1 Naloxegol and assessments of opioid-induced bowel dysfunction

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2 Study Synopsis

OBJECTIVES

The objective of this study was to investigate the effect of naloxegol on experimentally induced opioid-induced bowel dysfunction (OIBD) regarding gut motility, gut secretion, anal sphincter function, and subjective assessments of gastrointestinal (GI) symptoms.

METHODOLOGY

The study was designed as a two-armed randomized, double-blinded, placebo-controlled, cross-over trial in 24 healthy male subjects. The subjects participated in two separate 6-day periods, where they were randomized to oxycodone+naloxegol or oxycodone+placebo treatment in the first study period, and vice versa in the other study period. Oxycodone was used to induce experimental OIBD in the subjects.

1. Assessment of transit time, motility and secretion

To assess transit times and motility patterns of the GI tract we applied the Motility Tracking System-2 (MTS-2). The MTS-2 is not yet commercially available, but is used in our laboratory. Furthermore, dynamic imaging of the small and large bowel using magnetic resonance imaging (MR) was applied. The method provides assessment of both morphology and motility with high spatial and temporal resolution without any radiation risk for the patient. In contrast to other methods, both methods (MTS-2 and MR) provide insights into the movements of gut content in a very detailed way and are capable of differentiating between different motility patterns as well as retrograde movements that may occur during opioid treatment. The methods have recently been validated in drug studies and have high reliability. Additionally, to estimate the effect of naloxegol, faecal volume in the colon was also assessed, as this will provide insights into gut secretion. In collaboration with the imaging department at Aalborg University we have developed software capable of quantifying faecal content semi-automatically based on MR scans.

2. Assessment of anal sphincter function

Anal sphincter function is typically altered in OIBD due to the constrictive effect of opioids on smooth muscle. In this study, sphincter function was investigated assessing the recto-anal inhibitory reflex (RAIR). Furthermore, the functional lumen imaging probe (FLIP), a device capable of measuring the cross-sectional area of the anal sphincter, was used.

3. Subjective assessment

For subjective assessments, Bristol Stool Form Scale (BSFS), Gastrointestinal Symptom Rating Scale (GSRS) and Patient Assessment of Constipation Symptoms (PAC-SYM) were used. All have previously been validated and used in our laboratories.

INVESTIGATIONAL PRODUCT, DOSAGE, AND MODE OF ACTION

Oxycodone
Oxycodone is strictly used in this study as a mechanistic tool to experimentally induce OIBD in the subjects. Oxycodone treatment commenced of 10 mg twice on day 1 (daily dose 20 mg). The following days (day 2-4) they received 15 mg oxycodone twice (daily dose 30 mg) to maintain a steady plasma concentration and to induce effects from prolonged opioid treatment (e.g. constipation). On day 6 they were treated with oxycodone 15 mg once (daily dose 15 mg).

Naloxegol
Naloxegol (tradename Moventig) is administered orally as film-coated tablets. The active ingredient of naloxegol is a PEGylated naloxone molecule, making it a peripherally acting μ-opioid receptor antagonist. Subjects were treated with naloxegol 25 mg once a day orally (day 2-6) to maintain a steady plasma concentration and to affect opioid treatment. Placebo tablets were similar to naloxegol tablets only excluding the active ingredient.

CRITERIA FOR INCLUSION

- Signed informed consent.
- Able to read and understand Danish
- Male (to avoid influence of menstrual cycles on pain perception)
- Northern European descent (in order to minimize genetic variance influences on pain perception and drug metabolism)
- The researcher believes that the subject understands the study details, is compliant and is expected to complete the study
- Opioid naive (who have not taken opioid doses for 1 week or longer)
- Between 20 and 60 years of age
- Healthy

ENDPOINTS

Primary endpoint
- Total and segmental transit time (assessed by MTS-2)

Secondary endpoints
- Colonic volume (assessed by MR)
- Gut motility (assessed by MTS-2 and MR)
- Anal sphincter function (assessed by RAIR and FLIP)
- Subjective assessments of GI symptoms (assessed by questionnaires)

STATISTICAL METHODS

Unless otherwise stated, all data are presented as mean (95% confidence interval). All data were assessed for normality and handled accordingly with parametric or non-parametric statistics. *P*-values less than 0.05 were considered statistically significant, and analysis was carried out using Stata (version 14.0, StataCorp LP, Texas, USA).

For transit time data, Wilcoxon signed rank sum test was used to compare total and segmental transit times during the two treatments.

Total and segmental colonic volume data were baseline-corrected and analyzed with a multilevel mixed model with the factors treatment and colonic segment (ascending colon, transverse colon, descending colon, rectosigmoid colon, and total volume).

For the assessment of anal sphincter function, RAIR-data were baseline-corrected and analyzed using a multilevel mixed model with factors treatment and rectal distension volume. FLIP-data were compared using a Wilcoxon signed rank sum test.

Data from GSRS were baseline-corrected and analyzed with Wilcoxon signed rank sum test. Data from PAC-SYM (baseline-corrected), and BSFS questionnaire were analyzed by a two-way repeated measure analysis of variance with the factors treatment and day.

For verification of the analgesic effect of oxycodone, data from muscle pressure and cold pressor test were analyzed using a Student's t-test.

RESULTS

Twenty-four subjects completed the study. Compared to oxycodone+placebo, oxycodone+naloxegol decreased total GI transit time by 20% (*P*=0.02), and colorectal transit time by 25% (*P*=<0.01). No significant difference in colonic volume was found between the two treatments (*P*=0.62). Compared to oxycodone+placebo, there was an increased anal sphincter relaxation in response to rectal distention in the oxycodone+naloxegol arm (*P*=0.03). However, no significant difference between treatments was found in anal canal diameter (*P*=0.83) and pressure (*P*=0.68) after anal canal distention assessed by FLIP. Compared to oxycodone+placebo, fewer self-assessed GI symptoms were observed in oxycodone+naloxegol treatment, assessed by the applied questionnaires; GSRS in the abdominal pain subscale (*P*=0.04) and PAC-SYM (*P*<0.001). Compared to baseline, oxycodone increased pain detection threshold by 15% on day 6 (*P*= <0.001), confirming the analgesic effect of the chosen dose. All reported adverse effects were considered by the investigator to be well-known and non-harmful.

CONCLUSIONS

This study applied an experimental model of OIBD in healthy subjects to study the effect of naloxegol on gut motility, gut secretion, anal sphincter function and subjective GI symptoms. Compared to placebo, naloxegol not only decreased GI transit time, but also increased anal sphincter relaxation. This may be important to understand the effects of naloxegol on OIBD, as the beneficial effects were both on transit time and sphincter

function. This was reflected in the alleviation of various GI symptoms assessed by PAC-SYM and the abdominal pain subscale in GSRS. These results underline that naloxegol has an effect on several subjective and objective parameters important to alleviate OIBD, and that it may be used in the prevention and treatment of these symptoms. More comprehensive analysis on the effect of naloxegol on gut motility including MR of the small intestine will be very time demanding and will later follow this study report.

