Oral Delivery of Biologically Active Parathyroid Hormone

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Purpose. Parathyroid hormone (PTH), the only drug known to stimulate bone formation, is a peptide therapeutic indicated in the treatment of osteoporosis. Unfortunately, PTH is only effective when dosed by injection because it has no oral bioavailability. Herein we report the oral absorption of PTH in rats and monkeys facilitated by the novel delivery agent, *N*-[8-(2-hydroxy-4-methoxy)bensoyl]amino caprylic acid (4-MOAC).

Methods. 4-MOAC was selected from a group of 100 delivery agents based on *in vitro* chromotography studies and *in vivo* screening studies in rats. The PTH/4-MOAC combination was then tested in monkeys. The interaction of 4-MOAC and PTH was evaluated by nuclear magnetic resonance (NMR) spectroscopy.

Results. Monkeys were administered an aqueous solution containing 4-MOAC and PTH and mean peak serum PTH concentrations of about 3000 pg/mL were obtained. The relative bioavailability of oral PTH was 2.1% relative to subcutaneous administration. The biological activity of the orally-delivered PTH was further evaluated in a rat model of osteoporosis. These studies showed that the bone formed following oral PTH/4-MOAC administration was comparable to that formed following PTH injections. The 4-MOAC mediated absorption of PTH is hypothesized to be the result of a noncovalent interaction between 4-MOAC and PTH. The preliminary evaluation of this interaction by NMR is described.

Conclusions. 4-MOAC facilitates the absorption of PTH following oral administration to both rats and monkeys. The orally-absorbed PTH is biologically active as demonstrated in a rat model of osteoporosis.

KEY WORDS: parathyroid hormone; oral protein delivery; osteoporosis treatment.

INTRODUCTION

Over the past several decades a growing number of peptides and proteins have been made available as therapeutic agents. Unfortunately, the full therapeutic potential of these macromolecules has not been realized because they require administration by injection. Ideally, the oral route of administration would be preferred.

Therapeutic macromolecules represent a major challenge for oral delivery (1) because, by design, the gastrointestinal (GI) tract degrades proteins (2) and prevents their absorption (3) as intact entities. Acid-induced hydrolysis in the stomach, enzymatic degradation throughout the gastrointestinal tract, and bacterial fermentation in the colon are among the many chemical and biochemical barriers that can prevent the oral delivery of proteins and large peptides. Physical barriers to oral delivery include poor solubility in the intestinal environment and lack of permeation through the epithelial cells as a consequence of size, charge, and/or lipophilicity. Given these barriers, it is not surprising that the oral delivery of proteins and peptides has been considered impossible or, at best, extremely challenging.

One protein under investigation for its therapeutic potential is parathyroid hormone. It is an 84 amino acid protein (4) that is normally secreted by the parathyroid gland in response to low levels of circulating calcium. Parathyroid hormone regulates calcium homeostasis by a) stimulating bone resorption and release of bound calcium, b) increasing calcium absorption in the intestine, and c) increasing calcium and phosphate resorption in the kidneys. A high level of circulating parathyroid hormone, as observed with hyperparathyroidism, induces an increase in bone turnover and a subsequent reduction in bone mass caused by stimulation of osteoclastic resorption activity; and the absence of PTH results in a lack of bone growth. However, intermittent, once-daily, subcutaneous administration of low levels of parathyroid hormone stimulates the bone-formation activity of osteoblasts, replacing lost bone in both osteopenic, ovariectomized rats, and osteoporotic humans (5). Bone rebuilding of this type has not been observed for other postmenopausal osteoporosis therapies including estrogens, calcitonins, bisphosphonates, or selective estrogen receptor modulators (SERMs). These drugs are not anabolic agents but are inhibitors of bone turnover or osteoclastic resorption activity (6). The bone rebuilding activity of full-length, recombinant, human parathyroid hormone is thought to be contained in parathyroid hormone (1–34) [PTH], the thirty-four residue amino-terminal fragment (7). This fragment was used in all of the studies described herein.

We have designed and synthesized low molecular weight compounds called delivery agents that facilitate the gastrointestinal absorption of proteins and other macromolecular therapeutics (8–12). We now report the successful oral delivery of biologically active PTH in rats and primates from aqueous solutions using these novel delivery agents.

