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The limitation of lipidation: Conversion of semaglutide from once-weekly to once-monthly dosing

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The objective of this work was to develop a long-acting form of the lipidated peptide semaglutide that can be administered to humans once-monthly. Semaglutide was attached to hydrogel microspheres by a cleavable linker with an expected in vivo release half-life of about 1 mo. After a single subcutaneous dose, the pharmacokinetic parameters of released semaglutide and bodyweight loss were determined in mice, and results were used to estimate the dosing and pharmacokinetics of the released semaglutide in humans. The in vivo half-life of released semaglutide was ~36 d, and a single dose in diet-induced obese mice resulted in a lean-sparing body weight loss of 20% over 1 mo, statistically the same as semaglutide dosed twice daily. Simulations indicated the microsphere-semaglutide would permit once-monthly administration in humans; moreover, it could maintain the therapeutic minimum concentration (C_{min}) of once-weekly semaglutide with only 75% of the once-weekly maximum concentration (C_{max}), a feature that could reduce adverse side effects or allow higher dosing. The same approach should enable the conversion of other lipidated peptides from once-weekly to once-monthly administration.

semaglutide | obesity | weight loss | Glucagon-Like Peptide 1 (GLP-1) | hydrogel

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) are mainstays of treatments for type 2 diabetes and obesity and are potential therapies for metabolic-disfunction associated steatohepatitis (MASH) and age-related diseases such as Parkinson's and Alzheimer's (1). Most GLP-1RAs consist of short-lived peptides modified with a fatty acid to create longer-acting "lipidated" peptides (2, 3). The fatty acid reversibly binds to albumin and converts the parent peptide's half-life to ~1 wk by piggybacking on albumin (3). Notably, Novo Nordisk's tour de force optimization of peptide lipidation has likely achieved its practical upper limit.

It has been reported that persistence in antiobesity drug use is low (4, 5). Among drugs studied, semaglutide showed the highest 1-y persistence, yet only 40% were persistent with the medication (4). Also, gastro-intestinal side effects caused lower adherence and the rate of adherence was significantly higher in individuals treated with long-acting vs. short-acting GLP-1RA (5). A proven method to increase adherence to injectable drugs is by reducing the dosing frequency (6), so it seems important to increase the half-life of antiobesity peptides. Long-acting GLP-1RAs also address an unmet need in diseases and patient populations that would benefit from monthly or longer dosing that coincides with doctor visits, such as Parkinson's and Alzheimer's.

A yet untried approach to decrease the dosing frequency of antiobesity peptides is to "override" the half-life limit of lipidated peptides with an alternative half-life extension technology. Thus far, the 1-wk half-life barrier has not been overcome by attachment of polyethylene glycol (PEGylation), polymer encapsulation, or Fc fusion (7). However, the Amgen compound AMG133—an anti-glucose-dependent insulinotropic polypeptide receptor (GIPR) monoclonal antibody conjugated to a GLP-1RA—has a 2-wk half-life but can be used for once-monthly dosing because it is administered at a sufficiently high dose that can last two half-lives (8). Still, there are few technologies that can achieve dosing frequencies of 1 mo or greater.

We have developed an approach to half-life extension in which a macromolecular prodrug serves as a subcutaneous depot that slowly releases its drug over a designated, preprogrammed period. Here, a drug is covalently tethered to a long-lived carrier—50 μ hydrogel microspheres (MS)—by a cleavable linker that dictates the half-life (9, 10); the prodrug is administered subcutaneously and the depot slowly releases the drug by a base-catalyzed β-elimination over the predetermined period. This technology can reliably achieve in vivo half-lives of 1 mo or longer for peptides (11). Indeed, it has already produced an exenatide GLP-1RA with a half-life of 1 mo and with drug remaining above therapeutic levels for up to 3 mo (11). Here, we propose that the very same approach could be used to convert lipidated peptides—semaglutide and others—from once-weekly to once-monthly administration.

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In the present work, we attached semaglutide to our MSs by a linker with an expected in vivo cleavage half-life of ~1 mo. We determined the pharmacokinetic parameters and weight loss effects of a single dose in diet induced obese (DIO) mice. Finally, we simulated the pharmacokinetics of our once-monthly semaglutide in humans.