DATE OF THE REPORT

21.12.2016

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4 List of abbreviations

BSFS	Bristol Stool Form Scale
CSA	Cross sectional area
CRF	Case report form
FLIP	Functional lumen imaging probe
GCP	Good clinical practice
GI	Gastrointestinal
GSRS	Gastrointestinal Symptom Rating Scale
MR	Magnetic resonance imaging
MTS-2	Motility tracking system 2
OIBD	Opioid-induced bowel dysfunction
PAC-SYM	Patient Assessment of Constipation Symptoms
Oxy + nalox	Oxycodone + naloxegol treatment
Oxy + pla	Oxycodone + placebo treatment

5 Ethics

5.1 Independent ethics committee and ethical conduct of the study

This study and any amendments hereto were approved by the independent Ethics Committee "Region of Northern Jutland, Denmark" (reference no. N-20150014).

The study was performed according to the protocol and in compliance with the ethical principles originating from or derived from the Declaration of Helsinki, the International Conference on Harmonisation guidelines for Good Clinical Practice (GCP) and all other applicable regulatory requirements, including any subsequent amendments and in compliance with the Data Protection Act, 1998.

5.2 Subject information and consent

Written consent was obtained prior to the subjects entering the study and prior to initiation of any protocol-specific procedures. The investigator or designee explained the nature, purpose, and risks of the study to each subjects. Each subject was informed that he could withdraw from the study at any time and for any reason and was given sufficient time to consider the implications of the study before deciding whether to participate.

6 Health authority

This study and any amendments hereto were approved by the Danish Medicines Authority (reference no. 2015-000419-42).

7 Investigators and study administrative structure

This study was conducted at the Department of Gastroenterology, Aalborg University Hospital, by nurses and a PhD student. Naloxegol was delivered by Astra Zeneca A/S and the study was monitored by the GCP-unit at Aarhus University Hospital. Principal investigator and sponsor was Professor Asbjørn Mohr Drewes, Aalborg University Hospital, Denmark.

8 Background

Pain is one of the most frequently presented symptoms in patients in the primary as well as the secondary health care sector. In fact, one in five adults in the Western world suffers from chronic pain, which leads to insecurity, decreased quality of life, and comprehensive socioeconomic consequences (1–3). Of the patients presenting with moderate to severe non-malignant pain, opioids are often considered the best chance of achieving adequate pain relief (4). However, treatment with opioids is associated with a range of adverse effects. The adverse effects can be so severe that patients choose to discontinue treatment, which leads to inadequate pain management. Some adverse effects are caused by activation of opioid receptors in the gastrointestinal (GI) tract (5). Activation of opioid receptors in the GI tract will lead to increased tone of the circular muscle layer causing static, non-propulsive contractions. This motility pattern results in segmental contractions and decreased forward-movements. Hence, clinical doses of opioids decrease transit time of the entire GI tract including decreased gastric emptying and decreased peristalsis of the small and large bowel. Furthermore decreased secretion leads to decreased volume within the intestine, thereby further inhibiting the local reflexes within the gut that normally promotes motility. Moreover, opioids affect sphincter functions throughout the GI tract, leading to asynchronous and even retrograde gut motility. Taken together, the patients develop a range of adverse effects collectively known as opioid induced bowel dysfunction (OIBD) (6). The primary adverse effect associated with opioid treatment is OIBD. Approximately 80% of patients in opioid treatment experience constipation and this adverse effect is the most frequent cause of therapy discontinuation. OIBD significantly reduces quality of life (7) and affects normal functioning and work productivity (8), thereby carrying great socioeconomic impact.

The treatment options for OIBD are mainly symptomatic and rely heavily on laxatives although these often display poor efficacy and are therefore, used only by half of the patients. It is believed that the primary mechanism of action of laxatives relies on increasing the osmotic gradient and/or stimulating the colonic musculature. Because OIBD affects the entire GI tract, treatment with laxatives displays relatively poor efficacy. Also, the amount of literature on this subject is rather limited and a recent literature search came up with just three studies investigating the effect of laxatives in patients in opioid treatment and none of these provided insights into how and where these drugs affect the GI tract. New ways of targeting OIBD are being investigated, and have yielded a few novel treatment options in the recent years. One of these drugs is Moventig (where naloxegol is the active ingredient), which is a peripherally restricted opioid antagonist. Opioids provide pain relief whilst naloxegol reduces the adverse effects on the GI tract. The efficacy of naloxegol has been evaluated by means of change in number of spontaneous bowel movements from baseline, time from first dose of study drug to first laxation (9,10) and lactulose hydrogen GI motility test (used as a measurement of oral-cecal transit time) (11) as objective measures of OIBD. However, the physiological effects on the various segments of the GI tract have yet to be fully elucidated.

Due to the complicity of OIBD in patients, confounded by co-morbidities as well as therapeutics, it is difficult to assess the effect of a specific drug in this patient group. Experimental models in healthy can be used to evoke and assess OIBD under controlled circumstances, to encompass these problems. Our group has recently developed an experimental model of OIBD where it was demonstrated that OIBD can be induced in healthy subjects by 5 days of oxycodone treatment (12). Moreover, we have developed a group of comprehensive objective methods for assessment of symptoms of OIBD (13,14).

The objective of this study was to evaluate the effect of naloxegol on experimentally induced OIBD in healthy subjects in terms of gut motility, gut secretion, anal sphincter function and subjective GI symptoms. These

parameters will be assessed using the Motility Tracking System-2 (MTS-2) for GI transit time and detailed motility measurements, magnetic resonance imaging (MR) guided quantification of small bowel motility and deformation, MR assessment of colonic volume, the recto-anal inhibitory reflex (RAIR) and functional lumen imaging probe (FLIP) for assessment of anal sphincter function, and self-assessed questionnaires on GI symptoms.

8.1 Primary endpoint

The primary endpoints were total and segmental transit time (assessed by MTS-2)

8.2 Secondary endpoint

The secondary endpoints were:

- Colonic volume (assessed by MR)
- Gut motility (assessed by MTS-2 and MR)
- Anal sphincter function (assessed by RAIR and FLIP)
- Subjective assessments of GI symptoms (assessed by questionnaires)

9 Study population

Fifty-six subjects responded to the recruiting material and contact was made with these. Of these subjects, 31 were found to be non-eligible, of which 5 failed to respond to feedback, and 26 were excluded by the study personnel for various reasons. In total, 25 subjects fulfilled the inclusion criteria and were enrolled in the study. One subject was non-compliant and was excluded by the study personnel after the first study period, hence, 24 subjects completed both study periods (Figure 1).

