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https://doi.org/10.31925/farmacia.2024.6.13 **ORIGINAL ARTICLE**

A COMPARATIVE STUDY OF FOUR PERMEATION ENHANCERS FOR INCREASING THE TRANSPORT OF SALMON CALCITONIN ON CACO-2 CELL LINES

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Abstract

Over the past 50 years, research has led to the development of effective peptide-based macromolecules, yet only 4% are administered orally, despite patient preference. Oral delivery of peptides is challenging due to degradation by proteolytic enzymes and low gastric permeability. Co-formulation with permeation enhancers (PEs) offers a promising strategy to overcome these barriers and improve oral bioavailability. This study evaluates the efficacy and safety of 4 PEs - in 2 different concentrations - on the permeability of salmon calcitonin (sCT) in Caco-2 cell lines: S-nitroso-N-acetyl-DL-penicillamine (SNAP), sodium taurodeoxycholate (TDC), dimethyl-palmitoyl-ammonio-propane-sulfonate (PPS) and tetradecyl maltoside (TDM). Among the PEs tested, TDM 0.2 mg/mL significantly increased sCT permeability, with a 282% increase *versus* control (p = 0.017), 240% *versus* SNAP 0.002 mg/mL (p = 0.01) and 149% *versus* TDC 0.1 mg/mL (p = 0.036). All PEs significantly decreased the transepithelial electrical resistance (TEER) of the cell lines *versus* control, with the strongest effects observed within the first 15 minutes. TDM 1 mg/mL caused the most significant TEER reduction, followed by PPS 0.2 mg/mL and TDC 0.25 mg/mL. TEER values were lowest at 60 minutes, with partial recovery noted at two hours, suggesting potential reversibility. These findings could guide future research in selecting the optimal PE for co-formulation with sCT or other peptides with similar molecular characteristics.

Rezumat

În ultimii 50 de ani, au fost dezvoltate numeroase peptide terapeutice, însă doar 4% sunt administrate oral, deși aceasta este calea preferată de pacienți. Administrarea orală a peptidelor este dificilă din cauza degradării enzimatice și a permeabilității gastrice scăzute. Co-formularea cu potențiatori de permeație (PP) oferă o strategie viabilă pentru îmbunătățirea biodisponibilității obținute prin administrarea orală. Acest studiu evaluează eficacitatea și siguranța a 4 PP - în 2 concentrații diferite - asupra permeabilității calcitoninei de somon (sCT) pe liniile celulare Caco-2: S-nitrozo-N-acetil-DL-penicilamină (SNAP), taurodeoxicolat de sodiu (TDC), dimetil-palmitoil-amoniu-propansulfonat (PPS) și tetradecil maltozidă (TDM). Dintre PP investigați, TDM 0,2 mg/mL a crescut semnificativ permeabilitatea sCT, cu 282% față de control (p = 0,017), cu 240% față de SNAP 0,002 mg/mL (p = 0,01) și cu 149% față de TDC 0,1 mg/mL (p = 0,036). Toți PP au scăzut semnificativ rezistența electrică transepitelială (RET) a liniilor celulare față de control, cu cele mai mari efecte observate în primele 15 minute. TDM 1 mg/mL a produs cea mai mare reducere a RET, urmat de PPS 0,2 mg/mL și TDC 0,25 mg/mL. Valorile RET au fost cele mai scăzute la 60 de minute, cu o recuperare parțială observată după două ore, sugerând un efect reversibil. Aceste rezultate ar putea coordona cercetările viitoare privind selectarea PP pentru co-formularea cu sCT sau cu alte peptide similare.

Keywords: oral, calcitonin, permeation, enhancers

Introduction

During the last 50 years, extensive research efforts and investments have resulted in the development of peptide-based macromolecules that possess an acceptable safety profile and demonstrate high efficacy in treating a range of chronic diseases [1]. Among the 240 macromolecules developed from 1980 to the present day, only 4% are administered orally, while over 90% are administered parenterally [2]. This presents a significant challenge, as patients generally prefer oral administration, and patient preference is a crucial factor in achieving and maintaining therapeutic targets [3]. Salmon calcitonin is an active peptide with a molecular weight of 3432 Da, composed of 32 amino acids [4], and is currently used in clinical practice for the treatment of post-menopausal osteoporosis, hypercalcemia, Paget's disease and even bone pain due to its analgesic properties [5]. Salmon calcitonin (sCT) is preferred over human calcitonin because it has a higher affinity for the calcitonin receptors, and 100-fold the potency of human calcitonin [5]. However, like other peptide-structured macromolecules, sCT is administered parenterally and thus, it is associated a high degree of discomfort or even pain [6]. Consequently, patient adherence and persistence with injectable treatments are significantly reduced [7, 8]. To address these disadvantages, intra-

nasal salmon calcitonin was developed [5]. However, this administration route also has major drawbacks affecting patient compliance, such as nasal mucosa ulcers, epistaxis and an increased risk of rhinitis [5]. Significant efforts have been made to develop an oral formulation of sCT to overcome the challenges of its currently used pharmaceutical forms [6]. Unfortunately, successful results have not been reported so far [6]. In our previous research, we have detailed the challenges and the strategies used for developing oral calcitonin formulations, as well as the key reasons that led to the failures and eventual abandonment of the effort to develop an oral form of salmon calcitonin [6]. These reasons include the limited bioavailability of orally administered calcitonin [4], the distribution of calcitonin receptors in other tissues, which leads to limited availability of the active substance in bone tissue [9] the emergence of other technologies that prolong the half-life of calcitonin by up to 3 weeks (allowing for significantly increased intervals between administrations) [10], and the development of other classes of drugs with greater efficacy in treating osteoporosis [7]. Nevertheless, salmon calcitonin is still used in clinical practice, and the development of an oral calcitonin form by using the co-formulation technology with a gastric permeation enhancer has the potential to increase the accessibility of the treatment at an affordable price [6]. Permeation enhancers (PEs) or absorption enhancers are a heterogeneous class of excipients that, in addition to their primary role in formulations, allow macromolecules to penetrate the gastric membrane by transiently altering the epithelial intestinal barrier [11]. By temporary altering the gastric barrier, permeation enhancers can facilitate the transport of macromolecules *via* the paracellular pathway or the transcellular pathway [11]. Since permeation enhancers operate through a variety of mechanisms, numerous research projects have investigated their effects both *in vitro* (on cell lines) and *in vivo* (on laboratory animals) [12]. Due to its molecular characteristics, sCT is a feasible candidate for evaluating the efficacy and safety of absorption enhancers. Consequently, significant progress has been made in investigating its co-formulation with various permeation enhancers, even though only a few of the co-formulations have advanced to human trials [6]. In this study, we examine the comparative efficacy and safety of four permeation enhancers in two different concentrations: S-nitroso-N-acetyl-DL-penicillamine (SNAP) C1 = 0.002 mg/mL and C2 = 0.22 mg/mL, sodium taurodeoxycholate (TDC) C1 = 0.1 mg/mL and C2 = 0.25 mg/mL, dimethyl-palmitoyl-ammonio-propanesulfonate (PPS) C1 = 0.1 mg/mL and C2 = 0.2 mg/mL and tetradecyl maltoside (TDM) C1 = 0.2 mg/mL and C2 = 1 mg/mL. All the PEs were selected based on previous research findings [13, 16]. The PEs were assessed for their ability to enhance the permeability of sCT *in vitro*, using Caco-2 cell lines, while maintaining the integrity of the cells. The efficacy of each PE was quantified by measuring the apparent permeability coefficient (Papp), and safety was evaluated by monitoring the trans-epithelial electric resistance (TEER). Comparing the PEs in a single study provided the opportunity to rank them directly, according to the permeation enhancing and TEER decreasing effects.

