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## **EFFECT OF ITOPRIDE ON ESOPHAGEAL MOTILITY AND LOWER ESOPHAGEAL SPHINCTER FUNCTION IN MAN**

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**EFFECT OF ITOPRIDE ON ESOPHAGEAL MOTILITY AND LOWER ESOPHAGEAL SPHINCTER FUNCTION IN MAN**

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## **EFFECT OF ITOPRIDE ON ESOPHAGEAL MOTILITY AND LOWER ESOPHAGEAL SPHINCTER FUNCTION IN MAN**

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**Running title: Itopride and esophageal motility in man**

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Keywords: itopride, prokinetics, TLESRS, GERD.

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## SUMMARY

**Introduction:** Itopride is a new prokinetic agent which combines antidopaminergic and cholinesterase inhibitory actions. Previous studies suggested that itopride improves heartburn in functional dyspepsia, and decreases esophageal acid exposure in GERD. It is unclear whether this effect is due to effects of itopride on the LES. **Aims:** To study the effects of itopride on fasting and postprandial LES function in healthy subjects. **Materials and methods:** twelve healthy volunteers (5 males;  $32.6 \pm 2.0$  years) underwent three esophageal sleeve manometry studies after 3 days premedication with itopride 50 mg, itopride 100mg or placebo t.i.d. Drug was administered after 30 minutes and a standardized meal was administered after 90 minutes, with measurements continuing to 120 minutes postprandially. Throughout the study, 10 wet swallows were administered at 30-minute intervals, and gastrointestinal symptoms were scored on 100 mm visual analogue scales (VAS) at 15-minute intervals. **Results:** LES resting pressures, swallow-induced relaxations and the amplitude or duration of peristaltic contractions were not altered by both doses of itopride, at all time points. Itopride pretreatment inhibited the meal-induced rise of transient LES relaxations (TLESRs). **Conclusions:** Itopride inhibits TLESRs without significantly affecting esophageal peristaltic function or LES pressure. These observations support further studies with itopride in GERD.

## INTRODUCTION

Itopride is a novel prokinetic agent acting both as a dopamine D2 receptor antagonist and as an acetylcholine esterase inhibitor. Both animal (1-3) and human studies (5,6) have shown the ability of the drug to accelerate delayed gastric emptying, associated with anti-emetic properties. In a phase 2 controlled trial, itopride was superior to placebo in relieving symptoms of functional dyspepsia (6). In a phase 3 program, this superiority was not confirmed, and comparison of both studies suggested that the presence of heartburn, which was excluded in the phase 3 program, was a predictor of response in the phase 2 studies (7). Heartburn is a typical symptom of gastro-esophageal reflux disease (GERD) (8), and a pilot study in GERD patients confirmed the ability of itopride to decrease esophageal acid exposure (9). The mechanisms underlying a potential beneficial effect of itopride in GERD remain to be elucidated.

The pathophysiology of GERD is multifactorial and involves several well-known mechanisms such as failure of the anti-reflux barrier, impaired esophageal clearance, the presence of caustic factors in the refluxate (acid and/or non-acid) and defective esophageal mucosal resistance (10). The influence of itopride on the anti-reflux barrier has not been studied. Among the dysfunctions of the anti-reflux barrier, transient lower oesophageal sphincter relaxations (TLESRs) are the major mechanism underlying gastro-oesophageal reflux events in normal subjects, and in the majority of GERD patients. (10,11) TLESRs are controlled by a vago–vagal reflex pathway, triggered by activation of stretch receptors of the proximal stomach, and organized in the brain stem, leading to transient relaxation of the lower esophageal sphincter (LES) (12). Pharmacological inhibition of TLESRs is considered a relevant target for the control of GERD (10). Several pharmacological agents, including atropine, morphine, GABA-B agonists, cholecystokinin 1 receptor antagonists and nitric oxide synthase inhibitors, have been shown to inhibit the occurrence of transient lower oesophageal sphincter relaxations (12,13).