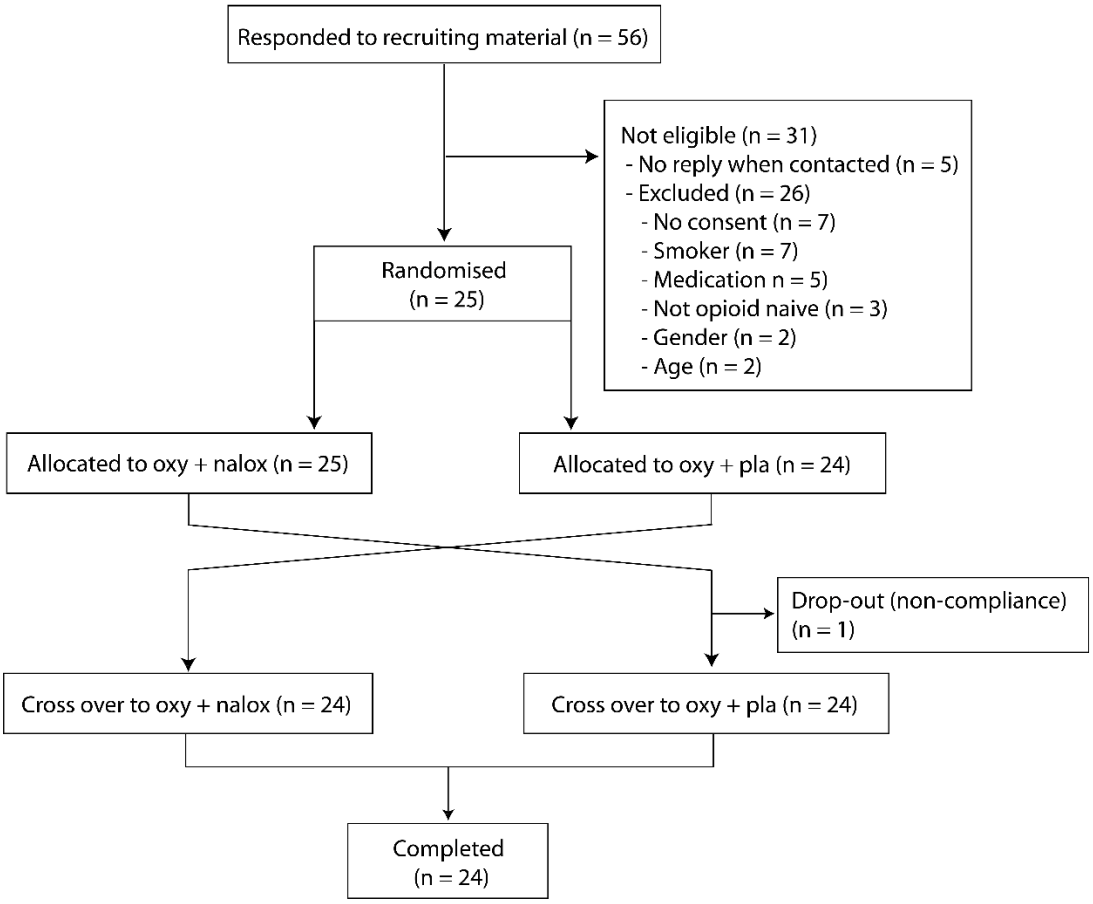

Figure 1 Flowchart for disposition of subjects.

10 Study design

The study was designed as a two-armed randomized, double-blinded, placebo-controlled, cross-over trial in 24 healthy male subjects. First, the subjects took part in an enriched enrollment process and if requirements for inclusion were fulfilled, the subject participated in two separate 6-day periods, where they were randomized to oxycodone+naloxegol or oxycodone+placebo treatment in the first study period, and vice versa in the other study period (Figure 2).

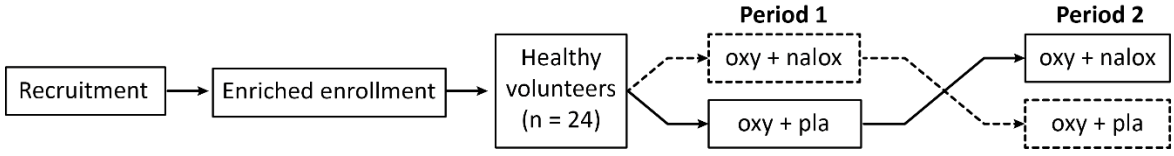

Figure 2 Schematic illustration of the study design. Period 1 and 2 were separated by a minimum 14 days.

10.1 Discussion of study design

The cross-over design was chosen to minimize the well-known heterogeneity in the healthy subjects. The strength of a randomized study is to ensure that the intervention and placebo groups are similar in all respects and double-

blinding ensures that the critical information on allocation of treatment is hidden from the subjects and investigator. Furthermore, the method of blinding is used to ensure that there are no differences in the way in which each group is assessed or managed, and therefore minimize bias.

10.2 In- and exclusion criteria

10.2.1 Inclusion criteria

- Signed informed consent.
- Able to read and understand Danish.
- Male (to avoid influence of menstrual cycles on pain perception)
- Northern European descent (in order to minimize genetic variance influences on pain perception and drug metabolism).
- The researcher believes that the subject understands the study details, is compliant and is expected to complete the study.
- Opioid naive (who have not taken opioid doses for 1 week or longer)
- Between 20 and 60 years of age.
- Healthy.

10.2.2 Exclusion criteria

- Known hypersensitivity or allergy towards the pharmaceutical compounds used in the study or pharmaceutical compounds similar to those used in the study.
- Participation in other studies within 14 days prior to first visit.
- Expected need of medical/surgical treatment during the course of the study.
- Any diagnosed disease, which investigator concludes will affect the trial (including all contraindicated complications: severe chronic obstructive pulmonary disease, pulmonary heart disease, severe bronchial asthma, paralytic ileus, hypercapnia, serious respiratory depression with hypoxia, moderate to severe decreased liver function, gastrointestinal obstruction or perforation, acute surgical abdominal complications such as appendicitis, Mb. Crohn's, ulcerative colitis, and toxic mega colon).
- History of substance abuse.
- Family history of substance abuse.
- Daily alcohol consumption
- Daily nicotine consumption (e.g. cigarette smoking, nicotine patch, etc.).
- Consumption of grapefruit juice or juice from Seville oranges (as there is a contraindication with Moventig and the use of strong CYP3A4 inhibitors present in grapefruit juice or juice from Seville oranges).
- Metal implants or pacemaker.
- Use of prescription medicine and/or herbal medicine.

10.3 Treatments

10.3.1 Identity of investigational product

Oxycontin: The active ingredient is oxycodone, which is a semisynthetic opioid agonist. It is administered as a prolonged-release oral tablet, releasing oxycodone hydrochloride. It exerts agonistic effects on both peripheral and central opioid receptors. Due to its effect on the peripheral opioid receptors, constipation is among the very commonly occurring adverse effects. Therefore, it was used mechanistically to induce OIBD.

Moventig: The active ingredient is naloxegol; a peripherally restricted opioid antagonist.

10.3.2 Treatment dosages

Oxycodone was administered in a dosage regimen in a total of eleven doses during both study periods, and given as an orally ingested tablet with prolonged release. Naloxegol was administered in a dosage regimen in a total of five doses during both study periods and given as an orally ingested tablet with prolonged release. Placebo matched the physical appearance of naloxegol, route of administration and frequency of dosing.

