Renal handling of calcium and phosphorus in experimental renal hyperparathyroidism in dogs

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Abstract – Twenty-four hour urinary excretion, fractional excretion and the filtered load of calcium and phosphorus were monitored as hyperparathyroidism evolved in a model of progressive canine renal failure. Thirteen beagles of both sexes aged four and a half months were used. Nine of them were subjected to a renal damaging schedule (neomycin, 60 mg/kg/48 h, IM, 32 weeks) in order to induce chronic renal failure leading to secondary hyperparathyroidism (2HPT group). The remaining four were kept as the control group. The experiment was conducted over 32 weeks. Blood and 24 h urine were collected every four weeks. Calcium, phosphorus and creatinine were analyzed. Plasma parathormone and calcitonin were determined at weeks 0, 12, 24 and 32. The level of renal function in the 2HPT animals was reduced to 25% of that of the controls (endogenous creatinine clearance was 0.45 ± 0.22 mL/min/kg as opposed to 1.81 ± 0.54 mL/min/kg). Hyperparathyroidism was confirmed by a progressive increase in the levels of the parathyroid hormone. Calcitonin levels were not modified. A tendency to hypocalcaemia was observed, reaching statistically significant levels from the twenty-eighth week of the study, when hyperphosphataemia also became significant. Daily urinary excretion of calcium and phosphorus remained at values considered normal throughout the experiment with no alteration imputable to the impaired renal function. This is explained by the decrease in the filtered load of these elements (in both cases statistically significant from the 24th week on) being associated with an increase in their fractional excretion. Thus, calcium and phosphorus urinary excretion values could be maintained in a normal range up to the end of the experiment, showing that renal calcium handling in dogs with experimentally induced renal failure seems to differ from that observed in human patients.

renal hyperparathyroidism / calciuria / phosphaturia / parathyroid hormone / calcitonin / dog

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1. INTRODUCTION

The metabolism of calcium and phosphorus is considerably disturbed during uraemia, and is involved in the development of renal hyperparathyroidism, although in the early stages of renal failure, hypocalcaemia and hyperphosphataemia are not analytically detectable [26].

In the kidney, the parathyroid hormone (PTH) acts to reduce both proximal and distal tubular reabsorption of phosphates, bringing about a greater fractional excretion (FEP) of them, and thus increasing the total urinary excretion. With regards to the urinary excretion of calcium, PTH seems to lead to an increase in total reabsorption both at the level of the thick ascending limb of the Henle’s loop and at the level of the distal tubule, with an overall effect on calcium retention [27].

These effects are not easy to identify when the disease process of hyperparathyroidism is secondary to renal failure, because in this case the physiological action of PTH interacts with the excretory and metabolic changes associated with the renal disorder. The renal handling of phosphorus (P) in renal failure has been widely studied in humans and animals, but this is not the case for the renal handling of calcium (Ca). In humans, it has been observed that although the overall urinary excretion of calcium is generally reduced, the fractional excretion of calcium (FECa) increases in advanced renal failure [4] but it does not happen the same in moderate renal failure [22]. The findings reported in animals [2, 20] have not been able to clarify this matter. For this reason, further studies are needed to throw light on the pathogenesis of hyperparathyroidism subsequent to renal failure.

With regards to calcitonin (CT), a large percentage of human patients with renal failure shows high levels in plasma [9, 12, 16]. In fact, CT has been included as one of the pathogenic factors involved in the development of renal secondary hyperparathyroidism, although its importance has not been established [13].

The purpose of this study was to determine the evolution of (a) the total and fractional urinary excretion of calcium and phosphorus, and (b) the plasma levels of parathyroid hormone and calcitonin, during the progression of an experimental hyperparathyroidism subsequent to renal failure in dogs.

2. MATERIALS AND METHODS

2.1. Animals and diets

Thirteen beagles of both sexes, reared in our facilities and kept in standard conditions in compliance with Spanish law [23] and European guidelines [5], were used. They were fed on commercial dog food (Hill’s Canine Maintenance, Hill’s Pet Nutrition, Topeka, Kansas, USA), with a calcium content of 1.1% and a phosphorus content of 0.8%, and had free access to water.