MATERIALS AND METHODS

Delivery Agent Synthesis

Delivery agents 1–33 were prepared using previously published methods (8,9). The structure of each delivery agent was confirmed by nuclear magnetic resonance (NMR) at 300 MHz in either D_2O or DMSO-d_6. The purity of each delivery agent was confirmed by high pressure liquid chromatography (HPLC) (>95 area%) and combustion analysis (CHN 0.4%). 4-MOAC is an acronym derived from the chemical name *N*-[8-(2-hydroxy-4-methoxy)benzoyl]amino caprylic acid.

IAM Chromatography

High pressure liquid chromatography was carried out on a 30 × 4.6 mm IAM.PC.C3/C10 column (Regis Technologies, Morton Grove, IL). The mobile phase was 10 mM sodium phosphate (pH 6.4) in 25% aqueous propylene glycol. The flow rate was 2 mL/min. Compounds for analysis were

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prepared at 100 μg/mL in mobile phase and the injection volume was 50 μL. Compounds were detected at 220 nm.

PTH Affinity Chromatography

High pressure liquid chromatography was carried out on a 21 × 0.5 cm glass column packed with sepharose covalently linked to PTH at a concentration of 43 mg PTH/mL resin. The mobile phase was 200 mM phosphate buffer (pH 6.5) with 1% sodium azide. The flow rate was 0.3 mL/min. Compounds for analysis were prepared at 1 mg/mL in mobile phase and the injection volume was 20 μL. Compounds were detected at 254 nm.

NMR Studies

Nuclear magnetic resonance spectroscopy was conducted using a Varian Unity 500 spectrometer at 25°C. For the pH and ligand titration experiments 3mM ^{15}N PTH was used. For the ligand binding experiments, the solution concentrations varied as described in the text.

Animal Studies

All animal protocols adhered to the "Principles of Laboratory Animal Care" and were approved by the Animal Care and Use Committees of Emisphere Technologies, Inc. or Eli Lilly and Company. Serum PTH concentrations were measured by radioimmunoassay (human PTH 1-34 kit, Peninsula Labs, San Carlos, CA). Dosing solutions were prepared by adding PTH to an aqueous solution of 4-MOAC in water.

Protocol for Rat Experiments

Male Sprague Dawley rats weighing 200–250 g were fasted for 24 h. Immediately prior to dosing, the rats were anesthetized with an intramuscular injection of ketamine (44 mg/kg) and chlorpromazine (1.5 mg/kg) and then administered the dosing solution by oral gavage using an 11 cm Rusch 8 French catheter. The catheter was placed down the esophagus and the dosing solution was expressed slowly into the stomach. For screening studies, the dose of PTH was 200 mcg/kg and the dose of delivery agent was 100 mg/kg. Blood samples were collected from the tail artery prior to dosing and at 15, 30, 45, 60, and 90 min after dosing. The serum was harvested and the samples assayed for PTH concentrations.

Protocol for Monkey Experiments

Six, male, conscious Rhesus monkeys ranging from 3–6 kg were fasted for 6 h and then dosed, by oral gavage, with 0.5 mL/kg of the 4-MOAC/PTH dosing solution. The dose of PTH was 400 mcg/kg and the dose of 4-MOAC was 200 mg/kg. Blood samples were collected from the saphenous vein prior to dosing and at 15, 30, and 45 min and 1, 1.5, 2, 2.5, and 3 h after dosing. The serum was harvested and the samples assayed for PTH concentrations.

Protocol for Efficacy Studies

Six-month-old virgin Sprague Dawley rats were ovariectomized. The animals were acclimated for 4 weeks following ovariectomy, weighed, subjected to bone mineral density measurement, and then administered daily doses of either PTH, 4-MOAC, 4-MOAC/PTH orally or PTH subcutaneously following a 10-h fast. After 21 days of dosing the animals were weighed and subjected to bone mineral density measurements. The rats were sacrificed and histomorphometry studies were conducted.

