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MODULATION OF TIGHT JUNCTIONS BY PEPTIDES TO INCREASE DRUG PENETRATION ACROSS BIOLOGICAL BARRIERS

Summary of the Ph.D. thesis

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1. INTRODUCTION

Biological barriers separate our bodies from the external environment and certain protected organs, like the brain, from the systemic circulation. Epithelial and endothelial cells connect by tight junctions (TJs) and form the anatomical basis of these barriers. TJs close the intercellular gap thus restrict the paracellular passage of cells, molecules or ions across epithelial and endothelial barriers and limit the penetration of drugs and determine the tightness of biological barriers. Therefore, the paracellular permeability is one of the most important determining factors of drug delivery across barriers and enhancing the penetration of certain drugs, especially hydrophilic large biopharmaceuticals is a great challenge in pharmaceutical research. In addition barriers especially the endothelial cells of the blood-brain barrier (BBB) provide a metabolic barrier by expressing enzymes that modify endogenous and exogenous molecules. Therefore peptide or neurotransmitter transport to the brain is restricted not only by this mechanism but also by the restricted paracellular route.

TJs of barrier cells are complex structures composed of integral membrane proteins, linker proteins connecting membrane proteins to the actin cytoskeleton and signaling molecules regulating paracellular tightness and transport. Knowledge of the amino acid sequence of these proteins and their secondary structure made it possible to design peptides as novel type of TJ modulators to open the paracellular barrier for drug delivery. A wide variety of modulator peptides were synthesized based on different junctional proteins and tested on barrier models *in vivo* and *in vitro*. Based on literature data we have selected for our study six different modulator peptides which act at different targets (Table 1).

Table 1. Characteristics of the selected TJ modulator peptides.

Peptide	Target	Amino acid sequence	Mechanism	Derived from
ADT-6	E-cadherin	Ac-ADTPPV-NH2	EC1 domain analog	designed to target ECL1
HAV-6	E-cadherin	Ac-SHAVSS-NH2	EC1 domain analog	designed to target ECL1
C-CPE	Claudin-3, -4	SSYSGNYPYSILFQKF-OH	Interact with ECLs	microbial toxin fragment
AT-1002	ZO-1	FCIGRL	Zot receptor	microbial toxin fragment
7-mer	unknown	FDFWITP	unknown	phage display
PN159	unknown	KLALKLALKALKAALKLA-amide	unknown	phage display

Abbreviation: ZO-1, zonula occludens protein-1; EC1, first extracellular domain; ECL, extracellular loop.

For our experiments two well characterized culture models were used. The human Caco-2 cell line, which resembles the epithelium of small intestine both from structural and functional aspects and the primary cell based rat BBB triple co-culture model.

2. AIMS

While the triple co-culture BBB model was already tested for several types of marker molecules and drugs, it was previously not examined for the penetration of bioactive peptides. Therefore the first aim of this work was to study the transfer of an unlabeled neuropeptide, the analgesic opiorphin on our BBB culture model.

Several types of TJ modulator peptides are described in the literature as discussed above, but detailed comparative studies were missing at the start of our work. For example, there are many observations on C-CPE or E-cadherin peptides but their efficacy was investigated on one type of barrier model only. No data were available on their barrier specificity or their efficacy compared to other types of TJ modulator peptides either. Thus we wanted to compare the six TJ modulator peptides shown in Table 1 that we selected based on their diversity of targets, mode of action and different sequence on two different barrier models.

Our specific aims were the following:

(i) Testing the effects of TJ modulator peptides on intestinal epithelial and blood-brain endothelial models for:

cell viability

barrier integrity

cell morphology

(ii) Comparing the efficacy, safety, reversibility and barrier specifity of TJ modulator peptides.

(iii) Investigation of PN159 peptide for:

dose dependence of efficacy

long-term effect on cell viability

reversibility of kinetic and morphological changes

penetration enhancement for marker molecules and drugs

(iv) Identification of the potential targets of PN159 TJ modulator peptide.

3. MATERIALS AND METHODS

3.1. Materials

All reagents were purchased from Sigma (Sigma-Aldrich, Ltd., Hungary), unless otherwise indicated.

3.2. Peptide synthesis

Opiorphin (QRFSR) and TJ modulator peptides peptides were synthesized manually by our cooperation partners (Dr. Géza Tóth, Institute of Biochemistry, HAS; Dr. Lívia Fülöp, Department of Medical Chemistry, University of Szeged).

3.3. Cell cultures – human Caco-2 cell line and primary brain endothelial cells

Human Caco-2 intestinal epithelial cell line was purchased from ATCC (cat.no. HTB-37) and used until passage 60 for the experiments. Caco-2 cells were grown in DMEM/HAM's F-12 culture medium with stable glutamine (Biochrom GmbH, Germany) supplemented with 10 % fetal bovine serum (FBS; Pan-Biotech GmbH, Germany) and 50 μg/ml gentamycin. All plastic surfaces were coated with 0.05 % rat tail collagen in sterile distilled water before cell seeding.

Primary cultures of brain endothelial cells, glial cells, and pericytes, and the *in vitro* BBB model were prepared according to the method described in our previous studies (Nakagawa et al., 2009; Walter et al., 2015). Brain endothelial cells were cultured in DMEM/F-12 (Gibco, Life Technologies, USA), 15 % plasma-derived bovine serum (First Link, UK), 100 μg/ml heparin, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 1 ng/ml basic fibroblast growth factor (Roche, Basel, Switzerland) and 50 μg/ml gentamycin. Pericytes and glial cells were cultured in DMEM/HAM's F-12 supplemented with 10 % FBS (Pan-Biotech GmbH) and 50 μg/ml gentamycin.