The aim of the present study was to investigate the effect of itopride on oesophageal and lower oesophageal sphincter function in healthy subjects, with a focus on the occurrence of TLESRS.

## MATERIALS AND METHODS

### Subjects

Studies were performed in 12 healthy volunteers (five men and seven women; mean age, 32.6±2.0 years; range, 23–41 years) with a mean body mass index of 22.2±0.9 kg/m<sup>2</sup>.

None of the subjects had symptoms or a history of gastrointestinal disease or upper gastrointestinal surgery, nor were they taking any medication. Written informed consent was obtained from each subject and the study protocol had been approved previously by the Ethics Committee of the University Hospital.

### Study design

All subjects underwent the studies after 3 days premedication with itopride 50 mg, itopride 100mg or matching placebo t.i.d. in a double-blind randomised cross-over design. Treatment periods occurred at least one week apart. On each day of measurements, subjects were studied after an overnight fast of at least 12 hours. A summary of the protocol is shown in Figure 1. Together with a stationary manometry probe, a pH assembly was passed through the nose under topical anaesthesia and positioned with the pH electrode at 5 cm above the LES. After placement of the assembly, the subjects remained in a sitting position for a habituation period of 20 minutes. This period allowed baseline assessment of esophageal peristalsis and LES function. Ten wet swallows of 5 ml of water were administered at 1-minute interval and followed by ingestion of itopride or placebo according to the double-blind, randomized cross-over design. During the 30 min after administration of the drug oesophageal and lower oesophageal sphincter pressure and oesophageal pH were continuously monitored. Sixty minutes after drug administration the subjects ingested a mixed liquid meal (400 mL, 600 kcal, 13% proteins, 48% carbohydrates, 39% lipids; Nutridrink®, Nutricia, Bornem, Belgium) and recordings continued for 2 hours after the meal. Throughout the study, 10 wet swallows of 5 ml of water were administered at 30-minute intervals. Throughout the study, the sensations of

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3 fullness, nausea, heartburn, belching, satiety, hunger, anxiety, dizziness, sleepiness and  
4 fatigue were measured every 15 min using validated 100-mm visual analogue scales.  
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## 8 **Recording methods**

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11 Following an overnight fast, an oesophageal manometric catheter fitted with a 6-cm Dent  
12 Sleeve was introduced through the mouth. Subsequently, the oesophageal catheter was  
13 positioned so that pressures could be recorded from the gastric fundus (side hole 2 cm  
14 below the sleeve), the LES (sleeve), oesophageal body (side holes 4, 7 and 10 cm  
15 proximal to the sleeve) and pharynx (side hole 28 cm proximal to the sleeve, to detect  
16 swallows). The oesophageal catheter was infused at a flow rate of 0.5 mL/min with distilled  
17 water using a low-compliance pneumo-hydraulic capillary infusion system (Arndorfer  
18 Medical Specialties, Milwaukee, WI, USA). The infusion system was connected to external  
19 pressure transducers, and signals were recorded on a polygraph (Synectics Medical,  
20 Stockholm, Sweden).  
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31 The oesophageal pH was measured with an antimony pH electrode (Synectics Medical,  
32 Stockholm, Sweden) positioned 5 cm above the proximal margin of the sleeve. The pH  
33 electrode was calibrated in buffers of pH 1 and pH 7 before and after each study. During  
34 the study period, the oesophageal pH was recorded continuously using an ambulatory  
35 data-logger (MicroDigitrapper, Synectics Medical, Stockholm, Sweden).  
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## 44 **Data analysis**