RESULTS AND DISCUSSION

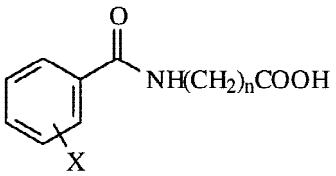
Oral PTH Delivery Agent Selection

Given the GI tract environment, it is difficult to envision the passive transcellular diffusion of proteins through epithelial cells (13). However, proteins are transported into or through lipid bilayers within and between cells as a normal biological process (14,15). We hypothesize a similar mechanism for delivery agent facilitated protein absorption through the GI epithelial cells (9,16). Thus, the delivery agents interact non-covalently with the macromolecule to alter its physicochemical properties, for example by increasing hydrophobicity.

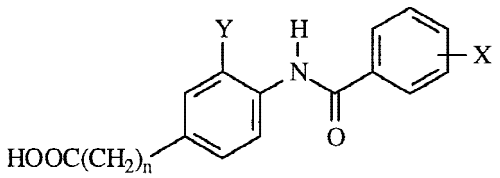
To test this hypothesis, approximately 100 delivery agent candidates were prepared using standard, synthetic organic reactions as well as novel chemistries. These delivery agents were screened for their membrane interaction potential using immobilized artificial membrane (IAM) chromatography (17). This *in vitro* technique, which mimics the partitioning of compounds into lipid membranes, was chosen as a model of the interaction between delivery agents and the lipophilic gastrointestinal membrane. In previously reported studies, IAM chromatography has been correlated to partitioning (log relative k') into liposomes, permeability through Caco-2 cells and inverted rat intestine, and oral bioavailability in rats (17). Earlier studies in our laboratories have shown that the interaction of a delivery agent with the IAM column is a good predictor of delivery agent activity (18). Delivery agents must transport proteins between aqueous environments through a lipophilic membrane, thus these compounds must exhibit both lipophilic and hydrophilic character. Previous studies have suggested that compounds having log relative k' values around 1 partition readily into intestinal epithelial cells (17). To bracket this value, delivery agents having log relative k' values between 0.6 and 1.4 were selected for additional studies. Using this criterion, 33 of the 100 delivery agents were chosen for *in vivo* screening in rats.

The delivery agents selected from the IAM analysis (Table I) were then tested *in vivo* for their ability to promote the gastrointestinal absorption of PTH in an animal model. Sprague-Dawley rats (5/group) weighing 200–250 g were administered an aqueous solution of the delivery agent and PTH by oral gavage. Blood samples were collected serially from the tail artery and the serum assayed by radioimmunoassay to quantitate circulating PTH concentrations. Table I also shows representative mean peak serum PTH concentrations (C_{max}) following a single, oral dose of PTH in combination with each of the 33 delivery agents selected by IAM chromatography. C_{max} was selected as the most appropriate pharmacokinetic parameter for ranking the delivery agents because it is a good indicator of drug activity (5) For comparison, a control group of animals was administered by oral gavage an aqueous solution of PTH alone. The data indicate that the gastrointestinal absorption of PTH, in the presence of delivery agents, is significantly enhanced above the level observed when PTH is

Table I. Chemical Structures an *In Vivo* Data for Delivery Agents 1–33



Compound	X	n	C max (pg/mL) ± SD
1	2-OMe	3	14 ± 8
2	2-F	5	0
3	2-COOH	9	37 ± 4
4	H	5	27 ± 10
5	2-Me	3	5 ± 5
6	2-Me	5	29 ± 14
7	2-OH	7	553 ± 37
8	2-OH, 4-OMe	7	600 ± 12
9	H	9	101 ± 18
10	2-OMe	9	17 ± 4
11	2-OH, 5-OMe	7	347 ± 39
12	2-OH, 5-Cl	6	463 ± 128
13	4-OH	8	32 ± 17
14	2-OMe	10	27 ± 18
15	2-OH, 4-OMe	9	68 ± 32
16	2-OH, 5-F	7	583 ± 48
17	2-OMe	7	114 ± 26
18	2-OMe	5	0
19	2-OH, 4-Me	7	288 ± 49
20	2-OH, 3-F, 5-Cl	7	337 ± 34
21	2-OEt	7	28 ± 14