3.4. Peptide treatment concentrations and intervals

For the comparative studies final concentrations of the peptides in treatment solutions were as follows: 2 mM for ADT-6, HAV-6 and AT-1002 peptides, 1 mM for C-CPE peptide, 100 μM for 7-mer peptide and 10 μM for PN159 peptide. For additional experiments with PN159 peptide final concentrations in treatment solutions were 1-100 μM.

3.5. Cell viability studies

Two methods were applied to determine the effects of TJ modulator peptides on cell viability: MTT dye conversion assay, and cell impedance measurement.

In the MTT assay the yellow dye is taken up by cells and converted by mitochondrial and cytoplasmic enzymes into blue formazan crystals. The dye conversion rate determines cell metabolic activity and viability. After treatments cells were incubated with MTT solution and the amount of formazan converted by cells was determined by absorbance measurement with a microplate reader (Fluostar Optima, BMG Labtechnologies, Germany).

Measurement of the impedance dynamically monitors living cells. The RTCA SP instrument (ACEA Biosciences, USA) measures impedance at 10 kHz, which derives from interaction between cells and electrodes of 96-well E-plates. This interaction correlates with cell proliferation, viability or with transcellular ion flux in real-time.

3.6. Measurement of the paracellular barrier integrity

3.6.1. Electrical resistance of cell layers

Transepithelial or transendothelial electrical resistance (TEER) reflects the tightness of the intercellular junctions. TEER was measured by an EVOM volt-ohmmeter (World Precision Instruments, Sarasota, FL, USA) combined with STX-2 electrodes, and was expressed relative to the surface area of the monolayers as $\Omega \times cm^2$. Resistance of cell-free inserts ($130\ \Omega \times cm^2$).

3.6.2. Permeability studies

Permeability marker molecules albumin (10 mg/ml; Mw: 65 kDa) labeled with Evans blue (167.5 μg/ml) and fluorescein (10 μg/ml; Mw: 376 Da) were used for the permeability experiments. For recovery experiments treatment solutions from both compartments were collected and changed the peptide solutions for culture medium, after one day the permeability experiments were repeated. The concentrations of the marker molecules of collected samples were determined by a fluorescence multi-well plate reader (Fluostar Optima). To measure the transfer of opiorphin across the BBB model 10 μM peptide solutions were added to the upper/donor compartment. To measure peptide flux from the upper to lower compartment the inserts were transferred at 30 and 60 minutes to new wells containing buffer solution. Samples were collected after the experiments and the opiorphin levels were detected by mass spectrometry. In permeability experiments with PN159 (10 μM) on Caco-2 cells fluorescein isothiocyanate (FITC)-labeled dextran marker molecules were used at 1 mM

concentrations, and fluorescence intensities of collected samples were measured using a Fluorolog FL3-22 spectrofluorometer (Horiba Jobin Yvon, Paris, France). Drugs were used at 10 μM concentrations and samples were measured by HPLC instrument. The apparent permeability coefficients (P_{app}) were calculated in upper to lower directions and the clearance of the molecules were also evaluated.

3.7. Immunohistochemistry

Morphological changes in epithelial and endothelial barrier integrity were followed by immunostaining for TJ proteins occludin, claudin--5, linker proteins β-catenin and ZO-1 after peptide treatments and one-day recovery. After treatments cells were washed with phosphate buffer (PBS), and fixed with 1:1 mixture of ice-cold acetone and methanol for 10 minutes at room temperature. After blocking with albumin in PBS cells were incubated with primary antibodies (Life Technologies, Carlsbad, USA) overnight. Incubation with secondary antibodies (Life Technologies, Invitrogen, USA) of samples was lasted for 1 hour. Hoechst 33342 was used to stain cell nuclei. After mounting the samples (Fluoromount-G; Southern Biotech, USA) staining was visualized by a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems GmbH, Germany).

3.8. Electron microscopy

Cells were treated with 10 μM PN159 peptide for 30 minutes and fixed immediately after treatment or after a 1-day recovery. After briefly washing with the buffer several times, cells were postfixed in OsO_4 and dehydrated. Finally, the membranes of the culture inserts with the cells were removed from their support and embedded in Taab 812. Following polymerization, ultrathin sections were cut perpendicularly for the membrane using a Leica UCT ultramicrotome (Leica Microsystems, UK) and examined using a Hitachi 7100 transmission electron microscope (Hitachi Ltd., Japan).

3.9. Molecular modeling

Protein structures were obtained by homology modeling using the MODELLER program package. Human claudins 1, 3, 4, 5 and 7 were homology modeled using the completed mouse claudin-15 as a template. Molecular dynamics simulations were performed by the program NAMD using the CHARMM27 molecular force field with CMAP correction. The results were visualized by VMD v1.9.1. The docking studies were performed by the

CABS docking server and the resulted C-alpha traces were reconstructed by MODELLER using the python script supplied by the server homepage.

