Transepithelial transport of poly(amidoamine) dendrimers across Caco-2 cell monolayers

Mohamed El-Sayed\textsuperscript{a}, Mark Ginski\textsuperscript{b}, Christopher Rhodes\textsuperscript{b}, Hamidreza Ghandehari\textsuperscript{a,\*}

\textsuperscript{a}University of Maryland School of Pharmacy, Department of Pharmaceutical Sciences, 20 N. Pine Street, Baltimore, MD 21201, USA
\textsuperscript{b}Guilford Pharmaceuticals Inc., Department of Pharmaceutics, 6411 Beckley Street, Baltimore, MD 21224, USA

Received 22 December 2001; accepted 22 March 2002

Abstract

The objective of this study was to investigate the influence of physiochemical parameters (such as size, molecular weight, molecular geometry, and number of surface amine groups) of poly (amidoamine) (PAMAM) dendrimers, on their permeability across Caco-2 cell monolayers. The permeability of a series of PAMAM dendrimers, generations 0–4 (G0–G4), was investigated across Caco-2 cell monolayers in both the apical to basolateral (AB) and basolateral to apical (BA) directions. The influence of PAMAM dendrimers on the integrity, paracellular permeability, and viability of Caco-2 cell monolayers was also monitored by measuring the transepithelial electrical resistance (TEER), mannitol permeability, and leakage of lactate dehydrogenase (LDH) enzyme, respectively. G0, G1 and G2 demonstrated similar AB permeabilities, which were moderate several fold higher than the AB permeability of higher generations. The AB and BA permeability of G0–G4 typically increased with the increase in donor concentration and incubation time. Permeability values are not reported at generations, concentrations or incubation times that the dendrimers were toxic to Caco-2 cells. TEER values decreased and mannitol permeability increased as a function of donor concentration, incubation time, and generation number. LDH results for G3 and G4 indicate that Caco-2 cell viability was reduced with increasing donor concentration, incubation time, and generation number. The appreciable permeability of G0–G2, coupled with their nontoxic effects on Caco-2 cells, suggest their potential as water-soluble polymeric drug carriers for controlled oral drug delivery. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Poly (amidoamine) dendrimers; PAMAM; Caco-2 cells; Transepithelial transport; Drug delivery

1. Introduction

Oral drug delivery is the most desirable route of administration for many drugs. The oral bioavailability of polymeric drug carriers, however, is often limited by their large size and molecular weight. Variation in the mechanism and net permeability of polymeric drug carriers has been attributed to the difference in their structural features such as hydrodynamic volume, molecular weight, molecular geometry, and charge [1–4]. For example, transport of neutral poly (ethylene) glycol (PEG) polymers across rabbit colonic epithelium appears to occur via the paracellular route and depends on the molecular
weight of the polymer where permeability is decreased as the molecular weight is increased [1]. Whereas, it has been shown that the transport of dendrimers, across everted rat intestinal sacs and MDCK cells, is due to a combination of factors including size and charge [2–4].

PAMAM dendrimers are a family of water-soluble polymers characterized by a unique tree-like branching architecture and a compact spherical shape in solution [5,6]. Their potential as drug carriers arises from the large number of arms and surface amine groups that can be utilized to immobilize drugs, enzymes, antibodies or other bioactive agents [7,8]. Initial studies have shown that PAMAM dendrimers can permeate across epithelial barriers suggesting their potential as oral drug carriers [2–4]. However, limited data are available for a systematic correlation of the structure of these polymers with their transport across biological barriers, specifically the intestinal epithelia. The present work is an attempt to systematically investigate the influence of physicochemical parameters such as size, molecular weight, molecular geometry, and number of surface amine groups of PAMAM dendrimers, on their permeability across Caco-2 cell monolayers. Collectively, the results of this research will aid in understanding the relationship between the structural features of PAMAM dendrimers and their intestinal permeability, with the prospect of designing novel polymeric carriers for oral drug delivery.

