MEN1-related hyperparathyroidism: response to cinacalcet and its relationship with the calcium-sensing receptor gene variant Arg990Gly

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Abstract

Objective: Primary hyperparathyroidism (PHPT) is a challenging problem in type 1 multiple endocrine neoplasia (MEN1) due to the high postsurgery recurrence rate. The aim was to evaluate the efficacy of cinacalcet in MEN1 patients in comparison with patients with sporadic PHPT (sPHPT) and the effect of Arg990Gly calcium-sensing receptor (CASR) polymorphism on the response to treatment.

Design: This is a randomized, crossover, double-blind study carried out in the University Hospitals.

Methods: Fifteen MEN1 patients with PHPT were randomized to two groups, one administered with 30 mg daily cinacalcet, titrated until calcium normalization, and one with placebo. After 3 months, patients were reassessed and after washout switched to the other treatment. For comparison, 20 sPHPT patients with similar calcium levels were administered with cinacalcet for 3 months. Ionized and total calcium, phosphate, and parathyroid hormone (PTH) were evaluated. CASR Arg990Gly was genotyped on blood DNA by direct sequencing.

Results: Cinacalcet normalized calcium, increased phosphate, and reduced PTH levels in all patients. Cinacalcet dosage required to normalize calcium in MEN1 and sPHPT was not significantly different (45 ± 21 vs 54 ± 25 mg/day). Few mild adverse events, not requiring drug withdrawal, were observed in both the groups. No association between Arg990Gly CASR polymorphism and response to cinacalcet was found.

Conclusions: This short-term prospective study demonstrated that the efficacy profile of cinacalcet in patients with MEN1-related PHPT and in those with sPHPT was similar and was not influenced by the 990 CASR variant. Although long-term safety and efficacy data are required, cinacalcet might be considered a treatment option in MEN1 patients who have contraindications to surgery or persistent PHPT after surgery.

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Introduction

Multiple endocrine neoplasia type 1 (MEN1, OMIM +131100) is an autosomal dominant disorder characterized by the combined occurrence of tumors of parathyroids, anterior pituitary, and pancreatic islet cells, due to inactivating mutations of MEN1 gene. Primary hyperparathyroidism (PHPT) is present in 100% of MEN1 patients at age 50 years, and renal and bone complications are more frequent in these patients than in patients with sporadic PHPT (sPHPT) (1, 2). Treatment options for PHPT are parathyroidectomy for patients meeting current criteria according to the National Institutes of Health’s Consensus (2001) (1) and regular monitoring of biochemical markers and disease complications. Due to the frequent occurrence of multiple gland hyperplasia and ectopic locations, the success rate of surgery in MEN1 patients is definitely lower than that of patients with sPHPT, with a recurrence rate of about 50%, even in the hands of experienced parathyroid surgeons (2, 3, 4).

Cinacalcet is an allosteric modulator of the calcium-sensing receptor (CASR) that sensitizes the receptor to the extracellular calcium. Cinacalcet is effective in reducing or normalizing serum calcium levels in patients with hyperparathyroidism of different origins (5, 6, 7, 8, 9). According to the European Medicines Agency (EMA) prescription labeling, cinacalcet is...
indicated for the treatment of patients with secondary HPT due to chronic kidney disease, patients with PHPT due to parathyroid carcinoma, and patients with PHPT meeting the criteria for parathyroidectomy who have contraindications to surgery or persistent PHPT after surgery. Although MEN1 patients are good candidates for a medical treatment, the effectiveness of cinacalcet has been so far reported in 11 patients described in three different studies (10, 11, 12).

