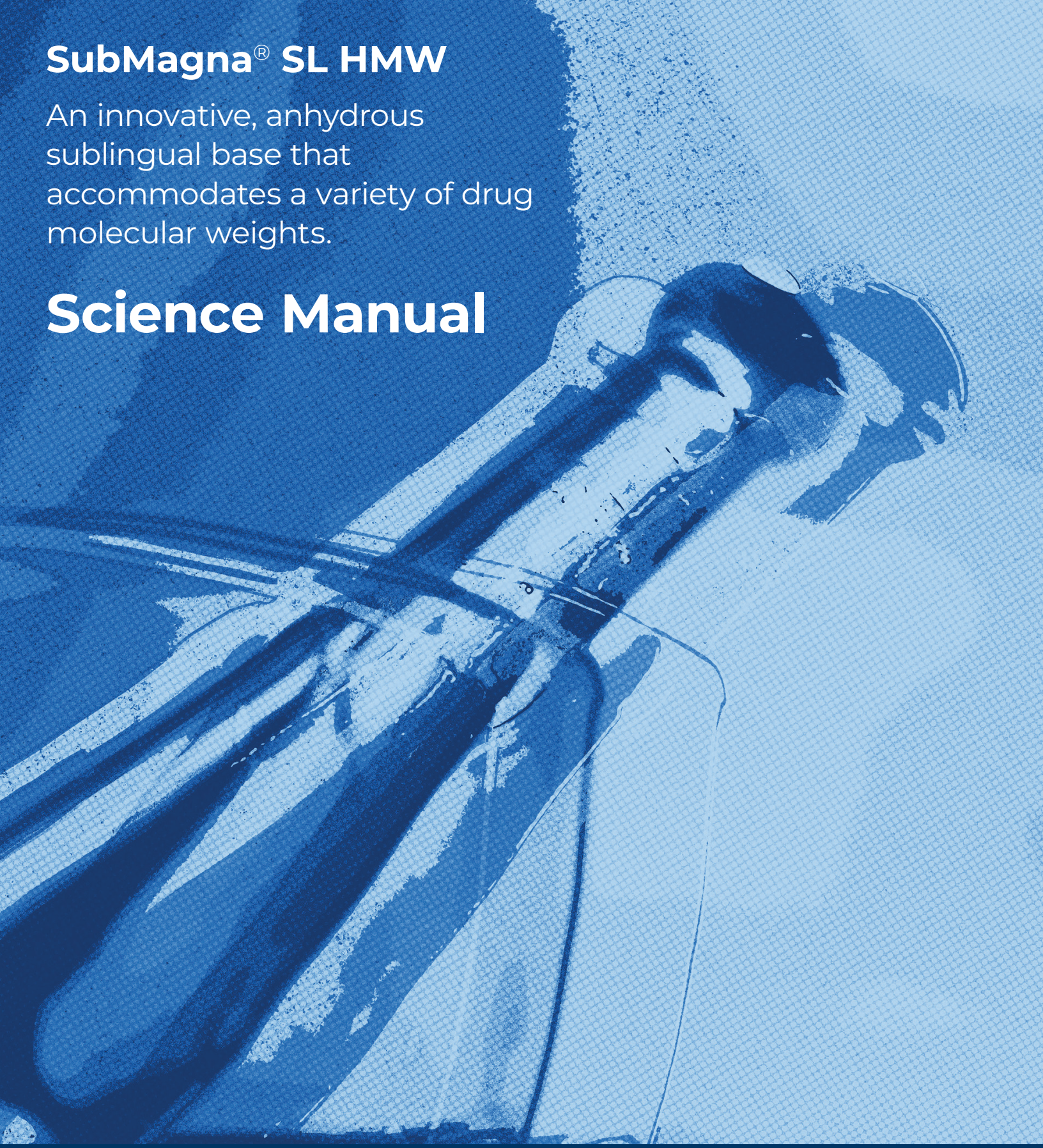
SubMagna® SL HMW

An innovative, anhydrous sublingual base that accommodates a variety of drug molecular weights.

Science Manual



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PCCA SubMagna® SL HMW **TECHNICAL REPORT**

A Single-Dose Pharmacokinetic Study of Sublingually Delivered Semaglutide in SubMagna® in Rats

SUMMARY: The previous pilot pharmacokinetic study has demonstrated the absorption of semaglutide via sublingual route using SubMagna. The objective of this study is to compare the pharmacokinetic profile of sublingually delivered semaglutide in SubMagna with that of oral and injectable products. Results showed that semaglutide in SubMagna resulted in less variability in plasma concentrations and improved bioavailability compared to oral commercial tablet.

Introduction:

SubMagna® SL HMW was designed to efficiently deliver a wide range of drugs with varying molecular weights — including high molecular weight (HMW) substances. SubMagna was initially developed as an alternative dosing form for commercially available semaglutide in nonsterile compounded preparations. This proof-of-concept study aims to demonstrate the sublingual absorption of semaglutide from SubMagna and to compare its pharmacokinetic profile with that of commercially available oral and injectable products.

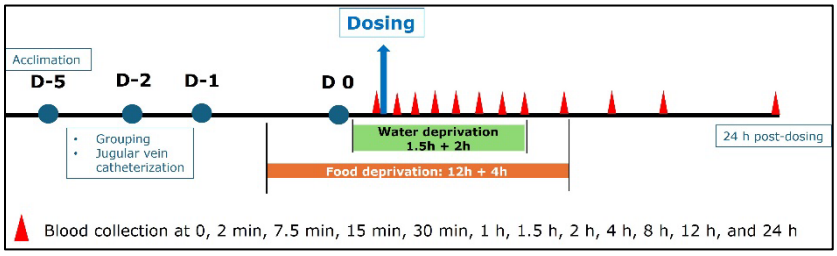

Figure 1. *Summary of the study timeline.*

Methodology:

The animal study was conducted by GemPharmatech Co., Ltd following ethics approval: animal protocol CDAP20240621-1#; project number PO-GJC0520240500076-02. The test product semaglutide (API from commercial Rybelsus® tablets) $6x10^6$ ng/mL compounded formulation (SubMagna SL HMW) was provided by PCCA. Sprague–Dawley rats (5-8 weeks old, male) (n=24) were randomly assigned to treatment groups as shown in Table 1. The dosing and sampling schedule is present in Figure 1. All rats were fasted 12 hours prior to dosing and for 4 hours post-dosing. Water deprivation started 1.5 hours before dosing and resumed 2 hours afterward. The plasma samples were analyzed for semaglutide using LC-MS/MS.

Treatment	Dose	Route	Frequency	Number of Rats
Commercial (Semaglutide injection)	0.01 mg/kg	Subcutaneous	Single dose	6
Semaglutide in SubMagna	1 mg/kg	Sublingual	Single dose	6
Commercial (Semaglutide tablets)	1 mg/kg	Oral	Single dose	6
Commercial (Semaglutide tablets)	20mg/kg	Oral	Single dose	6

Table 1. *Summary of the treatment groups.*

Results:

Semaglutide was detected in all rats as early as 2 minutes post-dosing. The variability in plasma concentrations among individual rats and pharmacokinetic parameters was analyzed.

Variability

Consistent with findings in humans, oral semaglutide produces large variability in plasma concentrations of semaglutide in rats. In contrast, sublingual dosing results in smaller variability as shown in Figure 2, with the smallest variability observed following subcutaneous injection.

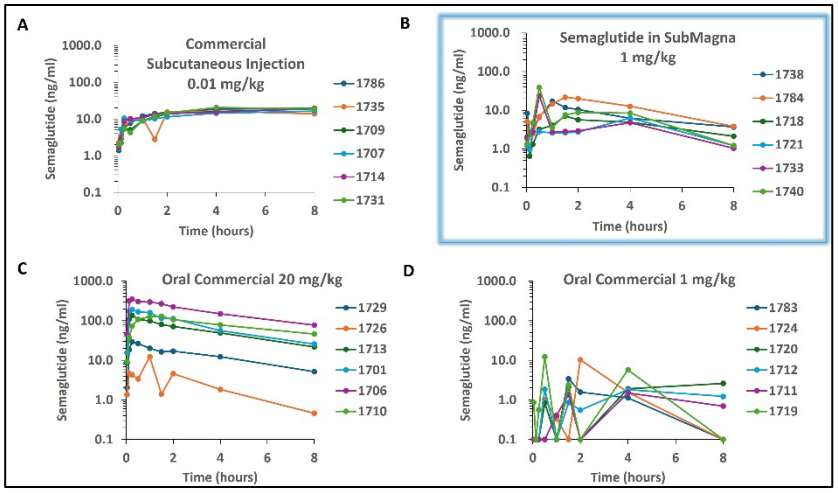

Figure 2. *Plasma concentrations of semaglutide in different treatment groups. Each line represents data from an individual rat, labeled with its ID.*

A Single-Dose Pharmacokinetic Study of Sublingually Delivered Semaglutide in SubMagna® in Rats

Absorption / AUC

When comparing the area under the plasma concentration-time curve (AUC) over 24 hours, as shown in Figure 3, the sublingual group exhibited a significantly higher AUC than the oral tablet 1 mg/kg group. The AUC in oral tablet 20 mg/kg group was not shown in the bar graph due to considerable variability ranging from 29.34 ng*h/ml to 2077.24 ng*h/ml.