Results

Semaglutide was carbamylated at the N terminus with N_3-linker-HSI to give N_3-linker-semaglutide in ~80% yield. The N_3-linker-semaglutide was then coupled to MS-bicyclo[6.1.0]non-4-yne (BCN) by strain-promoted azide–alkyne cycloaddition (SPAAC) to give conjugates with 2- and 4 µmol semaglutide/mL MS. Treatment of MS~semaglutide conjugates under accelerated release conditions at pH 9.4, 37 °C, released semaglutide with a $t_{1/2}$ of 13.3 ± 0.5 h, or 1,330 h at pH 7.4.

Concentration vs. time plots after single injections of 400 and 2,000 nmol/kg MS~semaglutide showed dose linearity and a $t_{1/2}$ ~ 36 d (865 h) (Fig. 1*A*). The C_{max} and AUC_{inf} dose-normalized to 1 nmol/kg were 1.2 nM and 1,080 nM h, respectively. The dose-normalized steady-state $AUC_{0\text{-}28d}$ once-monthly (QMo) MS~semaglutide is 1,370 nM h, compared to a reported steady-state $AUC_{0\text{-}30d}$ of 1,500 nM h for semaglutide (12), giving a relative bioavailability of 92%. As shown in Fig. 1*B* twice daily (BID) semaglutide at 10 nmol/kg over 1 Mo—a ~600 nmol/kg cumulative dose—or single doses of 660- and 2,000 nmol/kg MS~semaglutide gave ~20% weight loss in DIO mice, and differences were statistically insignificant. Dual-energy x-ray absorptiometry (DEXA) scan measurements at ~30 d showed loss of fat mass rather than lean mass (Fig. 1 *C* and *D*). As expected (12), both BID semaglutide and single dose MS~semaglutide lowered blood glucose levels in a dose-dependent manner and suppressed appetite/food intake.

The dose of MS~semaglutide at steady-state in humans is estimated from single doses in mice. First, the effective dose of semaglutide in humans (6 nmol/kg/wk) is 23-fold lower than in mice (140 nmol/kg/wk) (13, 14). From $t_{1/2}$ ~ 1 Mo, it would take 2.3 Mo for MS~semaglutide to reach steady-state; so, the equieffective dose in DIO mice would be ~2.3-fold lower than the single dose. Therefore, the steady-state dose in humans should be 2.3 × 23 or ~50-fold lower than in mice, so the 0.66 to 2.0 µmol/kg/mo MS~semaglutide in mouse is equivalent to 0.013 to 0.04 µmol/kg/mo in humans. Hence, for a 100 kg human, the effective dose should be ~0.33 to 1 mL of the 4 µmol/mL MS~semaglutide. Further, simulated C vs. t plots of QMo MS~semaglutide at doses of 5, 10, and 17 mg followed by fixed 17.5 mg show that the released semaglutide stays well within the reported C_{min}/C_{max} boundaries of once-weekly (QWk) semaglutide and have only 75% of the C_{max}.

Discussion

We have described an approach whereby a lipidated peptide with a half-life of ~1 wk can be converted to a peptide with a half-life of ~1 mo. The lipidated peptide is tethered to a microsphere depot by a releasable linker that overrides lipidation and dictates the half-life of the released peptide (11). Here, we demonstrated conversion of semaglutide—which has a half-life of 160 h in humans—to a prodrug in which the released semaglutide has a half-life of ~36 d, optimal for QMo administration. Further, since the half-life obtained with the technology is species-independent (9), it should directly translate from mouse to human.