2. Materials and methods

2.1. Materials

Aqueous solutions of PAMAM dendrimers (G0–G4), fluorescein isothiocyanate (FITC), and lactate dehydrogenase assay kits were purchased from Sigma-Aldrich Co. (St. Louis, MO). Superose 12 HR 10/30 column, Superose 12 preparative grade beads and HR 16/50 column were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Caco-2 cells were purchased from American Type Cell Culture (ATCC, Rockville, MD). [14C] Mannitol (specific activity 51.5 mCi/mmol) was purchased from NEN Life Sciences (Boston, MA).

2.2. Fluorescence labeling and fractionation of PAMAM dendrimers

PAMAM dendrimers (G0–G4, Table 1) were labeled using FITC following a previously reported method [9]. Briefly, PAMAM aqueous solutions were diluted 100-fold (v/v) in phosphate buffered saline at pH 7.4. The corresponding amount of FITC (polymer:FITC molar ratio=1:1) was dissolved in acetone (5 mg/ml), added to unlabeled PAMAM solutions, and allowed to stir overnight at room temperature. Fluorescently labeled PAMAM solutions were dialyzed against deionized water, fractionated on a Superose 12 HR 16/50 preparative scale column using a Fast Protein Liquid Chromatography System (FPLC) (Amersham Pharmacia Biotech), a mobile phase of 30%;70% (v/v) acetonitrile:Tris buffer (pH 8.0), and a flow rate of 1.0 ml/min. Eluting molecules were detected at 280 nm. Fractions corresponding to the size and molecular weight of each polymer were collected, dialyzed against deionized water to remove the mobile phase salts, and subsequently lyophilized and stored at 4°C for the permeability experiments.

2.3. Caco-2 cell culture

Caco-2 cells were grown at 37°C in T-75 flasks in an atmosphere of 5% CO2 and 95% relative humidity using Dulbecco’s Modified Eagle’s Medium (pH 7.4) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, and 0.05% penicillin–streptomycin. Cells were passaged at 80–90% confluence using a 0.25% trypsin/0.20% ethylene diamine tetraacetic acid (EDTA) solution. Media were changed approximately every 48 h. Caco-2
cells (passages 30–70) were seeded at 6.3×10^4 cells/cm² on polycarbonate 6-well or 12-well Transwell® filters (Corning Costar Corporation, Cambridge, MA) (3.0 μm mean pore size). Caco-2 cells were cultured on Transwells® under the same incubation conditions and used for transport experiments 21–28 days after seeding.

2.4. Caco-2 permeability of PAMAM dendrimers

The transport of fluorescently labeled PAMAM dendrimers (G0–G4) across Caco-2 cell monolayers was investigated in triplicate in both AB and BA directions at donor concentrations of 1.0 mM (6-well Transwells®, 4.7 cm² surface area) and 10.0 mM (12-well Transwells®, 1.0 cm² surface area). At 1.0 mM, 6-well Transwells® were used to permit quantitation of receiver samples (by increasing surface area). Permeability experiments were conducted at pH 7.0, 37 °C, 5% CO₂, 95% relative humidity, and shaking at 50 rev./min while maintaining sink conditions. Samples were collected at 30, 90, 150, and 210 min. The 30-min sample was discarded to ensure steady-state transport. The integrity of Caco-2 cell monolayers was monitored by measuring TEER before and after each experiment.

Permeability coefficients (P eff) of G0–G4 were calculated as previously reported [10]. Control transport experiments were also conducted across Transwell® filters without Caco-2 cells to determine the filter permeability (P filter). The permeability of Caco-2 cell monolayers (P m) was estimated by correcting the effective permeability (P eff) for filter permeability (P filter) according to:

\[ P_{\text{eff}} = P_{m}^{-1} + P_{\text{filter}}^{-1} \]

After permeating across Caco-2 cell monolayers, the dendrimers were randomly examined for the absence of low molecular weight fragments using size exclusion chromatography techniques (as described in Section 2.2).

