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Research paper

Enhanced nasal absorption of hydrophilic markers after dosing with AT1002, a tight junction modulator

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Abstract

AT1002 is a six-mer synthetic peptide, H-FCIGRL-OH, that retains the delta *G* and Zot biological activity of reversibly opening tight junctions and increases the paracellular transport of drugs. The objective of this study was to evaluate the possible use of AT1002 in enhancing the nasal availability of macromolecules using large paracellular markers as model agents. Male Sprague–Dawley rats cannulated in the jugular vein were randomly assigned to receive radiolabelled paracellular markers, [^{14}C]PEG4000 or [^{14}C]inulin, with/without AT1002, for each intranasal study. The plasma concentration of PEG4000 with AT1002 (10 mg/kg) was significantly higher than that from PEG4000 control over 360 min following intranasal administration. The $AUC_{0-360\ min}$ and C_{max} from the PEG4000/AT1002 (10 mg/kg) treatment were statistically ($p < 0.05$) increased to 235% and 357%, of control, respectively. When inulin was administered with AT1002 (10 mg/kg), the plasma concentration was significantly higher ($p < 0.05$) than control over 360 min, and increases ($p < 0.05$) of 292% and 315% for $AUC_{0-360\ min}$ and C_{max} over control were observed, respectively. AT1002 significantly increased the nasal absorption of molecular weight markers, PEG4000 and inulin. This study suggests that AT1002 may be used to enhance the systemic availability of macromolecules when administered concurrently.

Keywords: AT1002; PEG4000; Inulin; Intranasal administration; Zot

1. Introduction

The nasal mucosa has become an increasingly attractive site for the systemic administration of therapeutic agents. It has a relatively high surface area (160–180 cm^2, epithelium covered with microvilli), good vascularity, and lower enzymatic activity than the gastrointestinal tract [1–3]. Further, the nasal pathway is responsive to reversible modification using permeation enhancing formulations for safe and effective drug transport [1–3]. Thus, it has the potential to provide for a more rapid and complete drug absorption into systemic circulation and to allow for increased bioavailability. Nonetheless, many therapeutically active agents experience low bioavailability after oral or nasal administration due to poor permeation. An approach to improve the absorption of drugs may be to reversibly loosen the epithelial tight junctions so as to enhance their paracellular transport. Absorption enhancers are capable of improving the transport/absorption of low bioavailable drugs. Some absorption enhancers specifically loosen tight junctions and enhance paracellular permeability. Surfactants, chitosans, and calcium chelators are among the most well known absorption enhancers [4–6]. However, they may cause serious damage to epithelial integrity, morphology, and function [6–8].

As stated, a viable approach to enhance nasal drug delivery may be to exploit the physiological regulation of intercellular tight junctions in order to enhance paracellular drug transport. Reports have indicated that tight junctions are not static, rather they are dynamic and can be

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manipulated to enhance paracellular transport by a protein referred to as Zonula Occludens Toxin (Zot) [9–11]. Zot, a 45-kDa protein, elaborated from *Vibrio cholera* exerts its permeating effect by mimicking a eukaryotic analogue, referred to as Zonulin which is in charge of modulation of intercellular tight junctions [10,11]. Since Zot exploits the physiologic Zonulin pathway, it reversibly opens intercellular tight junctions after binding to the Zonulin receptor present on the surface of epithelial and endothelial cells [10,11].

