

DRUG DELIVERY

Week-long diabetes therapy

A subcutaneous depot of a diabetes drug fused to a thermosensitive biopolymer leads to blood-glucose control, for over one week after a single injection, in animal models of type-2 diabetes.

Omid Veisheh

The goal for the management of type-2 diabetes — a chronic metabolic disease characterized by persistent high blood-glucose levels (hyperglycaemia), and the leading cause of blindness, non-traumatic lower-limb amputations, and kidney and cardiovascular diseases¹ — is to restore glycaemic control while avoiding adverse episodes of hypoglycaemia¹. The aetiology of the disease is not fully understood, yet it is associated with poor diet, a lack of physical activity, and obesity. Physicians often start with a plan to address lifestyle changes, but for many patients this alone is insufficient, and therefore medications that improve insulin sensitivity (such as Metformin) and/or affect glucose metabolism and insulin concentration in blood (termed incretin-based therapies) are added to better regulate glycaemic levels.

One promising incretin-based therapy is a hormone that stimulates receptors of the glucagon-like peptide-1 (GLP-1) in many body tissues². Activation of GLP-1 receptors increases endogenous secretion of GLP-1, which in turn stimulates insulin secretion (in a glucose-dependent manner) while simultaneously suppressing glucagon, a hormone that increases blood-glucose levels². A number of clinical trials have shown that patients who receive constant high levels of GLP-1 through intravenous infusion are better able to achieve glycaemic control (normal blood-glucose levels both after fasting and after meals) without adverse incidences of hypoglycaemia^{1,2}. Unfortunately, naturally occurring GLP-1 is highly susceptible to enzymatic degradation in the blood, and thus has a very short blood half-life (about 2 minutes in human patients³). To overcome the burden of continuous intravenous infusions, researchers have identified long-acting analogues of GLP-1 receptor agonists (such as exenatide and liraglutide) that are most stable against degradation and thus have longer half-lives (longer than 2.4 hours) in blood, thus making them compatible for injection³. However, the need for frequent injections continues to remain a major barrier to the wider adoption and

compliance of incretin-based therapies by patients. Ashutosh Chilkoti and colleagues now report in *Nature Biomedical Engineering* that a long-acting formulation of GLP-1 can be achieved by fusing it with a thermosensitive biopolymer⁴.

Chilkoti and co-authors' formulation consists of an engineered analogue of the GLP-1 receptor agonist that is recombinantly fused to a thermosensitive elastin-like polypeptide (ELP). At room temperature, the GLP-1-ELP formulation is highly soluble and can readily be administered through a standard injection needle; when injected subcutaneously, the higher temperature in the body triggers the formation of a depot that releases GLP-1 with zero-order release kinetics (constant release over time) for at least one week after a single injection (Fig. 1).

To produce the formulation that yielded the most extended zero-order release kinetics of the GLP-1 analogue when administered *in vivo*, Chilkoti and co-authors examined the effects of various fusion-peptide sequences and molecular weights. The authors hypothesized that by altering the transition temperature (15–36 °C) of the thermosensitive ELP, they could modulate the duration of release of the drug from the subcutaneous depot. The ELPs that they first explored consisted of 120 repeating units of a pentapeptide amino acid sequence of valine–proline–glycine–X–glycine (VPGXG). In this model, the X guest-residue motif could be substituted with amino acids of varying hydrophobicity by incorporating different ratios of alanine, valine and leucine to produce ELPs with altered transition

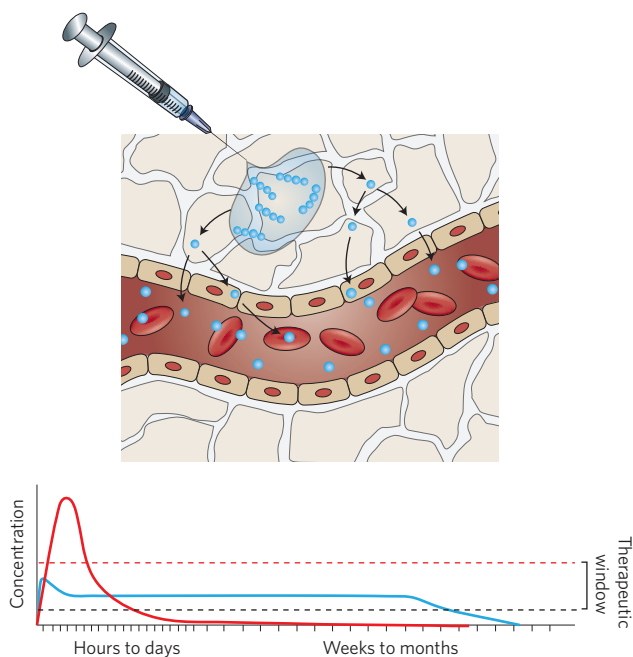


Figure 1 | Long-acting release of an analogue of the glucagon-like peptide-1 receptor agonist via fusion with a thermosensitive elastin-like polypeptide. On subcutaneous injection, the formulation creates a subcutaneous depot (top) that controls blood-glucose levels through zero-order release kinetics (bottom; blue line) in animal models of type-2 diabetes for longer than one week after a single injection. Constant drug release rates allow for extended release (blue line) and avoids peaks and valleys (red line) in drug concentration.

