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Note

Transport mechanism(s) of poly (amidoamine) dendrimers across Caco-2 cell monolayers

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Abstract

The objective of this research was to investigate the mechanism(s) of transport of generation 2 (G2) poly (amidoamine) dendrimers across Caco-2 cell monolayers. The contribution of an energy-dependent process such as adsorptive endocytosis was investigated by determining G2 permeability at 4 and 37 °C. The contribution of P-gp efflux to transport was examined by determining the apical to basolateral (AB) and basolateral to apical (BA) permeability of ^{14}C-paclitaxel in presence of G2, and by determining AB and BA permeability of G2 in presence of paclitaxel. The permeability of G2 and ^{14}C-mannitol was investigated in the presence of palmitoyl carnitine to determine the contribution of the paracellular pathway. Permeability of G2 at 4 °C was significantly ($P < 0.05$) lower than that observed at 37 °C. AB and BA permeability of ^{14}C-paclitaxel did not change in the presence of G2. AB and BA permeability of G2 did not change in the presence of paclitaxel. The permeability of G2 and ^{14}C-mannitol increased significantly ($P < 0.05$) in the presence of palmitoyl carnitine, and in addition, ^{14}C-mannitol permeability was increased in presence of G2. The permeability of G2 across Caco-2 cell monolayers appears to involve a combination of paracellular transport and an energy-dependent process, possibly adsorptive endocytosis. G2 dendrimers do not appear to be substrates for the P-gp efflux system.

Keywords: Drug delivery; Oral permeability; Poly (amidoamine) dendrimers

Among water-soluble polymers that have shown potential as oral drug carriers is a family of cascade polymers named poly (amidoamine), PAMAM, dendrimers (Wiwattanapatapee et al., 1998, 2000; El-Sayed et al., 2002, 2003). Our previous studies on the transport of cationic PAMAM-NH_2 dendrimers of generations 0–4 (G0–G4) showed that the smaller dendrimers, G0–G2, exhibited moderate permeability across Caco-2 cell monolayers (1×10^{-6}–10×10^{-6} cm/s) (El-Sayed et al., 2002). Of these three dendrimers, G2 appeared to be the most promising candidate for oral delivery due to its higher permeability, as well as its higher number of surface amine groups that can be used for immobilization of drug molecules. The objective of this research was an initial attempt to investigate the mechanism(s) of G2 transport across Caco-2 cell monolayers.

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PAMAM-NH_2 (G2) (Sigma-Aldrich Co., St. Louis, MO) was labeled using fluorescein isothiocyanate (FITC, Sigma-Aldrich Co., St. Louis, MO) following a previously reported method (El-Sayed et al., 2002). Caco-2 cell monolayers were prepared by standard procedures described previously and used for transport experiments 21–28 days after seeding (El-Sayed et al., 2002).

Permeability of FITC-labeled G2 across Caco-2 cell monolayers was investigated in triplicate at a donor concentration of 10.0 mM at pH 7.0, 37 °C, 5% CO_2, 95% relative humidity, and shaking at 50 revolutions per minute (RPM) while maintaining sink conditions. The contribution of adsorptive endocytosis was investigated by determining G2 permeability at a lower temperature of 4 °C. The permeability of ^{14}C-mannitol as control was not affected by temperature. It is assumed that the lower temperature will reduce the contribution of energy-dependent mechanisms such as adsorptive endocytosis. Contribution of the P-gp efflux system to G2 transport was also examined by determining the AB and BA permeability of ^{14}C-paclitaxel at a donor concentration of 200 nM (American Radiolabeled Chemicals, Inc., St. Louis, MO) in presence of G2 (10.0 mM) and by determining AB and BA permeability of G2 (10.0 mM) in presence of paclitaxel (200 nM) under similar experimental conditions.