2.2. Renal damage schedule

Two groups were randomly formed four and a half months after the animals were born: a control group (four normal untreated dogs) and a secondary hyperparathyroidism group (2HPT, nine dogs). Hyperparathyroidism secondary to chronic renal failure (CRF) was induced by administering neomycin sulphate (anhydrous assay 706 µg/mg and approximate value in use 686 µg/mg) at a dose of 60 mg/kg of body weight, IM, every second day [10] for thirty-two weeks.

2.3. Experimental protocol

Every four weeks, starting prior to the first administration of the nephrotoxic drug, and ending with the end of the experiment, samples of blood and urine excreted over 24 h were taken. All blood samples
were taken early in the morning, when the animals left the metabolic cage after fasting for 24 h. The samples were collected from the cephalic vein, using heparin to obtain plasma, which was stored in plastic tubes at –18 °C within an hour of being taken, and kept thus until biochemical and hormonal analysis.

To obtain the twenty-four-hour urine samples, metabolic cages were used, the dogs being allowed only water ad libitum for this period. Before the animals were put in these cages, the urinary bladder was catheterized and emptied, and the urine discarded. At the end of the 24 h, the bladder was catheterized again and the urine pooled with that gathered in the metabolic cage so as to obtain the total quantity of urine produced in the 24-h period time. A fixed quantity of urine was then stored at –18 °C until biochemical analysis.

2.4. Analysis and calculations

Reagent kits and an automated discrete analyzer (Hitachi 704, Boehringer Mannheim, Mannheim, Germany) were used to determine the concentrations of urine and plasma creatinine, inorganic phosphorus and calcium, in order to calculate the filtered load, fractional excretion and 24-h urinary excretion of calcium and phosphorus, and endogenous creatinine clearance.

Endogenous creatinine clearance (CCl) was expressed as a function of the animal’s weight and calculated by means of the following formula:

\[
CCl = \frac{[\text{urine creatinine}] \times (\text{urine volume/min})}{[\text{serum creatinine}]}
\]

FECa and FEP were calculated as:

\[
\text{FE (Ca/P)} = \frac{[\text{urine Ca/P}] \times [\text{serum creatinine}] \times 100}{[\text{urine creatinine}] \times [\text{serum Ca/P}]}
\]

Filtered load of calcium (FLCa) and phosphorus (FLP) was obtained thus:

\[
\text{FL (Ca/P)} = [\text{serum Ca/P}] \times \text{CCl}
\]

2.5. Statistics

A two-way variance analysis was carried out to make a comparative study of the control group and 2HPT group and to assess each group’s evolution over time. Whenever significant differences were found in any of the parameters, the Newman-Keuls test was used to check the differences between the two groups. The whole study was effected by means of the Statistica 5.1 program (StatSoft, Tulsa, Oklahoma, USA).

3. RESULTS

The two-way variance analysis revealed statistically significant differences between groups, between weeks and in the group/week interaction for all parameters except calciuria, phosphaturia and plasma calcitonin. The one-way variance analysis to assess the effect of the evolution of the different parameters revealed no statistically significant differences between the two groups for any of the three parameters mentioned (Tabs. I and II).

Regarding the creatinine clearance (Fig. 1), a progressive decrease was observed in the 2HPT group, which was statistically significant after the 16th week until the end of the experiment, when it had mean values (0.45 ± 0.22 mL/min/kg) of about 25% of those of the control animals (1.81 ± 0.54 mL/min/kg).

PTH levels in plasma (Tab. I) remained stable in the control animals while in the 2HPT there was a constant and progressive increase. The average figure at week 12
was double the average of week zero and, in some cases, by the end of the experiment had increased twenty-fold.

Plasma Ca levels (Fig. 2) in the control animals remained very stable throughout the experiment, while the 2HPT group showed similar values until week 24, thereafter tending to decrease slightly, with statistical significance since the week 28, although the values (2.13 ± 0.09 mmol/L) were not clearly hypocalcaemic at the end of the experiment.

Plasma P (Fig. 3) in the control animals decreased gradually from the outset until the end of the experiment. The 2HPT animals evolved similarly until week 20, whereafter the levels tended to rise, with statistical significance since week 28, and hyperphosphataemia was clearly present in the last week (3.71 ± 1.29 mmol/L).