Compound	X	Y	n	C max (pg/mL) ± SD
22	2-carboxyphenyl	H	2	504 ± 28
23	2-NHCHO	H	2	204 ± 52
24	2-OH, 3-Me, 5-F	H	2	427 ± 10
25	2-OH, 5-OMe	H	2	546 ± 234
26	2-OH, 4-OMe	H	2	59 ± 25
27	2-OH	F	2	417 ± 104
28	2-OH, 4-OMe	Cl	2	65 ± 21
29	2-NH_2, 5-F	H	2	28 ± 5
30	2,3-O(CH_2)O	H	2	76 ± 39
31	2-OH, 3-Cl, 5-F	H	2	572 ± 123
32	2-OEt	H	3	585 ± 118
33	2-OH, 5-OMe	H	3	568 ± 257
none	NA	NA	NA	0

administered alone. From the results of these studies, delivery agent 8 (4-MOAC, Fig. 1) was identified as the most effective oral PTH delivery agent.

4-MOAC was selected for testing in an oral dose-response study in rats where the dose of 4-MOAC was held constant and the dose of PTH was either 100, 200, or 400 mcg/kg. The data from these doses vs. area under the response curve studies (Fig. 2) show an essentially linear PTH dose-response relationship (correlation coefficient 0.987). The areas under the concentration vs. time curves are 12891, 37779, and 65054 for PTH doses of 100, 200, and 400 mcg/kg respectively. The bioavailability of this orally absorbed PTH relative to subcutaneous injection was 5%. This study demonstrates that significant oral absorption can be obtained from PTH doses as low as 100 mcg/kg. The oral administration of PTH alone does not produce any elevation in circulating PTH levels.

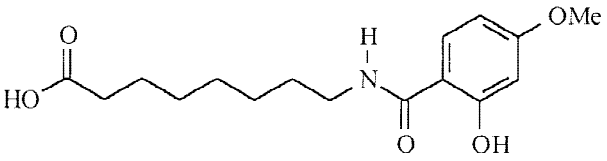


Fig. 1. Chemical Structure of 4-MOAC.

Delivery Agent Mediated Oral PTH Absorption

Based on the results of the rat studies, the 4-MOAC/PTH combination was chosen to study the mechanism of delivery agent mediated oral PTH absorption and to test the hypothesis that a non-covalent drug/delivery agent interaction is responsible for oral PTH delivery. The increased oral absorption of various drugs that has been achieved with "traditional penetration enhancers" has been reported to correlate directly with the extent of damage caused to the GI tract (19). Histological examination of the GI tracts of rats was performed following a single oral administration of 4-MOAC (2000 mg/kg) to ensure that the increased absorption of PTH observed in these studies was not due to tissue damage. At no time point was there detectable pathology caused by 4-MOAC confirming that the increased absorption of PTH in the presence of 4-MOAC was not due to disruption of the intestinal epithelium.

PTH Affinity Chromatography

The 33 delivery agents selected by IAM chromotography for *in vivo* testing were also evaluated using PTH affinity

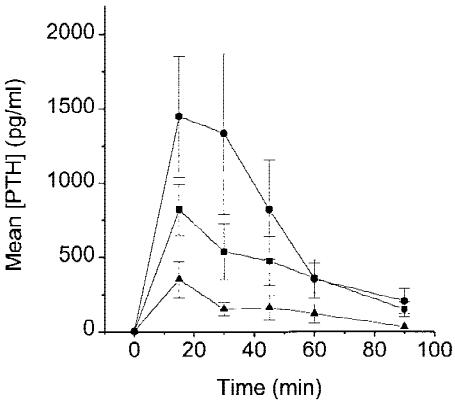


Fig. 2. Oral delivery of PTH in rats. Dose response study in rats following a single, oral administration of the 4-MOAC/PTH combinations. The dose of 4-MOAC is 300 mg/kg and the dose of PTH is varied. The PTH doses are 400 mcg/kg (circles), 200 mcg/kg (squares), and 100 mcg/kg (triangles).

