INTRODUCTION

Nearly 95 years after the first unsuccessful attempts to deliver a peptide orally, research in this area has resulted in technologies that produce a clinically relevant oral bioavailability of only 1-2% for the majority of peptides studied, despite pharmaceutical and biotechnology companies expending a considerable amount of resources on oral peptide delivery.

With the exception of small peptides such as taltirelin (Ceredist®) and desmopressin (Minirin®), which are commercially available as oral drugs, the majority of macromolecule-based biopharmaceuticals are currently administered parenterally either as intramuscular or subcutaneous injections, or as intravenous infusions, which is clearly less desirable for many patients, especially for chronic indications. Larger stable peptides, such as linaclotide (Linzess®) for irritable bowel syndrome or vancomycin (Vancocin® and fidaxomycin (Dificid®) for Clostridium difficile-associated diarrhoea, are marketed as oral drugs but they are for local gastro-intestinal (GI) targets and are not absorbed systemically.

There are numerous biological barriers that affect the stability, bioavailability and variability of oral peptide delivery. The physicochemical characteristics of the biomolecule may determine whether oral delivery or other non-invasive routes of administration, such as nasal, pulmonary, transdermal, rectal or vaginal delivery, may be more practical, however oral delivery offers the greatest patient acceptance and compliance, hence there is greater emphasis on this route of delivery. The ideal peptide candidate for systemic oral delivery is highly potent, stable, resistant to proteases, does not aggregate and has a wide therapeutic window.

TRANSCELLULAR DELIVERY

Unlike conventional drugs, which are generally lipophilic and are absorbed through enterocytes by partitioning between membrane lipid and an aqueous environment via the transcellular pathway, most naturally occurring peptides have a low log P, a molecular weight greater than 500 and other properties that make them poor candidates for oral delivery via this pathway. In order to utilise the transcellular pathway peptides either need to be lipophilic for passive diffusion, have a receptor on the cell surface for active transport or the presence of a surfactant(s) in close proximity to cells to destabilise their membranes reversibly and allow for peptide diffusion through the cells. Peptide lipophilicity can be increased by reversibly binding them to more hydrophobic molecules, like sodium olate, or molecules, such as derivatives of caprylic acid, that alter their conformation in such a way as to render them more hydrophobic.

“An earlier Phase IIa study carried out with the formulation that contained both citric acid and LLC in healthy postmenopausal women showed that LLC increased bioavailability by approximately three-fold.”

TECHNOLOGIES & CLINICAL STUDIES FOR THE ORAL DELIVERY OF CALCITONIN

In this piece, Nozer Mehta, PhD, Principal, Peptide Technologies, James P Gilligan, PhD, MSIB, Chief Scientific Officer, Tarsa Therapeutics, and William Stern, PhD, Consultant, Peptide Drug Development, summarise the different technologies that have been in development for oral delivery of peptides through the gastro-intestinal mucosal surfaces via the transcellular or the paracellular pathways and describe the results of several long-term clinical studies on the oral delivery of salmon calcitonin.
Receptor-mediated transport can be achieved by attaching a ligand like vitamin B12 or biotin to the peptide allowing receptors on the cell surface to transport them through enterocytes. Ideally these ligands are attached via a cleavable linker or the ligand has little effect on the bioactivity of the peptide. For peptides that require a surfactant to enhance transcellular absorption Whitehead and Mitragotri have screened a number of surfactants on Caco-2 cells for their effect on cell viability and transport properties.

One of the most advanced technologies using the transcellular pathway is the Emissphere Eligen® technology (Table 1) that uses “peptide carriers” such as caprylic acid derivatives. One such carrier, 5-CNAC (8-(N-2-hydroxy-5-chlorobenzoyl)-amino-caprylic acid), has been used to deliver salmon calcitonin (sCT) orally in a Phase III trial for the treatment of osteoporosis (OP) and in two separate Phase III trials for the treatment of osteoarthritis (OA). 5-CNAC binds non-covalently to sCT. In the acidic pH of the stomach the carrier/peptide complex is insoluble rendering the peptide resistant to degradation. Upon transit to the duodenum, where the pH rises to 5.5 or greater, the complex is soluble and the peptide is absorbed through the epithelial membrane into systemic circulation.