Materials and Methods

Materials

Salmon calcitonin (sCT), S-nitroso-N-acetyl-DL-penicillamine (SNAP), sodium taurodeoxycholate (TDC), dimethyl-palmitoyl-ammonio-propanesulfonate (PPS) and tetradecyl maltoside (TDM) were purchased from Sigma-Aldrich Chemie GmbH. The transwell Caco-2 assay system used was the CacoReady 96 Transwell (Corning) seeded with 21 days differentiated and polarised Caco-2 cells, designed for high-throughput experimentation and purchased from ReadyCell SL. The ELISA kit for the analysis of salmon calcitonin samples was obtained from Abbexa LTD. The voltmeter used for TEER measurements was the EVOM2, coupled with the STX100C96 electrode, designed for the Corning HTS 96-Well Plate system of the Caco-2 assay, as recommended by ReadyCell. Both the EVOM2 voltmeter and the STX100C96 electrode were purchased from World Precision Instruments LLC. The rest of the materials used were of analytical grade and were obtained from various providers.

Evaluation of PEs efficacy in increasing the permeability of sCT

The efficacy of SNAP, TDC, PPS and TDM in enhancing the permeability of sCT was evaluated across the Caco-2 cell lines, according to the protocol provided by ReadyCell. For the permeability studies, the working dilutions of sCT and PEs were prepared in HBSS buffer solution. 100 μL of each pair of test solutions (sCT and SNAP C1, sCT and SNAP C2, sCT and TDC C1, sCT and TDC C2, sCT and PPS C1, sCT and PPS C2, sCT and TDM C1, sCT and TDM C2) were added to the apical chambers. The final concentration of sCT in the apical chamber was 0.01 mg/mL. SNAP was added to the apical chambers at final concentrations of 0.002 mg/mL (C1) and 0.22 mg/mL (C2). TDC was added to the apical chambers at final concentrations of 0.1 mg/mL (C1) and 0.25 mg/mL (C2). PPS was added to the apical chambers at final concentrations of 0.1 mg/mL (C1) and 0.2 mg/mL (C2). TDM was added to the apical chambers at final concentrations of 0.2 mg/mL (C1) and 1 mg/mL (C2). The control arm consisted only in HBSS buffer solution. 25 μL samples from the apical inserts were collected at the start of the experiment (0 minutes) and the plate was incubated for 2 hours (at 37°C and 5% CO_2) without shaking. At the 120-minute mark 25 μL samples were taken from each basolateral chamber, to assess the amount of sCT transported across the cell lines. All

samples were collected in triplicate (n = 3), with the exception of the control samples which were collected in duplicate (n = 2). The quantitative detection of sCT in the samples was performed *via* ELISA.

After determining the sCT concentration in each study sample, the apparent permeability coefficient (Papp) was calculated using the formula below (according to the protocol provided by ReadyCell):

$$Papp = \frac{dQ}{dt} dt \ X \ \frac{1}{A \ X \ C_0},$$

where, dQ/dt represents the amount of product present in the basolateral chamber as a function of time (nmol/s); A represents the area of Transwell = 0.14 cm^2; C_0 represents the initial concentration of sCT applied in the apical chamber.

Evaluation of PEs safety in increasing the permeability of sCT

The safety of SNAP, TDC, PPS and TDM was evaluated by measuring the effect of each PE on the transepithelial electrical resistance (TEER) and assessing the reversibility of the effect. TEER measurements were conducted at pre-determined timepoints: 0 minutes, 15 minutes, 60 minutes and 120 minutes after incubation. The blank resistance was measured in the cell culture insert without the cells and was subtracted from the TEER values measured at the predetermined time-points, to obtain the true effects of the PEs on the resistance value of the Caco-2 cell monolayers.

Statistical analysis

Results were expressed by using the mean ± 1 standard deviation. The statistical significance for the comparison of all permeability datasets was assessed by using the Tukey Simultaneous test, at a level of significance of 5% (α = 0.05). Additionally, we compared each permeability dataset obtained for permeation enhancers with the control dataset by using the Dunnett Multiple Comparison test, at a level of significance of 5% (α = 0.05). To assess the statistical significance for the comparison of the TEER datasets with the control group, we also used the Dunnett Multiple Comparison test for each time point (15 minutes, 60 minutes and 120 minutes), at a level of significance of 5% (α = 0.05). For the statistical calculations we used the Minitab Statistical Software version 22.

Results and Discussion

TDM C1 (0.2 mg/mL) was the only permeation enhancer which had a statistically significant effect on the sCT Papp in comparison with control, SNAP C1 (0.002 mg/mL) and TDC C1 (0.1 mg/mL). TDM C1 (0.2 mg/mL) increased the sCT Papp with 282% *vs*. Control (p = 0.017), with 240% *vs*. SNAP C1 (0.002 mg/mL) (p = 0,01) and with 149% *vs*. TDC C1 (0.1 mg/mL) (p = 0.036). There were no other significant differences between the permeation enhancers regarding the effect on sCT Papp. However, we observed numerically higher values of sCT Papp obtained with all PEs in comparison with control, signifying a trend for increasing the permeability of sCT, as seen in Figure 1.