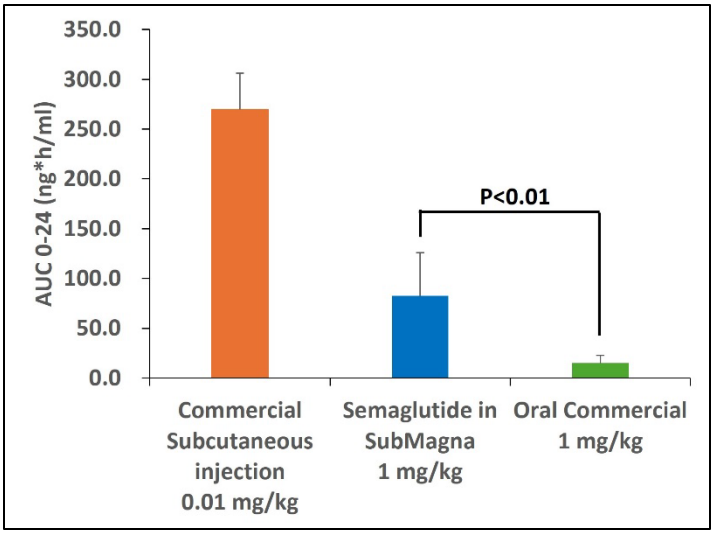

Figure 3. *AUC of semaglutide in different treatment groups.*

C_{max} & T_{max}

Semaglutide in SubMagna demonstrates a similar absorption profile compared to the oral group, both reaching C_{max} as early as 30 minutes post-dosing as shown in Figure 4 (right panel). This is distinct from the subcutaneous route, which reaches a prolonged peak at 8 hours (Figure 4**,** left panel).

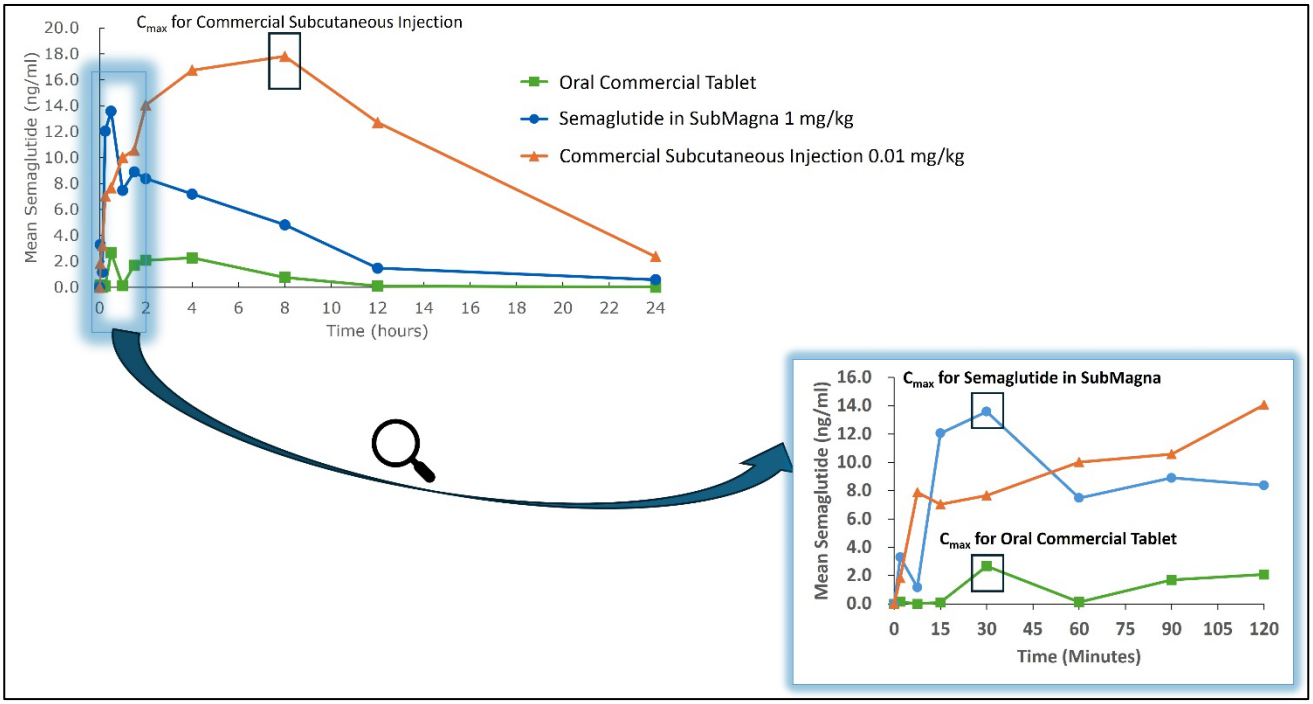

Figure 4. *Mean plasma concentration-time profile of semaglutide in different treatment groups. C_{max} of injection group was labeled in the left panel. C_{max} of sublingual and oral groups were labeled in a zoomed-in view of the first 120 minutes post-dosing (right panel).*

A Single-Dose Pharmacokinetic Study of Sublingually Delivered Semaglutide in SubMagna® in Rats

Relative Bioavailability

Relative bioavailability was calculated based on the absorption of semaglutide in different dosage forms versus semaglutide in commercial injection using the data in Table 2. Sublingual administration shows significantly higher bioavailability compared to both oral groups. There is no significant difference in bioavailability between the oral 1 mg/kg and 20 mg/kg groups.

Drug	Route	Dose (mg/kg)	T_{max} (h)	C_{max} (Range) (ng/mL)	AUC_{0-24} (Range) (ng*h/mL)	$t_{1/2}$ (h)	Relative Bioavailability (F) (%)
Commercial Injection	Subcutaneous	0.01	8	17.8 (16.3-20.3)	269.85 (211.97-302.77)	5.27	100
Commercial Tablet	Oral	20	0.25	131.3 (12.3-352.0)	798.02 (29.34-2077.24)	5.82	0.163
	Oral	1	0.5	2.7 (1.5-12.3)	15.08 (7.05-27.58)	5.22	0.061
Semaglutide	**Sublingual in SubMagna**	**1**	**0.5**	**13.6 (6.3-38.5)**	**82.53 (43.02-138.93)**	**5.88**	**0.336**

Table 2. *Summary of pharmacokinetic parameters and calculated relative bioavailability of semaglutide in different treatment*

Conclusion:

This single-dose pharmacokinetic study further validated SubMagna's ability to deliver semaglutide sublingually. Sublingual delivery by SubMagna demonstrated less variability in plasma concentrations and absorption compared to the oral commercial tablet. The findings suggested improved bioavailability for semaglutide in SubMagna versus the oral commercial tablet. The C_{max} /T_{max} profiles for sublingual and oral administration are similar, indicating that the dosing frequency for sublingual should be similar to that of oral dosing.

This proof-of-concept study is not intended to provide guidance on the sublingual dosing of semaglutide in SubMagna in humans, or dosing conversion from oral or injectable forms to sublingual administration. Further pharmacokinetic studies in humans, along with dose-finding studies, are necessary to determine the equivalent sublingual dose in humans

Evaluation of the Absorption of a Sublingual Semaglutide Compounded Formulation (SubMagna™ SL HMW) using the EpiGingival™ and EpiOral™ *In Vitro* Tissue Models

SUMMARY: GLP-1 agonists have been increasingly utilized in the treatment of type 2 diabetes and obesity. The semaglutide commercial oral tablets have extremely low absorption and an alternative sublingual compounded formulation is proposed: semaglutide in SubMagna SL HMW. The *in vitro* tissue models suggest that SubMagna SL HMW is able to deliver the peptide into and through human gingival and oral tissues.

Introduction:

There is a growing demand worldwide for glucagon-like peptide (GLP)-1 agonists, a class of medications utilized in the treatment of type 2 diabetes and obesity. Semaglutide, the active ingredient in the injectable medications Ozempic® and Wegovy® (Figure 1), is the most popular GLP-1 agonist and there are often shortages in the marketplace [1].

Many patients would prefer to avoid injections if possible, and there is an extremely low absorption of the oral tablets (less than 1% per the labeling for Rybelsus®). For these reasons, prescribers and patients may prefer a patent-pending compounded formulation of semaglutide for sublingual administration comprising Rybelsus tablets and SubMagna SL HMW [2]. SubMagna is an anhydrous, self-emulsifying drug delivery system intentionally developed to carry drugs of high molecular weight (HMW) in a sublingual route of administration. This innovative compounding base also benefits from mucoadhesive properties which increase the contact time of the drug in the sublingual space [3].

The purpose of this study was not to determine the appropriate sublingual dose of semaglutide but, instead, to evaluate the ability of the SubMagna to deliver the peptide into and through human gingival and oral tissues. This analysis is not a substitute for *in vivo* pharmacokinetic studies.

Figure 1. *Self-administration of semaglutide injection; stock illustration ID: 2403927641 (adapted from Caroline Ruda /Shutterstock.com).*

Methodology:

The EpiGingival and EpiOral tissues, manufactured by MatTek (Ashland, MA), were the models used to evaluate *in vitro* the absorption of the sublingual compounded formulation semaglutide 3 mg/mL in SubMagna SL HMW. Six tissues of each were incubated overnight at 37º C and 5% CO_2 for equilibration. The assay medium (Teer-Buffer-GLC buffer) was pre-warmed to 37° C and pipetted into 6-well plates. The tissues were transferred into the plates together with the assay medium. The semaglutide compounded formulation was then applied and, following 15 min of elapsed permeation time, the receptor media was collected for analysis. This procedure was repeated for 30 min of total elapsed permeation time.

The quantification of semaglutide was performed using the ELISA analysis, kit purchased from OriGene (Rockville, MD). The standards and test samples were loaded into the wells of the immunoplate. The antiserum was added, and the plate was incubated at room temperature for 1 hr. Following incubation, the rehydrated Bt-tracer was placed on each well and incubated for 2 hrs. After washing, Streptavidin-HRP was added to the plate and the color was then generated with TMB chromogenic solution. Absorbance was read at 450 nm following termination of enzymatic reaction, and the permeation flux of semaglutide was calculated.