3.10. Circular dichroism spectroscopy

Far-UV circular dichroism (CD) spectra of PN159 peptide were recorded at 25 °C temperature on a J-810 spectropolarimeter (JASCO International Co. Ltd., Japan). To test the stability of the peptide structure the CD spectra were recorded between 25 ^{o}C and 95 ^{o}C temperature with a ramp rate of 5 ^{o}C/min on a JASCO J-810 spectropolarimeter by using a Peltier sample holder. The CD spectra were measured between 260 nm and 185 nm with an optical pathlength of 1 mm, the peptide concentration was 0.1 mg/ml in Milli-Q water. The data were analyzed by CDSSTR method on the Dichroweb server.

3.11. Statistical analysis

For statistical analysis GraphPad Prism 5.0 software (GraphPad Software Inc., USA) was used. All data presented are means ± SD. Values were compared using analysis of variance followed by Dunnett's test and t-test. Changes were considered statistically significant at $p < 0.05$. All measurements were repeated three times, the number of parallel samples was minimum three.

4. RESULTS AND DISCUSSION

4.1. Opiorphin penetration across a BBB culture model

MS/MS characterization of the synthetic opiorphin peptide was performed using both collision induced dissociation and higher energy collisional activation. The observed m/z value of the protonated peptide ions and fragmentation pattern were in good agreement with those expected for the QRFSR sequence (Fig. 1A). Signal intensities in the receiver compartment samples were 1.5×10^{8} as opposed to $1\text{-}2 \times 10^{6}$ in the donor compartment samples. The relative quantity of opiorphin which penetrated through the BBB model was calculated to be 2.91 ± 1.13 % based on the area under curve of the doubly charged opiorphin peptide ion m/z 347.1932. As a comparison, 0.41 ± 0.03 % of fluorescein and 0.03 ± 0.01 % of albumin crossed the brain endothelial cell layers in parallel inserts from the same cell isolation under identical assay conditions. The clearance of opiorphin is shown on Fig. 1B. P_{app} of opiorphin was $0.53 \pm 1.36 \times 10^{-6}$ cm/s (n = 4).

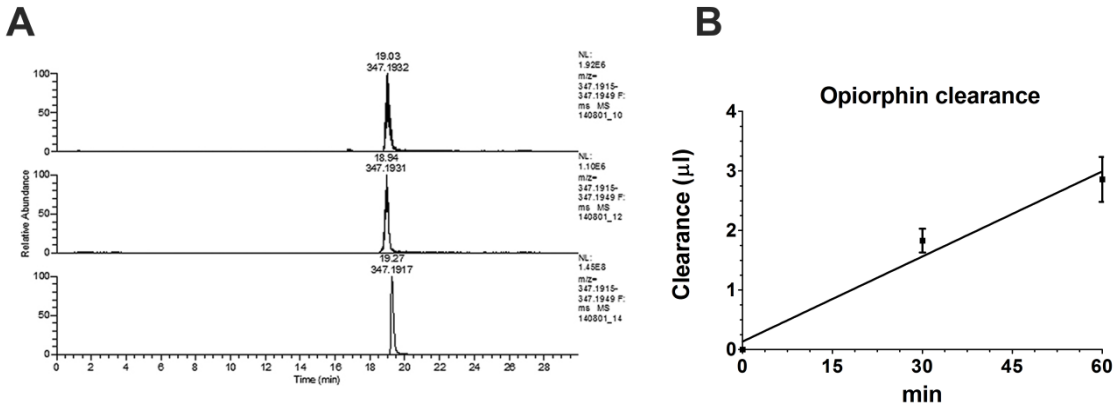

Figure 1. (A) Extracted ion chromatograms of m/z: 347.1932 ± 5 ppm corresponding to the doubly charged peptide ion of opiorphin. Upper panel: sample from the receiver compartment ("brain side") after 30 minutes incubation, middle panel: sample from the receiver compartment after 60 minutes incubation, bottom panel: sample from the donor compartment ("blood side") after 60 minutes incubation. Peak labels denote retention time and m/z value. (n = 4). (B) Clearance of opiorphin across the BBB model at 30 and 60 minutes (mean ± SD, n = 4).

4.2. Effect of TJ modulator peptides on barrier integrity

4.2.1. Effect of modulator peptides on electrical resistance

All six peptides significantly decreased the TEER of Caco-2 layers after 1-hour treatment in non-toxic concentration range (Fig. 2A).

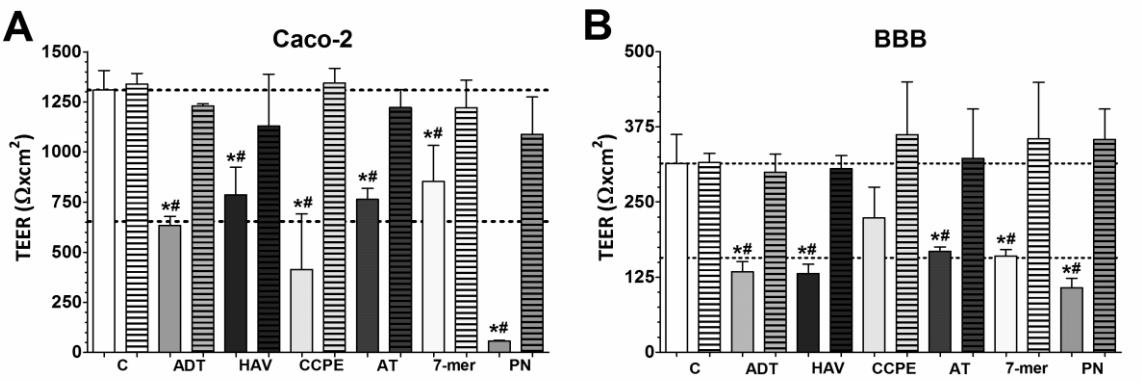

Figure 2. Effects of modulator peptides on the resistance of Caco-2 epithelial (A) and brain endothelial (B) cell layers. Empty columns represent the effect of 1-hour peptide treatment and striped columns show resistance after 24-hour recovery. Statistically significant differences are: *, $p < 0.05$, compared to control group; #, $p < 0.05$ compared to control group value 24 hours later.