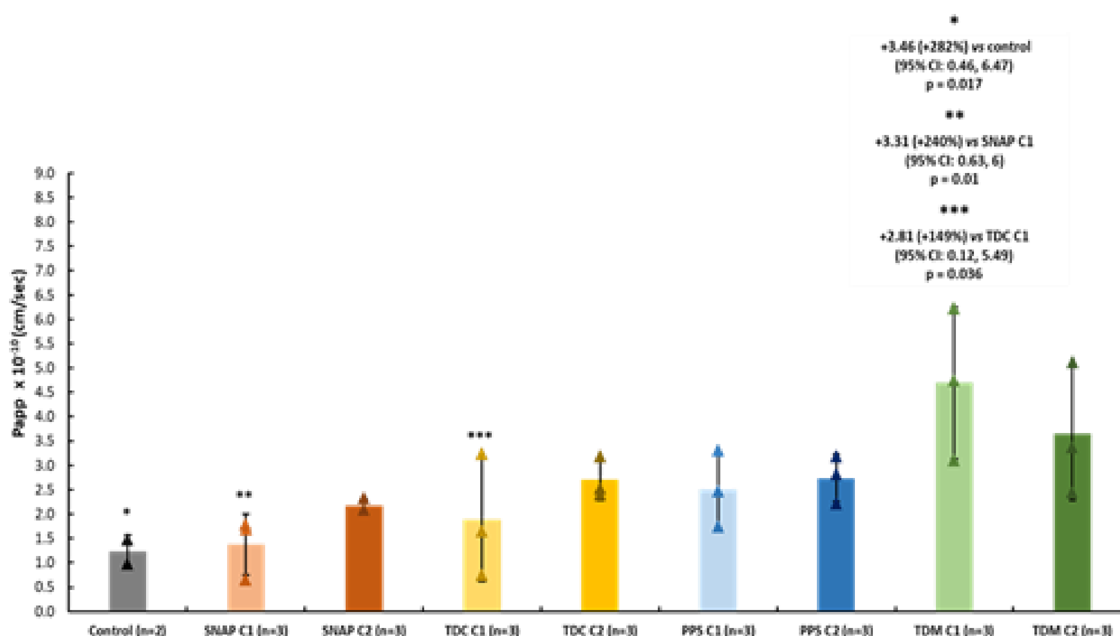

Figure 1.

The enhancement effects of SNAP (C1 = 0.002 mg/mL; C2 = 0.22 mg/mL), TDC (C1 = 0.1 mg/mL; C2 = 0.25 mg/mL), PPS (C1 = 0.1 mg/mL; C2 = 0.2 mg/mL) and TDM (C1 = 0.2 mg/mL; C2 = 1 mg/mL) on the permeability of sCT across the Caco-2 cell lines

Results are expressed as the mean ± SD of three or two determinations. Each triangle represents one measurement. (*): p < 0.05 compared with control; (**): p < 0.05 for TDM C1 compared with SNAP C1; (***): p < 0.05 for TDM C1 compared with TDC C1

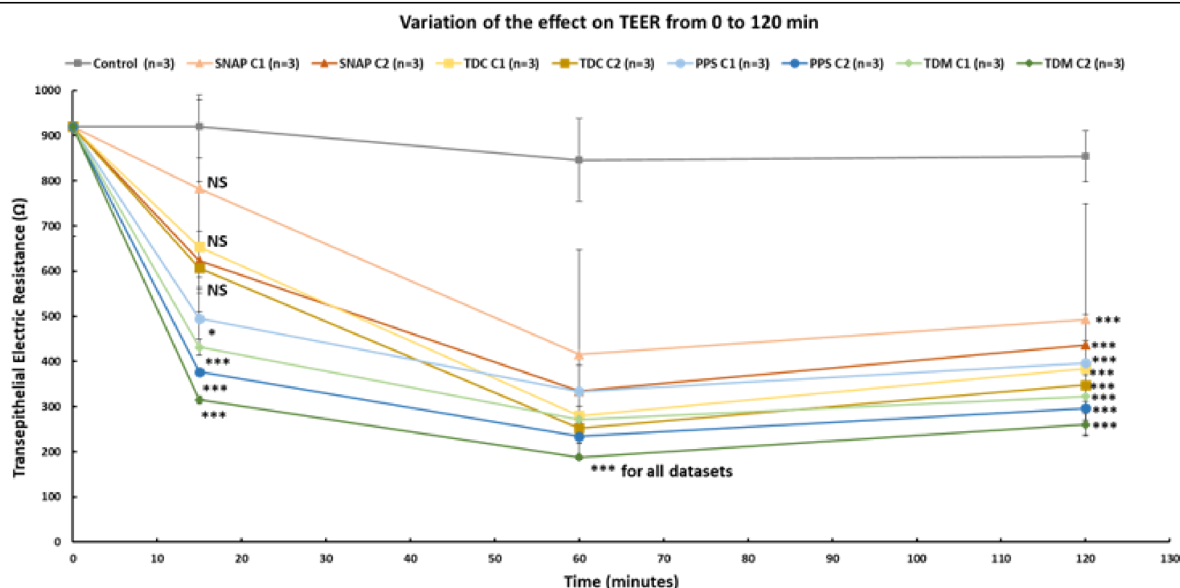

Figure 2.

The effects of SNAP (C1 = 0.002 mg/mL; C2 = 0.22 mg/mL), TDC (C1 = 0.1 mg/mL; C2 = 0.25 mg/mL), PPS (C1 = 0.1 mg/mL; C2 = 0.2 mg/mL), TDM (C1 = 0.2 mg/mL; C2 = 1 mg/mL) on the TEER of Caco-2 cell lines at 15 minutes, 60 minutes and 120 minutes

Results are expressed as the mean ± SD of three or two determinations. (NS): $p > 0.05$ compared with control; (*): $p < 0.05$ compared with control; (***): $p < 0.0001$ compared with control.

Compared to the control arm, all permeation enhancers showed a statistically significant effect on TEER measured two hours after exposure. However, there were no statistically significant differences between the PEs in their impact on TEER at the two-hour mark. The effect of all the PEs was concentration-dependent, with higher concentrations of each PE resulting in a numerically greater decreases in TEER. The decrease in TEER was most pronounced within the first 15 minutes after exposure, during which TDM C2 (1 mg/mL), PPS C2 (0.2 mg/mL), TDM C1 (0.2 mg/mL) and PPS C1 (0.1 mg/mL) produced statistically significant effects compared to control. The lowest TEER values were observed 60 minutes after exposure for all PEs, with the most significant decreases seen in TDM C2 (77.8% *vs*. control, $p < 0.0001$), followed by PPS C2 (72.3% *vs*. control, $p < 0.0001$) and TDC C2 (70.1% *vs*. control, $p < 0.0001$). The TEER values measured two hours after exposure show a slight recovery, with values for TDM C2 (69.6% *vs*. control, $p < 0.0001$), PPS C2 (65.3% *vs*. control, $p < 0.0001$), TDC C2 (59.3% *vs*. control, $p < 0.0001$) and the other PEs being numerically higher than those observed at 60 minutes. This suggests that the effects on TEER might be reversible. However, further investigation is needed to determine if and when TEER values fully return to baseline after exposure to each PE, which is crucial for assessing potential toxic effects. Overall, at the 15-minute, 60-minute and two-hour marks, TDM C2 (1 mg/mL) had the strongest effect on TEER reduction, while SNAP C1 (0.002 mg/mL) had the weakest effect (Figure 2).