2.5. HPLC analysis of PAMAM dendrimers

Fluorescently labeled PAMAM samples were analyzed using a HPLC system (Hewlett Packard, Palo Alto, CA) with fluorescence detection (Shimadzu Corporation, Kyoto, Japan). G0, G1 and G2 samples were analyzed using a LUNA C-18 column (Phenomenex, Torrance, CA), a mobile phase of 12:88 (v/v%) acetonitrile:phosphate buffer (pH 7.4), and a flow rate of 1.0 ml/min. G3 and G4 samples were analyzed using a PolySep GFC-P3000 column (Phenomenex), a mobile phase composed of 30:70 (v/v%) acetonitrile:Tris buffer (pH 8.0), and a flow rate of 1.0 ml/min. G0–G4 were all detected at an excitation wavelength of 495 nm and an emission wavelength of 518 nm.

2.6. Effect of PAMAM dendrimers on TEER across Caco-2 cell monolayers

The effect of PAMAM dendrimers (G0–G4) on the integrity of Caco-2 cell monolayers was investigated by monitoring the TEER across Caco-2 cell monolayers in the presence of PAMAM dendrimers at donor concentrations of 0.1, 1.0 and 10.0 mM upon both apical and basolateral incubation. A Millicell® ERS meter (Millipore Corporation, Bedford, MA) connected to a pair of chopstick electrodes was used to measure TEER values at 30, 60, 90, 120, 150, 180, and 210 min. All experiments were conducted in triplicate at pH 7.0, 37 °C, 5% CO₂ and 95% relative humidity while shaking at 50 rev./min. Control experiments, in HBSS, were also conducted at the same experimental conditions yielding mean TEER values in the range of 800–1200 Ω·cm².

2.7. Effect of PAMAM dendrimers on mannitol permeability across Caco-2 cell monolayers

The permeability of [14C] mannitol (3.2 μM) across Caco-2 cell monolayers was investigated in triplicate in both AB and BA directions at donor concentrations of 0.1, 1.0, and 10.0 mM PAMAM dendrimers. Control permeability of [14C] mannitol was studied in triplicate in blank HBSS in both the AB and BA directions. Permeability experiments were conducted at pH 7.0, 37 °C, 5% CO₂, 95% relative humidity, and shaking at 50 rev./min while maintaining sink conditions. Samples were collected at 30, 60, 90, 120, 150, 180, and 210 min. The 30-min sample was discarded to ensure steady-state transport. The integrity of Caco-2 cell monolayers was also monitored by measuring TEER before and after each experiment. Samples were analyzed by liquid scintillation counting (Beckman Coulter, Ful-
The monolayer permeability \( (P_m) \) of \([^{15}C]\) mannitol, in the presence and absence of PAMAM dendrimers, was calculated as described earlier for PAMAM dendrimers.

### 2.8. Lactate dehydrogenase (LDH) leakage assay

LDH is a cytosolic enzyme that is not normally secreted outside the cell. However, upon damage to cell membranes, it has been proven to leak into the culture medium [11]. LDH leakage into the apical compartment was used to measure the effect of PAMAM dendrimers (G0–G4) on the viability of Caco-2 cell monolayers. Caco-2 cell monolayers (12-well Transwells) were treated with unlabeled G0–G4 at apical donor concentrations of 0.1, 1.0 and 10.0 mM. Caco-2 cell monolayers were also treated with blank HBSS and 1% Triton X-100 as negative and positive controls, respectively. The effect of incubation time of each dendrimer on LDH leakage was investigated at 90, 150, and 210 min. Experiments were conducted in triplicate at pH 7.0, 37°C, 5% CO\(_2\) and 95% relative humidity while shaking at 50 rev./min to mimic the conditions of PAMAM permeability experiments. LDH leakage in the donor compartment was quantified using an LDH assay kit following the manufacturer’s specifications. A one-tailed \( t \)-test (Microsoft Excel) \((P<0.01)\) was used to identify significant differences between LDH results for PAMAM dendrimers and the negative control, blank HBSS.