In recent years, previous studies investigated the possible association between polymorphic variants of the CASR gene and clinical and biochemical characteristics of PHPT patients (13, 14). In a cohort of sPHPT patients previously reported, patients homozygous or heterozygous for the Arg990Gly polymorphism had a phenotype consistent with increased sensitivity of the variant 990Gly to extracellular calcium (15). At present, only one small cohort of patients with secondary HPT due to chronic kidney disease has been screened for possible association between CASR polymorphisms and different responsiveness to cinacalcet (16). In this study, patients bearing the 990Gly allele were more sensitive to cinacalcet compared with homozygotes for Arg at that location (16). This observation is consistent with in vitro functional data, showing increased sensitivity of HEK cells stably transfected with CASR 990Gly allele to the calcimimetic R-568 (17) and an increased antilipolytic effect of cinacalcet in primary cultures of normal adipocytes having 990Gly allele (18).

This study is a randomized, crossover, double-blind study aimed to test whether cinacalcet may represent a medical approach to PHPT in patients with MEN1, in analogy with that observed in patients with sPHPT. Moreover, the study will explore the possibility to identify patients with different responsiveness to the drug by a pharmacogenetic approach based on the polymorphic Arg990Gly variant of CASR gene.

**Patients and methods**

**Patients**

The study included 15 patients (eight females and seven males; age at presentation 42.3 ± 12.2 years) with genetically confirmed MEN1 affected by PHPT, between June and December 2010 (Table 1) and 20 consecutive unrelated patients (16 females and four males, age 61 ± 12.4 years, P < 0.001, vs MEN1 patients) with sPHPT matched with MEN1-related PHPT patients for serum calcium levels. Diagnosis of PHPT was made on the basis of high ionized calcium levels in the presence of elevated or inappropriately normal serum parathyroid hormone (PTH) levels in MEN1 and sPHPT patients (plasma ionized calcium 1.44 ± 0.5 and 1.43 ± 0.6 mmol/l. P = NS, n.v. 1.13–1.32; total serum calcium 11.5 ± 0.2 and
Study design

The study had the assessment of the efficacy on calcium–phosphorus metabolism and safety profile of cinacalcet as primary clinical objective and the evaluation of the possible role of CASR 990 Gly polymorphism on cinacalcet response in MEN1 patients as secondary objective. MEN1 patients were enrolled in a randomized, crossover, double-blind with AB/BA design study. After baseline assessment of clinical and biochemical parameters, patients with MEN1 were randomly divided into two groups (A/B) and treated with cinacalcet and placebo respectively. Cinacalcet treatment was started with 30 mg daily dosage and subsequently titrated according to the following algorithm: i) serum and ionized calcium was assessed after 1 week; ii) if not normalized, a dose of 30 mg was added, returning to point 1; and iii) if normalized, cinacalcet dosage was kept constant and exposition phase started.

After titration, treatment was maintained for 3 months and then patients were reassessed for clinical and biochemical parameters. After 1 month of washout, treatments were switched (B/A) and patients underwent a second exposure phase and were finally reassessed. Titraration dosage and clinical examination were carried out by different physicians in order to preserve double-blind protocol. As regards blinding procedure and placebo controlling, cinacalcet and placebo tablets were wrapped in anonymous capsules and confections were labeled with alphanumeric codes unknown to patients and personnel dedicated to clinical examination. Before enrollment, all patients were treated with orally administered cholecalciferol 300 000 units every 4–6 months and the last administration was between 1 and 2 months before initiation of the study. During the study phases, cholecalciferol replacement was suspended. None of the patients received bisphosphonates within 6 months.

For comparison, the efficacy of 3 months of cinacalcet treatment was evaluated in a cohort of 20 consecutive sPHPT patients, selected on the basis of pretreatment levels of serum calcium similar to those found in MEN1 patients. All patients were treated with orally administered cholecalciferol 300 000 units every 4–6 months and the last administration was between 1 and 2 months before the initiation of study. None of these patients took cholecalciferol during the first 3 months after cinacalcet starting. Thirteen patients (65%) regularly took bisphosphonates for osteoporosis for 9–48 months prior to assessment. After basal evaluation, all sPHPT patients were administered with cinacalcet 30 mg/day, and a dose of 30 mg was added until serum calcium normalization. Reassessment was carried out after 3 months, without treatment interruption.