Considering that the effect on TEER is a crucial component of the mechanism by which permeability of the Caco-2 cell lines is increased, the impact of each PE on sCT permeability and the 2-hour TEER values of the caco-2 cell lines, together with the variation in TEER from 0 to 120 minutes and the effects on sCT permeability across the Caco-2 cell lines, as correlated with TEER values measured 120 minutes after exposure, are illustrated in Figure 3 for SNAC, Figure 4 for TDC, Figure 5 for PPS and Figure 6 for TDM.

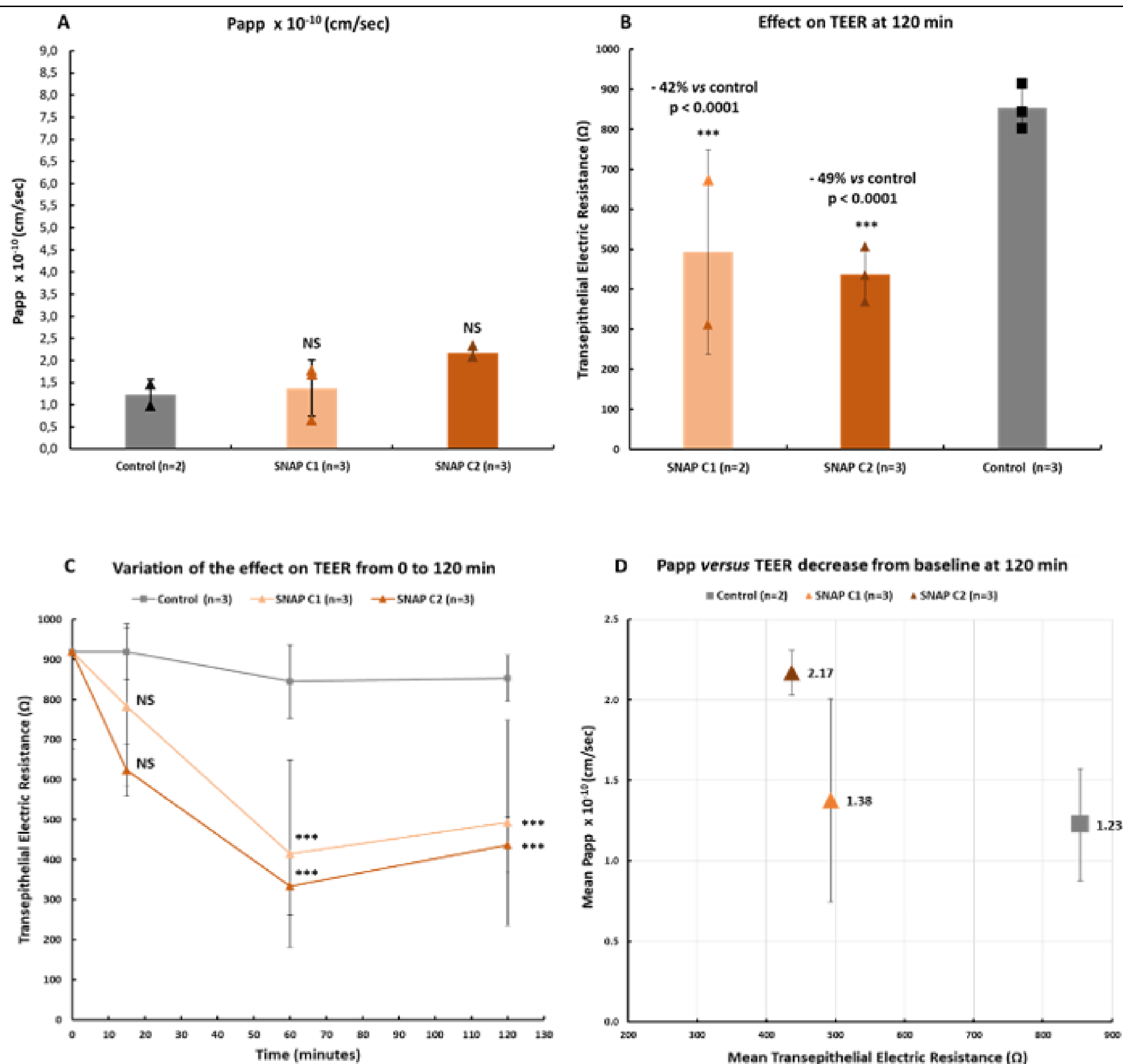

Figure 3.

The effects of SNAP C1 (0.002 mg/mL) and C2 (0.22 mg/mL) on the permeability of sCT across the Caco-2 cell lines (A) and on the TEER of Caco-2 cell lines measured at 120 minutes after exposure (B), the variation of the effect on TEER from 0 to 120 minutes (C) and the effects on the permeability of sCT across the Caco-2 cell lines according to the TEER values measured at 120 minutes after exposure (D)

(A) Results are expressed as the mean ± SD of three or two determinations. Each triangle represents one measurement. (NS): $p > 0.05$ compared with control

(B) Results are expressed as the mean ± SD of three or two determinations. Each triangle and each square represent one measurement. (NS): $p > 0.05$ compared with control. (***) $p < 0.0001$ compared with control

(C) Results are expressed as the mean ± SD of three or two determinations. (NS): $p > 0.05$ compared with control; (***) $p < 0.0001$ compared with control

(D) Results for Papp are expressed as the mean ± SD of three or two determinations and for TEER only as the mean