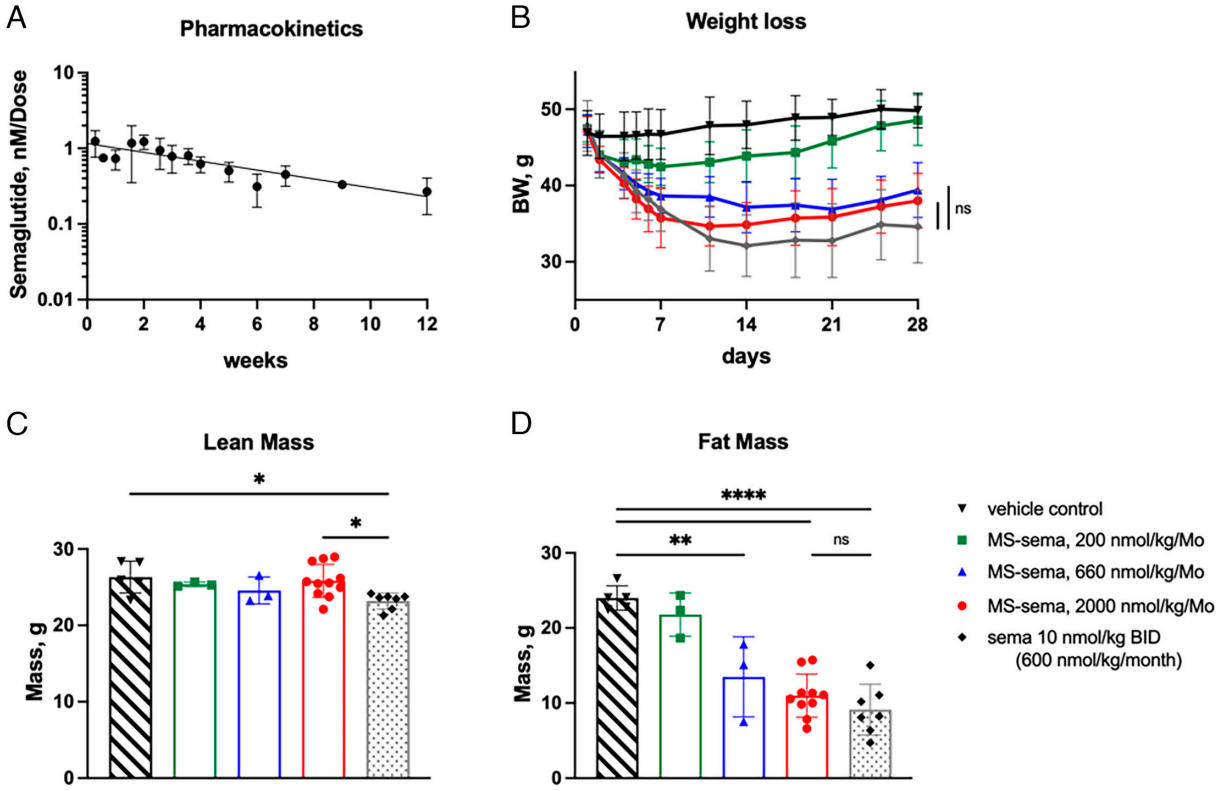

Fig. 1. Pharmacokinetics and weight loss effects of MS~semaglutide. (*A*) Dose-normalized concentration vs. time plot in the mouse of semaglutide released from MS~semaglutide containing 4 µmol semaglutide/mL. Individual data points are dose-normalized, mean ± SD (n = 3 to 6), from single doses of 400 and 2,000 nmol/kg MS~semaglutide. Data were fit using a one-phase decay model giving a $t_{1/2}$ = 36 d, 95% CI 30 to 44 d. (*B*) Body weight vs. time of DIO mice; mean ± SD, mixed-effects model and Tukey's post hoc. DIO mice were treated with vehicle (▼), 10 µmol/kg BID semaglutide (cumulative 600 nmol/kg/mo) (◆) and single doses of 200 (■), 660 (▲), or 2,000 nmol/kg (●) MS~semaglutide (n = 3/group). (*C* and *D*) Composition of weight loss after 28 d treatment; mean ± SD, 1-way ANOVA, and Tukey's post hoc. *P < 0.05, **P < 0.01, ****P < 0.0001 vs. vehicle.

In addition to being the second GLP-1RA with a month half-life, the QMo semaglutide shows that high synergy in half-life extension can be achieved by concomitant use of both lipidation and β-elimination technologies. The exposure and bioavailability of a single dose of MS~semaglutide vs. daily (QD) semaglutide in mice over 1 mo are nearly identical, indicating stability and efficient release of semaglutide from MS~semaglutide in the subcutaneous space. Thus, it is expected that the pharmacodynamics will also be long-acting. Indeed, a single dose of MS~semaglutide to DIO mice gave a 20% lean-sparing weight loss over 1 mo which is the same as that achieved by a similar cumulative dose of semaglutide given BID (13). Also, MS~semaglutide caused the appropriate decrease in food intake and glucose (12). Overall, the pharmacokinetic data and protracted period of weight loss show that the linker of MS~semaglutide—not the lipid—controls the in vivo duration of the drug. Using data obtained in the DIO mouse studies and the effective doses of semaglutide in mouse and man, we estimate the human dose of QMo MS~semaglutide to comprise ~5 to 15 mg semaglutide, which is contained in 0.3 to 1 mL of our 4 μmol/mL MS~semaglutide preparation. Simulations indicate that QMo MS~semaglutide would maintain the therapeutic C_{min} of QWk semaglutide with only 75% of the C_{max}, a feature that could improve tolerability or allow higher dosing (15). For the future, we posit that the very same approach could be used to convert other lipidated peptides of current interest from once-weekly to once-monthly administration. Finally, the availability of once-monthly antiobesity agents should mitigate the problem of low persistence, decrease the side effects of once-weekly dosing, and address an unmet need in patient populations that would benefit from less frequent dosing of a GLP-1RA.

