The interaction of PTH and dietary phosphorus and calcium on serum calcitriol levels in the rat with experimental renal failure

A. Martin-Malo1, M. Rodriguez1, M. E. Martinez2, A. Torres3 and A. J. Felsenfeld4

1Unit of Investigation and the Department of Nephrology, Hospital Universitario Reina Sofia Cordoba; 2Hospital La Paz, Madrid; 3Department of Nephrology, Hospital Universitario, Tenerife, Spain; and 4Department of Medicine, West Los Angeles VA Medical Center and UCLA, Los Angeles, CA, USA

Abstract

Background. Renal failure results in decreased calcitriol production, a key factor in the development of secondary hyperparathyroidism. Phosphorus accumulation and high parathyroid hormone (PTH) levels, both inherent to renal failure, have different effects on calcitriol production; moreover, dietary calcium loading may have a separate inhibitory effect on calcitriol production. This study was designed to evaluate the relative effects of PTH and dietary phosphorus and calcium on serum calcitriol levels.

Methods. Renal failure was surgically induced and rats were divided into normal, moderate renal failure, and advanced renal failure based on the serum creatinine. Each group was subdivided and received either a high-phosphorus diet (HPD, 0.6% Ca, 1.2% P) or high-calcium diet (HCaD, 1.2% Ca, 0.6% P) for 14-16 days to determine the relative effects of dietary calcium and phosphorus loading on serum calcitriol. In addition, the effect of PTH and phosphorus on calcitriol stimulation was determined with a 48-h PTH infusion combined with either a low (0.16%) or high (1%) phosphorus diet; both diets had negligible calcium (<0.05%).

Results. With decreasing renal function, PTH increased and was greater in rats fed the HPD than the HCaD; serum calcitriol decreased as renal function decreased and was lower in normal rats and rats with moderate renal failure fed a HCaD (P<0.01). The calcitriol response to a PTH infusion decreased as renal function decreased (P<0.05) but was greater on a low- (0.16%) than a high- (1%) phosphorus diet (P<0.05).

Conclusions. Dietary calcium loading either directly decreases serum calcitriol or acts by modifying the stimulatory effect of PTH; the stimulatory effect of PTH on serum calcitriol is modified by dietary phosphorus; in moderate renal failure, serum calcitriol levels depend on a complex interaction between PTH and dietary calcium and phosphorus; and in advanced renal failure, serum calcitriol levels are low and are difficult to stimulate, presumably because of the loss of renal mass.

Key words: calcitriol; calcium; phosphorus; PTH; rat; renal failure

Introduction

A calcitriol deficiency is an important pathogenic factor in the development of secondary hyperparathyroidism in renal failure [1,2]. Although calcitriol synthesis is decreased in renal failure because of a reduction in renal mass [3,4], clinical studies of patients with moderate renal failure have reported both low and normal serum calcitriol levels [3-6]. This may be due to the balance between the inhibition of calcitriol by an increased phosphorus load and without hyperphosphataemia [7,8] and stimulation of calcitriol by increased PTH levels [9,10]; moreover, although not usually considered as a factor, dietary calcium and changes in serum calcium may also affect calcitriol production [5,11,12].

While reducing the magnitude of dietary phosphorus absorption, a high-calcium diet may, as has been shown in azotaemic rats [11], independently reduce calcitriol levels. The daily amount of calcium consumed in a normal human diet is approximately 800 mg. Thus, the daily use of 2 g of a calcium-based phosphate binder will result in a doubling of calcium ingestion.

In patients with moderate renal failure, it is unclear to what extent PTH and the dietary content of phosphorus and calcium are capable of modifying calcitriol production. Clarification of the relative roles of these factors may help to explain the observed differences in serum calcitriol levels and may lead to a better understanding of the prevention and treatment of secondary hyperparathyroidism. The goal of this study was to evaluate in rats with different levels of renal function, the relative effects of secondary hyperparathyroidism...
artery in the hilum of the left kidney, followed 1 week later by right nephrectomy (2/3 Nx); and the third group, ligation of two branches of the main renal artery in the hilum of the left kidney, followed 1 week later by right nephrectomy (5/6 Nx). Rats were pair fed and randomly placed on either a high-phosphorus diet (HPD) containing 1.2% phosphorus and 0.6% calcium or a high-calcium diet (HCaD) containing 1.2% calcium and 0.6% phosphorus. Both diets contained 100 IU/100 g of vitamin D and had a similar caloric content.

Fig. 1. Schematic representation of the study protocol.

