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Effect of the biologically active fragment of zonula occludens toxin, ΔG, on the intestinal paracellular transport and oral absorption of mannitol

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Abstract

Objective: Many therapeutically active agents experience low bioavailability upon oral administration due to low permeability, low solubility, interaction with efflux transporters or first pass metabolism. In general, absorption enhancers are agents that can modulate the paracellular permeability of drugs, thus, potentially increasing oral bioavailability. The objective of this study was to examine the effect of the active fragment of Zonula occludens toxin (Zot), ΔG, on the transport of a paracellular marker, mannitol, using in vitro (Caco-2 cell monolayers) and in vivo (intraduodenal administration in rats) experimental methods. *Methods:* The transport of [^{14}C]mannitol with ΔG (0, 50, 80, or 100 μg/ml) was determined across Caco-2 cells. Male Sprague–Dawley rats were assigned to receive one of the following treatments: [^{14}C] mannitol (40 μCi/kg), [^{14}C]mannitol/ΔG (417 μg/kg), or [^{14}C] mannitol/ΔG/Protease inhibitors (PI). *Results:* The mean (±S.E.M.) apparent mannitol permeability coefficients (P_{app}) observed after incubation with 0, 50, 80, and 100 μg/ml ΔG were 3.5 (±0.4), 4.17 (±0.27), 4.33 (±0.61), and 9.94 (±0.24) × 10^{-6} cm/s. After oral administration, C_{max} (3.8 × 10^{-4} vs. 4.4 × 10^{-4} mM) and $AUC_{0-6\ h}$ (0.096 vs. 0.088 mM min), obtained for [^{14}C]mannitol and [^{14}C]mannitol/ΔG, respectively, were not statistically different. However, both C_{max} (7.6 × 10^{-4} mM) and $AUC_{0-6\ h}$ (0.25 mM min) were significantly higher for the [^{14}C]mannitol/ΔG/PI treatment. *Conclusions*: The 12 kDa fragment of Zot, ΔG, enhanced the in vitro transport and oral absorption of the paracellular marker, mannitol, in the presence of protease inhibitors (PI).

Keywords: Permeability; ΔG; Zot; Mannitol; Bioavailability; Absorption enhancer

1. Introduction

Many therapeutically active agents experience low bioavailability upon oral administration due to low permeability, low solubility, interaction

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with efflux transporters or first pass metabolism. An approach to minimize the former problem may be to reversibly loosen intestinal tight junctions (tj) so as to enhance paracellular transport as a means of increasing oral absorption. There are certain agents referred to as 'absorption enhancers' that specifically loosen tj and enhance paracellular permeability. The increase in tj diameter allows for a larger portion of the administered drug to cross the intestinal membrane and, hence, enter the blood stream. In general, for an absorption enhancer to function ideally, it should be non-toxic, functional and biocompatible. It should possess an immediate action, coincident with the presence of the drug at the absorption site and preferably have a specific and reversible effect on the junctional complex (Schipper et al., 1996; Thanou et al., 2000). Absorption enhancers are classified into several categories: Calcium chelators, surfactants/detergents, chitosans, etc. Despite the advantageous effect of absorption enhancers, the potential adverse reactions of many existing absorption enhancing agents on the epithelial integrity, morphology and function are of major concern. (e.g. exfoliation of the intestinal epithelium, diminished cell adhesion) (Schipper et al., 1996; Thanou et al., 2000).

Fasano et al. has identified Zonula occludens toxin (Zot), a 45 kDa protein located in the cell envelope of the bacterial strain Vibrio cholerae, which is capable of reversibly opening the tj between cells, thus, having the potential of increasing the paracellular transport of many drugs in a non-toxic manner (Fasano et al., 1991, 1995; Fasano and Uzzau, 1997; Fasano et al., 1997; Cox et al., 2001, 2002). Studies have shown that Zot enhances the transport of drug candidates of varying molecular weight (mannitol, PEG4000, inulin) or low bioavailability (paclitaxel, acyclovir, cyclosporin A) across Caco-2 cell monolayers without modulating transcellular transport (Cox et al., 2001, 2002). In addition, the transport enhancing effect of Zot is reversible and non-toxic (Fasano et al., 1991; Cox et al., 2002). Studies in Diabetic rats have shown that Zot allows for the effective oral administration of insulin, an agent exclusively administered subcutaneously (Fasano et al., 1997).