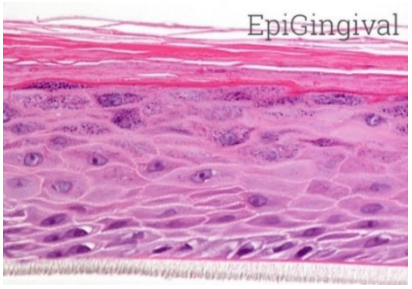

Figure 2. *Illustration of the EpiGingival™ tissue model (adapted from MatTek).*

Evaluation of the Absorption of a Sublingual Semaglutide Compounded Formulation (SubMagna™ SL HMW) using the EpiGingival™ and EpiOral™ *In Vitro* Tissue Models

Results and Discussion:

MatTek's EpiGingival and EpiOral tissues consist of normal, human-derived oral epithelial cells which have been cultured to form multilayered, highly differentiated models of the human gingival and oral phenotypes. These tissue models exhibit *in vivo*-like morphological and growth characteristics, which are uniform and highly reproducible. As such, these models are commonly used for *in vitro* testing of transbuccal delivery of drugs [4-7]. In this study, the absorption of semaglutide into and through the EpiGingival and EpiOral tissues was detected as early as 15 minutes post-application of the sublingual compounded formulation. The permeation flux of the sublingual semaglutide is shown in Figure 3 for the gingival tissues and in Figure 4 for the oral tissues.

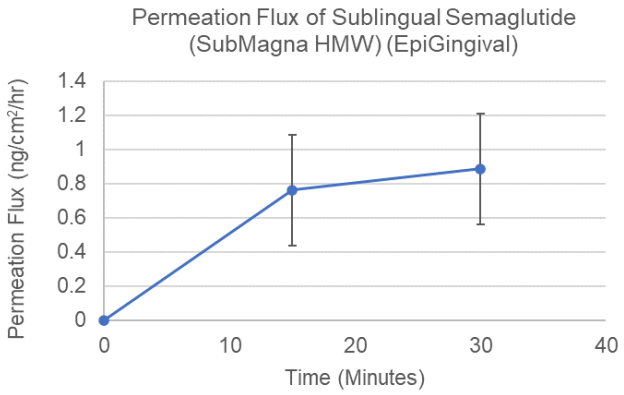

Figure 3. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

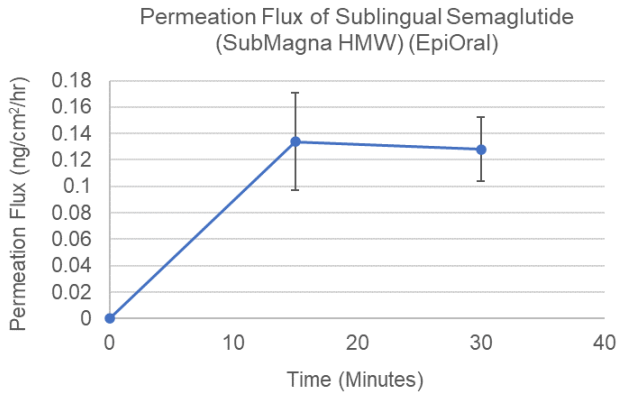

Figure 4. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

Conclusions:

The buccal mucosa is an attractive site to administer drugs, for either local or systemic delivery, because of its diminutive barrier properties, relatively neutral pH and limited enzymatic activity. Underneath the epithelium there is the mucosal tissue which includes blood and lymphatic vessels. When in the buccal region, drugs can be rapidly and directly absorbed into the systemic circulation by means of a venous drainage to the superior vena cava [8].

Considering that the semaglutide commercial oral tablets have extremely low absorption, the sublingual route of administration is a potentially interesting alternative. This *in vitro* study demonstrates that SubMagna SL HMW is able to deliver the peptide into and through human gingival and oral tissues.

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Evaluation of SubMagna™ SL HMW Micellar Formation Using Fluorescence Microscopy

SUMMARY: Green fluorescent protein (GFP) was used in this study to mimic the peptide semaglutide. When GFP is incorporated in SubMagna and the formulation is exposed to water, there is spontaneous formation of micelles which is a favorable attribute for the delivery of medications.

Introduction:

Green fluorescent protein (GFP) is a protein that exhibits bright green fluorescence when exposed to blue light. It is commonly used in scientific research as a marker to visualize proteins.

Micelles are lipid vesicles that can encapsulate drugs or other molecules, making them useful in drug delivery and research. When observing micellar formation, GFP may incorporate into the micellar membrane and/or encapsulate within the micelle. Using fluorescence microscopy, GFP is a valuable tool to track the localization and distribution of micelles.

Methodology:

GFP (Abcam, Boston, MA) was used in this study to represent the peptide semaglutide. GFP was mixed with SubMagna SL HMW to make a final concentration of 0.1 mg/mL. The mixture was added to water to make a 1:1 dilution with gentle mixing to mimic administration and contact of the formulation with saliva. The distribution of GFP in SubMagna was observed under microscopy using blue light or white light at 40x magnification.

Results and Discussion:

When SubMagna is exposed to water, there is spontaneous formation of vesicles (micelles), as displayed in Figure 1.

The white light evaluation shows the GFP inside the micelles, but it is not evident because the images are colorless. On the other hand, the blue light evaluation shows clearly the fluorescent protein encapsulated inside micelles and distributed on the membranes.

The spontaneous micellar formation of SubMagna when in contact with water is a favorable attribute for the delivery of medications. It avoids the instability issue often associated with micelles. Moreover, micelles contain lipid bilayers composed of phospholipids and cholesterols, mimicking the structure of cell membranes. Thus, micelles can fuse with cell membranes to release the drug instead of relying on endocytosis. This mechanism ensures rapid drug delivery, independent of drug molecular size, and reduces risk of drug degradation.

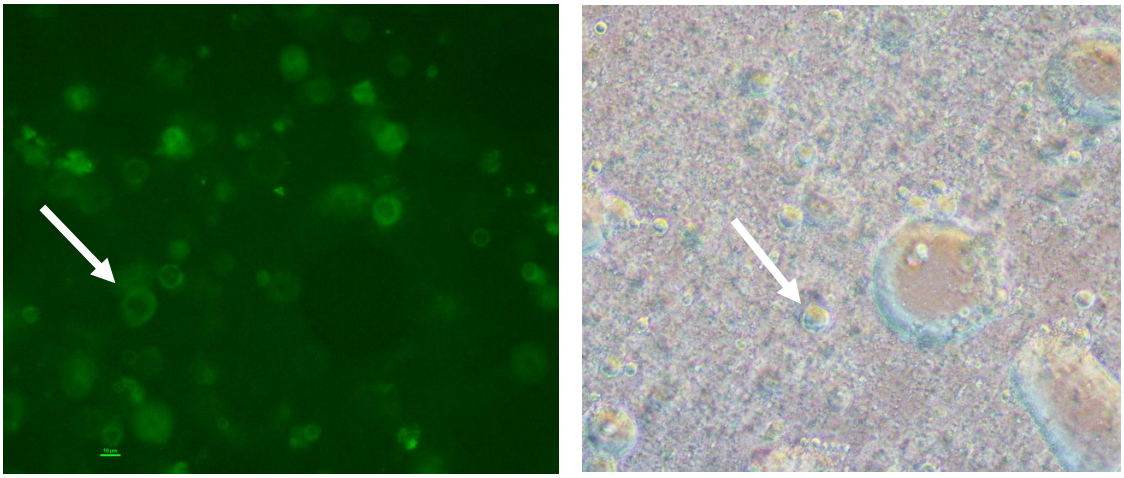


Figure 1. *Fluorescence microscopy: GFP 0.1 mg/mL in SubMagna using blue light (left) and white light (right), at 40x magnification; white arrows highlight selected micelles.*

Evaluation of the Content Uniformity of 7 SubMagna™ Sublingual Suspensions

Introduction:

Suspensions are pharmaceutical dosage forms consisting of insoluble active pharmaceutical ingredients (APIs) dispersed in a liquid medium (suspending vehicle). Sublingual suspensions are intended to be absorbed into the blood through the mucous membranes of the oral cavity. It is important to evaluate the content uniformity of sublingual suspensions to ensure that each dose is equivalent in the concentration/amount of APIs. The content uniformity is defined as the consistency in the amount of API(s) among dosage units. Content uniformity is highly dependent on the characteristics of the dosage forms. The purpose of this study is to evaluate if SubMagna, a viscous liquid dosage form, contributes to suspensions that are uniform in content. A total of 7 sublingual suspensions were selected based on popularity, each containing one API incorporated in SubMagna SL HMW.

Methodology:

The evaluation of the content uniformity was divided in two stages:

1. Elaboration of the 7 sublingual suspensions according to the corresponding PCCA formulas (Tables 1 and 2).

2. Potency testing by Ultra-Performance Liquid Chromatography (UPLC) assay. The test samples were stored at room temperature and were analyzed by the analytical laboratory in the PCCA Research & Development department or by Eagle Analytical Services, Inc. For each sample, 10 sampling points were taken for analysis and the value reported is the average of all sampling points.

Rx	***112 mL***
Rybelsus® (semaglutide) 14 mg Tablets	24 Tablets
Flavor, Natural Caramel	2.24 mL
Flavor, Banana Creme, Artificial	1.12 mL
Base, PCCA SubMagna™ SL HMW	q.s. 112 mL

Table 1. PCCA Formula 15041: Semaglutide (CADP) 3 mg/mL Sublingual Suspension (SubMagna™ SL HMW)

Results and Discussion:

The potency testing showed that all sublingual suspensions (SubMagna SL HMW) were within the 90.0%–110.0% potency specification (USP <621> chapter: Chromatography), as displayed in Table 2. As such, the innovative compounding base SubMagna successfully contributed to the content uniformity of sublingual suspensions with APIs in variable strengths.

