Expert Opinion

Delivery of biologics to select organelles – the role of biologically active polymers

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Biologics (i.e., nucleic acid and protein-based drugs) suffer from poor bioavailability, as membrane partitioning and intracellular targeting are a significant problem. Various strategies have been developed in an attempt to modulate biologics bioavailability by means of manipulating whole body pharmacokinetics and subcellular trafficking. Limited direct success has been observed. This review focuses on the components of nanomedicine systems rather than the whole, facilitating an overview of materials that may be of clinical relevance in the future. Some of the advantages and disadvantages associated with the use of soluble drug delivery systems are considered. Although the focus is on linear poly(amidoamine) polymers, emerging technologies capable of the delivery of large molecules to other specific intracellular compartments are also examined. The focus is maintained on cytosolic access for two reasons, initially because this intracellular compartment may be viewed as a 'gateway' to other intracellular organelles and also because this is where the greatest therapeutic benefit is likely to be found. It is likely that in the coming years and in combination with other existing, well-characterized drug delivery platform technologies, such as liposomal formulation or polymer conjugation, that the targeting of specific organelles will become more accessible.

Keywords: endocytosis, endomembrane, poly(amidoamine), toxin, trafficking

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1. Introduction

With only a few notable visceral (i.e., organs) and calciferous (i.e., bone) exceptions, humans are composed of sheets of cells folded into tubes [1]. This observation presents drug delivery scientists with a problem, in that these sheets and tubes form barriers composed of membrane. At both the gross and molecular levels these membranes have evolved to exclude, sort and compartmentalize material [2]. How are these barriers to be overcome in order to get our 'payload-*du-jour*' to its target? Here, access to various intracellular compartments is considered within the context of drug delivery.

1.1 Lysosomes

Fortunately, some targets are easier to assimilate than others. The late endocytic compartment consists of late endosomes, lysosomes and the hybrid late endosome/lysosome organelle (where digestion occurs) [3]. This compartment is, relative to the nucleus, easily accessible owing to the topological equivalence of the endosomal lumen with the outside of the cell. From the perspective of replacing missing, broken or lost lysosomal enzymes, this is a well-exploited phenomenon, as seen by the clinical success of products such as Ceredase®, Cerezyme®, Aldurazyme®, Fabrazyme® and Myozyme®, developed by Genzyme Corp, Cambridge, MA, USA [4]. These products are not nanomedicines but recombinant proteins;

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Article highlights.

- Non-viral gene delivery, although not as potent as viral gene delivery, is thought to be safer.
- Polycations have received a lot of attention as non-viral gene delivery systems.
- Since the 1950s, polycations have been known to be toxic.
- Alternative, non-viral technology platforms (such as detoxified protein toxins and poly(amidoamine)s) with the potential to mediate the translocation of material to various intracellular compartments are emerging and are discussed.

This box summarizes key points contained in the article.

however, they do highlight successful access to the late endocytic compartment. Further, these enzymes are able to take advantage of endogenous receptor trafficking pathways such as those used by the mannose 6-phosphate receptor, which can cycle between the Golgi and late endocytic compartment via the plasma membrane [5].

Beyond the obvious functions of the endocytic system, that is, attenuating activated signaling molecules such as the epidermal growth factor receptor [6], degrading protein toxins [7], bone readsorption/remodeling [8] and providing nutrients for the cell [9], there is another facet associated with the late endocytic system that should be considered. This is the compartmentalization of lysosomal content, keeping catabolic enzymes [10] away from vital cellular components. Indeed, the effect of compromising cell membranes and the cells's acute responses to these insults are well documented [11]. Of note is the work by Tam *et al.*, who has reported the profound reorganization of various membrane markers (i.e., lysosome-associated membrane protein (LAMP)-1) in response to mechanical damaging of the plasma membrane [12].

1.2 Access to the cytosolic compartment by means of the endocytic system

Some of the most widely studied non-viral delivery systems designed to deliver biologics to either the cytosol or the nucleus, contain cationic polymers such as poly(L-lysine) (PLL) or poly(ethylenimine) (PEI) [13].

Polycations can destroy membranes when exposed to either the exofacial leaflet on the outside of the cell (i.e., before internalization) [14-16] or the (previously) exofacial leaflet, forming the lumen of a vesicle (i.e., from the inside of a vesicle) [14,17,18] after the internalization of the polymer. Indeed, the propensity of polycations such as poly(L-lysine) to destabilize membrane has been known and documented since the 1950s [19,20]. The former observation is interesting in relation to the 'proton sponge hypothesis' [21], especially given the permeability of membranes to both ions (cf., nerve cells) and water. This is exemplified by the cellular requirement of transporters (and channels) for ions [22] and aquaporins for water [23]. Further, a recent commentary documents the likely contribution of osmotic pressure to vesicle rupture [24] and concludes that it may not be sufficient to facilitate the release of material into the cytosol. However, there is a further problem associated with the use of polycations as drug delivery systems. The pharmacokinetic properties of polycations are, at best, limited. Like most polycations, PLL, PEI and even chitosan when injected into rats intravenously are cleared from the systemic circulation rapidly, accumulating in the liver and lungs [15,16,25]. This may provide an opportunity for very efficient hepatic targeting if the problem of toxicity is overcome.

