TITLE PAGE

The combination of oligo- and poly-saccharides and reticulated protein for the control of symptoms in patients with irritable bowel syndrome: results of a randomized, placebo-controlled, double-blind, parallel group, multicentre clinical trial

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Keywords

Irritable bowel syndrome, reticulated protein, vegetable oligo-saccharides, vegetable poly-saccharides, stools, efficacy, mucosal protectors, abdominal pain, flatulence, quality of life.

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Abstract

Background A medical device containing the film-forming agent reticulated protein and a prebiotic mixture of vegetable oligo- and poly-sachharides has been developed, being recently received European approval as MED class III for the treatment of chronic/functional or recidivant diarrhea due to different causes including irritable bowel syndrome (IBS). In the present paper, we evaluated protein preparation containing these components in comparison with placebo in adult patients with diarrhea-predominant IBS.

Methods In a randomized, placebo-controlled, double-blind, parallel group, multicentre, clinical trial, patients were randomly assigned to receive the combination of oligo- and poly-saccharides and reticulated protein and placebo (4 oral tablets/day during 56 days). Demographic, clinical and quality of life characteristics and presence and intensity of abdominal pain and flatulence (7-point Likert scale) were assessed at 3 study visits (baseline, at 28 and 56 days). Stools emissions were recorded in the diary card using the 7-point Bristol Stool Scale.

Results A total of 128 patients were randomized to receive either tablets containing the combination (n=63) or placebo (n=65). Treatment with oligo- and poly-saccharides and reticulated protein was safe and well tolerated. A significant improvement in symptoms across the study was observed in patients treated with oligo- and poly-saccharides and reticulated protein between visit 2 and visit 3 in abdominal pain (p=0.0167) and

flatulence (p=0.0373). We also detected a statistically significant increase in the quality

of life of patients receiving the active treatment from baseline to visit 3 (p<0.0001).

Conclusions Treatment with oligo- and poly-saccharides and reticulated protein is safe,

improving IBS symptoms and quality of life of patients with diarrhea-predominant IBS.

Introduction

Irritable bowel syndrome (IBS) is the most frequently diagnosed functional gastrointestinal disorder in primary and secondary care (up to 50% of all office visits to gastroenterology clinics).[1-4] It is characterized by abdominal discomfort, pain and changes in bowel habits, in the absence of an organic cause.[1,2] Discomfort or abdominal pain relieved by defecation, associated with changes in stool form, is considered typical clinical manifestations in IBS.[3,5-9] IBS is a problematic disorder resulting in impaired quality of life and high healthcare costs and high resources´ utilization,[1-3,10] remaining a clinical challenge in the 21st century.[3]

The pathogenesis of IBS, not yet completely understood, is complex and multifactorial and includes physiological, emotional, cognitive and behavioural pathways (biopsychosocial model), a number of which implicate a role for the gastrointestinal microbiota.[1-3,11] Due to the heterogeneity of IBS, there is no standard or definitive treatment and the chronic use of drugs should be minimized as much as possible or even avoided.[3] Symptoms can be controlled by non-pharmacologic management during variable time periods and eliminating some exacerbating factors such as certain drugs, stressor conditions or changes in dietary habits.[3] In fact, the development of new effective treatments to control the symptoms represents a huge challenge currently.[4,12]

In this regard, since non-pharmacological options including film-forming agents and prebiotics could have a role in IBS, a medical device containing the film-forming agent reticulated protein and a prebiotic mixture of vegetable oligo- and poly-saccharides has been developed, being recently received European approval as MED class III for the treatment of chronic/functional or recidivant diarrhea due to different causes, including IBS.

This combination represents a new non-pharmacological option for the treatment of IBS based on the combination of a mucosal protector, with film-forming properties to control diarrhea,[13,14] and the prebiotic mixture of vegetable oligo- and poly-saccharides, which could have a role in the microbiota's composition, especially increase in bifidobacteria, that can be regarded as a marker of intestinal health.[15,16] The present randomized, placebo-controlled, double-blind, parallel group, multicentre, clinical trial was performed to evaluate the safety and efficacy of tablets containing oligo- and poly-saccharides and reticulated protein, in comparison with placebo in adult patients with diarrhea-predominant IBS.

Methods

The study protocol was approved by the Ethics Committee of Human Experimentation in Romania and procedures were in accordance with the ethical standards laid down in the Declaration of Helsinki, as revised in the year 2000. Written informed consent was

obtained from all subjects. Patients were recruited in different Romanian private offices of general practitioners (with the participation of 15 centres from Bucharest, Galati, Craiova and Gluj) in the context of their routine clinical practice.