Recent studies have identified a smaller 12 kDa fragment of Zot, referred to as ΔG (Di Pierro et al., 2001). These studies focused on identifying the Zot domain(s) directly involved in the protein permeating effect. Amino acid comparison between the Zot active fragment and Zonulin, an eukaryotic molecule that governs the permeability of intercellular tj (Di Pierro et al., 2001), combined with site-directed mutagenesis experiments, confirmed the presence of an octapeptide receptor-binding domain toward the amino terminus of the processed Zot. ΔG results in a cascade of intracellular events leading to actin cytoskeletal rearrangement, disengagement of junctional complex proteins and finally opening of tj. Hence, the objective of this report was to examine the effect of the active fragment of Zot, ΔG, on the transport of a paracellular marker, mannitol, using in vitro (Caco-2 cell monolayers) and in vivo (intraduodenal administration in rats) experimental methods. In this study, we used mannitol (mol. wt. 182), a straight chain sugar, as a paracellular marker to investigate intestinal permeability (Delahunty and Hollander, 1987; Pappenheimer, 1990; Ittel et al., 1996; Singhal et al., 1998). The in vivo effect of ΔG was also assessed in the presence of protease inhibitors (PI). A mixture of PI, consisting of bestatin, captopril, and leupeptin, was used.

2. Materials and methods

2.1. Materials

The Caco-2 cell line was obtained from ATCC (Rockville, MD). Ketamine HCl injection, USP, was purchased from Bedford Laboratories (Bedford, OH). Xylazine, captopril, bestatin, leupeptin, LDH kits were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were of analytical grade. All surgical supplies were purchased from World Precision Instruments (Sarasota, FL). Experimental supplies were purchased from Fisher Scientific (Fair Lawn, New Jersey). Polyethylene glycol (PE) 50 tubing was obtained from Clay Adams (Parsippany, NJ). D-Mannitol-1- [^{14}C] (55 μCi/mmol) was purchased from Sigma Chemical Co. (St. Louis, MO). Universol Scintil-

lation counting cocktail was purchased from ICN (Cost Mesa, CA). Caco-2 cell culture supplies (Dulbecco's modified Eagle medium, Non essential amino acids, fetal bovine serum, L-glutamate, trypsin (0.25%)-EDTA (1 mM) and Penicillin G-streptomycin sulfate antibiotic mixture, gentamycin, and fungizone) were purchased from Gibco laboratories (Lenexa, KS). Transwell clusters, 12 well (0.4 μm pores, surface area 1 cm^2) were purchased from Corning costar (Cambridge, MA).

2.2. Methods

2.2.1. Caco-2 transport studies with ΔG

Caco-2 cell lines (passages 50–57) were seeded at a density of 65 000 cells/cm^2 on transwell costar filters (1 cm^2, 0.4 μm pore size). The cells were grown for 21 days in Dulbecco's Modified Eagle medium (1X), containing 1% penicillin-streptomycin, 2% glutamine, 10% fetal bovine serum, 1% non essential amino acids, 0.2% gentamycin, and 0.4% fungizone, under standardized conditions (95% relative humidity, 5% CO_2, 37 °C) with change of medium every other day. On the day of the experiment, the integrity of tj was determined by measuring transepithelial electrical resistance (TEER) values before the study using a Millicell®-ERS meter (Millipore Corp., Bedford, MA) with chopstick electrodes.

Caco-2 cells were preincubated with phosphate buffer saline (PBS) or Δ*G* (0, 50, 80, or 100 μg/ml) for 30 min before the addition of [^{14}C]mannitol (9.1 μM, 0.5 μCi/ml) to the apical chamber. At the end of the preincubation period, a mixture of [^{14}C]mannitol/PBS (control) or [^{14}C]mannitol/Δ*G* (test), at the investigated concentration, was applied to the apical chamber. Samples were collected from the receiver compartment at the following time points; 10, 20, 30, 45, 60, 75, 90, and 120 min. The measurement of radioactivity was performed using Beckman Coulter LS 6500 multi-purpose Scintillation counter.