Oxycodone	mg/dose	Doses/day	Daily dose
Day 1	10 mg	2	20 mg
Day 2	15 mg	2	30 mg
Day 3	15 mg	2	30 mg
Day 4	15 mg	2	30 mg
Day 5	15 mg	2	30 mg
Day 6	15 mg	1	15 mg

Naloxegol	mg/dose	Doses/day	Daily dose
Day 1	0 mg	0	0 mg
Day 2	25 mg	1	25 mg
Day 3	25 mg	1	25 mg
Day 4	25 mg	1	25 mg
Day 5	25 mg	1	25 mg
Day 6	25 mg	1	25 mg

10.3.3 Method of randomization

A randomization list was generated by The Hospital Pharmacy Aalborg using approved statistical software as for example from the website www.randomization.com. Healthy subjects and all personnel involved in the study were blinded to the randomization.

10.4 Procedures in the study

10.4.1 Screening

Following informed consent, subjects underwent a screening session. At this visit, demographic details (height, weight, date of birth) were obtained and noted in the CRF. A medical doctor examined the subjects. This examination covered all inclusion and exclusion criteria. Vital signs (blood pressure and heart rate) were also noted in the CRF along with any other relevant information. Hereafter, the experimental procedures were performed in order to familiarize these to the subjects. The subjects were also asked to fill out two questionnaires, Bristol Stool Form Scale (BSFS) and Patient Assessment of Constipation Symptoms (PAC-SYM) at home for a week, prior to the first visit, in order to assess their normal GI function.

10.4.2 Overview of the two study periods

Figure 3 provides a schematic overview of assessment parameters on the different days.

Day 1: On day 1 of each study periods were baseline measurements recorded. MR small bowel and colonography were performed at the Department of Radiology before and after administration of approximately 1.5 L of water (for small bowel distension). When completed, the subjects returned to the Mech-Sense laboratory to complete the rest of the experimental procedures. These included the following: All psychological questionnaires, pain response

to muscle pressure and immersion of hand in ice-cold water (cold pressor test) and the experimental procedures on sphincter function: FLIP and RAIR. The subjects were hereafter treated with oxycodone twice a day.

Day 2: Subjects underwent MR on small bowel and colonography again. Data from this was analyzed shortly hereafter to evaluate if a 10% decrease in motility-index was achieved compared to Day 1. If this 10% decrease was achieved the subject would continue in the study. In the event that the decrease was <10%, the subject would be excluded from the study. Hereafter, the subjects were randomized to placebo or naloxegol. Muscle pressure and cold pressor test were performed. The first dose of naloxegol/placebo was administered. The MTS-2 was mounted and medication for the evening and 4 more days was handed out for self-administration at pre-specified time points. The MTS-2 capsule was handed out to swallow in the afternoon. Immediately hereafter, the subject would consume a standardized meal and thereafter fast for 6 hours.

Day 3: The subjects were at home, two physiological questionnaires were filled out: BSFS and PAC-SYM.
Day 4: The subjects were at home, two physiological questionnaires were filled out: BSFS and PAC-SYM.
Day 5: The subjects were at home, two physiological questionnaires were filled out: BSFS and PAC-SYM.

Day 6: The MTS-2 was removed. MR small bowel and colonography was performed along with the experimental procedures as described for day 1.

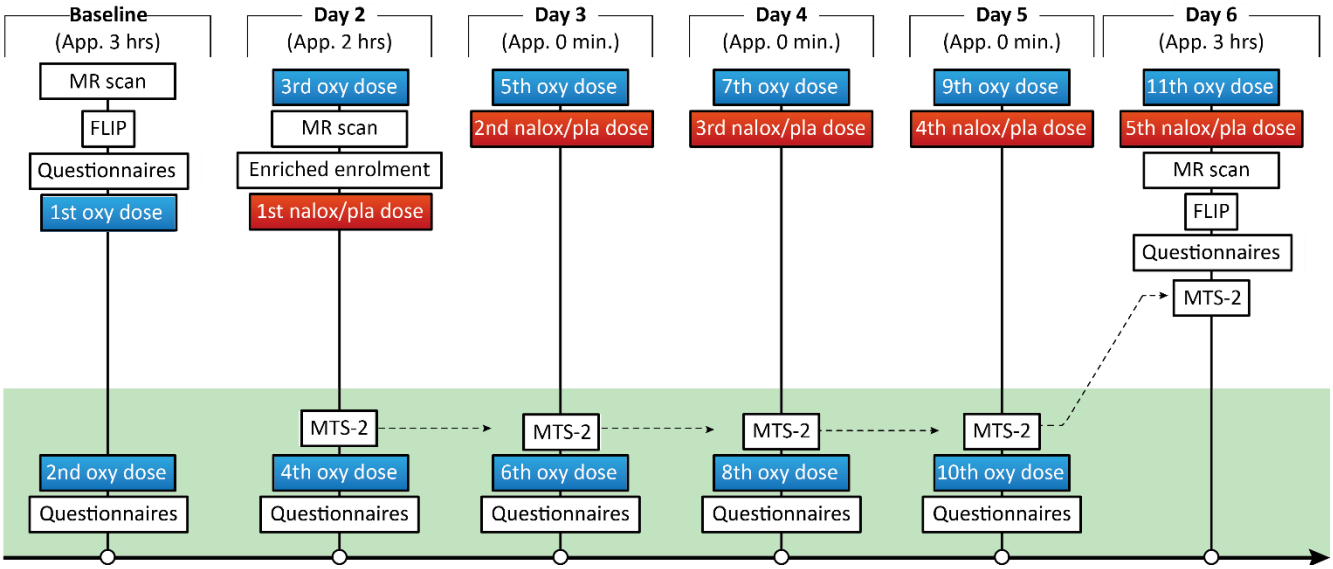

Figure 3 Schematic overview of assessment parameters. MTS-2 is the magnetic capsule for motility assessment and MR scans includes both small bowel motility measures and colonic volume. Additionally, muscle pressure and immersion of the hand in cold water were applied on day 1,2 and 6 (not shown in figure).

11 Overview on methods

11.1 Gastrointestinal transit time

The MTS-2 is a minimally invasive and non-radiant tool, which provides a valid measure of total and segmental GI transit time. The subjects swallowed a capsule which was tracked all the way through the GI tract. Information on transit time and motility patterns were obtained through a portable abdominal belt worn by the subjects (figure 4). Longer transit times indicates more constipation.

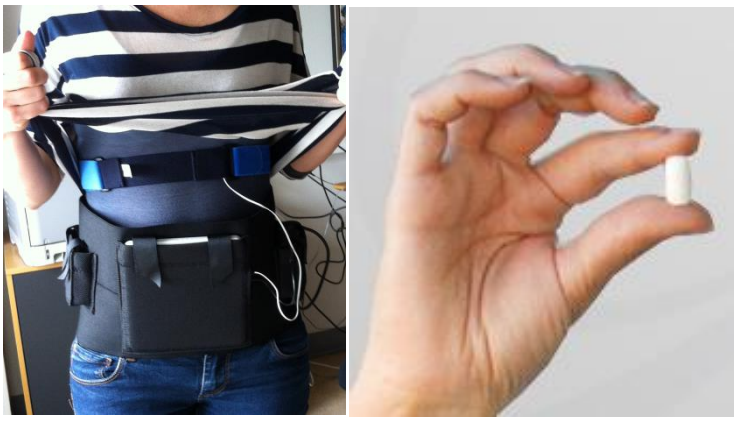

Figure 4 The portable MTS-2 belt and the MTS-2 capsule to swallow.

