QJM

Peptides as drugs

We are on the brink of a therapeutic revolution. There has been a rapid expansion in the use of peptides as drugs over the last decade, and this is likely to continue. Peptides regulate most physiological processes, acting at some sites as endocrine or paracrine signals and at others as neurotransmitters or growth factors. They are already being used therapeutically in such diverse areas as neurology, endocrinology and haematology.

Most peptides cannot be administered orally as they are rapidly inactivated by gastrointestinal enzymes, so that subcutaneous or intravenous administration is required. Therefore, research is focussing on alternative routes of delivery, including inhaled, buccal, intranasal and transdermal routes, as well as novel delivery systems such as the use of protective liposomes. Neuropeptide systems in the brain are being examined as potential targets for therapeutics, providing an exciting future development area. The dual problems of local targeted delivery and the blood-brain barrier, prevent administered peptides from readily gaining access to the required site of action, although as we will discuss, solutions are on the horizon.

Peptides act by binding to specific cell surface receptors. The perfect therapeutic agent would be a small-molecular-mass chemical mimic of the receptor ligand, which would be cheap to manufacture and could get to the site of action after oral administration. However, receptors are large with many binding sites, and peptides have a complex tertiary structure, both of which improve specificity as well as affording protection from simple invading molecules, like bacterial toxins. Consequently, production of successful peptide mimics using chemical libraries is largely unsuccessful and we still rely on the native peptide for therapeutics.

The list of peptides as potential drugs is huge; space does not permit discussion of them all. It is beyond the scope of this article to focus on older peptide therapies such as luteinizing-hormone-releasing hormone, growth hormone, arginine vasopressin or the very interesting peptide, cyclosporin. We will instead highlight some exciting new areas of research as well as recent developments in the use of more established peptide therapies.

Insulin was the first peptide to be isolated and administered therapeutically, and is still the most commonly prescribed peptide, having been used for over half a century. We have yet to find a chemical mimic; however, there is still ongoing research into novel analogues and methods of administration. Manipulation of the insulin molecule has allowed the development of shorter-acting insulins, such as Lispro insulin. This is rapidly absorbed, readily dissociates into insulin monomers, and produces plasma levels that more closely mimic the normal postprandial insulin profile. Thus, Lispro can be injected immediately prior to a meal, unlike conventional short-acting insulins that should be injected half an hour earlier.[1]

A recent development is the use of non-injectable forms of insulin. Daily injections up to four times per day, is a major source of distress in the diabetic population, for example children or the elderly. It comes as no surprise that inhaled, intranasal, buccal, rectal and sub-lingual preparations of insulin have all been investigated. Precise insulin dosage is critical for 'brittle' type 1 diabetics. However, for some, e.g. people with type 2 diabetes who have significant residual endogenous insulin secretion, less accurate forms of administration may be adequate. Results presented at the 1998 American Diabetes Association meeting, showed that inhaled insulin provided glycaemic control equivalent to the subcutaneous route, whilst allowing insulin to be taken immediately before a meal. Thus, the future seems to be more hopeful for the 'multiply-punctured diabetic'.

A number of other peptides have been investigated for the treatment of diabetes. For example, peptides that delay gastric emptying may be valuable in the treatment of both type 1 and type 2 diabetes.[2] If patients with glucose intolerance or early diabetes, ate small amounts of food frequently, decreasing the flux in intestinal glucose absorption, this would diminish beta-cell work, and may therefore slow the progression of the disease. Islet amyloid was originally noted to be present in the islets of patients with type 2 diabetes, and its precursor amylin is secreted

with insulin. A proposed action of amylin to improve insulin sensitivity was not confirmed[3] but it was found to be a potent inhibitor of gastric emptying.[2] Recent studies of pramlintide, a stable amylin analogue which does not form amyloid, indicate potential for the approach of delaying gastric emptying,[4] and it is undergoing phase 3 clinical trials.

