Why Current PTH Assays Mislead Clinical Decision Making in Patients with Secondary Hyperparathyroidism

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Keywords

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Abstract

Preclinical studies in cell culture systems as well as in whole animal chronic kidney disease (CKD) models showed that parathyroid hormone (PTH), oxidized at the 2 methionine residues (positions 8 and 18), caused a loss of function. This was so far not considered in the development of PTH assays used in current clinical practice. Patients with advanced CKD are subject to oxidative stress, and plasma proteins (including PTH) are targets for oxidants. In patients with CKD, a considerable but variable fraction (about 70 to 90%) of measured PTH appears to be oxidized. Oxidized PTH (oxPTH) does not interact with the PTH receptor resulting in loss of biological activity. Currently used intact PTH (iPTH) assays detect both oxidized and non-oxPTH (n-oxPTH). Clinical studies demonstrated that bioactive, n-oxPTH, but not iPTH nor oxPTH, is associated with mortality in CKD patients.

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The parathyroid hormone (PTH) is an 84-residue peptide secreted by the parathyroid gland, which regulates the blood Ca²⁺ level via GPCR binding and subsequent

activation of intracellular signaling cascades [1]. Experimental studies using animal models of chronic kidney disease (CKD) clearly demonstrated that both too low and also too high PTH concentrations in these CKD model are causal for the development of both bone and cardiovascular diseases [2–5]. These preclinical data were translated to humans. Low PTH concentrations, for example, after total parathyroidectomy as well as high PTH concentrations in CKD patients are associated with excess morbidity and mortality [6–9]. These studies led to the development of guidelines for the treatment of too low and in particular too high PTH status in CKD patients [10, 11] and subsequently to the development of pharmaceutical tools to treat these abnormal status such as PTH analogues [12] for conditions of low PTH-related situations as well as vitamin D analogues, calcimimetics, and phosphorus binders for conditions with high PTH [13–16]. The decision to use these drugs and the monitoring of the efficacy requires reliable analytical tools to measure PTH. However, this seems to be tricky. As of today, we are not able to define a clear cut-off value for PTH that is clearly associated with secondary hyperparathyroidism. We have instead very complicated guidelines how PTH in patients on dialysis should look like. PTH should be 2–9 times higher than the upper detection limit of the individual assay and physicians need to consider PTH trends when adjusting therapy [10, 11]. These wordings

Fig. 1. Parathyroid hormone (PTH) is an 84-amino acid hormone. The amino acids, such as methionine, at position 8 and 18 can be oxidized under conditions of oxidative stress. The first oxidation step is reversible, while the second one is not. The amino acids

8 and 18 in the PTH molecule are belonging to the PTH receptor binding site of the hormone. An oxidation of these methionone residues alters the hormone receptor interaction; for details see Hocher et al. [49].

clearly indicate that the current tools to measure PTH are insufficient. No cardiologist would accept such a definition for troponin T as biomarker of the acute coronary syndrome, and indeed in daily practice it is well known that PTH measurements in individual cases are often not fitting to the clinical situation [17]. One cause that might contribute substantially to the poor performance of current PTH assays is potential posttranslational modification of PTH – in particular, oxidation at Met8 and Met18 of the PTH-1–84 molecule (see also Fig. 1), since patients with CKD and in particular CKD patients on dialysis have a huge burden of oxidative stress [18].

There is clear evidence from studies performed by leading research groups worldwide about 2 decades ago that oxidized PTH (oxPTH) and non-oxPTH (n-oxPTH) have completely different biological properties [19–48]. Initial studies using classical receptor binding assays demonstrated that oxPTH has a much lower binding affinity to the PTH receptor [24–26, 28, 38]. Other studies focused on the generation of the second messenger of PTH-receptor cAMP. These studies indicated that oxPTH – in contrast to n-oxPTH – does not

stimulate the PTH receptor to generate cAMP [22, 24, 25, 37, 42, 45, 48]. In addition, it was demonstrated that oxPTH loses its biological action on smooth muscle cells contraction/vascular effects in tissues like trachea, aortic rings, and the uterus [32, 35, 36, 41, 42, 45]. Stimulation of alkaline phosphatase activity in cultured neonatal mouse calvarial bone cells by PTH [39] was seen only after incubation with n-oxPTH but not with oxPTH. Other studies demonstrated that only n-oxPTH but not oxPTH is able to regulate calcium and phosphate metabolism [40, 43].

These overwhelming data indicating that PTH oxidation (Fig. 1) are critical for the biological activity of PTH. PTH oxidation, however, has been ignored so far in the development of PTH assays that are used in the current clinical practice.

Therefore, the assay system of separating oxPTH from n-oxPTH was developed recently [49–51]. Children with chronic renal failure stage 2–4 had the highest mean n-oxPTH concentrations compared with adult patients (adults on dialysis, as well as kidney transplant recipients, see 49–51). Analyzing the subgroup of children

Fig. 2. a, b Baseline samples of children with chronic kidney disease stages 1–4 participating in the 4C study (Querfeld et al. [62]) and an intact parathyroid hormone (iPTH) >250 ng/L were analyzed. iPTH correlates very well with oxidized PTH (oxPTH) but to a much weaker extent with non-oxidized PTH. In patients with

high iPTH (SHPT) iPTH data can be mainly explained by oxPTH, a protein that does not interact with the PTH receptor but reflects protein oxidation; iPTH values in pediatric SHPT patients, thus, mainly describe oxidative stress.

with intact PTH (iPTH) >250 ng/L demonstrated a close to linear correlation between iPTH and oxPTH ($r^2 = 0.997$; $p < 0.001$), but a much weaker correlation between iPTH and n-oxPTH ($r^2 = 0.718$; $p < 0.05$; Fig. 2). The relationship between oxPTH and n-oxPTH of individual patients varied substantially in all 3 populations with renal impairment (children with CKD stage 2–4, adults on dialysis and adults after kidney transplantation). The analysis of n-oxPTH in healthy control subjects revealed that n-oxPTH concentrations in patients with renal failure were higher than those in healthy adult controls (2.25-fold in children with renal failure; 1.53-fold in adult patients on dialysis; 1.56-fold in kidney transplant recipients) [49].

