PCCA RESEARCH & DEVELOPMENT

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SubMagna™ SL HMW

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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.

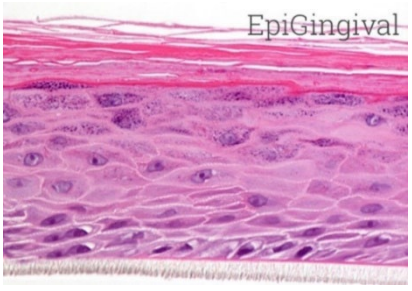

Figure 2. *Illustration of the EpiGingival™ tissue model (adapted from MatTek).*

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.

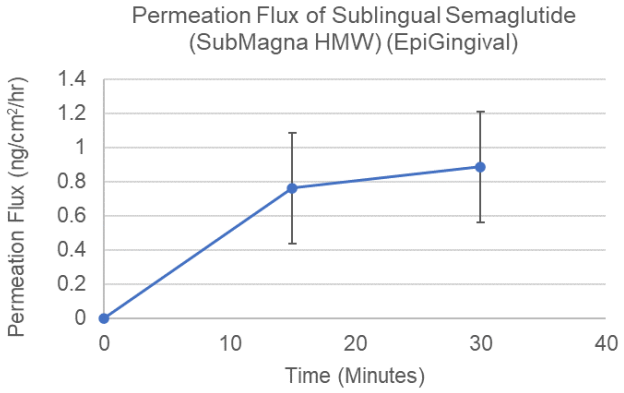

Figure 3. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

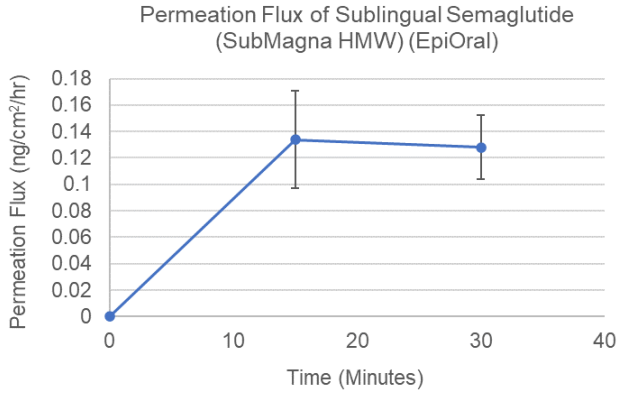

Figure 4. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

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.

References:

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Evaluation of SubMagna™ SL HMW Liposomal Formation Using Fluorescence Microscopy

SUMMARY: Green fluorescent protein (GFP) was used in this study to mimic the peptide semaglutide. When GFP is incorporated in SugMagna and the formulation is exposed to water, there is spontaneous formation of liposomes 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.

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

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 (liposomes), as displayed in Figure 1.

The white light evaluation shows the GFP inside the liposomes, 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 liposomes and distributed on the membranes.

The spontaneous liposomal 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 liposomes. Moreover, liposomes contain lipid bilayers composed of phospholipids and cholesterols, mimicking the structure of cell membranes. Thus, liposomes 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.

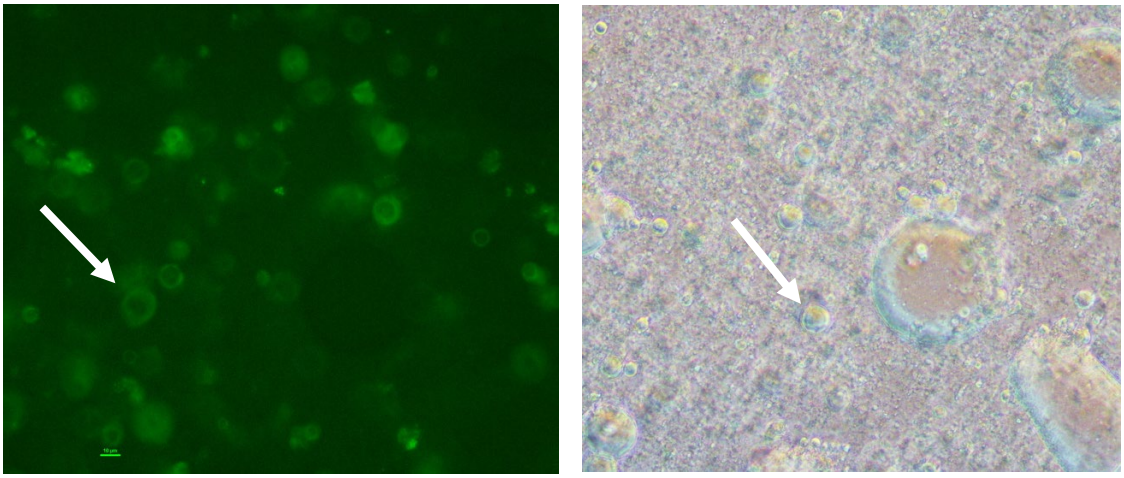

Figure 1. *Fluorescence microscopy: GFP 0.1 mg/mL in SubMagna using blue light (left) and white light (right), at 40x magnification; white arrows highlight selected liposomes.*

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.

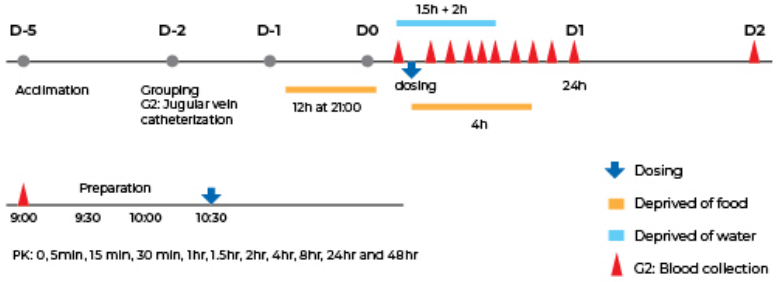

Figure 1. Study timeline and description for the *in vivo* pharmacokinetics evaluation of a sublingual semaglutide compounded formulation.

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.