2.2.2. Viability of Caco-2 cell monolayers

Caco-2 cell monolayers ($n = 3$) were incubated with Δ*G* (100 ug/ml) for a period of 30, 90, or 150 min. The activity of lactate dehydrogenase enzyme (LDH) was assessed as a function of time by the addition of LDH kit reagents to an aliquot of the solution in the apical chamber at the end of the incubation period following the manufacturer's instructions. Triton X-100 (1%) and PBS were used as positive and negative controls, respectively, and the absorbance was measured at $\lambda =$ 340 nm.

2.2.3. Animals

Jugular cannulated male Sprague–Dawley rats (250–275 g) were purchased from Harlan Laboratories (Indianapolis, IN). Rats were housed individually in cages and allowed to acclimate at least 2 days after arrival before the surgeries were performed. Rats were fed Rat Chow and water 'ad libitum' and maintained on a 12-h light:12-h dark cycle. The jugular vein cannula was kept patent by flushing with heparinized physiological saline and heparinized glycerol. The protocol for the animal studies was approved by the School of Pharmacy, University of Maryland IACUC.

2.2.4. Oral absorption studies with ΔG after intraduodenal dosing in rats

Jugular cannulated Sprague–Dawley rats were anaesthetized with an intraperitoneal injection of a ketamine/xylazine solution (80 mg/kg ketamine, 12 mg/kg xylazine) for at least 45 min. The duodenum was cannulated prior to the start of the study by a polyethylene (PE 50) tube. The cannula was anchored and exteriorized at the back of the neck. Perfusion solution (PBS) was infused very slowly to ensure the free flow of fluids through the intraduodenal cannula. Rats were allowed to recover from the surgery for 2 days prior starting the study.

The animals were fasted prior to the experiment, with free access to water. Rats ($n = 3$–4/group) were randomly assigned to receive the following treatments intraduodenally: (1)[^{14}C]mannitol (0.4 mM, 40 μCi/kg), (2) [^{14}C]mannitol (0.4 mM, 40 μCi/kg)/Δ*G* (417 μg/kg) or (3) [^{14}C]mannitol (0.4 mM, 40 μCi/kg)/Δ*G* (417 μg/kg)/PI. The doses of the PI administered were 30 mg/kg for captopril, 30 mg/kg for bestatin, and 67 mg/kg for leupeptin. The PI mixture was administered 5 min prior to Δ*G* and/or [^{14}C]mannitol administration. Bestatin, leupeptin and captopril were selected because of

their inhibitory effect on leucine aminopeptidase, aminopeptidase B, triaminopeptidase, angiotensin converting enzyme, serine and thiol proteases, calpain, cathepsin B, H and L and trypsin (Aoyagi and Umezawa, 1981; Knight, 1980; Kuramochi et al., 1979; Umezawa, 1976; Zimmerman and Schlaepfer, 1982; Zollner, 1993). The final solution volume administered intraduodenally was adjusted to 1 ml with PBS.

Blood samples (250 μl) were collected via the jugular cannula into heparinized syringes at: (predose) 10, 20, 30, 45, 60, 90, 120, 240, 360 min. Blood samples were centrifuged (13 000 rpm for 10 min) and plasma was obtained. Scintillation cocktail was added and samples were analyzed for radioactivity by Beckman Coulter LS 6500 multipurpose Scintillation counter. At the end of the experiment, each rat was sacrificed by CO_2 asphyxiation. The duodenum was inspected to ensure that the intraduodenal cannula was in place at the time of dosing.

2.3. Data analysis

Apparent permeability coefficients (P_{app}) were calculated according to the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{Vr}{ADo} \quad (1)$$

Where dQ/dt is equal to the linear appearance rate of mass in the receiver solution, A is the cross sectional area (1 cm^2), Do is equal to the initial amount in the donor compartment, Vr is equal to the volume of the receiver compartment (1.5 ml). All values are represented as mean and standard error of the mean of the values. The fold increase in transport, R, was calculated from the formula,

$$R = \frac{P_{app_{(+\Delta G)}}}{P_{app_{(Control)}}} \quad (2)$$

In the in vivo study, the amount of radiolabelled mannitol transported was converted to concentrations using the specific activity of the radiolabelled mannitol solution. The pharmacokinetic (PK) parameters (C_{max}, T_{max} and $AUC_{0-6\ h}$) were calculated. Data are presented as mean ± S.E.M. The fold increase, R, for the PK was calculated from the following equation:

$$R = \frac{PK_{parameter_{(\Delta G+/-PI)}}}{PK_{parameter_{(control)}}} \quad (3)$$

Permeability coefficients and PK were statistically compared with ANOVA using Dunnett's post-hoc analysis and statistical significance was set at $P < 0.05$.

