Supplemental Online Material

A Study Investigating the Effect of Omeprazole on the Pharmacokinetics of Oral Semaglutide

1.0 Eligibility Criteria

1.1 Inclusion criteria:

- Informed consent obtained before any trial-related activities.
- Male or female age 18-75 years.
- Body mass index 18-29.9 kg/m^2.
- A good general health based on medical history, physical examination, and results of vital signs, electrocardiogram, and laboratory safety tests performed during the screening visit, as judged by the investigator.

1.2 Exclusion criteria:

- Known or suspected hypersensitivity to trial products or related products.
- Previous participation in this trial.
- Female who is pregnant, breast-feeding or intends to become pregnant, or is of child-bearing potential and not using adequate contraceptive methods.
- The receipt of any investigational medicinal product within 90 days prior to screening.

- Unable to refrain from use of prescription or non-prescription medicinal and herbal products (including St John's wort and ginkgo biloba) or food supplements (including routine vitamins) within 3 weeks preceding the dosing and during the trial.
- History of, or presence of, cancer, diabetes or any clinically significant cardiovascular, respiratory, metabolic, renal, hepatic, gastrointestinal, endocrinological, hematological, dermatological, venereal, neurological, psychiatric diseases, or other major disorders, as judged by the investigator.
- Any disorder which, in the opinion of the investigator, might jeopardize subject safety or compliance with the protocol.
- Personal or family history of familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2.
- History of Crohn's disease, ulcerative colitis, or other inflammatory bowel diseases.
- Subjects with previous gastrointestinal surgery, except subjects that underwent uncomplicated surgical procedures such as appendectomy, hernia surgery, biopsies, as well as colonic and gastric endoscopy.
- Hypertension (defined as sitting supine systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg).
- Pulse >90 bpm, assessed in sitting position after 5 minutes rest.
- History of pancreatitis (acute or chronic).
- HbA1c >6.0% at screening.

- Any laboratory safety parameter at screening outside the below extended laboratory ranges (re-screening or re-sampling is not allowed if the subject has failed one of the exclusion criteria related to laboratory parameters):

  a) Sodium outside lower normal limit (LNL)–0% and upper normal limit (UNL)+5%

  b) Potassium outside LNL–5% and UNL+5%

  c) Creatinine outside LNL–5% and UNL+10%

  d) Albumin outside LNL–5% and UNL+5%

  e) Alanine transaminase outside LNL–100% and UNL+50%

  f) Hemoglobin outside LNL–5% and UNL+10%

  g) Bicarbonate outside LNL–5% and UNL+10%

  h) Leukocytes outside LNL–20% and UNL+20%

  i) Thrombocytes outside LNL–15% and UNL+15%

  j) Amylase ≥UNL+100%

  k) Lipase ≥UNL+100%

  l) Calcitonin ≥50 ng/L

  m) Positive by test for:

    - Hepatitis B virus as evidenced by Hepatitis B virus surface antigen
    - Hepatitis C virus as evidenced by Hepatitis C virus antibody
    - Human immunodeficiency virus (HIV) as evidenced by HIV-1 or HIV-2 antibodies and HIV-1 antigen (HIV 1/2 combi test).

- A subject who is smoking more than 5 cigarettes or the equivalent per day, including use of nicotine substitute products. Unable to refrain from smoking or the equivalent and use of nicotine substitute products during the in-house periods.
- Vulnerable subjects (e.g. persons kept in detention).
- Subject who is an investigator, or any sub-investigator, research assistant, pharmacist, trial coordinator, other staff, or relative thereof directly involved in the conduct of the clinical trial.
- Mental incapacity, unwillingness, or language barrier precluding adequate understanding or co-operation.
- Known or suspected abuse of alcohol (defined as regular intake of more than 21 units weekly for men and 14 units weekly for women – 1 unit = 300 mL of beer; 100 mL of wine; 25 mL of distilled spirits) or drugs, or a positive drug/alcohol test.
- Any blood draw in excess of 25 mL in the past month, or donation of blood or plasma in excess of 400 mL within the 3 months preceding screening.

2.0 Sampling Timings

For determination of plasma semaglutide concentrations, samples were collected on days 9 and 10 pre-dose and at 10, 20, 30 minutes, and 1, 1.5, 2, 2.5, 3, 4, 6, and 12 hours post-dose. In addition, one blood sample was collected on days 12, 14, 17, 21, 24, 31, and on day 43 ± 2 days for determination of the terminal half-life of semaglutide. For determination of plasma SNAC concentrations, samples were collected on days 9 and 10 pre-dose and at 10, 20, 30,

40, 50 minutes, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours post-dose. On day 10, additional samples were also collected at 2, 4, 6, 12, 14, and 16 minutes.

3.0 Imputation of Values Below the Lower Limit of Quantification (LLOQ)

3.1 Semaglutide

Pre-dose samples before the very first dose (on day 1) that were below LLOQ were set to 0.

For each profile (on days 9 and 10) used for the endpoint calculation:

- Pre-dose samples (after the very first dose on day 1) that were below LLOQ were set to LLOQ/2.
- Intermediate samples below LLOQ (after first dosing and before the time of last quantifiable observation) were set to LLOQ/2.