### 3. Results

#### 3.1. Fluorescence labeling and fractionation of PAMAM dendrimers

PAMAM dendrimers were fluorescently labeled at a 1:1 molar ratio with FITC. Fractionation of fluorescently labeled PAMAM dendrimers was based on the principles of size exclusion chromatography whereby molecules with larger hydrodynamic volumes elute earlier than those with smaller hydrodynamic volumes. The elution volumes of the fluorescently labeled PAMAM dendrimers (G0–G4) were used as indicators of their relative sizes. As expected the elution volumes of PAMAM dendrimers were in the order G0>G1>G2>G3>G4 and size exclusion chromatograms ruled out the presence of free FITC and any small or large molecular weight polymer fragments which could have potentially influenced permeability results (Fig. 1).

### 3.2. Caco-2 permeability of PAMAM dendrimers

At 1.0 mM, PAMAM dendrimers demonstrated an incubation time- and generation-dependent Caco-2 permeability profile (Fig. 2). Results indicate G0, G1 and G2, demonstrated similar AB permeabilities that were up to six fold higher than the AB permeability of larger G3 dendrimers. Under similar conditions, the BA permeability of each dendrimer was typically higher than its corresponding AB permeability. Permeability of G3 and G4 are not reported at incubation time points where cytotoxicity may have contributed to the observed permeability values as denoted by LDH results (Fig. 2 and Table 2). At 10.0 mM, PAMAM dendrimers demonstrated an incubation time- and generation-dependent permeability profile which was similar to that observed at 1.0 mM (Fig. 3). However, both AB and BA permeabilities for a given generation at 10.0 mM were typically higher than their corresponding values at 1.0 mM. At longer incubation times, the AB

---

*Fig. 1. Size exclusion chromatogram of fluorescently labeled PAMAM dendrimers (G0–G4) after fractionation on a Superose 12 HR preparative scale column (Amersham Pharmacia Biotech) using a mobile phase composed of 30:70 (v/v) acetonitrile:Tris buffer (pH=8) at a flow rate of 1 ml/min. Detection of eluting molecules was done using a UV detector at a fixed wavelength (\(\lambda = 280\) nm).*
permeabilities of G0–G2 were moderate (1×10\(^{-6}\) and 1×10\(^{-5}\) cm/s) and the BA permeabilities were high (greater than 1×10\(^{-5}\) cm/s), respectively, when compared to the permeability of a low permeable marker compound such as mannitol. Size exclusion chromatograms of the randomly selected receiver samples demonstrated elution volumes which were similar to those of the original fluorescently labeled PAMAM dendrimers without small molecular weight fragments or impurities (data not shown).

### 3.3. Effect of PAMAM dendrimers on Caco-2 cell monolayer integrity

TEER values were measured in the presence of donor concentrations of 0.1, 1.0, and 10.0 mM PAMAM dendrimers (G0–G4). To summarize this large amount of data, however, TEER values at all concentrations are only illustrated for G0 (Fig. 4A) and G4 (Fig. 4B). The decline in TEER for each generation was concentration-dependent in both the AB and BA directions. The onset and the extent of decline in TEER values also changed as a function of generation. The incubation of larger PAMAM dendrimers on the apical side (Fig. 4C) resulted in a faster, more pronounced, and extended decline in TEER values than smaller ones. For example, TEER in the presence of G0 declined slowly for the first 30 min and continued to decrease slowly with increase in incubation time. On the other hand, TEER dropped sharply within the first 30 min following exposure to larger PAMAM generations such as G3 and remained constant throughout the rest of the experiment. Basolateral incubation of PAMAM dendrimers also resulted in a faster and more pronounced decline in TEER compared to apical incubation under similar conditions (Fig. 4D).

