Effect of hydrochlorothiazide on 1,25-dihydroxyvitamin D₃-induced changes in calcium metabolism in experimental hypoparathyroidism in rats

R. RIZZOLI, K. HUGI, H. FLEISCH AND J.-P. BONJOUR
Department of Pathophysiology, University of Berne, Murtenstrasse 35, 3010 Berne, Switzerland

(Received 7 February/8 July 1980; accepted 28 July 1980)

Summary

1. Chronic administration of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] can normalize plasma calcium in human hypoparathyroidism and in thyroparathyroidectomized animals. The effect of 1,25(OH)₂D₃ on plasma calcium is associated with an increase in urinary calcium excretion. In an attempt to prevent this increase, thyroparathyroidectomized rats receiving 1,25(OH)₂D₃ were also treated with hydrochlorothiazide for 9-11 days.

2. Calcium clearance studies show that hydrochlorothiazide stimulated the tubular reabsorption of calcium in thyroparathyroidectomized rats treated with 1,25(OH)₂D₃.

3. Calcium balance and kinetic studies indicated that hydrochlorothiazide decreased 1,25(OH)₂D₃-induced hypercalciuria in thyroparathyroidectomized rats. Hydrochlorothiazide did not affect the 1,25(OH)₂D₃-induced increase in plasma calcium. The hypocalciuric effect of hydrochlorothiazide was not associated with significant changes in calcium deposition into or release from bone.

4. In thyroparathyroidectomized rats treated with 1,25(OH)₂D₃, the hypocalciuric effect of hydrochlorothiazide was associated with a fall in intestinal calcium absorption. Overall, the calcium balance was unaffected.

5. Thus it appears that hydrochlorothiazide reduces the 1,25(OH)₂D₃-induced hypercalciuria in parathyroid hormone-deficient animals by decreasing intestinal calcium absorption.

Despite the decreased absorption, hydrochlorothiazide does not reduce the 1,25(OH)₂D₃-induced increase in plasma calcium.

Key words: bone, calcium, calciuria, 1,25-dihydroxyvitamin D₃, hydrochlorothiazide, intestinal, thyroparathyroidectomy, tubular calcium reabsorption.

Abbreviation: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin DGRAY

Introduction

Vitamin D derivatives have been used for many years in the treatment of patients with hypocalcaemia due to deficient parathyroid function. However, the correction of the hypocalcaemia is accompanied by hypercalciuria with a risk of nephro lithiasis (Fourman & Royer, 1968; Potts & Deftos, 1969). More recently the production of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] has been shown to fall after removal of the parathyroid glands in rats (Garabedian, Holick, DeLuca & Boyle, 1972). The low plasma 1,25(OH)₂D₃ level in hypoparathyroid patients (Hausser, Baylink, Hughes, Brumbaugh, Wergedal, Shen, Nielsen, Counts, Bursac & McCain, 1976) is consistent with these findings. Therefore it appears rational to use 1,25(OH)₂D₃ in preference to other vitamin D derivatives in the management of hypoparathyroidism. Indeed clinical studies have clearly documented the potency of 1,25(OH)₂D₃ in elevating plasma calcium levels in hypoparathyroidism (Russell, Walton, Smith, Preston, Basson, Henderson & Norman, 1974). However, 1,25(OH)₂D₃ does not appear to be
superior to any other vitamin D derivative in avoiding the increase in calcium excretion which accompanies the elevation of plasma calcium. Therefore other forms of treatment have to be considered which might reduce this hypercalciuria.

Acute and chronic administration of thiazide diuretics have been shown to reduce calciuria in various experimental and clinical conditions by a mechanism which is still controversial (Yendt & Cohanim, 1978). We have reported that, in thyroparathyroidectomized rats, 1,25(OH)2D3, given in doses which correct the intestinal calcium absorption, tended to normalize the level of plasma calcium (Rizzoli, Fleisch & Bonjour, 1977). However, as in hypoparathyroid patients, 1,25(OH)2D3 led to a rise in urinary calcium excretion. This effect was not due to a change in the tubular handling of calcium but was entirely explained by an increase in the filtered load of calcium (Hugi, Bonjour & Fleisch, 1979).

