

**INTRAGASTRIC INFUSION OF THE BITTER TASTANT QUININE SUPPRESSES HORMONE
RELEASE AND ANTRAL MOTILITY DURING THE FASTING STATE IN HEALTHY FEMALE
VOLUNTEERS**

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Running title: Quinine inhibits gastrointestinal function

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Abbreviations: CCK: cholecystokinin; DB: denatonium benzoate; GLP-1: glucagon-like peptide-1; GOAT: ghrelin O-acyltransferase; MI: motility index; MMC: migrating motor complex; PYY: peptide YY; TAS2R: taste 2 receptor; QHCl: quinine-hydrochloride

ABSTRACT

Background: Intragastric administration of the bitter tastant denatonium benzoate inhibits the increase of motilin plasma levels and antral contractility. While these findings suggest that gastrointestinal bitter taste receptors could be new targets to modulate gastrointestinal motility and hormone release, they need confirmation with other bitter receptor agonists. The primary aim was to evaluate the effect of intragastric administration of the bitter tastant quinine-hydrochloride (QHCl) on motilin and ghrelin plasma levels. Secondly, we studied the effect on interdigestive motility.

Methods: Ten healthy female volunteers were recruited (33 ± 4 years; 22 ± 0.5 kg/m²). Placebo or QHCl (10 μ mol/kg) was administered intragastrically through a nasogastric feeding tube after an overnight fast in a single-blind randomized fashion. Administration started 20 min after the first phase III of the migrating motor complex. The measurement continued for another 2 hours after the administration. Blood samples were collected every 10 min with the baseline sample taken 10 min prior to administration.

Key results: The increase in plasma levels of motilin (administration; $p=0.04$) and total ghrelin (administration; $p=0.02$) was significantly lower after QHCl. The fluctuation of octanoylated ghrelin was reduced after QHCl (time by administration; $p=0.03$). Duodenal motility did not differ. The fluctuation of antral activity differed over time between placebo and QHCl (time by administration; $p=0.03$).

Conclusions: QHCl suppresses the increase of both motilin and ghrelin plasma levels. Moreover, QHCl reduced the fluctuation of antral motility. These findings confirm the potential of bitter taste receptors as targets for modifying interdigestive motility in man.

Key points:

- Intragastric administration of denatonium benzoate modulates gastrointestinal behaviour. The effect of other bitter taste receptor agonists on interdigestive gastrointestinal function is unknown.
- Intragastric administration of quinine-hydrochloride reduces the increase of both motilin and ghrelin plasma concentrations, as well as the fluctuation of antral motility.
- Gastric bitter taste receptors could be new targets to modulate gastrointestinal motility in health and disease.

INTRODUCTION

In 1916 *Carlson* first reported the inhibitory effect of intragastric bitter administration on fasting gastric contractility in man.(1) Eighty years later *Höfer et al.* provided the first evidence for gastrointestinal taste signalling by reporting the expression of α -gustducin in the gut of rats.(2) Further research showed that the bitter taste receptors, taste 2 receptors (TAS2Rs), are expressed in the human gastrointestinal tract.(3, 4) Their presence has stimulated research to modulate gastrointestinal physiology via the administration of bitter tastants.

In rats and mice, direct intraluminal administration of bitter agonists inhibited ongoing ingestive behaviour and gastric emptying.(4-6) Bitter sham feeding significantly reduced gastric emptying of a liquid meal in humans.(7) Gastric emptying of a solid meal was however not affected when a bitter tastant was administered directly into the stomach of healthy volunteers.(8) Intragastric administration of denatonium benzoate (DB) was shown to inhibit gastric accommodation and subsequently food intake.(4) A similar response was observed after the intragastric administration of quinine-hydrochloride (QHCl).(9)

In vitro studies have shown that DB increases the release of cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and insulin.(10-12) A similar result has been described for quinine on the release of GLP-1.(11) However, results obtained *in vivo* report contradictory results. *Van Avesaat et. al.*(13) reported no significant change in CCK, GLP-1 and PYY after intraduodenal infusion of quinine, while another study did report an increase in postprandial CCK release after intraduodenal quinine administration.(14) Ghrelin release was not altered after intragastric administration of DB in humans.(8) A similar result was obtained for ghrelin secretion after intraduodenal administration of quinine.(14) Our group has recently shown that intragastric administration of DB inhibited the increase in motilin plasma levels and

reduced the occurrence of gastric phase III contractions of the migrating motor complex (MMC) in man.(8)

While these findings suggest that gastrointestinal bitter taste receptors could be new targets to modulate gastrointestinal motility and hormone release, they need confirmation with other bitter receptor agonists. Therefore, the aim of this study was to evaluate the effect of intragastric administration of QHCl on gastrointestinal hormone release and motility during the interdigestive state.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Medical Ethics Committee of the Leuven University Hospital, Leuven, Belgium, and performed in full accordance with the Declaration of Helsinki. This trial was registered at clinicaltrials.gov as NCT02759926.