**PARACELLULAR DELIVERY**

Peptides that cannot be transported by the transcellular pathway are absorbed via the paracellular route, which involves peptide transport through tight junctions also known as zona occludens between epithelial cells in the GI tract. Tight junctions are maintained by a group of proteins that include cadherins, claudins, occludin and junctional adhesion molecules, which seal together adjacent cells and provide cytoskeletal anchorage.

Several technologies have been developed to open tight junctions transiently and allow passage of peptides into the systemic circulation, all in various stages of preclinical or clinical development. The leading technologies for paracellular transport are:

- **POD™** technology (Oramed Pharmaceuticals, Inc, Jerusalem, Israel)
- **TPE®** technology (Chiasma, Inc, Waltham, MA, US)
- **GIPET®** technology, which may also work partly by a transcellular mechanism (Merrion Pharmaceuticals, Dublin, Ireland (in administration))
- **Axcess™** delivery system (Proxima Concept, Ltd, St Helier, Jersey, UK)
- **Peptelligence™** technology (Enteris BioPharma, Inc, Boonton, NJ, US)

In order to enhance paracellular transport these technologies utilise a variety of permeation enhancers that are generally non-ionic surfactants, acyl carnitines, fatty acids, fatty acid esters, bile salts and alkyl glucosides. Other chemicals that have been found to enhance paracellular transport include calcium chelating agents, sodium salicylate, aspirin, non-steroidal anti-inflammatory drugs (NSAIDS), phenothiazines and chitosan.

The Peptelligence™ technology has been used successfully to deliver sCT orally in Phase II and Phase III trials. The technology employs an enteric-coated tablet that contains citric acid and in certain embodiments lauroyl-L-carnitine (LLC), an acylcarnitine (Table 1). The enteric coating protects the peptide from degradation in the stomach and allows the tablet to release its contents in the intestine. Citric acid enhances peptide absorption by lowering intestinal pH to inhibit proteolytic activity and also chelates intracellular calcium, while the acylcarnitine enlarges the pore size of tight junctions thus increasing their hydrodynamic radius.

**LATE-STAGE ORAL DELIVERY STUDIES WITH sCT**

Salmon calcitonin (sCT) is a 32 amino acid peptide hormone that inhibits osteoclasts and induces the suppression of degradation of collagen type II, the primary protein in cartilage.

Here follows a summary of clinical trials of various oral formulations of sCT, including Phase II studies in patients with osteopenia, Phase III studies in postmenopausal OP and Phase III studies in men and women with osteoarthritis (OA) of the knee. These studies were performed using two of the leading oral delivery technologies, namely the Eligen® technology for transcellular transport and the Peptelligence™ technology for paracellular transport (see Table 1).

**Studies with the Transcellular Eligen® Technology**

Several companies and research groups have attempted to develop oral delivery technologies for sCT. The Eligen® technology utilises carriers that bind covalently to a peptide and increase

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Table 1: Transcellular and paracellular delivery technologies employed in late-stage studies for oral delivery of salmon calcitonin.

“Different meta-analyses, including one conducted by the FDA, have indicated that there is little evidence of a causative relationship between calcitonin and cancer. ... Following a full review by the FDA no black box or bolded warning was issued for sCT products nor was a limitation on the duration of use imposed, as is seen with other drugs used in the treatment of OP.”

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its lipophilicity. This technology has been used for the oral delivery of several peptides, and a currently approved product for the oral delivery of vitamin B12.12 5-CNAC has been extensively studied in combination with sCT in clinical studies to determine bioavailability, efficacy, food effects, and interaction with water intake.18,39 A Phase II study in postmenopausal women demonstrated significant reductions in bone resorption as assessed by serum CTX-1, a marker for bone resorption, over three months.40

These early clinical studies were followed by a large randomised, double-blind, multicentre, placebo-controlled Phase III study to evaluate the efficacy and safety of this formulation in the treatment of OP in postmenopausal women taking calcium and vitamin D.14 In this three-year study a total of 4665 subjects were randomised into either the treatment or placebo group. Subjects were instructed to take a single oral tablet containing 0.8 mg sCT once daily in the evening 30-60 min before dinner, together with a maximum of 50 mL of water.

