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Short-term Effects of Carbetimer on Calcium and Bone Metabolism in Man

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Abstract—*Carbetimer is a new antineoplastic agent whose limiting toxicity consists of dose- and treatment duration-dependent hypercalcemia. We examined the short-term effects of Carbetimer on calcium metabolism on days*, 1, 3 *and* 5 *during* 11 5-*day courses* (6.5–8.2 g/m^2/*day given over daily* 2-h *infusions, q* 3–4 *weeks*). *Blood parameters were measured before and after Carbetimer, whereas urinary parameters were studied in three consecutive* 2-h *collections before, during and after Carbetimer infusions. Carbetimer effects were similar regardless of the infusion day. We found a consistent decrease of plasma ionized* Ca (Ca^{2+}) *levels from* 4.56 ± 0.05 mg/dl *before infusion to* 4.28 ± 0.06 mg/dl *after infusion* ($P < 0.001$) *whereas total serum* Ca (*corrected for protein levels*) *did not change. The fall of* Ca^{2+} *stimulated parathyroid function, as suggested by the increased plasma PTH levels, the decreased serum phosphorus and TmP/GFR index, or the increased urinary phosphate and cyclic AMP excretion. Carbetimer infusions also induced a marked increase in urinary* Ca *excretion* (*expressed as* mg Ca/mg *creatinine*) *from* 0.093 ± 0.011 *before to* 0.359 ± 0.042 *during and* 0.177 ± 0.031 *after infusion* ($P < 0.001$). *These changes were best explained by Carbetimer-induced* Ca *chelation that we confirmed* in vitro *by incubating Carbetimer at various concentrations in whole blood for* 2 h *at* 37°C, *e.g.* 2 mg *of Carbetimer*/ml *lowered* Ca^{2+} *from* 4.82 *to* 3.20 mg/dl *without changing total* Ca *levels. On the other hand, a direct effect of Carbetimer on bone cannot be excluded since we observed an increase of serum osteocalcin levels from* 2.0 ± 0.3 *to* 2.5 ± 0.4 ng/ml *after infusion* ($P < 0.001$). *In summary, the short-term effects of Carbetimer on calcium metabolism markedly differ from the long-term effects. They mainly consist of a dose-related calcium chelation leading to a decrease in* Ca^{2+} *levels, an increase in urinary* Ca *excretion and a stimulation of parathyroid function.*

INTRODUCTION

CARBETIMER is a low molecular weight (1590) polymer derived from ethylene and maleic anhydride with an unusual pattern of preclinical antitumor activity [1, 2]. Clinical trials have been initiated and interesting activity has been demonstrated, particularly against malignant melanoma [3]. The mechanism of action of Carbetimer is still unclear and could involve direct antiproliferative effects, immunomodulation, or an action on the uptake and the phosphorylation of pyrimidines [3, 4].

Carbetimer does not have the usual side-effects of chemotherapeutic agents. Indeed, marrow toxicity, alopecia or mucositis are absent or minimal, whereas neurotoxicity and hypercalcemia constitute the major side-effects. Hypercalcemia was actually the dose-limiting toxicity in phase I trials, and its magnitude and frequency appeared to be treatment duration-dependent [3, 5, 6]. During the phase I trial performed in our Institution, 11 out of 26 patients developed hypercalcemia. The highest serum calcium concentration was 12.9 mg/dl, but higher levels have been observed in other centers.

The short-term effects of Carbetimer on calcium metabolism have not been previously reported and we show here that they are quite different from the above-mentioned long-term effects.

MATERIALS AND METHODS

Patients

We investigated the short-term effects of Carbetimer in eight cancer patients during 11 therapeutic cycles (three patients were studied twice). There were five males and three females; their median age was 55 (range 39–74) years. Tumor types consisted of seven melanomas and one colon cancer. Carbet-

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imer was diluted in 500 ml of glucose 5% and administered over 2 h at a daily dosage of 6.5–8.2 g/m^2 for 5 consecutive days every 3–4 weeks. Studies were performed on days 1, 3, and 5 of the 11 treatment cycles.

Laboratory determinations

The measurements were performed with techniques previously applied and validated [7–9].