During the study, both MEN1-related and sporadic hyperparathyroidism patients were on free diet.

Assays

Blood samples for measurement of total and ionized calcium, phosphorus, PTH, and biochemistry were collected at baseline and at each follow-up visit after an overnight fasting and before the morning dose of cinacalcet. Twenty-four-hour urine samples were collected for calcium–phosphorous metabolism evaluation. Serum intact PTH was measured by a chemiluminescent method (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), with intra- and interassay coefficients of variation of < 4.5 and < 10.0% respectively. All other analytes were determined using standard methods. Calcium adjusted for albumin was calculated by the formula: total calcium + 0.8 × (4.4 – albumin).
DNA extraction and genotyping

All patients included in the study were genotyped to test the possible association between the polymorphic Arg990Gly variant (rs1042636, c.2977A>G, and p.Arg990Gly) of CASR and responsiveness to cinacalcet treatment, as previously reported (15). Briefly, blood DNA was extracted and PCRs were carried out in 50 μl of reaction mix with 500 ng of patient DNA, 25 pmol of each primer, and 2.5 U of AmpliTaq DNA polymerase (Applied Biosystems, Life Technologies Corp., Carlsbad, CA, USA). The PCRs were denatured for 3 min at 95 °C and followed by 35 cycles at 95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min. A final cycle at 72 °C for 10 min was carried out. The primer pair was 5′-GCAAGCAGAAGTCTCATTT-3′ and 5′-GTCTCTTGCAGACCCTGGTTC-3′. PCR products were sequenced by ABI-PRISM sequencer (Applied Biosystems, Life Technologies Corp.).

Statistical analysis

As the study design was a crossover AB/BA type, data pre- and posttreatment were analyzed with paired Student’s t-test (Gaussian parameters) or by McNemar’s test (categorical parameters). Comparisons with sPHPT patients were performed by unpaired Student’s t-test for continuous variables and χ² test for categorical variables. PTH and calcium vs cinacalcet dosage bivariate correlation was evaluated by Spearman’s rank correlation coefficient. P < 0.05 was considered statistically significant. Calculations were made by SPSS 18.0 software (IBM, Inc., Armonk, NY, USA).

Results

After randomization of MEN1 patients, seven patients received cinacalcet (group A) and eight patients were administered with placebo (group B). After titration, the mean cinacalcet dosage was 40 ± 16 mg/day. In this group, minimal cinacalcet dosage able to normalize calcium levels was reached in 1–2 weeks. One patient of group A experienced transient nausea for about 1 week (cinacalcet dosage: 30 mg/day), without necessity of treatment withdrawal. No patients of group A had hypocalcemia. During the first maintenance phase (3 months), one patient of group A and two of group B exited from the study due to personal reasons.

Subsequently, patients were reassessed for clinical and biochemical parameters and, after 1-month washout, treatments were switched between the two groups and the second maintenance phase started. After titration, the mean cinacalcet dosage was 48 ± 27 mg/day and the minimal effective dosage was reached in 1–3 weeks. One of the group B patients experienced transient nausea for few days, without treatment interruption or modification (cinacalcet dosage: 60 mg/day). No patients had hypocalcemia. At the first visit of the second maintenance phase, one patient of the placebo group exited from the study for personal reasons. After 3 months, patients were reassessed and the study ended.

Paired comparisons between cinacalcet and placebo exposures of MEN1 patients who fully completed the study are presented in Table 2. In particular, during cinacalcet phase, plasma ionized and serum albumin-corrected calcium were normalized in all patients and were accompanied by a significant increase in serum phosphate (Table 2 and Fig. 1). As far as PTH levels were considered, a statistically significant reduction of PTH levels occurred in all patients (median = 26.3%, interquartile range (IQR) 19.2–43.6%, P = 0.002), with normalization in five patients (45.4%) (Table 2 and Fig. 1). No significant change in 24-h urinary calcium (4.8 ± 1.2 mg/kg per day, P = NS), chromogranin A (47.0 ± 20.8, P = NS), and gastrin (81.3 ± 18.8 pg/ml).