A prospective observational study showed that the predictive power of n-oxPTH and iPTH on mortality of hemodialysis patients differed substantially. This study demonstrated an increased survival in the highest versus lowest n-oxPTH tertile (χ^2 , 14.3; $p = 0.0008$). Median survival was 1,702 days in the highest n-oxPTH tertile but only 453 days in the lowest n-oxPTH tertile. Multivariable-adjusted Cox regression showed that higher age increased odds for death, whereas higher n-oxPTH reduced the odds for death [50].

To confirm the hypothesis in a second independent cohort that n-oxPTH, but not oxPTH or iPTH, has a predictive value for cardiovascular events and all-cause

mortality, we analyzed baseline plasma samples from 2,867 participants of the EVOLVE trial (ClinicalTrials.gov: NCT00345839) [13]. The patients were followed for up to 64 months. The primary composite end point (PCEP) was the time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event [13]. Pearson's correlation analyses showed a very strong relationship between iPTH and oxPTH ($r = 0.996$; $p < 0.001$) and a weaker relationship between iPTH and n-oxPTH ($r = 0.82$; $p < 0.001$) [52]. A multivariate Cox regression model adjusted for patient characteristics, cardiovascular comorbidities and baseline labs on PCEP revealed that n-oxPTH, but not oxPTH or iPTH, was associated with the EVOLVE primary composite endpoint (time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event, see 13; hazard ratio 1.078; 95% CI 1.012–1.148; $p = 0.020$), cardiovascular mortality (hazard ratio 1.111; 95% CI 1.014–1.218; $p = 0.024$), and all-cause mortality (hazard ratio 1.113; 95% CI 1.038–1.193; $p = 0.003$) [52].

The linear correlation [52] between oxPTH and iPTH measures in patients with secondary hypertension (adults, see 52) and children (Fig. 2) suggests that the currently used iPTH assays primarily describe oxidative stress in CKD patients but not PTH bioactivity – for which these iPTH assays were originally developed.

Fig. 3. It is necessary to dissect the intact parathyroid hormone (iPTH)-mortality curve into a PTH bioactivity curve and an oxidative stress-mortality curve, since both conditions – PTH bioactivity and oxidative stress – will require different treatment approaches. An iPTH assay cannot distinguish between oxidative stress and real PTH bioactivity. Modifications adopted from Floege et al. [9].

In conclusion, a significant proportion of circulating PTH measured by current state-of-the-art iPTH assay systems is oxidized and thus not biologically active. The relationship between oxPTH and n-oxPTH of individual patients varied substantially. n-oxPTH concentrations are 1.5- to 2.25-fold higher in patients with renal failure as compared to healthy controls. Data from an observational trial and a phase 3 clinical study (EVOLVE study) indicate that the predictive power of n-oxPTH and iPTH on the mortality of hemodialysis patients differs substantially. Only n-oxPTH – but not oxPTH nor iPTH – was independently associated with cardiovascular morbidity and all-cause mortality in these studies [51, 52]. The iPTH measures most likely describe oxidative stress in patients with renal failure (Fig. 2), rather than the PTH hormone status/bioactivity. Measurements of n-oxPTH may reflect the PTH hormone status more precisely. We can only improve guidelines and hence patient treatment for CKD-MBD, when we are able to dissect the iPTH mortality curve into an oxidative stress-related mortality curve and an n-oxPTH-related mortality curve, since both conditions, that is, PTH bioactivity and oxidative stress, will require different treatment approaches (Fig. 3). Currently, we do not know what n-oxPTH concentrations are associated with

low and high mortality. Studies like the EVOLVE study would be an ideal tool to define n-oxPTH treatment targets. Measurements of n-oxPTH in daily clinical practice will be most likely a tool to improve patient's outcome. However, n-oxPTH assays will also have limitations. Potential other post-translational PTH modifications such as phosphorylation of certain amino acids of the PTH molecule [53, 54] will not be detected by this type of assay system [49–51]. What we really need is a true PTH bioassay suitable for routine testing – a challenge for scientists working in the field of assay development. A big step in this direction represents the development of assays to determine systemic calcification propensity [55]. The combination of this assay [55] with real assays describing the bioactivity of PTH and also other factors/hormones involved in the pathogenesis of CKD-MBD will be a useful clinical tool to improve patient's outcome in the future. It might be necessary to reevaluate clinical studies in patients with SHPT where iPTH was used as key inclusion criteria or read-out [13, 56–59].

The detection of the biological implications of PTH oxidation in humans [49–52] also affects endocrinology in general. Hormones with amino acids that might be subject to oxidation will most likely also alter their biological activity as well. Our studies [49–52] might stimulate others to analyze the effects of oxidative stress on other hormone's bioactivity. In patients with CKD-MBD, hormones such as Klotho [60, 61] are good candidates for post-translational modifications.

Disclosure Statement

The authors have no conflicts of interest to declare.

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