Subjects

Volunteers were eligible to participate if they were healthy, aged between 18 and 60 years old, had a BMI (in kg/m²) ranging from 19.0 to 25.0, and were recruited from an existing volunteer database in our group. Exclusion criteria were gastrointestinal diseases, abdominal surgery (appendectomy allowed), psychiatric illnesses, and usage of drugs affecting the gastrointestinal tract or central nervous system. Only female participants were included since it has been reported that sensitivity to intragastrically administered bitter tastants differs between men and women.⁽⁸⁾ Written informed consent was obtained from all volunteers before the start of the study. All subjects were studied after an overnight fast of 12 hours and were asked to refrain from smoking at least 1 hour before the start of the study.

Test compounds

QHCl was purchased from Fragon (Rotterdam, The Netherlands). Solutions of QHCl were prepared in Milli-Q water. The stock concentration for intragastric administration was 100 mM. A volume of 0.1 ml/kg bodyweight was administered. The dosage of QHCl (10 µmol/kg) was chosen based on its inhibitory effect on gastric accommodation in healthy volunteers.⁽⁹⁾ Vehicle was given during the placebo condition in a volume of 0.1 ml/kg bodyweight.

Study design

After an overnight fast of 12 hours, antroduodenal motility was measured until the occurrence of the first phase III contraction. Twenty minutes after the end of phase III, either placebo or QHCl (10 μ mol/kg bodyweight) was administered intragastrically in a single-blind randomized fashion. Intragastric administration was done through a nasogastric feeding tube (Flocare, Nutricia, Bornem, Belgium). The feeding tube was placed with its tip in the upper part of the stomach and the position was checked with fluoroscopy prior to the start of the study. The measurement continued for another two hours after the administration. Blood samples were collected every 10 min with the baseline sample taken 10 min prior to administration.

Antroduodenal manometry Activity of the MMC was measured using a high-resolution solid-state manometry catheter (36 channels, spaced 1 cm apart, Manoscan 360, Sierra Scientific Instruments, Los Angeles, CA, USA, Manoview analysis software v2.0) as described previously.⁽¹⁵⁾ Phases of the MMC were identified based on standardized definitions.^(16, 17) Motility index (MI) was calculated as follows: (number of contractions*average amplitude contractions*average duration contractions)/5min.^(15, 18, 19) An average MI was calculated for the antrum and duodenum by averaging the MI of six consecutive channels.

Blood collection Blood samples were collected via an intravenous catheter. Samples for motilin and ghrelin were collected in lithium heparin and EDTA tubes each containing 500 kIU/ml aprotinin (Roche Applied Science, Penzberg, Germany). Samples were centrifuged at 4°C for 10 min at 1400 x g. Ghrelin plasma samples were immediately acidified to a final concentration of 0.1 N HCl and extracted on Sep-Pak C18 columns (Waters Corporation, Milford, MA, USA) and vacuum-dried. All plasma samples were stored at -80°C until analysis. Motilin and ghrelin (total and octanoylated) plasma levels were determined by radioimmunoassay as fully described elsewhere.^(6, 15)

Statistical analysis

Significance was set at $p < 0.05$. SAS (Statistical Analysis System version 9.3; SAS Institute) and GraphPad Prism (Version 7; GraphPad) were used for the analysis. Data are represented as mean \pm SEM or median (IQR).

For the hormone plasma levels a percentage change from baseline level was calculated and used for the analysis. Mixed model analysis (SAS) was used to assess the main effects of time and administration (placebo and QHCl) and their interaction effect on the variables of interest (hormone concentrations and MI). Administration and time were entered as within-subject categorical variables. Time was also added as a random (subject-specific) intercept.

The time interval until the occurrence of phase III was compared between the two conditions using Wilcoxon signed rank test (GraphPad). The percentage origin of phase III was compared between placebo and QHCl with McNemar's test (GraphPad). For both hormone plasma concentrations and antral motility the peak values were determined to correlate the time of peak occurrence between antral motility and hormone concentrations. Spearman's rank correlation coefficient (one-tailed) was used for both conditions (GraphPad).

RESULTS

Ten healthy female volunteers were recruited (33 ± 4 years; 22 ± 0.5 kg/m²). None of the subjects reported any adverse events after the administration of QHCl.