The permeability of G2 and ^{14}C-mannitol was investigated in the presence of palmitoyl carnitine (0.3 mM) to determine the contribution of the paracellular pathway to G2 transport (Knipp et al., 1997). Permeability samples were collected from the receiver compartment at 30, 90, and 150 min. The 30-min sample was discarded to ensure steady state transport and sink conditions were maintained throughout the experiments. G2 permeability samples were analyzed using an HPLC system (Hewlett Packard, Palo Alto, CA) with fluorescence detection (Shimadzu Corporation, Kyoto, Japan) following a method reported earlier (El-Sayed et al., 2002). Permeability samples of radioactive nuclides were analyzed by liquid scintillation counting (Beckman Coulter, Fullerton, CA). The permeability of Caco-2 cell monolayers (P_m) of G2 and various radioactive nuclides was determined following an equation reported earlier (El-Sayed et al., 2002). *t*-test (Excel®) was used to establish statistical significance between different groups ($P < 0.05$).

AB permeability of G2 (10.0 mM), across Caco-2 cell monolayers, was investigated at 90 and 150 min at both 37 and 4 °C (Fig. 1). Permeability of G2 at 37 °C increased with the increase in incubation time that is similar to the trend reported earlier (El-Sayed et al., 2002). Permeability of G2 at 4 °C was significantly lower ($P < 0.05$) than that observed at 37 °C at both incubation times of 90 and 150 min. The observed decrease in G2 permeability at 4 °C in comparison to that observed at 37 °C suggests the contribution of adsorptive endocytosis to G2 transport across Caco-2 cell monolayers. It must be noted however, that this observation is suggestive since temperature changes may also result in changes in the diffusivity of the probes, membrane fluidity, transporter activity, intracellular trafficking and other factors that may contribute to changes in permeability.

The permeability of ^{14}C-paclitaxel, a well-known P-gp substrate, across Caco-2 cell monolayers was determined in both AB and BA directions in the presence of G2 (10.0 mM) at incubation times 90 and 150 min. Results at both time points were similar, therefore, only the results at the last time point (150 min) are reported (Fig. 2). In the control experiment, BA permeability of ^{14}C-paclitaxel was four-fold higher than the corresponding AB permeability demonstrating a functioning P-gp efflux system in the Caco-2 cell monolayers used in this study (Fig. 2A). Results indicate that AB and BA permeability of ^{14}C-paclitaxel did not change in the presence of G2 (10.0 mM) ($P < 0.05$) (Fig. 2A).

The BA permeability of G2 (10.0 mM) was higher than the corresponding AB permeability (Fig. 2B) and is similar to the profile reported earlier (El-Sayed et al., 2002). This suggested the contribution of possibly one or more of the efflux systems located at the apical surface of Caco-2 cells, such as P-gp and ABC transporters, to the observed G2 permeability profile. This prompted us to examine the potential contribution of P-gp efflux system on the directional permeability of these cationic dendrimers. Both AB and BA permeability of G2 did not change in the presence of paclitaxel (200 nM) suggesting that G2 permeability does not involve the P-gp efflux system. The observed directional permeability of G2 appears to be due to the reported differences in the structure of the tight junctions of Caco-2 cell monolayers