3. Results

3.1. Caco-2 transport studies with ΔG

The permeability of mannitol alone and in the presence of ΔG was determined to assess the ability of this protein to effectively open tj. Caco-2 cells were preincubated with ΔG (0, 50, 80, or 100 μg/ml) for 30 min before the addition of [^{14}C]mannitol (9.1 μM, 0.5 μCi/ml) to the apical chamber. The mean (±S.E.M.) [^{14}C]mannitol apparent permeability coefficients observed after incubation with 0, 50, 80, and 100 μg/ml ΔG were 3.5 (±0.4), 4.17 (±0.27), 4.33 (±0.61), and 9.94 (±0.24) × 10^{-6} cm/s. The increase in Papp after the 100 μg/ml ΔG was found to be significantly different ($P < 0.05$) from the control treatment. Fig. 1 shows the percent fold increase in mannitol P_{app} in the presence of ΔG 0, 50, 80, and 100 μg/ml. The fold increase associated with the ΔG treatments ranged from 1.20 to 2.84.

To determine if ΔG was cytotoxic, Caco-2 cell monolayers were incubated with ΔG (100 μg/ml) for a period of 30, 90, or 150 min and LDH activity was measured at the end of each incubation interval. Table 1 presents the LDH results after Caco-2 cell monolayer incubation with ΔG, PBS and TritonX-100. LDH activity in Caco-2 cells incubated with ΔG for 30, 90, and 150 min were not significantly different from control (PBS) incubation, however, both ΔG and PBS incubation were significantly different ($P < 0.05$) from Triton X-100. These results suggest that at a concentration of 100 μg/ml, ΔG is not cytotoxic.

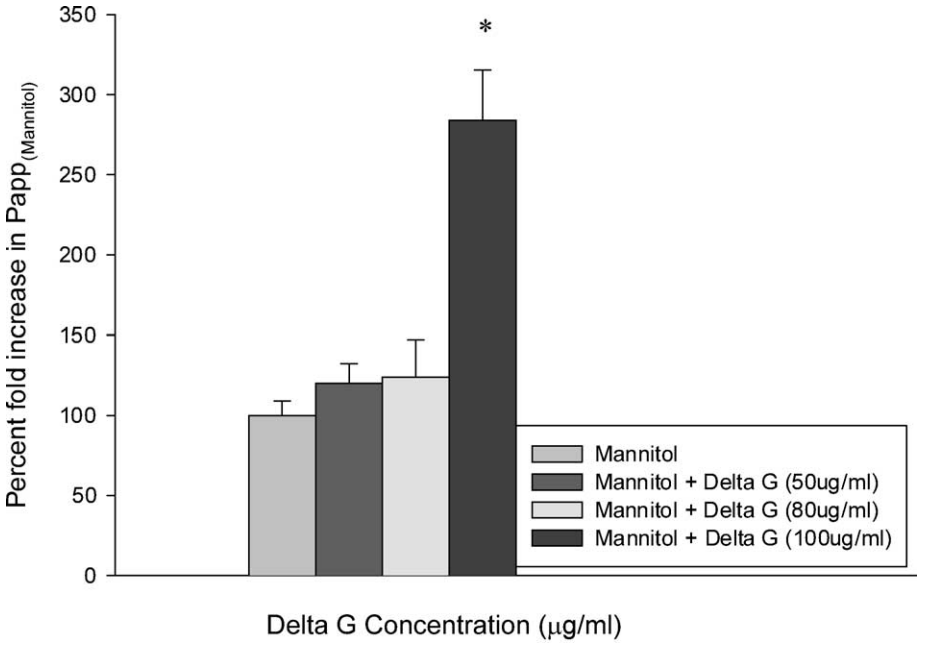

Fig. 1. The percent fold enhancement in the apparent permeability coefficient (Papp) of [^{14}C]mannitol in the presence of ΔG at 0, 50, 80, and 100 μg/ml across Caco-2 cell monolayers ($n = 3$). * Significant at $P < 0.05$ compared with mannitol alone. Papp of mannitol was 3.5×10^{-6} cm/s, TEER > 350 Ω cm^2. Data presented as mean (±S.E.M.).