11.2 Colonic volume

Assessment of colonic volume was performed at the MR unit in the hospital. Quantification of colonic volume was assessed using special developed software (figure 5). MR scans were not allowed when the subjects still retained the capsule on day 6, as this system has not been approved for MR. The outcome of this method was volume of feces (mL) in total and segmental colon.

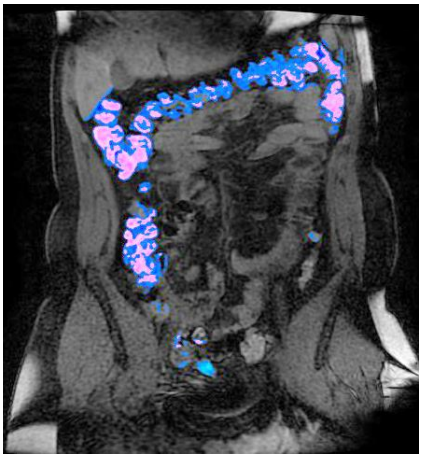

Figure 5 Faecal tagging using MR images of colon.

11.3 Gut motility

For small bowel motility, a method has been developed by Centre for Medical Imaging, Division of Medicine, University College London, to perform a dynamic imaging of the small bowel using MR (15). The method provides assessment of both morphology and motility with high spatial and temporal resolution without any radiation risk (figure 6). Before the MR scan, the subjects drank approximately 1.5 L of water for small bowel distension.

Furthermore, motility patterns of the entire gut were assessed with the MTS-2. The MTS-2 provides information on motility in a very detailed way and is capable of differentiating between different motility patterns as well as retrograde movements (figure 6).

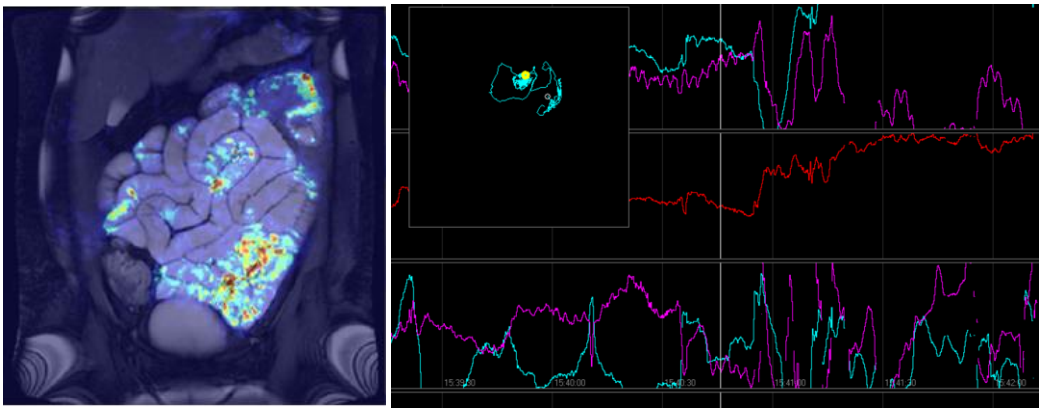

Figure 6 To the left, assessment of small bowel motility using MR images. To the right, a screenshot from a MTS-2 recording. Purple and blue lines indicates rotation and propulsion of the MTS-2 capsule providing insides of gut motility.

11.4 Anal sphincter function

11.4.1 RAIR

To assess RAIR, a balloon and a pressure catheter were placed in the internal anal sphincter. The balloon was filled with air volumes of 10-100 mL (10 mL stepwise increase with a short break in between), to assess anal sphincter relaxation in response to distension (figure 7). A larger drop in internal sphincter pressure equals more sphincter relaxation.

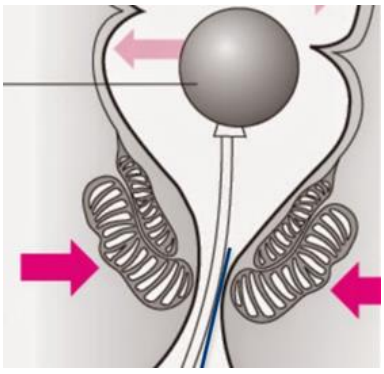

Figure 7 Illustration of the RAIR-method. A balloon is placed in the rectum and is distended to provoke the reflex.

11.4.2 FLIP

The FLIP consists of a probe which was positioned in the anal sphincter. The probe was slowly filled with water up to 50 mL (figure 8). Two measures from FLIP can be used to assess anal sphincter function; the cross-sectional area within the proximal anal canal (CSA), and anal sphincter pressure, at the point where the anal canal starts to give upon distention. A larger CSA and a lesser sphincter pressure equals a lesser constrict sphincter.

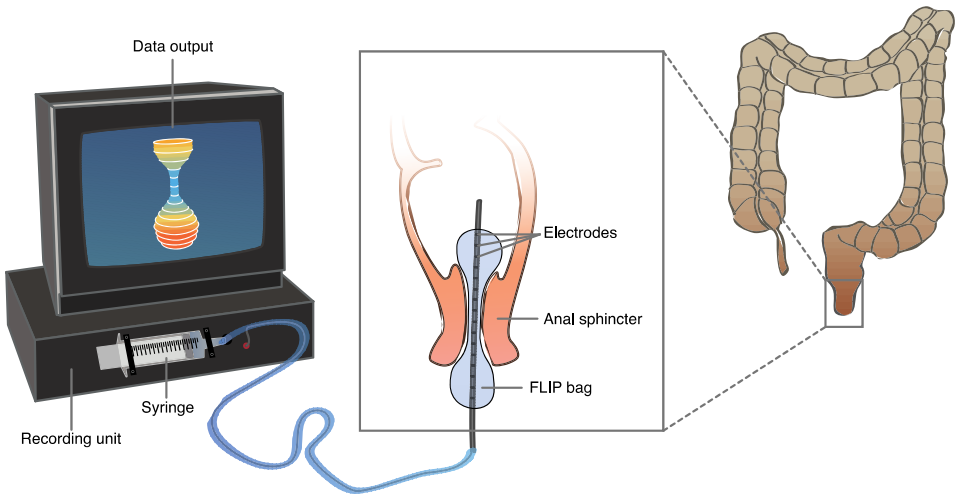

Figure 8 The FLIP system. A balloon is connected to a computer-controlled syringe with water. The probe measures the cross-sectional area of the anal sphincter.

11.5 Questionnaires

11.5.1 GSRS

The Gastrointestinal Symptom Rating Scale (GSRS) is a disease-specific instrument of 15 items combined into five symptom clusters depicting abdominal pain, reflux, indigestion, diarrhoea and constipation. In this study only two clusters, abdominal pain and reflux, were assessed. The GSRS has a seven-point graded Likert-type scale, where 1 represents absence of troublesome symptoms and 7 represents very troublesome symptoms.

