Chapter 3

EFFECT OF ALOSETRON ON LEFT COLONIC MOTILITY IN NON-CONSTIPATED IBS PATIENTS AND HEALTHY VOLUNTEERS

C.H.M. Clemens, M. Samsom, G.P van Berge Henegouwen, M. Fabri, A.J.P.M. Smout

Aliment Pharmacol Ther 2002;16:993-1002.
ABSTRACT

**Background:** Alosetron is a 5-HT$_3$ receptor antagonist reducing symptoms in female patients with diarrhea-predominant IBS, and is known to increase colonic transit time.

**Aim:** To study the effect of alosetron on left colonic phasic motility in ambulant non-constipated IBS patients and healthy volunteers.

**Methods:** In a double-blind, randomized, cross-over design, 10 IBS patients and 12 sex- and age-matched volunteers were treated for two 7-days periods with alosetron 4 mg bd or placebo bd. On day 6 of each treatment period a 6-channel solid-state manometric catheter was positioned in the left colon and 24 hour motility was studied on day 7. Periprandial phasic motility around dinner was evaluated in the descending and sigmoid colon. High-Amplitude Propagated Contraction (HAPC) frequency and characteristics were calculated.

**Results:** Alosetron appeared to increase overall periprandial frequency in the sigmoid colon ($p=0.043$) and mean amplitude of colonic contractions in the descending colon ($p=0.007$). HAPC frequency was higher on alosetron during the second half of the day for IBS patients ($p=0.002$) with increased mean HAPC propagation length ($p=0.001$). Stool frequency ($p=0.024$), and stool consistency score ($p=0.002$) were decreased by alosetron.

**Conclusions:** The 5-HT$_3$ receptor antagonist alosetron marginally increased left colonic periprandial phasic motility. Alosetron increased the number and propagation length of HAPCs which were paradoxically accompanied by a decrease in stool frequency and a firming of stool consistency.
INTRODUCTION

Although the pathogenesis of irritable bowel syndrome (IBS) is still poorly understood, altered intestinal motor function and visceral hypersensitivity have been shown to be important etiological factors. Abnormal gastrointestinal motor function was frequently reported in IBS, not only in the colon, but also in the small intestine. The literature about colonic motor abnormalities in IBS is partly conflicting but older publications suggest that the incidence of segmenting contractions is increased in constipation-predominant and decreased in diarrhea-predominant IBS. In addition to abnormalities in segmenting contractions, abnormalities in High-Amplitude Propagated Contractions (HAPCs) were found in IBS patients. In those patients with diarrhea an increased incidence of HAPCs was observed, whereas constipated patients had less HAPCs than normals.

In addition, colonic tone, as measured with the barostat technique, appears to be abnormal in IBS. In particular, the postprandial increase in tone was less prominent and shorter in duration in IBS patients than in healthy subjects.

Recent evidence supports the hypothesis that, in a subset of IBS patients, symptoms are related to visceral hypersensitivity. Rectal balloon distension has been used as a model to examine visceral sensitivity and showed that patients with IBS are more sensitive to rectal distension than healthy volunteers. However, the relationship between altered visceral sensitivity and abnormal motility has yet to be established.

5-hydroxytryptamine (5-HT) plays an important role in the regulation of gastrointestinal motility and perception. In diarrhea-predominant IBS patients, the postprandial increase in 5-HT plasma concentration was found to be significantly exaggerated. Alosetron is a potent and selective antagonist at the 5-HT receptor. Placebo-controlled clinical trials have shown that alosetron is of benefit in female patients with diarrhea-predominant IBS. In the clinical trials alosetron was well tolerated and improved abdominal pain and discomfort, urgency, stool frequency, and stool consistency. Alosetron increases the compliance of the descending colon to distension and could thereby contribute to changes in perception of colonic distension.
and improvement in symptoms of IBS.23 Alosetron has been shown to have no overall effect on orocaecal transit time, but it increases whole gut transit time as a result of increasing left colonic transit time.24 The effect of alosetron on phasic left colonic contractions and HAPCs has not previously been studied. The aim of this study was to examine the effects of orally administered alosetron (4mg twice daily) on left colonic motility in patients with non-constipated IBS and healthy volunteers. Since colonic motility in general is highly variable throughout a 24-hour period and HAPCs are infrequent colonic events, ambulatory colonic manometry over a 24-hour period was used.