Table 2 Placebo and cinacalcet effects on PTH, calcium/phosphorous metabolism, and symptoms in MEN1-related and sporadic PHPT patients.

<table>
<thead>
<tr>
<th>MEN1-related PHPT (n=11)</th>
<th>Sporadic PHPT (n=20)</th>
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<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td><strong>Cinacalcet</strong></td>
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<tr>
<td>PTH (pg/ml)</td>
<td>98.9 ± 21.5</td>
</tr>
<tr>
<td>Corrected total serum calcium (mg/dl)</td>
<td>11.5 ± 0.2</td>
</tr>
<tr>
<td>Ionized calcium (mEq/l)</td>
<td>1.44 ± 0.5</td>
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<tr>
<td>Serum phosphate (mg/dl)</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Alkaline phosphatases (U/l)</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Creatinine clearance (mg/min)</td>
<td>88.8 ± 16.2</td>
</tr>
<tr>
<td>Daily urinary calcium (mg/day)</td>
<td>323 ± 124</td>
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<tr>
<td>Gastrointestinal symptoms (%, n)</td>
<td>36.4 (4)</td>
</tr>
<tr>
<td>Neuromuscular symptoms (%, n)</td>
<td>27.3 (3)</td>
</tr>
<tr>
<td>Cinacalcet dosage (mg/day)</td>
<td>–</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>–</td>
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</table>

IQR, interquartile range. Statistical significance results are highlighted in bold.

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Similarly, no significant variations in gastrointestinal and neuromuscular symptoms, blood pressure, and antihypertensive drug requirement between cinacalcet and placebo groups were observed (Table 2 and data not shown). As far as quality of life was concerned, similar scale scores were recorded in cinacalcet and placebo treatments (data not shown).

In the sPHPT group, cinacalcet normalized ionized and total calcium, increased serum phosphate, and did not change urinary calcium excretion in all patients. PTH normalization was obtained in only one patient (5.0%), despite a percentage of PTH reduction (median – 20.6%, IQR 14.9–33.3%, \( P = 0.007 \) (Table 2 and Fig. 1) that was similar to that recorded in MEN1 patients. In particular, no difference in PTH and calcium during cinacalcet treatment was observed between patients taking bisphosphonates or not (PTH: 111.6 ± 32.7 vs 137.6 ± 46.1 pg/ml, \( P = \text{NS} \), calcium: 9.6 ± 0.4 vs 9.4 ± 0.4 mg/dl, \( P = \text{NS} \)). As regards adverse effects, four patients experienced nausea (one patient taking 30 mg/day, two 60 mg/day, and one 120 mg/day), which lasted for a few days in three of them, without cinacalcet discontinuation. In one patient (dosage: 120 mg/day), cinacalcet was maintained with the addition of ranitidine hydrochloride 150 mg/day for 5 weeks, with symptom improvement.

Mean cinacalcet dosage required to control calcium levels in sPHPT patients had a tendency to be higher than that administered to MEN1 patients (54 ± 25 vs 45 ± 21 mg/day, \( P = 0.314 \)). In particular, in the maintenance phase, hypercalcemia was controlled by 30 mg daily in seven of 11 MEN1 and eight of 20 sPHPT patients (\( P = \text{NS} \)). No significant correlations were found between cinacalcet dosage and both PTH and serum calcium reduction in MEN1, sPHPT, and MEN1 plus sPHPT groups (Fig. 2). No significant variations in SBP and DBP and the antihypertensive drug requirement between cinacalcet and placebo treatments were observed (Table 2 and data not shown).

