Expert Opinion





# Delivery of parathyroid hormone for the treatment of osteoporosis

Paul Morley
*Zelos Therapeutics, Inc., 1730 St. Laurent Blvd, Suite 400, Ottawa, Ontario K1G 5L1, Canada*

Parathyroid hormone (PTH), along with its fragments and analogues, potently restores bone mass and biomechanical strength in animal models of osteoporosis, and reduces fractures by up to 65% in clinical trials in osteoporotic patients. Despite this demonstrated efficacy, patient acceptance and compliance with PTH is limited by the need for daily subcutaneous injections. The development of an equally efficacious, noninjectable form of PTH would significantly expand the present market. A challenge to the development of an alternative delivery system is the requirement for low-dose, daily, intermittent pulses of PTH to induce the anabolic actions on bone. In this review, recent basic and clinical efforts to deliver PTH by oral, buccal, sublingual, transdermal, nasal and pulmonary approaches will be addressed.

Keywords: buccal, inhalation, nasal, oral, osteoporosis, PTH, pulmonary

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## 1. Introduction

Human parathyroid hormone (hPTH) is a linear 84-amino acid peptide hormone that regulates calcium homeostasis. PTH is secreted from the parathyroid glands when the blood calcium concentration falls below the normal range. PTH acts on PTHR1 receptors in bone, kidney and intestine to promote calcium reabsorption in the kidney, renal production of 1,25-$(OH)_2$-vitamin D3 and on bone to stimulate bone resorbing osteoclasts to release calcium and restore the blood calcium concentration to normal levels. Although high sustained levels of PTH are catabolic to bone, pulsatile low-dose administration is potently anabolic [1,2]. The anabolic actions of PTH, and of its fragments and analogues, have been extensively reported in both animal models and in human clinical studies [3-5]. Most of the activity of PTH is found in the 34 N-terminal amino acids [6], so the majority of preclinical and clinical studies have used the hPTH(1-34) fragment.

At the present time, the only marketed hPTH product is Eli Lilly's recombinant hPTH(1-34), which was approved for marketing in the US in 2002 under the name Forteo®, and later in Europe under the name Forsteo®, for the treatment of postmenopausal osteoporosis [7]. The goal of osteoporosis therapy is to increase the mass of mechanically normal bone, restore bone strength and to ultimately prevent fractures. Before 2002, all drugs in use to treat osteoporosis were antiresorptive therapies, such as oestrogen, bisphosphonates, selective oestrogen receptor modulators and calcitonin, which all reduce bone loss and prevent fractures by suppressing the activity of osteoclasts. These drugs work best as preventative therapies and they moderately increase bone mineral density (BMD) by up to 5% over 3 years and lower the risk of fracture by 30 – 50% [8-10]. However, patients with established osteoporosis may lose > 20% of their trabecular bone mass [11] and can still be at risk of fractures even with antiresorptive therapy, so there is a strong clinical need for anabolic therapies to rapidly restore bone mass and strength.

The Phase III clinical trial for Forteo was conducted in 1637 women, of 30 – 85 years of age and at least 5 years postmenopausal, exhibiting at least one vertebral fracture [12]. Patients received placebo or Forteo 20 or 40 µg/day. After 19 months mean exposure, there was a reduction in the relative risk of vertebral fracture by 65% and a 54%

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reduction in nontraumatic, nonspine fractures relative to the placebo group. Forteo was well tolerated, and at the end of the treatment patients showed increases in vertebral, femoral and total-body BMD. Although the increases in BMD were greater in the 40-µg dose group, the 20- and 40-µg groups had similar effects on the risk of fracture. Because there were more side effects in the 40-µg group, the marketed dose is 20 µg/day. Forteo is delivered as a daily subcutaneous injection for a maximum of 2 years using a multiple-use injection pen/cartridge system similar to an insulin pen. Injection pens are relatively small and convenient for patients to use and have finer needles that are less painful than standard syringes.

