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HAL Id: hal-00534999
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Investigations of inter- and intraindividual relationships between exposure to oral salmon calcitonin and a surrogate marker of pharmacodynamic efficacy

Morten A. Karsdal · Inger Byrjalsen · Kim Henriksen · Bente J. Riis · Claus Christiansen

Received: 14 May 2009 / Accepted: 21 September 2009 / Published online: 8 October 2009
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Abstract

Aims The aims of the study were to investigate interindividual variations in the bioavailability of salmon calcitonin (sCT) following single oral 0.8 mg doses at three different times of the day, and intraindividual variation in sCT bioavailability at each end of a 14-day treatment period. We also investigated correlations between exposure to sCT and levels of the bone resorption biomarker serum C-terminal telopeptide of collagen type I (CTX-I).

Methods Participants were from two randomized, double-blind, placebo-controlled studies. In study I, healthy postmenopausal women received a single dose of 0.8 mg of oral sCT or placebo at 08:00 (n=42), 17:00 (n=20), or at 22:00 (n=19). In study II, age-matched men or postmenopausal women with osteoarthritis received 0.8 mg oral sCT (n=26) or placebo (n=23) twice daily for 14 days, with dosing at 08:00 and 17:00. In both studies, drug exposure was assessed by plasma sCT concentrations, and bone resorption by CTX-I levels.

Results The variability in exposure between patients, measured as coefficient of variation (CV), was as follows: 22% for the morning dose, 30% for the predinner dose, and 34% for the evening dose. In study I, a high degree of correlation was seen between the level of exposure following a single 0.8 mg dose of sCT and suppression of serum CTX-I, with Pearson correlation coefficients of \( r = -0.74 \), \( -0.96 \), and \( -0.78 \), following doses at 08:00, 17:00, and 22:00, respectively. In study II, exposure to sCT varied widely within the same individuals between dosing days 1 and 14, with weak correlations of \( r = 0.40 \) and 0.38 at the dose times 08:00 and 17:00, respectively. As expected from this finding, the intraindividual response in serum CTX-I levels was non-significantly associated on dosing days 1 and 14 (\( r = 0.34 \) and \( r = 0.27 \) at dose times 08:00 and 17:00, respectively).

Conclusion Increased bioavailability of orally administered 0.8 mg sCT was highly correlated with increased suppression of the bone resorption marker serum CTX-I irrespective of the time of day. However, the high inter- and intraindividual variability in sCT exposure demonstrates the importance of determining the optimum conditions for ensuring the most beneficial sCT uptake.

Keywords Calcitonin · Oral · Formulation · CTX-I · Efficacy

Introduction

Salmon calcitonin (sCT) is approved for the treatment of osteoporosis and other diseases involving accelerated bone turnover [1]. In addition, sCT has been suggested as a possible intervention strategy for osteoarthritis [2, 3]. Calcitonin is a small, 32-amino-acid peptide, with potent antiresorptive capacities, and in its natural form in vertebrates is believed to be secreted in response to excess calcium in serum [4]. Clinical use of salmon calcitonin has been limited to either the subcutaneous or intranasal route [5]. However, oral administration remains the desired delivery route to ensure patient satisfaction and compliance. An increasing number of studies have evaluated novel oral formulations of calcitonin [2, 6–12].

Major challenges exist to developing an effective mechanism for oral delivery of proteins. Firstly, proteins
may exhibit low bioavailability after oral administration because of the high acid content of the digestive tract, the extensive array of proteases present there [13], and finally the proteins’ limited ability to cross the gastrointestinal epithelium [11, 14]. 5-CNAC [8-(N-2-hydroxy-5-chloro-benzoyl)-amino-caprylic acid], a molecule based on the Eligen technology, has proven useful in combination with calcitonin to facilitate calcitonin passage through the gastrointestinal tract resulting in enhanced absorption from the intestinal lumen into the bloodstream [1, 2, 15, 16]. A detailed description of the Eligen technology was recently published [17, 18]. The oral formulation of 5-CNAC in combination with sCT was demonstrated to be safe and effective in reducing levels of biomarkers of bone resorption in a 3-month phase II study in healthy postmenopausal women [1].