### 45 *Lower oesophageal motility*

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48 The basal lower oesophageal sphincter pressure was measured at end-expiration relative  
49 to the end-expiratory intra-gastric pressure. The basal lower oesophageal sphincter  
50 pressure was visually determined every 3 min and averaged over 30-min intervals. The  
51 influence of drug administration on the basal lower oesophageal sphincter pressure was  
52 assessed by comparing the value of the first with the value of the third pre-prandial 30-min  
53 interval. Transient lower oesophageal sphincter relaxations were defined according to  
54 published criteria (14): (i) absence of a swallowing signal for 4 s before to 2 s after the  
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3 onset of lower oesophageal sphincter relaxation; (ii) relaxation rate of  $\geq 1$  mmHg/s; (iii) time  
4 from onset to complete relaxation of  $\leq 10$  s; and (iv) nadir pressure of  $\leq 2$  mmHg. Excluding  
5 multiple swallows, lower oesophageal sphincter pressure falls that fulfil the last three  
6 criteria, but have a duration of  $>10$  s, can also be classified as TLESRs irrespective of the  
7 timing of lower oesophageal sphincter relaxation relative to swallowing.  
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### 12 13 14 15 16 *Oesophageal pH*

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19 The percentage of time with an oesophageal  $\text{pH} < 4$  and the number of acid reflux episodes  
20 were calculated. Acid reflux episodes were defined as a decrease in oesophageal pH to a  
21 value below pH 4 for at least 4 s or as a rapid drop of at least 1 pH unit if the pH was  
22 already below 4.  
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### 30 **Statistical analysis**

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33 Based on previous studies, the study had an 85% power to detect 30% difference in  
34 TLESR rate at the 5% significance level. The changes in the basal LES pressure were  
35 evaluated using analysis of variance for repeated measures. The changes in TLESRs  
36 were analyzed using analysis of variance for repeated measures and Tukey-Kamer  
37 multiple comparisons post-test correction.  
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44  $P < 0.05$  was considered to be statistically significant. Data are presented as the mean  $\pm$   
45 standard error of the mean (S.E.M.).  
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## RESULTS

### Conduct of the study

The positioning of oesophageal manometry catheter and pH probe were all well tolerated, and all subjects completed the three sessions of studies as planned.

### Lower esophageal sphincter pressure

Prior to drug administration, LES resting pressure and swallow-induced relaxations were similar for all 3 conditions (Table 1). However, both doses of itopride significantly decreased intra-gastric pressures compared to placebo (respectively  $16.0 \pm 1.3$  and  $17.3 \pm 1.5$  vs.  $20.4 \pm 1.7$  mm Hg, both  $p < 0.05$ ). After drug administration, no significant changes in LES pressure occurred, although there was a tendency towards decreased resting pressure after itopride 50 mg ( $15.8 \pm 2.8$  to  $11.8 \pm 1.8$  mm Hg,  $p = 0.07$ ). In the placebo condition and after itopride 100mg, ingestion of the meal was associated with a significant drop in LES pressure, which was already present during the first postprandial hour and persisted during the second postprandial hour. After itopride 50 mg a, LES pressure was significantly decreased during the first postprandial hour, but LES pressure had recovered to values that did not differ significantly from preprandial values during the second postprandial hour (Table 1).

### Oesophageal motility

Both the amplitude or duration of peristaltic contractions were not significantly altered by both doses of itopride in the pre-prandial and post-prandial periods (Table 1). Swallow-induced relaxations were not altered by either dose of itopride at any time point (Table 2).

### Swallowing rate

Both before and after the meal, the swallowing rate was significantly altered by itopride 100 mg. However, the 50 mg dose was associated with a significant rise in swallowing rate compared to placebo, both before and after the meal (Table 3).

## Transient lower esophageal sphincter relaxations

The numbers of transient lower oesophageal sphincter relaxations after the administration of placebo and itopride are summarized in Figure 2 and Table 4. After placebo, ingestion of the meal was associated with a significant increase in the rate of transient lower oesophageal sphincter relaxations during the first and the second post-prandial hour (ANOVA,  $p < 0.001$ ). After itopride 50 mg, no significant rise of TLESR rate was observed during the first postprandial hour (ANOVA,  $p > 0.05$ ), and in a paired comparison, the rate of TLESRs was significantly lower than after placebo during the first hour (t-test,  $p < 0.05$ ). After itopride 100 mg, no significant rise of TLESR rate was seen during the first and the second postprandial hour (ANOVA,  $p > 0.05$ ), and the rate of TLESRs was significantly lower than after placebo during the second hour (t-test,  $p < 0.05$ ). The duration of TLESRS did not differ significantly between the treatment conditions (Table 3).