At least two other PTH molecules are in development. NPS Pharmaceuticals has completed the Phase III clinical trials of recombinant hPTH(1-84) (PREOS®). The Phase I [13] and Phase II [14] clinical trial results for PREOS have been reported. Treatment of osteoporotic women with PREOS 50, 75 or 100 µg/day s.c. by injection for 12 months induced significant time- and dose-dependent increases in lumbar spine BMD and nonsignificant changes at the hip and total body. NPS filed a new drug application for PREOS in the US in May 2005. Zelos Therapeutics is in a Phase II clinical trial with the hPTH analogue (Leu27)-cyclo(Glu22-Lys26)-hPTH(1-31)$NH_2$, called Ostabolin-C™ [15]. This analogue has generated considerable interest as the 31-amino acid PTH analogues have been shown in animal [16] and human [17] studies to retain PTH's bone formation properties but not to induce hypercalcaemia, a side effect reported for both Forteo and PREOS. Unlike hPTH(1-34), the 31-amino acid analogues do not stimulate bone resorption and thus do not lead to elevated levels of serum calcium [16,17].

Although Forteo potently rebuilds bone lost due to osteoporosis, its use is limited by both its high cost and the need for daily subcutaneous injections. Consequently, many patients who could benefit from PTH therapy receive antiresorptive therapy with agents such as the orally available bisphosphonates. Patient compliance for osteoporosis medications in general is not very good [18], and it would be expected that compliance for injectable PTHs would be even worse. However, the clinical experience so far indicates that the aversion to self-injection may not be as large as some would predict. In the NPS PREOS 12-month Phase II clinical trial, which also used an injection pen system, patients administered, on average, 95% of their daily doses [14]. Patients with established osteoporosis with previous fractures worry about future fractures and are very aware of their need for an anabolic therapy such as PTH, regardless of how it is administered. PTH injection regimens may not require daily injections over a 2-year period as effective bone building is obtained when PTH is injected in 28-day cycles every 3 months [19] or following intermittent weekly administration [20].

The peak annual sales projections for injectable PTHs vary widely from US$500 million to US$2 billion annually, yet all agree that the availability of an equally efficacious, noninjectable version of the drug has the potential to significantly expand this market through greater patient acceptance and compliance. At this same time, there is no data to suggest that a noninjectable PTH product would make a better medicine, rather than just a more convenient one. One challenge for the development of an alternatively dosed PTH therapy is the essential requirement for low-dose, daily, intermittent pulses of PTH to induce a bone anabolic effect [1,2]. In modifying the PTH molecule for alternate delivery, one must be cautious. Long-acting, protease-resistant PTH analogues will not have the short plasma pulses needed for bone-building activity, but rather produce the prolonged pulses that stimulate osteoclastic bone resorption. In addition, changing the amino acid sequence of PTH could result in analogues with altered receptor binding specificity, giving rise to negative side effects through binding to other closely related G-protein-coupled receptors in the PTH receptor family.

Efforts to find noninjectable delivery systems for peptides began soon after the introduction of insulin in the 1920s, but there has been no success so far. This review will address the present landscape of alternate delivery options for PTHs, including oral (buccal, sublingual, enteric–gastrointestinal), transdermal, nasal and pulmonary approaches. This review does not address needle-free injectors, which are similar to injections except that they deliver drugs subcutaneously without needles. It is unclear if the discomfort experienced using these devices is any less than that for conventional injections, and the pharmacokinetic profiles of drugs delivered using these systems need to be carefully investigated to ensure equivalence with standard injections. The parathyroid hormone related peptides (PTHrP) act on the same PTHR1 receptor as PTH and various fragments have been shown to have anabolic effects on bone in animal and human studies [21]. This review does not specifically address PTHrP delivery; however, it would be expected that the issues would not be different than for PTH delivery.

## 2. Oral delivery of parathyroid hormone

The oral administration of a pill is unquestionably the preferred route of administration of any pharmaceutical agent. For peptides and proteins, oral delivery is not an option at this time, thus necessitating that these agents be delivered by injection. However, with the development of a plethora of new peptide therapeutics, the search for alternative delivery approaches has never been stronger. Insulin was the first widely used peptide therapeutic, being discovered in 1921. Due to the large worldwide market, most alternative delivery programmes for peptides have begun using insulin. Because insulin is essential to sustain life in diabetics, and failure to inject leads to rapid complications, insulin injections have been reasonably well tolerated by patients. Newer peptides, such as the PTHs, that treat non-life-threatening and sometimes asymptomatic diseases will require noninjection delivery systems to maximise patient acceptance.