What about the possibility of detoxifying or shielding these molecules by generating AB di-block systems (i.e., alternating discrete blocks of two types of polymer grafted onto one another), utilizing poly(ethylene glycol) (PEG) as one of the blocks? Here, the 'PEG dilemma' is encountered [26]. When PEG-PEI AB di-block systems were evaluated it was documented that toxicity was indeed reduced. However, the dilemma is such that with this reduction in toxicity comes also a reduction in transfection efficiency. Will it be possible to titrate these two facets, giving rise to the relatively non-toxic but still efficient transfection vehicle?

Recently, a study focusing on a well-characterized series of amphiphilic polymers was published, which examined the subcellular trafficking of linear poly(amidoamine)s (PAAs) *in vivo* [14]. This paper quantified the effect of time and dose on polymer subcellular localization as well as the cytosolic release of lysosomal hydrolases *in vivo*. Of note was a nonlinear, plateau effect relative to the cytosolic localization of both the polymer and the lysosomal enzyme, both in response to increased time and increased dose.

This may indicate that the documented membrane breach may be, in some instances, self-limiting, as evidenced by the dramatically reduced toxicity of the PAAs relative to other cationic polymers. Vesicular acidification, mediated by the V-ATPase, is required for both homotypic and heterotypic endocytic fusion [27]. Here macromolecular material (a synthetic polymer or a lysosomal protein) was documented moving from the lumen of a membrane-delimited compartment into the cytosol. This effect may be linked to the propensity of the synthetic polymer to protonate in response to a drop in pH, resulting in membrane rupture [15,28].

Consequently it is reasonable to assume, though this has not been measured directly yet, that during the transit of material into the cytosol there will be a concomitant loss of protons from the lumen of the vesicle. This drop in luminal pH would lead to the deprotonation of the polymer and an inhibition of further endocytic fusion events until the vesicle had reacidified. This conjecture marries well with another published observation, that the PAAs documented are very well tolerated both *in vitro* and *in vivo*, having been administered to rats intravenously at concentrations up to 100 mg/kg without any acute toxicity being observed [14]. Further, by manipulating the backbone chemistry of these molecules, they can be used to access pharmacokinetic

compartments other than the liver [15]. PAAs can even be selectively directed to solid tumor mass, presumably by means of the enhanced permeability and retention (EPR) effect [15]. The EPR effect is a phenomenon that results in the passive accumulation of macromolecules within solid tumor mass and is driven by 'leaky' neovascularization of the tumor mass in conjunction with limited tumor lymphatic drainage [29].

1.3 Mitochondria and the nucleus

The nucleus and mitochondria both contain DNA and the machinery necessary to express genes (i.e., RNA polymerases and associated proteins), making them attractive targets for therapeutic genes. Current knowledge suggests that the easiest way to access either of these compartments is by means of the cytosol. As material is moved from the cytosol to the nucleus during the everyday maintenance of the cell, exploiting import sequences is relatively trivial. An example of a nuclear import signal is employed by simian virus 40 (SV40). The SV40 nuclear localization signal (NLS) (amino acid sequence PKKKRKV) is utilized by SV40 large T antigen to access the nucleus via the nuclear pore complex [30]. The signaling mechanisms used to move material > 5 kDa into the mitochondria are more complex and less well defined than those necessary for nuclear import [31].

1.4 The endoplasmic reticulum, Golgi, MHC Class I and the Sec61p translocon

Ribosome-inactivating proteins (RIPs) may be described as nano-scaled *Trojan horses*, subverting the cell's membrane trafficking machinery to their own ends [32,33]. These entities have been used in a therapeutic context for well over 20 years, though their lethal domains have attracted more attention than the intricate architecture that allows these lethal domains to reach their cytosolic targets [34]. Ricin is an excellent example of such a RIP. It is of significant note that many RIPs also possess the necessary architecture to deliver a macromolecule to the lumen of the Golgi body, the endoplasmic reticulum (ER) and the cytosol of a cell [32]. Transit through the ER and Golgi has been identified directly by means of experiments requiring the organelle-specific sulfonation and glycosylation [35] of these (RIP) molecules, and although a relatively small proportion of a molecule such as ricin toxin evades lysosomal trafficking (an estimated 5 – 10%) [7], it is possible that the amount of 'modified' toxin reaching the Golgi, ER and cytosol is still enough to deliver a pharmacologically relevant dose of a biologic, whether this is an antigen to the MHC class I compartment [36] or an RNAi or antisense agent to the cytosol. This is particularly interesting from the perspective of drug delivery as any problems of toxicity associated with breaching the limiting membrane of late endosomes, are effectively circumvented [11]. Consequently, by separating the toxic or 'lethal' domains from the architecture that has evolved to move the lethal domain into the cytosol, it may be possible to use these molecules not only as potent toxins but also as 'cytosolic shuttles' for gene drugs such as antisense or RNAi agents [33].

2. Expert opinion

As has been discussed, a variety of technologies exists that can facilitate the organelle-specific targeting of material. It is likely that in the coming years and in combination with other existing, well-characterized drug delivery platform technologies, such as liposomal formulation or polymer conjugation, that the targeting of specific organelles will become more accessible.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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