## Oesophageal pH monitoring

The percentage of time  $\text{pH} < 4$  in the oesophagus did not differ between the itopride and placebo studies in the pre-prandial ( $0.3 \pm 0.1\%$ ;  $0.3 \pm 0.2\%$  and  $0.8 \pm 0.5\%$ , for placebo, itopride 50 mg and itopride 100 mg respectively; NS) and postprandial periods ( $0.3 \pm 0.1\%$ ;  $0.4 \pm 0.1\%$  and  $0.3 \pm 0.2\%$ , for placebo, itopride 50 mg and itopride 100 mg respectively; NS).

The number of acid reflux episodes during the post-prandial period after itopride was not significantly different from that after placebo (Figure 3). The number of acid reflux episodes was significantly increased postprandially in the placebo and itopride 100 mg groups, but not in the 50 mg group. Individual data showed a decrease in reflux episodes in all subjects after 100 mg, except for one subject who had a very high rate of reflux episodes under this dose.

## Symptoms and side effects

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3 No significant differences in symptom scores (calculated as area under the curve, AUC)  
4 during both the pre-prandial and post-prandial periods were found between the itopride  
5 and placebo studies (details not shown).  
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**DISCUSSION**

The analysis of phase 2 and phase 3 clinical trials with itopride in FD, as well as a small pH monitoring study in patients with heartburn suggested a beneficial effect of itopride in GERD (6,7,9). In order to elucidate the underlying mechanism, we studied the influence of 2 doses of itopride in a double-blind placebo-controlled cross-over study on pre- and postprandial esophageal motility in healthy volunteers. Our main finding was that itopride inhibited the post-prandial increase in the rate of TLESRS without significantly affecting post-prandial LES pressure. Esophageal body peristalsis was also not altered by itopride.

Inhibition of TLESRS is a now well-established therapeutic target in GERD, and this effect may help to explain clinical effects of itopride on heartburn and on esophageal acid exposure (6,7,9). On the other hand, itopride did not affect esophageal acid exposure in the present study, and the rise in postprandial acid reflux events was only significantly inhibited in the 50 mg group, with some evidence of large inter-individual variability in the 100 mg group. The lack of major effects on these reflux parameters is probably attributable to the fact that this study recruited healthy volunteers, and not GERD patients, and the relatively low sample size. Confirmation of these effects in a larger group of GERD patients seems warranted.

The mechanism underlying the inhibition of TLESRs by itopride remains to be established. In the absence of effects on esophageal peristalsis and LES resting pressure, esophageal motility does not seem to be the target for itopride in TLESR inhibition. TLESRs are controlled by a vago-vagal reflex pathway which is triggered by gastric distention, integrated in the brainstem and induces release of nitric oxide from intrinsic nerves at the LES (11-13). The absence of an influence of itopride on swallow-induced LES relaxations, which are also mediated by intrinsic nitrergic nerves, argues against an effect of itopride at this level. Itopride does have the potential to affect the occurrence of TLESRs at the level of triggering through distention of the proximal stomach. Both positron emission tomography studies and gastric barostat studies showed a decreased postprandial volume of the proximal stomach in healthy volunteers after itopride pretreatment (15,16). Finally, an effect of itopride, which is likely to cross the blood-brain-barrier (17), on integration of TLESRs in the brainstem cannot be excluded, but is difficult to study in man.