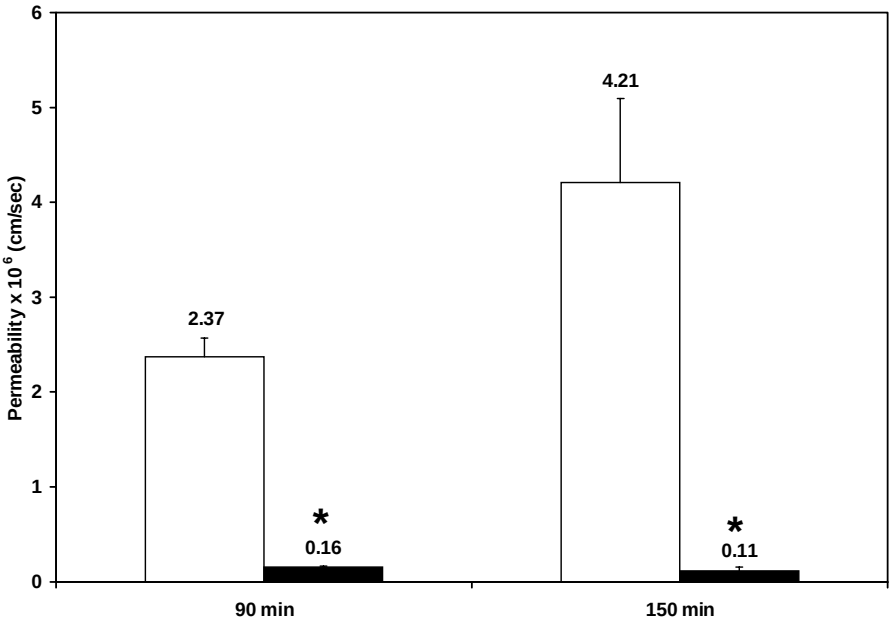

Fig. 1. AB permeability of G2 across Caco-2 cell monolayers at incubation times of 90 and 150 min at 37 °C (□) and 4 °C (■). Statistical difference at $P < 0.05$ is denoted by ∗. Results are reported as mean ± standard error of the mean.

where tight junctional proteins are present only at the apical but not at the basolateral membrane (Noach et al., 1993). However, it must be noted that since G2 at 10 mM causes the opening of tight junctions, this may result in a change in the activity of the cells with respect to efflux transport mechanisms. Moreover, transporters other than P-gp can potentially be involved in PAMAM efflux. Therefore, the contribution of efflux systems cannot be completely ruled out at this time and further experiments are needed.

The AB permeability of G2 increased significantly ($P < 0.05$) from 3.21×10^{-6} cm/s to 30.71×10^{-6} cm/s in the presence of palmitoyl carnitine at an incubation time point of 150 min (Fig. 3). Under similar experimental conditions, palmitoyl carnitine also caused a statistically significant ($P < 0.05$) increase in ^{14}C-mannitol AB permeability from 1.09×10^{-6} cm/s to 35.43×10^{-6} cm/s (Fig. 3). The increase in G2 and ^{14}C-mannitol AB permeability values was similar in the presence of palmitoyl carnitine suggesting the involvement of the paracellular pathway to G2 transport across Caco-2 cell monolayers. This conclusion is further supported by the observed incubation time-dependent decline in TEER values (Fig. 4A) and the corresponding increase in ^{14}C-mannitol permeability (Fig. 4B) upon apical and basolateral incubation of G2 (10.0 mM) with Caco-2 cell monolayers. These results suggest that G2 modulates the tight junctions of Caco-2 cell monolayers, thus causing an increase in the paracellular permeability.

Based on the macromolecular nature of PAMAM dendrimers in general, and the compact spherical geometry, hydrophilicity and positive charge of G2 in particular, we hypothesized that transport of G2 across Caco-2 cell monolayers may be attributed to a combination of transcellular transport by adsorptive endocytosis and paracellular transport via the tight junctions. G2 exhibited lower permeability at 4 °C compared to its permeability at 37 °C at a given incubation time point suggesting it could be transported in part by adsorptive endocytosis. It is important to note that reduced permeability at lower temperature can also be attributed to inhibition of carrier-mediated transport mechanisms, however the transport of G2