chromatography. This *in vitro* system was chosen as a model of the interaction between PTH and the delivery agents. In this technique, PTH is covalently bound to a sepharose resin and the delivery agents are eluted through the resin-bound drug. The rate at which the delivery agents move through the column is related to their interaction with PTH. Interacting delivery agents would move through the column more slowly than non-interacting delivery agents. Thus, it is proposed that active delivery agents will exhibit larger relative k' values than less active delivery agents because the more active delivery agents interact more effectively with PTH. Analysis of the relative k' values obtained from affinity chromatography with respect to the ability of these delivery agents to facilitate the oral PTH absorption in rats showed that delivery agents having relative k' values between 0.9 and 2.9 were the most effective (Fig. 3). Of the 9 delivery agents that mediate PTH absorption to produce C_{max} of >500 pg/mL, 5 fall within this range. The other 4 delivery agents fall outside this range. Upon closer examination, these data show that the in-range delivery agents facilitate more consistant (less variable) oral PTH absorption (Table I). These data support the hypothesis that an interaction (non-covalent) between PTH and the delivery agent is necessary for oral PTH absorption and that this interaction is maximized for delivery agents having the specified relative k' range.

The log relative k' from IAM were graphed against the relative k' values obtained from affinity chromatography for the 33 PTH oral delivery agent candidates. The intersection of the optimal log relative k' and relative k' values (Fig. 4, box) identifies nine delivery agents that were found to be active by both IAM and affinity chromatography. These nine delivery agents include 4-MOAC. Thus, the combination of these two techniques supported by limited *in vivo* studies to set parameter boundaries, appears to be useful as an *in vitro* screen for the selection of oral PTH delivery agents.

NMR

NMR spectroscopy is a sensitive tool for detecting weak interactions in exchanging systems like ligand-protein composites using chemical shift changes, selective line broadening, and nuclear Overhauser effects (NOEs). Mixtures of

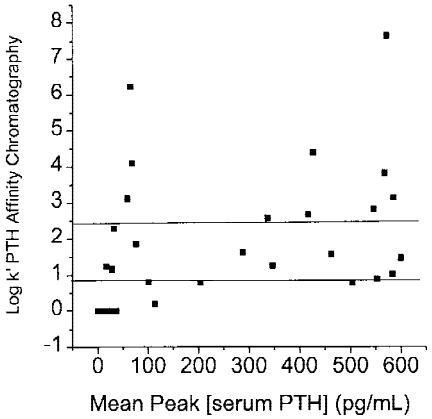


Fig. 3. Plot of relative k' (PTH affinity chromatography) vs. PTH C_{max}. Compounds that are the most effective and least variable facilitators of oral PTH absorption have relative k' values in the range 0.9–2.4.

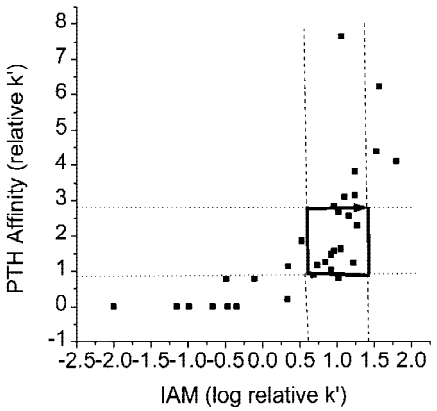


Fig. 4. Plot of log relative k' (IAM chromatography) vs. relative k' (PTH affinity chromatography). Compounds that are effective facilitators of oral PTH absorption have log relative k' values in the range 0.6–1.4 (dashed line) and relative k' values in the range 0.9–2.9 (dotted line). Those delivery agents that fall within both of these range limits are contained in the box defined by the two vertical and horizontal lines.

PTH and 4-MOAC provide a serious challenge for study by NMR spectroscopy. The solubility of 4-MOAC decreases as the pH drops below 7.4, and PTH has reduced solubility and suffers substantial line broadening in pH ranges near and above neutrality (20). Attempts to make direct assignments of the PTH resonances at pH 7.4, either alone or in the presence of 4-MOAC, by using three-dimensional techniques such as heteronuclear single-quantum coherence 2D nuclear Overhauser effect spectroscopy (HSQC-NOESY) and heteronuclear single-quantum coherence total correlated spectroscopy (HSQC-TOCSY) were thwarted by line-broadening effects. Nevertheless, most of the PTH resonances could be followed in HSQC titration experiments (pH 5-7.8), and then by ligand titration at pH 7.4 (no ligand up to ligand/PTH ratios of 82). The presence of increasing amounts of 4-MOAC improved the PTH line-width characteristics, but the lines remained broader than observed under acidic conditions.

