Measurement of Osteoanabolic Agents PTH (1-34) and PTHrP (1-36) in Therapeutic Studies and Clinical Diagnosis

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Introduction
Teriparatide, a recombinant human PTH (1-34) is an osteoanabolic agent for treatment of osteoporosis. PTH (1-34) can also be used a replacement therapy in hypoparathyroidism and to accelerate fracture healing. Abaloparatide, PTHrP (1-36) analogue is a novel anabolic drug for treatment of osteoporosis. Measurement of plasma PTH (1-34) has also been used to assess response to PTH in conditions such as pseudohypoparathyroidism (PHP) (Ellsworth-Howard test (EHT)).

Aims and Objectives

- To review the use of PTH (1-34) measurements in drug development studies, and in the diagnosis of patients with PHP
- To highlight the potential use of measurement of PTHrP (1-36) using our LC/MS/MS method for measurement of intact PTHrP (1-36), intact PTH (1-34) and its respective oxidised forms simultaneously.

Study Design and Method

Sample Collection

- PTH (1-34) was analysed in EDTA plasma obtained from human subjects given either single subcutaneous (sc) injection of 20 μg Teriparatide (n=10) or 0.69 mg (sc), 2.07 mg (sc) oral PTH (1-34) (Enterabio).
- Baseline samples were taken immediately before drug administration.
- Urine cAMP and P04 were analysed on samples voided every 30 min for 3 hours post PTH (1-34).

Oxidation of PTH (1-34) and PTHrP (1-36)

- Oxidation of the sulphur atom in methionine residues by peroxides is one of the major degradation pathways of therapeutic peptides. PTH (1-34) contains two methionine residues at position 8 (Met8) and position 18 (Met18).
- Three of the PTH (1-34) products were isolated, namely Met8 sulphoxide, Met18 Sulfoxide, and both positions Met Sulfoxide
- Oxidation of the methionine residues causes a change in the secondary structure of PTH (1-34), especially oxidation of Met8. The change in the secondary structure is greater when both methionine residues are oxidised.
- Double oxidised forms of PTH (1-34) possess reduced biological activity, which consequently reflected on the potency of the treatment.
- In contrast to human PTH (1-34), human PTHrP (1-36) does not contain methionine residues in its structure. Therefore, PTHrP (1-36) is not oxidised by hydrogen peroxide (H2O2).

Our data showing that oxidation contributes (23.9 ± 6.1%) to bias between our LC/MS/MS method for PTH (1-34) and immunoassays results.

- Due to the absence of methionine residues in human PTHrP (1-36) and analogues of hPTHrP (1-34) such as Abaloparatide they are resistant to oxidation, hence this may explain some of the difference in efficacy observed in Abaloparatide preclinical/clinical studies. However, further investigations are required to confirm this possibility.

The Use of PTH (1-34) Measurement in Pharmacokinetics Studies

Table: PK parameters for PTH (1-34) of 20 μg subcutaneous Enterabio injection and oral 0.69 and 2.07 mg administration.

Figure (3) MS spectrum of human PTH (1-34) (MW = 4131.7 Da) before and after 40 and 60 min oxidation with H2O2. 8B: represent +7 and 42 charged state of non-oxidised PTH (1-34) respectively. 8B: represent +6 and +7 charged state of PTH (1-34) oxidised at Met B or Met 8 respectively (single-oxidised form with an increase of 16 mass units). 6B: represent +6 and +7 charged state of PTH (1-34) oxidised at both Met B and Met 8 (dihydroxide-double oxidised form with an increase of 32 mass units).

Figure (4) Recovery efficiency:
- Standard solution recovery:
  - Mean recovery: 100 ± 6.2% (n=10), RSD: 6.2%
- Spiking recovery:
  - Mean recovery: 100 ± 3.6% (n=10), RSD: 3.6%

Figure (5) Chromatograms showing the separation of human PTH (1-34) and its respective single- and double-oxidised forms from human PTHrP (1-36) as well as the internal standard rat PTH (1-34) fragment.

The Use of PTH (1-34) Measurement in the Diagnosis of PHP

PHD disorders are characterized by impaired signalling of various hormones (mainly PTH) that activate cAMP-dependent pathways via Gα protein. Ellsworth-Howard test or PTH loading test has been used traditionally to confirm PHP. Measurement of serum and urinary cAMP concentrations after the injection of exogenous PTH plus PO4 measurement confirmed the diagnosis of PHP type 1 (PHP1), in which a blunted cAMP response is observed, from PHP type 2 (PHP2) in which the cAMP response to PTH is conserved but the phosphatidic response is deficient.

Conclusions

- Our method for measurement of non-oxidised and oxidised forms of PTH (1-34) as well as for PTHrP (1-36) may:
  1) offer new insights into the physiology and pathophysiology of PTH
  2) help investigate the therapeutic use/efficacy of osteoanabolic agents
  3) help in development of combination therapy with other anti-resorptive/anti-remodelling agents.