Secondly, some patients may respond better than others to oral therapies, which may be due in part to individual parameters in the gastrointestinal tract affecting drug uptake and hence drug efficacy [14, 19]. Thus, careful investigations of inter- and intraindividual variations are necessary for a thorough assessment of a new therapy’s efficacy and safety.

Thirdly, some drugs do not follow the expected dose-response pattern. Phase II studies with new chemical entities are conducted to identify the optimum dose that delivers maximum efficacy without toxic side effects. In these studies, the generally desired profile is a linear relationship between exposure and efficacy. However, the potential efficacy of a drug is not always in direct linear relationship to exposure. For example, sCT has been demonstrated to have a protracted effect on efficacy parameters in the gastrointestinal tract affecting drug uptake [15, 17, 20–22], resembling an indirect pharmacokinetic model [23]. Thus, alternatives to the linear relationship are needed to measure oral sCT efficacy and pharmacokinetic parameters.

Serological biochemical markers of surrogate efficacy are receiving increasing attention in pharmacological research [24, 25]. Osteoclast-mediated bone resorption is mainly mediated by cathepsin K [26]. The protease activity of cathepsin K results in a specific degradation fragment of collagen type I, C-terminal telopeptide of collagen type I (CTX-I) [25, 27]. This fragment has been extensively used as a surrogate marker of bone resorption for in vitro, preclinical, and clinical studies [25, 28].

The aim of the current study was to investigate interindividual variations in bioavailability of sCT following single oral 0.8 mg doses in the morning at 08:00, in the late afternoon at 17:00, or in the evening at 22:00, and the correlations between exposure to sCT and levels of the pharmacodynamic biomarker serum CTX-1. Furthermore we investigated intraindividual variation in the bioavail-

ability of the active substance following dosing on days 1 and 14.

Materials and methods

Drug substance

SMC021 A/C is an oral formulation of salmon calcitonin. The investigational drug consisted of 0.8 mg of recombinant sCT and 200 mg of 5-CNAC [8-(N-2-hydroxy-5-chloro-benzoyl)-amino-caprylic acid], a unimolecular enhancer of gastrointestinal peptide absorption developed by Emisphere Technology (NJ, USA) and licensed to Novartis Pharma (Basel, Switzerland). The same batch was used in study I and study II.

Study design

Participants were from two randomized, double-blind, double-dummy, placebo-controlled studies. In study I, healthy postmenopausal women aged 57–71 years were randomized to receive 0.8 mg sCT or placebo in a specific order, with blood samples being taken before drug intake and at 5, 10, 15, 30, 45 min, 1, 1.5, 2, 2.5, and 3 h, and every subsequent hour until 24 h after dosing [15]. In part 1, subjects (n=42) were given placebo or 0.8 mg sCT from either a pilot equipment batch or a full-scale production batch at 08:00 after overnight fasting. Meals were served at 09:00, 13:00, and 18:00. In the next two parts of the study, sCT from the same full-scale production batch was used. In part 2, subjects (n=20) received lunch at the study site at 13:00 and study drug at 17:00, with further meals at 18:00, 09:00, and 13:00. In part 3, subjects (n=19) received dinner at the study site at 17:00, with study drug at 22:00 and meals at 09:00, 13:00, and 18:00. In all three parts, no other food consumption was permitted, but intake of water was allowed from 1 h after dosing.

The study was conducted in accordance with Helsinki Declaration II and approved by local Ethics Committees (EudraCT number: 2006-002685-19; www.clinicaltrials.gov, registration number NCT00411125). Written informed consent was obtained from all participants.