The PTH ^{1}H and ^{15}N assignments show that PTH resonances are shifted in varying degrees and directions by the presence of a 30-fold excess of 4-MOAC at pH 7.4. These changes that accompany the addition of ligand at constant pH are greater than those observed for the pH titration in the absence of ligand, but some peaks change very little (W23, F34). These data suggest that PTH changes conformation as the ligand binds, because the changes in chemical shift are observed at diverse sites on the protein. The nature of the conformational change, whether folding or unfolding, is not apparent, however, protein/ligand complexation is clearly taking place.

In a transferred NOE experiment, the detection of weak interactions between small and large molecules depends on the variation of NOEs from small and positive for low molecular weight compounds to larger and negative for larger molecules and their complexes (21). When a ligand exchanges at an appropriate rate between free and bound states, the average NOE exhibited by the ligand resonance is dominated by the large negative effect acquired while a portion of the ligand population was associated with the protein. The change of sign of ligand NOEs from positive to negative in the presence of added protein is a reliable indication of binding

(22). We used the transferred NOE procedure to demonstrate the binding of 4-MOAC to PTH at pH 7.4 (Fig. 5). NOE crosspeaks were positive for 4-MOAC in all NOESY spectra of the ligand in the absence of PTH (maximum concentration 90 mM). Ligand NOEs remained positive at low PTH concentrations, suggestive of weak binding interactions, but at 2.5 mM PTH and 41.4 mM 4-MOAC strong negative NOEs were produced (Fig. 5). This change in sign and magnitude of the ligand NOEs does not characterize the 4-MOAC/PTH binding, but it illustrates clearly that binding does occur.

Oral PTH Efficacy

Encouraged that 4-MOAC promoted the oral delivery of PTH, and the serum PTH levels were comparable to those observed previously with subcutaneous injection and shown to induce bone apposition in rats (5), studies were initiated to evaluate skeletal effects. Efficacy was assessed in a rodent model of postmenopausal osteoporosis (23–25). Specifically, 6-month-old female Sprague Dawley rats were surgically ovariectomized (day 0) and permitted to lose bone for 30 days. An additional group of animals underwent the surgical procedure without ovary removal (sham-operated). Shown previously for this model system (6,26), the ovariectomized rats lost approximately 20% of the volumetric bone mineral density (BMD, mg/cc) of the proximal tibia metaphysis compared to the sham group as measured by quantitative computed tomography (QCT) using $0.148 \times 0.148 \times 1.2$ mm voxels (27). The sham-operated group suffered minimal bone loss (Fig. 5, day 30). On day 30, ovariectomized rats were randomized into three groups (8–9/group) for the next experimental stage. Group 1 (4-MOAC/PTH) was administered, following a 10 h fast, an aqueous solution of 4-MOAC (300 mg/kg) in combination with PTH (1 mg/kg) by oral gavage once daily for 2 months. The remaining groups of ovariectomized rats (Groups 2–4) and the sham-operated group were dosed with the following treatment regimens utilizing the dosing protocol described for Group 1. Group 2 (4-MOACpo) and the sham-operated rats were administered an aqueous solution of 4-MOAC (300 mg/kg) alone by oral gavage. Group 3 (PTHsc) was dosed subcutaneously with PTH (0.01 mg/kg).

QCT analyses performed on day 60 and day 88 (30 and 58 days after initiation of the treatment regimens) showed restoration of BMD and bone mineral content (BMC) in the proximal tibia metaphysis for the 4-MOAC/PTH oral gavage and PTHsc (positive control) groups (Fig. 6). The rate and extent of bone accumulation were similar in these two groups. The animals in Group 2 (4-MOACpo alone) did not show a change in BMD after 2 months of oral dosing. In a separate study, orally-administered PTH in the absence of the delivery agent were shown not to promote bone accumulation (data not shown). These data indicate that 4-MOAC is effective in facilitating the gastrointestinal absorption of pharmacologically active PTH in fasted rats.

