Paracelluar permeation-enhancing effect of AT1002 C-terminal amidation in nasal delivery

Keon-Hyoung Song¹
Sang-Bum Kim²
Chang-Koo Shim²
Suk-Jae Chung³
Dae-Duk Kim³
Guang J Choi¹
Chul-Hyun Kim³
Kiyoun Kim⁴

¹Department of Pharmaceutical Engineering, Soonchunhyang University, Asan, Republic of Korea; ²College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea; ³Department of Sports Medicine, Soonchunhyang University, Asan, Republic of Korea; ⁴Department of Medical Biotechnology, Soonchunhyang University, Asan, Republic of Korea

Correspondence: Keon-Hyoung Song
Department of Pharmaceutical Engineering, Soonchunhyang University, 22 Soonchunhyang Road, Asan, Republic of Korea
Tel +82 41 530 1656
Fax +82 41 530 3085
Email beophys@sch.ac.kr

Background: The identification of permeation enhancers has gained interest in the development of drug delivery systems. A six-mer peptide, H-FCIGRL-OH (AT1002), is a tight junction modulator with promising permeation-enhancing activity. AT1002 enhances the transport of molecular weight markers or agents with low bioavailability with no cytotoxicity. However, AT1002 is not stable in neutral pH or after incubation under physiological conditions, which is necessary to fully uncover its permeation-enhancing effect. Thus, we increased the stability or mitigated the instability of AT1002 by modifying its terminal amino acids and evaluated its subsequently biological activity.

Methods: C-terminal-amidated (FCIGRL-NH₂, Pep1) and N-terminal-acetylated (Ac-FCIGRL, Pep2) peptides were analyzed by liquid chromatography–mass spectrometry. We further assessed cytotoxicity on cell monolayers, as well as the permeation-enhancing activity following nasal administration of the paracellular marker mannitol.

Results: Pep1 was nontoxic to cell monolayers and showed a relatively low decrease in peak area compared to AT1002. In addition, administration of mannitol with Pep1 resulted in significant increases in the area under the plasma concentration–time curve and peak plasma concentration at 3.63-fold and 2.68-fold, respectively, compared to mannitol alone. In contrast, no increase in mannitol concentration was shown with mannitol/AT1002 or mannitol/Pep2 compared to the control. Thus, Pep1 increased the stability or possibly reduced the instability of AT1002, which resulted in an increased permeation-enhancing effect of AT1002.

Conclusion: These results suggest the potential usefulness of C-terminal-amidated AT1002 in enhancing nasal drug delivery, which may lead to the development of a practical drug delivery technology for drugs with low bioavailability.

Keywords: N-terminal acetylation, stability, permeation enhancer

Introduction

The identification of permeation enhancers has gained interest in the development of drug delivery systems to improve the bioavailability of therapeutic agents that need to reach the systemic circulation. Tight junctions between adjacent epithelial cells are major absorption barriers for therapeutic agents.¹ Studies of permeation enhancers have focused on modulating the tight junction barrier; ideal candidates should have potent, reversible, and nontoxic enhancing properties at the cellular membrane or cytoskeleton.²

The six-mer peptide H-FCIGRL-OH (AT1002) is a promising permeation enhancer that modulates tight junctions. AT1002 was identified from modifications of the zonula occludens toxin (Zot, 399 amino acids, 44.8 kDa), which binds to a specific receptor on the luminal membrane surface, and reversibly opens tight junctions between epithelial cells in a nontoxic manner.³¹ The Zot fragment of AT1002 (amino acid residues 288–293) enhances the transport of molecular weight markers or agents...
with low bioavailability such as mannitol, PEG4000, inulin, cyclosporin A, calcitonin, ritonavir, and saquinavir, with no cytotoxicity after intranasal, intraduodenal, and intratracheal administration.\textsuperscript{12–17} For example, the area under the curve (AUC\textsubscript{0–t}) and maximum concentration (C\textsubscript{max}) of molecular weight markers were significantly increased, respectively, by 2.35-fold and 3.57-fold for PEG-4000, and by 2.92-fold and 3.15-fold for inulin (P<0.05) following intranasal administration with 10 mg/kg AT1002.\textsuperscript{16}