The gastrointestinal (GI) tract is designed to digest peptides and proteins ingested in our foodstuffs, therefore, the bioavailability of oral peptide drug delivery is very low. The stomach and small intestine provide an acidic environment full of digestive enzymes to breakdown proteins into their amino-acid building blocks. In the digestion process, pepsins in the stomach hydrolyse the bonds between aromatic amino acids to produce polypeptides of various sizes. In the small intestine, these polypeptides are further digested by the endopeptidases (trypsin, chymotrypsin, elastase) and exopeptidases (amino and carboxypeptidases) from the pancreas and intestinal mucosa. The result of normal digestion is di- and tripeptides that are transported into intestinal cells by a combination of passive diffusion, facilitated diffusion and active carrier-mediated transport [22].

The oral delivery of peptides and proteins is made difficult by a variety of obstacles. First, peptide and protein drugs are large and susceptible to the acidic environment of the GI tract; this can be overcome by the use of enteric-coated capsules to prevent the peptide being degraded before it is absorbed. Second, peptides are susceptible to proteolytic enzymatic degradation in the small intestine [23,24]; this problem can potentially be solved by transiently limiting enzyme activity with protease inhibitors, transiently adjusting the local pH in the intestine so these enzymes are not active or by maintaining a concentration of drug so high that the enzymes are saturated [25]. Third, the transport of large charged molecules such as peptides across the intestinal epithelium is very difficult.

Taking all of these factors into account, the relative bioavailability of oral peptides can range from < 1% to a maximum of ~ 10% [26], depending on the molecular weight of the peptide, its charge and its proteolytic susceptibility. Major technical advances in the cost-effective manufacture of large quantities of pure peptides and proteins have been a critical factor in the advancement of alternate delivery development programmes for peptides. Most alternative delivery options have bioavailabilities that are only a fraction of that obtained following subcutaneous dosing, thus necessitating less expensive manufacturing to make the economics of the drug product viable.

Most oral peptide drug delivery development programmes have been directed towards insulin and calcitonin [27]. There are two main approaches to oral peptide delivery: those in which the protein drug remains unaltered but is encapsulated in enteric-coated capsules and those in which the drug is modified, for example, by attaching low molecular weight polymers, in order to protect it from the enzymes and the acidic environment of the GI tract until it gets to the area where it can be absorbed. The conjugations can theoretically enhance absorption across the GI wall and be used to alter the pharmacokinetic profile of the drug through modulating the pattern in which the drug appears in the blood, or its metabolism in the body. However, conjugated peptides are considered as new chemical entities and thus require safety toxicology studies in addition to those required for the peptide itself. At least four oral PTH delivery programmes have been reported and at least three of these are still active.

One approach uses lipid micelles as carriers in which lipids protect the peptide from peptidases and enable transport across biological membranes such as those in the intestinal epithelium. Emisphere Technologies and Eli Lilly have been developing an oral formulation of rhPTH(1-34) using Emisphere's proprietary Eligen® technology since 1997. At the time of writing this article, Emisphere and Eli Lilly were in litigation over the termination of the oral PTH project and, in December 2004, Emisphere also entered into an option and licence agreement with Novartis for the development and commercialisation of an oral form of PTH [28]. The Eligen technology, which comprises a large family of carrier agents, uses the body's passive transcellular processes to promote large molecules being transported across the GI tract where the drug and carrier dissociate. The Eligen carriers do not damage cell membranes or chemically modify the drug in this process [29,101].

Gastric gavage of rhPTH(1-34) 1 mg and 8-(*N*-2-hydroxy-4-methoxybenzoyl)amino caprylic acid (4-MOAC) 300 mg restores bone mass in ovariectomised (OVX) rats as effectively as of rhPTH(1-34) 10 mg/kg/day s.c. [29,30]. The study was carried out in 7-month-old OVX rats that were allowed to lose bone for 1 month before an 8-week treatment with PTH (10 mg/kg/day). Proximal tibia quantitative computerised tomography showed that the rhPTH/4–MOAC combination restored BMD and bone mineral content to baseline levels, with an efficacy slightly better than the subcutaneous hPTH(1-34) administration [29,30]. Histomorphometric analysis showed that the oral rhPTH(1-34) restored trabecular area and preserved trabecular number, whereas bone formation rate, mineral apposition rate and eroded perimeter were not different from OVX controls or the subcutaneous administration of hPTH(1-34). Biomechanical compression testing of vertebra and three-point bending of the femoral diaphysis showed that the oral PTH restored bone strength to at least baseline levels. Leone-Bay *et al.* [29] have reported additional data on the use of 4-MOAC for rhPTH(1-34) delivery in rhesus monkeys [29]. In monkeys, aqueous solutions of PTH/4-MOAC gave mean peak serum PTH concentrations of 3000 pg/ml with a bioavailability of 2.1% relative to subcutaneous dosing. Importantly, the pharmacokinetic profile was pulsatile, as required for anabolic actions. Unfortunately, no bone formation parameters were reported in this study. The low bioavailability suggests that an oral product would require the delivery of up to 50-fold more drug for each administration than would be needed for a subcutaneous injection. This could have a major impact on the affordability and ultimate commercial success of oral peptides.