Statistics

Comparisons of the means of more than two groups were performed by ANOVA followed by the Duncan test. The unpaired t test was used to compare two different groups. Results are shown as the mean ± standard error (SE).
Results

Serum levels of calcium, phosphorus, PTH and calcitriol after the high phosphorus diet (HPD) and high calcium diet (HCaD) (Table 1, Figure 2)

The HPD resulted in a decrease in serum calcium only in rats with advanced renal failure; by contrast, the serum calcium was normal in all rats on a HCaD including those with advanced renal failure. The HPD resulted in hyperphosphataemia in rats with moderate and advanced renal failure; with the HCaD, the serum phosphorus did not increase despite renal failure (Table 1).

The ingestion of a HPD induced marked secondary hyperparathyroidism in azotaemic rats (Figure 2A), while rats with the same degree of renal failure on a HCaD had only a moderate increase in the serum PTH level. As shown in Figure 2B, the serum calcitriol level decreased as renal function decreased. Except in advanced renal failure rats, serum calcitriol was greater in the HPD than the HCaD group. Thus, in rats with normal renal function, the basal serum calcitriol level was 179±15 and 112±8 pg/ml, (P<0.01) in the HPD and HCaD groups respectively and this difference was not associated with differences in serum phosphorus, calcium (Table 1) or PTH (Figure 2A). In rats with moderate renal failure, the serum calcitriol was greater in the HPD than the HCaD group (140±10 vs 77±6 pg/ml, P<0.01) and this was associated with a higher basal PTH level in the HPD group, (177±26 vs 70±6 pg/ml, P<0.01) (Figure 2A). Since basal serum phosphorus was also increased in the HPD group (Table 1), the higher serum calcitriol level in the HPD group should be due to the higher PTH level. Finally, in rats with advanced renal failure, serum calcitriol was similarly decreased in the HCaD and HPD groups.

The effect of PTH infusion on serum calcium and phosphorus (Table 1)

The 48-h PTH infusion increased the serum calcium in both the HPD and HCaD groups. However the calcaemic response to PTH was less in rats with renal failure and was further decreased by the ingestion of the 1% phosphorus diet. In advanced renal failure, the PTH infusion did not increase the serum calcium in rats ingesting the 1% phosphorus diet.

The combination of the PTH infusion and the ingestion of the 0.16% phosphorus diet resulted in serum levels of phosphorus that were similarly decreased in all groups of rats, including those with moderate and advanced renal failure. In contrast, the combination of the PTH infusion and the 1% phosphorus diet resulted in serum levels of phosphorus that were similarly decreased in all groups of rats, including those with moderate and advanced renal failure.

Table I. The serum calcium and phosphorus concentration in the HPD and HCaD groups before the PTH infusion and after the PTH infusion combined with either a 0.16 or 1% phosphorus diet.

<table>
<thead>
<tr>
<th>Serum calcium (mmol/l)</th>
<th>Cr&lt;0.5 mg/dl</th>
<th>Cr 0.5–0.7 mg/dl</th>
<th>Cr&gt;0.7 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPD</td>
<td>HCaD</td>
<td>HPD</td>
</tr>
<tr>
<td>BASAL</td>
<td>2.58±0.03</td>
<td>2.45±0.05</td>
<td>2.43±0.05</td>
</tr>
<tr>
<td>PTH+1% P</td>
<td>3.55±0.10</td>
<td>3.20±0.12</td>
<td>3.58±0.05</td>
</tr>
<tr>
<td>PTH+0.16% P</td>
<td>4.30±0.20</td>
<td>4.28±0.02</td>
<td>3.33±0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum phosphorus (mmol/l)</th>
<th>Cr&lt;0.5 mg/dl</th>
<th>Cr 0.5–0.7 mg/dl</th>
<th>Cr&gt;0.7 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASAL</td>
<td>2.5±0.1</td>
<td>2.7±0.3</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>PTH+1% P</td>
<td>1.6±0.1</td>
<td>2.0±0.2*</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>PTH+0.16% P</td>
<td>1.8±0.1</td>
<td>2.0±0.1</td>
<td>1.7±0.2</td>
</tr>
</tbody>
</table>

Mean±SE. *P<0.05 as compared with HPD. BASAL, values before the PTH infusion; PTH+1% P, values after the PTH infusion combined with a 1% phosphorus diet; PTH+0.16% P, values after the PTH infusion combined with a 0.16% phosphorus diet.
phorus diet resulted in an increase in serum phosphorus in all azotaemic groups, but levels were higher in the HPD group. Finally, in rats with normal renal function, the infusion of PTH plus the 1% phosphorus diet resulted in a higher serum phosphorus in the HCaD than HPD group, although the serum phosphorus was in the low normal range for both groups.