Treatments with ADT-6, C-CPE and PN159 reduced the resistance of cell layers by more than 50 %, while 7-mer, HAV-6 and AT-1002 caused a 30-45 % drop as compared to control ($1309 \pm 97\ \Omega \times cm^2$). The resistance of brain endothelial monolayers was also decreased by the peptides, except C-CPE peptide, which had no significant effect since its targets not expressed on brain endothelial cells (Fig. 2B). The TJ modulator peptides caused less than 50 % reduction of TEER compared to control ($314 \pm 48\ \Omega \times cm^2$). PN159 peptide

was the most effective on both models (Fig. 2). Barrier integrity was recovered in both models in all treatment groups after 24 hours.

4.2.2. Effect of modulator peptides on permeability of cell layers

The modulator peptides increased the permeability of Caco-2 layers for fluorescein and albumin markers (Figs. 3A and 3B). ADT-6, HAV-6 and especially PN159 peptides were the most effective. The 7-mer peptide had no effect on fluorescein flux, but increased the permeability of albumin.

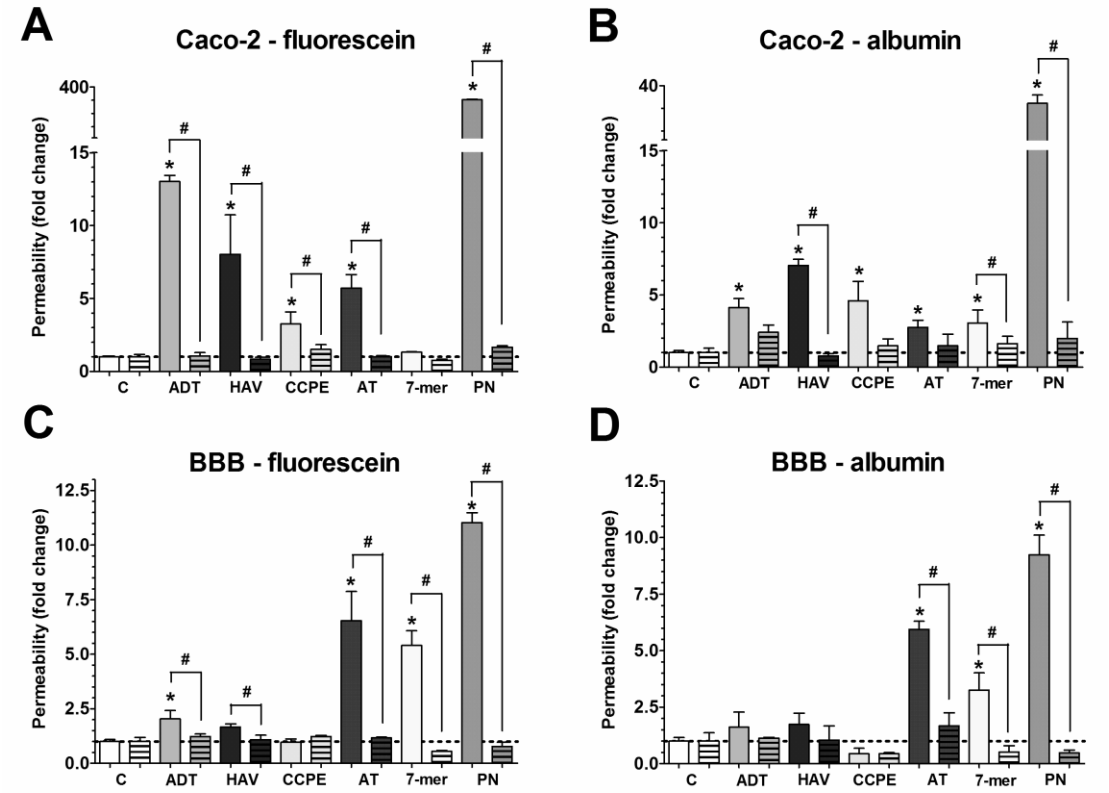

Figure 3. Evaluation of barrier integrity on Caco-2 epithelial (A and B) and brain endothelial cell layers (C and D). Permeability (A, C) for fluorescein and (B, D) for albumin after 1-hour treatment with modulator peptides and after 24-hour recovery. Empty columns represent the effect of 1-hour peptide treatment and striped columns show permeability after 24-hour recovery. Data is expressed in the % of control groups. Statistically significant differences were detected after treatment with peptides ($p < 0.05$) as compared with the control group (*) and compared with each peptides (#) after recovery.

On the BBB model AT-1002, 7-mer and PN159 peptides significantly enhanced the permeability of both markers (Figs. 3C and 3D). AT-1002 and 7-mer peptides increased the flux of markers 3.5-6-fold, while PN159 peptide showed the highest effect. In the BBB model E-cadherin peptides had a small effect on fluorescein, but not on albumin penetration, while C-CPE had no effect in concordance with the results of TEER measurement. Permeability

assays were repeated after 24-hour recovery and the TJ modulating effect of the peptides were reversible on both Caco-2 and BBB models.