In vitro and *in vivo* studies have shown that Zot significantly enhances the intestinal absorption of a variety of structurally dissimilar co-administered agents [11–17]. A smaller 12-kDa fragment of Zot, named delta *G* (Δ*G*), has been identified as its biologically active fragment [12]. Δ*G* significantly increased the *in vitro* transport and the *in vivo* absorption of low bioavailable therapeutic agents [15–17]. Even though promising results were obtained with the use of Δ*G*, the isolation/purification process does not yield sufficient amounts to allow for conduct of *in vivo* studies at higher doses. In an attempt to resolve this issue, several modifications of Δ*G* sequence were evaluated [12]. It was noted that the amino acid sequence 'IGRL', identified as part of the binding domain in Zot/delta *G*, is the same as that observed in the PAR-2 agonists (fur-LIGRL, FCIGRL) [12]. PAR-2 agonists have been reported to increase paracellular permeability [18]. As such, this led to the hypothesis that Zot/Δ*G* may act at these receptors and produce an increase in paracellular permeability. Recently, the sequence of AT1002, a six-mer synthetic peptide H-FCIGRL-OH, was isolated from the active fragment of Δ*G*, subsequently synthesized and assumed to retain Δ*G* or Zot permeating effect on intercellular tight junctions. FCIGRL is identical to the AA residues 288–293 of Zot and the XX-IGRL sequence is part of the putative receptor binding motif of Zot/Δ*G*, thus the peptide was assumed to have similar properties as Zot/Δ*G* (Fig. 1).

The purpose of this study was to evaluate the biological ability of AT1002 to enhance the systemic bioavailability of molecular weight hydrophilic markers (i.e., PEG4000, inulin) after intranasal administration. Increases in the absorption of molecular weight markers would suggest that AT1002 could be used to improve the nasal absorption of therapeutic macromolecules, such as peptides, or adjuvants for the nasal delivery of vaccines.

2. Materials and methods

2.1. Materials

AT1002 was purchased from Alba Therapeutics Corporation (Baltimore, MD). [^{14}C]PEG4000 (13.4 mCi/g, 50 μCi/ml) and [^{14}C]inulin (1.2 mCi/g, 100 μCi/ml) were purchased from Amersham Radiochemicals (Piscataway, NJ) and American Radiolabeled Chemicals, Inc. (St. Louis, MO), respectively. Dextrose injection solution was purchased from Baxter Healthcare Corporation (Deerfield, IL). Ketamine HCl injection, USP, was purchased from Bedford Laboratories (Bedford, OH). Xylazine was purchased from Sigma Chemical Co. (St. Louis, MO). All surgical supplies were purchased from World Precision Instruments (Sarasota, FL). All chemicals were of analytical grade.

2.2. Animals

Male Sprague–Dawley rats (280–300 g) were purchased from Harlan Laboratories (Indianapolis, IN). Rats were housed individually in cages and allowed to acclimate at least 2 days after arrival. Rats were fed Rat Chow (Harlan Teklad, Madison, WI) and water *ad libitum* and maintained on a 12-h light: 12-h dark cycle. The protocol for the animal studies was approved by the School of Pharmacy, University of Maryland IACUC.

2.3. Preparation of PEG4000 and inulin formulations

PEG4000 solutions for intranasal administration were obtained by mixing [^{14}C]PEG4000 with 5% dextrose solution to form the PEG4000 control dosing solution. After addition of an appropriate amount of AT1002 to the control, the AT1002 treatments were prepared as 5% dextrose-[^{14}C]PEG4000 (15 μCi/kg) with AT1002 (5 and 10 mg/kg) solutions. The dosing control solution for inulin was prepared by mixing [^{14}C]inulin with 5% dextrose and the treatment dosing solutions were prepared by combining 5% dextrose-[^{14}C]inulin (30 μCi/kg) with AT1002 (5 and 10 mg/kg). The control and treatment solutions were prepared immediately prior to dosing.

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1   MSIFIHHGAP  GSYKTSGALW  LRLLPAIKSG  RHIITNVRGL  NLERMAKYLK  MDVSDISIEF
61  IDTDHPDGRL  TMARFWHWAR  KDAFLFIDEC  GRIWPPRLTA  TNLKALDTPP  DLVAEDRPES
121 FEVAFDMHRH  HGWDICLTTP  NIAKVHNMIR  EAAEIGYRHF  NRATVGLGAK  FTLTTHDAAN
181 SGQMDSHALT  RQVKKIPSPI  FKMYASTTTG  KARDTMAGTA  LWKDRKILFL  FGMVFLMFSY
241 SFYGLHDNPI  FTGGNDATIE  SEQSEPQSKA  TAGNAVGSKA  VAPASFG FCIGRL CVQDGFV
301 TVGDERYRLV  DNLDIPYRGL  WATGHHIYKD  KLTVFFETES  GSVPTELFAS  SYRYKVLPLP
361 DFNHFVVFDT  FAAQALWVEV  KRGLPIKTEN  DKKGLNSIF
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Fig. 1. Amino acid sequence of Zot, delta *G*, and AT1002. Bold (265–399) is delta *G*, the biologically active fragment of Zot, and box (288–293) is AT1002, active domain of Zot.