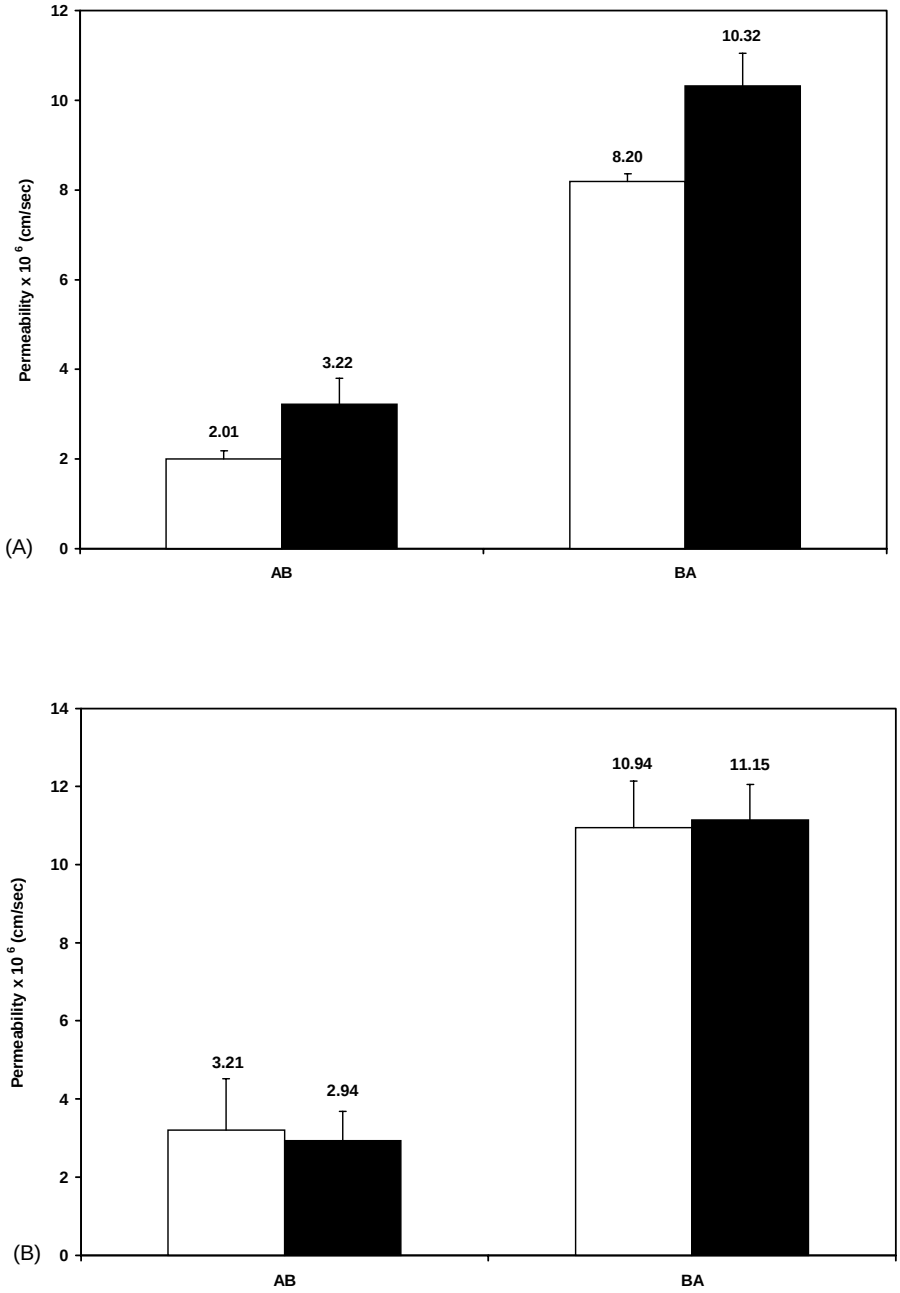

Fig. 2. (A) AB and BA permeability of ^{14}C-paclitaxel across Caco-2 cell monolayers in absence (□) and presence (■) of G2 (10.0 mM) at incubation time of 150 min. (B) AB and BA permeability of G2 (10.0 mM) across Caco-2 cell monolayers in absence (□) and presence (■) of paclitaxel (200 nM) at incubation time of 150 min. Results are reported as mean ± standard error of the mean.