Study population and study design

This randomized, placebo-controlled, double-blind, parallel group, multicentre, clinical trial was performed to evaluate the safety and efficacy of the active treatment (tablets containing a mixture of vegetable oligo- and poly-saccharides -750 mg-, reticulated protein -250 mg-, and the excipients corscarmellose sodium -133 mg- and magnesium stearate -17 mg-), in comparison with placebo (tablets containing corn starch, corscarmellose sodium and magnesium stearate) in adult patients with diarrhea-predominant IBS, with good general health status (normal physical and analytical conditions). Potential participants were excluded if they had organic gastrointestinal diseases.

The mixture of oligo- and poly-saccharides is manufactured by Beneo oralfi, GmbH (Germany) by the extraction from vegetable roots (chicory). Reticulated protein is manufactured by Laboratorios Argenol, SA (Spain) by the mixture of tannins and protein (gelatin)." The dose used in this clinical study is based on the results of previous preclinical studies performed by the company.

Diarrhea-predominant IBS was diagnosed following Rome III criteria,[2,17] according which recurrent abdominal pain or discomfort had to be present at least 3 days/month in the last 3 months, associated with two or more points: improvement with defecation, onset associated with a change in frequency of stools, onset associated with a change in form (appearance) of stool. In diarrhea-predominant IBS, the stool pattern includes loose (mushy) or watery stools (Bristol scale 6–7)[18,19] ≥25% and hard or lumpy stool <25% in the absence of antidiarrheal or laxative use.[2,17,20]

Subjects allocation to treatment group was determined by a computer generated randomization list, which was stratified by center. Double blinding procedures were applied during the whole period of the study.

Patients were randomly assigned to receive the combination (Novintethical Pharma, SA) and placebo at a ratio of 1:1 in the form of oral tablets at a posology of 4 tablets/day (2 before breakfast and 2 before dinner) during a period of 8 weeks (56 days).

The active and placebo tablets were manufactured by direct compression, following the processes of dispensing, mixing and packaging.

After intake of tablets, the active substances of the product, a mixture of vegetable oligo- and poly-saccharides and reticulated protein, are no absorbed at the gastrointestinal tract. Their effect is local on the intestinal mucosa.

Study chronogram and workflow are shown in Figure 1a and Figure 1b, respectively. During the first enrolment visit, patients from the 2 groups were given a 56-day treatment (with the first dose being administered at the time of recruitment) and were instructed to fulfill a patient´s diary card to daily register the time of treatment intake, the severity and frequency of adverse events (AEs), the number and type of stool emissions and the use of rescue medication for IBS symptoms. At this visit, demographic and clinical characteristics were also recorded.

During visit 1 and visit 3 (day 56), patients were interviewed using the IBS QoL questionnaire[21] and physical examination and blood sampling for haematological and biochemical analysis were performed (Figure 1a).

During visits 1, 2 (day 28) and 3, patients measured the presence and intensity of abdominal pain and flatulence in a 7-point Likert scale (7=very much better, 6=much better, 5=somewhat better, 4=same, 3=somewhat worse, 2=much worse, 1=very much worse) (Figure 1a).

Stools emissions (including number of emissions/day) were recorded in the diary card and consistency of each stool was assessed using the 7-point Bristol Stool Scale.[18,19]

At visit 3, patients returned the fulfilled diary card and the remaining medication and data were transferred to the case report form. Adherence of treatment was assessed by

The IBS QoL questionnaire is a 34-item measure constructed specifically to assess the subjective well-being of patients with IBS, including eight dimensions (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual dysfunction, and relationships). Each item is scored on a five-point scale (1=not at all, 5=a great deal). The summed total score is transformed to a zero to 100 scale ranging from zero (maximum quality of life) to 100 (minimum).[21] In the version we used, higher scores on the IBS-QoL indicated poorer quality of life. This version has been used to assess quality of life of IBS patients in other published studies.[22]

Primary variables

The primary safety variable was the prevalence of AEs in the both groups of patients. The frequency, intensity and relationship with the studied product were recorded during the 3 study visits, together with significant changes in vital signs and analytical parameters.

The primary efficacy variable was the rate of clinical remission at 4 and 8 weeks, defined as the disappearance of diarrhea, i.e. 2 or less nonwatery stools emissions per day (stool of type 5 or less on the Bristol scale).[18,19]

Statistical analysis

Sample size (n=130, n=65 in each group) was calculated to have a 80% of power to detect, with 95% probability, a non-inferiority margin difference between the group proportions of 0.1100.

The comparison between the active and the placebo group on the clinical remission was performed using Fisher's Exact test and by constructing a 95% Confidence Interval around the difference between the clinical remission rates showed by the active group and placebo group.

Non-parametric analyses were carried out on the abdominal pain and flatulence parameters to test the presence of any significant difference between the improvement rates of treatment group vs placebo group. An independent sample t-test was used to compare the changes from baseline on the IBS QoL summed scores of the two groups.