11.5.2 PAC-SYM

PAC-SYM is a sensitive and reliable instrument for monitoring the symptoms of OIBD. It contains 12 items assigned to 3 subscales: stool symptoms, rectal symptoms, and abdominal symptoms. Each question is rated from 0-4, 0 represents absence of troublesome symptoms, 4 represents very troublesome symptoms.

11.5.3 BSFS

BSFS is an objective assessment of the most frequently reported OIBD symptom; constipation. It can partly detect parameters such as bowel movement frequency and stool form. Stool form is rated from 1-7, 1 represents very hard stools, 7 represents very soft stools.

11.6 Verification of the analgesic effect of oxycodone

To verify that the chosen oxycodone dose regimen was sufficient to provide an analgesic effect, pressure algometry and the cold pressor test were included as a measure of analgesic effect. Muscle pressure was applied with a handheld pressure algometer (figure 9). Pressure was applied on the dorsal forearm 10 cm distal to the elbow, until the subjects reached their pain detection threshold (kPa). A larger pain detection threshold indicates a larger analgesic effect of oxycodone. For the cold pressor test, subjects immersed their non-dominant hand in cold water (2 °C) for 2 minutes, and continuously rated pain intensity on a VAS scale from 1 to 10, 5 being pain detection threshold (figure 9).

Figure 9 To the left, the pressure algometer. To the right, the icewater bath for the cold pressor test.

12 Overview on results

The terms naloxegol and placebo treatments in this result section is always referring to the two treatments oxycodone+naloxegol and oxycodone+placebo. This is abbreviated to oxy+nalox and oxy+pla in tables.

12.1 Demographic characteristics

The 24 subjects who completed the entire study were all men and Caucasians. Mean age was 25.3 ± 6.2 SD.

12.2 Vital signs

No significant differences between blood pressure and pulse between day 1, 2 and 6 within each treatment period were observed (all $P<0.05$), (Table 1).

Table 1 Blood pressure and pulse assessed at day 1, 2 and 6 during the two treatments. Data are presented as mean ± SD

Systolic (mmHg) (n=24)					
Oxy+nalox baseline	Oxy+pla baseline	Oxy+nalox day 2	Oxy+pla day 2	Oxy+nalox day 6	Oxy+pla day 6
127.5 ± 10.4	125 ± 9.2	124.3 ± 12.1	125.7 ± 13.4	127.7 ± 9.2	124.8 ± 11.2

Diastolic (mmHg) (n=24)					
Oxy+nalox baseline	Oxy+pla baseline	Oxy+nalox day 2	Oxy+pla day 2	Oxy+nalox day 6	Oxy+pla day 6
73.3 ± 8.1	70 ± 10.2	72 ± 6.3	70.3 ± 5.9	73.4 ± 7.7	71.7 ± 7.8

Pulse (beats pr. min) (n=24)					
Oxy+nalox baseline	Oxy+pla baseline	Oxy+nalox day 2	Oxy+pla day 2	Oxy+nalox day 6	Oxy+pla day 6
70.9 ± 12.0	70.2 ± 11.5	65 ± 8.9	68.1 ± 12.7	74.6 ± 15.1	72.8 ± 13.9

12.3 Gastrointestinal transit time

Capsule retention on day 6 occurred in 3/25 during naloxegol treatment and 8/25 recordings during placebo (*P*=0.08). Compared to placebo, naloxegol decreased colorectal transit time by 25% (Z= -2.77, *P*<0.01) and total GI transit time by 20% (Z= -2.4, *P*=0.02), (Table 2, Figure 10), indicating that naloxegol alleviates constipation in OIBD. However, naloxegol treatment did not decrease time to gastric emptying (Z= 1.4, *P*=0.14) or small bowel transit time (Z=0.4, *P*=0.64).

Table 2 Total and segmental transit times. Data are presented as mean (95% confidence interval).

Transit times (hours) (n=24)		
	Oxy+nalox	Oxy+pla
Gastric emptying	5.3 [3.1 – 7.4]	3.4 [3.4 – 4.5
Small bowel transit time	7.4 [6.3 – 8.5]	7.5 [5.4 – 9.6]
Colorectal transit time	45 [35.5 – 53]	59.7 [52 – 68.3]
Total transit time	56.8 [48 – 65.7]	71.3 [63 – 79.5]

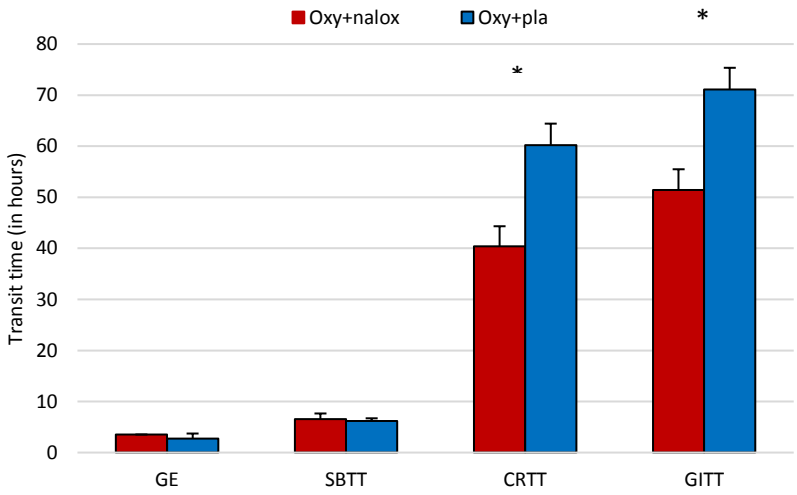

Figure 10 Total and segmental transit times. Data are presented as mean ± SEM. GE; Gastric emptying, SBTT; Small bowel transit time, CRTT; Colorectal transit time, GITT; Total gastrointestinal transit time.

12.4 Colonic volume

As MR scans were not allowed when the subjects still retained the capsule on day 6, 11 scans were missed due to this. In addition, 4 scans were discarded due to bad quality. This left 13 full dataset to be analyzed. No significant difference in colonic volume was found between naloxegol and placebo treatments (F=0.25; *P*=0.62), (Table 3).

Table 3 Colonic volumes. Data are presented as mean (95% confidence interval). Asc.; Ascending colon, Trans.; Transverse colon, Desc.; Descending colon, Sig.; Rectosigmoid colon.

Colonic volumes (mL) (n=13)				
	Oxy+nalox baseline	Oxy+nalox day 6	Oxy+pla baseline	Oxy+pla day 6
Asc.	196 [154 – 238]	234 [185-282]	188 [155 – 221]	231 [203-258]
Trans.	209 [166 – 251]	322 [250 – 394]	226 [166 – 286]	298 [258 – 337]
Desc.	147 [97- 197]	227 [188 – 267]	133 [98 – 168]	208 [167 – 249]
Sig.	185 [132 – 237	200 [137 – 263]	157 [125 – 190]	209 [153-265]
Total	738 [627 – 848]	984 [815 – 1154]	706 [588 – 824]	946 [854 – 1039]

12.5 Anal sphincter function

12.5.1 RAIR

Compared to placebo, naloxegol increased RAIR (measured in anal sphincter relaxation) in response to rectal distension (F=5.5, *P*=0.03), (Table 4, Figure 11). Post-hoc analysis showed a significant difference between treatments at volumes 40, 80, 90 and 100 mL (all *P*<0.05).

