Tunable Semaglutide Release from CHHA Nanogel Depots via Concentration Control

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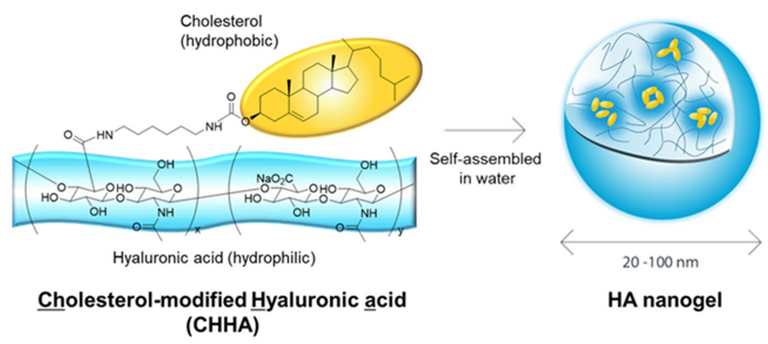
Introduction

Cholesterol-substituted hyaluronic acid (CHHA) is a novel excipient for long-acting injectable formulations. Our group has developed cholesterol-modified hyaluronic acid (CHHA), which forms nanosized hydrogels (HA nanogel) in water and exhibits versatile encapsulation properties for peptides, and proteins by simply mixing process[1)]. We are manufacturing CHHA at industrial scale. This study evaluated whether the sustained-release profile of a semaglutide depot formulation can be tuned by formulation concentration, focusing on the roles of free (non-associated) drug and CHHA concentration.



Method

Preparation CHHA-Semaglutide formulation

Semaglutide/CHHA formulations were prepared at varying semaglutide concentrations (2–10 mg/mL) and CHHA concentrations (10–25 mg/mL) by simply mixing.

Free semaglutide analysis

Free semaglutide in formulations was quantified by size-exclusion chromatography (SEC; TSKgel G2000SWXL, 10 mM phosphate buffer, UV 230 nm) using a calibration curve (formulations diluted five-fold).

PK study

Pharmacokinetics (PK) after subcutaneous administration were evaluated in Sprague–Dawley rats (6–8 weeks; 4 mL/kg) with plasma semaglutide quantified by LC–MS/MS.

Result

■ Representative SEC chromatograms used to distinguish free semaglutide from CHHA- and peptide-complex.

- At a fixed CHHA concentration of 25 mg/mL, increasing semaglutide concentration reduced loading efficiency only at 10 mg/mL semaglutide (56%), suggesting increased free semaglutide under high loading conditions.
- CHHA concentration-dependent increase in loading efficiency at a fixed semaglutide concentration of 4 mg/mL.

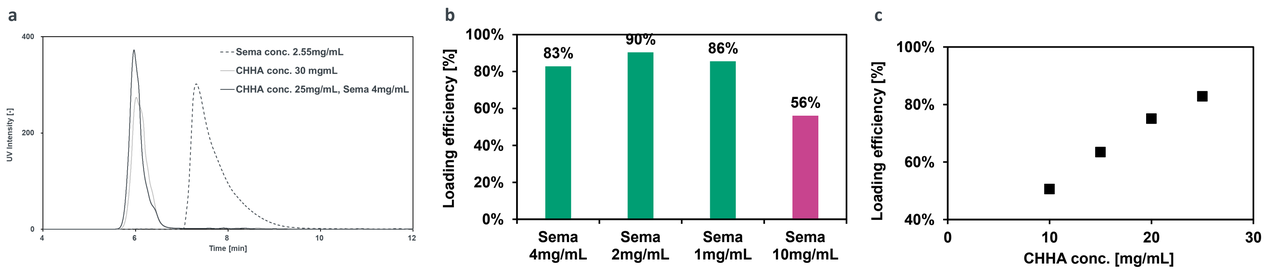


Figure 1. Formulation SEC analysis. a, Determination free semaglutide (unencapsulated by CHHA) using Size exclusion chromatography. **b**, Loading efficiency of semaglutide encapsulated by CHHA (fixed CHHA concentration 25 mg/mL). **c**, Loading efficiency of semaglutide encapsulated by CHHA (fixed semaglutide concentration 4 mg/mL).

■ PK parameter depends on CHHA concentration.

- Optimal CHHA concentration improved loading efficiency and prolonged residence time.
- CHHA concentration governed both formulation loading and sustained PK behavior.
- Increasing CHHA reduced peak exposure while extending semaglutide retention.

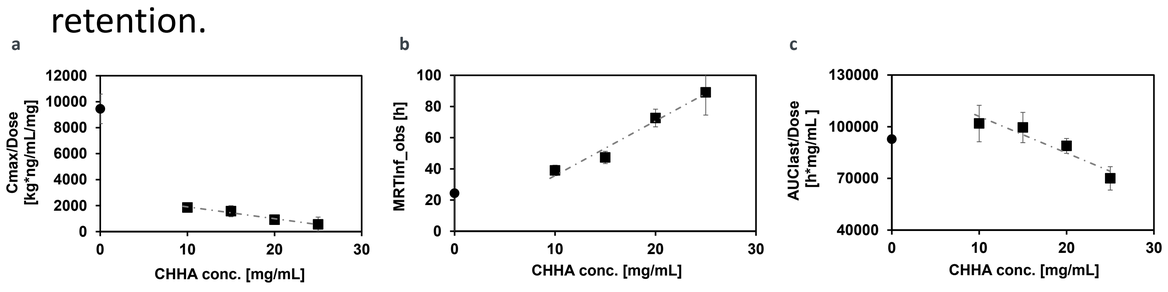


Figure 3. Effect of CHHA concentration on PK parameters at a fixed semaglutide concentration of 4 mg/mL. a, Cmax/Dose decreased with increasing CHHA concentration. **b**, MRTinf_obs increased with increasing CHHA concentration. **c**, AUClast/Dose showed a downward trend at higher CHHA concentrations.