2.4. Intranasal administration with AT1002 to Sprague–Dawley rats

Jugular vein cannulated Sprague–Dawley rats were anaesthetized with an intra-muscular injection of a ketamine/xylazine solution (80 mg/kg ketamine, 12 mg/kg xylazine). The following formulations were administered per radiolabelled compound intranasally to rats: (1) 5% dextrose solution of PEG4000 (15 μCi/kg), (2) 5% dextrose solution of PEG4000 (15 μCi/kg) and AT1002 (5 mg/kg), (3) 5% dextrose solution of PEG4000 (15 μCi/kg) and AT1002 (10 mg/kg) for the PEG4000 studies, and (1) 5% dextrose solution of inulin (30 μCi/kg), (2) 5% dextrose solution of inulin (30 μCi/kg) and AT1002 (5 mg/kg), (3) 5% dextrose solution of inulin (30 μCi/kg) and AT1002 (10 mg/kg) for the inulin studies. The rats were manually restrained in a supine position and the head was tilted back slightly while the formulation was instilled into the nostril. Doses were administered with a maximum administrated volume (50 μl/nostril, [19,20]) via a micropipette and disposable tip, considering the solubility of AT1002. Extreme care was taken during dosing to avoid tissue damage potentially resulting from contact of intranasal mucosa.

2.5. Sample analysis

Blood samples (250 μl) were drawn via the jugular cannula into heparinized syringes at 0, 10, 20, 30, 40, 60, 90, 120, 240, and 360 min into polypropylene tubes, centrifuged (13,000 rpm for 10 min) immediately and plasma was obtained. Scintillation cocktail was added and plasma samples were analyzed for radioactivity using Beckman Coulter LS 6500 multi-purpose Scintillation counter.

2.6. Data analysis

The amount of each radiolabelled compound absorbed was converted to concentrations using the specific activity of the radiolabelled stock solution. The pharmacokinetic parameters were calculated using the non-compartmental analysis Winnolin® pharmacokinetic software package (Pharsight Inc., Mountain view, CA). The area under the plasma concentration–time curve (AUC_{0-t}) was calculated using the linear trapezoidal method. Peak plasma concentration of each compound (C_{max}), time to reach the peak (T_{max}), mean residence time (MRT), clearance normalized by bioavailability (Cl/F), and distribution volume normalized by bioavailability (V_d/F) following intranasal administration were determined from the observed data. The percent enhancement ratio, ER (%), for the pharmacokinetic parameters was calculated from the formula

$$\mathrm{ER}\ (\%) = \frac{\mathrm{PK}_{\mathrm{parameter\cdot(treatment)}}}{\mathrm{PK}_{\mathrm{parameter\cdot(control)}}} \times 100 \quad (1)$$

All data were expressed as the mean and standard error of the mean of the values (mean ± SEM). The statistical significance of differences between treatments and/or controls was evaluated using the Student's *t*-test and ANOVA followed by Dunnett's post-hoc test where appropriate (SPSS for Windows versions 12.0., SPSS Inc., Chicago, IL) and the level of significance was set at $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Intranasal administration PEG4000 with AT1002 to Sprague–Dawley rats

