BIOTECHNOLOGY

Hanging around for longer

One of the most common methods to increase the *in vivo* half-life of therapeutic proteins is pegylation — the attachment of a polyethylene glycol (PEG) moiety. Writing in *Nature Biotechnology*, Schellenberger and colleagues describe a new method to increase drug half-life — fusion of an unstructured protein sequence to the drug — that has several advantages over pegylation, including the potential to substantially reduce dosing frequency.

To aid the design of a polypeptide with desirable characteristics for *in vivo* use, the authors focused on six amino acids (A, E, G, P, S and T). From an initial expression screen of 1,500 peptide segments comprising randomized sequences, a single 864-amino-acid sequence, named XTEN, was selected for further study.

The gene encoding XTEN was synthesized and fused to a synthetic gene (expressed in *Escherichia coli*) that encoded exenatide (Byetta; Amylin/Lilly), a 39-amino-acid peptide that is approved for the treatment of type 2 diabetes. It has a plasma half-life of 2.4 hours and requires twice-daily dosing. Fusion proteins that included green fluorescent protein (GFP), glucagon, factor VII or human growth hormone (HGH) were also formed with XTEN.

Initial characterization studies showed that exenatide–XTEN was a homogeneous chemical species that was not strongly immunogenic and showed no adverse effects in toxicity studies. In cynomolgus monkeys, the terminal half-life of the construct was 60 hours, with 80% bioavailability from a subcutaneous injection. A slow-absorption phase resulted in a maximum plasma concentration 24–48 hours after injection and a constant plasma concentration for ~100 hours before a linear elimination phase. Such a pharmacokinetic profile would be expected to reduce safety issues associated with peak-dose toxicity that is seen with many drugs, and to simultaneously increase the therapeutic window for the drug. A glucose challenge mouse model showed that exenatide–XTEN confers resistance to glucose challenge for at least 48 hours after a single dose.

Furthermore, studies using XTEN fused to GFP showed that the half-life of the construct could be reduced by shortening the length of the XTEN sequence, indicating the possibility of tailoring the half-life of such fusion proteins to the therapeutic purpose. Half-life tuning led to the generation of a HGH–XTEN construct with a 110-hour terminal half-life in monkeys. In addition, using allometric scaling and historical data, the authors calculated that a single dose of 100 mg exenatide–XTEN should provide therapeutic benefit in patients with type 2 diabetes for at least 30 days. Therefore, fusion with XTEN might enable monthly dosing of exenatide and other therapeutic proteins.

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ORIGINAL RESEARCH PAPER Schellenberger, V. *et al.* A recombinant polypeptide extends the *in vivo* half-life of peptides and proteins in a tunable manner. *Nature Biotech.* 15 Nov 2009 (doi:10.1038/nbt.1588)