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3 The effect of itopride on TLESRs seems to lack a clear dose-dependency. This could in  
4 part be due to some increased variability with the 100 mg dose, as illustrated in Figure 3.  
5 However, itopride has a dual pharmacological action as it acts both as a dopamine-2  
6 receptor antagonist and as an acetylcholine esterase inhibitor. It is unclear which one of  
7 these pharmacological properties underlies the observed effect on TLESRs. For  
8 domperidone, another dopamine-2 antagonist, conflicting data are found in the literature  
9 regarding its clinical efficacy in GERD (18-20), but its effects on TLESRs have not been  
10 studied to date. In a preliminary study, decreased postprandial gastric volumes, measured  
11 with the barostat, were also reported in healthy volunteers treated with domperidone (16).  
12 Besides D2 receptor antagonism, itopride also exerts cholinesterase inhibitory action. In a  
13 gastric barostat study in healthy volunteers, a cholinesterase inhibitor did not alter proximal  
14 stomach volume, but increased the number of phasic contractions (21). It is less likely that  
15 this would result in decreased triggering of TLESRs, but the effect of cholinesterase  
16 inhibition on TLESRs has not been investigated so far. Conversely, acetylcholine receptor  
17 antagonism has been shown to inhibit the occurrence of TLESRs, probably via a central  
18 site of action (22). It is conceivable, but unproven to date, that enhanced cholinergic  
19 activity, induced by a cholinesterase inhibitor, might thus lead to enhanced occurrence of  
20 TLESRs. If this is indeed the case, higher doses of itopride exerting higher cholinesterase  
21 inhibition levels might act to enhance rather than inhibit TLESRs, and this could have  
22 contributed to the lack of a dose-dependent effect of itopride in the present study. A  
23 decreased rate in swallowing has been implicated in the effects of baclofen on TLESRs  
24 and reflux events (23), but we only observed a modest but significant increase in  
25 swallowing rate with the 50 mg dose of itopride.

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46 The present study has a number of limitations. First, the number of reflux events and the  
47 number of TLESRs are generally low. This is probably attributable to the selection of  
48 asymptomatic healthy subjects with a normal body mass index, and the small size and  
49 caloric content of the meal. Second, the liquid meal may lead to more rapid buffering of  
50 intragastric acid, thereby under-estimating reflux events when pH monitoring is used.  
51 Taken together, it is conceivable that a larger therapeutic effect could be obtained in a  
52 population of GERD patients, using a larger meal. Furthermore, the study set-up used two  
53 independent systems to monitor manometry and reflux events. Hence, reliable analysis of  
54 the motor events underlying the reflux patterns is not possible.

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3 In summary, we demonstrated that the dopamine D2 receptor antagonist and  
4 cholinesterase inhibitor, itopride, is able to inhibit the meal-induced increase in transient  
5 lower oesophageal sphincter relaxations. The underlying mechanisms require further  
6 studies, but could be related to altered postprandial gastric volumes. Based on these  
7 observations, further studies of the impact of itopride in gastro-oesophageal reflux disease  
8 seem warranted.  
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## FIGURES

**Figure 1. Study protocol.** Healthy volunteers underwent esophageal sleeve manometry and pH measurement studies after 3 days premedication with itopride 50 mg, itopride 100mg or placebo. After placement of the assembly ten wet swallows of 5 ml of water were administered, followed by ingestion of the medication. After 60 minutes a standardized meal was administered and measurements continued for another 120 minutes. At 30-minute intervals, 10 wet swallows were administered. Throughout the study, at 15-minute intervals the intensity of 8 epigastric symptoms was scored on visual analogue scales.

**Figure 2.** Individual numbers of total transient LES relaxations for the 3 treatment conditions.

**Figure 3.** Acid reflux episodes for the 3 treatment conditions, before and after the meal. \* =  $p < 0.05$  compared to preprandial.

## TABLES

**Table 1.** Esophageal motility parameters for the 3 treatment conditions, before and after drug intake, and before and after the meal. No significant differences between groups occurred. \* $p < 0.05$  compared to basal.