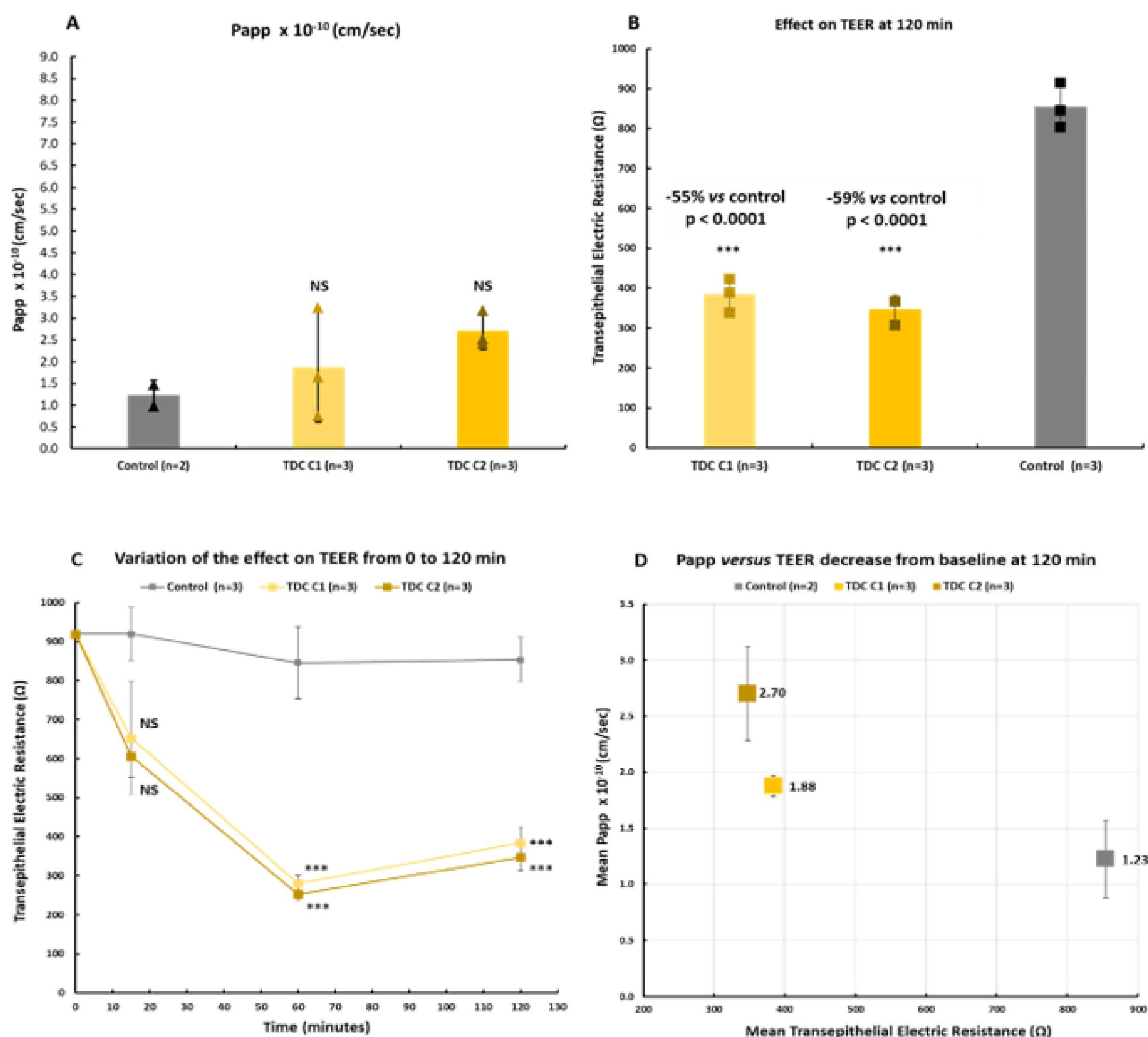

Figure 4.

The effects of TDC C1 (0.1 mg/mL) and C2 (0.25 mg/mL) on the permeability of sCT across the Caco-2 cell lines (A) and on the TEER of Caco-2 cell lines measured at 120 minutes after exposure (B), the variation of the effect on TEER from 0 to 120 minutes (C) and the effects on the permeability of sCT across the Caco-2 cell lines according to the TEER values measured at 120 minutes after exposure (D)

(A) Results are expressed as the mean ± SD of three or two determinations. Each triangle represents one measurement. (NS): $p > 0.05$ compared with control

(B) Results are expressed as the mean ± SD of three or two determinations. Each square represents one measurement. (NS): $p > 0.05$ compared with control. (***) $p < 0.0001$ compared with control

(C) Results are expressed as the mean ± SD of three or two determinations. (NS): $p > 0.05$ compared with control; (***) $p < 0.0001$ compared with control

(D) Results for Papp are expressed as the mean ± SD of three or two determinations and for TEER only as the mean

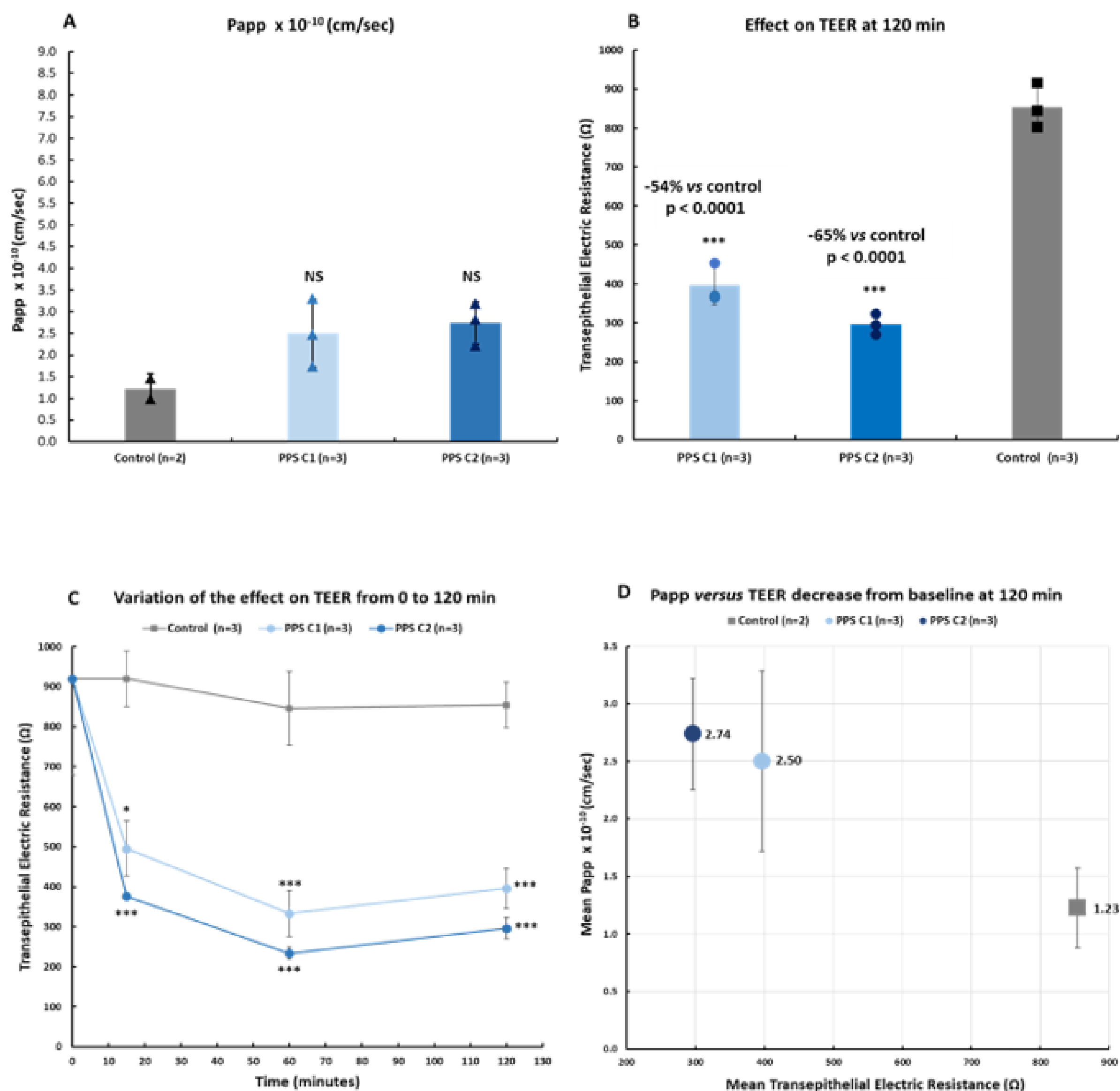

Figure 5.

