CLINICAL STUDY

Evaluation of a standardized short-time calcium suppression test in healthy subjects: interest for the diagnosis of primary hyperparathyroidism

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Abstract

Objective: The diagnosis of primary hyperparathyroidism (PHP) can be difficult in patients with normal plasma calcium or parathyroid hormone (PTH) levels. We perfected a standardized short-time i.v. calcium loading test in healthy controls (HC) and compared the results with those of patients with PHP. **Methods:** Sixteen HC received 0.33 mmol/kg calcium gluconate intravenously for 3 h. Plasma calcium and serum PTH levels (assayed with immunoluminescent sandwich methods) were measured before, at the end of the infusion and 3 h later. Results were compared with those of 16 PHP patients.

Results: In HC, basal total plasma calcium (mean $\pm$ s.e.m.) was 2.33 ± 0.02 mmol/l. At the end of calcium loading, calcemia reached 3.21 ± 0.05 mmol/l and decreased to 2.94 ± 0.08 mmol/l 3 h later. In PHP patients, basal plasma calcium was 2.54 ± 0.03 mmol/l and reached similar values as in HC during the testing. Basal serum PTH levels were 32.5 ± 3.3 ng/l in HC and 86.9 ± 6.3 ng/l in PHP. At the end of calcium loading, they dropped to 8.8 ± 0.6 ng/l (HC) and to 31.4 ± 4.2 ng/l (PHP). Three hours later, they were 11.6 ± 0.8 and 39.8 ± 4.0 ng/l respectively. There was a cut-off in serum PTH values between the two groups at the end of calcium loading and 3 h later.

Conclusion: The standardized short-time PTH suppression test appears reliable to differentiate healthy subjects from PHP whose serum PTH levels remain >14 and >23 ng/ml respectively at the end of loading and 3 h later. This well-tolerated and easily performed test could be used for the diagnosis of PHP in patients suspected for the disease despite the normality of some basal biological markers.

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Introduction

Primary hyperparathyroidism (PHP), the most frequent cause of hypercalcemia in outpatients, occurs in about 1 out of 1000 adults. Without treatment about 25% of patients have a progressive disease that can lead to severe complications such as kidney stones, renal failure or osteoporosis (1). By now, PHP is usually suspected in asymptomatic patients (2) on the basis of the association of incidentally discovered hypercalcemia and unsuppressed plasma parathyroid hormone (PTH) levels. However, the complete biological pattern of PHP is lacking in some cases. Some PHP patients display either normal calcemia (3, 4) or normal plasma PTH levels (5). In such cases, the diagnosis of PHP is not easily established on basal data but cannot be unrecognized on account of the potential complications of the disease (6–8). As in normal parathyroid cells, calcium-sensing receptors are present in the plasma membrane of parathyroid adenoma cells

(9–11). However, adenomatous parathyroid cells appear less sensitive to the negative feedback of calcium than normal parathyroid cells (12–14). The molecular basis of this relative insensitivity was attributed to either a decrease in the number of calcium-sensing receptors, an alteration in the transduction system, or both (10, 15, 16). On this basis, a PTH suppression test using calcium loading has been proposed in order to differentiate PHP patients from subjects with normal parathyroid function (17). Several modalities have been described (12, 18–23) and different markers have been proposed as phosphate clearance (24), urinary cyclic AMP (17), or immuno-reactive PTH (12, 18, 19, 21–23). However, no standardized test is presently available. The aim of the present study was to evaluate a standardized i.v. calcium loading for suppression of PTH secretion in a group of healthy volunteers. The results were compared with those obtained using a similar procedure in a group of patients with proven PHP.

Subjects and methods

This open study was approved by the Ethics Committee of Haute Normandie (CCPRB-HN). The subjects studied were divided into two groups.