		Placebo	Itopride 50 mg	Itopride 100 mg
<b>LES pressure (mm Hg)</b>	<b>Basal</b>	19.7±2.5	15.8±2.8	20.2±3.0
	<b>Post-drug</b>	17.3±3.0	11.8±1.6	19.8±3.7
	<b>Postprandial 1<sup>st</sup> hour</b>	10.3±1.5 *	8.3±1.5 *	11.1±1.8 *
	<b>Postprandial 2<sup>nd</sup> hour</b>	8.6±3.0 *	10.0±2.0	8.8±4.8 *
<b>Distal contraction amplitude (mm Hg)</b>	<b>Basal</b>	76.0 ±3.4	78.4 ± 4.3	76.9 ± 3.7
	<b>Post-drug</b>	83.0 ± 4.8	80.2 ± 4.2	86.2 ± 2.9
	<b>Postprandial 1<sup>st</sup> hour</b>	78.6 ± 3.6	76.8 ± 3.1	79.5 ± 3.9
	<b>Postprandial 2<sup>nd</sup> hour</b>	73.9 ± 4.3	73.3 ± 2.9	76.9 ± 4.1
<b>Distal contraction duration (sec)</b>	<b>Basal</b>	4.6 ± 0.3	5.2 ± 0.4	4.8 ± 0.3
	<b>Post-drug</b>	5.0 ± 0.3	5.3 ± 0.4	4.7 ± 0.2
	<b>Postprandial 1<sup>st</sup> hour</b>	5.1 ± 0.3	5.4 ± 0.3	4.7 ± 0.2
	<b>Postprandial 2<sup>nd</sup> hour</b>	4.9 ± 0.3	5.2 ± 0.3	4.6 ± 0.2

**Table 2.** Characteristics of swallow-induced relaxations for the 3 conditions, before and after drug intake, and before and after the meal. No significant differences occurred between groups or over time.

	Placebo	Itopride 50 mg	Itopride 100 mg
<b>Relaxation (%)</b>			
<b>Basal</b>	97.7±0.8	97.8±0.7	96.4±0.7
<b>Post-drug</b>	94.4±1.6	92.4±2.3	96.4±1.2
<b>Postprandial 1<sup>st</sup> hour</b>	92.2±3.9	95.5±1.1	96.8±0.6
<b>Postprandial 2<sup>nd</sup> hour</b>	95.9±1.2	93.3±1.7	93.0±3.0
<b>Duration (sec)</b>			
<b>Basal</b>	11.9±0.4	13.9±0.8	12.8±0.8
<b>Post-drug</b>	11.3±0.7	13.0±0.5	11.7±0.5
<b>Postprandial 1<sup>st</sup> hour</b>	11.6±0.6	13.4±0.7	11.7±0.7
<b>Postprandial 2<sup>nd</sup> hour</b>	11.7±0.5	13.0±0.5	12.9±0.6

**Table 3.** Number of swallows for the 3 treatment conditions, before and after the meal. \*  
p< 0.05 compared to placebo.

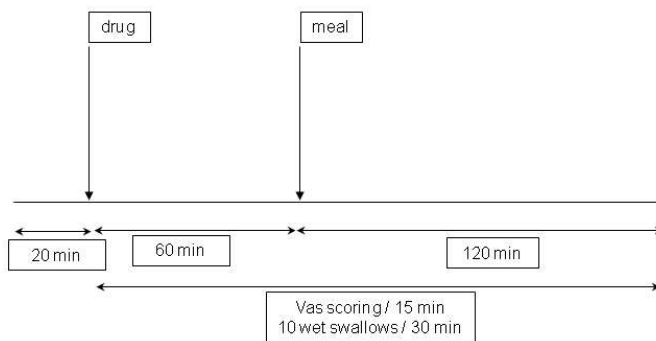
<b>Pretreatment</b>	<b>Pre-prandial</b>	<b>First post-prandial hour</b>	<b>Second post-prandial hour</b>	<b>TOTAL</b>
<b>Placebo</b>	25.2 ± 2.8	19.0 ± 1.1	20.5 ± 2.2	63.8 ± 5.1
<b>Itopride 50 mg</b>	27.3 ± 2.3	24.6 ± 2.1*	23.8 ± 1.7	75.8 ± 5.5*
<b>Itopride 100 mg</b>	25.3 ± 2.7	21.4 ± 2.5	21.9 ± 2.6	63.1 ± 8.4

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**Table 4.** Number of transient lower oesophageal sphincter relaxations for the 3 treatment conditions, before and after the meal. \* P < 0.05 compared to preprandial. # p< 0.05 compared to placebo.