### 3.4. Effect of PAMAM dendrimers on paracellular permeability

Mannitol permeability was investigated across Caco-2 cell monolayers in both AB and BA directions at donor concentrations of 0.1, 1.0 and 10.0 mM of each generation. Even though results indicate that both AB and BA mannitol permeability increased with incubation time, data are only reported at the last incubation time point, 210 min (Fig.
Table 2
Lactate dehydrogenase (LDH) assay of PAMAM dendrimers

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>LDH leakage % ( (90\text{ min}) )</th>
<th>LDH leakage % ( (150\text{ min}) )</th>
<th>LDH leakage % ( (210\text{ min}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSS</td>
<td>14.6±2.1%</td>
<td>16.1±1.2%</td>
<td>6.3±0.8%</td>
</tr>
<tr>
<td>PAMAM-G0 0.1 mM</td>
<td>11.9±1.6%</td>
<td>8.3±0.5%</td>
<td>5.1±1.8%</td>
</tr>
<tr>
<td>PAMAM-G0 1.0 mM</td>
<td>16.3±1.5%</td>
<td>8.0±0.4%</td>
<td>6.2±1.4%</td>
</tr>
<tr>
<td>PAMAM-G1 0.1 mM</td>
<td>15.9±3.0%</td>
<td>9.4±2.1%</td>
<td>4.7±0.2%</td>
</tr>
<tr>
<td>PAMAM-G1 1.0 mM</td>
<td>16.5±1.1%</td>
<td>10.3±1.4%</td>
<td>7.9±0.5%</td>
</tr>
<tr>
<td>PAMAM-G2 0.1 mM</td>
<td>19.9±1.2%</td>
<td>6.9±3.8%</td>
<td>8.5±0.7%</td>
</tr>
<tr>
<td>PAMAM-G2 1.0 mM</td>
<td>42.2±3.1%</td>
<td>20.9±1.5%</td>
<td>33.4±2.0%</td>
</tr>
<tr>
<td>PAMAM-G3 0.1 mM</td>
<td>16.3±1.2%</td>
<td>14.0±2.1%</td>
<td>11.0±0.5%</td>
</tr>
<tr>
<td>PAMAM-G3 1.0 mM</td>
<td>25.2±1.1%</td>
<td>19.8±0.7%</td>
<td>19.2±1.9%</td>
</tr>
<tr>
<td>PAMAM-G4 0.1 mM</td>
<td>46.0±3.0%</td>
<td>39.5±1.5%</td>
<td>29.6±0.9%</td>
</tr>
<tr>
<td>PAMAM-G4 10.0 mM</td>
<td>221.0±14.8%</td>
<td>210.9±11.1%</td>
<td>141.4±2.7%</td>
</tr>
<tr>
<td>Triton X-100 100.0 mM</td>
<td>100.0±23.2%</td>
<td>100.0±22.2%</td>
<td>100.0±22.2%</td>
</tr>
</tbody>
</table>

Italicized cells denote a significant increase in leakage of LDH compared to that in HBSS based upon a \( P \)-value < 0.01 using a one tail \( t \)-test.

\( ^* \) Results are reported as % of LDH leakage observed upon incubation with the positive control, Triton X-100.

5A–C). Mannitol permeability in the presence of PAMAM dendrimers is reported in reference to control AB and BA mannitol permeabilities, which were \( 1.26 \times 10^{-6} \text{ cm/s} \) and \( 1.96 \times 10^{-6} \text{ cm/s} \), respectively. At 0.1 mM, both AB and BA mannitol permeability increased as much as six fold (Fig. 5A). At 1.0 mM, AB and BA mannitol permeability increased as much as 4- and 16-fold, respectively (Fig. 5B). At 10.0 mM, AB and BA mannitol permeability increased as much as 8- and 13-fold, respectively (Fig. 5C). Results indicate that the BA mannitol permeability was typically higher than its corresponding AB permeability under similar experimental conditions. Both AB and BA permeability of mannitol increased with the increase in PAMAM generation number. Only the permeability values at non-toxic PAMAM concentrations, as evident by LDH results (Table 2), are reported.

3.5. Effect of PAMAM dendrimers on Caco-2 cell viability

The influence of PAMAM dendrimers on the viability of Caco-2 cell monolayers, as denoted by LDH leakage results, is reported in Table 2. There was no significant LDH leakage upon incubation of Caco-2 cell monolayers with G0 and G1 at concentrations of 0.1, 1.0 and 10.0 mM for an incubation time of up to 210 min. G2 induced a statistically significant leakage of LDH only at 10.0 mM at 210 min. G3 induced a statistically significant LDH leakage at all the concentrations and incubation times investigated.