Blood sampling was performed just before (= 0 h) and after (= 2 h) Carbetimer infusions. Measurements included:

- serum electrolytes, urea, creatinine, proteins, total calcium (Ca), which were all determined on a Technicon autoanalyzer (Tarrytown, NY, U.S.A.). Ca is also reported after correction for protein levels according to the following formula [10]:

$$\text{Corrected Ca (corr. Ca)} = \frac{\text{Ca measured}}{0.55 + \text{protein}/16};$$

  normal values 8.5–10.2 mg/dl.
- plasma ionized calcium (Ca^{2+}) measured with the Orion SS-20 electrode (Cambridge, MA, U.S.A.); normal values 4.2–5.0 mg/dl.
- serum phosphorus (Pi) measured by a classical colorimetric method; normal values 2.5–4.5 mg/dl.
- plasma immunoreactive parathyroid hormone (iPTH) measured by the PTH-MM assay of INCStar (Stillwater, MN, U.S.A.); normal values <30–77 pmol/l.
- serum osteocalcin (BGP) which estimates bone formation rate [7]; normal values 1–6 ng/l
- immunoreactive calcitonin (iCT) measured with an in-house assay on unextracted serum [11].

Urinary determinations included calcium, phosphate, hydroxyproline, creatinine and cyclic AMP (cAMP). We thus obtained the following ratios or indices: calcium/creatinine (Ca/creat.; Nl values <0.19 mg/mg), cAMP excretion/100 ml of glomerular filtrate (UcAMP; Nl values 1.4–6.3 nmol/100 ml GFR), and the renal phosphate threshold (TmP/GFR; Nl values 2.5–4.2 mg/dl).

All urinary parameters were determined during three consecutive 2-h urinary collections (before, during, and after Carbetimer infusions).

In vitro *effects of Carbetimer*

Carbetimer was added to 5 ml of blood at the following concentrations: 0, 100, 500, 2000, 10000 μg/ml of blood. After a 2-h incubation at 37°C, Ca^{2+} level was determined on the Corning 634 apparatus (Ciba-Corning, MA, U.S.A.) which corrects Ca^{2+} values for a pH of 7.4.

Statistical procedures

Data are reported as the mean ± S.E.M., except when indicated. Statistical significance was evaluated by two-tailed paired Student's *t* tests or analysis of variance.

RESULTS

Effects of Carbetimer on blood parameters of calcium metabolism (Table 1 and Fig. 1)

Carbetimer infusions caused a slight fall in total serum Ca levels which was no longer found when Ca was corrected for changes in protein levels (corr. Ca). Similarly, Carbetimer infusions (500 ml over 2 h) induced a slight but significant fall in hematocrit from 36.7 ± 1.5 to 34.2 ± 1.6% ($n = 13$; $P < 0.05$). Whereas corr. Ca did not change, there was a consistent and highly significant decrease in Ca^{2+} levels. We ruled out Carbetimer-induced alkalosis as a possible cause of this selective fall in Ca^{2+} levels; indeed, in the 10 infusions evaluable for this effect, we found no significant changes in serum bicarbonate levels (from 26.9 ± 0.9 before to 26.9 ± 0.8 mEq/l after infusion) or in blood pH (from 7.42 ± 0.01 to 7.41 ± 0.03 after infusion). There were also no detectable changes in serum iCT levels measured before and after five infusions.

The fall of Ca^{2+} was accompanied by an increase of iPTH and a decrease of Pi concentrations. We also observed a slight elevation of serum BGP levels (Table 1). As shown in Fig. 1 for Ca^{2+} and Pi, all these changes were consistently observed regardless of the infusion day. Similar results were obtained when the analysis was restricted to the very first infusion of Carbetimer in the eight studied patients, e.g. Ca^{2+} fell from 4.52 ± 0.12 to 4.22 ± 0.12 mg/dl ($P < 0.01$) and Pi fell from 3.5 ± 0.3 to 3.1 ± 0.3 mg/dl ($P < 0.05$).

Effects of Carbetimer on urinary parameters of calcium metabolism (Table 2 and Fig. 2)

Carbetimer infusions induced a considerable increase in urinary Ca excretion which was more important during the 2-h infusion than in the 2-h post-infusion collection (Table 2). As for plasma Ca^{2+}, these changes were observed regardless of the infusion day (Fig. 2). Similar results were again found when the analysis was restricted to the first Carbetimer infusion in the eight patients, since the Ca/creat. ratio (mg/mg) increased from 0.078 ± 0.018 before treatment to 0.322 ± 0.056 during Carbetimer infusion and to 0.167 ± 0.043 after infusion ($P < 0.001$). In absolute terms, the mean excretion of urinary Ca was 24.8 ± 5.6 mg higher during the 2-h Carbetimer infusion than during the preinfusion period. On the other hand, it is interesting to note that there were no significant changes in hydroxyproline excretion (Table 2).

