A comparison of the acute effects of subcutaneous and intranasal calcitonin

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SUMMARY
1. We studied the acute effects of intranasal and subcutaneous calcitonin in 40 patients with active Paget's disease of bone. Patients received a single dose of either 400 units of calcitonin delivered as a nasal spray, or 1, 10 or 100 units of subcutaneous calcitonin, or placebo.
2. Subcutaneous salmon calcitonin, administered at doses of 1, 10 and 100 units to nine patients with Paget's disease of bone, induced a dose-dependent fall in the serum calcium. This calcium-lowering effect was not seen with a second group of nine patients receiving placebo.
3. The lower doses of calcitonin had significant effects, and these were more pronounced in patients with lower rates of bone turnover.
4. Four hundred units of calcitonin administered as a nasal spray induced effects qualitatively similar to those seen with subcutaneous calcitonin, with an efficacy equivalent to approximately 30 units of subcutaneous calcitonin.
5. We conclude that the bioequivalence of calcitonin given by intranasal insufflation is low compared with its parenteral administration. The intranasal route may be more appropriate for managing patients with disorders associated with low bone turnover.

Key words: acute hypocalcaemic response, calcitonin, nasal spray.

INTRODUCTION
Calcitonin is used in the management of several disorders associated with an increased resorption of bone. These include Paget's disease of bone [1, 2], postmenopausal osteoporosis [3], algodystrophy [4] and hypercalcaemia due to malignancy [5, 6]. There are several problems, however, regarding the use of calcitonin. It is rapidly degraded by gastric secretions, and for this reason is usually administered parenterally [5]. In addition, unpleasant side-effects have been reported in up to 77% of patients [7, 8], and although usually mild and transient, they may be severe enough to stop treatment in up to 15% of patients [7, 8]. This has limited the use of calcitonin for long-term treatment, and has prompted the search for novel routes of administration without these problems.

There is now clear evidence that calcitonin is absorbed across the nasal mucosa [9-12], but there are conflicting data on its efficacy [13-17]. Difficulties with the assay of calcitonin when low doses are given and the paucity of dose-response data for the parenteral route has made it difficult to assess the bioequivalence of intranasal calcitonin. For this reason we studied the acute hypocalcaemic response to administered calcitonin. Since the fall in serum calcium induced by calcitonin is primarily due to its inhibitory effects on osteoclasts [18], and the degree of hypocalcaemia is proportional to the rate of bone turnover [19], we chose to investigate this in patients with Paget's disease of bone.

MATERIALS AND METHODS
We studied 40 patients with active Paget's disease of bone. These patients were divided into five treatment groups, which were matched for disease activity. Nine patients received, sequentially and in a random order, 1, 10 and 100 units of salmon calcitonin subcutaneously (Calsynar; Rorer). These tests were separated in time by at least 2 weeks. For the 1 and 10 unit tests, standard salmon calcitonin solution was diluted with saline (150 mmol/l NaCl) containing albumin, to minimize its adsorption to the plastic syringe. Nine further patients, matched for disease activity (±10%), were given a subcutaneous injection of saline. These two groups were compared with a third group of randomly selected 22 patients, who received 400 units of intranasal salmon calcitonin (SMC 20-051; Sandoz), administered as a nasal spray of 200 units into each nostril.
All patients were admitted to a Metabolic Unit for the period of the test. After an overnight fast and after voiding the bladder, the urine produced over the subsequent hour was collected and saved. Halfway through this period a venous blood sample was taken for serum estimations. At the end of the first urine collection, calcitonin or saline was administered to the patients, and hourly urine and blood collections were made for the next 6 h. The patients remained fasting throughout the 6 h of the test, but were allowed to drink distilled water. The urine samples were taken into acidified bottles and stored at 4°C until analysis.

Serum was analysed for calcium, phosphate, alkaline phosphatase, creatinine, hepatic transaminases, urea and electrolytes using the Technicon SMAC. Serum calcium values were adjusted for fluctuations in serum albumin (corrected to an albumin level of 42 g/l).

The urine was assayed for phosphate, hydroxyproline and creatinine by automated methods [20]. Urinary excretion of hydroxyproline was expressed as a molar ratio to creatinine excretion.

The significance of differences between treatments was assessed using one-way analysis of variance and Student's t-test for unpaired data. Changes in biochemical values during each treatment were studied using the t-test for paired observations. Changes in hydroxyproline and alkaline phosphatase, statistical comparisons were made on log-transformed values, but were antilogged for presentation. Results are expressed as means ± SEM, unless otherwise indicated.

The study had the prior approval of the local Ethical Committee and all patients gave informed consent before enrollment.

RESULTS

Before the start of this study there were no significant differences between the groups in any of the measured variables. In particular, the prevailing rates of bone turnover, as judged by the urinary excretion of hydroxyproline and the serum activity of alkaline phosphatase, were well matched in the placebo and each of the test groups (Table 1).