Table 4 Anal sphincter relaxation after rectal distension of 40,50,60,70,80,90 and 100 mL. Data are presented as mean (95 % confidence interval).

Recto-anal inhibitory reflex (mmHg) (n=22)				
	Oxy+nalox baseline	Oxy+nalox day 6	Oxy+pla baseline	Oxy+pla day 6
40 mL	-39 [-48 – -29]	-40 [-50 – -31]	-43 [-52 – -33]	-32 [-42 – -23]
50 mL	-41 [50 – -31]	-40 [-47 – -32]	-48 [-59 – -38]	-33 [-41 – -26]
60 mL	-48 [-55 – -40]	-50 [-58 – -40]	-54 [-64 – -44]	-42 [-60 – -33]
70 mL	-50 [-58 – -42]	-46 [-55 – -38]	-56 [-64 – -46]	-48 [-57 – -39]
80 mL	-51 [-60 – -43]	-53 [-70 – -43]	-62 [-73 – -52]	-44 [-52 – -36]
90 mL	-52 [60 – -44]	-58 [68 – -48]	-62 [-73 – -52]	-50 [-59 – -42]
100 mL	-66 [-78 – -53]	-58 [-66 – -48]	-54 [-63 – -45]	-50 [-58 – -42]

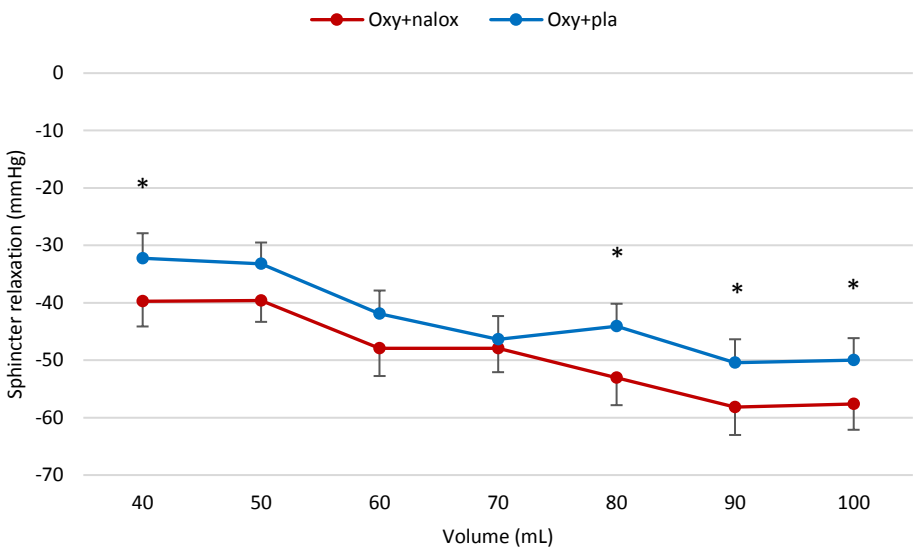

Figure 11 Anal sphincter relaxation after rectal distension at increasing volumes. Data are presented as mean ± SEM.

12.5.2 FLIP

No significant difference in luminal CSA of the proximal anal canal was found between naloxegol and placebo treatments, during anal canal distension (Z=0.21; *P*=0.83), (Table 5). Furthermore, no significant difference in pressure of the proximal anal canal was found (Z= -0.4, *P*=0.68).

Table 5 CSA and pressure data from the functional lumen imaging probe. Data are presented as mean (95% confidence interval)

Functional lumen imaging probe (n=24)				
	Oxy+nalox, baseline	Oxy+nalox Day 6	Oxy+pla baseline	Oxy+pla Day 6
CSA (mm)	7.2 [6.9 – 7.5]	7.4 [6.9 – 7.8]	7 [6.7 – 7.3]	7.5 [7.2 – 7.8]
Pressure (mmHg)	24.2 [21.4 – 27]	24.8 [21 – 28.5]	26.7 [22.7 – 30.6]	26.1 [22.2 – 30]

12.6 Questionnaires

12.6.1 GSRS

For the GSRS questionnaire, two clusters were assessed; abdominal pain and reflux symptoms. Compared to placebo, naloxegol decreased abdominal pain symptoms (Z= -2.0, *P*=0.04), (Table 6, figure 12). However, naloxegol treatment did not decrease reflux symptoms (Z= 0.22, *P*=0.82).

Table 6 GSRS scores. Data are presented as mean (95% confidence interval).

GSRS (score) (n=22)				
	Oxy+nalox, baseline	Oxy+nalox Day 6	Oxy+pla baseline	Oxy+pla Day 6
Abdominal pain	1.0 [0.4 – 1.6]	2.0 [0.8 – 3.2]	0.8 [0.3 – 1.4]	2.8 [1.6 – 3.9]
Reflux symptoms	0.4 [0.1 – 0.7]	0.7 [0.2 – 1.2]	0.3 [-0.1 – 0.7]	0.5 [0.05 – 1.0]

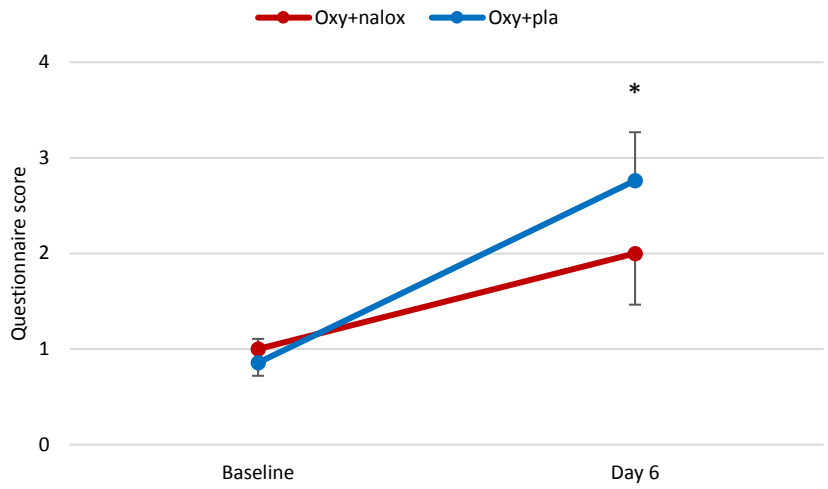

Figure 12 GSRS (abdominal pain) scores from baseline to day 6 in each treatment . Data are presented as mean ± SEM.

12.6.2 PAC-SYM

Compared to placebo, naloxegol decreased PAC-SYM scores (F=18.08; *P*<0.001), (Table 7, figure 13), indicating less GI symptoms following this treatment. Post-hoc analysis showed a significant difference between treatments on days 5 (*P*<0.001) and 6 (*P*<0.001).

Table 7 PAC-SYM scores. Data are presented as mean (95% confidence interval).