Table 1
The LDH assay results ($n = 3$–6) for ΔG (100 μg/ml) compared with PBS (negative control) and Triton X-100 (positive control) with the percent of LDH activity for the different treatments relative to Triton X-100 at 30, 90, and 150 min

Incubation period	Treatment	% (±S.E.M.) LDH leakage
30	PBS	7.84 (0.60)
	ΔG	1.68 (0.50)
	Triton X-100	100 (19.5)[a]
90	PBS	4.42 (0.55)
	ΔG	3.38 (1.30)
	Triton X-100	100 (9.1)[a]
150	PBS	2.03 (0.26)
	ΔG	2.86 (1.03)
	Triton X-100	100 (4.9)[a]

[a] Significant at $P < 0.05$ compared with PBS.

3.2. Oral absorption studies with ΔG after intraduodenal dosing in rats

Male Sprague–Dawley rats cannulated in the jugular vein and duodenum randomly received [^{14}C]mannitol, [^{14}C]mannitol/ΔG (417 μg/kg), or [^{14}C]mannitol/ΔG (417 μg/kg)/PIs treatments. Table 2 lists the bioavailability parameters for the different treatments. The bioavailability parameters, C_{max} (3.8×10^{-4} vs. 4.4×10^{-4} mM) and $AUC_{0-6\ h}$ (0.096 vs. 0.088 mM min), obtained after [^{14}C]mannitol and [^{14}C]mannitol/ΔG administration, respectively, were not statistically different. Control mannitol AUC was comparable with previous reported values (Terao et al., 2001). When PI were co-administered with ΔG there was a statistically significant increase in both C_{max} (7.6×10^{-4} mM) and $AUC_{0-6\ h}$ (0.25 mM min) for mannitol. The C_{max} and $AUC_{0-6\ h}$ fold-enhancement of the [^{14}C]mannitol/ΔG /PI treatment were 2 and 2.57 folds versus control ([^{14}C]mannitol), respectively. This enhancement was also apparent in the cumulative mannitol absorption profiles when comparing the [^{14}C]mannitol, [^{14}C]mannitol/ΔG and [^{14}C]mannitol/ΔG/PI as shown in Fig. 2. Statistically higher cumulative absorption was noted for [^{14}C]mannitol/ΔG /PI treatment after dosing for each sampled time point.

Table 2
Mean (±S.E.M.) PK parameters in male Sprague–Dawley rats after intraduodenal administration of [^{14}C]mannitol (40 μCi/kg)/Δ*G* (417 μg/kg), [^{14}C]mannitol (40 μCi/kg)/Δ*G* (417 μg/kg)/PI groups and [^{14}C]mannitol (40 μCi/kg)

Treatment	C_{max} (mM) ($\times 10^{-4}$)	T_{max} (min)	$AUC_{(0-6\ h)}$ (mM min)
[^{14}C] mannitol	3.8 (0. 3)	100 (6.3)	0.096 (0.006)
[^{14}C] mannitol/Δ*G*	4.4 (0. 07)	120 (0)	0.088 (0.002)
[^{14}C]mannitol/Δ*G*/PI	7.6[a] (0. 77)	75[a] (15)	0.247[a] (0.003)

[a] Significant at $P < 0.05$ compared with control. PI administered were bestatin (30 mg/kg), captopril (30 mg/kg) and leupeptin (67 mg/kg).

4. Discussion

Low oral bioavailability of effective therapeutic agents continues to be a major problem that affects the drug development process. In some cases, poor oral absorption may be the deciding factor on whether or not a potent agent is developed. Theoretically, absorption enhancement allows for an increase in the amount of drug that can potentially reach the systemic circulation after oral dosing. Absorption enhancers, including surfactants, fatty acids, glycerides, acyl carnitines and chitosan derivatives, act by either disruption of the cell membrane or modulation of the tj (Aungst, 2000). In general, the optimal absorption enhancer should possess the following qualities; its effect should be reversible, it should provide a rapid permeation enhancing effect on the intestinal cellular membrane, it should be non-cytotoxic at the effective concentration level without deleterious and/or irreversible effects on the cellular membrane or cytoskeleton of the tj.