In the present study the thyroparathyroidectomized rat was used as an experimental model of human hypoparathyroidism to determine whether hypercalciuria complicating 1,25(OH)2D3 administration could be prevented by treatment with hydrochlorothiazide. Preliminary experiments indicated that hydrochlorothiazide decreased the 1,25(OH)2D3-induced hypercalciuria without reducing the effect of the vitamin D metabolite on plasma calcium. We have therefore investigated in thyroparathyroidectomized rats supplemented with 1,25(OH)2D3 the effect of hydrochlorothiazide on calcium fluxes in the kidney, intestine and bone.

Methods

Renal clearance of calcium

From weaning, male Wistar rats from our own breeding colony were fed on a commercial chow (Altromin no. 1314) containing 27.5 mmol (1.1 g) of calcium/100 g, 32.6 mmol (1.0 g) of inorganic phosphate/100 g and 280 i.u. of vitamin D3/100 g. They were used when weighing 200–220 g. During the 10 day period preceding the clearance study, they were pair-fed a vitamin D-depleted diet (Altromin no. 1730), to which calcium gluconate and K2HPO4/KH2PO4 (7:3) were added, giving a chemically verified concentration of 2.5 mmol (1.0 g) of calcium/100 g and 25.8 mmol (0.8 g) of phosphorus/100 g. Ten i.u. of vitamin D3 (650 nmol = 0.25 µg), dissolved in vegetable oil, was added to the daily ration. The rats had free access to distilled water. One day after starting the experimental diet, which was given for 9 days, all rats were injected intraperitoneally twice a day with 13 pmol of 1,25(OH)2D3 (kindly supplied by F. Hoffmann-La Roche, Switzerland) dissolved in 25 µl of 95% ethanol. They received subcutaneously either hydrochlorothiazide (2 x 30 µmol day−1 kg−1, kindly supplied by Ciba, Switzerland) or its solvent vehicle (0.15 mol/l NaCl, pH 10.5) in a volume of 4 ml/kg. The last injections of both 1,25(OH)2D3 and hydrochlorothiazide were given on the experimental day, 1 h before starting the clearance study. Forty-four to 48 h before this study, the animals were anaesthetized with pentobarbital (40 mg/kg body weight). They were thyroparathyroidectomized and a subtotal cystectomy was made in order to reduce the dead space of the urinary tract (Hugi, Bonjour & Fleisch, 1979). A catheter (PE 50) was implanted into the left jugular vein and fixed to the skin of the neck for delivering solutions during the clearance experiment.

On the experimental day, the rats were weighed and put into restrictive cages specially designed for studying renal function in conscious rats. Urine was collected in weighed test tubes. Blood samples were taken from the tip of the tail into heparinized plastic tubes.

A priming dose of [3H]inulin (5 µCi/kg) with unlabelled inulin (Fluka) (80 µg/kg) dissolved in NaCl solution (0.15 mol/l) was injected intravenously through the jugular vein catheter in a volume of 5 ml/kg. A solution of NaCl (0.15 mol/l) containing 500 µCi of [3H]inulin and 12 g of unlabelled inulin/l was then infused at a rate of 4.0–4.2 ml/h. After 2 h, urine was collected during four consecutive intervals (periods 1 to 4) of 30 min each. After the first interval the infusion of NaCl (0.15 mol/l) was replaced by a solution containing calcium gluconate (0.06 mol/l) and NaCl (0.10 mol/l) to progressively increase the concentration of plasma calcium (Hugi et al., 1979). The osmolality of both solutions was 290–300 mosmol/kg water. Blood samples were taken before the infusion started and at the beginning and end of each clearance period.

At the end of the clearance study the rats were fed on the usual laboratory chow and allowed tap water ad libitum for 5 days. They were then starved overnight and plasma calcium concentration was determined the next morning. Only rats with a plasma calcium concentration below 1.88 mmol/l were kept in the study.