Table 2. Mean potency (percentage of recovery) at room temperature for 7 SubMagna sublingual suspensions.

Sublingual Suspensions (SubMagna SL HMW)	***PCCA Formula***	***Mean Potency (%)***	***Standard Deviation***
Ketotifen 4 mg/mL	15258	98.629	0.993
Loperamide HCl 8 mg/mL	15260	94.076	0.588
Naltrexone HCl 7.5 mg/mL	15254	100.989	2.042
Promethazine HCl 100 mg/mL	15255	96.413	0.577
Semaglutide (CADP*) 1 mg/mL	15043	109.277	1.474
Semaglutide (CADP*) 3 mg/mL	15041	97.215	1.480
Testosterone 1 mg/0.1 mL	15031	104.512	2.337

*CADP: Commercially Available Drug Product

Conclusion:

This study has demonstrated that all 7 SubMagna sublingual suspensions were uniform in content. By following the corresponding PCCA formulas, compounding pharmacists are likely to meet the requirements of content uniformity and, as a result, dispense innovative sublingual suspensions (SubMagna SL HMW) in accordance with the corresponding labeled claims.

In Vivo Pharmacokinetic Evaluation of a Sublingual Semaglutide Compounded Formulation (SubMagna™ SL HMW): Pilot Study in an Animal Model

SUMMARY: A compounded formulation of sublingual semaglutide in PCCA SubMagna SL HMW was prepared for *in vivo* pharmacokinetics evaluation. In this pilot study, it was observed that semaglutide is detected in the blood plasma as soon as 5 minutes after sublingual administration. There were no adverse effects observed.

Introduction:

Semaglutide delivered sublingually is a potential alternative route of administration that overcomes the extremely low absorption of the oral tablets and the inconvenience of the injectable medications. A compounded formulation of sublingual semaglutide in PCCA SubMagna SL HMW was prepared for *in vivo* pharmacokinetics evaluation. The purpose of this study was not to determine the appropriate sublingual dose of semaglutide but, instead, to evaluate the ability of the proprietary base to deliver the peptide into the blood system by means of a sublingual dosage form.

Methodology:

The *in vivo* evaluation test was conducted by GemPharmatech Co., Ltd following ethics approval: animal protocol CDAP20240621-1#; project number PO-GJC0520240500076-01. The test product semaglutide (Rybelsus®) $6x10^6$ ng/mL compounded formulation (SubMagna SL HMW) was provided by PCCA. SD (Sprague–Dawley) rats (5-8 weeks, male) (n=3) were divided in two groups according to body weight: G1 (control group, n=1, SubMagna SL HMW only) and G2 (test group, n=2, sublingual semaglutide compounded formulation). Following 12 hr of fasting and 1.5 hr of deprivation of water, the rats were administered 1 mg/kg of SubMagna SL HMW (G1) or the sublingual semaglutide compounded formulation (G2) (day 0). All rats were fasted for 4 hr and deprived of water for 2 hr after administration. Blood collection by jugular vein catheter occurred at the following time-points: 0 min (pre-dosing), 5 min, 15 min, 30 min, 1 hr, 1.5 hr, 2 hr, 4 hr, 8 hr, 24 hr (day 1), and 48 hr (day 2), as shown in Figure 1. The plasma samples were analyzed for LC-MS/MS detection of semaglutide. All rats were observed for signs of adverse effects after administration. The observations included general activity, fur, head, feces, body weight and body temperature. The rat in G1 was monitored for 7 days.

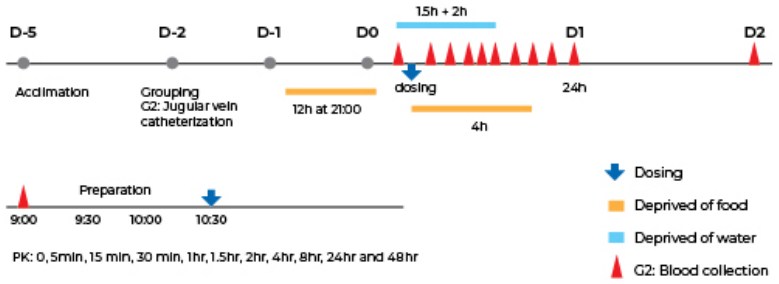
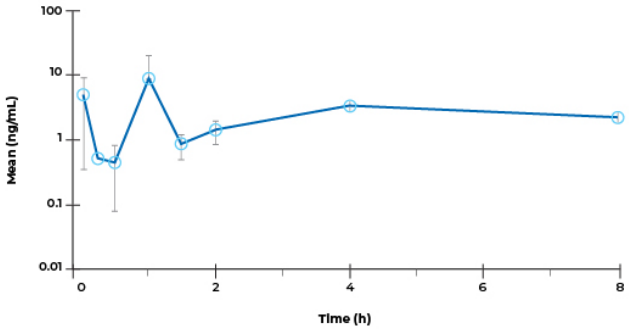


Figure 1. Study timeline and description for the *in vivo* pharmacokinetics evaluation of a sublingual semaglutide compounded formulation.

Results and Discussion:

Semaglutide was detected in the blood plasma of the rats in the test group (G2) as soon as 5 minutes after sublingual administration. As shown in Figure 2, the highest levels of semaglutide were registered at 1 hr post-administration (8.8 ng/mL). The calculated half-life from the two rats was 6.3 hr, which is similar to the reported half-life in a published study using these animals. All rats increased in weight throughout the study according to the expected growth curve of the SD strain. This is an important parameter that indicates a good health profile in both groups. All rats completed the study and there were no adverse effects observed. This *in vivo* pharmacokinetics evaluation of a sublingual semaglutide compounded formulation, although preliminary, shows clear evidence to support that semaglutide is effectively absorbed sublingually. This is a pilot study due to the limited number of rats tested. As such, statistical analysis was not performed, and further studies are needed for a quantitative evaluation. A full-scale single-dose pharmacokinetics study in rats is currently ongoing and the results will be available soon.



PCCA SubMagna™ SL HMW **TECHNICAL REPORT**

Evaluation of the Absorption of a Sublingual Testosterone Compounded Formulation (SubMagna™ SL HMW) using the EpiOral™ *In Vitro* Tissue Model

SUMMARY: Beyond semaglutide, PCCA SubMagna™ SL HMW accommodates a broad range of active pharmaceutical ingredients (APIs) to be delivered under the tongue (sublingual). In this study, the *in vitro* tissue model suggests that PCCA SubMagna is able to deliver testosterone into and through human oral tissues.

Introduction:

PCCA SubMagna is an anhydrous, sublingual base designed to deliver high molecular weight (HMW) drugs under the tongue. This innovative compounding base also benefits from mucoadhesive properties which increase the contact time of the drugs in the sublingual space.

Beyond semaglutide, PCCA SubMagna accommodates a broad range of active pharmaceutical ingredients (APIs). The purpose of this study was to evaluate the ability of PCCA SubMagna to deliver testosterone through *in vitro* human oral tissues.

Methodology:

The EpiOral tissue (ORL-606), manufactured by MatTek (Ashland, MA), was the model used to evaluate *in vitro* the absorption of the sublingual suspension testosterone 1 mg/0.1 mL in PCCA SubMagna SL HMW (Table 1).

Six tissues were incubated overnight at 37° C and 5% CO_2 for equilibration. The assay medium (Teer-Buffer-GLC buffer) was pre-warmed to 37° C and pipetted into 6-well plates. The tissues were transferred into the plates together with the assay medium. The testosterone compounded formulation was then applied and, following 15 min of elapsed permeation time, the receptor media was collected for analysis. This procedure was repeated for 30 min of total elapsed permeation time.

Rx	
Testosterone USP Micronized (Yam) CIII	1 g
Flavor, Creme DeMenthe	0.4 mL
Base, PCCA SubMagna™ SL HMW	q.s. 100 mL

Table 1. *Testosterone 1 mg/0.1 mL Sublingual Suspension (SubMagna SL HMW): PCCA Formula 15031.*

The quantification of testosterone was performed using Ultra-Performance Liquid Chromatography (UPLC): column Acquity UPLC CSH Phenyl-Hexyl 1.7 um 2.1 x 100 mm; target column temperature of 45.0°C; gradient solvent mixture of water and acetonitrile at a flow rate of 0.5 mL/min.

Results and Discussion:

In this study, the absorption of testosterone into and through the EpiOral tissue model showed a rapid penetration upon application of the sublingual suspension. The permeation flux values for testosterone across EpiOral tissues are: 4.999±0.121 µg/cm^2/hr at 15 min and 5.528±0.0.163 µg/cm^2/hr at 30 min, as displayed in Figure 1.

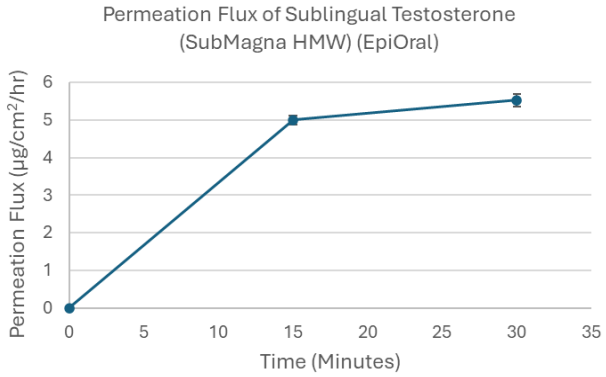


Figure 1. *Permeation flux of the sublingual testosterone compounded formulation over time for 30 minutes.*

Conclusions:

Sublingual formulations offer the potential for improved absorption compared to buccal formulations, such as troches, because the epithelium layer is much thinner under the tongue. The sublingual route is particularly promising for the delivery of hormones. This *in vitro* study demonstrates that PCCA SubMagna SL HMW is able to deliver testosterone into and through human oral tissues, offering compounding pharmacists a key alternative formulation for testosterone.