4.3. Effect of PN159 modulator peptide on the morphology of intercellular junctions

In both culture models the cells were tightly apposed and all junctional proteins were localized at the intercellular connections forming pericellular belts in control groups (Fig. 4).

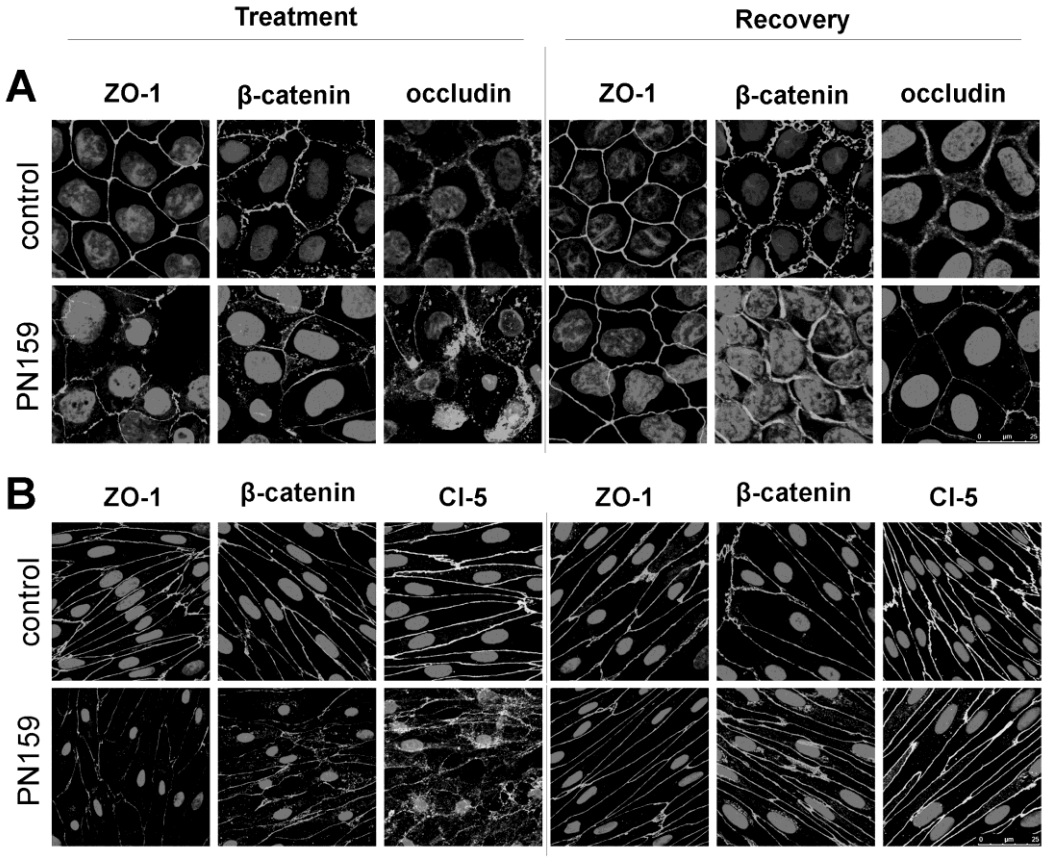

Figure 4. Effects of PN159 modulator peptide on junctional morphology of (A) Caco-2 cells and (B) brain endothelial cells. Immunostaining for zonula occludens-1 (ZO-1), β-catenin, occluding and claudin-5 proteins after treatment and 24-hour recovery. Bar = 25 μm.

Epithelial (Fig. 4A) and endothelial (Fig. 4B) junctional morphology was drastically changed by the most effective PN159 peptide. All junctional proteins partially disappeared from cell borders. A strong decrease of the staining at the cell border was accompanied by an increase in staining over cell bodies. The morphological changes caused by the peptides were reversible after 24-hour recovery in both models (Fig. 4).

4.4. Effect of PN159 peptide on Caco-2 cell viability

As a follow-up work PN159 was further examined for safety and efficacy on Caco-2 epithelial cells. The colorimetric endpoint dye conversion MTT assay reflects the metabolic activity and viability of cells. MTT test was performed at three different time points after 1-hour treatment with different PN159 peptide concentrations: (i) immediately after the 1-hour treatment, (ii) at one-day recovery and (iii) at one-week recovery (Fig. 5A). Low concentrations of the peptide (1-10 μM) did not decrease cell viability, while cell damage was found at higher 30 and 100 μM concentrations. The cytotoxic effect of PN159 at 100 μM concentration was not reversible after one day or one week.