Zot, a toxin produced by the bacterial strain V. cholerae, is capable of binding to a specific receptor on the luminal surface of the intestine and reversibly opening the tj between intestinal

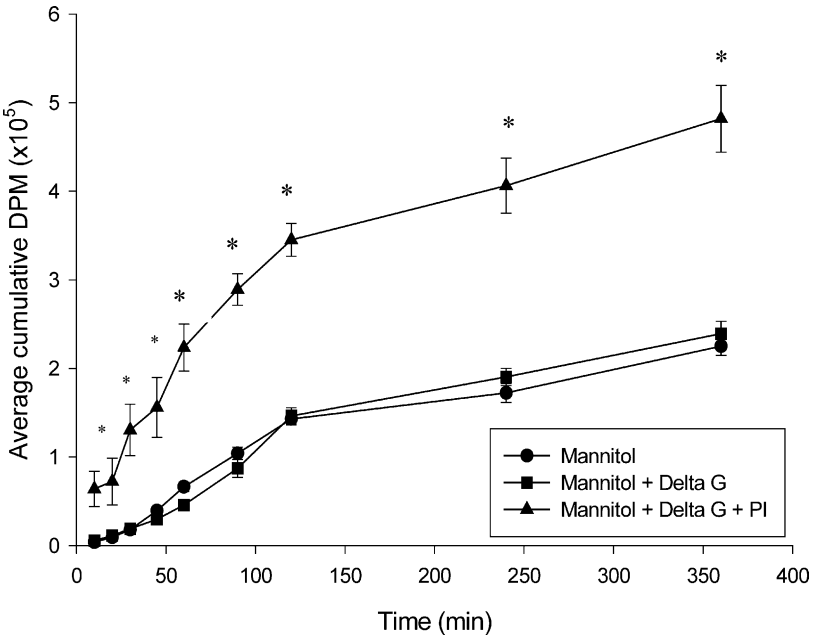

Fig. 2. The Mean±S.E.M. cumulative oral absorption of [^{14}C]mannitol after control (●), Δ*G* (■), and Δ*G*/PI (▲) intraduodenal dosing to male Sprague–Dawley rats ($n = 3$–4/group). PI administered were bestatin (30 mg/kg), captopril (30 mg/kg) and leupeptin 67 (mg/kg). * Significant at $P < 0.05$ compared with mannitol alone.

epithelial cells (Fasano et al., 1991, 1995; Fasano and Uzzau, 1997; Fasano et al., 1997; Cox et al., 2001, 2002). Recent studies identified a 12 kDa fragment of Zot, referred to as ΔG, which possesses the intrinsic activity of modulating tj, thus, increasing the paracellular transport of drugs (Di Pierro et al., 2001). In the study reported herein, ΔG was capable of increasing the transport of mannitol by 1.24 and 2.84-fold at 80 and 100 μg/ml, respectively, in comparison with the trans-epithelial transport of mannitol in absence of ΔG. Previous reports with Zot (4 μg/ml) displayed a 10-fold increase in mannitol permeability (Cox et al., 2001, 2002). In comparison, the *N*-trimethyl chitosan and chitosan derivatives have displayed fold enhancements with mannitol ranging from 11 to 60 using Caco-2 cells (Kotze et al., 1997, 1998, 1999) An approximate 3-fold increase in mannitol transport was observed with palmitoyl carnitine (1 mM) across Caco-2 cells (Duizer et al., 1998). It would appear that the absorption enhancing effect of Zot/ΔG, at the concentrations studied, is less than that of chitosan derivatives but comparable with other enhancers such as palmitoyl carnitine. However, the results that we report (2-fold enhancement) would be a significant increase in bioavailability for compounds such as HIV PI ($F \sim = 5–15\%$) or other low bioavailable agents. A 2-fold increase in bioavailability would allow for a higher systemic bioavailability and may decrease the total amount of drug required for effective therapy.

