moghimi use the metaphor of playing Russian roulette. They clearly believe that certain synthetic polymers have the potential for significant toxicity in medical applications, and I think they are correct to bring attention to these possible toxic effects.

Biomedical polymers include DNA/cationic polyplexes for non-viral gene transfer, nanoparticles or liposomes for drug delivery, perfluorocarbon emulsions for oxygen delivery and blood substitutes, and many others. The potential toxicity of the pharmaceutical polymers has not received much attention, mostly because the medical applications of these molecules are still the subject of intensive investigation. However, in several cases, there are already indications that certain polymers are toxic if the appropriate formulation is not used; for example, DNA/cationic polyplexes and nanoparticles.

Non-viral gene transfer with cationic polyplexes

Gene transfer with viral vectors is highly problematic as the viruses either invoke toxic inflammatory reactions (e.g. adenovirus, herpes virus) or permanently and randomly alter the host genome (e.g. adeno-associated virus, retrovirus). Thus, it is clear that non-viral gene transfer methods must be developed. The principal non-viral gene transfer method uses complexes of plasmid DNA and cationic polymers. The DNA/cationic polyplex is made in low salt solutions, because the complexes form micron-sized aggregates in either physiological saline or in blood. These polymers are toxic both in cell culture [2] and in animals *in vivo* [3,4] at concentrations that are less than an order of magnitude higher than the concentrations yielding therapeutic effects. This therapeutic index is unacceptable for applications in humans, and alternative formulations for non-viral gene transfer need to be developed.

Nanoparticles

Several synthetic polymers can be formulated into nanoparticles of ~100–300 nm in diameter for drug delivery. The nanoparticles aggregate in the absence of surfactants [5], so detergents such as cholic acid or polysorbate-80 (Tween-80) must be added to stabilize the nanoparticle formulation. However, such detergents can be toxic *in vivo*. Tween-80 causes disruption of the blood–brain barrier at systemic doses as low as 3 mg/kg [6], and it has been suggested that nanoparticles might mediate drug delivery to the brain *in vivo* just by disrupting the blood–brain barrier [7].

The potential toxicological effects of pharmaceutical polymers must be considered from the earliest stages of the development process that yields the final molecular formulation selected for *in vivo* applications. Gene therapy could benefit by the development of non-viral gene transfer methods, but this will require the proper formulation of pharmaceutical polymers that are both effective *in vivo* and non-toxic. Targeted delivery of drugs could ultimately use nanoparticle technology, but these formulations must be free of detergents or surfactants that are toxic *in vivo*. Russian prisoners of the nineteenth century might have been coerced into risking their lives at odds of 1 in 6. However, twenty-first century drugs and gene therapies must offer much better chances for longevity.

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Genetic approach to chemical genetics ▼

Many drugs on the market today were initially identified because they demonstrated activity in patients, animal models or cellular assays that were believed to be physiologically relevant. The advent of molecular biology allowed more detailed dissection of biological processes into their component biochemical processes. Thus, clear links between the physiological effects of compounds and their molecular mechanisms are relatively recent in the history of the pharmaceutical industry. Some of the clearest associations between genes and disease processes come from analyses of naturally arising mutations in the human population. Only in very few cases, however, is there a tight link between a single gene and a particular human disease that identifies a clear target for pharmaceutical intervention.

There are a number of molecular biology approaches that can help to delineate the functions of individual genes at the gene, transcript or protein level. These include genetic tools such as transgenic animals where only one (or a group) of genes are altered. Current techniques enable the expression of the gene to be restricted to specific tissues, and there is some degree of regulation possible by exogenous intervention, but in the vast majority of transgenic animals available today, this type of regulation was not engineered in. Biochemical approaches such as RNA interference (RNAi) can be use to reduce specific protein levels by interfering with the transcript, but it is technically difficult to apply these approaches to a broad range of cells or in whole animals. Alternatively, dominant–negative mutants, either virally encoded or stably transfected, can be used to probe gene function by reducing protein activity.

In their recent paper, Skokat and Velleca [1] describe a hybrid of these approaches where they introduce a mutant copy of a gene of interest that possesses wild-type activity but is engineered to bind to specific unique ligands. Replacement of the wild-type copy of this gene by homologous recombination with this engineered version results in mice where the activities of the encoded mutant proteins can be examined specifically by dosing with exogenous low molecular weight ligands. Because the engineered proteins are wild type in every other respect, these recombinant mice provide excellent models of what might be expected if unique selective inhibitors were developed as therapeutic agents. Not only can the overall physiological response to inhibition be measured, but more detailed analyses can also be performed; for example, assessing changes in transcriptional and/or proteomic profiles or direct quantitation of downstream products.

As with any method designed to be a surrogate for the effects of a novel drug, there are drawbacks. Creating the mutants, although no longer scientifically challenging, is technically demanding. In addition, the high degree of selectivity that can be achieved with compounds interacting with mutants is in many cases extremely difficult if not impossible to achieve using drug-like molecules designed to interact with the endogenous target. Despite these limitations, Shokat and Velleca have demonstrated the potential power that can result when genetics and chemistry are ingeniously combined.

Reference

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Mining the human ‘kinome’

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Whenever there is a major advance in science, new tools and paradigms change and accelerate the pace of discovery. The sequencing of the human genome has had a major effect on the way we pursue the discovery and development of new drugs.

The now commonly used ‘omics’ terminology has come to refer to the paradigm shift that genomics has had on the way we see biology. At the *2nd International IBC Conference on Protein Kinases* held in Boston (MA, USA; 9–10 September 2002), use of the terms ‘kinomics’ and ‘phosphonomics’ reflect the impact that

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