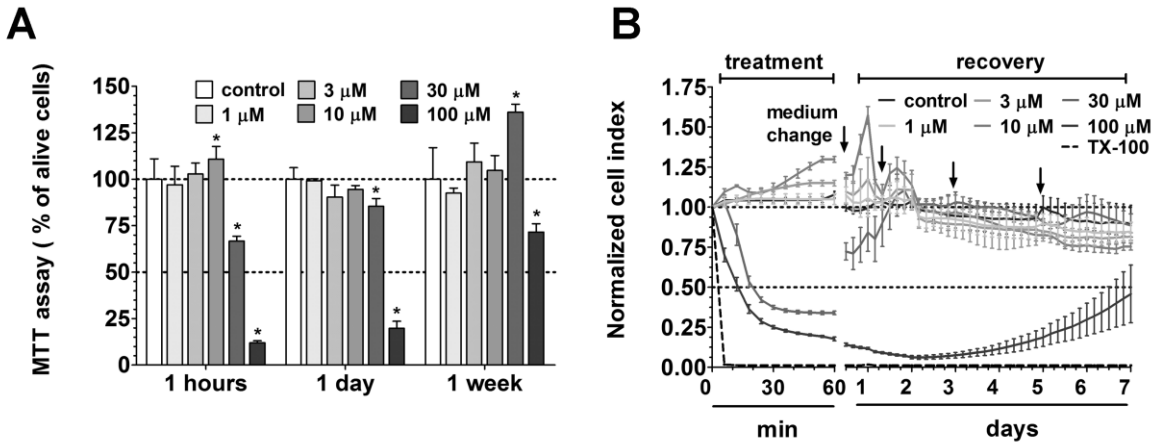

Figure 5. (A) MTT assay after 1-hour treatment with PN159 peptide followed by 1 hour, 1 day or 1 week recovery. The MTT values are given as percent of control (100 % viability). $p < 0.05$ as compared with the control groups. (B) Impedance measurements after 1-hour treatment followed by a recovery phase of 1 week. The effects of PN159 peptide on the impedance were shown as cell index. Values are presented as means ± SD, n = 3-8. Arrows indicate change of culture medium.

Kinetics of PN159 effects on Caco-2 cells were followed by real-time impedance measurements (Fig. 5B). Only the two highest peptide concentrations decreased the cell impedance after 1-hour treatment indicating cell damage (Fig. 5), similarly to the results of the MTT assay.

4.5. Effect of PN159 peptide on barrier integrity ultrastructure of Caco-2 cell junctions

The two highest 10 and 30 μM concentrations opened the paracellular barrier the most and increased the paracellular permeability for both fluorescein and albumin (Fig. 6A). Because the peptide was safe for the cells but effectively opened the barrier at 10 μM, this concentration was selected to reveal the kinetics of the reversibility of barrier opening, and to investigate its effects on drug penetration. The effect PN159 peptide was very rapid, the electrical resistance dropped already to 42 % of control value after 1 minute treatment (Fig. 6B), this decreasing tendency continued until the end of the 30-minute treatment. After the

removal of the peptide complete recovery of the barrier function could be observed at 20 hours. Intact TJs providing the morphological basis of barrier functions were visualized

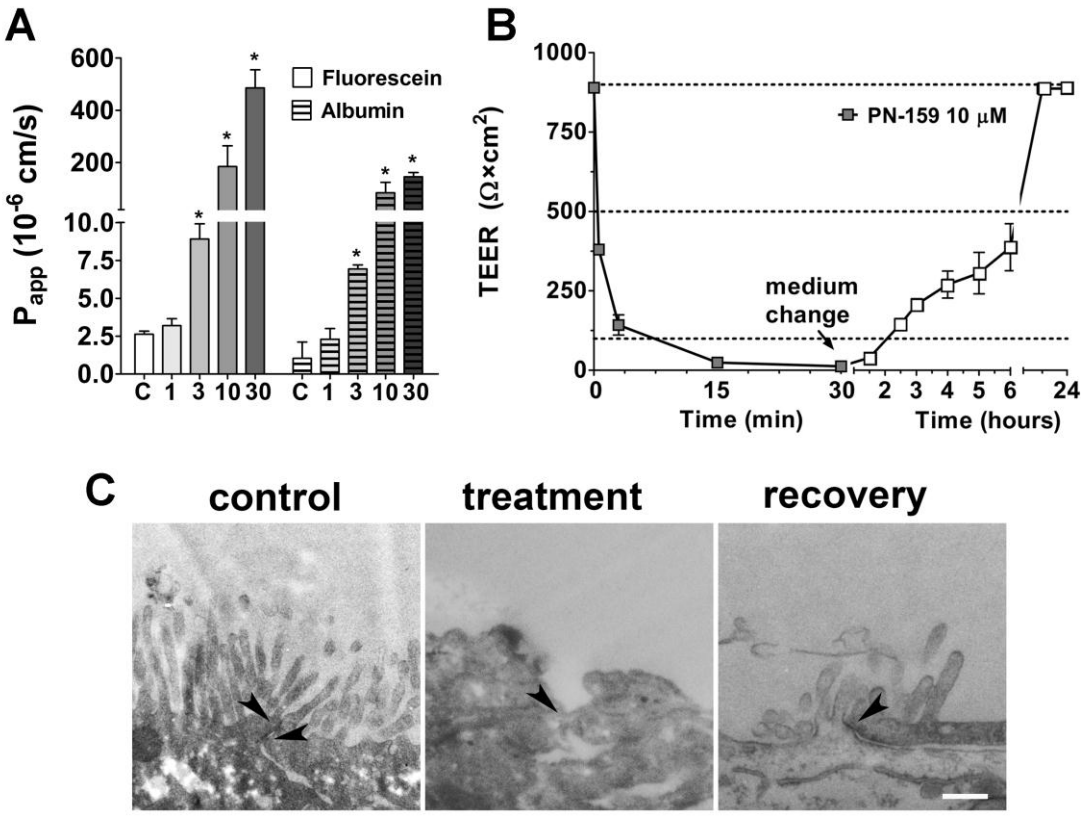

Figure 6. (A) Caco-2 cell layer permeability for fluorescein and albumin marker molecules (P_{app} A-B 10^{-6} cm/s), $p < 0.05$. (B) Kinetic analysis of transepithelial electrical resistance (TEER) after PN159 peptide treatment. (C) Transmission electron micrographs of cell-cell connections (arrowheads); bar = 400 nm.

between Caco-2 epithelial cells in the control group by transmission electron microscopy, but no junctions were observed following treatment with PN159 peptide (Fig. 6C). The disappearance of intercellular TJs was reversible, because after 1-day recovery.