Another peptide in which there is currently much interest is the endogenous hormone, glucagon-like peptide–1 (GLP–1).[5] This not only has insulin-releasing properties, but also suppresses glucagon levels, and delays gastric emptying. The insulinotropic action of GLP–1 is glucose-dependent, thus, it acts more potently after a meal than in the fasting state, protecting against hypoglycaemia. GLP–1 has been reported to increase peripheral glucose disposal, another desirable effect in diabetes, and a target for non-peptide treatments such as the thiazolidinediones. GLP–1 has been shown to decrease appetite in the rat,[6] and more recently in man,[7] particularly relevant for the 85% of patients with NIDDM who are overweight. The results of a recent small double-blind placebo-controlled cross-over study using subcutaneous injections of GLP–1 for three weeks, has demonstrated therapeutic promise in early type 2 diabetes.[8] Indeed, development of a novel buccal tablet,[9] with good bioavailability has ensured at least two pharmaceutical companies are exploring the possible use of GLP–1 as therapy. Longer-acting peptide agonists of the GLP–1 receptor are also under clinical trial.

In 1980, 8% of the population in Britain were clinically obese (body mass index >30); by 1994 this had increased to 15%. Obesity is a major risk factor for ischaemic heart disease, diabetes and stroke. As all those who see obese patients know, exercise and dieting does not work for the majority. The recent withdrawal from the market of phentermine and fenfluramine, indicates the difficulty in finding a safe and effective therapy. The number of new obesity treatments recently licensed demonstrates the magnitude of the need and the desperate desire for an effective medical treatment.

The discovery of the adipose tissue peptide hormone, leptin,[10] has opened a new chapter in the search for an anti-obesity agent. Leptin, a signal of the degree of body adipose tissue mass, decreases appetite and increase metabolic rate in animal models. Exogenous administration of this peptide could treat obesity. Conversely an antagonist could treat anorexia. In obese humans, leptin levels are high, yet they keep eating. This may reflect the fact that the transport system into the central nervous system is saturable, producing functional leptin resistance.[11] However, it is still believed by many that exogenous administration of leptin will cause weight loss. The first clinical evaluation of daily injection of recombinant methionyl leptin in obese volunteers, treated for 6 months, demonstrated an 8 kg loss in weight. This occurred with minimal side-effects. We await with interest to see whether this weight loss continues on further treatment. Certainly patients with the rare condition of obesity due to genetically absent leptin,[12] respond well.

The discovery of one adipose tissue hormone that regulates body weight may lead to the identification of others. These may hold greater therapeutic hope. With over 100 million obese people globally, and the incidence of obesity climbing, this is an area which will continue to be a major focus for research.

The long acting stable analogue of somatostatin, octreotide, has a number of therapeutic indications. Octreotide is very effective at treating acromegaly as well as the symptoms from gastro-enteropancreatic endocrine tumours, particularly from carcinoid syndrome, Zollinger-Ellison syndrome and VIPomas. Octreotide has also been used as a therapy for a variety of other disorders including upper gastrointestinal bleeding, acute pancreatitis, dumping syndrome, gastrointestinal fistulae and secretory diarrhoea. Recently, two new analogues and delivery systems making octreotide more stable and thus longer-acting have been licensed, such that subcutaneous injections are only necessary every 2 weeks (Lanreotide) or 4 weeks (Sandostatin LAR), providing an improvement in the quality of life of these patients. Interestingly, an oral preparation of somatostatin demonstrated good bioactivity,[13] however, it has not yet been developed commercially.

Interest has focussed on the use of radiolabelled octreotide for imaging, and its potential therapeutic use. Expression of particular somatostatin receptors on certain tumours allows imaging of these tissues, and specific binding and internalization of higher dose ^{111}In-pentetreotide, produces cell death and tumour regression.[14] ^{111}In-pentetreotide therapy is in its infancy and has only been used in a few patients, but has resulted in good objective evidence of tumour response. Longer-term studies are required to assess the exact usefulness of this novel weapon in our therapeutic armament. Hopefully it will lead the way for other such peptides to allow specific targeting of a cytotoxic dose of radioactivity.

When the interferons were discovered they were hailed as a panacea for many conditions, but this has not proven to be the case. Recombinant interferon beta–1b has been licensed for use in relapsing-remitting multiple sclerosis following the results of a large multicentre randomized, placebo-controlled trial in 1993.[15] This showed a 34% decrease in the annual rate of exacerbations as well as a decrease in their severity after 2 years therapy with interferon beta–1b (8 MIU subcutaneously every second day). Unfortunately, there was no evidence for any effect

on the level of disability. Interferon beta–1a, being glycosylated, is closer to natural interferon. This has also been shown to reduce relapse rate but, in addition, demonstrated a delay in progression of disability.[16] Since the introduction of these drugs, a highly charged argument has waged on the cost-benefit ratio for such expensive treatments with relatively little evidence of long-term benefit.