Male Sprague–Dawley rats cannulated in the jugular vein were randomly assigned to receive radiolabelled paracellular markers with/without AT1002. The pharmacokinetic profiles were characterized for each group and the pharmacokinetic parameters were calculated using non-compartmental analysis. Fig. 2 illustrates the mean plasma concentration versus time profiles for [^{14}C]PEG4000 in rats following intranasal administration of PEG4000, PEG4000/AT1002 (5 mg/kg), and PEG4000/AT1002 (10 mg/kg). Each PEG4000 plasma concentration from the AT1002 10 mg/kg dose was found to be higher ($p < 0.05$) than control over a 360 min time period (Fig. 2), indicating a significant enhancement in intranasal absorption of PEG4000 by AT1002. At 20 min post-dosing, the enhancement ratio for the plasma concentration of PEG4000/AT1002 (10 mg/kg) was 439% higher ($p < 0.05$) than control. The pharmacokinetic profile for PEG4000 when co-administered with AT1002 (5 mg/kg) displayed enhancement at multiple times in comparison to the control plasma concentrations. As shown in Table 1, the administration of PEG4000 with AT1002 (10 mg/kg) produced a statistically significant increase in $AUC_{0-360\ min}$ (15.74 ± 2.32 min μg/ml) as compared to control (6.70 ± 0.22 min μg/ml) along with an enhancement ratio (ER %) of 235%. The C_{max} (95.46 ± 14.63 ng/ml) achieved after

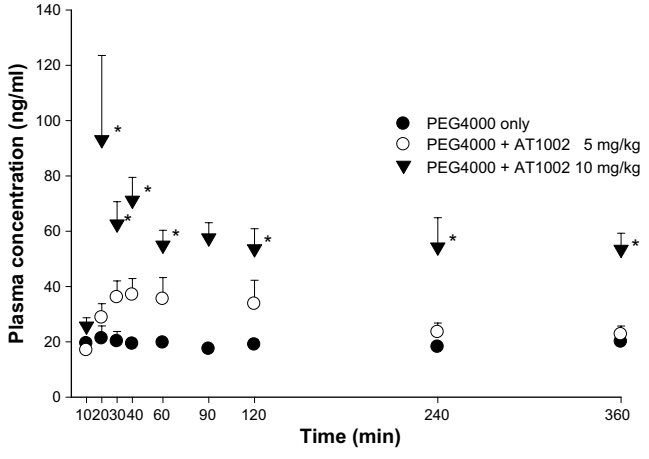

Fig. 2. Average plasma concentration versus time profile for [^{14}C]PEG4000 in jugular cannulated Sprague–Dawley rats following the intranasal administration of treatments. i.e., PEG4000 (●), PEG4000/AT1002 (5 mg/kg) (○), and PEG4000/AT1002 (10 mg/kg). Each data point represents mean ± SEM of 4–5 rats. *Significant at $p < 0.05$ compared to PEG4000 only of each same time point.

Table 1
Mean ± SEM bioavailability parameters for paracellular markers ([^{14}C]PEG4000, 15 μCi/kg; [^{14}C]inulin, 30 μCi/kg) after intranasal administration to jugular vein cannulated Sprague–Dawley rats ($n = 4$–5) alone and/or with AT1002

Treatment	$AUC_{0\text{-}360\ min}$ (min μg/ml)	C_{max} (ng/ml)	T_{max} (min)	MRT (min)	V_d/F (l/kg)	Cl/F (l/min/kg)
[^{14}C]PEG4000 only (control)	6.70 ± 0.22	26.72 ± 3.12	35.00 ± 8.66	182.81 ± 3.12	47.75 ± 2.69	21.85 ± 6.92
[^{14}C]PEG4000 + AT 1002 (5 mg/kg)	9.99 ± 1.71 (149%)	43.66 ± 6.82 (163%)	46.00 ± 6.00 (131%)	167.52 ± 11.30 (92%)	23.10 ± 3.20 * (48%)	68.08 ± 8.64 * (312%)
[^{14}C]PEG4000 + AT 1002 (10 mg/kg)	15.74 ± 2.32 * (235%)	95.46 ± 14.63 (357%)	32.50 ± 4.79 (93%)	145.63 ± 25.34 (80%)	14.37 ± 0.23 * (30%)	18.92 ± 5.38 (87%)
[^{14}C]inulin only (control)	203.43 ± 33.94	656.45 ± 100.68	47.50 ± 7.50	179.88 ± 1.70	40.65 ± 5.69	26.00 ± 10.52
[^{14}C]inulin + AT 1002 (5 mg/kg)	224.15 ± 56.00 (110%)	828.28 ± 259.40 (126%)	152.00 ± 53.67 (320%)	183.01 ± 5.22 (102%)	34.70 ± 7.45 (85%)	39.69 ± 12.29 (153%)
[^{14}C]inulin + AT 1002 (10 mg/kg)	594.86 ± 168.91 * (292%)	2065.73 ± 517.80 * (315%)	167.50 ± 80.35 (353%)	189.04 ± 8.23 (105%)	15.35 ± 2.24 * (38%)	17.98 ± 1.41 (69%)