In study II, age-matched men (n=36) and postmenopausal women (n=37) suffering from osteoarthritis [29] were randomized to receive oral sCT doses of 0.6 mg (n=24, 12 women and 12 men), 0.8 mg (n=26, 13 women and 13 men), or placebo (n=23, 12 women and 11 men) twice daily for 14 days, with one dose at 08:00 and the other before dinner at 17:00. The drug substance was from the same batch. On treatment days 1 and 14, blood samples were taken before drug intake, and at 10, 15, 30, 45 min,
and 1, 2, 4, 6, and 8 h after dosing at 08:00, and before drug intake and at 10, 15, 30, 45 min, and 1, 2, and 4 h after dosing at 17:00. On days 1 and 14, subjects remained fasting until lunch was served at 13:00. Coffee with a light snack was served at 15:30 and dinner at 18:00. No other food consumption was allowed.

To enable comparisons across studies I and II, only data from the common active dose—0.8 mg sCT—were included in the analysis. High exposures were defined as the 50% of the subjects having AUC of plasma sCT above the median, and low exposures being the other half having AUC below the median. The subjects were divided into those two groups for the purpose of investigating the dependency of the level of absorption of sCT on the pharmacokinetics and pharmacodynamics patterns.

The study was conducted in accordance with Helsinki Declaration II and approved by local Ethics Committees (EudraCT number: 2006-005532-24; www.clinicaltrials.gov, registration number NCT00486369). Written informed consent was obtained from all participants.

Biochemical measurements

Plasma and serum samples were stored at −20°C until analysis. For the pharmacokinetic assessment, plasma sCT was measured in the blood samples collected 0–4 h after drug intake. The concentration of plasma sCT was measured by a chemiluminescence-based immunoassay as previously described [1]. For the pharmacodynamic assessment, the efficacy of oral sCT was assessed by serum levels of bone resorption biomarker CTX-I in blood samples collected 0–24 h after drug intake (study I), and 0–8 h after 08:00 dosing and 0–4 h after 17:00 dosing (study II). The concentration of serum CTX-I was determined by Serum CrossLaps One Step ELISA (Nordic Bioscience Diagnostics, Herlev, Denmark) [30]. The interassay coefficients of variation of the plasma sCT and serum CTX-I measurements were 10 and 11%.

Statistical analysis

The trapezoidal method was applied for calculation of the area under the curve (AUC) of plasma sCT\textsubscript{0–4h}, relative change in serum CTX-I\textsubscript{0–12h} after dosing, relative change in serum CTX-I\textsubscript{0–8h} after 08:00 dosing, and CTX-I\textsubscript{0–4h} after 17:00 dosing. The relative value of serum CTX-I was calculated as the percentage of the individual predose value. A noncompartmental pharmacokinetic analysis was performed for calculation of the elimination half-life (T\textsubscript{1/2}) associated with the terminal slope (λ\textsubscript{z}) of a semilogarithmic concentration-time curve using WinNonLin version 5.2. The elimination half-life was estimated as ln2/λ\textsubscript{z}. Plasma sCT values below the lower limit of quantification of 2.5 pg/ml were assigned the value of 2.5 pg/ml in the calculation of the AUC of plasma sCT\textsubscript{0–4h} and C\textsubscript{max}.

The AUC of plasma sCT, C\textsubscript{max}, and the time and the time course data of the relative values of serum CTX-I were logarithmically transformed to obtain normality and symmetry of variances. The nonparametric Wilcoxon test was used for comparison of AUC of plasma sCT\textsubscript{0–4h}, C\textsubscript{max}, and T\textsubscript{max} values between high and low absorbers. The two-tailed Student’s t-test was applied for comparison of the elimination rate constant ke and AUC of serum CTX-I\textsubscript{0–12h} between high and low absorbers. The two-tailed paired Student’s t-test was applied for comparison of AUC of serum CTX-I\textsubscript{0–12h} between placebo and 0.8 mg oral sCT groups. The Pearson correlation coefficient was calculated to assess the association between the exposure of sCT and the efficacy response of AUC of relative change in serum CTX-I\textsubscript{0–12h}.