The effects of PPS C1 (0.1 mg/mL) and C2 (0.2 mg/mL) on the permeability of sCT across the Caco-2 cell lines (A) and on the TEER of Caco-2 cell lines measured at 120 minutes after exposure (B), the variation of the effect on TEER from 0 to 120 minutes (C) and the effects on the permeability of sCT across the Caco-2 cell lines according to the TEER values measured at 120 minutes after exposure (D)

(A) Results are expressed as the mean ± SD of three or two determinations. Each triangle represents one measurement. (NS): $p > 0.05$ compared with control

(B) Results are expressed as the mean ± SD of three or two determinations. Each dot and each square represent one measurement. (NS): $p > 0.05$ compared with control. (***) $p < 0.0001$ compared with control

(C) Results are expressed as the mean ± SD of three or two determinations. (NS): $p > 0.05$ compared with control; (***) $p < 0.0001$ compared with control

(D) Results for Papp are expressed as the mean ± SD of three or two determinations and for TEER only as the mean

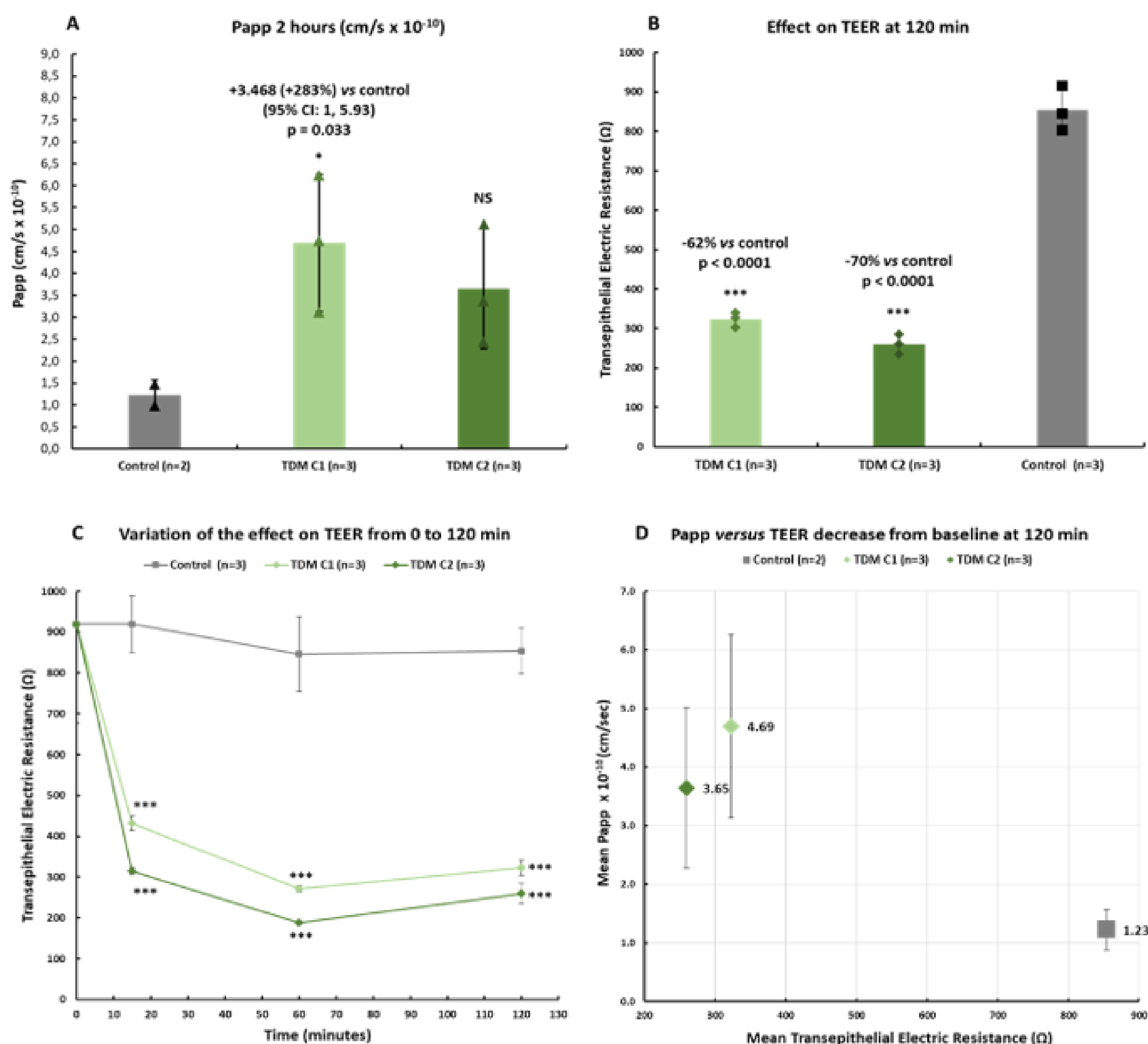

Figure 6.

The effects of TDM C1 (0.2 mg/mL) and C2 (1 mg/mL) on the permeability of sCT across the Caco-2 cell lines (A) and on the TEER of Caco-2 cell lines measured at 120 minutes after exposure (B), the variation of the effect on TEER from 0 to 120 minutes (C) and the effects on the permeability of sCT across the Caco-2 cell lines according to the TEER values measured at 120 minutes after exposure (D)

(A) Results are expressed as the mean ± SD of three or two determinations. Each triangle represents one measurement. (NS): $p > 0.05$ compared with control

(B) Results are expressed as the mean ± SD of three or two determinations. Each rectangle and each square represent one measurement. (NS): $p > 0.05$ compared with control. (***) $p < 0.0001$ compared with control

(C) Results are expressed as the mean ± SD of three or two determinations. (NS): $p > 0.05$ compared with control; (***) $p < 0.0001$ compared with control

(D) Results for Papp are expressed as the mean ± SD of three or two determinations and for TEER only as the mean

Due to its molecular characteristics, sCT is an ideal candidate for evaluating the efficacy and safety of absorption enhancers, and numerous studies have been conducted for this purpose. However, to our knowledge, this is the first study to directly compare the effects of SNAP, TDC, PPS and TDM on the permeability of sCT across Caco-2 cell lines, enabling a ranking of the investigated PEs, based on their impact on sCT Papp and TEER of the Caco-2 cell lines. As shown in Figure 7, TDM C1 had the greatest effect on increasing sCT Papp, followed by TDM C2, PPS C2, TDC C2, PPS C1, SNAP C2, TDC C1 and SNAP C1, respectively.

