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GENE DELIVERY

Preferential liver gene expression with polypropylenimine dendrimers

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Abstract

Previously, the lower generation (DAB 8–generation 2 and DAB 16–generation 3) polypropylenimine dendrimers have been shown to be effective gene delivery systems in vitro. In the current work, we sought to: (a) test the effect of the strength of the carrier, DNA electrostatic interaction on gene transfer and (b) to study the in vivo gene transfer activity of these low molecular weight (<1687 Da) non-amphiphilic plain and quaternary ammonium gene carriers. Towards this aim, methyl quaternary ammonium derivatives of DAB 4 (generation 1), DAB 8, DAB 16 and DAB 32 (generation 4) were synthesised to give Q4, Q8, Q16 and Q32, respectively. Quaternisation of DAB 8 proved to be critical in improving DNA binding, as evidenced by data from the ethidium bromide exclusion assay and dendrimer–DNA colloidal stability data. This improved colloidal stability had a major effect on vector tolerability, as Q8–DNA formulations were well tolerated on intravenous injection while a similar DAB 8–DNA dose was lethally toxic by the same route. Quaternisation also improved the in vitro cell biocompatibility of DAB 16–DNA and DAB 32–DNA dendrimer complexes by about 4-fold but not that of the lower generation DAB 4–DNA and DAB 8–DNA formulations.

In contrast to previous reports with non-viral gene delivery systems, the intravenous administration of DAB 16–DNA and Q8–DNA formulations resulted in liver targeted gene expression as opposed to the lung targeted gene expression obtained with the control polymer-Exgen 500 [linear poly(ethylenimine)] and a lung avoidance hypothesis is postulated. We conclude that the polypropylenimine dendrimers are promising gene delivery systems which may be used to target the liver and avoid the lung and also that molecular modifications conferring colloidal stability on gene delivery formulations have a profound effect on their tolerability on intravenous administration.

Keywords: Polypropylenimine dendrimers; Dendrimers; Gene delivery; Quaternary ammonium dendrimers; Liver targeting; Liver; Particles

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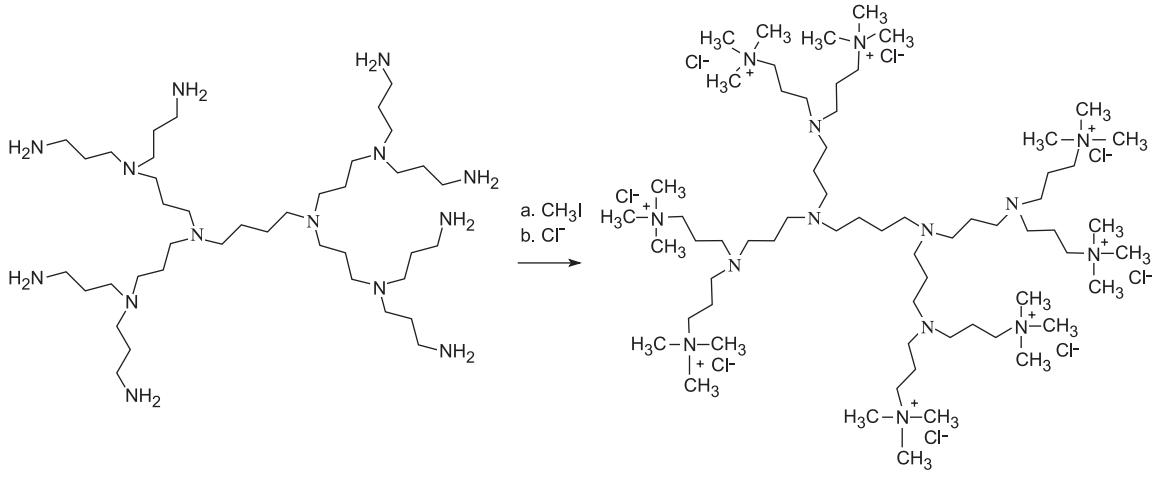
1. Introduction

Advances in gene therapy are only ever likely to make a clinical impact once effective gene delivery systems are developed. Our focus is on the development of synthetic gene delivery systems [1,2] as there is still some uncertainty as to the safety of some of the viral gene delivery systems [3,4]. While most efforts aimed at preparing synthetic gene delivery systems are focused on the use of amphiphilic [5] or polymeric [6] cations, we have previously established that the lower generation (generation 2–DAB 8 and generation 3–DAB 16) amine rich polypropylenimine dendrimers are effective gene transfer systems in vitro [7] and provided the first evidence that a relatively low molecular weight (DAB 8, MW=773) non-amphiphilic compound may be used for in vitro gene transfer. Prior to our work, relatively poor gene transfer activity had been reported with the higher generation DAB 32 (generation 4), DAB 64 (generation 5) and DAB 128 (generation 6) dendrimers [8,9]. Other groups working on the structurally related polyamidoamine dendrimers, in which a combination of amine and amide bonds are used to build up the dendrimer, have found gene transfer activity to only reside within the higher generation materials (>generation 7, MW G7 polyamidoamine dendrimer=87, 000) with the lower generation materials showing diminished activity [10]. The current work describes the evaluation of the polypropylenimine dendrimers and their quaternary amine derivatives as in vivo gene delivery systems and reports on the intrinsic ability of these dendrimers to distribute gene expression preferentially to the liver rather than the lung after intravenous injection. It is important to systemically deliver genes to specific organs and most other synthetic gene carriers devoid of homing ligands (e.g. galactose) do not target the liver, a major site of protein production.

2. Materials and methods

2.1. Synthesis and characterisation of quaternary ammonium dendrimers

Polypropylenimine dendrimers (DABs, 500 mg, Sigma-Aldrich, UK) were reacted in *N*-methylpyrrolidone (50 mL, Sigma-Aldrich) with methyl iodide (3 g, Sigma-Aldrich) in the presence of sodium iodide (150 mg, Sigma-Aldrich) and under a stream of nitrogen for 3 h (Scheme 1). The products were recovered as yellow solids by precipitation in diethyl ether, washing with absolute ethanol, passage over an amberlite ion exchange column as described previously [11] and lyophilisation. Products were characterised using elemental analysis (Perkin Elmer 2400 analyser) and ^{1}H NMR in D_20 (Bruker 400 MHz spectrometer). The yields of Q4, Q8, Q16 and Q32 were 324, 331, 387 and 364 mg, respectively.



Scheme 1. The synthesis of quaternary ammonium dendrimers (Q4, Q8, Q16 and Q32 from DAB 4, DAB 8, DAB 16 and DAB 32, respectively) as exemplified by the synthesis of Q8.