Sixteen healthy controls (HC), 13 men, 3 women aged 19–69 years, were included after written informed consent. They had no history of the disease and were not taking any medication. They were under normal diet containing 800–1200 mg calcium per day. The total and ionized plasma calcium, phosphorus, serum albumin, 24 h calciuria, creatinine clearance (calculated using the Cockcroft and Gault formula), plasma vitamin D, and serum PTH levels were normal.

The PHP group included 16 patients (4 men and 12 women) aged 31–74 years, placed under a normal diet as in controls. They were taking no medication. Among the 10 postmenopausal women, none were taking a hormone substitution. PHP was suspected on either mild hypercalcemia with normal serum PTH levels or on elevated serum PTH despite normal plasma calcium levels. An increase in 24 h calciuria was found in each case. Because they had a dissociated biological pattern, a calcium loading was included in their investigation testing. Their creatinine clearance (calculated as in healthy volunteers) was normal for age. 25-OH vitamin D plasma levels, measured in half of the patients, were in the normal range (30–120 nmol/l). The diagnosis of PHP was confirmed by the pathological examination of the parathyroid tissue removed during surgery. PHP was linked to a single parathyroid adenoma (adenoma size: 8–25 mm) in 12 patients or to parathyroid hyperplasia in 4 patients. Three of the latter patients suffered from a multiple endocrine neoplasia type 1 (MEN1).

Calcium loading test was performed in non-fasting subjects placed in recumbency. At 0800 h two catheters were placed in peripheral veins of the right and left arms for blood sampling and calcium infusion respectively. Then 0.33 mmol/kg of 10% calcium gluconate were added to 0.9% NaCl in order to obtain a total volume of 500 ml which was infused in 3 h. Blood was drawn before and at the end of calcium infusion, and 3 h later to measure plasma calcium, phosphorus, and serum PTH levels.

The plasma calcium and phosphorus levels were measured using the usual automated techniques of the laboratory. Plasma ionized calcium level was measured in HC with an electrochemical method using a selective electrode specific for calcium. Serum PTH levels were determined using an immuno-luminescent sandwich assay (Advantage I-PTH, Nichols, San Clemente, CA, USA). This immunoassay detects equally both 1–84 PTH and its large fragment 7–84. In controls and in seven PHP patients, the serum PTH levels were also measured using the bio-intact immunoassay exclusively directed against the intact 1–84 PTH (Bio-intact-PTH (bi-PTH), Nichols). The sensitivity of these two assays is 1 ng/ml and the normal ranges proposed by the

manufacturer are between 10 and 60 ng/l and 10 and 46 ng/l respectively. PTH assays were performed between 2001 and 2005 ends in PHP patients and in December 2004 in HC.

Statistical analysis

Statistical analysis used the Mann–Whitney test for a comparison between groups of the values of the biological parameters measured at each time. The Bonferroni correction was used after performing three successive tests. The level of statistical significance was $0.05/3=0.017$ for each test. A receiver operating characteristic (ROC) curve was established and Fisher’s exact test was used for the statistical evaluation of the cut-off values between the two groups studied. Correlations between plasma calcium and serum PTH levels were examined by linear regression study after verification that distribution of sample values was not precluded. The level of statistical significance was 0.05.

Results

The overall clinical tolerance of the test was good. Three HC complained of mild nausea at the end of the calcium infusion. Blood pressure moderately and transiently increased in three controls at the same time. These symptoms, linked to hypercalcemia, disappeared rapidly and spontaneously.

Basal calcemia (mean $\pm$ S.E.M.), corrected as a function of plasma albumin levels, was 2.33 ± 0.02 and 2.54 ± 0.03 mmol/l in HC and in PHP respectively (Table 1). Out of the 16 PHP patients, 11 had a serum calcium level in the normal range (2.2–2.6 mmol/l, Fig. 1). At the end of the calcium infusion, calcemia rose to 3.21 ± 0.05 mmol/l (HC) and 3.44 ± 0.06 mmol/l (PHP). Three hours after the calcium infusion was stopped, calcemia decreased to 2.94 ± 0.08 mmol/l (HC) and 2.96 ± 0.08 mmol/l (PHP). There was no statistical difference in the values of calcemia between

Table 1 Biological parameters (individual lowest and highest value) measured in basal conditions in healthy volunteers and in patients with primary hyperparathyroidism. Median is indicated in brackets.