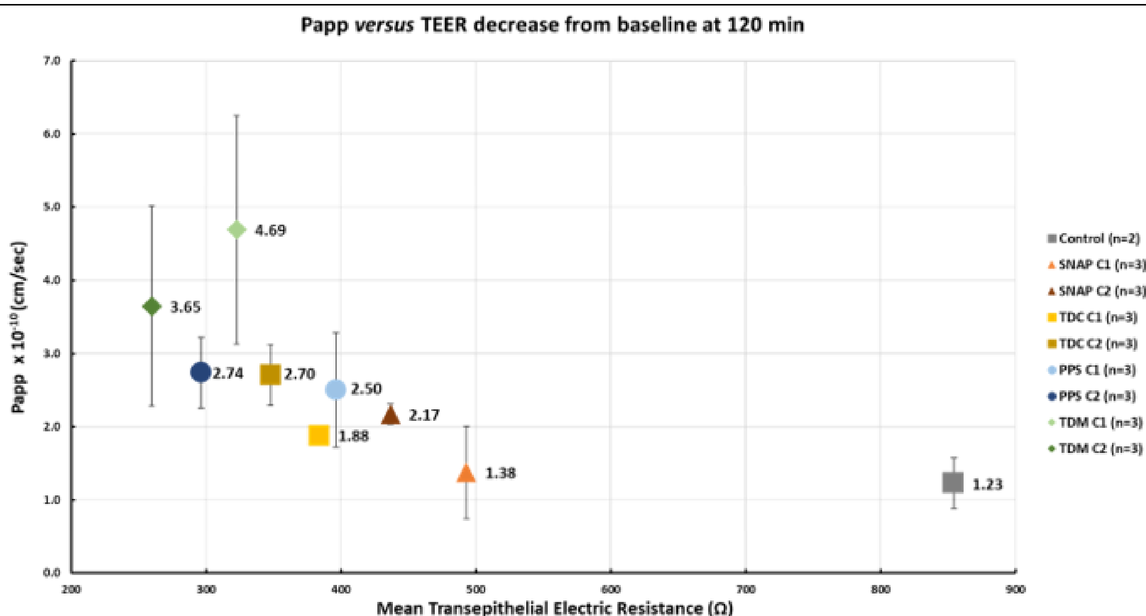

Figure 7.

Effects of SNAP (C1 = 0.002 mg/mL; C2 = 0.22 mg/mL), TDC (C1 = 0.1 mg/mL; C2 = 0.25 mg/mL), PPS (C1 = 0.1 mg/mL; C2 = 0.2 mg/mL), TDM (C1 = 0.2 mg/mL; C2 = 1 mg/mL) on the permeability of sCT across the Caco-2 cell lines (vertical axis) and on the TEER of Caco-2 cell lines at 120 minutes (horizontal axis)

Results for Papp are expressed as the mean ± SD of three or two measurements and for TEER only as the mean

It remains to be investigated if and when the TEER values return to normal after exposure to each PE, as this is important for the assessment of potential toxic effects. Our study has several strengths, notably the comparison of PEs using standardised Caco-2 cell lines, which represents a fundamental method for understanding and predicting the intestinal absorption of pharmaceutical agents [17]. Permeability screening using Caco-2 cells has been extensively employed in the pharmaceutical industry for many years, despite the fact that these cells originate from the colon rather than the small intestine [18, 19]. Additionally, the PEs examined in this study were selected based on prior research, and each has proven efficacious in enhancing the permeability of calcitonin in various settings. SNAP, a nitric oxide donor, proved to be highly effective in enhancing the intestinal transport of insulin and eel calcitonin in rats [13]. TDC is a bile acid salt which demonstrated promising effects in enhancing the absorption of orally administered sCT in rats, particularly when combined with a mucolytic agent and a non-ionic surfactant [14]. PPS is a zwitterionic surfactant shown to effectively and safely enhance the intestinal absorption of therapeutic macromolecules such as insulin and salmon calcitonin [15]. While TDM is a mild surfactant which significantly enhanced sCT permeation across intestinal epithelial monolayers and increased absolute bioavailability of sCT in the rat colon [16]. Our study offers a ranking of the investigated PEs, but further research is needed to validate these findings. The results of this study could guide future research in selecting the optimal PE for co-formulation with sCT or other therapeutic peptides with similar molecular characteristics, such as pramlintide – a derivative of the pancreatic hormone amylin, from the same family of peptides as calcitonin [20] – currently used for the treatment of diabetes [21]. The development of an oral formulation of pramlintide would be particularly beneficial, as patients often develop resistance to treatment due to the need for co-administration with separate insulin injections [22]. Additionally, dual amylin and calcitonin receptor agonists (DACRAs), which currently are being researched in the treatment of obesity and type 2 diabetes [23] might also be feasible for co-formulation with PEs. Cagrilintide, a DACRA derived from an amylin backbone [24], has shown promising results in the treatment of obesity, either as a standalone therapy [25] or in combination with semaglutide [26], which in the subcutaneous once weekly formulation showed additional cardiovascular benefits in people with type 2 diabetes [27]. Semaglutide is also available in an oral formulation with promising outcomes in managing type 2 diabetes associated with obesity [28]. This formulation is achieved through co-formulation with sodium N-(8-[2-hydroxybenzoyl]-amino) caprylate (SNAC) as a permeation enhancer [28]. Our study also has several limitations, particularly the small sample size which reduces the statistical power, making it more difficult to detect true effects and increasing the risk of type II errors, where significant effects might go unnoticed. Also, with fewer data points, individual variability can have a greater impact on the results, potentially skewing the findings and leading to less reliable conclusions [29].

Conclusions

Our study demonstrates that tetradecyl maltoside is a particularly effective permeation enhancer for salmon calcitonin on Caco-2 cell lines, significantly outperforming S-Nitroso-N-acetyl-DL-penicillamine and sodium taurodeoxycholate and achieving numerically higher Papp values compared with dimethyl-palmitoyl-ammonio-propanesulfonate, however without reaching statistical significance. The impact on TEER was observed for all permeation enhancers evaluated, consistent with the known class effect of these active excipients. Additionally, our study underscores the importance of conducting TEER evaluations for longer than 2 hours after initial exposure to assess the reversibility of this effect. Given that permeation enhancers remain a promising approach for formulating orally administered peptides, these findings could guide future research in selecting the optimal enhancer for co-formulation with salmon calcitonin or other therapeutic peptides with similar molecular characteristics, potentially increasing oral bioavailability.

Conflict of interest

The authors declare no conflict of interest.