The Complexing Agent Drug Delivery System has been reported to facilitate the oral delivery of PTH(1-38) in rats and monkeys, with ~ 5% bioactivity relative to subcutaneous injection [31]. No information on bone-building

efficacy was reported but the orally delivered PTH was biologically active as it caused a drop in serum phosphate concentration [31].

Unigene Laboratories is also developing an oral PTH in collaboration with GlaxoSmithKline that began in 2002. They have not disclosed the exact PTH fragment or analogue in development. The Unigene approach involves enteric-coated solid dosage forms and excipients that modulate intestinal proteolytic activity by decreasing local pH below that required for protease function or by specifically inhibiting the primary enzyme that degrades the peptide. Their formulations also contain detergents to increase peptide solubility and enhance peptide paracellular transport. Unigene uses reagents that are generally regarded as safe, such as citric acid to lower intestinal pH, and taurodeoxycholic acid and lauroylcarnitine as absorption enhancers that can significantly enhance peptide absorption in the gut. Unigene has successfully delivered calcitonin orally [32] and has applied the same technology to PTH delivery. In 2001, Unigene announced the successful oral delivery of PTH to dogs as measured by blood levels of PTH. The Unigene PTH131A was 10-fold more able than hPTH(1-34)OH to enter the bloodstream from the duodenum [33]. Oral administration in an enteric-coated capsule to eight dogs gave a mean $C_{max}$ of 379 ± 152 pg/ml standard error of the mean (SEM)/mg of hPTH(1-34) peptide, whereas the PTH131A gave a mean $C_{max}$ of 2155 ± 456 SEM/mg. Importantly, the serum pharmacokinetic profile showed the needed pulsatile profile required for PTHs in order to mediate anabolic effects on bone. No bone formation parameters were reported, therefore, it is still unknown whether daily oral delivery can match the efficacy seen with daily subcutaneous dosing. In 2004, Unigene reported the completion of a Phase I human trial of an oral PTH formulation. The preliminary results showed that PTH can be delivered from an oral formulation to the blood and that the molecule was intact and biologically active [34], although the full data set has not been released.

The Australian company Bone Medical has also announced an oral PTH delivery programme [102]. In January 2005, Bone Medical reported the start of a human Phase I safety and tolerability trial in 18 healthy postmenopausal female subjects with its oral PTH product BN003. The data are expected in the second quarter of 2005. Bone Medical had previously conducted preclinical studies with BN003 that showed PTH levels in the blood and elevated blood calcium levels [102].

Because many biotechnology companies in collaborations with large pharmaceutical companies are currently developing oral formulations of insulin and calcitonin, it is likely that similar formulations of PTH will be developed if the significant hurdles of oral peptide delivery are overcome. At the moment, the significant intersubject variability in bioavailability and the safety of the excipients used in oral formulations remain to be resolved. As of now, despite the huge investment, the time frame for the commercialisation of oral peptides seems, at best, to be a decade away.

## 3. Buccal and sublingual parathyroid hormone delivery

There are several benefits of the oral mucosa for the delivery of peptide therapeutics such as PTH [35,36]. In addition to its accessibility and robustness to injury, the oral cavity has a large surface area, a very rich blood supply and there is little proteolytic activity. On the other hand, the multilayered squamous epithelium and the continuous flow of saliva present barriers to buccal drug delivery. Peptides are absorbed across the oral mucosa by passive diffusion and there is little evidence for carrier-mediated transport processes [36]. At the time of the preparation of this review, the author is not aware of any buccal or sublingual PTH delivery development programmes; however, work has been reported for insulin [37,38]. In these studies, absorption enhancers such as surfactants, bile salts, chelators, alcohol or fatty acids have been used to improve bioavailability. These are sometimes combined with adhesive materials such as patches or gels [39]. Most buccal delivery studies have been preclinical, although there is some human data for insulin. Insulin has been administered by the buccal route to Type 1 diabetics and reductions in blood glucose were observed; however, the responses were quite variable [37-39]. The low bioavailabilities also make multiple administrations necessary. Despite these efforts, the challenges of buccal administration have so far not been fully addressed. If these challenges can be addressed for insulin there is no reason that similar delivery approaches would not be effective for PTH.