	Healthy controls	Primary hyperparathyroidism
Total plasma calcium	2.2–2.45 (2.32)	2.35–2.68 (2.53)
Ionized plasma calcium	1.1–1.27 (1.21)	
Plasma phosphorus	0.87–1.5 (1.36)	0.58–1.39 (0.89)
Serum 1–84 + 7–84 PTH	20–62 (34)	46–139 (86)
Serum 1–84 PTH	8–26 (18)	42–76 (51)
Plasma 25 OH vitamin D	62–128 (87)	27–47 (42)
Plasma creatinine	66–115 (80)	56–109 (73)

Figure 1 Plasma calcium and serum PTH levels in healthy volunteers (open squares) and patients with primary hyperparathyroidism (dark squares) before (basal), at the end of calcium loading (3 h), and 3 h after the end of the calcium infusion (6 h). On basal graph, horizontal and vertical lines indicate the limits of normal values for serum PTH and plasma calcium levels.

the two groups, neither at the end of calcium infusion nor 3 h later.

In HC, ionized calcium level rose from 1.20 ± 0.015 to 1.78 ± 0.02 mmol/l and was $1.50.025$ mmol/l at the end of the test. Ionized and total calcemia were strongly correlated ($r=0.99$, $P<0.001$, Fig. 2).

Basal plasma phosphorus levels were 1.25 ± 0.05 (HC) and 0.87 ± 0.04 (PHP; $P<0.01$). They rose to 1.55 ± 0.04 and 1.15 ± 0.03 mmol/l respectively during calcium loading.

Figure 2 Correlation between total and ionized plasma calcium in healthy controls.

Basal serum PTH levels (mean $\pm$ s.e.m.) were 32.5 ± 3.3 and 86.9 ± 6.3 ng/l in the control group and PHP respectively ($P<0.01$). Of the 16 PHP patients, 13 had a serum PTH level above the normal range (10–60 ng/l, Fig. 1). At the end of calcium infusion, serum PTH level decreased to 8.8 ± 0.6 ng/l (HC) and to 31.4 ± 4.2 ng/l (PHP; $P<0.01$). There was no overlap in individual values between controls and subjects with sporadic PHP whose serum PTH levels remained respectively lower and higher than 15 ng/l at the end of calcium loading. In the three patients with MEN1-related PHP, serum PTH levels dropped to 14 ng/l. Three hours after the end of calcium infusion, serum PTH rose slightly to 11.6 ± 0.8 ng/l (HC) and to 39.8 ± 4.0 ng/l (PHP). Again, there was no overlap in individual values between controls and patients with sporadic PHP whose serum PTH levels were below 20 and above 26 ng/l respectively. In the three patients with MEN1, serum PTH levels reached 22, 23, and 23 ng/l. Using PTH levels, the ROC curve constructed with the data obtained at the end of the calcium infusion (third hour of testing) gave a specificity of 93% and a sensitivity of 100% for the cut-off value of 12 ng/l. With a cut-off value of 14 ng/l both sensitivity and specificity were 92%. The ROC curve established with the data obtained at the end of the test (sixth hour of testing) gave a specificity and a sensitivity of 100% for the cut-off value of 20 ng/l (Fig. 3). Considering the number of subjects in each group, the confidence interval ranged from 80 to 100%.

In subjects considered as a whole, serum bi-PTH (1–84) and i-PTH (1 and 7–84) levels were strongly correlated ($r=0.98$, $P<0.001$, Fig. 4).