Calcitriol stimulation during the PTH infusion in rats with normal renal function (Figure 3)

The PTH infusion together with the ingestion of 0.16% phosphorus diet increased the serum calcitriol ($P < 0.01$) to a similar level in the HPD and HCaD groups ($401 \pm 38$ vs $417 \pm 46$ respectively). The infusion of PTH combined with the 1% phosphorus diet also resulted in a similarly increased serum calcitriol level ($P < 0.05$) in the HCaD and HPD groups. However, in both groups, the magnitude of the increase in serum calcitriol was less during the ingestion of the 1% than the 0.16% phosphorus diet despite no difference in serum phosphorus levels (Table 1).

Calcitriol stimulation during a PTH infusion in rats with moderate renal failure (Figure 4)

After the PTH infusion, the serum calcitriol was greater ($P < 0.05$) in the HCaD than the HPD group and in each group the 1% phosphorus diet resulted in a lower calcitriol level than the 0.16% phosphorus diet (Figure 4). In rats with moderate renal failure, differences in the dietary content of phosphorus (0.16 and 1%) during the PTH infusion led to differences in serum phosphorus (Table 1). Since the amount of PTH infused was the same, the difference in the calcitriol response between the 0.16 and 1% phosphorus diet should be attributable to serum phosphorus.

In the HPD group, the PTH infusion with a 1% phosphorus diet failed to increase calcitriol (Figure 4). Moreover, even the 0.16% P diet did not result in a significant increase in serum calcitriol during the PTH infusion. Despite the fact that as compared with baseline, neither the 0.16% nor the 1% P diet increased serum calcitriol during the PTH infusion, serum calcitriol was greater with the 0.16% diet than the 1% phosphorus diet ($163 \pm 28$ vs $96 \pm 23$ pg/ml, $P < 0.05$).

In the HCaD group, serum calcitriol levels after the PTH infusion with the 0.16% and 1% phosphorus diets were $245 \pm 19$ and $179 \pm 24$ pg/ml respectively ($P < 0.05$).

Calcitriol stimulation during a PTH infusion in rats with advanced renal failure (Figure 5)

In the HPD group, the PTH infusion with either the 0.16% P or 1% P diet did not increase serum calcitriol (Figure 5). During the PTH infusion in the HCaD group, serum calcitriol was unchanged during the ingestion of the 1% P diet but increased on the 0.16% P diet ($47 \pm 7$ vs $106 \pm 31$ pg/ml, $P < 0.05$).

Discussion

We have studied the respective roles of PTH and dietary calcium and phosphorus loading on the production of calcitriol and how this effect is modified by renal failure. Our results indicate that in addition to the effect of the residual renal mass, calcitriol production was stimulated by high PTH and inhibited by
high dietary phosphorus and calcium. On the HPD, the inhibitory effect of phosphorus loading was counterbalanced by the concurrent increase in PTH. The HCaD resulted in an inhibition of calcitriol despite normal PTH levels in normal rats and elevated PTH levels in rats with moderate renal failure. Except in advanced renal failure, calcitriol production was stimulated by PTH and inhibited by phosphorus loading.

**Rats with normal renal function**

In normal rats on a HCaD, PTH and serum calcium were normal; nevertheless the serum calcitriol level was approximately 60% of that in the HPD group. In a previous study [11], the serum calcitriol level in normal rats on a HPD was similar to rats on a 0.6% calcium and 0.6% phosphorus diet; thus the calcitriol level in the normal rats on HPD would appear to represent normal serum values. Thus a high-calcium diet would seem to decrease calcitriol levels by a mechanism that is independent of changes in the serum levels of PTH, calcium and phosphorus. The administration of the same amount of PTH to the HPD and HCaD groups led to a similarly increased serum calcitriol concentration despite concomitant increases in serum calcium; thus the stimulatory effect of high PTH overcame any inhibitory effect of hypercalcaemia. In both groups a thus the stimulatory effect of high PTH overcame any inhibition despite concomitant increases in serum calcium; led to a similarly increased serum calcitriol concentration.