[3H]Inulin in plasma and urine was measured by scintillation counting. Calcium in urine and plasma was determined by EGTA titration (Corning Calcium Analyzer no. 940). Sodium concentrations in plasma and urine were determined by flame photometry (EEL Flame Photo-
Thiazide and 1,25(OH)₂D₃ in hypoparathyroidism

Calcium balance and ⁴⁵Ca kinetics

The balance of stable calcium and kinetics of injected ⁴⁶Ca were measured as in the model described by Aubert & Milhaud (1960). The details of the method used in our laboratory have been previously published (Gasser, Morgan, Fleisch & Richelle, 1972). Female Wistar rats raised as described above were used. When the animals were 42 ± 1 days old they were placed into single metabolic cages and pair-fed until the end of the experiment the same diet as described above (Altromin no. 1730), but containing 12.5 mmol (0.5 g) of calcium/100 g instead of 25 mmol (0.1 g)/100 g. Ten i.u. of vitamin D₃ were added to the daily ration as mentioned above. The animals had free access to distilled water. Surgical thyroparathyroidectomy was performed under ether anaesthesia. Four days later a blood sample was taken for plasma calcium determination. Only animals with a plasma calcium lower than 1.88 mmol/l were retained in the study and a daily subcutaneous injection of 2 pg of L-thyroxin (Fluka) dissolved in NaOH (1 mmol/l) was given. From the age of 51 ± 1 days, the rats were injected intraperitoneally twice a day with either 13 pmol of 1,25(OH)₂D₃ dissolved in 25 μl of 95% ethanol or with the vehicle alone. Half of the animals of each group were injected subcutaneously with 30 pmol of hydrochlorothiazide/kg twice a day (injected in a volume of 4 ml/kg) and the other half received an equivalent volume of the solvent vehicle.

The calcium balance was made between days 11 and 13 of therapy and combined with a ⁴⁵Ca kinetic study. Thus 30 μCi of ⁴⁵CaCl₂ with a specific radioactivity of 10–25 mCi/mg was injected into a tail vein. Blood was taken for radioactivity counting 2, 4, 6, 24, 48 and 72 h after the ⁴⁵Ca injection. Plasma ⁴⁵Ca radioactivity was determined in a scintillation counter. Chemical analysis and calculation of the variables of calcium metabolism were made as previously described (Gasser et al., 1972).

Statistics

Results are presented as means ± SEM. Difference between means was evaluated by the two-sided Student's 𝑡-test. Linear regressions were calculated by the least-squares method and relationships were treated by covariance analysis.

Results

Renal handling of calcium

The influence of hydrochlorothiazide on the renal handling of calcium in 1,25(OH)₂D₃-treated rats is presented in Table 1 and Figs. 1 and 2. Clearance of inulin tended to be lower in the group receiving hydrochlorothiazide than in the rats given 1,25(OH)₂D₃ alone, but urine flow was similar in both groups. As expected rats receiving hydrochlorothiazide showed a significant increase in the fractional excretion of sodium throughout the experiment. In all periods of the clearance study, a mean increase of 0.2–0.3 mmol of plasma calcium/l was observed in rats.

Table 1. Effect of hydrochlorothiazide on the renal handling of calcium in thyroparathyroidectomized rats treated with 1,25(OH)₂D₃

All animals were injected during 9 days with 2 × 13 pmol of 1,25(OH)₂D₃/day, intraperitoneally. During the same period they were injected with either 2 × 30 μmol of hydrochlorothiazide day⁻¹ kg⁻¹ or the sodium chloride vehicle subcutaneously. * 𝑃 < 0.05; ** 𝑃 < 0.01 as compared with the corresponding period of the group receiving the sodium chloride vehicle. All values are the mean ± SEM; n = number of animals.