Evaluation of the Bioactivity of Semaglutide Compounded in SubMagna™ SL HMW using a Cell-Based Assay

SUMMARY: Semaglutide (GLP-1 agonist) induces the production of cAMP upon activation of the GLP-1 receptor. The cAMP Hunter™ bioassay was used to compare this bioactivity of semaglutide released from a compounded formulation using SubMagna™ SL HMW and semaglutide powder dissolved in PBS. The induction of cAMP was comparable with no statistical differences.

Introduction:

There is a growing demand worldwide for glucagon-like peptide (GLP)-1 agonists, a class of medications utilized in the treatment of type 2 diabetes and obesity. Semaglutide, the active ingredient in the injectable medications Ozempic® and Wegovy®, is the most popular GLP-1 agonist and there are often shortages in the marketplace. A compounded formulation of semaglutide for sublingual administration comprising Rybelsus® tablets and SubMagna SL HMW has been developed as an alternative to the injectable medications. An investigational cell-based study was hypothesized to evaluate and compare ligand-receptor activation by semaglutide released from the compounded formulation and semaglutide powder.

Methodology:

The cAMP Hunter™ semaglutide bioassay provides a robust, and highly sensitive, functional, cell-based assay to monitor 3'-5'-cyclic adenosine monophosphate (cAMP) production in cells, as a result of ligand-mediated activation of the GLP-1 receptor. The cyclic AMP is a derivative of adenosine triphosphate (ATP) and it is used for intracellular signaling. In this study, semaglutide was used as the ligand of GLP-1 receptor to evaluate the production of cAMP by the cells (Figure 1).

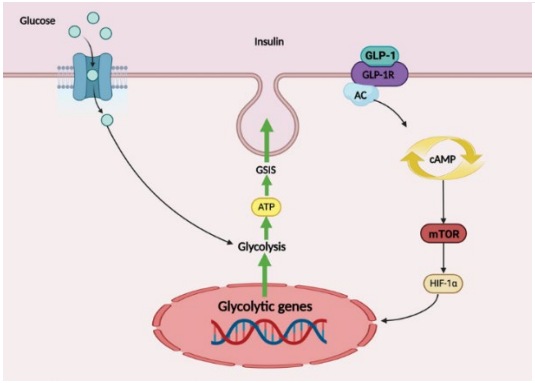


Figure 1. Graphic illustration of GLP-1 (e.g., semaglutide) binding to the receptor GLP-1R and stimulating the production of cAMP.

A bioassay kit was used for its conveniency and ready-to-use format. CHO-K1 GLP1R cells were seeded to each well of 96-well assay plate and incubated at 37° C and 5% CO_2 for 24 hrs before proceeding with the assay.

Serial dilutions from 3 ng/mL to 0.0123 ng/mL with PDB-B2 dilution buffer were made for semaglutide (powder, dissolved in PBS), semaglutide 3 mg/mL (SubMagna SL HMW) compounded, and positive control Exendin-4. The cells were treated with the diluted solutions for 30 min. cAMP antibody and cAMP working solution were added to each well and incubated for 1 hr at room temperature. The relative light units were read using a plate reader

Results and Discussion:

The dose response curve for semaglutide powder and semaglutide released from SubMagna was comparable with no significant statistical differences (*p* value=0.062), as shown in Figure 2. This result implies that Semaglutide (CADP) 3 mg/mL Sublingual Suspension (SubMagna SL HMW) functionally induced cAMP as well as the semaglutide powder.

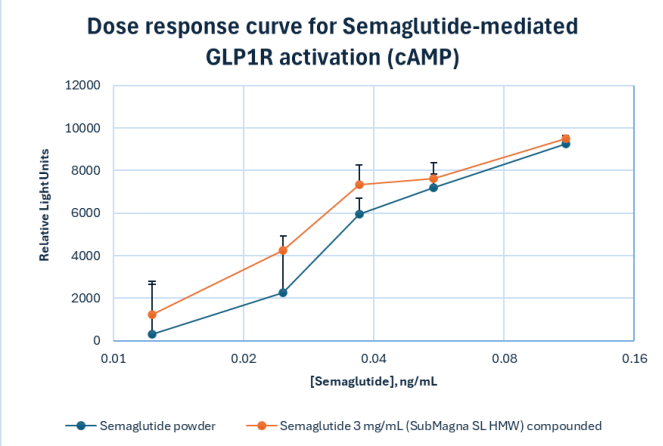


Figure 2. Dose response curve (relative light units) for semaglutide powder and semaglutide 3 mg/mL (SubMagna SL HMW) compounded.

References:

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2. SubSema (2023) "Understanding Compounded Semaglutide Making and Informed Decision". Available at: https://subsema.com/ (Accessed: October 2, 2024).

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Single-dose pharmacokinetics of sublingual semaglutide in rats

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ABSTRACT

This study aims to compare the single-dose pharmacokinetic profiles of semaglutide administered via sublingual, oral, and injectable routes in Sprague–Dawley rats. Semaglutide was delivered sublingually in a proprietary anhydrous suspension vehicle. Rats were randomized into five groups and received the following treatments: subcutaneous injection (0.011 mg/kg), sublingual suspension (1 mg/kg, prepared from either commercial tablets or peptide powder), and oral tablets (1 mg/kg and 20 mg/kg). Semaglutide was detectable in plasma within 2 minutes post-dosing in all groups except the oral 1 mg/kg group. Sublingual administration demonstrated lower variability in plasma concentrations compared to oral dosing. At 1 mg/kg, the sublingual route achieved a significantly higher area under the curve (AUC) than oral (82.53 *vs.*15.08 ng*h/ml, p=0.004), indicating improved bioavailability. The maximum plasma concentration (C_{max}) was reached within 30 minutes for oral and sublingual routes, and at 8 hours for subcutaneous injection. The relative bioavailability was 0.06% for oral 1 mg/kg, 0.16% for oral 20 mg/kg, and 0.34% and 0.29% for sublingual 1 mg/kg using tablets or powder, respectively. No significant difference in AUC was observed between sublingual semaglutide prepared from oral tablets *versus* powder. These results highlight the potential of sublingual delivery of semaglutide and suggest this route may improve absorption while reducing variability. This proof-of-concept study supports further development of sublingual semaglutide formulations and pharmacokinetics research in humans.

1. Introduction

Semaglutide, a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist, has been approved for the treatment of type 2 diabetes, overweight, and associated comorbidities. As a synthetic analog of endogenous GLP-1, it enhances glucose-dependent insulin secretion, suppresses glucagon release, delays gastric emptying, and promotes satiety, thereby improving glycemic control and supporting weight loss (Shoemaker et al., 2022; Teixidor-Deulofeu et al., 2025). Semaglutide has gained widespread recognition as a breakthrough therapy, especially offering safe and effective options for weight management.

Currently, semaglutide is commercially available as a once-weekly subcutaneous injection (*e.g.*, Wegovy® and Ozempic®) or as a once-daily oral tablet (Rybelsus®). Subcutaneous injections achieve high bioavailability - 76.65 to 82.85 % in rats (Lee et al., 2023) and approximately 89 % in humans (Ozempic® (semaglutide) [prescribing information], 2020) – but the need for regular injections may affect patient adherence (Bolge et al., 2015; Salaffi et al., 2020). Oral semaglutide offers a needle-free alternative but presents challenges such as low bioavailability (0.02–0.9 % relative to subcutaneous injection) (US FDA, 2019; Overgaard et al., 2021), gastrointestinal side effects, and strict fasting requirements. These limitations bring the need for alternative non-invasive delivery routes that improve patient convenience.

Sublingual administration has emerged as a promising strategy. By utilizing the highly vascularized sublingual mucosa and bypassing first-pass metabolism enzymatic degradation in the gastrointestinal tract, this route has the potential to enhance drug absorption (Yoo et al., 1999). While sublingual semaglutide delivery has not yet been characterized in humans, several preclinical studies have demonstrated its feasibility for peptide therapeutics (Park et al., 2025; Wu et al., 2024). For example, sublingual liraglutide encapsulated in milk-derived extracellular vesicles significantly reduced blood glucose levels in rodents, whereas oral administration of the same formulation showed no therapeutic effect, suggesting sublingual route may be an efficient alternative for peptide drugs that are not orally bioavailable (Xu et al., 2022). Another study showing measurable plasma concentrations of exenatide following sublingual dosing in rats further supports this route's potential (Gedulin et al., 2008).

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Interest in clinical utilization of sublingual GLP-1 delivery is also growing. A Phase I trial (NCT05268237) is currently investigating the safety, tolerability, and preliminary efficacy of sublingual liraglutide in patients with type 2 diabetes. However, comparative pharmacokinetic data across different delivery routes, especially for semaglutide, remain limited.

The objective of this study is to compare the single-dose pharmacokinetic profiles of sublingual semaglutide, which were prepared in an anhydrous suspension vehicle, with conventional oral and subcutaneous formulations in a preclinical model. This proof-of-concept study aims to inform future clinical development of sublingual semaglutide as a viable alternative to existing dosage forms.

