Parathyroid hormone and anaemia – an erythrocyte osmotic fragility study in primary and secondary hyperparathyroidism

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Summary: Parathyroid hormone (PTH) has been shown *in vitro* to enhance erythrocyte osmotic fragility (EOF) and has been incriminated as a factor in the anaemia seen in patients with primary hyperparathyroidism and in patients with renal disease and secondary hyperparathyroidism. Enhanced EOF has also been shown in patients with chronic renal failure but did not correlate with PTH levels. We studied a group of patients with primary hyperparathyroidism with and without anaemia, and patients with secondary hyperparathyroidism and anaemia. We found that EOF studies in these patients did not differ from normal control groups and that there was no relation between PTH, EOF, and haematocrit in either study group. We conclude that PTH over a range of concentrations seen *in vivo* does not affect erythrocyte osmotic fragility or cause anaemia.

Introduction

Patients with primary hyperparathyroidism are occasionally found to have a normochromic, normocytic anaemia.^{1–4} This anaemia has been the subject of interest because the same mechanisms producing anaemia in patients with primary hyperparathyroidism may be operative in patients with chronic renal failure who also have elevated parathyroid hormone levels. Parathyroid hormone (PTH) has been reported to cause both bone marrow suppression⁵ and increased erythrocyte osmotic fragility (EOF) *in vitro*.^{6,7} We therefore studied patients with mild primary hyperparathyroidism, primary hyperparathyroidism requiring surgery, and secondary hyperparathyroidism to determine if *in vivo* effects of PTH on EOF could be demonstrated.

Methods

Two sets of patients were selected prospectively for investigation of EOF. One was a group of patients

(*n* = 14) with primary hyperparathyroidism undergoing a longitudinal study of the effects of PTH on renal function and the other was a group of haemodialysis patients (*n* = 20) who were stable and had been maintained on haemodialysis for at least 6 months (46 ± 0.8 months). Control data were obtained from 15 healthy volunteers aged 28–35 who were either staff physicians or physicians in training who took no chronic medications. The research protocol was approved by the Brooke Army Medical Center Clinical Investigation and Human Use Committees in accordance with the Helsinki Declaration of 1975.

No patients were taking medications known to suppress PTH.⁸ All blood specimens were obtained between 07.00–08.30 hours in the fasting condition. All specimens were drawn pre-dialysis in the dialysis patients. Multi-channel blood chemistry analysis was done with the Technicon SMAC-2 Auto-analyser and complete blood count and red blood cell (RBC) indices by Coulter Counter. EOF was determined by using the Becton-Dickinson Unopette[®] system (Becton-Dickinson, Rutherford, NJ, USA), which is based on the method of Dacie and Vaughan,⁹ with the phosphate buffer modification described by Parpart *et al.*¹⁰ All EOF testing was completed within 20 minutes of RBC dilution. EOF studies of the control group and each experimental group were performed simultaneously. PTH was measured using antisera against the amino- and carboxy-terminal portions of the PTH molecule in the patients with primary hyperparathyroidism. PTH in the dialysis patients was measured using antisera against the carboxy-terminal

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portion of PTH, the mid-molecule portion, and the intact hormone. All PTH testing was done by the Nichols Institute, San Juan Capistrano, California, USA. These two assays are comparable in specificity and they differ with respect to their normal ranges (Nichols Institute, data on file). Median osmotic fragility was calculated as that percent NaCl solution at which 50% haemolysis had occurred.

All patients with primary hyperparathyroidism had hypercalcaemia and an inappropriately elevated PTH level, biochemical parameters consistent with primary hyperparathyroidism,^{4,11,12} and had been followed by one of the authors (CJF) for over 5 years. All but one of the haemodialysis patients had significant secondary hyperparathyroidism.¹³ In addition, the dialysis patients had normal hepatic transaminases, ferritin and bilirubin levels, reticulocyte (<1.6%), and RBC indices.

Even though the PTH assays are comparable, the units differ. Accordingly, the PTH values are presented with the units and ranges appropriate for the assays. Data are expressed as the mean $\pm$ s.d. The median osmotic fragility, PTH, and haematocrits were compared using the Pearson Product-Moment Coefficient, and the Multiple Correlation Coefficient. The median osmotic fragility of control and experimental groups was also compared using the Wilcoxon rank sum test. Statistical significance was accepted as $P < 0.05$.

Results

EOF studies and median osmotic fragility values were not different between either the primary hyperparathyroid patients and the normal control group or between the dialysis patients and the normal control group (Table I). Only two of the patients with primary hyperparathyroidism were anaemic (haematocrit < 38%), but all of the dialysis patients were anaemic. There was no difference in EOF or median osmotic fragility between the anaemic and non-anaemic patients. There was no correlation between PTH, haematocrit, or median osmotic fragility in any group (Table I).