Continuing studies have demonstrated that the permeation-enhancing effect of AT1002 is synergistically increased by the bioadhesive polymer carrageenan. The AUC\textsubscript{0–t} and C\textsubscript{max} of mannitol, inulin, calcitonin, saquinavir, or ritonavir were significantly increased by 2.17-fold/3.14-fold, 6.60-fold/6.89-fold, 4.08-fold/6.06-fold, 3.51-fold/5.77-fold, and 2.48-fold/2.55-fold, respectively (P<0.01), after intranasal administration with AT1002 (5 mg/kg) in the presence of carrageenan (1%, weight per volume [w/v]).\textsuperscript{12–14} By contrast, 5 mg/kg AT1002 alone did not significantly increase the AUC\textsubscript{0–t} and C\textsubscript{max} of inulin, calcitonin, saquinavir, or ritonavir (1.10-fold/1.26-fold, 1.43-fold/1.49-fold, 1.02-fold/1.02-fold, and 1.06-fold/1.15-fold, respectively).\textsuperscript{12–14} It was assumed that the synergistic permeation-enhancing effect resulted from the bioadhesive activity of carrageenan, which might increase the membrane adhesion of AT1002 and drugs. In addition, the synergistic effect between AT1002 and carrageenan was due to reduced instability of AT1002 by the viscosity of carrageenan, which protected the functional state of the peptide under stressful environmental conditions. The relationship between the effects and stability of AT1002 might be supported by results that show that AT1002 is unstable in neutral to basic pH conditions and with increasing incubation time.\textsuperscript{13}

An unstable peptide or protein, due to degradation or aggregation, may have reduced activity and solubility. Chemical degradation and protein aggregation may be induced by a variety of physical factors such as temperature and ionic strength, or with time;\textsuperscript{18} degradation products such as C-terminal fragments can change significantly with varying pH.\textsuperscript{19} In addition, the stability of proteins can be modified by soluble and nontoxic excipients, such as dextrose, or by changing their structural characteristics towards those of thermophilic proteins by introducing negatively charged residues at the N-cap or positively charged residues at the C-cap of the helix.\textsuperscript{18} The latter approach is effective because the helical structure of short peptides and proteins is stabilized by capping interactions between side chains and unfulfilled peptide groups at the N- and C-terminals.\textsuperscript{20,21} The hypothesis of this study is that the stability and permeation-enhancing activity of AT1002 can be further enhanced by modifying the amino acids of AT1002. In the experiments described, peptide terminal modification, C-terminal amidation, and N-terminal acetylation were applied to improve the stability of AT1002 in the nasal delivery of mannitol as a paracellular marker.

Materials and methods

Materials

Peptides (>95%) of FCIGRL (H-FCIGRL-OH, NH\textsubscript{2}-FCIGRL-COOH, AT1002), FCIGRL-NH\textsubscript{2} (H-FCIGRL-NH\textsubscript{2}, NH\textsubscript{2}-FCIGRL-CO\textsubscript{2}H\textsubscript{3}, Pep1), Ac-FCIGRL (Ac-FCIGRL-OH, CO\textsubscript{2}H\textsubscript{3}-NH-FCIGRL-COOH, Pep2), and Ac-FCIGRL-NH\textsubscript{2} (Ac-FCIGRL-OH, CO\textsubscript{2}H\textsubscript{3}-NH-FCIGRL-CO\textsubscript{2}H\textsubscript{3}, Pep3) were purchased from Peptron (Daejeon, Republic of Korea), and stored at −70°C before use. Citric acid, dextrose, hydrochloric acid, sodium phosphate monobasic, sodium phosphate dibasic, sodium tetaborate decahydrate, trifluoroacetic acid (TFA), Dulbecco’s Modified Eagle’s Medium, nonessential amino acid solution, penicillin–streptomycin, and Hank’s balanced salt solution (HBSS) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Methanol and acetonitrile were high-performance liquid chromatography (HPLC)-grade (Thermo Fisher Scientific, Waltham, MA, USA). All of the solutions and buffers were prepared with HPLC-grade water purified with a Zeneer Power I (Human Corp., Seoul, Republic of Korea) and cellulose nitrate membrane filters (47 mm, 0.2 μm, Whatman, Little Chalfont, UK).