* Significant ($p < 0.05$) compared to PEG4000 and inulin only control, respectively.

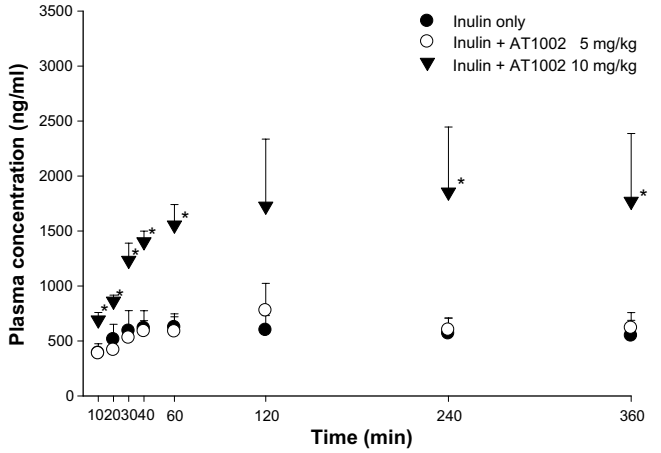

Fig. 3. Average plasma concentration versus time profile for [^{14}C]inulin in jugular cannulated Sprague–Dawley rats following the intranasal administration of treatments, i.e., inulin (●), inulin/AT1002 (5 mg/kg) (○), and inulin/AT1002 (10 mg/kg). Each data point represents mean ± SEM of 4–5 rats. *Significant at $p < 0.05$ compared to inulin only of each same time point.

AT1002 (10 mg/kg) was also higher ($p < 0.01$) than control (26.72 ± 3.12 ng/ml) as indicated by an ER of 357%. PEG4000/AT1002 (5 mg/kg) led to a 149% increase in the $AUC_{0\text{–}360\ min}$ (9.99 ± 1.71 min μg/ml) and 163% (43.66 ± 6.82 ng/ml) increase in C_{max} with non-significant differences as compared to the PEG4000 control.

3.2. Intranasal administration of inulin with AT1002 to Sprague–Dawley rats

The mean plasma concentration versus time profiles for [^{14}C]inulin in male Sprague–Dawley rats following the intranasal administration of inulin and inulin/AT1002 (5 or 10 mg/kg) are shown in Fig. 3. The inulin plasma versus time profile after AT1002 (10 mg/kg) was significantly higher than control over 360 min. At 240 min post-dosing, inulin levels associated with the AT1002 (10 mg/kg) dose were 328% higher ($p < 0.05$) than control. Significant enhancement in the rate and extent of absorption was observed with 292% increase in $AUC_{0\text{–}360\ min}$ (594.86 ± 168.91 min μg/ml) and 315% increase in C_{max} (2065.73 ± 517.80 ng/ml) compared to control ($AUC_{0\text{–}360\ min}$, 203.43 ± 33.94 min μg/ml; C_{max}, 656.45 ± 100.68 ng/ml). The enhancement for the AT1002 (5 mg/kg) treatment on $AUC_{0\text{–}360\ min}$ (110%) and C_{max} (126%) was not statistically different as compared to inulin alone.