4.6. Effects of PN159 on the penetration of dextran marker molecules and drugs

All these previous functional and morphological results pointed to the TJ opening effect of PN159 in Caco-2 cells which potentially can be exploited to increase drug penetration across the intestinal barrier. The permeability of Caco-2 monolayers was measured in the apical to basal (intestine to blood) direction for four water soluble dextran marker molecules of different size and four drugs, the hydrophilic atenolol and cimetidine, and lipophilic quinidine and verapamil. All four drugs are substrates of active efflux transporters.

Table 2. Apparent permeability coefficients of dextran marker molecules and drugs in the apical-basal direction (P_{app} A-B, 10^{-6} cm/s) in control and PN159 treated cultures. The differences between control and treated groups were expressed in fold changes.

		P_{app} A-B (10^{-6} cm/s) ± SD		Fold change
		control	PN159	
markers	FD-4	0.021 ± 0.001	4.2 ± 0.1	**42**
	FD-10	0.025 ± 0.01	4.8 ± 0.7	**48**
	FD-20	0.022 ± 0.002	3.5 ± 1.0	**35**
	FD-40	0.015 ± 0.003	6.0 ± 0.3	**60**
drugs	atenolol	1.2 ± 0.3	36.3 ± 1.9	**30.3**
	cimetidine	0.9 ± 0.2	34.5 ± 4.5	**38.3**
	quinidine	45.0 ± 13.3	72.6 ± 2.4	**1.6**
	verapamil	46.2 ± 3.9	86.7 ± 18.8	**1.9**

Abbreviation: FD, fluorescein isothiocyanate-labeled dextran.

Apparent permeability coefficients of the large FD macromolecules were very low in control conditions but they were elevated by 35-60 fold following PN159 treatment. The permeability of atenolol and cimetidine was the lowest from the tested molecules, while the highest penetration was measured for quinidine and verapamil on Caco-2 cells. PN159 treatment caused more than 30 fold changes for atenolol and cimetidine which penetrate slowly across the cells layers. In contrast, the peptide increased about 2 fold the permeability of the intestinal culture model for the lipophilic quinidine and verapamil.

4.7. Characterization of the secondary structure of PN159

According to the obtained CD spectra the secondary structure of PN159 peptide is predominantly unordered structure with β-sheet motifs (Fig. 7A). This secondary structure of PN159 peptide was relatively stable between 25 and 95 °C (Fig. 7B).

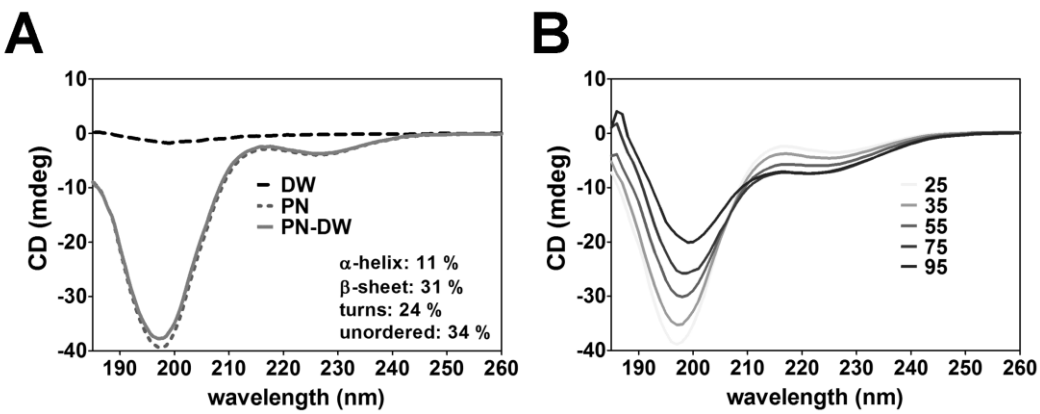

Figure 7. (A) Circular dichroism (CD) spectroscopy of PN159 peptide at 25 °C. (B) Thermostability measurement of the peptide structure.

4.8. Structure and interaction of claudin-7 with PN159

Claudin protein structures were obtained by homology modelling using the MODELLER program package. Docking of PN159 peptide to full length homology modelled human claudin monomers was performed on the CABS server. Docking energies ("total energy") were decomposed to "ligand energy", "interaction energy" and "receptor energy" parts (Table 3). Based on the modeling, energetically highly favorable interactions were found between PN159 and the ECLs of claudin-1, -4, -5 and -7, but not that of claudin-3.

Table 3. Docking energy components of PN159 to selected human claudin monomers

	Claudin-1	Claudin-3	Claudin-4	Claudin-5	Claudin-7
E_{lig}	-34	-17	-61	-100	-32
E_{int}	-63	-59	-79	-76	-186
E_{tot}	-1228	-1163	-1229	-1439	-1798

Abbreviation: E_{tot}: total energy; E_{lig}: ligand energy; E_{int}: interaction energy

Comparing the docking energy values of PN159 to claudins the following rank order in the strength of interaction can be established: claudin-3 < claudin-1 < claudin-4 < claudin-5 < claudin-7. After docking of the peptide disappearance of β-strands in ECLs of claudins was observed, that seems to be correlated with the strength of interaction. Docked poses of PN159 with ECLs of claudin-7 are shown on Fig. 8B which was the strongest interaction.