Having demonstrated that the orally-delivered PTH promoted bone accumulation (BMD and BMC), the structural and dynamic characteristics of this newly-formed bone were examined by histomorphometry. Trabecular bone area for

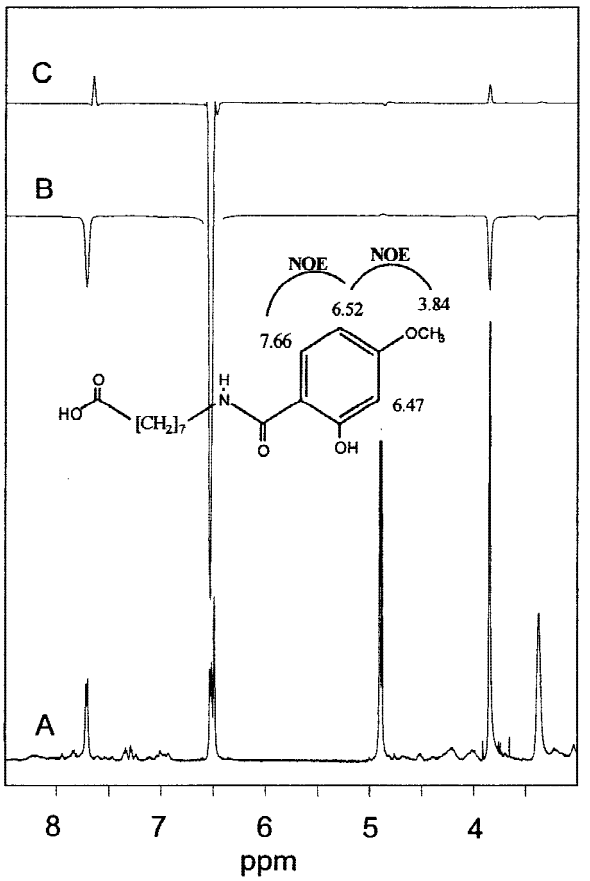


Fig. 5. NMR spectra of 4-MOAC in combination with PTH. (A) 1D spectrum of a solution containing 2.5 mM PTH and 41.4 mM 4-MOAC in H_2O at pH 7.4. (B) NMR trace along the T_2 dimension at T_1 = 6.52 ppm from a T_2-flitered NOESY spectrum of the solution in A (mix = 0.2 sec). (C) NMR trace along the T_2 dimension at T_1 = 6.52 ppm from a T_2-flitered NOESY spectrum of an aqueous solution of 4-MOAC only (mix = 0.5 sec).

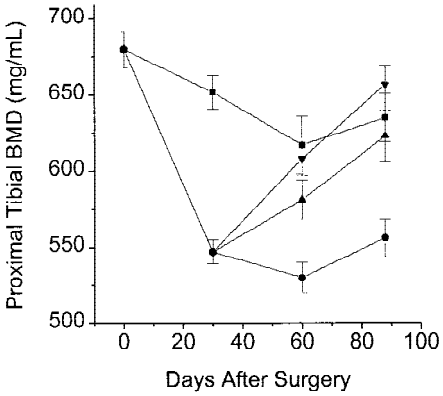


Fig. 6. Oral 4-MOAC/PTH efficacy *in vivo.* Longitudinal QCT analysis of volumetric BMD osteopenic, ovariectomized rats. The sham animals were subjected to surgery without ovary removal, while all other rats were ovariectomized. One month after surgery, all of the animals were dosed daily for 2 months. The 4-MOAC dose was 300 mg/kg. The dose of PTH was1 mg/kg (orally) and 0.003 mg/kg (subcutaneously). The diamonds represent the response of sham animals dosed orally with 4-MOAC alone in water. The down triangles represent the response of animals (Group 1) dosed orally with 4-MOAC/PTH combination in water. The circles represent the response of animals (Group 2) dosed orally with 4-MOAC alone in water. The up triangles (Group 3) represent the response of animals dosed subcutaneously with PTH. The data are plotted as mean ± SE with n = 8/9 per group.

the 4-MOAC/PTH group (10.52%) was comparable to that of the sham animals dosed subcutaneously with 0.003 mg/kg PTH (2.94%). The trabecular bone loss caused by ovariectomy was restored by the thickening of existing trabecular spicules without altering their number. This response is consistent with a stimulation of bone apposition. Mineralized surface, mineral apposition rate, bone formation rate, and eroded perimeter in the 4-MOAC/PTH group were not different from ovariectomized (OVX) or sham controls. The latter indicates that oral administration did not unduly stimulate osteoclast activity. Lamellar bone was observed in all samples by polarized light analysis with no evidence of woven bone formation or fibrosis.