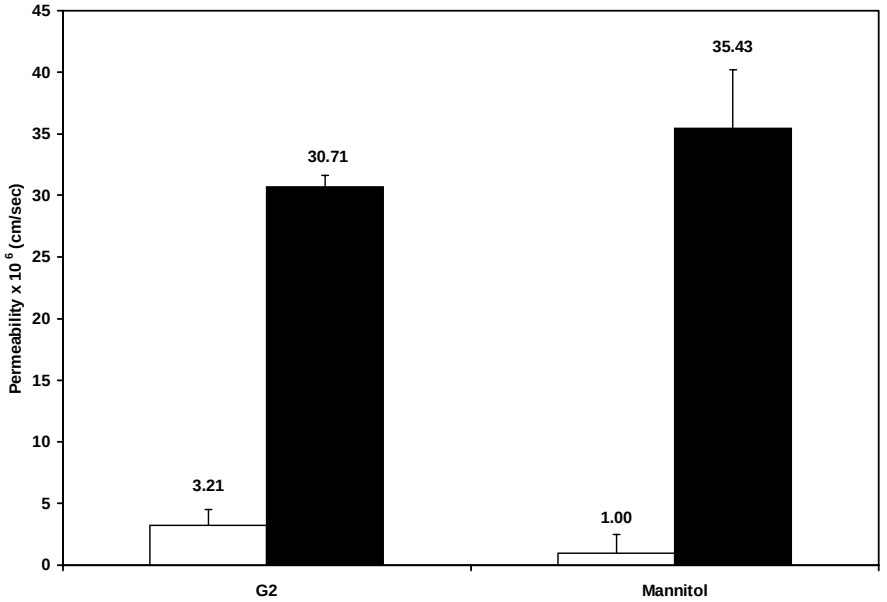

Fig. 3. AB permeability of G2 and ^{14}C-mannitol across Caco-2 cell monolayers in absence (□) and presence (■) of 0.3 mM palmitoyl carnitine. Results are reported as mean ± standard error of the mean.

is unlikely to involve such systems due to their large macromolecular nature and lack of structural similarity to the established substrates for such carrier systems.

Our previous studies showed that the BA permeability of G2 was higher than the corresponding AB permeability under the same experimental conditions (El-Sayed et al., 2002) which suggested the contribution of an efflux system. Based on the results of this study, it appears that the observed directional permeability of G2 is due to the differences in the structure of the tight junctions of Caco-2 cell monolayers where tight junctional proteins are present at the apical but not at the basolateral membranes (Noach et al., 1993).

The hydrophilic and cationic nature of G2 (resulting in modulation of the tight junctions) coupled with its compact spherical geometry in solution suggest that G2 may also permeate across Caco-2 cell monolayers via the paracellular route. Application of permeation enhancers such as palmitoyl carnitine has been shown to increase Caco-2 permeability for molecules that are transported via the paracellular route by dilation of the paracellular space (Knipp et al., 1997). Our results show that palmitoyl carnitine caused an increase in AB permeability of G2 that was similar to the observed increase in ^{14}C-mannitol permeability, a known paracellular permeability marker (Fig. 3). This suggests that G2 is in part transported via the paracellular route. This observation is further supported by the time-dependent decline in TEER and the corresponding increase in permeability of ^{14}C-mannitol across Caco-2 cell monolayers upon incubation with G2 (10.0 mM) (Fig. 4).

In conclusion, our initial observations suggest that the permeability of G2 across Caco-2 cell monolayers occurs by a combination of paracellular transport and an energy-dependent process such as adsorptive endocytosis. In addition, G2 dendrimers do not appear to be substrates for the P-gp efflux system. While these observations elucidate potential mechanisms of PAMAM transport across intestinal epithelial barriers, additional studies allowing the quantification and visualization of paracellular and transcellular components are needed to further clarify the mechanisms of transport of these macromolecular drug carriers.

