Hormone delivery: small synthetic molecular mimics

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Since the experimental intravenous injections of pancreatic extracts into animal models of diabetes in 1912 by Scott (1), and after the purification of insulin by Banting, Best and Collip in 1921, it remained unclear why oral administration of these extracts or insulin had no effect, until finally in 1925 Macleod showed that insulin was a peptide destroyed by proteolytic enzymes in the digestive tract. Today many proteins such as insulin, erythropoietin (EPO) and growth factors are still administered intravenously or subcutaneously to treat diabetes, anaemia and neutropenia respectively. Intense efforts have been made in the last decades to try to develop novel methods for drug delivery. Intranasal hormone therapy has been a success for calcitonin (32 amino acids) and glucagon (29 amino acids). Intranasal insulin and growth hormone therapy required intranasal doses up to 20 times higher, as well as the adjunction of ‘enhancers’ to improve mucosal absorption. Metabolic control deteriorated in diabetic patients (2), and the ‘enhancers’ proved to be too irritating. Projects on nanocapsular drug carriers, and coadministration of Vibrio cholerae toxins, aimed at enhancing enteral absorption, remain so far without convincing clinical results (3, 4). In 1995 Mitragotri et al. were able to show increased transdermal permeability to proteins with ultrasound (5). They used low frequency ultrasound to enhance the transport of proteins across skin; a phenomenon referred to as sonophoresis. The ultrasound induces growth of air pockets (cavitation) between keratinocytes, thus reversibly disorganising the stratum corneum. Insulin, interferon γ, and EPO transport was enhanced by sonophoresis and a decrease in blood glucose concentration was observed in rats following the administration of insulin. However, the ultrasound has to be applied for lengthy periods and no clinical application has been developed so far. The most recent innovations are inhaled insulin in the form of aerosols (6) or as large porous particles capable of long-term drug release and increased bioavailability (7). Clinical trials have proven the efficacy of liquid insulin aerosols, and the prospect of being able to inhale therapeutic doses of dried powdered insulin in one puff is attractive. Phase III trials are scheduled for this year. Despite these advances in technology, protein hormones, due to their size and rapid degradation, still rely on injections for optimal delivery and therapeutic effect.

An alternative to finding novel routes to administer protides is the development of non-peptide or small peptide analogues. A first breakthrough took place in 1996 when Wrighton et al. (8) and Livnah et al. (9) showed that a 20-residue cyclic peptide dimer, with no homology to the primary sequence of EPO, bound the extracellular domain of the EPO receptor (EPO-ERP) and induced signal transduction. To isolate small peptides that bound specifically to the EPO, Wrighton et al. screened a random filamentous phage library using the EPO extracellular domain as bait. They isolated an eight-amino acid peptide sequence that served as a starting point to screen for similar peptides with higher affinity in a mutagenesis library. The five clones obtained during this second selection were cyclic, all expressed a disulphide bond, and had median effective concentrations (EC50) ranging from 200 nmol/l to 1 μmol/l. X-ray crystallography of the EPO–EPO mimetic peptide complex (9) revealed two peptide molecules, each in contact with a receptor, and interacting between themselves in a symmetrical array, resulting in receptor dimerisation. Dimerisation activates the receptor, which then activates the cytoplasmic signalling cascades (Fig. 1). This was the first example of a small 2 kDa peptide being able to mimic the actions of the 34 kDa EPO molecule. Wrighton and Livnah were followed shortly by Cwirla et al. (10), who isolated a 14-amino acid peptide, totally unrelated to thrombopoietin (TPO), that was able, as a dimer, to bind the TPO receptor, inducing its dimerisation which results in activation of this receptor and stimulation of in vitro proliferation and maturation of human megakaryocytes. In vivo this peptide increased the platelet count in mice when administered subcutaneously. The EC50 of this peptide, 100 pmol/l, is identity to that of recombinant human TPO. With these results proving that these minimal hormones, with no homology to EPO or TPO, could mimic their physiological effects, the next step was to find a non-peptide compound capable of such actions. This of course with the idea of synthesising orally bioavailable drugs that can simulate peptide hormone.

A small non-peptide molecule capable of activating the granulocyte-colony stimulating factor (G-CSF) receptor was recently identified by Tian et al. (11). Binding of G-CSF to the extracellular domains of its receptor triggers homodimerisation and then activation of the Janus kinase and signal transducer and activator of transcription (JAK-STAT) signalling cascade. Using a murine myeloid cell line with a G-CSF responsive reporter, Tian et al. screened for compounds capable of

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binding and activating the G-CSF receptor (11). One compound, SB 247464, had 30% of the activity of G-CSF, activated the JAK-STAT cascade, and was specific to the G-CSF receptor. In mice, subcutaneous injection of SB 247464 induced a fourfold increase in peripheral neutrophils.

SB 247464 has a twofold rotational symmetry that probably plays an important role in the homodimerisation of G-CSF receptor chains. The peptides mimicking EPO and TPO also depend on their symmetrical structure, as seen by a decrease of three orders of magnitude in the EC₅₀ when peptide dimerisation (Fig. 1) was induced by carboxy-terminal linkage (8–10).

Despite the fact that this synthetic molecule only binds murine G-CSF receptors, the progress achieved by this discovery is remarkable. Receptors are often activated by dimerisation and in many cases the main role of the ligand is to induce dimerisation. It is now clear that large transmembrane receptors can be bound by small non-peptide molecules on sites other than normal ligand-binding domains, enhancing homodimerisation, and thus activating the receptor and its downstream signalling cascade. The insulin receptor is somewhat similar to the C-GSF receptor and is also activated by dimerisation on binding insulin. The development of a non-peptide synthetic compound, able to stimulate insulin receptors, administrable by mouth, seems a very exciting prospect, since its consequences for the current therapy of insulin-dependent diabetes could be tremendous. Analogues of EPO, TPO and growth hormone, interferon and cytokines could also all be given one day as tablets, facilitating the tasks of nephrologists, immunologists and oncologists. Certain previously abandoned methods of hormone delivery may be revived by smaller non-peptide molecules. Despite a multitude of techniques being developed for drug delivery, the prospect of one day being able to just swallow a tablet remains appealing.

**References**