In addition to displaying the ability to enhance paracellular transport, absorption enhancers should be non-toxic. The opening of tj with Zot has been shown to be non-toxic in cell and tissue culture experiments (Fasano et al., 1991; Cox et al., 2002). In this study, Caco-2 cells were incubated with ΔG (100 μg/ml) over a 150 min interval and ΔG was found not to be cytotoxic at the concentration examined. Other absorption enhancers such as *N*-Trimethyl chitosan displayed no toxicity to Caco-2 cells when tested by the Trypan Blue Dye exclusion test (Kotze et al., 1997). However, for chitosan chloride (0.1% w/v), a slight increase in the extracellular LDH activity after 30 min was reported along with a slight perturbation in the plasma membrane (Dodane et al., 1999). Sodium deoxycholate (0.1% w/v) and sodium caprate (0.5% w/v), two absorption enhancers causing 80% TEER decline, were significantly toxic to the plasma and nuclear membranes when tested by Trypan Blue Dye exclusion, protein release, the neutral-red assay, and the DNA-propidium iodide (Sakai et al., 1998). When tested with LDH assay, a lytic effect was reported with palmitoyl carnitine to almost the same extent as observed with Triton X-100. The leakage of the enzyme was significantly higher at palmitoyl carnitine concentrations above 0.2 mM in Caco-2 cells, whereas, mannitol absorption enhancement was achieved at a concentration of 0.4 mM (Duizer et al., 1998). Dodecylphosphocholine did not show extensive damage to the cell membrane at the concentration, which caused decrease in TEER and increase in mannitol transport when tested by MTT assay (Liu et al., 1999). It would appear from these studies that ΔG, as with some other absorption enhancers, might be safe at the effective concentrations, which leads to opening of the tj. As stated, the absorption enhancing effect of Zot/ΔG, at the concentrations studied, appears to be less than some absorption enhancers. Nonetheless, it should be noted that Zot/ΔG does not display the toxicity observed with other absorption enhancers. The lack of toxicity along with its reversibility supports the potential of Zot/ΔG as a viable drug delivery technology (Fasano et al., 1991; Cox et al., 2002).

The goal of absorption enhancers is to significantly increase the oral absorption of agents that display low bioavailability. Our studies examined the absorption characteristics of a paracellular molecular weight marker, mannitol, after intra-duodenal co-administration with ΔG. Based on the low molecular weight and structure of ΔG, PI were included in one of the treatment arms to minimize enzymatic degradation secondary to proteases or peptidases. After oral administration, ΔG displayed high intrinsic biological activity after pre-administration with PI. An approximate 2-fold higher C_{max} and a 2.75-fold higher $AUC_{0-6\ h}$ were observed after co-administration of ΔG with mannitol and PI. When mannitol was administered with only ΔG, no significant differences were observed for the PK parameters. ΔG (12 kDa) is

apparently more sensitive to enzymatic degradation as compared with Zot and, thus, PI are needed to ensure activity, an approach that was found not to be necessary with Zot (45 kDa). It is hypothesized that the tertiary structure of Zot minimized its enzymatic degradation in both the cell culture and animal experiments. Due to the metabolism of ΔG, it would seem reasonable that a higher concentration would be required to elicit a comparable biological activity.

Studies in our laboratory have also evaluated the effect of both Zot and ΔG on enhancing permeability across the blood-brain barrier (BBB). Based on our results, we have observed a BBB permeation enhancing effect with both Zot and ΔG. However, in both in vitro and in vivo studies, the effect appears to be transient and reversible (Karyekar et al., 2002a,b). A study examining in vivo transport of sucrose found that at 5 min after dosing of 400 and 800 μg/kg, there were 1.2–1.5 folds higher sucrose levels in the CNS (Karyekar et al., 2002a). However, this effect returns to baseline after 30 min. These studies along with the results of the current paper suggest that after oral dosing, ΔG (417 μg/kg) effect would be localized at the GIT and will not lead to significant effects at the BBB level.

In summary, this study provided information on the effectiveness of the active fragment of Zot, ΔG, in enhancing in vitro permeability and in vivo oral absorption. The enhancing effects observed in vitro were found to be significantly higher than our control, however, its effect as compared with reported absorption enhancers is much lower. The in vivo oral absorption study illustrates ΔG's potential usefulness in enhancing oral drug delivery. Formulation of the peptide to minimize the adverse effects related to the physiology of the GI tract, will be useful when ΔG proceeds to that stage. The metabolism of ΔG will be controlled by formulation techniques (e.g. enteric coating methods to prohibit acid degradation in the stomach), when it reaches clinical drug development. As such, studies are underway to examine higher doses of ΔG, pre-administration of ΔG versus co-administration and chemical modification of ΔG to enhance in vivo stability.

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