The intraindividual association between AUC of plasma sCT\textsubscript{0–4h} on dosing days 1 and 14 and the intraindividual association between the responses of AUC of relative change of serum CTX-I on dosing days 1 and 14 were assessed. The statistical calculations were performed using the SAS software package (release 9.1, SAS Institute, Cary, NC, USA).

Results

In study I, the age distribution among the 81 healthy, 57- to 71-year-old postmenopausal women taking part was comparable across the three parts of the study [21]. In study II, the 0.8 mg oral sCT group included 13 postmenopausal women aged 60 to 75 years and 13 males aged 58 to 75 years with osteoarthritis. Among study II subjects, 9 had a Kellgren Lawrence index score of 1, 12 had a score of 2, and 5 had a score of 3 [29].

The pharmacokinetic parameters of a single 0.8 mg dose of sCT for exposures below the median and above the median at the three dosing times in study I are given in Table 1. Irrespective of dosing time, oral sCT was rapidly absorbed, and C\textsubscript{max} was achieved between 15 and 30 min after dosing. After reaching C\textsubscript{max}, sCT was eliminated from plasma with a short half-life (T\textsubscript{1/2}) between 9 and 15 min. The half-life was significantly shorter in subjects with low exposure as compared to subjects with high exposure at the morning and predinner dosing times (p=0.03 and p=0.03), whereas no difference was observed at the evening dosing time (p=0.85).

Serum levels of the biomarker CTX-I, which indicate the efficacy of oral sCT in suppressing bone resorption, are shown in Fig. 1. The levels depicted are relative to the predose value up to 24 h postdose. Table 2 gives the mean AUC\textsubscript{0–12h} of serum CTX-I in study I. Overall, in healthy postmenopausal women, dosing with 0.8 mg oral sCT
resulted in significant suppression of serum CTX-I compared with placebo, irrespective of dosing time and level of exposure of sCT. A linear relationship existed between sCT AUC plasma exposure and the response in AUC of serum CTX-I, with a correlation coefficient of $r = -0.96$ ($p < 0.001$). Following the morning dose of oral sCT, the subjects with sCT exposure above the median showed suppression in serum CTX-I for up to 12 h postdose whereas in subjects with sCT exposure below the median, serum CTX-I returned to the placebo level 5 h postdose. An even more pronounced difference in suppression of serum CTX-I between sCT exposure groups was observed for the predinner dosing at 17:00. Serum CTX-I was suppressed about 16 h after dosing in those with the highest exposure in comparison with 5 h in those with lowest exposure. The same pattern of prolonged suppression of serum CTX-I was observed in those with high exposure following the evening dosing at 22:00.

Figure 2 shows the association between the level of exposure to sCT and the effect on serum CTX-I, measured as AUC of the relative change during the 12-h period following dosing. Irrespective of dosing time, statistically highly significant associations were found between exposure and serum CTX-I levels with a Pearson correlation coefficient $r$ value of $-0.74$ and $-0.78$ for the morning dose and evening dose, respectively, and $-0.96$ for the predinner dose.

The intraindividual association between the bioavailability of sCT on dosing day 1 and dosing day 14 in study II is shown in Fig. 3. Drug exposure levels were only weakly correlated, with low $r$ values of 0.40 for the 08:00 dose and 0.38 for the 17:00 dose. The difference in exposure was statistically significant at dosing time 08:00 ($p = 0.05$) but of borderline significance at dosing time 17:00 ($p = 0.06$). Consequently, and as expected from the high intrapatient variability in exposure, the intraindividual response in serum CTX-I levels was statistically nonsignificantly associated on dosing day 1 and dosing day 14 with $r = 0.34$ and $r = 0.27$ at the dose times 08:00 and 17:00 as shown in Fig. 4. Lastly, the variability in exposure to sCT between patients expressed as coefficient of variation (CV) was 22% for the morning dose, 30% for the predinner dose, and 34% for the evening dose.

