Therapeutic synthetic polymers: a game of Russian roulette?

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Synthetic polymer-based drug-delivery systems have been applied in drug delivery for the past 50 years. So why are there so few examples of these macromolecules being used successfully in the clinic? It is our view that many products are failing because of a neglect of the fundamental science surrounding the architectural control of the molecules present, their behaviour following *in vivo* administration and host response. Adverse events following parenteral administration of approved synthetic polymer-based systems have resulted in unpredictable and fatal responses in a significant number of individuals. Acceptance of the importance of immunotoxicological factors in response to the presence of these macromolecules must be addressed if emergent technologies, such as polymer-based gene-delivery systems, are going to succeed.

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▼ Synthetic polymers are a highly versatile and diverse group of macromolecules, many of which have been applied specifically in the arena of drug delivery, for example, solubilizing agents, nanoparticulate formation, surface modification, macromolecular drug carriers, diagnostic imaging agents and implants [1,2]. In addition, the majority of these polymers show a multitude of biological activities in their own right (e.g. antitumour, antibiotic, antiviral and antithrombotic activities, as well as inhibition of efflux pumps such as P-glycoprotein) [3–5]. At present, few of these polymers are licensed for use clinically, and those such as poly(ethylene glycol) (PEG), poly(lactide-co-glycolide) and poloxamer-188, which have FDA (http://www.fda/gov/) approval, have been problematic *in vivo* [3]. Although there is an increasing desire to use these materials in therapeutics, there are neglected areas of research that include biological and pharmacological activity, immunotoxicity and cytotoxicity. We will discuss the reasons why the full clinical potential of these materials in parenteral drug delivery has not been achieved *in vivo*, highlighting the potential areas, including cancer research and gene therapy, in which there is a significant knowledge gap, and indicating potential drawbacks and dangers in some strategies that are currently being used to exploit these molecules.

The polymer: characterization and degradation

The absolute chemical characterization of small drug molecules is straightforward; however, in the case of polymers, this is not possible with current analytical and separation techniques. At present, all polymers that are approved for clinical uses are polydisperse systems; that is, they exist as a molecular weight range. Breslow [5] demonstrated distinct and diverse biological activity, which is linked to the specific molecular weight of a polymer. The present inability to resolve these therapeutic systems into a monodisperse population represents a technological barrier with respect to the understanding of SARs, and also compromizes the understanding of precise biological interactions that are observed *in vivo*. A further complication is the presence of low molecular weight contaminants, either generated during synthesis or added during or post-production, which are difficult to separate from the polymer. These include antioxidants, UV stabilizers and products of thermal degradation, all of which have been suggested to exert undesirable immunotoxicity [2]. It is perhaps not surprising that a range of biological activities are observed with synthetic polymers when compared to endogenous biopolymers (e.g. peptides, proteins and polysaccharides) that are synthesized *in vivo* as monodisperse, with an absolutely defined structure affording precise biological function and fate. There is therefore a necessity to produce a wide range of monodisperse polymers,

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which can be used as molecular probes to map activity in both healthy and diseased individuals. Some of the above issues might be solved with the current synthetic technology being applied to tailor-made polymer surfactants [6]. Significantly, advances in controlled and/or living radical polymerization chemistry, such as atom transfer radical polymerization (ATRP), in protic solvents facilitate fast rates of polymerization at 20°C [7] and can achieve highly controlled architecture affording low polydispersity [8]

A plethora [9] of synthetic biodegradable polymers are currently being applied to both drug delivery and biomaterials research. Here, the carrier will degrade (e.g. enzymatically) or erode, thus releasing the drug payload. It is believed that the exposed polymer is degraded to a molecule, which is found endogenously with no undesirable side effects. Unfortunately, rates of degradation and erosion can not, at present, be fully controlled *in vivo* and depend on the environment encountered, which is dynamic. Hence, there is potential for incomplete polymer degradation, which could result in the release of varying molecular weight polymer chains with potential biological and/or immunological responses. This scenario might be supported by the pharmaceutical product Zoladex® (AstraZeneca; http://www.astrazeneca.com), which is formulated for the release of Goserelin from a blend of high and low molecular weight lactide-glycolide biodegradable polymers. Occurrences of hypersensitivity reactions, including anaphylaxis, have been recorded using this depot injection [10]. This could be a warning that the administration of biodegradable polymers by other routes, such as intravenous, could result in significant immunotoxicity. Therefore, differential rates of host recognition could result depending on the disease state, route of drug administration and the microenvironment encountered.

Importance of immunotoxicology

Immuntoxicology can be defined as an assessment of adverse events comprizing immunosuppresion, immunogenicity, adverse immunostimulation, autoimmunity and hypersensitivity. Fifty years ago saw the initial application of polymers and polymer drug-conjugates in therapeutic medicine [3]. Toxicity issues were recognized as a major impediment to the use of these materials. In the following years, the factors underlying the importance of immunotoxicology in relation to therapeutic polymers have been given only a cursory examination. For further advancement of this field, immunologically driven adverse events must be investigated and treated with equal importance to other clinically important adverse drug reactions. For example, complement-activation related pseudoallergy is responsible for 420,000 clinically significant allergic reactions and 20,400 fatalities through anaphylactic shock following drug administration in the USA each year [11]. Such reactions have been observed with advanced drug-delivery systems, such as Doxil® (ALZA Corporation; http://www.alza.com), a PEG-grafted liposomal carrier for doxorubicin, despite the currently held view that PEG is believed to be immunologically inert [12]. Other examples of immunologically problematic synthetic polymers include the polyethoxylated ether cremophor EL – the solubilizing agent for both paclitaxel and cyclosporin – and a range of poloxamers [2,11], including poloxamer 188, which is used to stabilize Fluosol DA. This product has now been withdrawn from the market because of severe anaphylactic shock mediated through complement-activation. Nonetheless, a purified form of poloxamer 188, CRL-5861, is now being developed as the vehicle for paclitaxel [13]. Research from our laboratory has demonstrated clear interindividual and unpredictable variation in complement activation in the sera of healthy individuals following challenge with clinical samples of poloxamer 188 (Hunter, A.C. *et al.* unpublished results). The circumvention and mechanistic understanding of this highly sensitive, tactically advanced, evolutionary refined immunological barrier is not a trivial task, but is fundamental to the future use of a diverse spectrum of synthetic polymers in drug delivery. Conversely, the immunological responses with polymers have been highly beneficial in the design of immunomodulators [14], as well as adjuvants (e.g. polymeric colloids) for vaccine engineering [15].