METHODS

Subjects
IBS patients were recruited from the outpatient clinic of the department of Gastroenterology of the University Medical Center Utrecht. After exclusion of organic disease IBS patients, diagnosed by Rome I criteria, who were non-constipated were enrolled. Based on medical history non-constipated was defined as having a stool consistency of \( \geq 2.5 \) on a five point scale: 1 = very hard, 2 = hard, 3 = formed, 4 = loose, 5 = watery. Age- and sex-matched healthy volunteers were recruited by advertisement and from our own files. They had to be free from cardiac, pulmonary, gastrointestinal, hepatic, renal, hematological, neurological and psychiatric disease, as determined by history, physical examination and laboratory tests (hematology, biochemistry, urine analysis). Written informed consent was obtained from each subject and the Research Ethics Committee of the University Medical Center Utrecht approved the study protocol.
Study protocol

In a randomized, double-blind, placebo-controlled, two-way cross-over study (S3BB1007) the effects of alosetron 4 mg bid on colonic motility and defecation were evaluated. Each subject was treated with alosetron or placebo for 7 days (day 1-7) and then switched to the alternate treatment after a 2-4 week washout period. Each dose was taken before breakfast and evening meals. The manometric study of the left colon was performed from day 6 until the morning of day 8.

Ambulatory manometry of the colon was performed with a 6-channel solid-state catheter (Sentron, Roden, The Netherlands). In the afternoon of day 6 the left colon was cleaned by means of administration of an enema (20g soap in 2L water, Driehoek zeep, Hartman Intradal B.V., Veenendaal, The Netherlands). After cleaning of the left colon the manometric catheter incorporating 6 pressure transducers at 10-cm intervals, was placed endoscopically. The procedure was performed without sedation and with minimal insufflation of air.

The tip of the manometric catheter was attached to the colonoscope and introduced until the tip of the catheter reached the mid-transverse colon. Under fluoroscopic control the catheter was pulled back until the distal sensor was located in the rectosigmoid, 10 cm above the anal verge and the most proximal sensor was in the distal transverse or proximal descending colon. The catheter was then secured to the peri-anal skin with tape. The catheter was connected to a portable data logger with 4Mb of random access memory (MMS, Enschede, The Netherlands) and recordings continued until the removal of the catheter on day 8. A sampling rate of 4 Hz was used for each of the six channels.

After placement of the catheter the subjects returned home. Subjects were requested to maintain their normal daily routines as much as possible with the exception of performing strenuous exercise. During the motility study subjects were asked to maintain a standard diet (see below). Smoking, drinking alcohol or coffee was prohibited for 24 hours prior to and during the manometric study.
On day 8, the subjects returned to the unit and the position of the catheter was checked using fluoroscopy. The catheter was then removed and the data transferred from the data logger to a personal computer. After the washout period the subjects switched to their second treatment period of 7 days. On the morning of day 6 and day 8 of the second treatment period, subjects returned again to the gastrointestinal research unit and the procedures described above were repeated.

**Defecation characteristics**

For 7 days proceeding each treatment period, and during treatment with alosetron and placebo, all subjects recorded their defecation characteristics, such as stool consistency and frequency, in a diary. The consistency of every stool was scored by the subjects on a 5-point scale: 1=very hard, 2=hard, 3=formed, 4=loose, 5=watery. During the colonic motility studies the subjects kept another diary in which meals, physical activities, urge to defecate, defecation characteristics, abdominal pain and discomfort were recorded. The start and finish of these events were also registered by pressing an event marker on the data logger.