## 4. Transdermal parathyroid hormone delivery

The transdermal delivery of peptide and protein drugs could potentially provide a noninvasive alternative to injections. Many companies are currently working on means to painlessly disrupt the skin to deliver large water-soluble charged peptides across the skin. The major barrier to transdermal delivery is the stratum corneum, and many different chemical and physical methods have been employed to overcome it, including iontophoresis and electroporation. Iontophoresis uses low-level electrical current to promote the movement of charged molecules through the skin. Electroporation uses high-voltage electrical pulses of very short duration to reversibly enhance skin permeability to macromolecules. Both of these approaches have been tried in animal studies for the delivery of hPTH(1-34).

Suzuki *et al.* [40] demonstrated that pulsatile patterns of serum hPTH(1-34) could be achieved with repeated on and off iontophoretic current in rats and dogs [40]. The same group then compared the iontophoretic and subcutaneous delivery of hPTH(1-34) in OVX rats [41]. Iontophoretic administration of hPTH(1-34) was achieved using three 30-min pulses of 0.1 mA/cm$^2$ separated by 45-min rest intervals. Each pulse produced a rapid peak in serum hPTH(1-34) levels and the concentration was proportional

to the dose from 40 to 400 µg/patch. Triple-pulse iontophoretic application three-times/week for 4 weeks produced dose-related increases in BMD in the distal femurs of rats. The iontophoretic dose of 120 µg/patch gave equivalent BMD changes to 5 µg/kg/day of subcutaneous dosing [41]. The average bioavailability using this iontophoretic delivery approach was only ~ 2%, again raising the issue of cost of goods of the active pharmaceutical ingredient and affordability of a commercial product.

Medi and Singh [42] studied electroporation, iontophoresis and electroporation followed by iontophoresis for the transdermal delivery of hPTH(1-34) *in vitro* using dermatomed porcine skin [42]. Electroporation and iontophoresis both significantly increased the percutaneous absorption of hPTH(1-34). The electroporative flux of hPTH(1-34) varied with the pulse amplitude and it was demonstrated using light microscopy that the stratum corneum was perturbed by the pulses [42]. When iontophoresis was used following electroporation, the flux was increased by up to 10-fold relative to the use of electroporation alone [42]. In another report, it seems that a 24-h delivery would be required using a 5 $cm^2$ patch to achieve a therapeutic dose of hPTH(1-34) using electroporation and iontophoresis; however, the pharmacokinetic profile would be too prolonged for the PTH to elicit an anabolic response [43]. There are a number of challenges facing the electroporation and iontophoresis approaches, including the regulatory approval of both the PTH peptide and the delivery device, determining if the approach has any better patient acceptance than daily subcutaneous injection and a better understanding of the recovery of the skin following the perturbation.

At least two delivery companies have reported transdermal PTH delivery programmes. Altea Therapeutics has developed the PassPort™ Patch that uses thermal energy to create hundreds of tiny channels that allow the movement of peptides through the skin. Although they have not reported data on the delivery of PTH, they have reported data on insulin delivery with this system [103]. The profile for insulin shows that serum insulin concentrations peak 4 – 6 h after the insulin patch is turned on. This slow pharmacokinetic profile would induce a catabolic, rather than an anabolic, response for PTH delivery unless the speed of delivery was increased.