**Discussion**

In the clinical use of calcitonin, the oral route is likely to be the preferred route of administration. However oral delivery

| Table 1 | Pharmacokinetic parameters of healthy postmenopausal women receiving a single 0.8 mg dose of oral salmon calcitonin (study 1) |
|------------------|------------------|------------------|
| **Dosing at 08:00** | **Low exposure** | **High exposure** | **p-Value** |
| AUC$_{0-4h}$ (pg·min/ml)$^a$ | 986 | 3,119 | – |
| Q1–Q3 range | (861–1,255) | (2,346–4,038) | |
| $C_{max}$ (pg/ml)$^a$ | 24 | 86 | $<0.0001$ |
| Q1–Q3 range | (16–31) | (64–132) | |
| $T_{max}$ (min)$^a$ | 15 | 30 | $<0.0001$ |
| Q1–Q3 range | (15–30) | (30–45) | |
| $T_{1/2}$ (min)$^b$ | 9.6 | 11.8 | 0.03 |
| ± 1SD range | (7.9–12.1) | (9.4–15.8) | |
| **Dosing at 17:00** | **n=10** | **n=10** | |
| AUC$_{0-4h}$ (pg·min/ml)$^a$ | 709 | 4,474 | – |
| Q1–Q3 range | (627–1,560) | (3,147–10,122) | |
| $C_{max}$ (pg/ml)$^a$ | 12 | 136 | $<0.0001$ |
| Q1–Q3 range | (5–45) | (102–205) | |
| $T_{max}$ (min)$^a$ | 15 | 30 | 0.10 |
| Q1–Q3 range | (15–30) | (30–30) | |
| $T_{1/2}$ (min)$^b$ | 9.3 | 14.6 | 0.02 |
| ± 1SD range | (8.6–10.1) | (10.9–22.0) | |
| **Dosing at 22:00** | **n=10** | **n=9** | |
| AUC$_{0-4h}$ (pg·min/ml)$^a$ | 771 | 3,794 | – |
| Q1–Q3 range | (600–858) | (1,699–8,909) | |
| $C_{max}$ (pg/ml)$^a$ | 12 | 102 | $<0.0001$ |
| Q1–Q3 range | (3–14) | (42–151) | |
| $T_{max}$ (min)$^a$ | 30 | 30 | 0.08 |
| Q1–Q3 range | (15–30) | (30–45) | |
| $T_{1/2}$ (min)$^b$ | 14.4 | 15.2 | 0.85 |
| ± 1SD range | (11.5–19.1) | (11.0–24.8) | |

$^a$Median (Q1–Q3 range)  
$^b$Mean (± 1SD range)
poses challenges in terms of ensuring robust drug uptake and efficacy.

Recently a new oral formulation of calcitonin in combination with the carrier molecule 5-CNAC has been tested clinically [1, 2]. An extensive clinical study program with this oral formulation has been undertaken, including but not limited to investigation of possible interaction with food [17], amount of water consumed [15], dosing at different time points during the day to assess the effect on the strong circadian variation in bone resorption [21], and finally a synthetic versus a recombinant form [22]. However, a systematic study of the individual response to this oral formulation has not been presented.

The present data clearly demonstrate that in healthy postmenopausal women significantly higher suppression of serum CTX-I was observed in subjects with the highest
intestinal exposure to sCT, regardless of the time of day of dosing. These interindividual observations are in line with previous findings in groups of patients receiving different doses of oral sCT that demonstrated a clear dose response to sCT.

Table 2 Pharmacodynamic effect of a single dose of 0.8 mg of oral salmon calcitonin on the bone resorption biomarker serum C-terminal telopeptide of collagen type I (CTX-I) in healthy postmenopausal women (study I)