temperatures. When they tested the various combinations of GLP-1–ELP fusion-peptide formulations in a diet-induced obese (DIO) mouse model of type-2 diabetes, they learned that a VPGXG sequence where X was substituted with A, V and L at a ratio of 5:5:0 yielded a fusion peptide with a transition temperature of 30.5 °C, and that this formulation outperformed all other fusion peptides tested. Significantly, this sequence-optimized formulation led to release activity for up to 144 hours. To further improve and achieve even longer release activity, the authors also varied the molecular weight of the ELP by using a similar screening approach while maintaining a constant fusion-peptide sequence. By increasing the number of VPGXG repeats, and thus the molecular weight of the fusion peptide, the authors found that those with a molecular weight of 67.5 kDa produced even longer activity, for up to 10 days.

To verify the results obtained in the DIO mouse model, Chilkoti and co-authors tested their optimized formulation in two additional rodent models of type-2 diabetes and in healthy non-human primates. In all rodent models, the optimized GLP-1–ELP formulation yielded release activity for at least 10 days, making this formulation useful for a once-weekly injection. Moreover, the formulation produced significant long-term health benefits to the mice, as indicated by weight loss, improved glycaemic control, a reduction in glycated-haemoglobin levels and other indicators, all monitored for 56 days. In healthy non-human primates, drug-release activity lasted for up to 17 days. The authors note that in humans the formulation would produce release

activity for up to three weeks (according to allometric scaling, and assuming similar bioavailability across species). The authors also note that it should be feasible to further optimize the injectable GLP-1 formulation to achieve glycaemic control in human patients with a once-monthly injection.

Incretin-based therapies are becoming the lead second-line therapy in the management of type-2 diabetes patients, with those who consistently receive regular drug injections achieving significant long-term health benefits⁴. However, compliance and adoption have been significant barriers to the widespread use of incretin-based therapies. In recent years, various pharmaceutical companies have invested substantial resources towards developing ultralong-acting GLP-1 receptor agonist drugs. Currently, five approved formulations exist on the market: exenatide (marketed by AstraZeneca as Bydureon), liraglutide (marketed by Novo Nordisk as Victoza), lixisenatide (marketed by Sanofi-Genzyme as Lyxumia), albiglutide (marketed by GlaxoSmithKline as Tanzeum) and dulaglutide (marketed by Eli Lilly as Trulicity). Bydureon, Tanzeum and Trulicity are available as once-weekly injections. However, Bydureon uses polymer microparticles that require a special large-bore needle for injection. Tanzeum is a GLP-1 dimer that is fused to human albumin so as to increase its size in order to achieve prolonged circulation and half-life in blood, and Trulicity uses a unique GLP-1 analogue that is fused to a large Fc fragment of the IgG4 antibody. Chilkoti and colleagues' approach towards developing ultralong-acting GLP-1-based therapeutics would, however, require less-frequent

injections, and would need smaller, standard insulin syringes.

Drug formulations that focus on extending the drug's circulation half-life in blood typically lead to drug-concentration profiles with peaks and valleys. These can affect the drug's long-term efficacy; for example, during peaks, patients could be more susceptible to developing neutralizing antibodies against the therapies. By combining a more stable GLP-1 analogue with an extended-release ELP that further enhances the stability of GLP-1 against enzymatic degradation in blood, Chilkoti and co-authors have developed a formulation that could potentially provide treatments for patients with type-2 diabetes through a once-monthly injection. The formulation's zero-order release kinetics would ensure that patients are able to maintain constant high levels of GLP-1 agonist activity in their blood. Although more extensive preclinical safety and toxicity studies would be necessary to ensure that the GLP-1–ELP formulation is both safe and effective prior to eventual clinical trials in humans, the authors' approach promises to deliver peptide drugs for a range of diseases for which frequent administration is a major burden. □

*Omid Veisheh is in the Department of Bioengineering, Rice University, 6100 Main Street, Houston, Texas 77030, USA.
e-mail: omid.veisheh@rice.edu*

References

1. Ismail-Beigi, F. N. *Engl. J. Med.* **366**, 1319–1327 (2012).
2. Meloni, A. R., DeYoung, M. B., Lowe, C. & Parkes, D. G. *Diabetes Obes. Metab.* **15**, 15–27 (2013).
3. Gupta, V. *Indian J. Endocrinol. Metab.* **17**, 413–421 (2013).
4. Luginbuhl, K. M. *Nat. Biomed. Eng.* **1**, 0078 (2017).