FECa (Fig. 4) remained under 0.7% throughout the experiment in the control group. In the 2HPT group, a constant and progressive increase was observed from week 4, although statistically significant differences were only reached after week 12, those of the last reading being highly significant, when they were up to six times higher than those of the control animals (2.93 ± 1.08% as opposed to 0.45 ± 0.17%).

Table I. Mean ± SD values of plasma parathyroid hormone (PTH) and calcitonin (pg/mL) in the control (n = 4) and secondary hyperparathyroidism (2HPT) groups (n = 9).

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>2HPT</th>
<th>Control</th>
<th>2HPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.85 ± 2.51</td>
<td>8.21 ± 2.27</td>
<td>8.16 ± 1.65</td>
<td>8.76 ± 0.56</td>
</tr>
<tr>
<td>12</td>
<td>7.16 ± 1.15</td>
<td>17.38 ± 3.82 ***</td>
<td>8.24 ± 1.29</td>
<td>8.58 ± 1.95</td>
</tr>
<tr>
<td>24</td>
<td>7.62 ± 1.94</td>
<td>37.49 ± 11.09 ***</td>
<td>7.81 ± 1.34</td>
<td>6.54 ± 1.66</td>
</tr>
<tr>
<td>32</td>
<td>10.37 ± 1.00</td>
<td>98.67 ± 70.07 *</td>
<td>8.13 ± 2.29</td>
<td>6.67 ± 1.97</td>
</tr>
</tbody>
</table>

Significant differences between groups are expressed as: * P < 0.05; ** P < 0.01; *** P < 0.001.

Table II. Mean ± SD values of urinary calcium and phosphorus excretion (mg/24 h) in the control (n = 4) and secondary hyperparathyroidism (2HPT) groups (n = 9) during the experiment. The differences between the groups were not significant.

<table>
<thead>
<tr>
<th>Week</th>
<th>Urinary calcium</th>
<th>Urinary phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2HPT</td>
</tr>
<tr>
<td>0</td>
<td>10.86 ± 2.14</td>
<td>10.66 ± 5.50</td>
</tr>
<tr>
<td>4</td>
<td>12.55 ± 4.82</td>
<td>11.23 ± 2.50</td>
</tr>
<tr>
<td>8</td>
<td>10.48 ± 4.28</td>
<td>12.90 ± 5.90</td>
</tr>
<tr>
<td>12</td>
<td>12.41 ± 5.78</td>
<td>11.25 ± 4.32</td>
</tr>
<tr>
<td>16</td>
<td>10.63 ± 5.55</td>
<td>12.67 ± 5.22</td>
</tr>
<tr>
<td>20</td>
<td>11.06 ± 6.58</td>
<td>15.01 ± 5.97</td>
</tr>
<tr>
<td>24</td>
<td>15.91 ± 5.14</td>
<td>14.23 ± 5.16</td>
</tr>
<tr>
<td>28</td>
<td>12.68 ± 6.61</td>
<td>12.20 ± 5.86</td>
</tr>
<tr>
<td>32</td>
<td>6.58 ± 2.13</td>
<td>8.92 ± 3.13</td>
</tr>
</tbody>
</table>
Urinary calcium and phosphorus in renal hyperparathyroidism

FEP (Fig. 5) was quite homogeneous in the control group throughout the experiment, but in the 2HPT group there was a constant and progressive tendency to increase, statistically significant from week 16 on, so by the end of the experiment the values were three times those of the control group (51.38 ± 20.24% as opposed to 15.22 ± 1.15%).

FLCa (Fig. 6) varied in both groups in a way comparable to the CCI, so in the 2HPT group, a progressive decrease was observed, reaching statistical significance from week 16 until the end of the experiment.

FLP (Fig. 7) evolved in almost exactly the same way as the FLCa, but in the last reading the values for the 2HPT group rose
slightly in comparison with those of the previous one.

4. DISCUSSION

One of the procedures used for assessing the glomerular filtration rate (GFR) is the CCl [1]. The progressive and constant decrease in this parameter in the 2HPT animals, down to values indicating an average loss of kidney function of over 75% together with a simultaneous increase in PTH levels, allowed us to conclude that these animals developed secondary hyperparathyroidism subsequent to CRF.