Bone quality was evaluated for skeletal sites enriched for cancellous and cortical bone, including assessment of whole bone parameters and material parameters. The mechanical integrity of the restored bone was evaluated by compression analysis of lumbar vertebra L6, sheer analysis of the femoral neck, and three-point bending analysis of the femoral midshaft. Oral 4-MOAC/PTH restored vertebral strength and stiffness above OVX to sham levels. Although significant differences between groups were not observed for the femoral neck, strength (ultimate load) of the oral 4-MOAC/PTH combination group was not less than OVX, baseline, sham, or PTHsc controls. Tissue analysis of the midshaft showed significant increases in cortical bone thickness for the oral group, resulting in ultimate load and stiffness that exceeded ovariectomized and baseline controls. Material analyses showed restoration of strength to sham levels, and Young's modulus that exceeded the PTHsc controls. Toughness was intermediate and not significantly different between ovariectomized or sham controls. In all cases, the analysis of bone from the 4-MOAC/PTH animals gave results comparable to or greater than the PTHsc controls, indicating efficacy in cancellous and cortical bone sites. The control groups dosed with oral 4-MOAC alone or oral PTH alone showed no significant changes in bone quality when compared to the untreated ovariectomized animals.

The results obtained from the rodent postmenopausal osteoporosis model show that PTH is absorbed following the oral administration of a solution of PTH in combination with 4-MOAC; and the PTH delivered from the oral solution retains its full biological activity.

Oral PTH Delivery to Monkeys

Finally, oral PTH absorption following dosing with the 4-MOAC/PTH combination was assessed in an animal model more closely associated with predicting oral delivery in humans. Following Institutional Animal Care and Use Committee protocol approval, four, conscious rhesus monkeys (Macaca mulatta) were administered a single, oral dose of 4-MOAC/PTH, containing 200 mg/kg 4-MOAC and 400 mcg/kg PTH. Blood samples were collected serially for 3 hours and the serum assayed using a radioimmunassay validated for PTH quantitation in primate serum. The data obtained from oral dosing were compared to data obtained after a subcutaneous injection of PTH (10 mcg/kg) in the same animals. Figure 7 illustrates the pharmacokinetic profile obtained from this study. The oral bioavailability of PTH when coadministered with 4-MOAC in this unformulated, unoptimized dosing solution was 2.1% relative to subcutaneous administration.

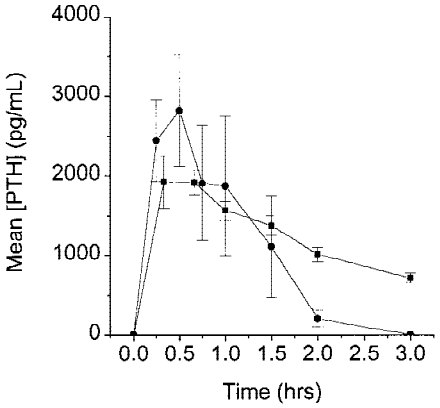


Fig. 7. Serum PTH concentration time profile in rhesus monkeys. The circles represent the response following a single, oral dose of 4-MOAC (200 mg/kg) in combination with PTH (400 mcg/kg) in water. The squares represent the response following a single, subcutaneous injection of PTH (10 mcg/kg). The 4-MOAC-facilitated oral bioavailability of PTH relative to subcutaneous administration is 2.1%.

CONCLUSION

The data presented here demonstrate that 4-MOAC facilitates the gastrointestinal absorption of biologically-active PTH in rats following oral dosing of an 4-MOAC/PTH aqueous solution. The effectiveness of the orally-delivered PTH was confirmed in a rat model of postmenopausal osteoporosis. These data are consistent with the absorption of orally administered PTH throughout the two-month study period. To our knowledge, this is the first demonstration of the reproducible, oral delivery of a biologically active protein drug in a randomized, controlled experiment in an animal model of human disease.

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