Materials and Methods

Detailed synthetic, analytical, and in vitro kinetic procedures, as well as in vivo pharmacokinetic and pharmacodynamic methods are provided in *SI Appendix*. All animal handling and care were performed by MuriGenics (Vallejo, CA) and conformed to Institutional Animal Care and Use Committee (IACUC) recommendations.

Data, Materials, and Software Availability. All data are available in the main text or *SI Appendix*.

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Supplemental Information

The limitation of lipidation: conversion of semaglutide from once-weekly to once-monthly dosing

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Methods

Chemistry

Preparation of N_3-linker-semaglutide. Semaglutide-Na (43 mg, 10.5 μmol) and N,N-diisopropylethylamine (9 mL, 52 μmol) in 1 mL 9:1 DMF/H_2O was treated with 5-azido-3,3-dimethyl-1-(methylsulfonyl)-2-pentyl succinimidyl carbonate (1), N_3-linker($MeSO_2$-)-HSI (11.8 mg, 31.4 μmol). After 3 h, HPLC indicated acylation on the Nα and imidazole of His (30% Nα, 9% imidazole, 59% diacylated). A 0.5 M solution of NH_2OH-HCl, pH 7 (200 μL), was added and after 16 h HPLC indicated 88% Nα-acylated N_3-linker($MeSO_2$-)-semaglutide. Preparative HPLC (Phenomenex Jupiter 5 μm 300Å C_{18} 250 x 21.4 mm column, 20- to 100% MeCN in H_2O + 0.05% TFA) gave purified N_3-linker($MeSO_2$-)-semaglutide (36 mg, 8.2 mmol, 78%). One peak by HPLC (280 nm); MS $[M+3H]^{3+}$ 1458.4379 (calc. 1458.3979).

Preparation of MS~semaglutide. Exemplary, N_3-linker($MeSO_2$-)-semaglutide (31 mg, 7.1 μmol) in 500 μL of DMF was added to BCN-microspheres (700 μL, 5.9 μM BCN) (2) in MeOH and rotated at 225 rpm for 42 hours, 37 °C. Unreacted N_3-linker($MeSO_2$-)-semaglutide was removed by washing with 4 mL of DMF, then buffer (10 mM NaOAc, 143 mM NaCl, 0.05% Tween 20, 10 mM Met, pH 5.0), and then exchanged into buffer containing 1.2% of 40k hyaluronic acid. To quantify loading, measured aliquots of slurry were dissolved in 50 mM NaOH, neutralized with 125 mM HEPES, pH 7.4, then assayed for peptide using ϵ_{280} = 6790 $M^{-1}cm^{-1}$.

Pharmacology

All animal handling and care was performed by MuriGenics (Vallejo, CA) and conformed to IACUC recommendations.

Pharmacokinetics in C57BL/6 mice. Male ~8 week-old C57BL/6 mice (6/group), were dosed SC in the flank with 50 μL MS~semaglutide to deliver 400 and 2,000 nmol/kg. Blood samples were drawn from the tail vein at various times to give 3 replicates per timepoint, and processed to plasma with K_2EDTA/protease inhibitors; semaglutide was quantified by ELISA (BMA Biomedicals #S-1530).

MS-semaglutide in DIO mice. Male ~22 week-old C57BL/6 DIO mice (3/group; average weight 47 g), were dosed SC with 50 μL of MS-semaglutide to deliver 200, 660 and 2000 nmol/kg. Body weights, food intake and glucose levels were measured at intervals over 28 d. At study termination, DEXA scan was used to determine the fat and lean mass.

Supplemental References

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