There was a significant decrease in CTX-1 in the treatment group compared with placebo, similar to what was seen in the earlier Phase II study. The primary endpoint required a reduction in new vertebral fractures however, and there was no significant difference in the incidence of new vertebral fractures between the treatment and placebo groups. The mean increase in bone mineral density (BMD) at the lumbar spine (LS) in the treatment group was 1.02%, which was significantly higher, by 0.83%, than the placebo group.

The authors of the study believe that the primary reason for lack of significant anti-fracture efficacy was the lower than expected blood exposure $C_{\text{max}}$ of sCT of 28 pg/mL and 22 pg/mL at the beginning and end of the study respectively, which was at least four times lower than seen in the earlier Phase I and Phase II studies. The authors suggested that there was a technical failure of the formulation that led to the lower than expected exposure to the drug.

With regard to safety, the study medication was generally well tolerated, though there were higher incidences of GI disorders and vascular disorders in the treatment group compared with placebo. Importantly, in light of the potential safety issue of sCT discussed later, no differences in cancer events were observed between the two groups.

Two Phase III studies were also carried out with oral sCT for the treatment of knee OA using the 5-CNAC enhancer.41 In these two double-blind, randomised, placebo-controlled, multicentre studies, 0.8 mg sCT or matching placebo was given twice daily for 24 months. Approximately 1200 patients were randomised in each of the two studies and divided equally between the treatment and placebo arms. The primary endpoints were the change in joint space width (JSW) over 24 months in the signal knee measured by X-ray, compared with placebo, and also change in pain and function using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) questionnaire.

Neither of the studies demonstrated a significant treatment effect of change in JSW at intervals during the study or at the 24-month study endpoint. The WOMAC questionnaire scores at the 24-month endpoint demonstrated a treatment effect in one of the two studies but the effect was considered non-significant due to the hierarchical testing procedure. In this study as well, there was a four-fold decrease in sCT exposure compared with the earlier phase studies at comparable doses, and the authors suggest that the Phase III failure is the result of a flawed hypothesis and a technical failure of the oral formulation that might have occurred as a scale-up issue in the manufacture of the tablets.

Studies with the Paracellular Peptelligence™ Technology
Tarsa Therapeutics has carried out a 48-week Phase III study for the use of oral sCT in the treatment of OP, and a Phase II study for the treatment of postmenopausal women with osteopenia using its Peptelligence™ technology. It should be noted that, although these studies utilised the components of the technology previously described, they did not include LLC as one of the active excipients. The Phase III OP study (ORACAL) was a randomised, double-blind, double-dummy, active- and placebo-controlled, multiple-dose study, enrolling 565 postmenopausal osteoporotic women to assess the efficacy and safety of oral recombinant calcitonin.42

The primary endpoint of the study was to determine the increase in LS BMD following treatments compared with baseline and Miacalcin (sCT) nasal spray. Oral treatments were with identical appearing tablets containing either 200 µg (1200 IU) of sCT or placebo, and nasal spray treatments contained 33 µg (200 IU) sCT.

The study met its primary endpoint and it was concluded that orally administered sCT resulted in improvement in LS BMD that was superior to that obtained with commercial nasal sCT spray or placebo after 48 weeks of treatment, with significant improvement in LS BMD observed after six months of treatment (Figure 1). Few women in any group reported any serious adverse events (AEs), and overall the safety findings were not dissimilar in the different treatment groups, although the women in the oral group did report greater incidences of nausea and dyspepsia, a side effect that has also been reported for women receiving injectable sCT. Interestingly there was a significantly

Figure 1: Increase in lumbar spine BMD in osteoporotic patients* at one year following treatment with oral sCT, compared with nasal sCT or placebo, utilising the Peptelligence™ technology. Oral sCT was superior to nasal CT and placebo at primary endpoint.