Table 1. Acute effects of Carbetimer infusion on blood parameters of calcium metabolism

	No. of evaluable infusions	Before infusion	After infusion	*P* value
Total Ca, mg/dl	33	9.3 ± 0.1	9.1 ± 0.1	<0.05
Corr. Ca, mg/dl	33	9.9 ± 0.1	9.9 ± 0.1	NS
Ca^{2+}, mg/dl	30	4.56 ± 0.05	4.28 ± 0.06	<0.001
Pi, mg/dl	32	3.9 ± 0.1	3.4 ± 0.1	<0.001
iPTH, pmol/l	21	44 ± 2	49 ± 2	<0.05
BGP, ng/ml	30	2.0 ± 0.3	2.5 ± 0.4	<0.001

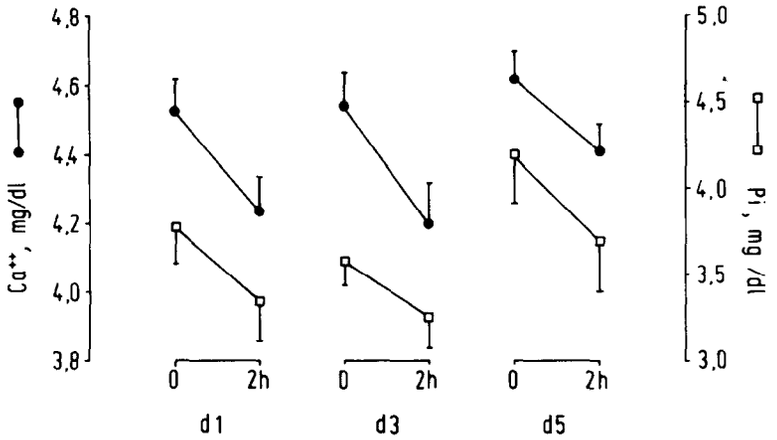

Fig. 1. Effects of Carbetimer on plasma Ca^{2+} and serum Pi levels evaluated during 11 5-day cycles in eight cancer patients. Sampling was performed before and after daily 2-h infusions of Carbetimer on days 1, 3 and 5 (d 1, d 3, d 5). Indicated values represent the mean ± S.E.M.

Table 2. Acute effects of Carbetimer infusion on urinary parameters of calcium metabolism

	No. of evaluable infusions	Before infusion	During infusion	After infusion
Ca/creat., mg/mg	32	0.093 ± 0.011	0.359 ± 0.042**	0.177 ± 0.031*
Hydroxyproline, mg × 100/mg creat.	28	4.7 ± 0.8	4.9 ± 0.7	4.8 ± 0.8
Pi/creat., mg/mg	27	0.26 ± 0.03	0.42 ± 0.04**	0.41 ± 0.03**
TmP/GFR, mg/dl	24	4.1 ± 0.1	3.5 ± 0.2**	3.2 ± 0.2**
UcAMP, nmol/100 ml GFR	21	4.8 ± 0.4	6.2 ± 0.7	6.0 ± 0.7

*$P < 0.01$, **$P < 0.001$ vs. baseline.

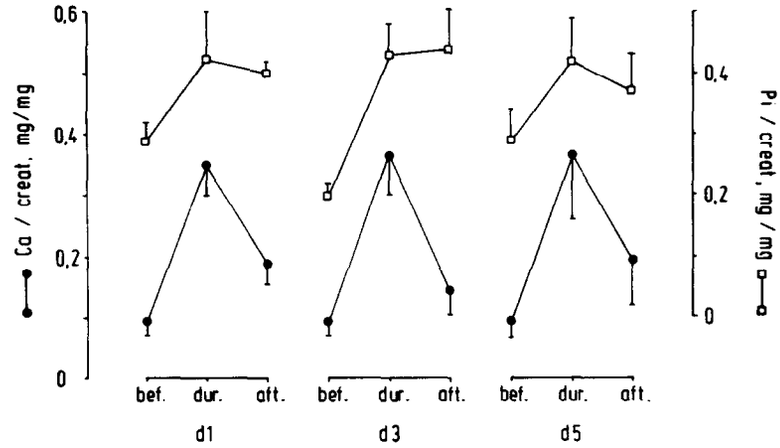

Fig. 2. Effects of Carbetimer on urinary Ca and Pi excretion, related to creatinine, measured in three consecutive 2-h urinary collections (before, during and after Carbetimer infusions).