Theratechnologies has reported a programme to deliver ThPTH, a transdermal formulation of synthetic hPTH(1-34), in collaboration with Alza Corp. using the Alza Macroflux® transdermal patch technology. The Macroflux technology uses a thin titanium screen with precision microprojections that create superficial pathways to overcome the skin barrier. In May 2004, Theratechnologies released data from a Phase I proof-of-concept clinical study designed to show safety and to determine the pharmacokinetic and pharmacodynamic profile of patch delivery [44]. In the crossover design, 31 female subjects received a single 1-h application of a 2 $cm^2$ Macroflux-PTH patch loaded with ThPTH 30 µg and subcutaneous injections of Forteo 20 or 40 µg. The 30 µg Macroflux-PTH patch delivered ThPTH at blood levels similar to those obtained with Forteo 40 µg but showed higher maximum concentrations and more rapid delivery times. Urinary cAMP, a biomarker of PTH activity, increased within 2 h for both routes of administration. Serum ionised calcium levels increased at 4 h postdose and serum phosphate levels decreased over the first hour for both routes of administration. Headache, nausea and dizziness were reported as adverse events in 50% of the subjects taking Macroflux-ThPTH 30 µg. Even if a suitable pharmacokinetic profile could be obtained with a regulatory approved delivery device, it is still unclear whether any of these patch approaches will be any more convenient than the use of a 30-day injection pen.

## 5. Nasal parathyroid hormone delivery

Companies are also exploring PTH delivery using nasal sprays. There is particular interest in nasal PTH delivery as the well-established antiresorptive drug salmon calcitonin is delivered nasally. Nasal delivery is noninvasive relative to injections and avoids first-pass hepatic metabolism of drugs. The nasal mucosa is readily accessible, highly vascularised and has a high epithelial permeability [45]. There are also factors that limit intranasal drug absorption, including the mucus and epithelial barriers, mucociliary clearance, and protease and peptidase activity in the mucus. Depending on the physicochemical properties of the particular drug, intranasal absorption can occur by transcellular or paracellular routes [45]. Many strategies use absorption enhancers such as cyclodextrins, phospholipids and chitosan to improve nasal delivery [46]. Enhancers can be irritating and can also damage the nasal membranes with long-term use [46]. Some nasal delivery technologies also make use of bioadhesive or mucoadhesive agents to increase residence time of the drug in the nasal cavity to overcome mucociliary clearance and to improve absorption [45,46].

Nasal PTH delivery has been reported in rats and humans. Anesthetised rats dosed nasally with aqueous solutions of hPTH(1-34) showed rapid intranasal absorption with maximum plasma concentrations obtained within 15 min [47]. The intranasal bioavailability was as high as 17.6% in the presence of 1% bovine serum albumin without any apparent adverse effects on the airway epithelium [47].

Ohnuma and colleagues have delivered a nasal formulation of hPTH(1-34) into healthy male volunteers at doses of 50, 125, 250 and 500 mg/spray [48]. The pharmacokinetics from inhalation were similar to those from 5, 10 or 20 µg s.c. injection. The delivered PTH was biologically active as indicated by increased urinary cAMP and blood calcium concentrations [48]. Increases were seen in BMD and bone biomechanical strength with no effect on bone resorption. There was no irritation of the nasal mucosa and bioavailability was ~ 17% that of subcutaneous administration.

Daiichi Suntory Pharma developed a low-cost method for making high-purity recombinant hPTH(1-34) and in 1999, a

lyophilised formulation of the peptide for nasal administration entered Phase I clinical trials in healthy male volunteers in Japan. In October 2004, Daiichi, in collaboration with Chugai Pharmaceutical, presented the results of a randomised, open-label Phase II clinical trial, carried out in Japan, of intranasal PTH in 92 patients [49]. The subjects, aged 52 – 84 years received hPTH(1-34) 250, 500 or 1000 µg/day for 3 months, in addition to with calcium 300 mg and vitamin D 200 IU. Similar peak serum concentrations of hPTH(1-34) to those obtained with hPTH(1-34) 20 µg s.c. injection were achieved with nasal spray of hPTH(1-34) 1000 µg. Lumbar spine BMD increased in a dose-dependent manner, although only patients in the 1000-µg/day group showed a significant 2.4% increase in BMD after 12 weeks of treatment. The bone formation markers osteocalcin and P1NP also increased in a dose-dependent manner, with significant changes seen only in the 1000-µg dose group. Urinary C-telopeptides and N-telopeptides, which are markers of bone resorption, were suppressed after 3 months. Although transient increases in blood calcium levels were seen, no clinically significant adverse events were reported.

Nastech Pharmaceutical Company announced in May 2004 the start of a Phase I trial of intranasal hPTH(1-34) using Nastech's proprietary tight junction technology. The trial was to study the nasal absorption and safety of the Nastech formulation versus subcutaneous injection. The results of the trial have not yet been reported, although Nastech has announced the continued clinical development of the programme.