[\textsuperscript{3}H]-mannitol (1 mCi/mL) was purchased from American Radiolabeled Chemicals (St Louis, MO, USA). Universal scintillation counting cocktail was purchased from ICN (Cost Mesa, CA, USA). Rompun for injection was purchased from Bayer Korea (Seoul, Republic of Korea). Zoletil 50 was purchased from Virbac (Carros, France). Polyethylene tubing (PE-50) was obtained from BD (Franklin Lakes, NJ, USA). All of the surgical supplies were purchased from Professional Hospital Furnishers (Punjab, Pakistan). The Caco-2 cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). Trypsin–EDTA (Life Technologies Inc., Gaithersburg, MD, USA) and fetal bovine serum (HyClone Laboratories, Logan, UT, USA) were used as purchased. Transwells were purchased from Corning Inc. (Corning, NY, USA). All of the other reagents were of analytical grade or better.

Peptide stability study

Stock solutions (1 mg/mL) were prepared by dissolving each peptide in 70% methanol. Aliquots of the stock
solution containing specified amounts of each peptide were immediately placed in polypropylene tubes and evaporated using a centrifugal evaporator (CVE-3000; Eyela, Tokyo, Japan). Reconstitution solution (1 mL) was added to each tube followed by vortexing for 5 seconds to yield a peptide concentration of 10 µg/mL.

The reconstitution solution was prepared with various buffer solutions and dextrose solution using a citrate–phosphate buffer containing 0.02 M citric acid and 0.02 M sodium phosphate dibasic at a pH of 2.5, 4.0, and 5.0; phosphate buffer containing 0.01 M sodium phosphate monobasic/0.02 M sodium phosphate dibasic at pH 7.4; and borate buffer containing 0.01 M hydrochloric acid/0.02 M sodium tetraborate decahydrate at pH 9.0. Dextrose solutions were prepared at concentrations of 0.5%, 2%, and 5% (w/v). The effect of incubation time on the peak area of AT1002 was assessed at every hour from 0 hour to 5 hours. The capped reaction vial tube was then placed into a tray at a constant temperature within the autosampler. Samples were periodically removed from each tube, and directly analyzed by liquid chromatography–mass spectrometry (LC-MS).

LC-MS conditions
The HPLC system (Shimadzu Corp., Kyoto, Japan) consisted of a binary pump (LC-20AD), autosampler (SIL-20A), and degasser. Aliquots (2 µL) of the reconstituted solutions were injected onto the LC column. Separation of each peptide was performed on a C18 reversed-phase column (2.1×150 mm, 3.5 µm particle size; Vydac, Hesperia, CA, USA) using gradient elution at a flow rate of 0.3 mL/minute for 15 minutes. The mobile phases were composed of acetonitrile containing 0.1% TFA (eluent A), and water containing 0.1% TFA (eluent B). Eluent A was increased from 20% to 34% for 7 minutes for the gradient program for AT1002. The outlet from the HPLC column was directly connected to the mass analyzer. Mass spectra were recorded on an LCMS-2020 mass spectrometer system (Shimadzu Corp.) equipped with an electrospray ionization (ESI) source. Nitrogen (>99.999%) produced from a GeniSys NitroGenerator (Texol Gasgen, Glasgow, Scotland) was used as the nebulizing and drying gas. The MS instrument was operated in positive-ion, selected-ion monitoring (SIM) mode with LabSolutions software (Shimadzu Corp.) under the following MS tuning conditions: desolvation line temperature of 250°C; heat block at 200°C; nebulizing gas of 1.5 L/minute; drying gas of 20 L/minute. The area of the mass peak of AT1002 in SIM chromatograms was calculated and compared to that of each control.