4. Discussion

Considering the reported molecular weight/diameter and the size exclusion profiles of fluorescently labeled PAMAM dendrimers (Table 1, Fig. 1), one would expect their Caco-2 permeability to decrease with the increase in generation number. However, the Caco-2 permeability of G0–G4 did not fit a typical size- and/or molecular weight-restricted permeability profile [1]. The AB permeability of smaller PAMAM dendrimers, G0, G1 and G2, showed similar and moderate permeability values despite the incremental increase in their molecular weights. The
moderate permeability of these dendrimers may be attributed to structural features such as charge, molecular weight and geometry. Compared to neutral molecules (such as urea and mannitol), or anionic molecules (such as formate and lactate), positively charged molecules (such as methylamine and atenolol) have been reported to permeate at a higher rate across Caco-2 cell monolayers due to a favorable electrostatic interaction with the negatively charged epithelial surfaces [12]. Similarly, positively charged polymers, such as chitosan derivatives, have been shown to enhance paracellular permeability across Caco-2 cell monolayers through modulation of tight junctions [13–17]. The extent of this modulation has also been shown to depend on the number of positively charged groups available to interact with the negatively charged epithelium [17]. According to the Henderson–Hasselbalch equation, 41.5% of the surface primary amine groups in PAMAM dendrimers are positively charged at pH 7.0, while the interior tertiary amine groups are almost un-ionized. Therefore, it is possible that the cationic nature of PAMAM dendrimers could have facilitated their interaction with Caco-2 cell epithelia, resulting in modulation of the tight junctions and a subsequent increase in their permeability, probably via the paracellular route. The interaction between PAMAM dendrimers and Caco-2 epithelial surface would be expected to increase with generation number, due to the increase in the number of positively charged surface amine groups (Table 1). Since the permeability of mannitol increased with increase in generation number (Fig. 5), results would support a direct interaction of PAMAM dendrimers with Caco-2 epithelia.

In addition to polymer charge, the change in molecular weight of the polymer has an impact on Caco-2 permeability. Results indicate that for smaller PAMAM dendrimers (G0–G2), Caco-2 permeability did not decrease with an increase in generation number, despite the relatively high molecular weight of G1 and G2. This may be attributed to the increase in the number of positively charged amine groups as the generation number increased. In fact, the AB permeability of G0–G2 was higher than the permeability of commonly used neutral polymeric drug carriers of corresponding molecular weights, such as PEG 900 and PEG 4000 [1], although the per-

Fig. 3. AB (□) and BA (■) Caco-2 permeability of PAMAM dendrimers (G0–G4) at a donor concentration of 10.0 mM and incubation times of: (A) 90 min, (B) 150 min, and (C) 210 min. AB and BA permeability values are not reported (**) at toxic incubation time points. Results are reported as mean ± standard error of the mean (S.E.M.).
meability of these polymers was assessed across rabbit colonic epithelium. Despite the higher number of positively charged surface amine groups, at nontoxic donor concentrations, permeability of G3 was much lower than G0–G2 probably due to its higher molecular weight.

