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ScienceDirect
Advanced Drug Delivery Reviews 58 (2006) 1523–1531
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Molecular hurdles in polyfectin design and mechanistic background to polycation induced cytotoxicity ☆

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Received 9 August 2006; accepted 13 September 2006
Available online 28 September 2006

Abstract

Synthetic polymer based Polyfectins (cationic polymer-DNA complex) have received intensive scientific research as they can potentially circumvent problems associated with viral vectors for gene therapy. These cationic macromolecules can readily condense DNA or RNA into stable nanostructures for use in gene delivery. Recently two commonly used polycations, poly (ethylenimine) (PEI) and poly(L-lysine) have demonstrated their ability to induce apoptosis in a range of human cell lines. This may be the explanation for short-term gene transfection observed with polyfectins. It is the aim of this review to discuss these and other factors behind observed toxicities including the inherent polydisperse nature of polymeric macromolecules and their behaviour in vivo. Strategies for reduction of toxicity are included such as new polymeric synthetic technologies and vector pegylation. There is a clear and immediate need for understanding of the mechanisms which cause polyfectin toxicity which will ultimately facilitate improved vector design and safer gene delivery.

Keywords: Polyfectin; Polymer toxicity; Gene therapy; Apoptosis; MTT assay; Poly(ethylenimine); Poly(L-lysine); Biodegradable polymer; Gene transfection

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Particulate Nanomedicines", Vol. 58/14, 2006.

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doi:10.1016/j.addr.2006.09.008

1. Introduction

Successful gene therapy with nano-delivery vehicles that are free of toxicity, have defined biological interactions and result in efficient gene transfer is one of the great scientific challenges in modern medicine. This multidisciplinary field has experienced intense research fuelled by the prospect of a revolutionary therapy for incurable diseases. Early clinical trails using recombinant viral vectors indicated significant problems primarily as they demonstrated short-lived transgene expression, an inability to persist in host cells and toxicity [1]. In 1999 an extreme adverse interaction occurred resulting in patient death following direct administration of an adenovirus vector into the hepatic artery. Analysis of inflammatory cytokine profile indicated that the vector had caused systemic inflammatory response syndrome [2] symptoms of which include intravascular coagulation, acute respiratory distress and multiorgan failure [3,4]. Subsequent primate studies have indicated that the adenoviral capsid proteins induced the inflammatory cytokine cascade and not the genetic package [5]. In contrast the first and most successful treatment of a human disorder was reported in 2000 where children suffering the fatal form of X-linked severe combined immunodeficiency disease (SCID-X1 syndrome) were able to develop functional immune systems following stem cell gene therapy [6]. Unfortunately two of the subjects developed a leukaemia-type disorder due to insertional mutagenesis [7–9]. Further studies revealed a significant risk of cancer and human trails were halted in 2003 [10] in spite of excellent clinical outcomes in the majority of those treated [11] with this retroviral stem cell therapy. This and other work have highlighted the need for fundamental research to improve viral vector design if site specific targeting enabling safe, controlled transgene insertion to avoid adverse activation of cellular genes [12] is to be achieved. Furthermore issues regarding the effects of patient age [13], availability of suitable animal models [12,14] and the generation of new technology and comparison of outcome databases for gene insertion profiles [15] will be essential to the clinical success of all types of gene therapy in the future.

In response to the observed problems with viral vectors a wide range of nonviral vectors has been developed such as cationic lipids (lipofectins) and polycations (polyfectins). Examples of these include linear, branched and dendritic vectors based on poly(ethylenimine) (PEI), poly(L-lysine) (PLL) and a range of poly(ethylene glycol) and ligand tagged constructs [16–21].

Cationic macromolecules readily condense genetic material into nanoconstructs for transfection and some, notably PEI attain excellent transfection efficiencies [16–18]. PEI is capable of condensing DNA and RNA into stable toroidal and globular nanostructures primarily via electrostatic interactions as supported by a range of biophysical studies [22–25] as have mechanisms of polyfectin cellular uptake [24] and internalisation [26–29].

Both PEI and PLL have shown cytotoxicity in a range of cell lines (18, 20, 30, 31). The aim of this review is to examine the underlying modes and mechanisms of toxicity inherent to synthetic cationic polymer based polyfectins, the understanding of which will help in the design of safer and more efficient nonviral vectors for gene therapy. Toxicity issues and their partial resolution related to lipofectins including (cellular toxicity, inflammatory toxicity, immunomodulation and pharmacokinetics) have already been covered in detail [32–41] however aspects of this review may be pertinent to the study of toxicity associated with cationic lipid vectors for delivery of genetic material.

2. Nature of synthetic polymeric materials and their biological interactions

The unique biological behaviour of individual polymers is diverse (e.g. antitumour, antibiotic, anticoagulant, *P*-glycoprotein inhibition) [42–45] and is difficult to predict. The situation is further complicated due to the outcome of synthesis of macromolecules, in that they are not monodisperse (i.e. a single molecular weight). Their polydisperse nature may therefore result