In the control group, phosphataemia decreased continuously from the beginning of the experiment, in line with the well-known physiological fact that
phosphataemia in immature dogs is higher than in adults [19]. In the 2HPT group, the initial evolution of phosphataemia is the same as in the control group, but from week 20 on, a slight and continuous increase was noticed, which gave rise to obvious hyperphosphataemia at the end of the experiment. The development of hyperphosphataemia in the final stages of CRF is well known, having been widely described both in clinical cases and in experimental models [7, 20, 30, 31]. This is explained by the inability of the mechanisms of kidney adaptation to keep up normal levels as the lesion worsens [3].

Literary references show that the P balance is maintained as GFR and the filtered load decrease, at the expense of an FEP increase. In more advanced renal failure, the P excretion is maintained thanks to an additional FEP rise, accompanied by an increase of plasma P [15]. Our results also confirmed this fact, because the plasma P increase seemed to be responsible for the disappearing of the FLP tendency to decrease in the last sampling.

Calcaemia values in the 2HPT group remained normal until week 24, with a slight hypocalcaemia being noted thereafter, becoming significant towards the end of the experiment. This fact seems to show that hormonal regulation of Ca in animals with iatrogenic chronic renal insufficiency functions effectively, thus maintaining normal levels of calcaemia over a long period of time. This has been attributed to the increased production and release of PTH, observable in the early stages of this disease model, and the adaptation of the remaining renal mass to the new situation [21]. High circulating levels of PTH have been detected in the early stages of CRF [14, 24], and have been attributed to a reduction in serum calcitriol and moderate decreases of ionized Ca [25].

Calciuria values were similar to those of healthy beagles [6] and were kept stable during the whole experiment in the 2HPT group, despite the filtered load decrease, at the expense of an elevation of the FECa values. This fact has also been observed in experimental models obtained by renal ablation in rats [8, 18].

With regards to dogs suffering renal hyperparathyroidism, we were only able to find figures for total urinary excretion of both Ca and P in the experimental work of Norrdin et al. [17], who recorded increased excretion of both in animals with marked uraemia. These data should, however, be treated cautiously, as in this study, there was not only hyperphosphataemia, but also a considerable hypercalcaemia. The author suggests that this fact could be a result of the experimental method, related to the destruction of subcortical nephrons, still developing in neonates, and a shift of the blood flow to inner-cortical and yuxtamedullary glomeruli with a differing tubular and vascular pattern.
Nevertheless, the increase in FECa has also been described in dogs in both clinical and experimental cases [11, 20, 30]. The increase in FECa, in spite of the hyperparathyroidism, remains to be elucidated, but has been suggested to be the result of an increase in the FLCa by the remaining nephrons [11, 28].

In humans, however, FECa and calciuria diminish with moderate renal failure [22]. This difference has been explained as a consequence of a better renal maintenance of calcitriol production by the remnant kidney in animals subjected to renal ablation. Another explanation could be the higher quantity of Ca and vitamin D provided by commercial animal diets [15].

In human patients with advanced renal failure, an FECa increase has been observed. The mechanism mediating this FECa elevation in humans remains unclear but it could be associated with acidosis, severe suppression of calcitriol production, an increase in the rate of distal nephron flow and expansion of the extracellular fluid volume [15].

In human patients with kidney failure, CT has been related to disorders in the metabolism of P and Ca during the development of renal secondary hyperparathyroidism [9, 12, 16]. In this experimental model of CRF in dogs, we were not able to prove the role of CT since no alteration was observed in the levels of this hormone during the evolution of CRF. We could not determine whether this was due to a difference imputable to the species, to the duration of the renal failure or to the treatment received [9], because all human patients who showed increased CT levels were undergoing dialysis or conservative treatment.

In conclusion, we must point out that dogs with drug-related renal secondary hyperparathyroidism in a moderate degree presented a renal P handling similar to human patients with moderate uraemia, but a different renal Ca handling. The factors involved in this different pattern must be studied in depth.

ACKNOWLEDGEMENTS

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REFERENCES


[23] Real Decreto 223/1988, de 14 de marzo, sobre protección de los animales utilizados para experimentación y otros fines científicos, BOE No. 67 de 18/03/88, pp. 8509–8512.