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Low exposure</th>
<th>High exposure</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing at 08:00</td>
<td>n=42</td>
<td>n=21</td>
<td>n=21</td>
</tr>
<tr>
<td>AUC0–12h (%·h)a</td>
<td>−443</td>
<td>−582***</td>
<td>−740***</td>
</tr>
<tr>
<td>± 1SD range</td>
<td>(−553 to −333)</td>
<td>(−670 to −494)</td>
<td>(−814 to −666)</td>
</tr>
<tr>
<td>Dosing at 17:00</td>
<td>n=20</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>AUC0–12h (%·h)a</td>
<td>270</td>
<td>24**</td>
<td>−551***</td>
</tr>
<tr>
<td>± 1SD range</td>
<td>(26 to 514)</td>
<td>(−107 to 156)</td>
<td>(−670 to −494)</td>
</tr>
<tr>
<td>Dosing at 22:00</td>
<td>n=19</td>
<td>n=10</td>
<td>n=9</td>
</tr>
<tr>
<td>AUC0–12h (%·h)a</td>
<td>681</td>
<td>404**</td>
<td>−185**</td>
</tr>
<tr>
<td>± 1SD range</td>
<td>(181 to 1,181)</td>
<td>(−20 to 829)</td>
<td>(−782 to 412)</td>
</tr>
</tbody>
</table>

Values given are mean (± 1SD range). Significance of difference from placebo: *p<0.05, **p<0.01, ***p<0.001. Comparison of low exposure versus high exposure is given in the p-value column.
to sCT and reduced serum CTX-I. However, those findings were only analyzed on a group basis [1]. The present controlled analysis of pharmacodynamic and pharmacokinetic relationships indicated almost no variable individual sensitivity to the drug.

The wide intrapatient variations in exposure to sCT could reasonably account for the low correlations in bioavailability between morning or late afternoon dosing on days 1 and 14. As expected from the high variability in drug exposure, the intraindividual levels of serum CTX-I on dosing days 1 and 14 were also weakly correlated.

The present studies suggest that subjects with relatively low exposure to oral sCT on the first day of dosing also did not necessarily have similarly low exposure on subsequent dosing days. The lower level of exposure resulted in an equally lower pharmacodynamic response, due to the strong linear relationship with efficacy markers. The data show a high variability in exposure between subjects. However this needs to be further investigated in larger clinical settings. Whether these differences arise from genetic dispositions or daily living patterns such as food consumption, exercise, and other nongenetic determinants remains to be investigated. These present data may aid other scientists involved in the oral formulation of peptides.

Osteoarthritis (OA) is the most common form of arthritis [31]. Bone and cartilage degradation are normally tightly coupled in the pathogenesis of OA [31, 32], in which subchondral bone turnover, sclerosis of the subchondral plate, trabecular thinning, and articular cartilage loss are coupled processes [32]. At present it is not well understood or documented which parameters are initiators or drivers of this disease. In view of this interaction between the bone and cartilage compartments of the articular joint, an optimum approach to counter the progression of OA may be to target both bone and cartilage degeneration. Calcitonin has been suggested to have direct effects on chondrocytes in addition to the well-described effects on osteoclasts [33, 34]. sCT has been demonstrated to have positive effects on OA clinical signs and symptoms under preliminary clinical settings [33, 35]. Whether sCT may be a structure-modifying treatment for OA is currently under investigation in phase III clinical settings, with this version of oral sCT.

This current study has some limitations. The study was not powered to take into account the potential influence of gender, differences in subjects’ renal function, the effect of exercise, or impact of diseases other than osteoarthritis, on serum levels of CTX-I. Even though CTX-I is an extensively used surrogate marker of bone resorption [24, 36], the present data need to be confirmed in long-term clinical settings. In addition, whether a similar dose-response relationship between oral calcitonin and cartilage parameters exists, as compared to that of bone parameters, needs to be further investigated under long-term clinical settings, although preliminary dose-response relationships have been presented [2].

In conclusion, increased bioavailability of orally administered sCT is highly correlated with increased suppression of the bone resorption marker serum CTX-I. The variable exposure of the drug demonstrates the importance of determining the optimum conditions for ensuring the most beneficial drug uptake. These current observations need to be evaluated in longer-term clinical studies with a higher number of participants presenting with the appropriate disease characteristics.

Acknowledgments The authors are very grateful to Bonnie Malloy for editorial assistance. The study was supported by Den Danske Forskningsfond, The Danish Research Foundation.
Reference