The permeability of the nasal mucosa to peptides decreases with increasing molecular weight, therefore, smaller hPTH fragments may be more suitable than the larger fragments. Caution is required, however, as PTH receptor activation is known to play a major role in the growth of cartilage [50]. If PTH stimulates growth of the nasal cartilage, it would be likely to preclude this delivery option. Other issues with nasal irritation and the large inter- and intraindividual variations in bioavailability due to variable mucus production will also need to be addressed.

## 6. Pulmonary parathyroid delivery

Of all the alternate delivery strategies for peptides, the pulmonary route is the most advanced. It has been appreciated for a long time that small nonpolar drugs such as nicotine can cross the alveoli into the systemic circulation. The first pulmonary delivered aerosol insulin product, Exubera®, was developed by Nektar Therapeutics, Pfizer and Aventis SA. Exubera has completed Phase III clinical trials and the new drug application has been submitted to the FDA in 2005 [51-53]. The regulatory approval and marketing of Exubera would be likely to open the door to many other inhaled peptide products using similar technologies.

The lung is an interesting site for peptide drug delivery as it provides a large absorptive surface area, has relatively permeable membranes and a rich blood perfusion [51,54]. In addition, the lung lacks some of the peptidases that degrade peptide drugs in the GI tract and first-pass hepatic metabolism is avoided. Mucocilliary clearance in the lung is a barrier to peptide drug absorption as it is in nasal delivery. Due to the thin epithelial lining of the lung, inhaled peptides up to 30 kDa can be rapidly absorbed into the bloodstream without the need for penetration enhancers [55]. Absorption through the alveolar membrane is likely to occur by transcytotic and paracellular mechanisms and absorption is typically more rapid than following subcutaneous administration [53]. Some pulmonary delivery technologies use absorption enhancers such as protease inhibitors, surfactants, lipids or cyclodextrins to increase systemic bioavailability [56]. These agents can act to stop drug degradation, alter the mucus layer or to increase paracellular transport by opening the tight junctions between epithelial cells [56]. The long-term pulmonary safety of all these excipients has not been demonstrated and it is possible that new safer enhancers will need to be developed.

All pulmonary delivery products require specialised inhalers. Devices such as dry-powder inhalers, metered-dose inhalers and nebulisers used for the delivery of asthma drugs are not suitable for the delivery of peptides to the deep lung alveoli where peptide absorption occurs. Hence, new breath-actuated inhalers have been developed to efficiently deliver peptide drugs to the deep lung [52]. Many factors affect intrapulmonary absorption, such as the formulation of the drug, the efficiency of the delivery device, breathing patterns, drug deposition in the throat and bronchioles, patient compliance, smoking and the presence of concomitant lung disease. Smoking has been shown to enhance the bioavailability of inhaled insulin [57]. The size and velocity of the aerosol particles are critical for deep lung delivery of peptides. Optimal aerosol particles are 1 – 5 µm in diameter and have low velocities [52,53]. These characteristics can be attained using either liquid or dry-powder formulations, although the delivery characteristics are in large part dependent on the inhaler device [53]. Dry powder formulations are not susceptible to microbial growth due to the low water content and they are suitable for both soluble and insoluble drugs [53].

hPTH(1-34) has been delivered to rats, dogs and monkeys by the pulmonary route and relative bioavailabilities in the range of 21 – 40% have been reported [54,58-60]. Intratracheal instillation of hPTH(1-34) in rats gave a 40% bioavailability [56] and ultrasonic nebulisation in rhesus monkeys reached an absolute bioavailability of 29% [59]. An absolute bioavailability of 34% was obtained in rats after insufflation of a dry powder of hPTH(1-34) [58]. It is not known if there are species differences in PTH metabolism, tissue binding or dosing that could explain the variability in bioavailability. In animal experiments, hPTH(1-34) is about twice as bioavailable as hPTH(1-84) [60]. High variability in patients would not be acceptable as it could lead to either no efficacy at the low end or increased toxicities at the high end. In all of these studies, plasma hPTH(1-34) levels peaked at ~ 15 min and decreased rapidly like the absorption profile seen with subcutaneously

injected PTH [54,58,59,61]; no acute inflammatory responses were observed [58]. It has yet to be demonstrated that the intermittent PTH pulses that occur following inhalation delivery stimulate bone-building activity.