Cationic vectors in gene delivery

Although modified viruses are known to efficiently smuggle genetic material into the cell nucleus, they often induce unusual or deadly immune responses [16]. As a result, attention has been focussed on the design of synthetic cationic vectors for DNA condensation and delivery. These strategies also minimize DNA degradation following internalization, for example by destabilizing the endosomal membrane. However, the ultimate fate of these polymers or their potential degradation products has been ignored. It could be speculated that the cytoplasmic presence of positively charged polymers could interfere with the physiological function of cellular constituents. These could include cytoplasmic enzymes, molecular chaperones, proteasomes, immunoproteasomes and mitochondria, and hence could explain the chronic outcome of gene therapy and DNA vaccination strategies with synthetic vectors. For instance, the interaction with the mitochondrial membrane could lead to membrane depolarization and early release of proapoptotic factors (e.g. caspase activating factors). Could this be a possible explanation for the transient

expression of gene products from such experimental strategies? In support of this statement, it has recently been demonstrated that the cationic macromolecule poly(ethyleneimine) in both free and complexed forms, when used to transfect endothelial cells, has resulted in cell death [17]. Despite these problems, the bulk of the research is being concentrated on the design and engineering of the polymeric vectors, whereas the fundamental basis of their potential toxicity is, in general, being ignored.

P-glycoprotein

An interesting and effective approach to increasing the concentration of antineoplastic agents in tumours has been through the use of polymers that inhibit members of the ATP-binding cassette (ABC) family of transporters; these include P-glycoprotein (Pgp) and the multidrug resistance protein (MDRP) family [18]. However, non-specific inhibition of these energy dependent pumps could result in significant side effects. Several sites, which contain Pgp, can be readily accessed from the circulatory system. For example, Pgp efflux pumps are present in polarized endothelial cells that form the exterior of the blood–brain barrier. Their action is generally perceived as unidirectional and functionally is responsible for the removal of by-products of CNS metabolism and the inhibition of drug and molecular transport into the brain. The only endogenous compounds actively transported by Pgp into the brain are the steroids aldosterone, corticosterone and cortisol [19]. Evidence has come to light that Pgp might have a modulatory role in transporting active opioids, including β-endorphin, to the periphery [20]. Long-term blockade of such functions by synthetic polymers might inhibit not only opioid neurotransmitters but also a range of as yet undiscovered modulators and homeostatic mediators. In terms of drug delivery into the brain, Pgp inhibition appears appealing; however, this approach again neglects the fact the Pgp is expressed in the brain parenchyma, such as microglia and astrocytes [18]. Therefore, it is perhaps not surprising to see that the survival rate of patients with astrocytomas and glioblastomas is extremely low. Fundamentally, this is a result of a high degree of MDRP expression resulting in the therapeutic failure of anticancer agents. From a therapeutic point of view, it seems fundamental to unravel the physiological function of the efflux pumps first.

Lack of appropriate model systems

Extreme caution must be employed with the choice of model system in which to assess polymer activity. *In vitro* cell-based systems for assessing polymer toxicity are of arguable value. Such systems are frequently used to assess toxicity by cell death. Although this might be of value for spotting extreme toxicity, the frequent conclusion given in the absence of cell death is that the material is 'biocompatible'. This is an erroneous conclusion because none of these systems contain a functional immune system and will express cell- and environment-specific receptors on their surface, which are not translatable to the human.

Care must also be taken when translating results observed in animal models because there have been distinct intra and inter-species variation. For example, early studies with poly(ethylene sulfonate) [4] clearly demonstrated a broad spectrum of antitumour activity in mice; however, the tumericidal activity of this polymer was significantly reduced in humans. Synergism of the foot and mouth disease vaccine was observed in mice following concomitant administration with pyran copolymer [5] but, unfortunately, this activity was not carried over to cattle and pigs. The future should incorporate the knowledge gained from the Human Genome Project, and specifically immunogenomics, to identify new methods of assessing immunotoxicity and understanding the basis of interindividual variation and responses. Examples could include the development of predictive gene-driven toxicogenomic databases [21] and appropriate humanized transgenic animal models [22].

Conclusion

There is a clear and urgent need for a paradigm shift in the thinking needed to the current approaches being applied to synthetic polymers in parenteral medicine. This will be required to resolve the range of host responses to these materials to optimize their therapeutic potential. Future studies must include the investigation of immunotoxicology, membrane biochemistry, extracellular and intracellular fate, and signal transduction responses to therapeutically active polymers. Coupled with this is a strong requirement for the development of polymer synthetic strategies and separation technology to facilitate scientific investigation with a fully characterized starting material. This, in turn, can be applied to pathophysiology of the disease state, the harnessing of which will enable smart drug delivery as opposed to the 'hit and hope' approach, which appears to be endemic at present. The immunotoxicological issues presented here are not currently identified in 'important areas of research', and have not been highlighted as a priority for funding in UK experimental drug-delivery research programmes [23].

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