Samples after the time of last quantifiable observation below LLOQ were imputed based on $t_{½}$.

3.2 SNAC

Pre-dose samples before the very first dose (on day 1) that were below LLOQ were set to 0.

For each profile (on days 9 and 10) used for the endpoint calculation:

- Pre-dose samples that were below LLOQ were set to 0.
- Intermediate samples below LLOQ (after first dosing and before the time of last quantifiable observation) are set to LLOQ/2.

- First sample after the time of last quantifiable observation was set to LLOQ/2 and the remaining to 0.

Supplemental Table S1. Pharmacokinetic endpoints for semaglutide and SNAC after the 9th dosing

	Oral semaglutide (N = 28)	**Oral semaglutide + omeprazole (N = 26)**
Semaglutide		
$AUC_{0–24h,semaglutide,Day9}$, nmol·h/L	251 (50.6)	285 (70.0)
$C_{max,semaglutide,Day9}$, nmol/L	13.8 (52.6)	16.0 (71.6)
$t_{max,semaglutide,Day9}$, h	1.0 (0.5, 4.0)	1.0 (0.5, 3.0)
SNAC		
$AUC_{0–24h,SNAC,Day9}$, ng·h/mL	1081 (30.4)	1194 (36.6)
$C_{max,SNAC,Day9}$, ng/mL	836 (83.8)	987 (78.0)
$t_{max,SNAC,Day9}$, h	0.5 (0.2, 5.0)	0.7 (0.3, 4.0)

Data are geometric means (coefficient of variation) except for t_{max} where median (minimum, maximum) values are presented.

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; t_{max}, time to maximum concentration.

Supplemental Table S2. Associations of $AUC_{0–24h}$ of semaglutide and C_{max} of semaglutide with gastric pH parameters

Day for PK endpoint	Gastric pH parameter	N	Estimate of coefficient	95%CI	p-value
Association of $AUC_{0–24h}$ of semaglutide with gastric pH parameters					
Day 9	$AUC_{0–3h,pH}$/time	53	0.0018	[–0.0797, 0.0832]	0.966
	$pH_{max,0–3h}$	53	–0.0159	[–0.1168, 0.0851]	0.754
	$iAUC_{0–3h,pH}$/time	53	0.0286	[–0.0903, 0.1474]	0.631
Day 10	$AUC_{0–3h,pH}$/time	53	–0.0032	[–0.0824, 0.0760]	0.935
	$pH_{max,0–3h}$	53	–0.0235	[–0.1215, 0.0745]	0.632
	$iAUC_{0–3h,pH}$/time	53	0.0699	[–0.0442, 0.1840]	0.224
Association of C_{max} of semaglutide with gastric pH parameters					
Day 9	$AUC_{0–3h,pH}$/time	53	0.0090	[–0.0747, 0.0928]	0.829
	$pH_{max,0–3h}$	53	–0.0118	[–0.1156, 0.0921]	0.821
	$iAUC_{0–3h,pH}$/time	53	0.0346	[–0.0875, 0.1567]	0.572

Day 10	$AUC_{0–3h,pH}$/time	53	0.0034	[–0.0762, 0.0831]	0.931
	$pH_{max,0–3h}$	53	–0.0158	[–0.1146, 0.0829]	0.749
	$iAUC_{0–3h,pH}$/time	53	0.0796	[–0.0347, 0.1939]	0.168

PK endpoints were logarithmic transformed and analyzed using a linear regression model with gastric pH parameter and gender as explanatory variables. Estimated parameter coefficients with corresponding 95%CIs for the pH parameters from the above model are reported.

AUC, area under the plasma concentration–time curve; iAUC, incremental AUC; CI, confidence interval; PK, pharmacokinetics.

Supplemental Table S3. Associations of $AUC_{0–24h}$ of SNAC and C_{max} of SNAC with gastric pH parameters

Day for PK endpoint	Gastric pH parameter	N	Estimate of coefficient	95%CI	p-value
Association of $AUC_{0–24h}$ of SNAC with gastric pH parameters					
Day 9	$AUC_{0–3h,pH}$/time	53	0.0263	[–0.0133, 0.0659]	0.189
	$pH_{max,0–3h}$	53	0.0154	[–0.0344, 0.0652]	0.538
	$iAUC_{0–3h,pH}$/time	53	0.0384	[–0.0195, 0.0964]	0.189
Day 10	$AUC_{0–3h,pH}$/time	53	0.0233	[–0.0117, 0.0583]	0.187
	$pH_{max,0–3h}$	53	0.0032	[–0.0410, 0.0473]	0.886
	$iAUC_{0–3h,pH}$/time	53	0.0173	[–0.0346, 0.0691]	0.507
Association of C_{max} of SNAC with gastric pH parameters					
Day 9	$AUC_{0–3h,pH}$/time	53	0.0752	[–0.0118, 0.1621]	0.089
	$pH_{max,0–3h}$	53	0.1080	[0.0012, 0.2147]	0.048
	$iAUC_{0–3h,pH}$/time	53	0.0625	[–0.0672, 0.1922]	0.338

Day 10	$AUC_{0–3h,pH}$/time	53	0.0281	[–0.0629, 0.1192]	0.538
	$pH_{max,0–3h}$	53	0.0618	[–0.0502, 0.1737]	0.273
	$iAUC_{0–3h,pH}$/time	53	0.0308	[–0.1025, 0.1642]	0.644

PK endpoints were logarithmic transformed and analyzed using a linear regression model with gastric pH parameter and sex as explanatory variables. Estimated parameter coefficients with corresponding 95%CIs for the pH parameters from the above model are reported.