<table>
<thead>
<tr>
<th>Period</th>
<th>NaCl soln. (n = 5; body wt. 221 ± 4 g)</th>
<th>Hydrochlorothiazide (n = 5; body wt. 218 ± 6 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clearance of inulin (ml/min)</td>
<td>Urine flow (μl/min)</td>
</tr>
<tr>
<td>1</td>
<td>1.97 ± 0.10</td>
<td>31 ± 0.58</td>
</tr>
<tr>
<td>2</td>
<td>1.75 ± 0.10</td>
<td>29 ± 0.82</td>
</tr>
<tr>
<td>3</td>
<td>1.77 ± 0.10</td>
<td>39 ± 0.28</td>
</tr>
<tr>
<td>4</td>
<td>1.65 ± 0.10</td>
<td>41 ± 0.26</td>
</tr>
</tbody>
</table>

receiving hydrochlorothiazide (Table 1). In the hydrochlorothiazide group the absolute excretion of calcium was reduced throughout the experiment despite a higher plasma calcium level. Fig. 1 presents the relationship between the plasma concentration and the urinary excretion of calcium in the hydrochlorothiazide-treated and in untreated rats. The slopes of the two regression lines were not statistically different. Covariance analysis, however, indicated that the absolute excretion of calcium/ml of glomerular filtration rate was significantly reduced in the hydrochlorothiazide group at equivalent plasma calcium concentrations ($F = 25.06, P < 0.01$). In $1,25(OH)_2D_3$-supplemented thyroparathyroidectomized rats hydrochlorothiazide also decreases significantly ($F = 57.88, P < 0.01$) the clearance of calcium for a given clearance of sodium. The rise in the clearance of total calcium as a function of the increase in clearance of sodium was less pronounced in the animals given hydrochlorothiazide. Assuming a linear relationship between the two clearances, the slope of the calculated linear regression (see legend of Fig. 2) was significantly ($P < 0.001$) smaller in the rats receiving hydrochlorothiazide than in the untreated animals.

**Calcium balance and $^{45}$Ca kinetics**

The $1,25(OH)_2D_3$-induced hypercalciuria in thyroparathyroidectomized rats was significantly reduced (Table 2) by administration of hydrochlorothiazide. The decrease in the urinary calcium was associated with a 70 µmol decrease in net intestinal calcium absorption. It appears that the $1,25(OH)_2D_3$-induced increase in intestinal calcium absorption was considerably lessened in animals given hydrochlorothiazide. Thus, whereas the $1,25(OH)_2D_3$-induced increase in the net intestinal calcium absorption was statistically significant in rats not receiving hydrochlorothiazide, this was not the case in animals receiving the diuretic agent (Table 2).

Confirming a similar study by Rizzoli et al. (1977), the results presented in Table 2 also indicate that in thyroparathyroidectomized rats $1,25(OH)_2D_3$ leads to an increase in the deposition and release of calcium into and from bone. Hydrochlorothiazide had no effect on bone either in the presence or absence of $1,25(OH)_2D_3$. No significant difference in the plasma calcium level between hydrochlorothiazide-treated and non-treated animals was seen whether or not they received $1,25(OH)_2D_3$ (Table 2).

**Discussion**

The present study confirms that $1,25(OH)_2D_3$ given to thyroparathyroidectomized rats, in doses which have been shown to normalize intestinal calcium absorption (Rizzoli et al., 1977), tend to correct the low plasma calcium concentration and promote a marked increase in the urinary calcium....
Thiazide and 1,25(OH)₂D₃ in hypoparathyroidism

TABLE 2. Effect of hydrochlorothiazide in thyroparathyroidectomised rats treated with 1,25(OH)₂D₃ on calcium balance and kinetics

The animals were injected during 14 days with either 2 x 13 μmol of 1,25(OH)₂D₃/day or the ethanol vehicle intraperitoneally, and with either 2 x 30 μmol of hydrochlorothiazide day⁻¹ kg⁻¹ or the sodium chloride vehicle subcutaneously. Calcium balance combined with "Ca kinetics was made during the last 3 days of treatment. Values are means ± SEM.