Table 1. Initial values of serum alkaline phosphatase activity and the urinary hydroxyproline/creatinine ratio

<table>
<thead>
<tr>
<th>Alkaline phosphatase (i.u./l)</th>
<th>Hydroxyproline/creatinine ratio of (µmol/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 units of subcutaneous CT</td>
<td>263 (224-309)</td>
</tr>
<tr>
<td>10 units of subcutaneous CT</td>
<td>263 (224-309)</td>
</tr>
<tr>
<td>1 unit of subcutaneous CT</td>
<td>269 (219-316)</td>
</tr>
<tr>
<td>Placebo</td>
<td>245 (257-295)</td>
</tr>
<tr>
<td>400 units of nasal CT</td>
<td>288 (257-322)</td>
</tr>
</tbody>
</table>

Fig. 1. Serum calcium concentration (mean ± SEM) after the subcutaneous injection of a single dose of 100 units of salmon calcitonin in nine patients with Paget's disease of bone.
Acute effects of calcitonin

1.5
1.0
1.0

T
T

Subcutaneous CT
Nasal CT

400 units of nasal CT

Placebo

1 unit

10 units

100 units

Subcutaneous CT

400 units of nasal CT

Placebo

10 units of subcutaneous CT

Fig. 2. Cumulative fall in serum calcium after nasal calcitonin, placebo and three doses of subcutaneous calcitonin. The fall in serum calcium was calculated from the summed decrement in values observed between 4 and 6 h. Abbreviation: CT, calcitonin.

No significant changes in serum calcium were seen in the placebo group, and intermediate responses were observed with the lower doses of parenteral calcitonin and with nasal calcitonin (Table 2). When the summed calcium drop from 4 to 6 h was plotted for each group a significant dose-response was evident (analysis of variance: \( P<0.01 \); Fig. 2). As expected, there was a significant correlation between hydroxyproline excretion before the test and the fall in serum calcium in patients given 100 units of subcutaneous calcitonin (\( r=0.7; P=0.038 \)). No significant correlation was observed, however, at any other dose, since the hypocalcaemic response appeared to be blunted particularly in those patients with the more marked disease activity. In order to explore this further we computed the relationship between initial disease activity and the hypocalcaemic response by bivariate regression in those patients given 100 units of subcutaneous calcitonin. In this way we calculated the expected maximal hypocalcaemic response (\( E \)) in the other tests from the initial urinary hydroxyproline. The observed (\( O \)) and expected hypocalcaemic responses for each patient were then compared by plotting:

\[
\frac{(O - E)}{E}
\]

When patients were subdivided into ‘low’ and ‘high’ bone-turnover groups based on their initial hydroxyproline excretion (values for all patients were ranked in increasing order and then divided into two equal groups about the median, i.e. \( < \) or \( > 58 \mu\text{mol/mmol of creatinine} \)), there was a significant difference between the hypocalcaemic responses of the patients with low and high turnover when nasal calcitonin was administered (\( P<0.05 \); Fig. 3). Thus in the high turnover group the observed hypocalcaemic effect of nasal calcitonin was less than expected, but in the low turnover group the mean effect was at least as marked as that induced by 100 units of subcutaneous calcitonin. A similar pattern was seen with 10 units of subcutaneous calcitonin, but it was not statistically significant. With placebo and 1 unit of subcutaneous calcitonin no difference was noted between the low- and high-turnover groups, and values were consistently less than expected.

To produce an estimate of the bioequivalence of intranasal calcitonin we plotted the mean summed calcium drop from 4 to 6 h for placebo and each dose of subcutaneous calcitonin against the logarithm of the dose used. The curve thus obtained was used to determine the dose of subcutaneous calcitonin that would have induced the fall in serum calcium observed in each patient receiving intranasal calcitonin (summed calcium drop from 4 to 6 h). Four hundred international units of intranasal calcitonin was equivalent to a mean of 29.9 i.u. of subcutaneous calcitonin, but the variation was wide (95% confidence limits 0–111.2 i.u.). This suggests a bioequivalence of around 7.5% (95% confidence limits 0–27.8%).

Other effects

After the administration of 100 units of subcutaneous calcitonin there was an increase in the urinary hydroxyproline excretion (\( P=0.049 \) at 2 h), and this was followed by a significant fall to below starting values from 4 to 6 h (\( P<0.05 \)). During this period (4–6 h), as with the serum
observed in patients given the larger dose of calcitonin (100 i.u.). The mechanism for this is unknown, but small
tonin (75.9±34.2 and 67.2±29.6 pmol of hydroxy-
the failure to match patients for disease activity. Indeed, a
not been consistently observed [lo, 21,22], perhaps due to
calcitonin in Paget's disease is the degree of disease
bone.

