The effects of vapreotide, a somatostatin analogue, on gastric acidity, gallbladder emptying and hormone release after 1 week of continuous subcutaneous infusion in normal subjects

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Aims Somatostatin analogues (e.g. vapreotide) are used for treatment of acromegaly, endocrine tumours and variceal bleeding. The pharmacodynamic effects of vapreotide have, however, not been documented in the gastrointestinal tract. The aim of this study was to investigate the effects of continuous vapreotide administration on gastric acidity, gallbladder contraction and hormone release.

Methods Ten healthy males participated in this randomised, placebo-controlled, double-blind, crossover trial. A constant vapreotide (or placebo) infusion (1.5 mg day$^{-1}$ s.c.) was given for 7 days with a portable pump. Intragastric pH was monitored on days 2 and 7. Gallbladder volume was sonographically assessed and the maximal ejection fraction was calculated. In addition basal and postprandial plasma levels of gastrin and cholecystokinin (CCK) were measured.

Results After an initial increase in the median 24 h intragastric pH to a value of 2.6 on day 2, vapreotide’s effect on pH decreased: (day 7: median pH = 1.9; respective placebo values were 1.7 and 1.5). On the same days with vapreotide treatment, gallbladder contraction and plasma levels of CCK were reduced; maximal ejection fractions after meal stimulation were 18% and 20% (respective placebo values were 57% and 62%). Plasma gastrin levels were not changed with vapreotide treatment.

Conclusions The short lasting effect of vapreotide on intragastric acidity suggests a down-regulation of somatostatin receptors during treatment. The lack of effect on gastrin indicates that the effects on gastric pH are not mediated by gastrin. Constant vapreotide infusion (but not placebo) reduced gallbladder contraction suggesting a long-lasting effect on biliary function.

Keywords: gut hormone, upper gastrointestinal bleeding, neuroendocrine tumour, octastatin, subcutaneous infusion

Introduction

The inhibitory effects of natural and synthetic somatostatin analogues on pituitary functions and on the gastrointestinal tract have been well documented in the last decade [1]. These compounds are used for the treatment of acromegaly [2], various endocrine tumours [3] and upper gastrointestinal bleedings [4]. Short-term systemic application of somatostatin has inhibitory effects on gastric acid secretion, gallbladder contraction and other gastrointestinal functions [5]. One main problem in chronic treatment with natural somatostatin is its short half-life and the need for intravenous application. The development of synthetic analogues has solved some of these problems. Vapreotide is an octapeptide analogue of natural somatostatin with a half-life of 164 ± 46 min after a single subcutaneous injection (N. Lahlou, unpublished results). Repetitive subcutaneous injections three times daily are therefore needed for long-term treatment, a method poorly tolerated by patients and, with repetitive plasma peaks, not imitating a physiological hormonal state. However, during long-term treatment with octreotide, another somatostatin analogue, it has been noted that certain inhibitory effects are reduced after repeated application [6] suggesting a down-regulation of receptors. A systematic evaluation of the effect on different
gastrointestinal organ systems in the same subject at stable
plasma concentrations has, however, not yet been carried
out. Therefore, throughout the course of 1 week, we
followed the effect of vapreotide during chronic treatment
on intragastric pH and gastrin, the main regulatory
hormone of gastric acid production. Parallel to this, we
also evaluated the effect of vapreotide on postprandial
gallbladder contraction during chronic treatment in order
to indirectly estimate the risk of promoting gallstone
formation. Since vapreotide could play this role by
inhibiting cholecystokinin (CCK), the main regulating
hormone of meal-induced gallbladder contraction [7], we
simultaneously measured it during the study. To obtain
stable plasma concentrations, vapreotide was given as a
continuous subcutaneous infusion using a small portable
infusion pump that allows delivery of 1 ml day−1 for
1 throughout
the entire infusion period [9].

On day 1 after overnight fasting, the subcutaneous
infusion pump was fixed by use of an infusion set-up to
the left upper quadrant of the abdomen. On day 2 and day 7 of each treatment period, 24 h intragastric pH, gallbladder contraction and plasma concentra-
tions of hormones were measured. To assess intra-
gastric pH, a miniature combined glass electrode for
continuous monitoring of intragastric pH (Ingold,
CH-8920 Urdorf, Switzerland) was inserted transnasally
in the fundus-corpus area of the stomach after calibration
at 37°C using a commercial buffer solution, and
temperature correction to 37°C. The electrode was
connected to a portable data-recorder (MIC, CH-4500
Solothurn, Switzerland) with a sampling rate of one
sample every 6 s. After overnight fasting, pH monitoring
started at 08.30 h. Standardised meals were given after
correct placement of the electrode (breakfast), at 14.30 h
(500 ml of a liquid meal, Ensure®) and at 20.00 h
(supper). The subjects were allowed to drink additional
water.