**Standardised meals**

During the manometric study the subjects used a standard diet. On day 7 a breakfast was taken containing 2218 kJ; protein 25 g, carbohydrate 53 g and fat 24 g. Lunch on day 7 consisted of chicken and rice and contained 2270 kJ; protein 30 g, carbohydrate 60 g, fat 20 g and 200 ml water. The evening meal contained 2370 kJ; protein 26 g, carbohydrate 60 g, fat 25 g.

**Analysis of colonic motility**

Only the motility data recorded on day 7 were analyzed, i.e. from midnight on the day the catheter was positioned until 24 hours later. The colonic motility recordings were considered a failure when more than one of the 6 manometric sensors had failed or when less than 18 hours of continuous colonic motility had been recorded. Manometric
data were analyzed both visually and automatically using a dedicated software programme.\(^{25}\) This software programme calculated the frequency, amplitude and motility index (\(\text{MI} = \ln ((n \times \Sigma \text{amplitudes}) + 1)\)) of all pressure waves detected at the 6 pressure sensors after base-line correction and elimination of artifacts.

In the analysis of the segmenting pressure waves, one signal recorded from the sigmoid -and one from the descending colon were selected. This was done on the basis of fluoroscopy images obtained before and after the ambulatory recording period. Analysis of phasic pressure waves was confined to signals recorded during four 15-min periods before -and eight 15-min periods after dinner from the sensors located in the descending colon and sigmoid. In each of these 15-min periods, mean frequency, amplitude and motility index were calculated.

The periprandial period was divided into three periods of one hour each: the four 15-min periods before dinner are called preprandial period, the first four 15-min periods after dinner are the early postprandial period and the last four 15-min periods are called late postprandial period. The mean frequency, amplitude and the motility index in these periprandial hours were calculated from the total number of contractions and the sum of amplitudes in four 15-min periods.

HAPCs, defined as pressure waves that propagate distally across at least 3 sensors, with a speed of more than 0.3 cm/sec and amplitude of at least 100 mmHg in 2 sensors and at least 75 mmHg in one other sensor, were analysed visually. HAPC characteristics that were recorded during day-time on day 7 (defined as the period that started when the subject arose in the morning and ended when the subject went to bed in the evening) were used for subsequent analysis. After identification of the HAPCs, their number, frequency, amplitude, propagation velocity, propagation distance and duration were calculated during this day-time period.

**Analysis of stool characteristics**

Mean stool frequency was determined during the first pre-treatment period of seven days. During treatment, a mean stool frequency was calculated from day 1 up to day 5 of each treatment period. Day 6 was the day of colonic cleaning and catheter placement.
while day 7 was the analyzed period for colonic motility. The mean consistency/stool was calculated for the first pre-treatment period of seven days. During treatment a mean stool consistency was calculated from day 1 up to day 5 of each treatment period.

**Statistical analysis**
The mean contractile frequency, mean amplitude and motility index in the three periprandial hours were calculated and subsequently analyzed for all subjects together using the MIXED effects modeling procedure in SAS(R) software (version 6.12). The effect of alosetron on colonic motility was also investigated separately in IBS patients and in healthy subjects using analysis of variance. All analyses were done for both locations of the colon; the descending colon and the sigmoid colon.

Data from all six sensors were integrated in order to derive the HAPC data. The HAPC characteristics, duration (sec), amplitude (kPa), propagation length (cm), propagation velocity (cm/sec), and the incidence of HAPCs, were also analyzed as described above. The mean stool frequency and consistency data collected prior to the treatment period were analyzed for group differences using independent student t-tests. Treatment effects on stool frequency and consistency were tested by paired t-tests.

**RESULTS**

**Study group**
Thirty-six subjects (18 patients, 18 healthy controls) were randomized to a treatment sequence. Data from 8 patients and 6 healthy volunteers could not be analyzed for various reasons (inadequate use of medication, incomplete follow-up, failure to position manometric catheter, catheter expulsion, and technical insufficiencies).