**DISCUSSION**

In this study we have shown a dose-dependent fall in
serum calcium concentration after the administration of
subcutaneous calcitonin to patients with Paget's disease of
bone. A dose-dependent decrease in serum calcium has
not been consistently observed [10, 21, 22], perhaps due to
the failure to match patients for disease activity. Indeed, a
major determinant of the hypocalcaemic response to
calcitonin in Paget's disease is the degree of disease
activity and by matching the treatment groups for disease
activity we hoped to reduce the importance of this factor
in our assessment.

The major mechanism for an acute fall in serum
calcium is thought to be due to the inhibition of
bone resorption [18] and this view is supported by the
correlation we noted between the hypocalcaemic
response to the larger dose of calcitonin (100 i.u.) and the
prevailing rate of bone turnover. Whereas hydroxyproline
excretion also fell, it is of interest that a small increase in the
hydroxyproline/creatinine excretion ratio was observed in patients given the larger dose of calcitonin
(100 i.u.). The mechanism for this is unknown, but small
increases have been observed by others, particularly in patients with lower rates of bone resorption [23, 24]. This
might suggest that calcitonin actively decreases tubular
reabsorption and clearance of hydroxyproline, but a non-
renal effect is also plausible.

Surprisingly, low doses of calcitonin appeared to have
acute effects qualitatively similar to those observed with
the highest dose used (100 i.u.) which are commonly
recommended for the treatment of bone disease. The
daily endogenous secretion rate for calcitonin has been
estimated at 20 i.u., but this is almost certainly an over-
estimate [25]. It therefore seems possible that doses such
as 10 i.u. might be exerting pharmacological effects. The
physiological role of calcitonin in man is still speculative
[26], but the small responses evoked with only 1 unit of
calcitonin suggest it may still prove to have a role in extra-
cellular calcium homeostasis.

In this study 400 units of intranasal calcitonin, given as
a single dose, appeared to have biological effects similar
to those observed with the use of subcutaneous calcitonin.
The activity of the nasal spray was, however, low, since
the hypocalcaemic effect of 400 units of intranasal calcito-
nin was similar to that of 10 units given subcutaneously.
We did not study any dose–response of nasal calcitonin,
but the sub-optimal response of serum calcium suggests a
bioequivalence of approximately 7.5%.

It is of particular interest that the response to the inter-
mediate dose of parenteral calcitonin (10 i.u.) and to the
nasal spray differed according to disease activity. Thus
whereas maximal effects were observed in patients with
the less extensive disease activity, in patients with the
higher disease activity the hypocalcaemic effect was
incomplete. Since the hypocalcaemic action of calcitonin
is primarily related to osteoclastic inhibition, and this is
also the mechanism by which calcitonin induces long-
term suppression of disease activity in Paget's disease, it is
likely that at this dosage nasal calcitonin would be unable
to provide long-term disease suppression except in
patients with mild disease activity (e.g. a urinary hydroxy-
proline/creatinine ratio <58 μmol/mmol of creatinine).
Nevertheless the efficacy of small doses of calcitonin suggests that at low rates of bone turnover the nasal spray,
even with its low bioequivalence, might be suitable for
long-term use in bone disorders associated with lower
rates of bone turnover, such as osteoporosis. This ability

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>100 units of subcutaneous CT</td>
<td>53.7</td>
<td>61.7</td>
<td>72.4*</td>
<td>47.9</td>
<td>39.8*</td>
<td>34.7*</td>
<td>32.4*</td>
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<tr>
<td>10 units of subcutaneous CT</td>
<td>60.3</td>
<td>58.9</td>
<td>66.1</td>
<td>56.2</td>
<td>50.1</td>
<td>36.3*</td>
<td>26.3*</td>
</tr>
<tr>
<td>1 unit of subcutaneous CT</td>
<td>51.3</td>
<td>56.2</td>
<td>57.5</td>
<td>49.0</td>
<td>49.0</td>
<td>46.8</td>
<td>57.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>60.3</td>
<td>56.2</td>
<td>61.7</td>
<td>72.4</td>
<td>70.8</td>
<td>63.1</td>
<td>67.6</td>
</tr>
<tr>
<td>400 units of nasal CT</td>
<td>70.8</td>
<td>74.1</td>
<td>79.4</td>
<td>54.9*</td>
<td>50.1*</td>
<td>50.1*</td>
<td>52.5*</td>
</tr>
</tbody>
</table>

Abbreviation: CT, calcitonin. Statistical significance: *P<0.05, 1P<0.01 compared with baseline.
of small doses of calcitonin to inhibit bone resorption in the presence of moderate increases in bone resorption may explain its apparent efficacy in delaying the bone loss of postmenopausal osteoporosis [27].

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REFERENCES