In addition to the observed generation-dependent permeability of PAMAM dendrimers, AB and BA permeability also demonstrated dependence on incubation time and concentration (Figs. 2 and 3). Incubation time-dependent permeability may be due to a prolonged interaction of these dendrimers with the Caco-2 epithelia, resulting in a more pronounced modulation of tight junctions. This hypothesis is supported by the observed decrease in TEER with time for all generations (Fig. 4). Concentration-dependent permeability is not normally observed for passively absorbed molecules. Even though the mechanism of permeation of PAMAM dendrimers has not yet been fully investigated, it is possible that
increasing the concentration of positively charged amine groups in solution increased the degree of interaction of PAMAM with Caco-2 epithelia, resulting in the higher observed permeability at higher concentrations. This interpretation is supported by similar observations that increasing the concentration or the degree of quaternization of N-trimethyl chitosan, a positively charged water-soluble polymer, results in greater modulation of the tight junctions and a subsequent increase in the mannitol permeability [14,17]. The BA permeabilities of PAMAM dendrimers were also typically higher than their corresponding AB permeabilities under similar conditions (Figs. 2 and 3). This may be attributed to either differences in tight junctional structure at the apical and basolateral membranes or efflux by secretory transporters. Caco-2 cell monolayers possess tight junctional proteins which are present at the apical but not at the basolateral membrane. These proteins not only serve to connect adjacent cells, but are also responsible for maintaining the integrity of tight junctions [18]. Similar observations have been reported upon application of EDTA to the apical and basolateral membranes of Caco-2 cell monolayers, whereby basolateral application of EDTA resulted in a more pronounced decline in TEER and higher paracellular permeability compared to the apical application of equivalent EDTA concentrations [18]. This directional permeability could also be due to the potential affinity of the studied dendrimers for efflux transporters, such as P-glycoprotein (P-gp), since it has been demonstrated that highly cationic molecules tend to have high affinity for P-gp [19]. However, efflux cannot be the only factor contributing to the directional permeability of these dendrimers since mannitol, which is not a substrate for P-gp, also exhibited a higher BA versus AB permeability under similar experimental conditions (Fig. 5). Additional studies are warranted to evaluate the mechanism of transport of these dendrimers across Caco-2 cell monolayers.

TEER values (Fig. 4) and mannitol permeability (Fig. 5) were used to assess the effect of PAMAM dendrimers (G0–G4) on the integrity and paracellular permeability of Caco-2 cell monolayers, respectively. Results indicate that TEER values decrease with PAMAM concentration and generation number. Low TEER values and high mannitol permeabilities...
across Caco-2 cell monolayers are typically observed to be indicative of cell monolayer disruption via cytotoxicity. PAMAM dendrimers have been previously identified to demonstrate generation-dependent in vitro hemolytic [20] and myotoxic effects [21]. Consistent with these findings, LDH leakage results indicate that small PAMAM dendrimers (G0–G2) were generally non-toxic to Caco-2 cell monolayers (except for G2 at 10.0 mM at 210 min), while larger dendrimers (G3 and G4) exhibited toxicity (Table 2). PAMAM dendrimers caused a decrease in TEER values and increase in mannitol permeability at concentrations that were shown to be non-toxic to Caco-2 cell monolayers. The effects of these dendrimers on TEER and mannitol permeability could be attributed to tight junctional modulation due to the cationic nature of PAMAM dendrimers. The progressive decline in TEER values or increase in mannitol permeability with the increase in incubation time, would also support this hypothesis due to the prolonged interaction of the positively charged amine groups with the Caco-2 epithelial surface. It is currently unclear how these results would correlate to intestinal permeability in humans, since it has been shown that the comparative biology of the tight junctions of Caco-2 monolayers and the human small intestine are different [22,23]. More specifically, Caco-2 monolayers are generally recognized as epithelium with more ‘restrictive’ overall tight junctional structure than human small intestinal epithelium [22,23]. Clearly additional studies to identify the mechanism of permeation for these dendrimers would assist in interpreting these results.

5. Conclusions

The permeability of PAMAM dendrimers across Caco-2 cell monolayers appears to depend on a combination of structural features such as their size, molecular weight, geometry and number of surface amine groups. PAMAM dendrimers enhanced their own permeability, as well as the permeability of mannitol. At higher concentrations and incubation times, G3 and G4 demonstrated toxic effects to Caco-2 cell monolayers. The moderate permeability of PAMAM generations G0, G1 and G2 across Caco-2 cell monolayers, and the lack of cellular toxicity, suggests their potential utility in controlled oral drug delivery. Results suggest that by adjusting the geometry, molecular weight, surface charge, and residence time of PAMAM dendrimers in the GI tract it is possible to control their transepithelial transport.

References

[12] G.T. Knipp, N.F.H. Ho, C.L. Barsuhn, R.R. Borchardt, Paracellular diffusion in Caco-2 cell monolayers: effect of perturbation on the transport of hydrophilic compounds that