\* P < 0·05; \* P < 0·01; \* P < 0·001, as compared with the animals injected with the ethanol and sodium chloride vehicles. § P < 0·05: II P < 0·01; \* P < 0·001, as compared with the animals injected with the ethanol vehicle and hydrochlorothiazide. ** P < 0·025 as compared with the animals injected with 1,25(OH)₂D₃ and the sodium chloride vehicle.

<table>
<thead>
<tr>
<th>Treatment . . . . .</th>
<th>Ethanol vehicle. NaCl soln. (n = 8)</th>
<th>Ethanol vehicle, hydrochlorothiazide (n = 6)</th>
<th>1,25(OH)₂D₃ NaCl soln. (n = 7)</th>
<th>1,25(OH)₂D₃ hydrochlorothiazide (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake (μmol/day)</td>
<td>1272 ± 42</td>
<td>1307 ± 35</td>
<td>1347 ± 40</td>
<td>1342 ± 30</td>
</tr>
<tr>
<td>Net intestinal absorption (μmol/day)</td>
<td>515 ± 37</td>
<td>570 ± 32</td>
<td>710 ± 57*</td>
<td>640 ± 52</td>
</tr>
<tr>
<td>Fractional absorption (μmol/day)</td>
<td>0-52 ± 0-03</td>
<td>0-54 ± 0-02</td>
<td>0-65 ± 0-03†</td>
<td>0-61 ± 0-02§</td>
</tr>
<tr>
<td>Urinary excretion (μmol/day)</td>
<td>17 ± 1</td>
<td>11 ± 2*</td>
<td>174 ± 34†</td>
<td>76 ± 19†**§</td>
</tr>
<tr>
<td>Retention (μmol/day)</td>
<td>500 ± 37</td>
<td>560 ± 32</td>
<td>537 ± 60</td>
<td>565 ± 42</td>
</tr>
<tr>
<td>Deposition into bone (μmol/day)</td>
<td>835 ± 75</td>
<td>952 ± 82</td>
<td>1222 ± 152*</td>
<td>1307 ± 107†§</td>
</tr>
<tr>
<td>Release from bone (μmol/day)</td>
<td>335 ± 70</td>
<td>392 ± 97</td>
<td>682 ± 125*</td>
<td>742 ± 105‡§</td>
</tr>
<tr>
<td>Plasma concentration (μmol/ml)</td>
<td>1·51 ± 0·05</td>
<td>1·49 ± 0·05</td>
<td>2·26 ± 0·12‡</td>
<td>2·50 ± 0·05†</td>
</tr>
</tbody>
</table>

In addition we have shown that the calciuric effect of 1,25(OH)₂D₃ can be significantly reduced by the concomitant administration of hydrochlorothiazide.

The hypocalciuric effect of thiazide diuretics when given chronically to humans, first described by Lamberg & Kuhlback (1959), has been confirmed many times (for review see Yendt & Cohanim, 1978). Until recently the thiazide-induced hypocalciuria was observed in man only in the presence of normal parathyroid function (Brickman, Massry & Coburn, 1972; Parfitt, 1972). However, Porter, Cox, Heaney, Hostetter, Stinebaugh & Suki (1978) reported that chlorothalidone, a thiazide-like diuretic, caused hypocalciuria and hypercalcemia in hypoparathyroid patients, although this effect required simultaneous restriction of sodium intake. In rats the presence of the parathyroid glands is not required for hypocalciuria to develop during chronic thiazide administration (Jørgensen, 1971).