PAC-SYM (score) (n=24)		
	Oxy+nalox	Oxy+pla
Baseline	0.9 [04. – 1.6]	0.6 [-0.1 - 1.4]
Day 2	2.5 [1 - 4]	1.7 [0.7 – 2.6]
Day 3	2.8 [1.2 – 4.4]	4.4 [2.6 – 6.1]
Day 4	3.8 [1 .7- 5.8]	6.2 [3.2 – 9.2]
Day 5	3.4 [1.7 – 5.1]	8.3 [5.8 – 10.6]
Day 6	2.1 [1.2 – 3.]	6.6 [4 – 9.2]

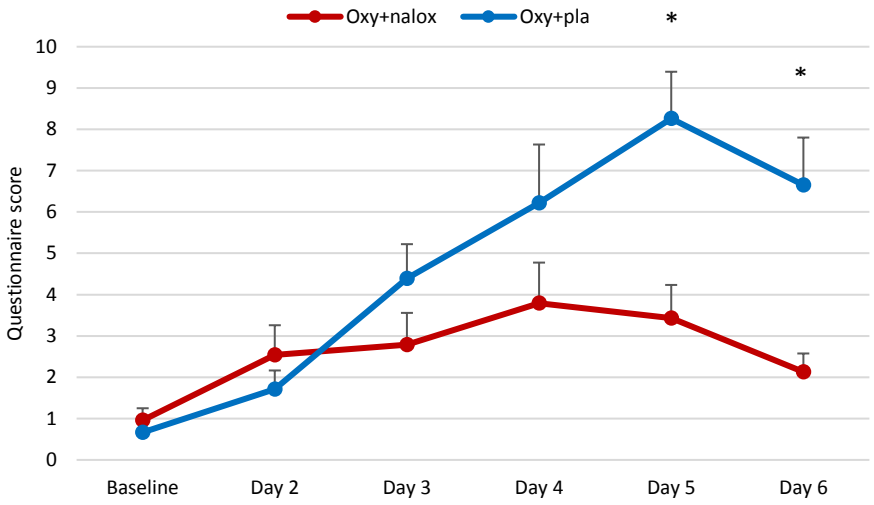

Figure 13 PAC-SYM scores for all 6 days of each treatment. Data are presented as mean ± SEM

12.6.3 BSFS

No significant difference in bowel movement frequency was found between naloxegol and placebo treatments (F=1.32, *P*=0.29), (Table 8). Furthermore, no significant difference was seen for stool form (Z=0.63, *P*=0.52), (Table 8). Despite insignificant results, compared to placebo, a tendency for an increase in bowel movements frequency and softer stools during naloxegol treatment was observed.

Table 8 BSFS scores of bowel movement frequency and stool form. Data are presented as mean (95% confidence interval).

BSFS (score)				
	Bowel movement frequency (n=24)		Stool form	
	Oxy+nalox	Oxy+pla	Oxy+nalox	Oxy+pla
Baseline	0.3 [0.1 – 0.5]	0.4 [0.2 – 0.6]	(n=7) 4.2 [3.3 – 5.3]	(n=9) 3.9 [3 – 4.8]
Day 2	0.5 [0.2 – 0.7]	0.4 [0.1 – 0.7]	(n=10) 2.6 [1.5 – 3.6]	(n=6) 3.3 [1.2 – 5.5]
Day 3	0.7 [0.3 – 1.1]	0.5 [0.2 – 0.9]	(n=10) 4.1 [3.1 – 5]	(n=8) 2.5 [1.4 3.6]
Day 4	0.7 [0.3 – 1.0]	0.6 [0.3 – 0.8]	(n=11) 3.5 [2.7 – 4.4]	(n=11) 2.3 [1.6 – 3]
Day 5	0.9 [0.6 – 1.2]	0.6 [0.3 – 0.8]	(n=16) 3.4 [2.9 – 4]	(n=12) 2.6 [1.9 – 3.4]

12.7 Verification of the analgesic effect of oxycodone

The analgesic effect of oxycodone was validated in the OIBD model with a 15% increase in pain detection threshold to muscle pressure (*P*=<0.001). However, the analgesic effect of oxycodone could not be validated with the cold pressor test, as no significant difference in pain scores between baseline and day 6 was found in this test (*P*=0.13).

12.8 Adverse effects

During naloxegol treatment, 24/25 had an adverse effect of any kind, and 23/25 experienced an adverse effect during placebo treatment (Table 9). The most common reported adverse effects during the two study periods were tiredness, constipation, nausea and dizziness. There was no significant difference between treatments in the number of subjects reporting adverse effects (*P*=0.55). All adverse effects were considered by the investigator to be well-known and non-harmful.

Table 9 Number of subjects with adverse effects during the two study periods.

Adverse effects (n)		
	Oxy+nalox	Oxy+pla
Any adverse effect	24	23
Nausea	10	15
Constipation	13	14
Vomiting	3	4
Dizziness	9	14
Headache	10	10
Tiredness	16	17
Stomach pain	3	2
Diarrhea	0	0
Dry mouth	10	9
Skin itch	11	12

13 Discussion of results and conclusions

In this study, an experimental model of OIBD was established by treating healthy subjects with oxycodone for 6 days. During this, subjects also received either naloxegol or placebo, to investigate the effect of naloxegol on OIBD. The primary endpoint was GI transit time. Oxycodone+naloxegol decreased total GI transit time by 20% and colorectal transit time by 25%, compared to oxycodone+placebo. This indicates that naloxegol alleviates the dampening effect of opioids on propulsive gut motility. The finding is consistent with results from an earlier study, showing that naloxegol decreased orocecal transit time assessed by a hydrogen breath test (16). MR colonography was used as a surrogate measure of gut secretion. This parameter failed to show any significant difference in colonic volume between oxycodone+naloxegol and oxycodone+placebo treatments. However, the low number of observations, due to a large number of capsule retention on day 6, is a clear limitation. Furthermore, oxycodone+naloxegol did not increase number of spontaneous bowel movements, compared to oxycodone+placebo, although this has been shown in earlier studies (17,18). However, these studies investigated the effect of naloxegol in patients with pain, and for a much longer period of time. The effect of naloxegol on anal sphincter function was assessed using FLIP and a rectal distention method to evaluate RAIR. FLIP failed to show a significant difference between oxycodone+naloxegol and oxycodone+placebo. However, compared to

oxycodone+placebo, oxycodone+naloxegol increased anal sphincter relaxation to rectal volume distention. This result indicate that naloxegol is able to alleviate the constrictive opioid effects on the anal sphincter, which may be of clinical importance for patients with OIBD. Moreover, oxycodone+naloxegol decreased subjective GI symptoms in various questionnaires, compared to oxycodone+placebo. No serious adverse effects of the study medication occurred during the study.

Up until now, the efficacy of naloxegol has merely been based on studies investigating transit time and change in number of spontaneous bowel movements. This study finds a beneficial effect of naloxegol on both transit time and anal sphincter function. This was reflected in the alleviation of various GI subjective GI symptoms. These results underline that naloxegol has an effect on several subjective and objective parameters important to relieve OIBD, and that it may be used in the prevention and treatment of these symptoms.

More comprehensive analysis on the effect of naloxegol on gut motility including MR of the small intestine will be very time demanding and will later follow this study report.

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