2. Materials and methods

2.1. In vivo study

2.1.1. Animals

Male Sprague Dawley (SD) rats, aged 5–6 weeks and weighing 150–170 g, were used in this study. Only male rats were used was to reduce variability and hormonal influence in this initial preclinical study. All animals were housed under specific pathogen-free conditions with controlled temperature (20–26 °C), relative humidity (30–70 %), and a 12-hour light-dark cycle (light period: 08:00–20:00). Rats had unrestricted access to autoclaved corncob bedding, standard irradiated feed, and tap water, tested per standards GB14924.3–2010 and GB5749–2006. Animals were acclimated for at least three days before experimental procedures. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of GemPharmatech Co., Ltd., and conducted in accordance with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International guidelines.

2.1.2. Test articles

The test articles included:

- Semaglutide injection (Ozempic®, 1.34 mg/mL, Novo Nordisk)
- Oral tablets (Rybelsus® 14 mg, Novo Nordisk)
- Two semaglutide sublingual formulations in SubMagna® SL HMW (Professional Compounding Centers of America Inc., Houston, TX)
  - 0.6 % Semaglutide using Rybelsus® tablets
  - 0.6 % Semaglutide using research-grade semaglutide powder (Professional Compounding Centers of America Inc., Houston, TX)

Immediately prior to administration, the injection solution of Ozempic® was diluted with saline to a final semaglutide concentration of 0.002 mg/mL. A standardized subcutaneous dosing volume of 5 μl/g body weight was administered to each animal to achieve a final subcutaneous dose of 0.01 mg/kg.

Oral tablets were also freshly prepared by suspending them in distilled water to obtain 0.1 mg/mL and 2 mg/mL suspensions. Rats received an oral dosing volume of 10 μl/g body weight to achieve final doses of 1 mg/kg and 20 mg/kg of semaglutide, respectively. Due to the presence of the excipient salcaprozate sodium (SNAC) in Rybelsus®, these rats also received corresponding SNAC doses of 21.4 mg/kg and 428.6 mg/kg.

Sublingual suspensions were prepared in a proprietary anhydrous suspension vehicle. Rybelsus® tablets or peptide powder were pulverized in a mortar, followed by the incremental addition of the suspension vehicle. The final mixture was transferred to a calibrated beaker to reach the desired final volume, with intermittent rinsing and mixing to ensure complete incorporation. The resulting suspensions were mixed using an Electronic Mortar & Pestle (EMP) at medium speed for 2 min to ensure homogeneity. The final formulation is a faint yellow suspension, stored in amber, airtight, light-resistant containers at room temperature. Uniformity and potency of the two sublingual formulations were verified using a validated ultra-performance liquid chromatography (UPLC) method. Sublingual dosing of 30 μl per 180 g rat was administered to achieve a final dose of 1 mg/kg. Rats that were given sublingual suspension containing Rybelsus® also received corresponding SNAC dose of 21.4 mg/kg sublingually.

2.1.3. Experimental design

Thirty rats were randomized into five groups ($n = 6$ per group). Animals were fasted for 12 h and water-deprived for 1.5 h prior to dosing. Post dosing, food and water deprivation were maintained for 4 h and 2 h, respectively. Sublingual dosing (30 μl per 180 g rat) was performed under Zoletil. Rats were positioned face down to prevent spontaneous swallowing, with anesthesia lasting about 30 min. Oral and subcutaneous administration were conducted without anesthesia.

2.1.4. Jugular vein catheterization and blood sampling

Jugular vein catheterization was performed one day before dosing. Blood samples (100 μl each) were collected at pre-dose (0 min) and at 2-, 7.5-, 15-, 30- min, and at 1-, 1.5-, 2-, 4-, 8-, 12-, and 24-hours post-dosing into K2 EDTA tubes. Plasma was separated by immediate centrifugation and stored at −80 °C until processing.

Plasma samples were preprocessed by adding 50 μL plasma to 120 μL of terfenadine solution (5.33 ng/mL in acetonitrile: methanol, 2:1). The mixture was vortexed for 1 min, centrifuged at 4 °C and 15,400 g for 10 min, and the supernatant was collected for analysis.

2.1.5. Pharmacokinetic analysis

Plasma semaglutide concentrations were quantified using a validated LC-MS/MS method using a Shimadzu LC-20A liquid chromatography system coupled with a Triple Quad 6500+ mass spectrometer. Chromatographic separation was performed on a ChromCore 300 C18 column (4.6 × 150 mm, 5 μm) at 40 °C. The mobile phase consisted of 0.1 % formic acid in water with 5 mM ammonium acetate (Phase A) and 0.1 % formic acid in acetonitrile (Phase B), with a gradient elution at a 1.0 mL/min flow rate over 5 min. The injection volume was 5 μL. Semaglutide was detected using an ESI+ mode in multiple reaction monitoring (MRM) scan type. The ion spray voltage was 6500 V with the following parameters: CAD (8 psi), CUR (35 psi), Gas1 (55 psi), Gas2 (55 psi), ISV (5500 V), and TEM (550 °C). The Q1/Q3 transitions monitored for semaglutide were 1029.2 → 1238.2. Terfenadine granules were used as the internal standard to normalize variability during LC-MS/MS quantification of semaglutide in plasma (King-Ahmad AJ, et al., 2018), and were monitored at 472.4 → 436.4. Calibration curves were prepared with nominal semaglutide concentrations ranging from 0.5 to 500 ng/mL, showing a linear regression coefficient of $r = 0.9965$. Quality control samples at 1.0, 100, and 400 ng/mL demonstrated accuracy within 92.5 % to 108.8 %.

Pharmacokinetic parameters including maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the concentration-time curve (AUC_{0-24}), elimination half-life ($t_{1/2}$), and relative bioavailability (F) were determined using non-compartmental analysis (Phoenix WinNonlin). Coefficient of variation (CV %) was derived from standard deviation and mean values.

2.2. In vitro dissolution-permeation testing

The combined dissolution and permeation behavior of semaglutide from each sublingual formulation was tested and compared using Franz diffusion cells (surface area:1.77 cm^2). Physiological phosphate-buffered saline (PBS) at 37 ± 1 °C was used as the receptor medium under continuous magnetic stirring. One mL of each formulation was applied onto a 0.45 mm hydrophobic Polytetrafluoroethylene (PTFE) membrane (Foxx Life Sciences, Salem, NH). In this setup, the drug first underwent dissolution from the sublingual suspension into the donor compartment of the Franz Cells, followed by permeation across the membrane into the receptor compartment. Therefore, both dissolution

and membrane permeability represented rate-limiting steps. At predetermined intervals (5, 10, 30 and 60 min), samples (0.5 mL) were withdrawn from the receptor compartment and analyzed using an ELISA kit (BMA Biomedicals, Augst, Switzerland). Absorbance was measured at 450 nm, and cumulative permeation was calculated from the standard curve.

2.3. Statistical analysis

Data were presented as mean ± standard deviation (SD). Pharmacokinetic comparisons between groups were conducted using unpaired, unequal variance, two-tailed *t*-test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Pharmacokinetic profiles

Semaglutide was detectable in plasma as early as 2 min post-dosing in all treatment groups except the oral 1 mg/kg group. At early time points (2, 7.5 and 15 min), only 1 of 6 rats in the 1 mg/kg group showed detectable plasma semaglutide levels.

Plasma concentration-time curves revealed clear distinctions between administration routes. Oral dosing resulted in high inter-individual variability (CV %= 88.29 % for 1 mg/kg; CV %=86.95 % for 20 mg/kg), while sublingual delivery demonstrated reduced variability as can be visualized in Fig. 1 and is summarized in Table 1 (CV % in tablet-based sublingual: 62.7 %; CV % in powder- based sublingual: 59.88 %), with the smallest variability observed following subcutaneous injection (CV %=27.20 %).

Subcutaneous semaglutide produced steady, prolonged absorption with a delayed T_{max} at 8 h, reflecting its long-acting pharmacologic profile (Fig. 2). Oral and sublingual formulations exhibited rapid absorption and elimination, achieving C_{max} before or around 30 min post-dosing, which suggested short-acting behavior. Due to extreme variability and error bars, data from the oral 20 mg/kg group are not plotted in Fig. 2.

Systemic exposure was lowest in the oral 1 mg/kg group (15.08 ± 7.65 ng*h/ml). The oral 20 mg/kg group demonstrated highly inconsistent bioavailability, with AUC values ranging from 29.34 to 2077.24 ng*h/mL – a >70-fold variation.

Notably, the powder-based sublingual formulation showed earlier T_{max} (2 - 7.5 min in 5 / 6 rats) and higher C_{max} (116.22 ± 69.59 ng/ml) compared to the tablet-based formulation (19.09 ± 11.97 ng/ml), indicating more rapid early phase mucosal absorption. Following the peaks, semaglutide plasma concentrations declined sharply and converged with levels observed in the tablet-based sublingual group within 1–2 h, resulting in comparable AUCs between the two sublingual treatments (powder-based: 82.53 ± 43.42 ng*h/mL; tablet-based: 70.25 ± 27.45 ng*h/mL; $p = 0.5736$)

As summarized in Table 1, both sublingual groups achieved significantly greater AUC compared to the same dose of oral group (oral *vs.* powder-based sublingual: $p = 0.004$; oral *vs.* tablet-based sublingual: $p = 0.01$). These findings support improved systemic absorption via sublingual compared to oral delivery. While total absorption was similar, powder-based sublingual formulation may be associated with faster initial absorption.

3.2. Relative bioavailability

Relative Bioavailability (F) was calculated using the subcutaneous group as the reference (Table 1). The oral 1 mg/kg formulation exhibited a relative bioavailability of 0.061 %, while the tablet-based and powder-based sublingual formulations showed relative bioavailability of 0.336 % and 0.286 %, respectively. Both sublingual groups demonstrated significantly higher bioavailability than oral 1 mg/kg dosing. No significant difference was observed between the two sublingual formulations ($p = 0.5713$) or between the two oral doses ($p = 0.1633$). Additionally, the oral 20 mg/kg group did not differ significantly from either sublingual formulation (oral 20 vs. tablet-based sublingual: $p = 0.0976$; oral 20 vs. powder-based sublingual: $p = 0.1398$). The high variability in the oral 20 mg/kg group limited meaningful comparisons.