The present report shows that the hydrochlorothiazide-induced hypocalciuric effect can be demonstrated in thyroparathyroidectomized rats chronically supplemented with 1,25(OH)₂D₃. Our study also shows that the urinary calcium excretion, measured at various plasma calcium concentrations, remains markedly depressed in rats given hydrochlorothiazide. Thus an increase in the net tubular reabsorptive capacity for calcium accompanies the hypocalciuric effect of hydrochlorothiazide in 1,25(OH)₂D₃-supplemented thyroparathyroidectomized rats. This tubular effect explains the acute influence of thiazide on urinary calcium excretion, which in man and animals does not require the presence of functional parathyroid glands (Duarte & Bland, 1965; Costanzo & Weiner, 1974; Costanzo, Moses, Rao & Weiner, 1975; Quamme, Wong, Sutton & Dirk, 1975). However, the tubular action of thiazides obviously cannot account for the hypocalciuria of chronically treated animals or humans. Only a decrease in the net calcium inflow from extra-renal sources into the extracellular compartment can explain the chronic hypocalciuria induced by thiazides.

A decrease in the net calcium inflow to plasma could be due to alterations in the intestinal and/or skeletal calcium fluxes. Thus the hypocalciuria could be maintained by an increase in bone calcium retention. Such an effect could result from either an increase in calcium deposition into the skeleton and/or a decrease in bone resorption. In our experiment the deposition and the release of calcium into and from bone were not significantly affected by the administration of thiazide. Thus it is unlikely that the hypocalciuria is due to an increased bone calcium retention.

A decrease in net intestinal calcium absorption could explain the hydrochlorothiazide-induced chronic hypocalciuria (Table 2). The balance studies indicate that the 1,25(OH)₂D₃-induced rise in net intestinal calcium absorption was significantly less pronounced in rats given hydro-
chlorothiazide. The effect of hydrochlorothiazide upon intestinal absorption in the 1,25(OH)2D3-treated thyroparathyroidectomized rats could explain why the level of plasma calcium did not rise despite an increase in the tubular calcium reabsorption. Previous studies on the effect of thiazides on intestinal calcium transport have provided conflicting results. Thus, in humans with intact parathyroid glands, treatment with these compounds led to no change, an increase or even a decrease in intestinal calcium absorption (for review see Yendt & Cohanin, 1978). To our knowledge there is no information on the effect of thiazides on intestinal calcium absorption in hypoparathyroid patients treated with vitamin D derivatives.

In our experiments hydrochlorothiazide did not lead to a decrease in intestinal calcium absorption of thyroparathyroidectomized rats. Only the 1,25(OH)2D3-stimulated increment was affected by the diuretic. In patients with hypercalcemia, long-term thiazide therapy was reported to decrease both the high intestinal calcium absorption and the elevated plasma concentration of 1,25(OH)2D3 (Zerwekh & Pak, 1980).

The mechanism whereby hydrochlorothiazide reduces the 1,25(OH)2D3-induced increase in calcium absorption in parathyroid hormone-deficient animals is unknown. The effect may be secondary to increased tubular calcium reabsorption, causing a rise in plasma calcium and thus a suppression of the endogenous 1,25(OH)2D3 production. Absence of parathyroid hormone does not exclude the possibility of such a regulatory loop. Indeed it has been recently demonstrated (Trechsel, Eisman, Fischer, Bonjour & Fleisch, 1980) that the plasma level of 1,25(OH)2D3 is influenced by calcium in both thyroparathyroidectomized and intact rats.

Whatever the mechanism, our study shows that hydrochlorothiazide reduces 1,25(OH)2D3-induced hypercalcemia without altering the action of the vitamin D metabolite on plasma calcium. Whether 1,25(OH)2D3 and hydrochlorothiazide given together would maintain a normal plasma calcium whilst reducing hypercalcemia and the risk of nephrolithiasis in hypoparathyroid patients remains unanswered. Nevertheless the present work gives further support to the idea (Porter et al., 1978) that thiazides might be of benefit in the management of human hypoparathyroidism.

Acknowledgments
We are grateful to Miss M. Bachmann for her expert technical help. We acknowledge Mrs B. Gyger for typing the manuscript. Mrs C. Stieger for drawing the figures, and Dr M. Bishop for reading the manuscript. This investigation was supported by the Swiss National Science Foundation (grant 3.725.76) and by F. Hoffmann-La Roche and Co. AG, Basel, Switzerland.

References