4. Discussion

The low bioavailability after oral dosing for potent therapeutic drugs, peptides or macromolecules continues to be an impediment in the drug development process. The nasal route for systemic drug delivery has been the focus of significant research as an alternative to oral drug administration. Intranasal delivery is a convenient, reliable, rapid, and

non-invasive delivery approach for low molecular weight compounds. Studies have demonstrated that molecular size and weight are determining factors that control efficient nasal delivery [1–3]. For example, the increased absorption of larger peptides is limited by their molecular weight, surface charge and enzymatic degradation [21]. A strategy used to minimize these issues is the co-administration of therapeutic macromolecule with absorption enhancers [4,21]. Hence, the use of the AT1002 technology as an adjuvant to increase the nasal delivery of peptides and vaccines seems to have significant potential in light of its mechanism of modulating tight junctions [22].

The objective of this study was to evaluate the ability of AT1002 to enhance the systemic bioavailability of the hydrophilic markers, inulin and PEG4000 after nasal administration. AT1002 is an active fragment of Zot and ΔG, representing the amino acid sequence that presumably retains their permeating effects (Fig. 1) [13–17]. Both Zot and ΔG have been shown to bind to a surface receptor and trigger a cascade of intracellular events mediated by protein kinase C with polymerization of soluble G-actin, subsequent displacement of proteins from the junctional complex, and loosening of tight junctions [12,23]. Zot has been reported to be capable of reversibly opening the tight junctions between cells and increasing the paracellular transport of many drugs in a non-toxic manner [10,11,13–17]. Intensive investigation of the biological activity of Zot as an absorption enhancer was triggered by reports of effective oral administration of insulin with Zot in diabetic rats [11]. Studies in our laboratory have shown that Zot ($0.22–0.89 \times 10^{-10}$ mol/ml) enhances the intestinal transport of macromolecules of varying molecular weight (mannitol, PEG4000, inulin, and sucrose) or low bioavailability compounds (paclitaxel, acyclovir, cyclosporin A, and doxorubicin) across Caco-2 cell monolayers in a non-toxic manner [13–17]. In addition, ΔG ($0.83–1.50 \times 10^{-8}$ mol/ml) significantly increased the *in vitro* transport of paracellular markers (mannitol, PEG4000, and inulin) in a non-toxic manner and the *in vivo* absorption of low bioavailable therapeutic agents (cyclosporin A, ritonavir, saquinavir, and acyclovir) and mannitol over a dose range of $3.48–6.00 \times 10^{-8}$ mol/kg [13–17]. AT1002 is a six-mer modified synthetic peptide H-FCIGRL-OH that was isolated on the basis of the biological activities of Zot and ΔG and is assumed to retain their tight junction modulating activity [12].

Recently, this laboratory examined the effect of AT1002 on the oral administration of the low bioavailable agent, cyclosporin A (CsA) [22]. A significant enhancement in the absorption of CsA was observed in this study after dosing with AT1002, suggesting that the six-mer peptide retained the Zot domain directly involved in the permeating effect. AT1002 significantly ($p < 0.05$) increased the $AUC_{0–120\ min}$ of CsA over a range of 164–214%, and C_{max} over a range of 177–256% after a dose of 10 (1.41×10^{-5} mol/kg) and 40 mg/kg (5.65×10^{-5} mol/kg). It should be noted that protease inhibitors (a mixture of bestatin, captopril, and leupeptin) were used to minimize enzymatic degradation of AT1002 in these studies. Further, we evaluated the biological activity of a peptide with a similar structure to AT1002 and did not observe enhancement after co-administration with cyclosporin A absorption [22].