Peptide cytotoxicity study
Cytotoxicity was determined using a lactate dehydrogenase (LDH) bioassay (Cytotoxicity Detection Kit PLUS; Hoffman-La Roche Ltd., Basel, Switzerland) as previously described. Briefly, Caco-2 cells were grown as monolayers at 37°C in an atmosphere of 5% CO2 and 90% relative humanity. The integrity of the cell monolayers was evaluated by measuring the transepithelial electrical resistance with an epithelial voltohmrometer (EVOM; World Precision Instruments, Sarasota, FL, USA). Caco-2 cell monolayers were incubated with AT1002, Pep1, and Pep2 in phosphate-buffered saline (PBS) for 1 hour, respectively. LDH activity was assessed as a function of time by the addition of LDH kit reagents to an aliquot of the solution in the Transwell plate at the end of each incubation period according to the manufacturer’s manual. PBS and Triton X-100 (5%) were used as the negative and positive control, respectively, and the absorbance was measured at 490 nm.

Animals
Male Sprague Dawley (SD) rats weighing 280–290 g were purchased from Orient Bio (Seongnam, Republic of Korea). Rats were housed individually in cages and allowed to acclimate for at least 2 days after arrival. Rats were fed standard rat chow and water ad libitum and maintained on a 12-hour light/dark cycle. Prior to the study, rats were fasted overnight with water ad libitum. The protocol for animal studies was approved by the Soonchunhyang University and Seoul National University Institutional Animal Care and Use Committee.

Intranasal administration of formulations with each peptide as a permeation enhancer
The formulation for [1H]-mannitol (20 µCi/kg) was obtained by mixing [1H]-mannitol with 5% dextrose solution (mannitol alone). After the addition of an appropriate amount of each peptide (2.5 mg/kg) to mannitol solution alone, peptide treatment solutions were prepared as mannitol/AT1002, mannitol/Pep1, and mannitol/Pep2. The formulation with Pep3 for nasal administration could not be prepared because Pep3 was not completely soluble in 5% dextrose solution at the concentration for nasal administration (2.5 mg/kg) to rats. It was assumed that terminally modified Pep3 increased the hydrophobic nature of the phenylalanine and leucine of AT1002. All of the dosing solutions were prepared immediately prior to intranasal administration to the femoral vein-cannulated rats. Male SD rats were anesthetized with an intramuscular
injection of Zoletil 50 (20 mg/kg) and Rompun (2.3 mg/kg), and the femoral vein was cannulated using PE-50. The following formulations were prepared for intranasal administration to rats (n=3–4 per group): 1) mannitol alone; 2) mannitol/AT1002; 3) mannitol/Pep1; and 4) mannitol/Pep2. The rats were manually restrained in a supine position, and the head was tilted back slightly while the formulations were instilled into the nostrils. The dosing solutions of 35 μL per nostril were administered. Blood samples (250 μL) were drawn via the femoral cannula into heparinized syringes at 10, 20, 40, 60, 120, 180, 240, and 360 minutes for the mannitol study, and were immediately centrifuged (15,000×g for 10 minutes) to obtain the plasma (100 μL). Scintillation cocktail for the samples of radiolabeled mannitol was added, and samples were analyzed for radioactivity by Beckman Coulter LS6500 multipurpose scintillation counter (Brea, CA, USA).

Data analysis
The area of the peak for each peptide from LC-MS was converted into the relative peak area (%) compared to that of each peptide in buffer (pH 2.5) after 0 hours’ incubation when comparing the stability of each peptide. For intranasal administration, the amount of absorbed radiolabeled mannitol was converted into a concentration using the specific activity with pH changes compared to AT1002. A peptide including one cysteine can be easily oxidized to form dimerized disulfide bonds, which can result in peptide instability due to aggregation or polymerization. However, we did not observe dimerization of AT1002, Pep1, Pep2, or Pep3 in each full-scan spectra due to the instability of AT1002, as shown in our previous report, and due to its degradation in solution. We considered that peptide dimerization was not due to the hydrophobic characteristics of phenylalanine and isoleucine flanking each cysteine in AT1002, Pep1, Pep2, and Pep3.