Pretreatment	Pre-prandial	First post-prandial hour	Second post-prandial hour	TOTAL
Placebo	0.3 ± 0.1	1.3 ± 0.2 *	1.7 ± 0.3 *	3.0 ± 0.5 *
Itopride 50 mg	0.4 ± 0.3	0.5 ± 0.3 #	1.2 ± 0.4 *	1.7 ± 0.7 *
Itopride 100 mg	0.7 ± 0.2	1.0 ± 0.3	0.4 ± 0.2 #	1.7 ± 0.4 *

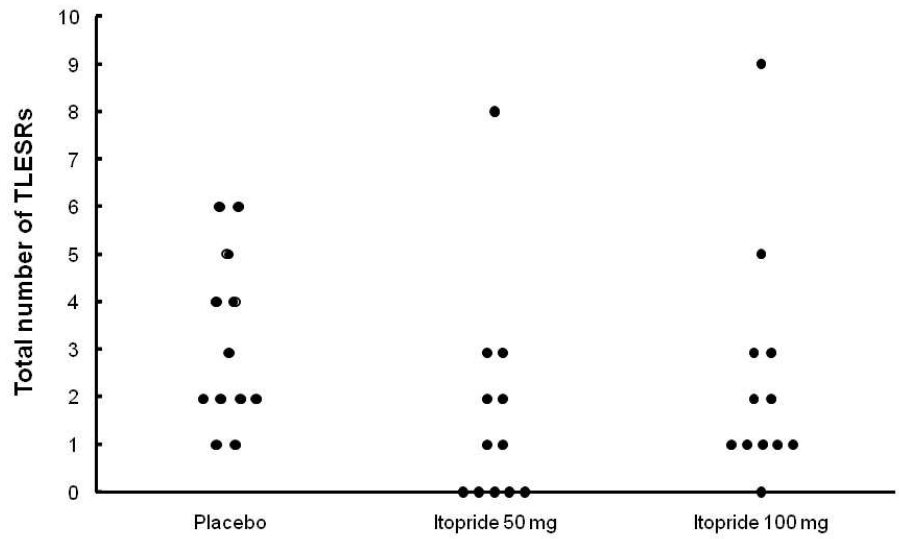
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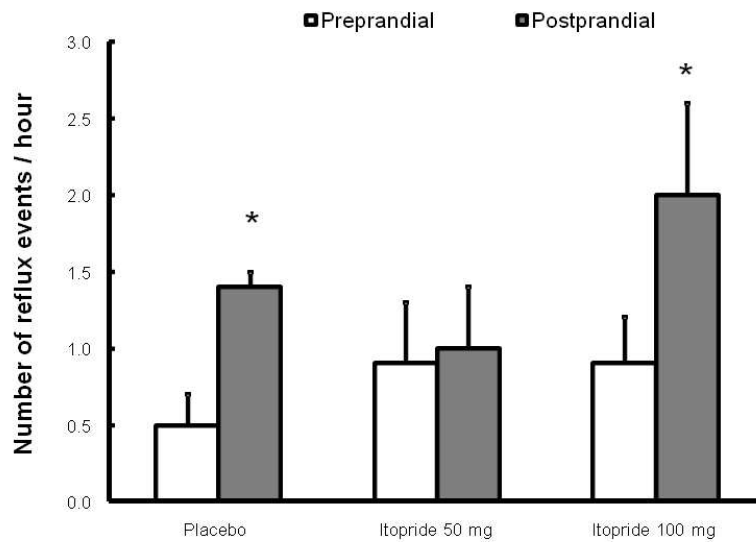
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8 Reviewer: 1

9 Comments for Transmission to the Authors

10 It's a bit odd that, four years after a clinical trial in NEJM about this drug, it's still not approved in the EU or USA...  
11 does it have a problem? Is it still being developed?

12  
13 The current status seems to be that development for US or EU in the functional dyspepsia indication has been  
14 abandoned.