**Rats with moderate renal failure**

The basal serum calcitriol was lower in rats with moderate renal failure than normal rats which indicates that a decreased renal mass limited calcitriol production. However, serum calcitriol was considerably greater in the HPD than the HCaD group; thus the stimulatory effect of PTH predominated over the inhibitory effect of phosphorus loading which even resulted in hyperphosphataemia. Furthermore, the inhibitory effect of the high-calcium diet was also evident since despite PTH levels that were approximately twice normal, calcitriol levels were less than in the HPD group. An extrapolation of the results of our study to the clinical setting would suggest that the use of a calcium-based phosphate binder, which in recommended doses may double the daily intake of calcium, has the potential to reduce serum calcitriol levels; whether this sequence of events does occur in patients with moderate renal failure receiving a calcium-based phosphate binder needs to be evaluated.

In both the HPD and HCaD groups, the serum calcitriol levels were increased after the PTH infusion but were greater when rats ingested a low-phosphorus (0.16%) than a high-phosphorus (1%) diet. These data indicate that rats with moderate renal failure have the capability to increase calcitriol production in response to PTH; moreover, for a similarly high PTH level, the dietary load of phosphorus with corresponding changes in serum phosphorus, becomes an important regulator of calcitriol production. However for the same amount of PTH infused and the same dietary phosphorus load (1%), the rats on HCaD had a higher serum calcitriol than the HPD group in which serum calcitriol did not increase despite the PTH infusion; this may be explained by the higher serum phosphorus in the HPD group. This result would indicate that proportional changes in PTH and phosphorus may neutralize each other resulting in no change in calcitriol.

The PTH infusion combined with 0.16% phosphorus diet resulted in higher calcitriol levels in the HCaD than the HPD group. This difference cannot be attributed to serum levels of calcium or phosphorus; however, it is possible that the HPD rats with higher calcitriol levels and higher PTH at baseline had a downregulation of the PTH receptor due to the high PTH levels [16–18]. In addition one must also consider the possibility that the greater phosphorus burden present in the HPD group at the end of the study diet may have contributed to the lower calcitriol level in the HPD group during the PTH infusion.

The results of the PTH stimulation of calcitriol in the groups with moderate renal failure may help to explain some of the conflicting results of recent clinical studies in patients with moderate renal failure [5,6]. Prince et al. reported that patients with moderate renal failure (mean GFR 44 ± 20 ml/min) had serum calcitriol levels that were approximately one-half normal;
these patients increased serum calcitriol levels by more than 50% after receiving a calcium restricted diet [5]. Thus these investigators were able to demonstrate that a reduction in dietary calcium either directly or in conjunction with PTH was able to stimulate calcitriol. Our rats on a HCaD also increased calcitriol in response to a combination of calcium restriction and an increase in PTH. Conversely Ritz et al. [6] have reported an attenuated calcitriol response to exogenous PTH infusion in patients with moderate renal failure (mean GFR 53 ml/min). Before the PTH infusion, these patients had a normal serum calcitriol and almost a threefold increase in intact PTH; after the PTH infusion, serum calcitriol increased by less than 14% in the azotaemic patients as opposed to a 100% increase in the normal controls. In essence, these azotaemic patients may be analogous to the rats in the HPD group in which baseline serum calcitriol approached normal with a fourfold increase in PTH; moreover these rats failed to further increase calcitriol during a PTH infusion. Thus it is possible that calcitriol production may be maximally stimulated by a three- to fourfold increase in PTH and higher PTH levels do not further stimulate calcitriol production. This may not be an unreasonable assumption since in normal humans, the maximal PTH level is three to four times greater than the basal PTH [19].

Rats with advanced renal failure

In rats with advanced renal failure, serum calcitriol levels were decreased in both HPD and HCaD groups. Except for a modest increase to subnormal levels in the HCaD groups, calcitriol could not be stimulated despite the combination of PTH infusion with a phosphorus restricted diet. These findings resemble results from clinical studies in patients with advanced renal failure [3,4,7,20]. The results of this study are summarized as follows: (1) calcitriol production decreases with renal failure; (2) calcitriol production can be modulated in normal rats and in rats with moderate renal failure; by contrast in advanced renal failure, calcitriol production is invariably low; (3) calcitriol production decreases with dietary calcium loading and this effect can be observed even in the absence of hypercalcaemia and despite high PTH levels; and (4) PTH stimulates and dietary phosphorus inhibits calcitriol production and both can counteract the effect of the other.

In conclusion, the serum calcitriol concentration in moderate renal failure may be low, normal, or even high depending on a complex interaction between the different effects of PTH, and dietary phosphorus and calcium.

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References


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