Inhaled hPTH(1-34) has been tested by Mannkind Biopharmaceuticals in humans using the pulmonary Technosphere™ drug delivery system that captures and stabilises peptides in small particles [62]. An organic molecule, 3,6-*bis*(*N*-fumaryl-*N*-[n-butyl]amino)-2,5-diketopiperazine, self assembles in a mild acidic environment into microspheres with diameters of 2 µm [62]. The PTH is encapsulated into the microsphere, which dissolves in the deep lung and facilitates the rapid absorption of the PTH in the systemic circulation. A total of 10 healthy volunteers with a mean age of 25.6 years received either hPTH(1-34) 400 IU s.c. or hPTH(1-34) 1600 IU in a crossover design using the Technosphere pulmonary delivery system. Pulmonary delivery gave a faster and more pronounced increase in serum hPTH(1-34) concentrations (maximum time [$T_{max}$] pulmonary 10 ± 5 min versus 28 ± 8 min following subcutaneous delivery and $C_{max}$ pulmonary 309 ± 215 pmol/l versus subcutaneous 102 ± 45 pmol/l). The relative bioavailability was 48% [62]. The pharmacokinetic profile of inhaled hPTH(1-34) was similar to that for subcutaneous administration with a $T_{max}$ of 30 min and a half-life of 75 min [62]. The inhaled hPTH(1-34) was well tolerated, although no lung function parameters were reported in the study. In addition, no bone formation parameters were measured in this short-term pilot study. Questions of the dose–response relationships, intrapatient variability, safety and efficacy will need to be answered in long-term clinical trials.

Studies with pulmonary insulin have shown that patients prefer inhaled insulin to the standard regimen of subcutaneous insulin [63]. For PTH, convenience and ease of use of inhalers will need to be assessed in the elderly osteoporotic population who may have limitations with dexterity and the ability to breath deeply enough to use inhalers. The impact of ageing on lung function such as a reduction in alveolar area, low-level inflammation and increased residual volume may also be expected to reduce the bioavailability of inhaled PTH peptides [64]. Three other concerns have been raised from the inhaled insulin clinical trials. First, patients treated with inhaled insulin developed increases in serum insulin antibody levels; however, these were subsequently shown not to be inactivating antibodies [52,65]. Second, standardised lung function testing is required in long-term studies to assess the safety of inhaled peptides. Third, the presence of insulin receptors in the lung [66] initially raised concerns about potential growth effects of insulin in the lung, although these have not materialised. For PTHs delivered by the pulmonary route similar questions of antigenicity and lung function will need answering, as PTHR1 receptors are also found in the lung [67].

## 7. Conclusion

Although significant advances have been made in many areas of noninjectable peptide delivery, so far there have been no successes and there does not appear to be much possibility of a marketed noninjection form of PTH therapy for many years. An orally available form of PTH remains the 'holy grail'; however, the best opportunity for PTH seems to be pulmonary delivery, if the more advanced inhaled insulin products are successfully approved. For all noninjectable routes of PTH delivery (oral, nasal, transdermal and pulmonary) the basic science advances still need to be subjected to clinical proof-of-concept trials.

## 8. Expert opinion

It is an exciting time for the noninjection delivery of peptide drugs as improved cost-effective manufacturing processes make new products commercially viable despite still low bioavailabilities. PTHs are ideal candidates for noninjection delivery clinical development programmes. PTH should be much easier to formulate and deliver than insulin, as there is no requirement to exactly replicate the physiological secretion patterns for PTH to be efficacious. The once-a-day administration with a rapid pharmacokinetic profile fits the profile for the ideal pulmonary drug, so this may represent the best option for PTH. However, significant challenges remain. For all of the delivery options discussed, overcoming the issues of the low and in some cases highly variable bioavailability, the impact of low bioavailability on cost of goods, and the long-term safety of excipients will need to be addressed before a noninjection PTH delivery becomes a commercial reality. Although many of these approaches have demonstrated proof-of-principle in animal studies, expensive long-term human clinical trials will ultimately be required to demonstrate their efficacy, tolerability and safety.

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## Affiliation

Paul Morley PhD
Zelos Therapeutics, Inc., 1730 St. Laurent Blvd, Suite 400, Ottawa, Ontario K1G 5L1, Canada
Tel: +1 613 993 9212; Fax: +1 613 747 0265;
E-mail: pmorley@zelostherapeutics.com