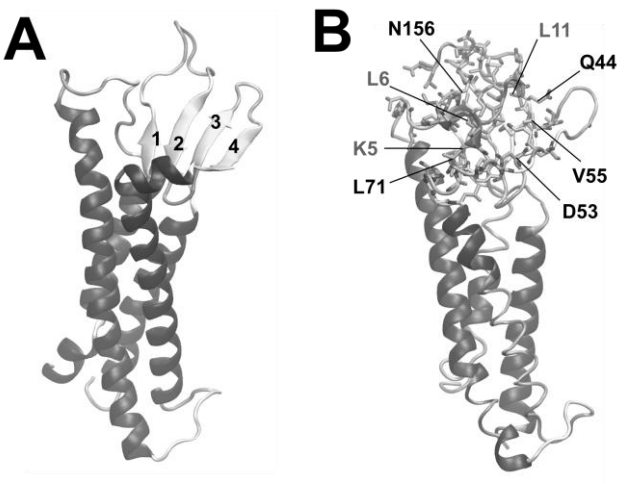

Figure 8. (A) Modelling human claudin-7 protein. (B) Docking of PN159 peptide to claudin-7. Interacting residues are shown as sticks.

5. SUMMARY

Epithelial and endothelial cells form the anatomical and functional basis of biological barriers. The penetration of drugs across these barriers is limited due to closed intercellular gaps by tight junction proteins, and metabolic enzymes expressed in cells. These important barrier properties which on one hand provide protection, on the other hand prevent the effective treatment of several diseases. The paracellular permeability is one of the most important determinants of drug delivery across biological barriers and enhancing the permeability of drugs, especially large biopharmaceuticals is a great challenge in pharmaceutical research. In the case of biopharmacon delivery beside tight junctions enzymes of the metabolic barrier represent the most limiting factor, particularly at the BBB. We demonstrated the transfer of opiorphin, a bioactive peptide across a well characterized and validated co-culture BBB model. Our data also support, that opiorphin may have a potential for further development as a centrally acting novel drug for the treatment of pain or depression.

One of the strategies to increase the penetration of large hydrophilic compounds is opening the paracellular gate by targeting TJ proteins. We studied six tight junction modulator peptides, ADT-6, HAV-6, C-CPE, AT-1002, 7-mer PN-78, PN159, which act on different targets, and compared their effects on intestinal epithelial and brain endothelial barriers. All peptides induced reversible opening of tight junctions as confirmed by different methods, but selectivity and differences in efficacy were observed. The targets of C-CPE, ADT-6 and HAV-6 peptides are expressed on epithelial cells which resulted in selective effects on epithelial cells. AT-1002 and 7-mer peptides caused enhanced permeability on both models but they were less effective on the intestinal barrier model. The selectivity of these peptides offers a great potential for innovative targeted drug delivery. PN159 peptide was the most effective permeability enhancer on both models: a rapid and reversible effect was found in low, non-toxic concentrations without permanent morphological changes. Potential targets of PN159 peptide were identified as claudin-1, -4, -5 and -7 but not claudin-3 by affinity measurement and molecular modeling. The secondary structure and the high thermostability of PN159 were also revealed, which can be important for the development of new pharmaceutical formulations and drug delivery systems. The presented results indicate that these peptides can be effectively and selectively used as potential pharmaceutical excipients to improve drug delivery across biological barriers.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Bocsik A**., Darula Z., Tóth G., Deli M.A., Wollemann M.
Transfer of opiorphin through a blood-brain barrier culture model
Archives of Medical Research 46, 502-506 (2015)
IF: 2.645 (2014)

II. **Bocsik A**., Walter R. F., Gyebrovszki A., Fülöp L., Blasig I., Dabrowski S., Ötvös F., Rákhely G., Veszelka S., Deli M.A.
Reversible opening of intercellular junctions of intestinal epithelial and brain endothelial cells with tight junction modulator peptides
Journal of Pharmaceutical Sciences 105, 754-765 (2016)
IF: 2.59 (2014)

III. **Bocsik A**., Kiss L., Gróf I., Ötvös F., Zsíros O., Fülöp L., Vastag M., Kittel Á., Szabó-Révész P., Deli M.A.
PN159 peptide increases drug penetration across Caco-2 intestinal barrier model by reversibly modulating tight junctions
(manuscript submitted for publication)

OTHER PUBLICATIONS

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PRESENTATIONS RELATED TO THE SUBJECT OF THE THESIS

Oral presentation

Bocsik A, Walter F, Veszelka S, Nagy IL, Vastag M, Fülöp L, Szabó-Révész P, Deli MA. Sejtek közötti szoros kapcsolatok nyitása peptidekkel endotél- és epitélsejteken. A Magyar Mikroszkópos Társaság Éves Konferenciája (Hungarian Society of Microscopy Meeting), Siófok, 2013. május 23-25.

Poster presentations

Bocsik A, Walter1 FR, Veszelka S, Blasig EI, Nagy L, Vastag M, Fülöp L, Szabó-Révész P, Deli MA. Opening the intercellular junctions of brain endothelial with peptides. 17th International Symposium on Signal Transduction at the Blood-Brain and Blood-Retina Barriers, Dublin, Írország, 2014. szeptember 11-13.

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