3.3. In vitro dissolution-permeation study

To investigate the differences in early absorption between tablet-based and powder-based sublingual formulations, the *in vitro* permeation of semaglutide was evaluated using a Franz diffusion cell system. Both formulations demonstrated similar semaglutide dissolution-

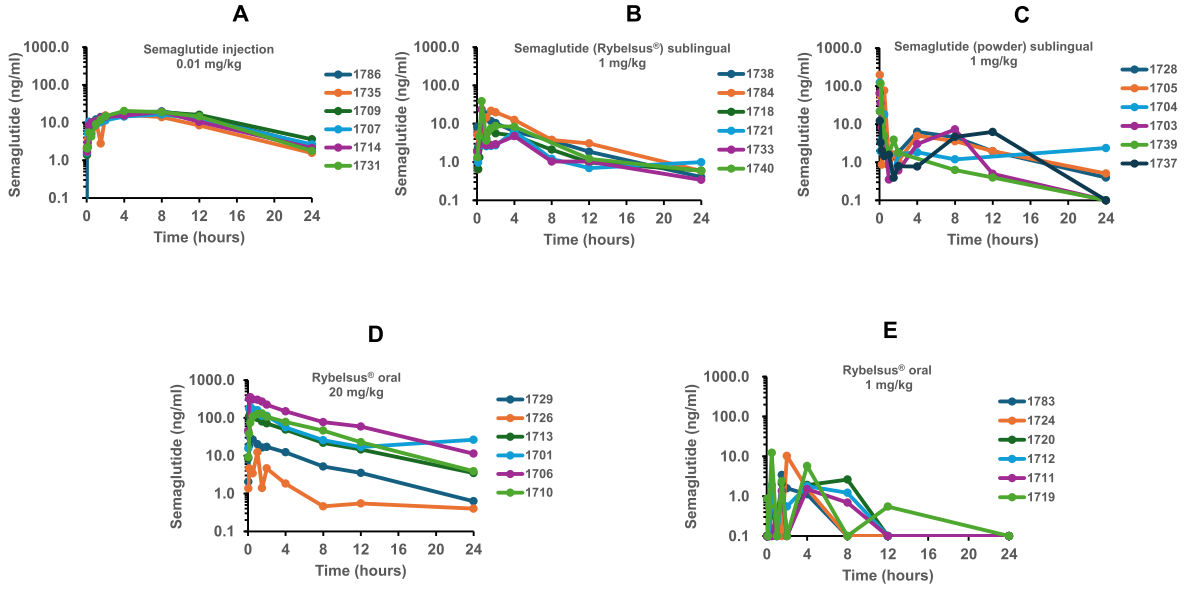

Fig. 1. Individual plasma centration-time profiles of semaglutide. Plasma concentrations – time curves for individual rat in each treatment group. Each line represents a single animal, labeled by ID, showing variability in semaglutide absorption across different routes and formulation.

Table 1
Pharmacokinetic parameters and relative bioavailability. Summary of key pharmacokinetic parameters (Tmax, Cmax, AUC0–24, t1/2) and calculated relative bioavailability (F %) of semaglutide and coefficient of variation (CV %) across different treatment groups. Statistical comparisons of AUC values are indicated ($p_{a\ vs.\ c} = 0.0119$; $p_{b\ vs.\ c} = 0.0035$; $p_{a\ vs.\ b} = 0.5713$).

Treatment	Route	Dose mg/kg	T_{max} h	C_{max} ng/mL		AUC_{0-24} ng*h/mL		$t_{1/2}$ h	Relative Bioavailability (F) %
				Mean ± SD	CV %	Mean ± SD	CV %		
Ozempic®	Subcutaneous Injection	**0.011**	**8**	**20.37 ± 5.54**	**27.20**	**269.85 ± 36.24**	**13.43**	**5.27**	**100**
Semaglutide (Rybelsus®) Sublingual suspension	**Sublingual**	**1**	**0.50**	**19.09 ± 11.97**	**62.70**	**82.53 ± 43.42** [a]	**52.61**	**5.88**	**0.34**
Semaglutide (Powder) in Sublingual suspension	**Sublingual**	**1**	**0.033**	**116.22 ± 69.59**	**59.88**	**70.25 ± 27.45** [b]	**39.07**	**4.85**	**0.29**
Rybelsus®	**Oral**	**1**	**0.50**	**5.34 ± 4.71**	**88.29**	**15.08 ± 7.65** [c]	**50.71**	**5.22**	**0.06**
	Oral	**20**	**0.25**	**141.95 ± 123.43**	**86.95**	**798.02 ± 738.67**	**92.56**	**5.82**	**0.16**

Note: Statistical comparisons of AUC value: $p_{a\ vs.\ c} = 0.0119$; $p_{b\ vs.\ c} = 0.0035$; $p_{a\ vs.\ b} = 0.5713$.

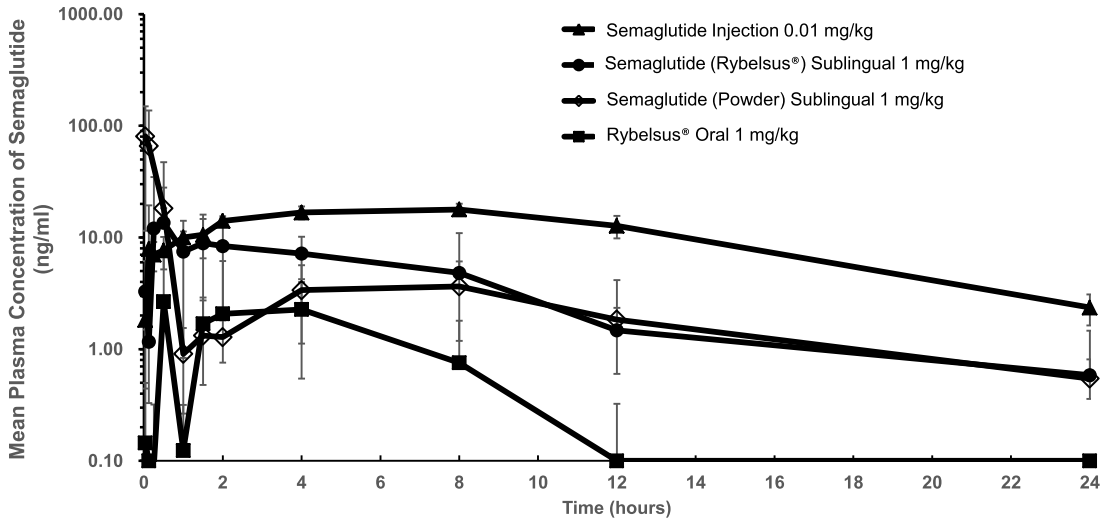

Fig. 2. Mean plasma concentration-time profile of semaglutide in different treatment groups. Data represents the mean ± SD of six rats per group.

permeation profiles across the artificial membrane (Fig. 3). However, the powder-based formulation exhibited slightly faster, yet statistically significant, permeation at 10 min (1.824 ± 0.197 ng/cm^2 vs. 4.956 ± 0.408 ng/cm^2; $p = 0.009$), which may be correlated with the earlier T_{max} observed *in vivo*. By 30 and 60 min, cumulative permeation between the two formulations was no longer significantly different (30 min: 22.868± 18.998 ng/cm^2 vs. 40.127 ± 12.835 ng/cm^2, $p = 0.3902$; 60 min: 141.641± 51.122 ng/cm^2 vs. 101.728 ± 40.022 ng/cm^2; $p = 0.4719$), suggesting similar overall dissolution-permeation potential despite different early kinetics.

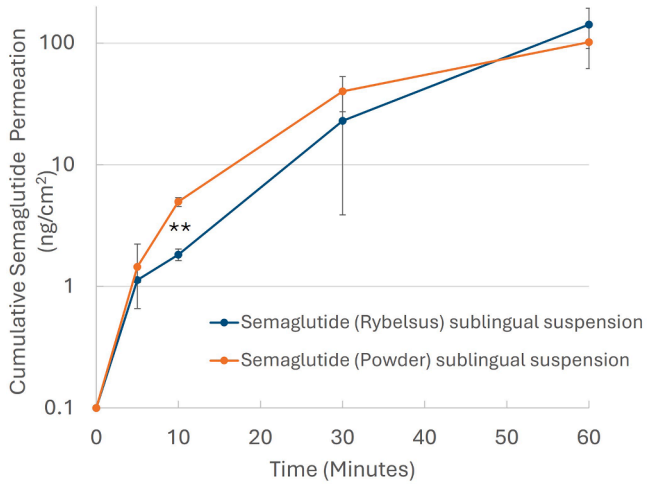

Fig. 3. Cumulative permeation of semaglutide over time across PTFE membranes from two different sublingual formulations in an *in vitro* diffusion model. ** $p = 0.009$.

4. Discussion

This study is the first to directly compare the pharmacokinetics of sublingual semaglutide formulations with oral tablets and subcutaneous injections, and uniquely, to evaluate two sublingual formulations using different drug sources. Our findings demonstrate that semaglutide can be effectively delivered via the sublingual route in rats using the anhydrous suspension vehicle, with reduced inter- individual variability compared to oral administration, suggesting more consistent systemic absorption. Furthermore, both sublingual formulations resulted in significantly higher mean AUC values than oral tablets given at the same dose, indicating enhanced bioavailability through sublingual delivery.