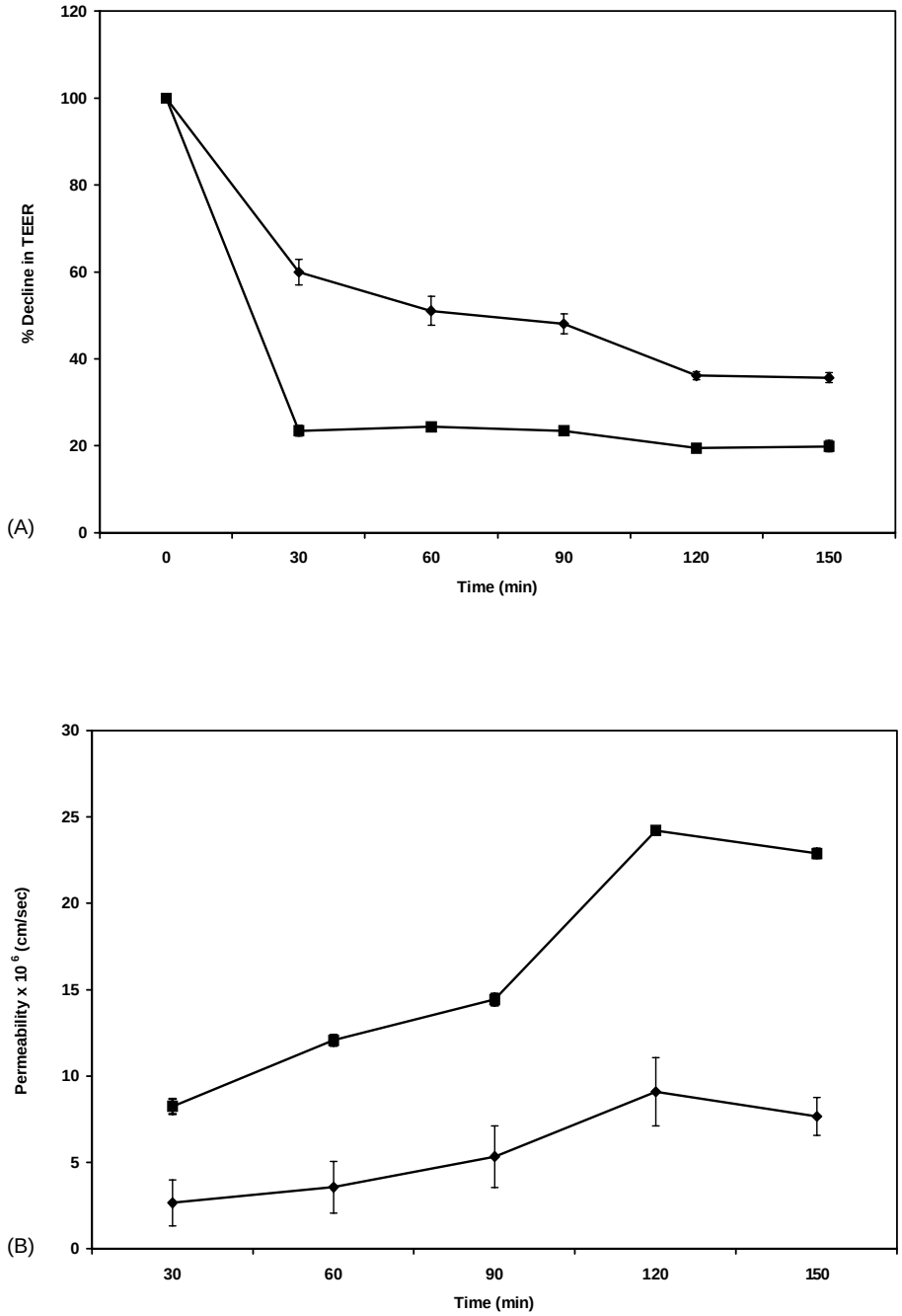

Fig. 4. Effect of apical (◆) and basolateral (■) incubation of G2 on: (A) transepithelial electrical resistance (TEER) and (B) ^{14}C-mannitol permeability across Caco-2 cell monolayers. Results are reported as mean ± standard error of the mean.

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