In this study, a significant enhancement in the nasal absorption of both paracellular markers, PEG4000 and inulin (mw 5000 Da), was observed after dosing with AT1002. The plasma concentration of PEG4000 when administered with AT1002 (10 mg/kg, 1.41×10^{-5} mol/kg) was 439% higher ($p < 0.05$) than the control at 20 min. Moreover, $AUC_{0–360\ min}$ and C_{max} for PEG4000 was increased by 235% and 357%, respectively, over the control treatment in the presence of AT1002 (10 mg/kg). When inulin was administered with AT1002 (10 mg/kg), the plasma concentration of inulin was 328% higher ($p < 0.05$) than that for the inulin control at 240 min. Further, the pharmacokinetic profile displayed significant increases in both the extent and rate of inulin absorption for a period of 6 h with 292% increase in $AUC_{0–360\ min}$ and 315% increase in C_{max} for the AT1002 treatment versus control.

The extent of nasal absorption observed for inulin was found to be higher (292%) as compared to PEG4000 (235%). Previous *in vitro* Caco-2 studies with Zot also noted a significant increase in the permeability of inulin from 0.7×10^{-6} cm/s (control) to 4.37×10^{-6} cm/s (4 μg/ml Zot) compared to PEG4000 from 0.15×10^{-6} cm/s to 0.33×10^{-6} cm/s [13]. Studies have suggested that inulin has a more compact cylindrical configuration relative to the extended chain configuration associated with PEG4000 [24,25]. Size exclusion chromatography analysis retention times of 44.8, 48.2, and 49.6 min for PEG4000, inulin and PEG900, respectively. These results suggest that despite the higher molecular weight of inulin, it has a molecular configuration that contributes to its retardation in size exclusion chromatography [26]. In addition, it has been reported that there are low molecular weight polysaccharide impurities in inulin which may influence its transport. Taken together, the higher permeability and/or nasal absorption of inulin versus PEG4000 observed in this study may be due to its compact cylindrical configuration as well as the presence of low molecular weight components.

The highest dose of AT1002, 10 mg/kg, for this study was determined based on preliminary studies addressing its solubility and in consideration of the volume limitations associated with nasal dosing to rats. The biological activity associated with the highest dose of AT1002 was found to take place 20 min after dosing and was maintained approximately for the duration of the sampling period. AT1002 produced its first significant difference in plasma PEG4000 concentrations 20 min after dosing, and this difference was maintained for 360 min. On the other hand, the most significant indication of AT1002 permeating activity was observed 240 min after inulin dosing; however, higher plasma levels were obtained 10 min after the dosing com-

pared to the control treatment. These results observed with both inulin and PEG4000 suggest that this six-mer peptide produces its biological activity promptly and continuously after dosing.

It should be noted that the enhancement of inulin and PEG4000 by AT1002 is assumed to be related to protease activated receptor-2 (PAR-2) receptor. PAR-2 agonists are six-mer peptides, with four of the amino acids being identical to that of the Zot/Zonulin receptor binding motif (XX-IGRL) [12]. This suggests that AT1002 (H-FCIGRL-OH) may possess similar biological activity at the PAR-2 receptors. The PAR-2 receptor belongs to a class of G-protein coupled receptors that are activated by cleavage of their N-terminal by a proteolytic enzyme. Studies are underway to determine definitive role of PAR-2 receptors in the biological activity of AT1002.

Previous studies were performed with epithelial cells to determine if AT1002 produced a cytotoxic response after exposure [22]. The Caco-2 epithelial cell line was used to investigate cytotoxicity and cells were exposed to AT1002, PBS (negative control) and Tritox X-100 (positive control) for up to 60 min. No significant differences were observed with the AT1002 incubations as compared to PBS, indicating that AT1002 did not produce cytotoxicity.

The promising results observed with AT1002 after intranasal dosing of high molecular weight hydrophilic markers suggest that this peptide may be a useful enhancer for peptides or as an adjuvant for nasal vaccine delivery. This study provides evidence of the effectiveness of the active modified and synthesized fragment of Zot/Δ*G*, AT1002, in enhancing the absorption of molecular weight markers (PEG4000, inulin) after intranasal administration. The permeation enhancing effect of AT1002 observed *in vivo* was found to be significantly higher than controls. The *in vivo* intranasal absorption study illustrates the peptide's potential usefulness in enhancing drug delivery, and positions it to be an effective technology for enhancing the systemic bioavailability of therapeutic agents and macromolecules.

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