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The influence of surface modification on the cytotoxicity of PAMAM dendrimers

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Abstract

The influence of surface modification on the cytotoxicity of PAMAM dendrimers was examined using Caco-2 cells. Dendrimers were modified by conjugating either lauroyl chains or polyethylene glycol (PEG) 2000 onto the surface of cationic PAMAM dendrimers (G2, G3, G4).

The cytotoxicity of unmodified dendrimers towards Caco-2 cells was appreciably higher for cationic (whole generation) compared with anionic (half generation) dendrimers and for both types increased with increasing size (generation) and concentration. A marked decrease in the cytotoxicity of cationic PAMAM dendrimers was noted when the surface was modified, with the addition of six lauroyl or four PEG chains being particularly effective in decreasing cytotoxicity. This decrease in cytotoxicity is thought to be due to a reduction/shielding of the positive charge on the dendrimer surface by the attached chains. The cytotoxicity of dendrimer-based delivery systems is likely to be very different from the parent dendrimer.

Keywords: Dendrimers; PAMAM; Surface modification; Cytotoxicity; Viability; Caco-2 cells; Lauroyl chloride; Polyethylene glycol

Dendrimers are highly branched macromolecules that have specific size, shape and chemical functionality. Dendrimers have found several pharmaceutical applications including the encapsulation and solubilization of drugs, use as carriers for the delivery of drugs via the gastrointestinal tract, the delivery of DNA and oligonucleotides, and the development of targeted delivery systems (D'Emanuele et al., 2002).

Starburst® polyamidoamine (PAMAM) dendrimers are a specific family of dendritic polymers, which are based on an ethylene diamine core and an amidoamine repeat branching structure (Tomalia, 1995) and commercially available (Dendritech) as whole (cationic) or half (anionic) generation polymers.

Despite the extensive interest in the pharmaceutical applications of dendrimers, there is conflicting evidence regarding their biological safety, (Roberts et al., 1996; Malik et al., 2000) and indeed, cationic PAMAM dendrimers have been shown to be haemolytic, a property that was associated with their cationic nature. In both of the above studies, unmodified PAMAM dendrimers were evaluated.

The present study compares the effect of whole and half generation PAMAM dendrimers on the viability of Caco-2 cells and investigates the effect on cytotoxicity of modifying the dendrimer surface by conjugat-

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ing with either a C12 chain fatty acid or polyethylene glycol.

Lauroyl chloride was attached to the surface of cationic PAMAM dendrimers (G2, G3, G4) using triethylamine as a coupling agent. The reaction was carried out for 4 days at 50 °C, and the conjugates were purified using liquid phase extraction and membrane dialysis. Characterization included high performance liquid chromatography (HPLC) and proton nuclear magnetic resonance (^{1}H NMR). The PEGylation (PEG 2000) of PAMAM dendrimers was achieved by adding freshly prepared tresylated polyethylene glycol monomethyl ether (mPEG-OT) to an aqueous solution of PAMAM dendrimer (G4), with the pH adjusted to 8.0 using 0.1 M HCl. The reaction was allowed to continue for 4 h at room temperature. Conjugates were purified by precipitation of unreacted PEG in methanol at −25 °C followed by dialysis. Conjugates were characterized by ^{1}H NMR and gel permeation chromatography (GPC).

Cell viability studies were performed on the human intestinal adenocarcinoma cell line Caco-2. The cells are of human origin and they exhibit many characteristics of the human small intestinal epithelium (Artursson, 1990). Briefly, Caco-2 cells (passage 40) were seeded at 30,000 cells/well in 96-well plates and maintained at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, 1% non-essential amino acids, 50 IU/ml penicillin and 50 mg/ml streptomycin. The medium was changed on alternate days, and after 3 days (∼90% confluency) DMEM was removed, the cells were washed in phosphate buffered saline and Hanks Balanced Salt Solution (HBSS) containing unmodified or modified PAMAM dendrimers (0–1000 mM) was added. Following 3 h incubation at 37 °C, 3-(4,5-dimethythiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (20 μl, 5 mg/ml) was added and cells incubated for a further 4 h. HBSS was removed, dimethylsulfoxide (DMSO) added and the optical density at 550 nm measured. MTT is a tetrazolium salt that is oxidized by mitochondrial dehydrogenase in living cells to give a dark blue formazan product (Mosmann, 1983). Damaged or dead cells show reduced or no dehydrogenase activity.

The cell survival versus dendrimer concentration is shown in Fig. 1 for both anionic and cationic PAMAM dendrimers over a range of generations. The cytotoxicity was a function of surface charge, size and