Notably, semaglutide was detectable in plasma within just 2 min

post-dosing in the sublingual groups, but not in the oral group using the same dose. Given that the rats were anesthetized and positioned facing down to prevent swallowing, this early plasma presence likely reflects direct sublingual mucosal absorption. This rapid absorption is particularly relevant for clinical translation, as patients typically hold sublingual medication under tongue for only 90 s to 5 min, which requires efficient drug uptake within a short timeframe.

While both sublingual formulations resulted in comparable total drug exposure, they exhibited subtle differences in early absorption kinetics. The powder-based formulation showed an earlier T_{max} and sharper C_{max}, suggesting distinct dissolution and/or permeation behaviors of semaglutide between the powder and tablet forms when delivered in the anhydrous suspension vehicle, but the data is not able to differentiate whether dissolution or permeation drives the differences. Similarly, the *in vitro* dissolution-permeation testing captures the combined contribution of both processes. Therefore, the measured cumulative semaglutide permeation represents an integrated outcome of the two sequential processes. Although the use of artificial membrane cannot fully reflect the *in vivo* permeation profile and may only show passive diffusion of drug under less efficient conditions, it still provides valuable comparative insight into the relative dissolution-permeation behavior of the two formulations. The similar trends observed both *in vitro* and *in vivo* highlight the need to consider these differences when designing formulations in future. The cause of the differences is unclear but is likely related to the excipients present in the commercial tablets, which may alter the dissolution behavior or local microenvironment to have impact on both dissolution and permeation. This implies that the source of active drug can have an impact on mucosal uptake.

Additionally, the early T_{max} and rapid elimination of semaglutide observed in both oral and sublingual groups contrasts with the prolonged profile seen with subcutaneous administration. The subcutaneous route provides a sustained release of semaglutide from the fatty tissue at the injection site, enabling less frequent administration. In contrast, sublingual formulations release semaglutide directly to systemic circulation through oral mucosa without forming a localized reservoir, leading to more rapid decline in plasma concentration after absorption. This difference suggests that the dosing schedule for subcutaneous injections is not suitable for sublingual delivery. The dosing frequency for sublingual semaglutide is expected to be more frequent than subcutaneous injection and may resemble the once-daily schedule of oral dosing. Future pharmacokinetic and dose-finding studies in humans involving assessments of glycemic control and weight management outcomes will be necessary to determine accurate dosing frequency for sublingual administration and establish dose conversions between sublingual, oral, and injection.

Although this study did not directly assess clinical relevance, the 1 mg/kg sublingual dose was selected based on preclinical data from regulatory reports on Ozempic® and Rybelsus® (U.S. FDA, 2017; EMA, 2020, 2019). Subcutaneous doses ranging from 0.0012 – 0.86 mg/kg have demonstrated efficacy in reducing body weight without showing toxicity in rats (EMA, 2020). The 0.011 mg/kg subcutaneous dose used in this study was chosen based on doses used in prior studies and their association with the maximum recommended human dose (MRHD), suggesting it is likely within a clinically relevant range. To ensure comparable pharmacokinetic profiles across routes, the 1 mg/kg oral and sublingual doses were selected based on the estimated ~1 % bioavailability of oral compared to injectables. While oral semaglutide at 20 mg/kg is known to decrease weight and food consumption in rats, data on lower oral doses in rats are limited in these regulatory reports. Therefore, the 20 mg/kg dose was included hoping to establish a reference range for clinically effective absorption levels. In our results, the pharmacokinetic profile of subcutaneous semaglutide aligns with previously reported data, supporting the validity of our model and methodology (U.S. FDA, 2017). The observed half-life and plasma concentrations following oral semaglutide administration are also aligned with findings from the FDA report (U.S. FDA, 2019).

The observed half-life of semaglutide in rats is much shorter than in humans (approximately 6 h *vs.*160 h), primarily due to the faster metabolic rate in rats, which leads to faster albumin turnover. Semaglutide's prolonged elimination half-life depends on albumin binding, however, the albumin turnover is approximately 2 days in rats compared with 25 days in human (Schreiber et al., 1971; Levitt and Levitt, 2016). As a result, semaglutide is cleared much more rapidly in rats than in humans, leading to a shorter apparent half-life. Because of the intrinsic species differences, the rat serves as a proof-of-concept model to compare routes of administration and relative bioavailability, rather than to predict human half-life or clinical dosing. The key translational relevance of the present study is demonstrating that semaglutide can be absorbed sublingually, and sublingual delivery increases systemic exposure compared to oral tablets at an equivalent dose, supporting the feasibility of this route for evaluation in humans.

Our results are also consistent with prior studies reporting poor and variable bioavailability of oral semaglutide in preclinical models and in human clinical trials (Buckley et al., 2018; Hellriegel et al., 1996; Yang and Yang, 2024). In our study, oral administration, particularly at the 20 mg/kg dose, was associated with extreme inter-individual variability, with AUC values differing by >70-fold. In humans, variability in oral semaglutide absorption originates from factors such as food, drink, body weight, and other health conditions. In rats, even under a strictly controlled fasting schedule, intrinsic differences in gastric pH, gastric emptying rate, gastrointestinal enzyme activities, and intestinal permeability, are likely to contribute to inconsistent systemic exposure. Formulation factors, including incomplete dissolution of tablets, precipitation, and subsequent re-solubilization and reabsorption may further explain the multiple C_{max} peaks observed in individual oral profile. In contrast, sublingual administration bypasses the gastric variabilities and first-pass metabolism, resulting in faster and more consistent plasma exposure. Although the sublingual route still showed moderate variability, the absence of major contributors in oral dosing-such as enzymatic degradation, tablet dissolution variability, and pH dependence- likely accounts for the improvement observed. The remaining variability from sublingual route may be attributed to physiological and formulation factors such as the differences in keratinization and vascularization of sublingual mucosa, variations in saliva volume, and the rate and completeness of emulsification of the anhydrous suspension vehicle. These findings point out the intrinsic limitations of current oral semaglutide, highlight the advantages of bypassing the gastrointestinal process, and call for the need for alternative delivery approaches that offer more predictable pharmacokinetics.

These findings also highlight the promise of the anhydrous suspension vehicle used in this study for sublingual delivery. This vehicle is a proprietary base developed to deliver drugs of varying molecular weights, and features an anhydrous, mucoadhesive system with self-emulsifying and permeation-enhancing properties. Similar to a previously published anhydrous self-emulsifying oral suspension system (Banov et al., 2023), upon contact with saliva, the sublingual suspension forms an emulsion that potentially improves the solubility and dispersibility of drug compounds. Combined with the unique mucoadhesive and penetration-enhancing properties, rapid mucosal absorption becomes possible. The anhydrous nature potentially enables extended shelf-life and eliminates the need for preservatives. Its compatibility with both tablet and powder forms adds flexibility for use in a variety of settings.

This study has several limitations. First, the findings are based on a small number of animals and should be interpreted with caution. Second, as a preclinical proof-of-concept study, the pharmacokinetics observed in rats may not directly translate to humans. In addition to the species differences in albumin turnover, the rat oral mucosa is more keratinized than that of humans (Clausen et al., 1986; Sa et al., 2016). The pronounced keratinization may result in a physical barrier to slow down peptide penetration (Souto et al., 2022), suggesting less physical barrier and possibly easier penetration in humans. While the differences

in mucosa prevent accurate prediction of sublingual absorption in humans, rats remain a widely accepted model for early pharmacokinetic studies (Bishnoi et al., 2021; Patil and Devarajan 2016; Ren et al., 2019; Sztuk et al., 2023). Additionally, we were unable to compare sublingual and oral delivery at the 20 mg/kg dose due to volume restriction in rat sublingual space. The maximum volume for sublingual delivery in rat (<180 g) is 30 μl, compared to >1 mL for oral delivery. While this limits high-dose comparisons, it is not expected to be a barrier in human applications, where the sublingual space is larger and the required dose is lower due to slower human metabolism (Nair and Jacob, 2016). Finally, the single-dose pharmacokinetics profile may not represent the steady-state profiles after repeated dosing.

Building on these promising results, future studies could consider larger-scale preclinical studies using repeated dosing to validate clinical efficacy of sublingual semaglutide in weight loss. Human pharmacokinetic and dose-finding studies are also necessary to assess the translational potential of sublingual semaglutide. Additional investigations should also explore pharmacodynamic outcomes and formulation optimization to maximize clinical benefits. Moreover, the anhydrous suspension delivery system may be applicable to other peptide drugs with similar challenges in oral bioavailability, offering a broader opportunity for needle-free peptide therapeutics.

5. Conclusions

This study demonstrates the feasibility and effectiveness of delivering semaglutide via the sublingual route using an anhydrous suspension vehicle in a rat model. Both tablet-based and powder-based sublingual formulations achieved significantly higher systemic exposure and reduced inter-individual variability compared to oral administration, highlighting the potential of sublingual delivery to improve the pharmacokinetic profile of semaglutide. The findings from this proof-of-concept study support the continued development of sublingual semaglutide as a promising, non-invasive alternative to injectable and oral formulation. The anhydrous suspension vehicle, with its compatibility with both tablets and peptide powder, and its potential to enhance mucosal absorption, offers a versatile and patient-friendly approach to peptide drug delivery. Future clinical studies are desired to validate these preclinical results, determine optimal dosing strategies, and further explore the therapeutic potential of sublingual GLP-1 drugs.

CRediT authorship contribution statement

Yi Liu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Guiyun Song:** Writing – review & editing, Resources, Methodology, Investigation, Data curation, Conceptualization. **Daniel Banov:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Jennifer Denison:** Project administration. **Courtaney Davis:** Resources, Investigation. **Kendice Ip:** Validation.

Data availability

Data will be made available on request.

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