Copolymer 1 (Cop–1) is a mixture of synthetic peptides composed of the amino acids alanine, glutamic acid, lysine and tyrosine. It was originally designed to mimic myelin basic protein, one of the components of myelin, which is now thought to have only a minor role in the pathogenesis of multiple sclerosis. Despite this, Cop–1 injections have been shown to reduce relapse rate by a similar degree to beta-interferon and may be better tolerated.[17] Cop–1 appears to work via a different mechanism to interferon, making dual therapy a likely future development.

Peptide neurotrophic factors such as nerve growth factor (NGF), have been suggested to be valuable in the therapy of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. NGF is hypothesized to rescue dying neurons and stimulate nerve terminal outgrowth. Direct infusion of NGF into the putamen has produced some benefit when used as a support to autografts of adrenal medulla in Parkinson's disease. Infusion of NGF into the third cerebral ventricle of patients with Alzheimer's disease for 3 months resulted in cognitive improvement as well as increased cerebral blood flow and EEG changes.[18] Obviously these methods of drug delivery are only appropriate for a very select patient group. However, the studies do point to another potential use for peptides as drugs in the future, and linking a neurotrophin to a carrier molecule to facilitate transport across the blood-brain barrier may allow intravenous usage.

Recombinant human erythropoietin (EPO) was first licensed as a therapeutic agent in 1988, remarkably within 5 years of cloning of the human gene.[19] It was an immediate success in treating the anaemia of chronic renal failure. At present, subcutaneous administration is the route of choice in Europe. Erythropoietin is one of a number of drugs, including insulin and interferon, for which encapsulation in liposomes to allow oral delivery has been investigated.[20] Pharmacological availability is extremely variable (0.74–31%), dependent on liposome composition and particle size, however, such studies indicate potential for this delivery system. Therapy with EPO has also been considered for the anaemia associated with many disorders including cancer, multiple myeloma, myelodysplasia, HIV infection and chemotherapy. It seems to be relatively free of side-effects, aside from a significant increase in blood pressure in about one third of patients in one study of renal patients, all but one being controlled with further anti-hypertensive treatment.[21] Indeed, it has become a drug of abuse amongst athletes who derive benefit from the increased oxygen carrying capacity of the resultant polycythaemia—time will tell as to the real prevalence of this problem.

Recombinant human growth factors such as granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) stimulate the production of neutrophils or granulocytes and monocytes. They reduce the severity of chemotherapy induced neutropenia and accelerate haematopoietic recovery,[22] and have also been used for neutropenia associated with other disorders. A number of studies have demonstrated a significant reduction in infections with these treatments given in the form of subcutaneous injections. They are now frequently used as part of the therapeutic protocol for a number of haematological malignancies. In particular, combination with EPO seems to improve its efficacy, at least in myelodysplastic syndromes.[23] After development of peptides to increase red-cell and white-cell numbers, it comes as no surprise that thrombopoietin is the latest replacement peptide being investigated as a treatment for thrombocytopenia.

Peptide antibiotics have been under investigation for a number of years. In this country there are currently two antibiotic peptides licensed, the polymyxins, polymyxin B and colistin (polymyxin E). Colistin is occasionally given by injection to treat *Pseudomonas aeruginosa* in patients with cystic fibrosis, though more frequently aerosol preparations have been used.[24] It is also used orally in bowel sterilization regimens for neutropenic patients, as it is not absorbed. Polymyxin B is just used as a topical preparation for local eye and ear infections. Both antibiotics are prescribable for local skin infections. Several classes of other antibiotic peptides including defensins, protegrins, magainins, tachyplesins, cecropins, mutacins and clavanins are under investigation. They are isolated from animal or bacterial sources as diverse as *Xenopus* skin, mudfish and streptococci. The inability of present non-peptide antibiotics to kill certain bacteria, make it likely that antibiotic peptides will form an important part of our fight to defeat multi-resistance in the twenty-first century.

We have described some of the present diversity of uses for peptide therapies and future potential development. Manipulation of the structure of some of these peptides has allowed development of receptor ligands with longer action. Peptides active via non-injected routes are beginning to appear. Gene therapy is likely to provide the basis for novel delivery systems in the future. Production of orally active preparations is more likely with the encapsulation of

peptides in liposomes or polymers allowing protection from digestion. This is an active area of research with much therapeutic potential; the 'holy grail' of orally active peptides will soon be realized, and with it a rapid expansion of peptide therapy will ensue.

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