The LC conditions were slightly modified for analysis of all peptides compared to our previous report. Under the described conditions, AT1002, Pep1, Pep2, and Pep3 had peak retention times of 4.9, 3.5, 8.8, and 7.7 minutes, respectively, in the SIM mode, and no interference was observed by the aqueous solutions.

**Effect of pH and dextrose on stability of AT1002 terminally modified peptides**

The peak areas of peptides in buffer solutions over a range of pH values (2.5–9.0) and dextrose solutions at concentrations of 0.5%, 2%, and 5% (w/v) were monitored by LC-MS. Stability was expressed as the relative peak area of each peptide, and each point was reported as a peak area ratio compared to the initial peak area of the same peptide at pH 2.5 with time 0 incubation as the control (100%). The AUC was calculated using the linear trapezoidal method; the Cmax of each peptide and time to reach the peak (Tmax) following intranasal administration was determined from the observed data. All of the data are expressed as the mean and standard error of the mean (mean ± SEM). The statistical significance of differences between treatments and/or controls was evaluated using Student’s t-test and one-way analysis of variance (ANOVA) followed by Dunnett’s post-hoc test where appropriate (SPSS for Windows; v12.0; SPSS, Chicago, IL, USA), and the level of significance was set at P<0.05 or P<0.01.

**Results and discussion**

**LC-MS chromatograms of AT1002 terminally modified peptides**

Figure 1 shows that each single-charged ion with a protonated molecule [M + H]+ appeared at m/z 708.4, 707.4, 750.4, and 749.4 in the full-scan spectra under positive ESI, which corresponded precisely to the molecular mass of AT1002 and the terminally modified AT1002 peptides (Pep1, Pep2, Pep3).
The effects of dextrose on the stability of AT1002 terminally modified peptides are shown in Figure 3. Dextrose is a stabilizing excipient of proteins, which is used as an additive for nasal administration solutions during in vivo studies. Our previous report indicated that dextrose has stabilizing effects on AT1002. Dextrose increased the peak areas of Pep1, Pep2, and Pep3 in the same manner as AT1002, indicating that it is effective at increasing stability or at least reducing the instability of the terminally modified peptides. The relative peak areas of all of the peptides increased with increasing dextrose concentration. For example, the peak area ratios of AT1002 were 68.8% ± 4.85%, 79.9% ± 6.36%, 94.3% ± 7.02%, and 99.5% ± 2.80%, and those of Pep1 were 75.0% ± 4.67%, 82.9% ± 5.63%, 91.2% ± 4.84%, and 97.2% ± 3.12%, at dextrose concentrations of 0.0%, 0.5%, 2.0%, and 5.0% (w/v), respectively. No significant differences in relative peak areas of terminally modified peptides compared to those of AT1002 at the same concentration of dextrose were found.

The reduced instability of terminally modified peptides by dextrose was confirmed in an incubation study of each peptide in a 5.0% dextrose solution for 5 hours (Figure 4). Pep1 showed a relatively low decrease in peak area compared to AT1002 at each incubation time point. The peak
area ratios of Pep1 with 3, 4, and 5 hours’ incubation time were 96.3%±6.77%, 94.7%±5.70%, and 95.7%±5.10%, which were significantly higher than those of AT1002 with 3 hours (76.1%±1.95%), 4 hours (71.7%±3.57%), and 5 hours (68.7%±4.61%) of incubation time, respectively ($P<0.05$), compared to the initial peak area of the same peptide at time 0 incubation time. In addition, when comparing only the change in the peptides themselves over incubation time, Pep1 showed no significant difference in peak areas, indicating that the peptide remained stable for at least 5 hours. Consequently, the stabilizing effect of dextrose was shown to be more effective in peptides modified with C-terminal amidation.