Change in hormone plasma levels after quinine-hydrochloride administration

The effect of intragastric QHCl administration on the change of plasma concentrations of motilin and ghrelin (total and octanoylated) was evaluated. Motilin levels were lower after QHCl, with a significant main effect of administration ($p=0.04$) on the percentage change of motilin plasma concentrations (Figure 1A). There was no significant effect of time ($p=0.2$) or a significant interaction effect between time and administration ($p=0.5$). QHCl had a similar effect on the change from baseline for total ghrelin plasma levels (Figure 1B). Administration had a significant effect ($p=0.02$), but both time ($p=0.7$) and the interaction effect between administration and time ($p=0.1$) were non-significant. Although there was no significant effect of both administration ($p=0.5$) or time ($p=0.3$) on the change of octanoylated ghrelin, there was a significant interaction effect between time and administration ($p=0.03$) (Figure 1C).

Change in antroduodenal motility after quinine-hydrochloride administration

Antroduodenal motility was measured using high-resolution manometry (Figure 2). The average duration until the occurrence of phase III prior to administration did not differ between placebo and QHCl (63 (40-87) min vs 21 (16-90) min; $p=0.07$). Fifty-six percent of the phase III contractions prior to placebo administration and 50% prior to QHCl were of gastric origin ($p=0.6$).

There was a significant time by administration effect ($p=0.03$) for antral motility with no significant effect of time ($p=0.06$) or administration ($p=0.4$) (Figure 3A). Duodenal motility

(Figure 3B) was not affected by administration ($p=0.2$), time ($p=0.5$) or their interaction effect ($p=0.6$).

Correlation between motility and peptide plasma levels

There was a significant positive correlation ($r=0.58$; $p=0.04$) between the occurrence of antral peak activity and the peak concentration of motilin during the placebo administration (Figure 4A). A similar result was obtained for octanoylated ghrelin plasma levels ($r=0.60$; $p=0.03$). There was no significant correlation between the occurrence of antral peak activity and the total ghrelin plasma peak ($r=-0.42$; $p=0.1$). The positive correlations with the peak concentrations of both motilin ($r=0.1$; $p=0.4$) and octanoylated ghrelin ($r=0.06$; $p=0.4$) were lost after QHCl administration (Figure 4B). There was however a significant positive correlation between the occurrence of antral peak activity and the peak concentration of total ghrelin ($r=0.58$; $p=0.04$).

DISCUSSION

Our study showed that intragastric administration of QHCl suppresses interdigestive gastrointestinal motor function and release of motilin and ghrelin. These results confirm our previous findings that intragastric bitter administration alters interdigestive functions of the gastrointestinal tract.(8)

Quinine binds to 9 different TAS2Rs (TAS2R4, TAS2R7, TAS2R10, TAS2R14, TAS2R39, TAS2R40, TAS2R43, TAS2R44, TAS2R46). Five of these receptors also bind DB (TAS2R4, TAS2R10, TAS2R39, TAS2R43, TAS2R46).(20) The difference in receptor activation might be an explanation for the different effect of QHCl and DB on ghrelin release. We previously reported that intragastric administration of DB inhibited the release of motilin, but had no effect on ghrelin.(8) Intragastric administration of QHCl however inhibited the release of both motilin and ghrelin. A discrepancy between intragastric DB and QHCl administration on ghrelin release has already been described in mice.(6) However, oral gavage of QHCl had no effect on ghrelin release in mice, while DB caused an increase. These results show that bitter agonists might have different effects in different species.

Our study showed that intragastric administration of 10 $\mu\text{mol/kg}$ QHCl inhibited the release of total (desoctanoyl and octanoyl) ghrelin and reduced the fluctuation of octanoylated ghrelin. Two cell types have been described that secrete ghrelin, a cell type containing both octanoylated and desoctanoylated ghrelin and another cell type containing only desoctanoylated ghrelin.(6) The octanoylation of ghrelin is caused by a posttranslational esterification of the third serine residue by ghrelin O-acyltransferase (GOAT).(21) A possible explanation for the different effect of QHCl on the plasma concentration of octanoylated vs. unacylated ghrelin could be a difference in the expression of TAS2Rs on the ghrelin secreting

cell types. So far, the expression of TAS2R4 and TAS2R10 has been shown in mucosal tissue from the fundus and small intestine in humans.(22) However, co-expression of TAS2Rs and ghrelin secreting cells has not been shown to date. Another possibility could be that the transduction pathway after QHCl administration also affects the expression of GOAT causing a discrepancy between total and octanoylated ghrelin. Further research is needed to specify the mechanism by which QHCl influences ghrelin secretion.