In total 10 patients with IBS (5 M, 5 F; age: 39.3 ± 8.0 yr.; height: 174.0 ± 9.8 cm; weight: 78.5 ± 16.9 kg) and 12 healthy age- and sex-matched controls (6 M, 6 F; age: 37.9 ± 8.9 yr.; height: 175.2 ± 8.8 cm; weight: 70.0 ± 12.5 kg) were studied successfully and only data obtained from these subjects were included in the statistical analysis.
Effect of alosetron on left colonic motility

Manometric data

Periprandial motility

During the periprandial period as a whole, alosetron slightly increased contractile frequency (p=0.043) in the sigmoid colon, whereas no effect of alosetron was observed in the descending colon. Also during the periprandial period as a whole, alosetron increased the mean amplitude of colonic contractions in the descending colon (p=0.007), whereas no effect was seen in the sigmoid colon (Table 1, Figure 1).

<table>
<thead>
<tr>
<th>Table 1: Effect of alosetron on periprandial colonic motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>DINNER</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Frequency (contractions/min)</strong></td>
</tr>
<tr>
<td>Pre</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Late</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td><strong>Amplitude (kPa)</strong></td>
</tr>
<tr>
<td>Pre</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Late</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td><strong>Motility index (1-h periods)</strong></td>
</tr>
<tr>
<td>Pre</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Late</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Data = mean ± standard deviation

a) P = 0.043

b) P = 0.007

c) P = 0.044

High-Amplitude Peristaltic Contractions

Alosetron significantly increased the frequency of day-time HAPCs in the total study population (p =0.021). Analysis of the HAPC frequency data showed that IBS patients had a significant increase in the number of HAPCs during treatment with alosetron only in the second half of the day (p = 0.002) whereas alosetron had no significant affect on the number of HAPCs in healthy volunteers (Figure 2).
Alosetron significantly increased the mean propagation length of daytime HAPCs (p =0.001) (Table 2). There was no evidence of a difference between IBS patients and healthy volunteers (p=0.415). However, alosetron did not significantly affect HAPC duration, amplitude or propagation velocity.

Figure 1  (a) Mean periprandial motility indices ± S.E.M. (15 minutes intervals) in all subjects (patients and volunteers) during treatment with alosetron (closed rectangles) or placebo (open circles) in the descending colon. (b) Mean periprandial motility indices ± S.E.M. (15 minutes intervals) in all subjects (patients and volunteers) during treatment with alosetron (closed rectangles) or placebo (open circles) in the sigmoid colon. Minor increase of motility indices during the preprandial period on alosetron ( * p=0.044).
Effect of alosetron on left colonic motility

Stool frequency and stool consistency
During the pre-treatment period a significantly higher mean stool frequency (2.3 ± 1.1 / day versus 1.1 ± 0.3 / day; p=0.003) was observed in the IBS patients compared to healthy volunteers. No difference in mean consistency score was observed between patients and volunteers during the pre-treatment period (3.2 ± 0.6 versus 3.0 ± 0.2).
Alosetron significantly decreased the stool frequency in the total study population from 1.8 ± 1.3 / day during placebo treatment to 1.4 ± 0.8 / day during alosetron treatment (p=0.024). Alosetron did not have a significant effect on stool frequency in IBS patients and healthy volunteers when each group was analyzed separately.
Alosetron significantly decreased the stool consistency score (i.e. stools became harder) in the total study population (from 3.0 ± 0.4 in the placebo group to 2.5 ± 0.9 in the alosetron group; p=0.002). When analyzed separately, alosetron significantly decreased stool consistency score in the control group (from 2.9 ± 0.2 to 2.2 ± 0.8; p=0.010) but not in the IBS patient group (from 3.2 ± 0.5 to 2.8 ± 1.0; p=0.112).