AUC, area under the plasma concentration–time curve; iAUC, incremental AUC; CI, confidence interval; PK, pharmacokinetics; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

Supplemental Table S4. Treatment-emergent adverse events by system organ class and preferred term

	Oral semaglutide n (%) E	**Oral semaglutide + omeprazole n (%) E**	**Total n (%) E**
Number of subjects	28	26	54
Adverse events	17 (60.7) 39	10 (38.5) 35	27 (50.0) 74
Gastrointestinal disorders	11 (39.3) 16	6 (23.1) 13	17 (31.5) 29
Nausea	2 (7.1) 2	3 (11.5) 8	5 (9.3) 10
Abdominal distension	4 (14.3) 5	1 (3.8) 1	5 (9.3) 6
Abdominal pain upper	2 (7.1) 2	1 (3.8) 1	3 (5.6) 3
Vomiting	1 (3.6) 1	2 (7.7) 2	3 (5.6) 3
Constipation	1 (3.6) 1	1 (3.8) 1	2 (3.7) 2
Abdominal pain	2 (7.1) 2	0	2 (3.7) 2
Diarrhea	2 (7.1) 2	0	2 (3.7) 2
Dyspepsia	1 (3.6) 1	0	1 (1.9) 1

Metabolism and nutritional disorders	5 (17.9) 5	7 (26.9) 7	12 (22.2) 12
Decreased appetite	5 (17.9) 5	7 (26.9) 7	12 (22.2) 12
Infections and infestations	4 (14.3) 4	3 (11.5) 3	7 (13.0) 7
Nasopharyngitis	3 (10.7) 3	3 (11.5) 3	6 (11.1) 6
Acute tonsillitis	1 (3.6) 1	0	1 (1.9) 1
Nervous system disorders	4 (14.3) 4	2 (7.7) 2	6 (11.1) 6
Headache	4 (14.3) 4	1 (3.8) 1	5 (9.3) 5
Dizziness	0	1 (3.8) 1	1 (1.9) 1
Respiratory, thoracic, and mediastinal disorders	4 (14.3) 4	1 (3.8) 3	5 (9.3) 7
Cough	2 (7.1) 2	1 (3.8) 1	3 (5.6) 3
Oropharyngeal pain	2 (7.1) 2	1 (3.8) 1	3 (5.6) 3
Dysphonia	0	1 (3.8) 1	1 (1.9) 1
General disorders and administration site conditions	2 (7.1) 2	2 (7.7) 4	4 (7.4) 6
Fatigue	2 (7.1) 2	2 (7.7) 3	4 (7.4) 5

Chills	0	1 (3.8) 1	1 (1.9) 1
Musculoskeletal and connective tissue disorders	1 (3.6) 1	2 (7.7) 2	3 (5.6) 3
Pain in extremity	0	1 (3.8) 1	1 (1.9) 1
Neck pain	1 (3.6) 1	0	1 (1.9) 1
Back pain	0	1 (3.8) 1	1 (1.9) 1
Psychiatric disorders	1 (3.6) 1	1 (3.8) 1	2 (3.7) 2
Dysphoria	1 (3.6) 1	0	1 (1.9) 1
Depressed mood	0	1 (3.8) 1	1 (1.9) 1
Skin and subcutaneous tissue disorders	2 (7.1) 2	0	2 (3.7) 2
Skin reaction	1 (3.6) 1	0	1 (1.9) 1
Cold sweat	1 (3.6) 1	0	1 (1.9) 1

E, number of adverse events; n, number of subjects with adverse event; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; %, proportion of subjects having an adverse event.

Supplemental Figure S1. Scatter plots for association of $AUC_{0–24h,Day\ 9}$ for semaglutide with gastric pH parameters

A) $AUC_{0–3h,pH,Day9}$/time

B) $pH_{max,Day9}$

C) $iAUC_{0–3h,pH,Day9}$/time

AUC, area under the plasma concentration–time curve; iAUC, incremental AUC; EM, extensive metabolizer; PM, poor metabolizer.

Supplemental Figure S2. Scatter plots for association of $AUC_{0–3h,Day\ 9}$ for SNAC with gastric pH parameters

A) $AUC_{0–3h,pH,Day9}$/time

B) $pH_{max,Day9}$

C) $iAUC_{0–3h,pH,Day9}$/time

AUC, area under the plasma concentration–time curve; iAUC, incremental AUC; EM, extensive metabolizer; PM, poor metabolizer; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.