Both MEN1 and sPHPT patients were genotyped for Arg990Gly polymorphism. Among MEN1 subjects who completed the study, nine patients (81.8%) were Arg990 homozygotes and two were heterozygotes, while in sPHPT group, 17 patients (85.0%) were Arg990 homozygotes and three were heterozygotes. No striking differences in PTH, serum calcium, phosphorous, nephrolithiasis, and osteoporosis were observed either at baseline or after treatment between 990Gly carriers and noncarriers, both in MEN1 and sPHPT groups considered separately or together. Moreover, no significant difference was found in cinacalcet dosage required for calcium normalization and PTH reduction between patients carrying the Gly allele or not (Fig. 2).

At the end of the study, four out of six patients (two females), who took cinacalcet during the second maintenance phases, agreed to continue the treatment for an unblind observational period of 12 months and were regularly assessed likewise the previously described maintenance phase. No variation in cinacalcet dosage was needed as calcium and PTH remained stable and no adverse effects appeared. Lumbar spine and
femur bone densitometry, which was repeated after 12 months of continuous treatment, did not show any significant variation in bone mass density.

Discussion

This short-term prospective study confirms that cinacalcet is effective in controlling hypercalcemia in patients with PHPT and extends this observation to patients with MEN1-related PHPT. It is well established that cinacalcet, a drug able to sensitize CASR to extracellular calcium, is a treatment option for patients with PHPT for whom parathyroidectomy would be indicated according to the National Institutes of Health’s Consensus, but in whom surgery is refused or contraindicated. Due to the frequent occurrence of multiple gland hyperplasia and ectopic locations, the success rate of parathyroid surgery in MEN1 patients is definitely lower than that of sPHPT, with a recurrence rate of about 50% of patients in the hands of experienced parathyroid surgeons (2, 3, 4). Although MEN1-related PHPT patients are likely the best candidates for a medical treatment, the effectiveness of cinacalcet in MEN1 has been poorly investigated, probably due to low frequency of the disorder that is estimated in two to ten cases per 100 000 in the general population. Indeed, three different studies described the efficacy of cinacalcet in a total of 11 patients (10, 11, 12).

In this study, 15 MEN1 patients were enrolled in a randomized, crossover, double-blind with AB/BA design study, and the efficacy and safety profile of cinacalcet were compared with that found in a group of 20 sPHPT patients, who were matched for calcium levels and not for age and sex due to the well-known difference between MEN1-related and sPHPT in these parameters. The short-term administration (3 months) of cinacalcet induced the normalization of ionized and total calcium that was accompanied by a significant increase in serum phosphate, no change in urinary calcium excretion, chromogranin-A, and gastrin in all MEN1 patients, while PTH levels were reduced in all patients, reaching the normal range in about half the number of patients. Admittedly, parameters associated with hypercalcemia, such as gastrointestinal and neuromuscular symptoms, blood pressure, and quality of life, did not improve during cinacalcet, possibly due to the short treatment duration. Normalization of calcium and PTH levels was confirmed in a small subgroup of four patients who agreed to continue cinacalcet for an additional unblind observational phase of 12 months. As previously reported in sPHPT patients (19), no significant variations of bone mass density were observed in these patients.

In the sPHPT group, while calcium normalization was obtained in all patients, PTH reduction within the normal range was observed in one only patient. These data are consistent with the previous observation of a more impressive reduction of PTH levels observed in individual MEN1 patients in comparison with that obtained in sPHPT (11, 12). The underlying pathology may be of importance in determining the different response to cinacalcet, as the expression level of CASR, which is the drug target, might be different in MEN1-associated chief cell hyperplasia in comparison with sporadic adenoma. However, as in this study the percent PTH decrease reached during cinacalcet treatment was similar in MEN1 and sPHPT patients, the difference in the rate of PTH normalization between the two groups
was probably related to pretreatment PTH levels that were significantly higher in sPHPT in comparison with MEN1-related PHPT. In fact, as reported in a previous multicenter study on a large series of sporadic and MEN1-related PHPT, in addition to young age, the presence of normal, but inappropriate, PTH levels is a hallmark of MEN1 (19). Despite the rare occurrence of PTH normalization in sPHPT patients, the percent PTH decline observed in this cohort of patients (33%) was greater than that previously reported (8, 9, 19), probably related to a less severe disorder in our series. Indeed, in order to comparatively assess the efficacy of cinacalcet on calcium normalization in MEN1 patients, sPHPT patients were selected on the basis of pretreatment levels of serum calcium similar to those found in MEN1 patients. Moreover, the possible contribution of vitamin D administration on PTH reduction in MEN1 and sPHPT patients was ruled out as deficient patients were appropriately supplemented every 4–6 months before the enrollment.