Carbetimer infusions also caused a significant decrease of the TmP/GFR index paralleled by an increase in urinary phosphate and a slight elevation of UcAMP excretion. Unlike the observed increase in urinary Ca excretion, these changes were quite similar in the 2-h collections performed during and after Carbetimer infusion (Table 2 and Fig. 2).

In vitro *effects of Carbetimer on* Ca^{2+} *levels (Fig. 3)*

Carbetimer caused a marked and dose-related decrease in Ca^{2+} levels starting at the concentration of 500 μg Carbetimer/ml, whereas total Ca fell only slightly at the highest concentration of 10,000 μg/ml.

DISCUSSION

We were very surprised at first by the short-term effects of Carbetimer which are in complete contradiction to the long-term hypercalcemic effects of the drug. Indeed, we mainly found a highly reproducible decrease in serum Ca^{2+} levels, no change in corrected total Ca concentrations and a marked increase in urinary Ca excretion. Classical causes of decrease in Ca^{2+} levels, such as alkalosis, acute hypoparathyroidism or even calcitonin release, can be eliminated. The best explanation of our *in vivo* findings obviously was a drug-induced calcium chelation. Indeed, the classical calcium chelator EDTA similarly causes a drop in serum Ca^{2+} without initially changing total serum Ca levels, which transiently stimulates parathyroid activity and increases urinary Ca excretion [12]. Our *in vitro* observations clearly confirm that Carbetimer is indeed a potent calcium chelator. The drop in Ca^{2+} concentrations was dose-dependent and probably of a degree comparable to the *in vivo* effects when one takes into account the administered dose (10–15 g) and the circulating volume. However, lack of pharmacokinetic data on Carbetimer makes it hazardous to draw precise correlations between active *in vitro* concentrations on Ca chelation and achieved *in vivo* serum levels of Carbetimer.

These short-term effects clearly cannot explain the long-term hypercalcemic effects of the drug. The stimulation of parathyroid function by a calcium chelator is only transient and repeated administrations are not able to induce permanent 'secondary' hyperparathyroidism leading to a hypothetical 'tertiary' hyperparathyroidism causing hypercalcemia as observed in some cases of chronic renal insufficiency. The long-term hypercalcemic effects of Carbetimer are more likely related to its effects on bone. In related experiments, we have indeed shown that Carbetimer is a very potent bone resorbing agent in the classical neonatal mouse calvaria bioassay [13]. In these experiments, bone resorption was only evaluated after a 48-h incubation with Carbetimer. On the other hand, animal data have shown that Carbetimer binds to bone [1] and an acute bone resorbing effect might have been masked by the potent chelating activity on serum calcium. The lack of increase in urinary hydroxyproline, a classical marker of bone resorption [14], argues against this hypothesis but the increase in serum BGP concentrations that we have observed is compatible with an acute effect of Carbetimer on bone. BGP is a bone-specific protein made by the osteoblasts; it is entrapped in bone and its serum concentration is considered to reflect bone formation activity. However, only *in vitro* experiments on bone cells could discriminate between possible stimulatory effects of Carbetimer on osteoblasts or simply effects on the bone matrix. The interpretation is

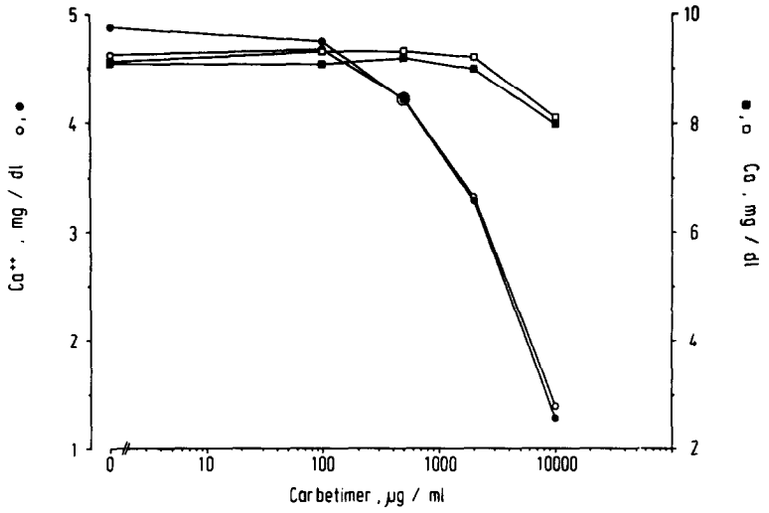

Fig. 3. In vitro *effects of various concentrations of Carbetimer on total calcium* (Ca: □, ■) *and ionized* Ca (Ca^{2+}; ○, ●) *levels. Results of two separate experiments are given (see Materials and Methods). Note the log scale for Carbetimer concentrations.*

further complicated by our ignorance of the effects of acute hypocalcemia on BGP release by bone cells.