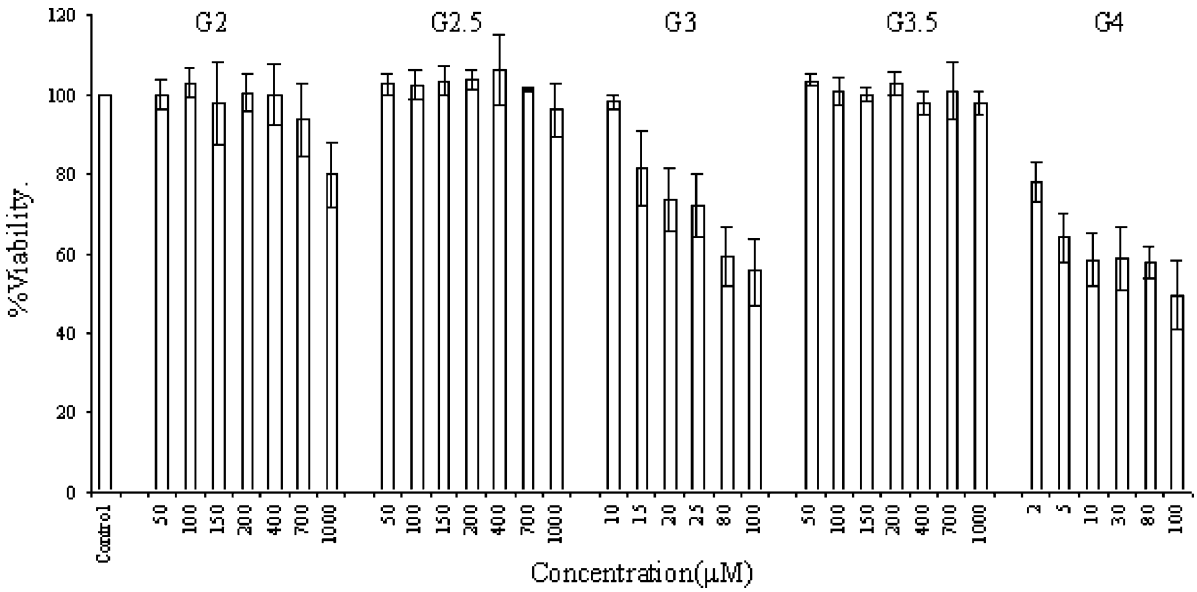

Fig. 1. The effect of dendrimer generation and concentration on the survival of Caco-2 cells (mean ± S.D., $n = 5$) exposed to control (HBSS), cationic (G2, G3, G4) and anionic (G2.5, G3.5) PAMAM dendrimers.

concentration. Anionic PAMAM dendrimers (G2.5, G3.5) exhibited no measurable cytotoxicity up to 1 mM concentration. In contrast, the cationic G2 PAMAM dendrimers showed cytotoxicity toward Caco-2 cells at concentrations above 700 μM, and G3 and G4 were significantly cytotoxic at all concentrations examined. El-Sayed et al. (2002) reported the effect of PAMAM dendrimers on the viability of Caco-2 cells using the lactate dehydrogenase (LDH) release assay. In that study G2 and G3 PAMAM dendrimers exhibited toxicity at concentrations above 10 and 0.1 mM respectively, much higher values than in our study. The discrepancies may be due to the fact that the LDH assay measures the release of enzyme upon damage of cell membranes whilst the MTT assay measures a reduction in mitochondrial dehydrogenase activity and does not depend on the measurement of severe cell damage to detect cytotoxicity.

A marked decrease in the cytotoxicity of the cationic PAMAM dendrimers was noted when cells were exposed to lauroyl- and PEGylated-PAMAM dendrimers. To quantitatively compare the cytotoxicity of dendrimers and conjugates, the IC_{50} values (concentration at which 50% inhibition of mitochondrial dehydrogenase activity was measured) were determined (Table 1). The IC_{50} values of G3 and G4 PAMAM dendrimers with six attached lipid chains were more than 7 fold greater compared to the equivalent unmodified dendrimer. Dendrimer-lipid conjugates with a higher number of attached chains were less effective at reducing the cytoxicity of the cationic dendrimers. Similarly, conjugation of G4 PAMAM dendrimers with 4 PEG chains resulted in a marked reduction in cytotoxicity.

Table 1
The effect of surface modification on the cytotoxicity of PAMAM dendrimers as determined by IC_{50} (mean ± S.D., $n = 5$)

Dendrimer[a]	IC_{50} (mM)
G2	>1
G2L6	>1.5
G2L9	1.06 ± 0.03
G3	0.14 ± 0.00
G3L6	>1
G3L9	0.31 ± 0.00
G3L13	0.22 ± 0.00
G4	0.13 ± 0.02
G4L6	>1
G4L9	0.10 ± 0.00
G4L15	0.04 ± 0.02
G4PEG2	0.12 ± 0.01
G4PEG4	0.79 ±0.01
G2.5	>1
G3.5	>1

[a] G*x*L*y* or G*x*PEG*z*, where *x* represents dendrimer generation, *y* represents the number of attached lipid groups, and *z* represents the number of attached PEG chains.

The cytotoxicity of cationic PAMAM dendrimers is thought to be the result of the interaction between positively charged dendrimers and negatively charged cell surfaces (Roberts et al., 1996; Malik et al., 2000). The modification of cationic dendrimers with lipid or PEG is likely to decrease/shield the positive charge on the dendrimer surface and lead to a decrease in cytotoxicity.

The biological profile of a dendrimer-based delivery system (with surface modifiers and a payload of drug) is likely to be different from that of an unmodified dendrimer. Indeed, it has been shown that DNA/PAMAM dendrimer complexes are less mytotoxic than unmodified dendrimer, possibly due to the fact that the complex reduces the overall positive charge of the dendrimer (Brazeau et al., 1998). Oligonucleotide-dendrimer complexes have also been shown to be less cytotoxic than unmodified dendrimer (Yoo and Juliano, 2000).

In conclusion, although cationic PAMAM dendrimers exhibit cytotoxicity toward Caco-2 cells, surface modification may lead to a marked reduction in toxicity. The cytotoxicity of dendrimer-based delivery systems is likely to be very different from the parent dendrimer. The influence of surface modification on the transport of dendrimers through epithelial cells is currently under investigation.

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