References

1. Fosgerau K, Hoffmann T, Peptide therapeutics: current status and future directions. *Drug Discov Today.*, 2015; 20(1): 122-128.
2. Lennernäs H, Abrahamsson B, The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension. *J Pharm Pharmacol.*, 2005; 57(3): 273-285.
3. Hamdy RC, Daley DN, Oral calcitonin. *Int J Womens Health.*, 2012; 4: 471-479.
4. Brayden DJ, Hill TA, Fairlie DP, Maher S, Mrsny RJ, Systemic delivery of peptides by the oral route: Formulation and medicinal chemistry approaches. *Adv Drug Deliv Rev.*, 2020; 157: 2-36.
5. McLaughlin MB, Jialal I, Calcitonin. StatPearls Treasure Island, Florida: *StatPearls Publishing*, 2020.
6. Hamza A, Șaramet G, Actualities in Endocrine Pharmacology: Advances in the Development of Oral Formulations for Calcitonin and Semaglutide. *Acta Endocrinol (Buchar).*, 2020; 16(3): 383-387.
7. Drucker DJ, Advances in oral peptide therapeutics. *Nat Rev Drug Discov.*, 2020; 19(4): 277-289.
8. Yu M, Xie J, Fernandez LL, Kabul S, Swindle RW, Liraglutide *versus* Exenatide Once Weekly: Persistence, Adherence, and Early Discontinuation. *Clin Therap.*, 2016; 38(1): 149-160.
9. Liu Y, Yu P, Peng X, Huang Q, Ding M, Chen Y, Jin R, Xie J, Zhao C, Li J, Hexapeptide-conjugated calcitonin for targeted therapy of osteoporosis. *J Control Release.*, 2019; 304: 39-50.
10. Lipp L, Sharma D, Banerjee A, Singh J, Controlled Delivery of Salmon Calcitonin Using Thermosensitive Triblock Copolymer Depot for Treatment of Osteoporosis. *ACS Omega*, 2019; 4(1): 1157-1166.
11. Maher S, Geoghegan C, Brayden DJ, Intestinal permeation enhancers to improve oral bioavailability of macromolecules: reasons for low efficacy in humans. *Expert Opin Drug Deliv.*, 2021; 18(2): 273-300.
12. Maher S, Mrsny RJ, Brayden DJ, Intestinal permeation enhancers for oral peptide delivery. *Adv Drug Deliv Rev.*, 2016; 106: 277-319.
13. Fetih G, Habib F, Okada N, Fujita T, Attia M, Yamamoto A, Nitric oxide donors can enhance the intestinal transport and absorption of insulin and [Asu (1,7)]-eel calcitonin in rats. *J Control Release.*, 2005; 106(3): 287-297.
14. Takatsuka S, Morita T, Koguchi A, Horikiri Y, Yamahara H, Yoshino H, Synergistic absorption enhancement of salmon calcitonin and reversible mucosal injury by applying a mucolytic agent and a non-ionic surfactant. *Int J Pharm.*, 2006; 316(1-2): 124-130.
15. Gupta V, Hwang BH, Doshi N, Mitragotri S, A permeation enhancer for increasing transport of therapeutic macromolecules across the intestine. *J Control Release.*, 2013; 172(2): 541-549.
16. Petersen SB, Nielsen LG, Rahbek UL, Guldbrandt M, Brayden DJ, Colonic absorption of salmon calcitonin using tetradecyl maltoside (TDM) as a permeation enhancer. *Eur J Pharm Sci.*, 2013; 48(4-5): 726-734.
17. Peng Y, Yadava P, Heikkinen AT, Parrott N, Railkar A, Applications of a 7-day Caco-2 cell model in drug discovery and development. *Eur J Pharm Sci.*, 2014; 56: 120-130.
18. Hidalgo IJ, Raub TJ, Borchardt RT, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology*, 1989; 96(3): 736-749.
19. Peng Y, Yadava P, Heikkinen AT, Parrott N, Railkar A, Applications of a 7-day Caco-2 cell model in drug discovery and development. *Eur J Pharm Sci.*, 2014; 56: 120-130.
20. Hay DL, Garelja ML, Poyner DR, Walker CS, Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR Review 25. *Br J Pharmacol.*, 2018; 175(1): 3-17.
21. Kern KA, DiBrog AM, Kaur K, Przybysz JT, Mietlicki-Baase EG, Chronic pramlintide decreases feeding *via* a reduction in meal size in male rats. *Peptides*, 2024; 176: 171197.
22. Kommera SP, Kumar A, Chitkara D, Mittal A, Pramlintide an Adjunct to Insulin Therapy: Challenges and Recent Progress in Delivery. *J Pharmacol Exp Ther.*, 2024; 388(1): 81-90.
23. Larsen AT, Mohamed KE, Melander SA, Karsdal MA, Henriksen K, The enduring metabolic improvement of combining dual amylin and calcitonin receptor agonist and semaglutide treatments in a rat model of obesity and diabetes. *Am J Physiol Endocrinol Metab.*, 2024; 327(2): E145-E154.
24. Larsen AT, Mohamed KE, Sonne N, Bredtoft E, Andersen F, Karsdal MA, Henriksen K, Does receptor balance matter? – Comparing the efficacies of the dual amylin and calcitonin receptor agonists cagrilintide and KBP-336 on metabolic parameters in preclinical models. *Biomed Pharmacother.*, 2022; 156: 113842.
25. Lau DCW, Erichsen L, Francisco AM, Satylganova A, le Roux CW, McGowan B, Pedersen SD, Pietiläinen KH, Rubino D, Batterham RL, Once-weekly cagrilintide

for weight management in people with overweight and obesity: a multicentre, randomised, double-blind, placebo-controlled and active-controlled, dose-finding phase 2 trial. *Lancet*, 2021; 398(10317): 2160-2172.
26. Frias JP, Deenadayalan S, Erichsen L, Knop FK, Lingvay I, Macura S, Mathieu C, Pedersen SD, Davies M, Efficacy and safety of co-administered once-weekly cagrilintide 2·4 mg with once-weekly semaglutide 2·4 mg in type 2 diabetes: a multicentre, randomised, double-blind, active-controlled, phase 2 trial. *Lancet*, 2023; 402(10403): 720-730.
27. Li L, Wang A, Wu S, Lv Y, Impact Of GLP-1 Receptor Agonist Semaglutide on Carotid Intima-Media Thickness in Diabetes Mellitus. *Farmacia*, 2024; 72(4): 816-825.
28. Tilinca MC, Antal C, Balint A, Săculean A, Varga A, The Newest Therapeutically Approach of “Diabesity” Using GLP-1 RA Molecules: Impact of the Oral Formulation. *Farmacia*, 2023; 71(1): 1-10.
29. Hoerl R, Snee R, Statistical thinking and methods in quality improvement: a look to the future. *Quality Engineering*, 2010; 22(2): 119-129.