■ Effect of CHHA concentration on semaglutide PK parameters after subcutaneous administration.

- At a fixed CHHA concentration of 25 mg/mL, CHHA/semaglutide formulations markedly prolonged semaglutide exposure compared with the control SC injection, maintaining apparent half-life values of 101–115 h and MRT values of 76–89 h versus 8.63 h and 24 h, respectively; increasing semaglutide concentration mainly shifted Tmax earlier only at the highest loading condition (10 mg/mL).
- In contrast, at fixed semaglutide concentration (4 mg/mL), decreasing CHHA concentration substantially accelerated the PK profile, demonstrating that CHHA concentration is the dominant driver of sustained semaglutide release.

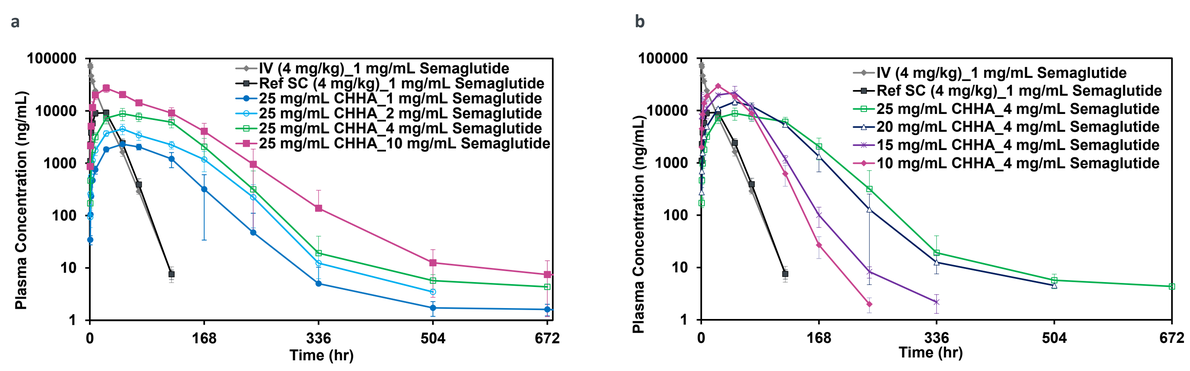


Figure 2. PK profile of CHHA-based semaglutide formulation (mean±S.D., n=4). a, Effect of semaglutide concentration on the PK profile at a fixed CHHA concentration (25 mg/mL). **b**, Effect of CHHA concentration on the PK profile at a fixed semaglutide concentration (4 mg/mL).

Table 1. PK parameter of CHHA / semaglutide formulation (mean±S.D., n=4).

PK parameters	Unit	Control SC (without CHHA)	Semaglutide 1 mg/mL	Semaglutide 2 mg/mL	Semaglutide 4 mg/mL	Semaglutide 10 mg/mL
Semaglutide conc.	mg/mL	1	1	2	4	10
Dose	mg/kg	4	4	8	16	40
	mL/kg	4	4	4	4	4
API/CHHA ratio	-		0.04	0.08	0.16	0.4
$T_{1/2}$	H	8.63	104	101	113	115
T_{max}	H	16	54	42	54	24
Cmax/Dose	ng*kg/(mL*mg)	2362	577	567	554	678
AUC_{last}	h*ng/mL	370996	257611	527259	1120231	2570432
MRT_{inf_obs}	h	24	80	89	89	76
AUC_{last}/D	h*mg/mL	92749	64403	65907	70014	64261
F	%	51	35	36	38	35

PK parameters	Unit	Control SC (without CHHA)	CHHA 25 mg/mL	CHHA 20 mg/mL	CHHA 15 mg/mL	CHHA 10 mg/mL
Semaglutide conc.	mg/mL	1	4	4	4	4
Dose	mg/kg	4	16	16	16	16
	mL/kg	4	4	4	4	4
API/CHHA ratio	-	-	0.16	0.2	0.27	0.4
$T_{1/2}$	h	8.63	113	151	24.2	13.1
T_{max}	h	16	54.0	48.0	30.1	24.0
C_{max}/Dose	ng*kg/(mL*mg)	2362	554	919	1569	1850
AUC_{last}	h*ng/mL	370996	1120231	1421563	1559696	1629542
MRT_{inf_obs}	h	24	89	73	47	39
AUC_{last}/D	h*mg/mL	92749	70014	88848	99520	101846
F	%	51	38	49	53	56

Conclusions

- Semaglutide release from CHHA depots is governed primarily by CHHA concentration, whereas free drug plays a secondary role, mainly influencing Tmax. These findings establish CHHA concentration as a practical formulation lever for tuning peptide long-acting injectables.
- Relative to semaglutide alone, CHHA depot formulations markedly prolonged apparent half-life and extended MRT by approximately 4-fold, supporting the feasibility of monthly sustained-release semaglutide with further formulation optimization.

REFERENCE

1) Macromolecular Bioscience. 2012. 475-483.

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