A low rate of adverse events, usually mild and transient nausea that did not require treatment withdrawal, was observed in both MEN1 and sPHPT patients. These results were probably due to the low dosage of cinacalcet (45 ± 21 mg/day in MEN1 and 54 ± 25 mg/day in sPHPT) effective in restoring normocalcemia, as greater frequency and severity of side effects have been reported in patients requiring high dose (up to 60 mg twice daily) to control hypercalcemia (8, 20, 21). As far as cinacalcet dosage was concerned, this study did not confirm the higher sensitivity to cinacalcet of MEN1 patients, which has been suggested on the basis of clinical and biochemical data of case reports (11, 12). In fact, the percentage of patients requiring 30 mg cinacalcet daily to restore normocalcemia did not significantly differ between the MEN1 and sPHPT groups.

This study investigated the possible association of the Arg990Gly variant of the CASR gene to the clinical and biochemical response to cinacalcet. Several polymorphisms within the human CASR gene have been described in healthy populations and patients with disorders in calcium metabolism. In particular, the contribution of Arg990Gly to the clinical course and severity of PHPT has been extensively investigated (13, 14). Although data are not conclusive, there is evidence to suggest that patients with the 990Gly allele had a phenotype consistent with increased sensitivity of the receptor to extracellular calcium (15, 17, 22). Indeed, Arg990Gly is a nonconservative polymorphism located in the CASR carboxyl-terminal tail, a domain that impacts on several receptor properties, such as cell surface expression, intracellular signaling activation, and desensitization (23). Moreover, the gain of function of this variant has been confirmed by in vitro experiments showing the increased sensitivity of HEK cells stably transfected with CASR 990Gly allele to the calcimimetic R-568 (17).

At present, only a cohort of seven patients with secondary HPT due to chronic kidney disease has been screened for possible association between CASR polymorphisms and responsiveness to cinacalcet (16). In that study, patients bearing the 990Gly allele showed a more marked inhibition of PTH levels in response to cinacalcet compared with those homozygous for Arg at that location (16).

The data reported here did not support an influence of the variant receptor on the response to cinacalcet (16, 24), although the small set of patients investigated by this study as well as by the previous one did not allow definitive conclusions.

In conclusion, this short-term study demonstrated that the efficacy and safety profile of cinacalcet in patients with MEN1-related PHPT and in patients with sPHPT was similar and that the response to cinacalcet treatment was not associated with 990 CASR variant. Due to the low occurrence of successful parathyroid surgery and the high recurrence rate in MEN1 patients, cinacalcet might be considered a second choice treatment option for calcium normalization in these patients. However, it is worth noting that normalization of calcium and PTH levels was not associated with improvements in calciuria, bone density, symptoms, or quality of life, possibly due to the short-term treatment. Moreover, the cost of this option should be taken into account, also considering the rather young age of many MEN1-related PHPT patients. Finally, the lack of safety data of long-term cinacalcet treatment and its poor, if any, beneficial effect on bone mass are important limitations that require further clinical investigations. Similarly, global, double-blind, randomized, placebo-controlled trials are very eagerly awaited for the evaluation of whether normalization of calcium and PTH by long-term cinacalcet treatment reduces the increased cardiovascular morbidity and mortality in PHPT patients.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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