Gallbladder volume was assessed by high resolution
real-time sonography using a 3.5 MHz sector scanner
(Aloka SSD650). The probe was first placed horizontally
in the right upper quadrant of the abdomen while the
subject was in supine position, then the probe was rotated
to obtain the maximal possible diameter of the gallbladder
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Methods
Subjects
Ten healthy male volunteers, with a median age of 25
years (range 23–39), having a median body weight of
83 kg (range 64–93) and a median height of 183 cm
(range 178–197), were selected for this trial. None of
the subjects had any history of previous gastrointestinal,
metabolic or endocrine disorders, and each had a normal
screening physical examination and normal laboratory
blood tests including serum chemistry, complete blood
count and an electrocardiogram. All subjects were H.
pylori negative based on a negative 13C-urea breath test
out period of at least 1 week (range 7 to 23 days). as at 6 and 12 h later, and, in addition, in the morning
on intragastric pH and gastrin, the main regulatory
the left upper quadrant of the abdomen.

Experimental design
The study was carried out as a randomised, double-blind,
cross-over trial. In brief, each subject had to take part in
two treatment periods with continuous subcutaneous
infusion of either placebo or vapreotide (1.5 mg day−1) for 1
week from a reservoir. This administration maintains
plateau concentrations of about 2000 pg ml−1 throughout
the entire infusion period [9].

To achieve constant drug plasma levels, the drug was
infused subcutaneously with a Chronoclip®, a new
infusion pump that allows delivery of 1 ml day−1 for 1
week from a reservoir. This administration maintains

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Values was characterised for each treatment group by median pH as well as 5th, 25th, 75th and 95th percentiles and the data are presented as box Whisker plots.

Basal gallbladder volumes in ml were compared using Student’s t-test. For further calculations, basal gallbladder volumes were taken as 100% and postprandial changes in gallbladder volume are given as % changes (mean±s.e.mean) from basal volume. Plasma concentrations of gastrin, CCK, GH and IGF-1 are given as mean±s.e.mean.

For statistical analysis, the area under the curve (AUC) of each hormone parameter as well as the maximum and the steady state plasma concentration of vapreotide were calculated for each individual subject and experiment. For comparison of parameters between vapreotide and placebo treatment and, on the other hand, between the first and last treatment day within a treatment group, data were first tested for normal distribution by the Wilk-Shapiro test. When normal distribution could not be rejected, data were analysed by analysis of variance (ANOVA), otherwise ANOVA was applied on rank-transformed data. In cases of significant differences, ANOVA was followed by the Duncan multi-comparison test for pair-wise comparisons. For all statistical analysis, SPSS for Windows Software was used. The level of statistical significance was P=0.05.

**Results**

**Intragastric acidity**

Median intragastric pH profiles over 24 h obtained during placebo and vapreotide treatment are shown in Figure 1. The pH-distribution-curve of vapreotide and placebo is shown in Figure 2; additional to the median value (50th percentile), the 5th, 25th, 75th and 95th percentile of 24 h pH-metry are shown in Whisker box plots (Figure 3). As can be seen, with placebo similar results were obtained of vapreotide was measured using a radioimmunoassay method adapted from Mason-Garcia [12].

**Materials**

Vapreotide acetate was provided by Debiopharm (CH-1000 Lausanne 9, Switzerland); Ensure® (a standard liquid test meal with 16.7% protein, 30.1% lipid and 53.2% carbohydrates) was purchased from Abbott AG (CH-6330 Cham, Switzerland). Chronoclip®, a new portable infusion pump (40 g when filled) which can deliver small amounts of a drug (1 ml day⁻¹), was provided by Debiotech, CH-1000 Lausanne 9, Switzerland. For each experiment, the reservoir was filled with 10 ml of sterile infusion solution and connected through the motor unit to a set-up for subcutaneous infusion (Soft Set®).

**Calculations and statistical analysis**

Intragastric pH measurements were recorded every 6 s over 24 h. Median pH value was calculated for each subject as previously described [13]. Distribution of these values was characterised for each treatment group by median pH as well as 5th, 25th, 75th and 95th percentiles and the data are presented as box Whisker plots.

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![Figure 1](image1.png) Median 24 h pH-profiles during subcutaneous infusion of placebo (a) or vapreotide (b) in 10 healthy volunteers.