* Compared with baseline. Modified intention to treat population, last observation carried forward.
reduced (approximately five-fold lower) immune response in subjects receiving oral sCT compared with nasal sCT. Based on the data from this study a NDA has been filed and accepted by the US FDA.

The Phase II study was conducted to investigate the effect of oral sCT on BMD of the spine in postmenopausal women with low bone mass and at increased risk of fracture, but who did not meet the BMD criteria for OP.

A total of 129 women were randomised between oral sCT and placebo and treated with a daily tablet for 54 weeks.

The study results demonstrated an increase in LS BMD, a reduced bone resorption marker CTX-I and a reduced total proximal femur BMD loss in women taking oral sCT (Figure 2). Few women in either group experienced serious AEs, although mild GI AEs were common in both groups and resolved upon discontinuation. This study also demonstrated a lack of a food effect for this formulation.

**DISCUSSION**

It appears that the Eligen® formulations based on 5-CNAC may have encountered a problem when scaling up the tablet manufacturing for the large Phase III studies, since the C_{max} values were 4-5 times lower than expected from the early phase studies. With the lower exposure there was no-reduction in vertebral fractures. However, there was some evidence of efficacy with regards to the secondary measures that may respond to lower exposure to sCT. In the OP study there was a small but significant increase in LS BMD and significant reductions in the markers for bone resorption urinary CTX-I and CTX-II. Similarly in the two OA studies there was some effect on pain, stiffness, function and a small decrease in the marker for cartilage degradation.

The studies carried out with the Peptelligence™ technology for OP and osteopenia both demonstrated a highly significant increase in LS BMD and a reduction in the primary marker for bone resorption, serum CTX-1, and this should translate into preservation of bone density in osteoporotic and osteopenic women.

A direct correlation with reduction in vertebral fractures cannot be made since these studies were not designed or powered to measure fracture prevention efficacy. However, the data suggest that 200 µg tablets of oral calcitonin may provide more consistent and greater exposure to calcitonin than the currently marketed nasal calcitonin formulations, which could translate to reduced fracture risk.

As previously mentioned, the Peptelligence™ formulation used in these studies did not include the active excipient LLC and no PK measurements were performed. However, an earlier Phase IIa study carried out with the formulation that contained both citric acid and LLC in healthy postmenopausal women showed that LLC increased bioavailability by approximately three-fold.

Salmon calcitonin has been marketed for over 30 years as injectable and nasal formulations. In 2012, following a meta-analysis of a variety of clinical studies and marketing data, the EMA suspended calcitonin nasal spray from the market and limited the duration of use of other calcitonin products due to a putative association with cancer. However, different meta-analyses, including one conducted by the FDA, have indicated that there is little evidence of a causative relationship between calcitonin and cancer. The combined safety data from the two one-year clinical trials with the Peptelligence™ oral sCT formulation demonstrated no signal of carcinogenicity.

“... The data from the studies described here hold out the promise that an oral formulation of sCT will eventually be approved for bone disorders such as OP or as a potential disease modifying drug for the treatment of OA, which is a large unmet medical need.”
here hold out the promise that an oral formulation of sCT will eventually be approved for bone disorders such as OP or as a potential disease modifying drug (DMOAD) for the treatment of OA,14 which is a large unmet medical need. Also based on the evidence from the OA studies that there was efficacy in the pain scores and a decrease in cartilage markers, an appropriate oral sCT formulation could also be developed for pain and mobility in patients with knee OA.

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Nozer Mehta, PhD, Principal, Peptide Technologies, received Bachelors and Masters degrees from the University of Bombay, India, and a Doctorat d’Université with Honours from the Université Louis Pasteur in France. His early work experience was at the CNRS laboratories in Strasbourg, at the Cancer Research Institute in Mumbai and at the University of Nebraska in Lincoln. He joined Unigene Laboratories in 1982 and advanced to Chief Scientific Officer and served next, also as CSO, at Enteris BioPharma. At both companies he and his team developed programs and technologies for oral delivery and recombinant expression of peptides. He then worked at MonoSol Rx as the Vice-President for Biologics, where he led research efforts on the buccal delivery of peptides. Dr Mehta currently consults for pharmaceutical companies and venture capital groups.

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