Discussion

The spontaneous evolution of PHP, the most frequent cause of hypercalcemia in outpatients, is responsible for severe complications such as osteoporosis (25, 26), kidney stones, and renal involvement in about one-fifth

Figure 3 ROC curves obtained for serum PTH levels measured either at the end (dark squares) or after the end of calcium loading (open squares).

of cases (1). The main goal of the treatment is to prevent the occurrence of such complications. Consensus conferences established the criteria for surgical treatment of PHP. They include calcemia 0.25 mmol/l above the upper limit of normal range or calciuria higher than 10 mmol/24 h (27–29). Most of the PHP are currently suspected in asymptomatic patients (2) on routine laboratory analysis revealing mild hypercalcemia and unsuppressed PTH levels. However, the complete biological pattern of PHP is lacking in some cases despite normal creatinine clearance and vitamin D status. Indeed, patients with PHP may present with either normocalcemia (3, 4) or more frequently normal serum PTH levels (5). In such cases, a dynamic PTH suppression test, using a calcium load, can provide further information for the diagnosis of PHP (12, 18–23). Indeed, calcium-sensing receptors are present in both normal and pathological (i.e. parathyroid adenoma or hyperplasia) parathyroid cells (9–11, 15), and both cell types reduce their PTH secretion in response to

Figure 4 Correlation between serum 'bio-intact' (1–84 PTH) and 'intact' parathyroid hormone (1–84 and 7–84) levels in healthy controls (open squares) and patients with primary hyperparathyroidism (dark squares).

a rise in serum calcium either *in vitro* (30) or *in vivo* (11, 22, 30–33). However, cells from patients with PHP appear less sensitive to calcium-loading suppression than normal parathyroid cells (30, 32, 34, 35). On this basis calcium-loading suppression tests were proposed as tools for the diagnosis of PHP with various modalities (oral or i.v. loading), timing, and biological criteria (12, 18–23). At the moment, no short-time standardized test is available. The present study was performed to assess the effects of a 3-h standardized i.v. calcium loading on serum PTH levels in a group of healthy volunteers and compare the results to that obtained in a group of patients with PHP.

In another study, using a similar calcium loading procedure, we observed that total plasma calcium levels rose linearly during the infusion and was above 3 mmol/l an hour after starting the test (unpublished data). Such a plasma calcium level is high enough to suppress normal parathyroid secretion (36). Half-lives of circulating 1–84 and 7–84 PTH are 3–5 and 10 min respectively (37, 38). Thus, measuring serum PTH levels 3 h after the beginning of calcium load appears to be a relevant timing to evaluate the result of the suppression. Serum i-PTH (1–84 and 7–84) and bi-PTH (1–84) levels are strongly correlated, demonstrating that a PTH assay identifying both 1–84 and 7–84 PTH can be appropriately used to assess parathyroid cell suppression. Similarly, total and ionized calcium are closely correlated, showing that measurement of corrected total plasma calcium is enough to obtain reliable results. The proposed procedure avoids the technical requirements needed for ionized calcium measurements and allows easy performance of this low-cost testing.

HC and PHP patients exhibit different mean ages and sex ratios. This should be taken in account for the comparison between the two groups studied. Indeed, serum/plasma PTH physiologically rises by about 35% with age (39–41), which could introduce a limitation to the value of the results. However, the serum/plasma PTH level remains in the normal range of adults and the ability to suppress PTH secretion by calcium does not change with age (41). On the other hand, gender does not appear to significantly modify age-induced changes in PTH secretion (42, 43). Our postmenopausal women were taking no hormonal treatments, and hormonal substitution with estrogens has been shown to modulate PTH secretion (44–47). Estrogen therapy reduces the set point of response of PTH secretion to calcium changes by 0.20 mmol/l (36). A slight difference in parathyroid cell sensitivity to calcium between postmenopausal women with PHP and controls is not excluded but is likely overcome by the rise of 0.9 mmol/l in plasma calcium levels during the suppression testing. Furthermore, PTH secretion appeared similarly suppressed by increasing calcium level in treated and non-treated women (36). Another potential confounding variable is vitamin D concentration. Plasma vitamin D levels as measured in one half of the patients were in the lowest half of normal