![Figure 2](image2.png) pH-distribution-curve of vapreotide (~ day 2, —— day 7) and placebo (— day 2, —— day 7) in 10 healthy volunteers.
on both days (median pH over 24 h was 1.7 on day 2 and 1.5 on day 7, NS). During the daytime, postprandial buffer effects were seen and during the night, typical individual variability was measured with a median pH slightly higher than during daytime. However, median pH values on both day 2 and day 7 were significantly ($P<0.01$) higher in the vapreotide group than those measured during placebo administration (day 2: median 2.6 and day 7: median 1.9; Figure 1 and Whisker box plots in Figure 3).

On day 7, however, the median pH in the vapreotide group was significantly lower than on day 2 ($P<0.01$). The dose of vapreotide used in this study did not raise the median intragastric pH above 4 in any of the 10 subjects (Figure 2).

**Gallbladder volume**

In the control period during placebo administration, basal gallbladder volumes were comparable on both days: $17 \pm 2\text{ ml on day 2, respectively } 15 \pm 2\text{ ml on day 7 (Ratio 1.2; 95\% CI = 0.9–1.5).}$ No significant differences were found postprandially with placebo treatment between day 2 and day 7. After ingestion of 500 ml of a liquid meal, gallbladder volume reached its minimal residual volume in the placebo group within 60 min, as can be seen in Figure 4. The ejection fraction at the time of maximal contraction was $63 \pm 10\%$ on day 2 and $64 \pm 9\%$ on day 7, respectively (NS).

During vapreotide treatment, there was a significant enlargement of basal gallbladder volume to $25 \pm 3\text{ ml}$ (day 2, $P<0.05$ vs placebo) and $29 \pm 2\text{ ml}$ (day 7, $P<0.01$ vs placebo). There was no significant difference between the two latter volumes. A significant reduction of the residual volume 60 min after the meal, compared with basal gallbladder volume ($21 \pm 3\text{ ml on day 2, respectively } 24 \pm 3\text{ ml on day 7$) was observed in the period with vapreotide treatment, but this reduction was much smaller than that observed during placebo treatment. Both values during vapreotide infusion on day 2 and day 7 were significantly ($P<0.01$) higher than each of the corresponding values in the placebo group. The maximal ejection fraction was similar on day 2 and on day 7 ($18 \pm 4\%$ and $20 \pm 6\%$, respectively). During both treatment periods, gallbladder volume did not reach normal basal values within 2 h after the meal (Figure 4).

**Hormone secretion**

Plasma hormone levels of gastrin and CCK were measured parallel to the postprandial intragastric pH and gallbladder contraction after a liquid lunch meal. There was no difference in postprandial gastrin secretion in both groups (Table 1).

The fasting baseline concentrations of gastrin did not differ in the placebo group comparing day 2 and day 7 ($35 \pm 2\text{ pg ml}^{-1}; 33 \pm 2\text{ respectively).}$. In the group treated with vapreotide, however, the basal concentrations of gastrin were significantly higher on day 2 ($40 \pm 3\text{ pg ml}^{-1}; P<0.05$ vs placebo and $P<0.01$ vs day 7) but no longer differed from the placebo group on day 7 ($34 \pm 2\text{ in the vapreotide group).}$

Fasting secretion of CCK did not differ between the treatments or within each treatment between day 2 and day 7. Postprandial secretion of cholecystokinin was, however, slightly reduced during treatment with vapreotide (Table 1). While this difference was not significant, a similar trend could be shown on both days (Figure 5).

In the control period during placebo administration, the AUC of plasma concentration of GH was $112 \pm 163$, $112 \pm 163$, $112 \pm 163$.
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Table 1 Postprandial gastrin and cholecystokinin (CCK) secretion during vapreotide or placebo infusion in 10 healthy volunteers (data are given as AUC (0, 120 min), mean ± s.e.mean) and ratio with 95% confidence intervals (CI).

<table>
<thead>
<tr>
<th></th>
<th>Day 2 (pmol l⁻¹)</th>
<th>Day 7 (pmol l⁻¹)</th>
<th>Ratio (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrin secretion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>4398 ± 222</td>
<td>4389 ± 91</td>
<td>1.00 (0.92–1.09)</td>
</tr>
<tr>
<td>Vapreotide</td>
<td>4395 ± 128</td>
<td>4178 ± 238</td>
<td>1.07 (1.00–1.13)</td>
</tr>
<tr>
<td>Ratio (CI)</td>
<td>1.00 (0.91–1.09)</td>
<td>1.07 (0.97–1.17)</td>
<td></td>
</tr>
<tr>
<td><strong>CCK secretion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1680 ± 236</td>
<td>1510 ± 111</td>
<td>1.08 (0.92–1.25)</td>
</tr>
<tr>
<td>Vapreotide</td>
<td>1367 ± 88</td>
<td>1336 ± 128</td>
<td>1.06 (0.94–1.19)</td>
</tr>
<tr>
<td>Ratio (CI)</td>
<td>1.23 (0.96–1.51)</td>
<td>1.17 (1.02–1.32)</td>
<td></td>
</tr>
</tbody>
</table>

Side effects

Some subjects complained of steatorrhoea and abdominal pain of a mild to moderate degree, but only one of them had to be treated with a spasmolytic drug for abdominal cramps, most probably due to an irritable colon (same complaints in verum and placebo phases).