The drop in serum Pi levels, the decreased TmP/GFR index, the increased UcAMP excretion are all consistent with a stimulation of PTH secretion in response to the decrease of biologically active Ca. The increase of plasma iPTH levels was modest, but this is probably partly due to hemodilution and the relative lack of sensitivity of 'mid-molecule' PTH assays compared to newer 'intact PTH' assays [15]. The increased PTH secretion was, however, not able to counteract the elevation of urinary calcium induced by drug chelation. Lastly, infusion of glucose may have contributed to the decrease of serum Pi levels.

In summary, the effects of Carbetimer on calcium and bone metabolism are quite complex and short-time effects clearly differ from long-term effects. This work demonstrates that short-term effects mainly consist of a decrease in serum Ca^{2+} levels, an increased urinary Ca excretion and a stimulation of parathyroid activity which all appear to be the consequence of a drug-induced calcium chelation. In addition, an acute effect on bone forming cells or on bone matrix cannot be excluded, and bone resorbing effects may become predominant after long-term administration.

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REFERENCES

1. *Investigational Brochure on Carbetimer: An Antineoplastic Agent.* Skokie, Illinois, GD Searle, 1988.
2. Kisner D, Paget E, Metha P, Von Hoff D. Carbetimer: activity in the human tumor cloning assay and report of a phase I clinical trial. *Invest New Drugs* 1984, **2**, 105.
3. Dodion P, De Valeriola D, Body JJ *et al.* Phase I clinical trial with Carbetimer. *Eur J Cancer Clin Oncol* 1989, **25**, 279–286.
4. Arladan B, Paget GE. Mechanism of action of a new antitumor agent, Carbetimer. *Cancer Res* 1986, **46**, 5473–5476.
5. Grunberg SM, Ehler E, Mitchell MS. Administration of a 5-day course of Carbetimer. *Proc Am Assoc Cancer Res* 1987, **28**, 224.
6. Hanauske A, Melink T, Clark G *et al.* Phase I clinical trial of Carbetimer. *Proc Am Soc Clin Oncol* 1987, **6**, 25.
7. Body JJ, Cleeren A, Pot M, Borkowski A. Serum osteocalcin (BGP) in tumor-associated hypercalcemia. *J Bone Miner Res* 1986, **1**, 523–527.
8. Body JJ, Borkowsi A, Cleeren A, Bijovet OLM. Treatment of malignancy-associated hypercalcemia with intravenous aminohydroxypropylidene diphosphonate (APD). *J Clin Oncol* 1986, **4**, 1177–1183.
9. Body JJ, Pot M, Borkowsi A, Sculier JP, Klastersky J. Dose/response study of amino-hydroxypropylidene bisphosphonate in tumor-associated hypercalcemia. *Am J Med* 1987, **82**, 957–963
10. Parfitt AM. Investigation of disorders of the parathyroid glands. *Clin Endocrinol Metab* 1974, **3**, 451–474.
11. Body JJ, Demeester-Mirkine N, Borkowski A, Suciu S, Corvilain J. Calcitonin deficiency in primary hypothyroidism. *J Clin Endocrinol Metab* 1986, **62**, 700–703.
12. Parfitt AM. Study of parathyroid function in man by EDTA infusion. *J Clin Endocrinol* 1969, **29**, 569–580.
13. Body JJ, Nejai S, Magritte A, Fernandez G, Dodion P. Effects of Carbetimer on calcium metabolism: *in vivo* and *in vitro* studies. *Calcif Tissue Int* 1989, **44**, S81.
14. Kivirikko KI. Urinary excretion of hydroxyproline in health and disease. *Int Rev Connect Tissue Res* 1970, **5**, 93–163.
15. Blind E, Schmidt-Gayk H, Scharla S *et al.* Two-site assay of intact parathyroid hormone in the investigation of primary hyperparathyroidism and other disorders of calcium metabolism compared with a midregion assay. *J Clin Endocrinol Metab* 1988, **67**, 353–360.