### Cytotoxicity of AT1002 terminally modified peptides

An LDH assay was used to examine the toxic effects of terminally modified AT1002 peptides on epithelial cells. An increase in LDH released from the cytosol of cells resulted from an increase in dead or plasma membrane–damaged cells. Caco-2 cell monolayers were incubated with AT1002, Pep1, and Pep2 in PBS for 1 hour. The viability of cells was not significantly different for each peptide compared to the PBS negative control (Figure 5). These results suggest that the terminally modified peptides of AT1002 did not produce a cytotoxic response to Caco-2 cells during the incubation...
time. AT1002 was also not cytotoxic, which correlates with our previous results.\(^{17}\)

**Intranasal administration of mannitol with AT1002 terminally modified peptides**

The formulations of \[^{3}H\]-mannitol in 5% dextrose solution with or without AT1002, Pep1, and Pep2 (2.5 mg/kg) were administered intranasally to femoral vein-cannulated SD male rats.

The pharmacokinetic profiles were characterized for each formulation, and the parameters were calculated using noncompartmental analysis. The mean (± SEM) plasma concentration versus time profiles following the intranasal administration of mannitol formulations are shown in Figure 6. Significantly higher permeation of mannitol was found in the formulations of mannitol/Pep1 compared to the control formulation of mannitol alone after 40 minutes, indicating a significant enhancement in the intranasal absorption of mannitol with Pep1 (\(P<0.01\)). The administration of mannitol with Pep1 produced a significant increase (3.63-fold) in AUC\(_{0–360\text{ min}}\) (22.08±3.54 min ng/mL) and an increase (2.68-fold) in C\(_{\text{max}}\) (82.06±12.10 pg/mL) over the control formulation of mannitol alone (AUC\(_{0–360\text{ min}}\)=6.08±1.33 min ng/mL, C\(_{\text{max}}\)=30.58±6.02 pg/mL; \(P<0.01\); Table 1). However, there were no significant differences in mannitol concentration at each time point between the formulations of mannitol/AT1002, mannitol/Pep2, and mannitol alone. The enhancement of mannitol by Pep1 was in accordance with no statistically significant difference over all of the incubation times in the peak areas of Pep1 compared to AT1002 or Pep2 (Figure 4), suggesting that C-terminal amidation of AT1002 increased the stability or at least reduces the instability of AT1002, which exposed the inherent permeation-enhancing effect of AT1002.

In addition, the enhancement of mannitol by Pep1 was more pronounced at lower doses of Pep1 and with the absence of carrageenan, which promoted the effect of AT1002. The permeation-enhancing effect of Pep1 further increased the AUC\(_{0–360\text{ min}}\) and C\(_{\text{max}}\) of mannitol (20 \(\mu\)Ci/kg) despite the lower dose of Pep1 (2.5 mg/kg). These data were in contrast to those from our previous study, which showed that AT1002 (5 mg/kg) increased the absorption of mannitol (40 \(\mu\)Ci/kg) with a 2.17-fold increase in AUC\(_{0–360\text{ min}}\) and a 3.14-fold increase in C\(_{\text{max}}\) compared to the control of mannitol/carrageenan, and compared to the study of mannitol without carrageenan, which showed that the permeation of mannitol did not change or increase with AT1002 (5 mg/kg).\(^{15\text{a}}\)

Enhanced mannitol permeation with Pep1 was comparable to that seen with the delta G fragment of Zot (amino acid residues 265–399) when the differences in the administration dose and route were disregarded. Delta G enhanced the transport of mannitol (40 \(\mu\)Ci/kg) by 2.57-fold of AUC\(_{0–360\text{ min}}\) and 2.00-fold of C\(_{\text{max}}\) respectively, after intraduodenal administration to rats.\(^{27\text{a}}\) Therefore, these data indicate that the C-terminal amidation of AT1002 increased the intranasal permeation of mannitol.