Table 2: Effect of alosetron on HAPCs during daytime period

<table>
<thead>
<tr>
<th>HAPC</th>
<th>Volunteers</th>
<th>IBS patients</th>
<th>Between treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Alosetron.</td>
<td>Placebo</td>
</tr>
<tr>
<td>Frequency  (no./HAPCs/h)</td>
<td>0.6±0.7</td>
<td>0.7±0.7</td>
<td>0.6±0.4</td>
</tr>
<tr>
<td>Velocity  (cm/s)</td>
<td>1.3±0.4</td>
<td>1.2±0.5</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Amplitude  (kPa)</td>
<td>22.4±6.5</td>
<td>24.2±5.5</td>
<td>24.9±5.9</td>
</tr>
<tr>
<td>Duration  (s)</td>
<td>16.3±3.2</td>
<td>21.2±8.1</td>
<td>17.1±2.7</td>
</tr>
<tr>
<td>Propagation distance  (cm)</td>
<td>28.4±7.0</td>
<td>33.4±4.7</td>
<td>29.6±6.4</td>
</tr>
</tbody>
</table>

Data = mean ± standard deviation

Adverse Events
Fourteen of the 16 subjects who experienced adverse events whilst receiving alosetron reported events which were classified as gastrointestinal in nature, of which 12 subjects (10 healthy volunteers, 2 IBS patients) experienced constipation, and 9 (8 volunteers, 1
patient) experienced abdominal discomfort and pain. Headaches were reported by 2 subjects whilst receiving alosetron, and by 1 subject on placebo. All adverse events resolved rapidly.

Figure 2. Number of HAPCs in individual IBS patients and volunteers during treatment with placebo and alosetron in the first and second half of the daytime period. The mean number of HAPCs is represented by filled black triangles. Increased HAPC frequency in second half of the daytime period in the patient group on alosetron (* p=0.002).

**DISCUSSION**

This is the first study evaluating the effect of alosetron on left colonic phasic motility in non-constipated patients with irritable bowel syndrome and healthy volunteers using prolonged ambulant manometry. Although multiple comparisons were performed in this study and comparisons were not adjusted for multiplicity, we feel that the following conclusions are justified within the context of the exploratory analyses: 1) alosetron affects left colonic motility in the peri-
Effect of alosetron on left colonic motility

prandial period in IBS patients as well as in healthy volunteers, 2) alosetron increases the HAPC frequency in IBS patients and propagation length in all subjects, 3) treatment with alosetron is accompanied by a decrease in stool frequency and consistency score, and 4) non-constipated IBS patients on alosetron appear to have less constipation and report less abdominal pain and discomfort compared to healthy volunteers.

Most studies on colonic motility in IBS patients have been performed in a laboratory setting, during a short period of time, and after a total colonic lavage. In the present study we used a prolonged ambulant manometric technique with the advantage of recording multiple HAPCs in each subject after refilling of the colon.

This study demonstrates that 24-hour colonic manometry is feasible and well-tolerated. However, the failure rate of about 40% remains one of the major problems of this technique. Half of this failure rate was caused by technical problems such as failure of catheter placement, transducer failure, catheter expulsion and peri-anal pain.

Periprandial motility is thought to be changed in IBS patients and other studies suggest a postprandial increase in 5-HT plasma concentration in diarrhea-predominant IBS patients. Periprandial motility was studied during the evening meal on day 7 because this meal was more than 26 h after partial colonic cleaning and was combined with alosetron or placebo.

HAPCs were analyzed during the daytime period on day 7, because it is known that hardly any HAPCs occur during sleep. During the 24-h period of day 7 only 2 % of the HAPCs were counted during the night-time in the placebo treatment period and 2.3 % of HAPCs were counted during the night-time in the alosetron treatment period. We were especially interested in the effect of alosetron on the diurnal occurrence of HAPCs: in healthy volunteers, HAPCs occur more often in the first part of the day. For this reasons we analyzed the total daytime period as well as the first and second half of this daytime period separately.

We did not take the menstrual cycle into account for practical reasons. In our opinion there is not sufficient evidence to support the view that the influence of hormones on left colonic motility significantly increases the intrinsic variability as is measured in men.
We found no significant differences between non-constipated IBS patients and healthy volunteers in terms of the periprandial motility index or 24-h HAPC frequency, regardless of whether the treatment received was placebo or alosetron. This might be due to patient selection, because non-constipated IBS patients can be considered as a mixture of diarrhea-predominant, alternating diarrhea and constipation, as well as pain-predominant IBS patients.