Discussion

Mallette and colleagues first drew attention to the problem of anaemia in patients with primary hyperparathyroidism, noting that 12 of their 57 patients were anaemic.¹ Falko and associates² and Boxer *et al.*³ noted as did Mallette *et al.*¹ that the anaemia directly correlated with higher calcium, PTH, and alkaline phosphatase levels and concluded that PTH-induced myelofibrosis was responsible for the anaemia.

Chronic renal failure routinely causes both anaemia

Table I Haematocrit, PTH, and median osmotic fragility

	<i>Haematocrit (a)</i>	<i>PTH (b)</i>	<i>Median osmotic fragility (c)</i>
Primary hyperparathyroidism (<i>n</i> = 14)			
Mean	39.9%	195†	0.43 % NaCl
s.d.	5.4%	282	0.02
Range	(26.2–46.0)	(83–1113)	(0.39–0.47)
Secondary hyperparathyroidism (<i>n</i> = 20)			
Mean	27.8%	4912‡	0.42 % NaCl
s.d.	5.7%	3570	0.03
Range	(18.5–34.5)	(371–14730)	(0.37–0.48)
Control (<i>n</i> = 15)			
Mean	44.1%	—	0.436
s.d.	2.7%	—	0.02
Range	(36.0–49.0)	—	(0.39–0.47)
P-HPTH* Pearson product-moment coefficient			
Haematocrit vs PTH	<i>r</i> = −0.0760		a vs b
Haematocrit vs MOF	<i>r</i> = 0.0011		a vs c
MOF vs PTH	<i>r</i> = −0.0036		b vs c
Multiple correlation coefficient	<i>r</i> = 0.4072		
S-HPTH* Pearson product-moment coefficient			
Haematocrit vs PTH	<i>r</i> = 0.00034		
Haematocrit vs MOF	<i>r</i> = −0.00016		
MOF vs PTH	<i>r</i> = −0.00150		
Multiple correlation coefficient	<i>r</i> = 0.00038		

†PTH $\mu\text{LEq/ml}$, normal range 20–100;

‡PTH pg/ml , normal range 430–1860;

*P-HPTH = Primary hyperparathyroidism;

*S-HPTH = Secondary hyperparathyroidism.

and secondary hyperparathyroidism and several studies have identified patients with severe anaemia and bone disease in whom amelioration of hyperparathyroidism either by subtotal parathyroidectomy or calcitriol therapy was followed by an improvement in haematocrit.^{7,14–17} PTH could cause anaemia by resulting in bone marrow fibrosis as suggested by Wainer and associates¹⁸ or by causing a dose-related suppression of peripheral blood burst forming units^{5,7} or by causing enhanced EOF as reported by Bogin and co-workers.⁶ However, Delwiche and colleagues¹⁹ who used highly purified intact bovine PTH could not reproduce these results and suggested that the crude PTH extract used by Meytes *et al.*⁵ may have contained some impurity responsible for their results. These observations also apply to the results of Bogin *et al.*⁶ and the results of Grutzmacher and colleagues.⁷ Godal and co-workers have demonstrated that minor changes in temperature, pH, and buffer strength may affect the results of osmotic fragility testing.²⁰

Three other groups of investigators have studied EOF in patients with renal failure.^{21–24} Two of these

studies are flawed by the facts that the patients had severe renal failure and were not dialysed or had active haemolytic anemias.²² Recently Docci and co-workers reported enhanced osmotic fragility in dialysis patients that did not correlate with secondary hyperparathyroidism and did not change after successful therapy of hyperparathyroidism.^{23,24} They felt that the enhanced osmotic fragility may have been due to aluminium accumulation in erythrocytes since their patients were taking aluminium gels.

Our primary hyperparathyroid patients are typical of those seen in clinical practice since they generally have mild disease and only three have required surgery.^{4,25} Anaemia was unusual and mild when present. The range of PTH levels seen in these patients was less than that reported by Meytes *et al.*⁵ and osmotic fragility was not different from control. Therefore, mild elevations in PTH do not alter EOF.

The dialysis patients, on the other hand, had elevations of PTH far in excess of that noted by Meytes *et al.*⁵ and yet had normal osmotic fragility studies. Our

dialysis patients were taking aluminium gels and are seemingly comparable to the patients described by Docci *et al.*^{23,24} with the exception that our patients had far higher PTH levels (1,465% elevation vs 700%). The aluminium levels in our patients were as elevated as those in Docci's work.^{13,23,24} We, therefore, do not believe that aluminium affected the results. We agree with Docci *et al.* that there is no relationship between PTH and enhanced EOF. Data concerning transfusion and reticulocyte counts is not available from the work of Docci and co-workers and this may have influenced our conflicting results.

EOF studies were normal in patients with primary hyperparathyroidism with and without anaemia, including those patients who subsequently required surgery. Dialysis patients with anaemia and marked secondary hyperparathyroidism also had normal EOF studies. There was no relationship in either group between the level of anaemia and the concentration of PTH. We conclude that PTH found in various *in vivo* states had no effect on either anaemia or EOF.

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