Discussion

Intragastric pH was markedly elevated by vapreotide, but only at the beginning of a long term treatment, while the elevation was much lower after 1 week. The efficacy concerning gallbladder contraction, however, did not disappear after 1 week of treatment with vapreotide. Basal gastrin concentrations also changed only at the beginning of the vapreotide phase, being significantly higher than in the placebo group on day 2, while there was no change after 1 week of treatment. Postprandial gastrin levels were not affected by the treatments. Postprandial cholecystokinin levels were slightly reduced during subcutaneous administration of vapreotide. The latter value was also significantly lower than IGF-1 concentration on day 7 in the placebo group.

Vapreotide plasma levels

During the 7 days of treatment with a constant subcutaneous infusion of 1.5 mg day⁻¹, steady state plasma concentration of 2062 ± 180 pg ml⁻¹ could be achieved. Maximal plasma concentration was 3366 ± 527 pg ml⁻¹, the area under the curve 406 ± 35 ng ml⁻¹ h.

The reason for the loss of efficacy after several days of treatment with a synthetic somatostatin analogue remains unclear.

Since plasma gastrin levels were not reduced under
treatment conditions, it seems that the inhibitory effect of vapreotide on intragastric pH is not mediated by suppression of gastrin, the main regulatory peptide of gastric acid secretion. This suggests a direct inhibition of histamine producing cells and/or parietal cells in the stomach, and this inhibition shows a tachyphylaxis.

The initial rise in basal gastrin levels on day 2 in the vapreotide group is explained by the lack of negative feedback due to the elevated intragastric pH. On day 7, however, when intragastric pH in both groups was almost similar, plasma gastrin levels no longer differed. Postprandial plasma gastrin levels were not affected by the treatment since they are directly stimulated by intragastric calcium and amino acids.

A second aim of the present study was to investigate the behaviour of the gallbladder during chronic treatment with vapreotide. In contrast to the effect on intragastric pH, no tachyphylaxis occurs and the contraction of the gallbladder is equal on day 2 and on day 7 of the treatment. These data are in contrast to findings of the group who also showed an enlargement of basal gallbladder volume at the beginning and at the end of 1 week of octreotide treatment twice daily, but no further difference in kinetics of gallbladder contraction after stimulation with exogenously administrated CCK [14].

These differences can best be explained by the different kind of stimulation: while Creutzfeldt used exogenous CCK, we measured gallbladder contractions after a meal which acts as a physiological stimulant. In our experiments, plasma CCK levels were inhibited postprandially in the vapreotide group, but these results were not significant. This is supported by additional experiments reported by the same group [14] after 1 week of pre-treatment with octreotide, when the stimulant for gallbladder contraction was a meal. These data also show a marked inhibition of the contractile biliary response and, at the same time, an abolished CCK secretion. Therefore, CCK plays a major role in postprandial gallbladder contraction, and its inhibition, even if not significant in the present study, is important in regulating gallbladder contraction by somatostatin analogues.

In summary, it can be concluded that gallbladder stasis occurs not only at the beginning of treatment with somatostatin analogues, but also during prolonged use of a constant amount. Gallbladder stasis is a high-risk factor for gallstone formation and provides important clinical implications, not only for the chronic treatment of oesophageal varices as a potential indication for vapreotide, but also for its therapeutic use in endocrine active tumours. We could also demonstrate that with the Chronochip® pump, a subcutaneous infusion of very small drug amounts can be constantly infused 24 h a day, and that sufficient and stable plasma levels of vapreotide can be produced throughout 1 week. These plasma levels were able to suppress GH during the entire period of vapreotide infusion showing a pharmacological activity of the drug. No major side effects appeared in the verum period and the system was well tolerated by the volunteers.

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References
7 World Medical Association Declaration of Helsinki: 18th world medical assembly (Helsinki, Finland 1964); Amended by the 29th (Tokyo, Japan 1975), the 35th (Venice, Italy 1983) and the 41st world medical assembly (Hong Kong 1989).
11 Mason-Garcia M, Vaccarella M, Horvath J, et al. Radioimmunosassay for octapeptide analogs of somatostatin: measurement of serum levels after administration of long-