Figure 5 Product of the nadir of serum PTH level (ng/l) by the peak value of plasma calcium (mmol/l) in healthy volunteers (open squares), patients with sporadic hyperparathyroidism (dark squares) and patients with MEN1-related hyperparathyroidism (open triangles). The cut-off value between controls and hyperparathyroid patients as a whole is 43. HC, healthy controls. Hyp, MEN1-related hyperparathyroidism. Ad, adenoma.

range. A vitamin D deficiency, frequently found in patients with PHP (48, 49), cannot be excluded in the other patients. This would lead to a further rise in serum PTH levels. However, previous studies showed that in such cases plasma calcium level was not significantly modified (48, 50) and that the ability to suppress PTH secretion by calcium is unchanged (41).

At the end of calcium loading, serum PTH level clearly separates healthy subjects from patients with sporadic PHP (cut-off value = 14 ng/l). At this time, the patients with parathyroid hyperplasia related to MEN1 are less easily separated from normal subjects. This is possibly due to a lower sensitivity of adenomatous cells than hyperplastic parathyroid cells to calcium. Among the different mechanisms proposed to explain the relative insensitivity of parathyroid adenomas to calcium (10, 15, 16, 51), the changes could be less profound in parathyroid cells from MEN1 patients than in sporadic PHP. However, a study of calcium-sensing receptor content or expression was not performed and this hypothesis cannot be confirmed in our cases. Irrespective of the involved mechanism of PTH insensitivity to calcium, serum PTH levels measured 3 h after calcium loading rose more rapidly in all PHP patients than in normal subjects. In particular, this pattern was observed in MEN1 patients likely by the occurrence of a rebound in PTH secretion due to parathyroid hyperplasia (22). At this time, controls and all hyperparathyroid patients were clearly separated by PTH levels (cut-off value = 20 ng/l). However, it should be emphasized that these cut-off values are only applicable to the tested assay (Advantage iPTH), which is unfortunately no longer available. In addition, a significant shift was observed in this assay between 2003 and 2005 (52), implicating that the cut-off values should be regarded with some caution. To improve the value of these

measurements, Hagag *et al.* proposed to calculate the product $P = \text{peak of } \text{Ca}^{2+} \times \text{nadir of PTH}$ (18). In our series, such calculations did not better separate the two populations than serum PTH level alone (Fig. 5). As to whether measurement of serum PTH value performed at the end of calcium infusion ($t = 3$ h) appears sufficient to identify sporadic hyperparathyroidism, a determination of serum PTH 3 h later ($t = 6$ h) should be preferred in patients suspected for MEN-related hyperparathyroidism.

In conclusion, we describe a standard short-time calcium loading test that is easily performed and has a low cost. The test is well tolerated. However, taking into account the peak of calcemia reached at the end of the infusion, it appears reasonable to keep the patient safely in the department until plasma calcium level has decreased to more physiological value. On the basis of the data obtained in this study such level should be reached 6 h after the end of the calcium infusion. The measurement of simple parameters, including only corrected total plasma calcium and serum PTH (with an assay identifying PTH 1–84 and its 7–84 fragment) appears sufficient to a relevant performing of this test. It can be carried out in adults without consideration of age and sex, which have a low incidence on the sensitivity of parathyroid secretion to calcium suppression. It is theoretically possible to choose the time of the measurements as a function of the context (sporadic hyperparathyroidism: + 3 h, or MEN: + 6 h). However, in the lack of clear context of MEN, both times of measurement (+ 3 h and + 6 h) should be preferred. This test appears to have good sensitivity and specificity for the diagnosis of PHP when the basal biological data (serum PTH level, serum calcium and 24 h calciuria) do not allow a clear diagnosis.

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