A previous study, where quinine (18 mg) was administered intraduodenally, showed that there was no effect on ghrelin release.(14) The dosage given in the current study is higher, which may account for some of the different effects. Moreover, ghrelin secreting cells are mainly located in the oxyntic mucosa of the stomach, so bypassing the stomach via intraduodenal administration could abolish the effects of quinine on ghrelin secretion.(23)

The inhibition of motilin release after intragastric bitter administration is seen after both DB and QHCl administration. Moreover, the correlation between motilin and antral motility is lost after both bitter administrations.(8) Interdigestive antral motility in man is regulated by motilin.(15) The inhibitory effect of bitter administration on antral motility might therefore be mediated by its effect on motilin release. However, in the present study we also found a significant correlation between peak activity of antral motility and octanoylated ghrelin, which was lost after QHCl administration. Although endogenous ghrelin plasma concentrations were not found to fluctuate with the different phases of the MMC, exogenous administration of octanoylated ghrelin is known to induce a gastric phase III contraction.(24) Nevertheless, the effect of desoctanoylated ghrelin was not investigated. In rats it has been shown that desacyl ghrelin disrupts fasting gastrointestinal motility.(25) In *suncus murinus*, a small laboratory animal model, ghrelin has been identified as an essential factor for the motilin-induced gastric

contractions.(26) A similar synergistic effect between motilin and ghrelin might therefore be present in humans. After QHCl administration antral motility seems to be controlled by total ghrelin since the positive correlation with motilin and octanoylated ghrelin was abolished. Although this correlation shows that the timing of total ghrelin correlates with the timing of antral peak activity, it does not say anything about the strength of antral contractility. Moreover, a direct effect of QHCl on smooth muscle cells of the gastrointestinal tract cannot be excluded. The presence of TAS2Rs and their associated G-proteins has been shown in human gastric smooth muscle cells.(4) Further research is necessary to determine via which pathway bitter agonists modulate gastrointestinal behavior.

MMC cycle length varies significantly between subjects.(16) Also in the current study antral MI varied significantly between subjects, as seen by the high error flags in the placebo condition. This is due to the different onset of phase III contractions between subjects. The variability in antral MI after QHCl administration was diminished compared to placebo. This decrease in variability is probably due to a delay in the occurrence of phase III. However, with the current protocol we did not consistently continue to measure until the next phase III after administration so this cannot be confirmed just on the basis of the current study.

In conclusion, our study confirms the inhibitory effect of intragastric bitter administration on interdigestive gastrointestinal behavior in humans. Although future studies are warranted to determine the pathway by which bitter agonists modulate gastrointestinal function, these findings show that bitter taste receptors could be used as new targets to modulate gastrointestinal activity.

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ED wrote the paper; ED and MC performed the research; ED, MC, LVO, ID and JT interpreted and analysed data; ED, MC, LVO, ID and JT performed a critical revision of the manuscript; ID and JT designed the research; JT provided funding. All the authors read and approved the final manuscript. None of the authors reported any conflicts of interest.

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FIGURE LEGENDS

Figure 1. The effect of intragastric QHCl administration on motilin and ghrelin plasma concentrations. Plasma concentrations were measured using hormone-specific radioimmunoassays. Data are represented as percentage change from baseline (10 min prior to administration). (A) Percentage change of motilin plasma levels differed between the two conditions ($p=0.04$). (B) Percentage change of total ghrelin plasma levels differed between the two conditions ($p=0.02$). (C) Percentage change of octanoylated ghrelin plasma levels. Significant time by administration effect ($p=0.03$). Data are represented as mean \pm SEM. *: $p<0.05$

Figure 2. High-resolution manometry of antroduodenal contractility. Pressure topography image of a measurement during intragastric placebo (A) and quinine-hydrochloride (B) administration. Pressure intensity is colour coded with low pressures represented by the blue spectrum and high pressures by the red spectrum. Intragastric administration (vertical line) started 20 min after the end of phase III. Antroduodenal motility was measured for another 2 hours after the administration. Arrows indicate phase III contractions. Both conditions represent measurements of antroduodenal activity in the same subject on separate occasions.

Figure 3. The effect of intragastric QHCl administration on antral and duodenal motility. Motility index was calculated by averaging the motility index of six consecutive antral or duodenal channels. (A) Antral motility showed a significant time by administration interaction effect ($p=0.03$). (B) Duodenal motility was not affected by the administration of quinine-hydrochloride. Data are represented as mean \pm SEM.

Figure 4. Correlation between antral peak activity and plasma peak concentration. Time interval until the maximum value was determined for both antral motility and hormone

concentrations. (A) Correlation for the placebo condition. (B) Correlation for the quinine-hydrochloride condition. Solid line represents regression curve for octanoylated ghrelin. Striped line represent regression curve for total ghrelin. Dotted line represents regression curve for motilin. MI: motility index