15  
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17  
18 I think we need one figure showing the data for individuals - perhaps the total TSELRs for placebo and the 2 doses,  
19 with the data points joined... how consistent is the effect? I'd prefer this approach for the final figure - it tells the  
20 reader so much more than histograms.

21  
22 We agree and added graphs with individual data  
23  
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27 Reviewer: 2

28 Comments for Transmission to the Authors

29 The authors investigated the effects of the new prokinetic agent, itopride, on esophageal and LES function in healthy  
30 subjects. The study was conducted in a double blind crossover control trial with a one week washout period in  
31 normal volunteers. The authors show that this agent inhibits TLESRs but has little to no effect on esophageal  
32 peristalsis or LES pressure. The experimental protocol is well described and the data is convincingly shown. The  
33 figure quality is poor and detracts from the relative importance of these findings.

34  
35 As also pointed out by reviewer 3, Figure and Table 3 overlap. We have deleted Figure 3 and we have replaced it by  
36 individual numbers figures to better illustrate the effects of the drug.

37  
38 The statistical analysis should be better described given the small sample size of ten subjects.

39  
40 We have elaborated on power calculations and statistical analysis. We have now also added ANOVA with repeated  
41 measure analysis.

42  
43 The major drawback from this study design is that the authors have selected normal volunteers to test this agent,  
44 however the authors recognize this relative weakness to the study design and have included this in the discussion  
45 section.

46  
47 We agree and have included this in the discussion. (for your information: a patient study will start before the end of  
48 this year).

49  
50 A proposed mechanism of action of the D2 antagonist/anticholinesterase would enhance the discussion section.

51  
52 We have added a section on the relative contribution of both pharmacological mechanisms in the discussion section,  
53 highlighted in the revised version.  
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3 Reviewer: 3

4 Comments for Transmission to the Authors

5 Aim of this study was to evaluate the role of itopride on fasting and postprandial oesophageal and LES motility in  
6 healthy subjects. Oesophageal pH was also recorded with a ambulatory data logger.

7 Results have shown that itopride inhibited meal-induced rise in TLESRs. Acid reflux was unaffected.

8 Interesting study. A few issues should be raised:

9 - A dose effect of itopride on rate of TLESRs was not seen. The authors should discuss this.

10  
11 We agree and have added a section in the discussion

12  
13  
14 - Comparisons among fasting, first and second postprandial hour should be done with tests for multiple comparisons  
15 and not t-test or the Wilcoxon test.

16  
17 We agree and have adapted the statistics; we used ANOVA with repeated measures and Tukey Cramer post test  
18 correction for multiple comparisons.

19  
20  
21 - The liquid formula used is not an optimal meal: substantial intragastric acid buffering induced by this type of meal  
22 decreases occurrence of acid reflux, making it harder to see differences between different experimental conditions.  
23 Furthermore the postprandial rate of TLESRs is low even during placebo compared to similar studies in healthy  
24 volunteers. The authors should discuss these points.

25  
26 We agree and this was addressed discussion section.

27  
28  
29 - a limitation of this study was that manometry and pH recording were performed with two independent systems,  
30 therefore analysis of motor events underlying reflux episodes could not be done. The authors should acknowledge  
31 this.

32  
33 We agree and this was added to the discussion section.

34  
35  
36 - the rate of swallowing has been shown to be decreased by drugs affecting the rate of TLESRs. The authors should  
37 report the rate of spontaneous swallowinedg in order to better interpret the results.

38  
39 We have added data on swallowing rate.

40  
41  
42 - BMI instead of body weight should be used

43  
44 This is corrected

45  
46  
47 - Table 3 should be avoided

48  
49 We agree that Table 3 and figure 3 are identical. We have kept the table, and have replaced Figure 3 with individual  
50 data points for the first and second postprandial hour

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52  
53 - Fig 2, the evaluated variable is “number of TLESRs” and not “number of TLESRs/hour”

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55 This is corrected  
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- minor point, page 6, line 19: “or as a rapid drop in pH of at least 1 unit if pH already below 4”

This is corrected.

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