Our healthy subjects had a somewhat higher number of HAPCs during the control arm of the study. However, we had a wide spread of HAPC number in the healthy volunteer group with 2 subjects having a very high number of HAPCs (28 and 30) on day 7. We decided not to exclude these two outliers. Without these two subjects the effect of alosetron on HAPC frequency would have been more convincing.

Recently, colonic transit through the ascending and transverse colon has been shown to be related to stool weight. Whole gut transit time, which is correlated to the stool form, and the stool frequency were significantly different in IBS patients reporting constipation compared with those reporting diarrhoea. Houghton et al. showed that alosetron increases left colonic transit time. Our results concerning stool characteristics (decreased stool frequency and consistency during alosetron treatment) are in line with the observed slowing of colonic transit.

The literature suggests that shortened colonic transit time, increased stool frequency and decreased stool consistency can be explained by a higher incidence of anally directed mass movements produced by a greater number of HAPCs and less segmenting non-propagated colonic contractions.

Serotonin plays a role in physiological and pathological states in the human colon. Bearcroft et al. showed an increase in serotonin release in response to a meal in female patients with diarrhea-predominant IBS. In a study with the 5-HT3 receptor antagonist ondansetron, it was found that selective blockade may blunt the postprandial tonic and phasic motor response in healthy volunteers. In contrast, in this study, it appears that alosetron slightly increased the frequency and amplitude of left-colonic contractions.
HAPCs are the major motor events in the colon producing mass movements. HAPCs are related to defecation and the feeling of urge. The highest frequency is noted after meals and after awakening in the morning. Less HAPCs are recorded in the late afternoon and during the night. Fewer HAPCs were counted in constipated patients, while a higher number was seen in patients with functional diarrhea. At present, no studies exist describing the effect of 5-HT₃ receptor antagonists on HAPC frequency. The results of our study show that 5-HT₃ receptor blockade seems to increase HAPC frequency and propagation length, and that there may be more HAPCs in non-constipated IBS patients on alosetron during the second half of the day. The paradox of a higher HAPC frequency and greater propagation distance accompanied by a decreased stool frequency and stool consistency, suggesting a delay in colonic transport, is difficult to explain. One might speculate that the incidence of non-propagating, segmenting contractions is increased by alosetron, leading to a longer colonic transit time and a higher stool consistency. This might further be promoted by retardation of proximal colonic emptying by alosetron, which was shown in patients with carcinoid diarrhoea. Furthermore, alosetron increases the compliance of the descending colon to distension, which might have a negative effect on fecal transport. Finally, the observed change in consistency may also be caused by an alosetron-induced decrease in water secretion in the small bowel. More HAPCs might just be needed to transport the high viscosity fecal mass across the highly resistant left colonic region.

The most frequently reported adverse effect during alosetron treatment was constipation. Ten out of 12 subjects who experienced constipation on alosetron were healthy volunteers. This suggests that the positive results of alosetron in non-constipated IBS patients might partly be related to a shift to a normal defecation frequency and consistency.

The reduction in the number of days with urgency that was reported in a large placebo-controlled study is likely to be related to a decreased faecal mass, a decreased rectal compliance, restoring the reservoir function of the colon and rectum, and a reduced rectal sensory score.
Alosetron has been shown to reduce abdominal pain in patients with irritable bowel syndrome, particularly in those with loose or watery stools. The effect of alosetron on visceral perception might be accomplished by an increase in compliance or by directly influencing colonic afferents.

In conclusion, the 5-HT₃ receptor antagonist alosetron appears to marginally increase left colonic periprandial phasic motility. Alosetron also increases the number and propagation distance of HAPCs, which is paradoxically accompanied by a decrease in stool frequency and a firming of stool consistency.
REFERENCES