**Conclusion**

Terminal modification of AT1002 affected the stability and effectiveness of paracellular permeation enhancement. Pep1 is a C-terminal-amidated peptide of AT1002 that is nontoxic to cell monolayers and showed a relatively low decrease in peak area compared to AT1002 in an incubation study. Furthermore, Pep1 produced a statistically significant increase in AUC\(_{0–360\text{ min}}\) (by 3.63-fold) and C\(_{\text{max}}\) (by 2.68-fold) compared to the control in the nasal administration of mannitol, whereas no significant difference in the mannitol concentration was observed for the formulations of mannitol/AT1002 or mannitol/Pep2 compared to the control of mannitol alone. Thus, Pep1 increased the stability or at least reduced the instability of AT1002, which exposed the inherent permeation-enhancing effect of AT1002. These results suggest the potential usefulness of C-terminal-amidated AT1002 in enhancing nasal drug delivery, and may lead to the development of a practical drug delivery technology for drugs with low bioavailability.

**Acknowledgment**

This research was supported by the Soonchunhyang University Research Fund.
Table 1  Pharmacokinetic parameters in femoral vein-cannulated SD rats after intranasal administration of [3H]-mannitol (20 μCi/kg) with AT1002 and terminally modified AT1002 (2.5 mg/kg)

<table>
<thead>
<tr>
<th></th>
<th>AUC_{0-360 min} (min ng/mL)</th>
<th>C_{max} (pg/mL)</th>
<th>T_{max} (min)</th>
<th>V/F (L/kg)</th>
<th>CL/F (ml/min/kg)</th>
<th>T_{1/2} (min)</th>
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<tr>
<td>Mannitol + FCIGRL-NH_{2} (Pep1)</td>
<td>22.08±3.54**</td>
<td>82.06±12.10**</td>
<td>60.00±0.00**</td>
<td>2.30±0.36**</td>
<td>9.65±1.25**</td>
<td>177.01±24.18</td>
</tr>
<tr>
<td>(3.63-fold)</td>
<td>(2.68-fold)</td>
<td>(1.71-fold)</td>
<td>(0.22-fold)</td>
<td>(0.29-fold)</td>
<td>(0.82-fold)</td>
<td></td>
</tr>
<tr>
<td>Mannitol + Ac-FCIGRL (Pep2)</td>
<td>5.87±1.59</td>
<td>22.63±5.72</td>
<td>66.67±29.06</td>
<td>8.90±2.59</td>
<td>39.27±1.54</td>
<td>176.48±49.29</td>
</tr>
<tr>
<td>(0.96-fold)</td>
<td>(0.74-fold)</td>
<td>(1.90-fold)</td>
<td>(0.86-fold)</td>
<td>(1.17-fold)</td>
<td>(0.82-fold)</td>
<td></td>
</tr>
<tr>
<td>Mannitol + FCIGRL (AT1002)</td>
<td>9.02±3.31</td>
<td>38.30±12.48</td>
<td>60.00±30.55</td>
<td>6.05±1.52</td>
<td>32.46±15.71</td>
<td>161.66±33.52</td>
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<td>(1.48-fold)</td>
<td>(1.25-fold)</td>
<td>(1.71-fold)</td>
<td>(0.58-fold)</td>
<td>(0.97-fold)</td>
<td>(0.75-fold)</td>
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<tr>
<td>Mannitol alone (control)</td>
<td>6.08±1.33</td>
<td>30.58±6.02</td>
<td>35.00±9.57</td>
<td>10.37±2.16</td>
<td>33.48±7.04</td>
<td>215.45±3.09</td>
</tr>
</tbody>
</table>

Notes: Each data represent the mean ± SEM of 3–4 rats. Each value in the bracket represents the enhancement ratio of each pharmacokinetic parameter compared to mannitol alone (*P<0.05, **P<0.01)

Abbreviations: AUC, area under the curve; CL/F, clearance; C_{max}, maximum concentration; min, minutes; SD, Sprague Dawley; SEM, standard error of the mean; T_{1/2}, half-life; T_{max}, time to reach the peak; V/F, volume of distribution.

Disclosure

The authors report no conflicts of interest in this work.

References