The difference between the frequency of AEs occurrence was assessed using Fisher´s Exact test and by constructing a 95% confidence intervals around the difference between the mean frequency of AEs occurrence in both groups.

In all cases, $p < 0.05$ were considered significant. According to CPMP/ICH/363/96 and CPMP/EWP/482/99 guidelines, both two-sided (at 95% significance level) and one-sided tests (at 97.5% significance level) were performed. Statistical analyses were performed using IBM SPSS 19 for Windows.

Results

A total of 130 Caucasian patients were recruited (n=65 in each group) and 128 patients were randomized to receive active treatment (n=63) and placebo (n=65), being 2 patients excluded.

Demographic characteristics were homogeneous in both groups (Table 1), with more women than men in the whole sample (69.35% vs 30.65%) and in both groups and with a mean age of 48.29±13.95 years in the active group and 47.72±14.21 years in the placebo group (Table 1).

Parameters recorded at baseline, including vital signs and analytical analyses, were comparable in both groups (Table 1). Similar profiles of concomitant and previous diseases were recorded in both groups, with similar percentages of patients having previous and underlying diseases (72.13% in the active group vs 76.56% in placebo) (Table 1).

Concomitant medications taken during the study period were similar in both groups (Table 2).

Results of adherence showed that all 128 patients included in the analysis were 100% complaint with the study treatment.

Regarding the primary study variable, i.e, safety assessment, in the whole sample, a total of 15 and 9 AEs were reported at visits 2 and 3, respectively. At visit 2, 9 AEs (14.75% of patients) were reported in the active group and 6 (10.00%) in the placebo

group, without statistically significant differences in the AE's prevalence between both groups (p=0.1533). At visit 3, the occurrence of AEs decreased, with 4 events (6.67%) in the active group and 5 (8.47%) in the placebo group, with statistically significant differences between both groups (p=0.0361).

In the both groups, main reported AEs included gastrointestinal events as abdominal and stomach ache, difficulties to evacuate and bloating. In the active group headache was also reported, while, in the placebo group, insomnia, agitation and itching were also observed. All of them were of mild or moderate intensity and no serious AEs were reported during the whole study period. Only gastrointestinal events were considered as possibly related to the study treatment.

Active treatment did not produce relevant changes in clinical signs, without statistically significant differences between baseline and visit 3 values.

Likewise, no statistically significant changes were observed in the analytical parameters after active treatment.

Regarding the primary efficacy variable, i.e., the rate of clinical remission at 4 and 8 weeks, defined as the disappearance of diarrhea, i.e. 2 or less nonwatery stools emissions per day (stool of type 5 or less on the Bristol scale), we observed a statistically significant increase in the rate of clinical remissions across the study in the active group (also higher than in the placebo group) (66.66% vs 46.15% at visit 2 and

76.19% vs 47.69% at visit 3, respectively) (p<0.0001 among visits, Kruskall Wallis test) (Figure 2).

In patients treated with oligo- and poly-saccharides and reticulated protein, the mean (± SD) number of stools emitted at days 1, 28 and 56 was 2.91 (± 1.63), 2.43 (± 1.41) and 2.25 (± 1.47), respectively. Patients treated with oligo- and poly-saccharides and reticulated protein during 28 or 56 days reduced the number of stools emissions compared to patients treated with placebo during the same period of time, in a statistically significant manner (Student-t test; p=0.031 at day 28 and p=0.001 at day 56). The mean reduction of stool emissions was 0.53 (CI95%: 0.05 to 1.02 stool emissions) at day 28 and 0.84 (CI95%: 0.34 to 1.33 stool emissions) at day 56. A higher reduction of stool emissions was observed at longer periods of treatment with oligo- and poly-saccharides and reticulated protein: 28 days of treatment reduced the stool emissions in a mean average of 0.48 (CI95%: 0.17 to 0.78 stool emissions) compared to day 1 (p=0.02; paired-t test), whereas 56 days of treatment reduced the stool emissions a mean average of 0.656 (CI95%: 0.29 to1.02 stool emissions) compared to day 1 (p=0.01).

In the whole sample, we reported an improvement in the intensity of abdominal pain and flatulence measured in a 7-point Likert scale, with significant increases in the percentages of patients feeling very much better from visit 2 to visit 3 (8.80% vs 26.23% for abdominal pain and 8.94% vs 26.23% for flatulence). In the active group,

we also detected a significant improvement in symptoms across the study, with statistically significant differences between visit 2 (day 28) and visit 3 (day 56) in the abdominal pain (p=0.0167, visit 2 vs visit 3, Mann Whitney test) and flatulence assessment (p=0.0373, visit 2 vs visit 3) (Figures 3a and 3b).

At the end of the study, the percentage of patients with abdominal pain was significantly lower in the active group than in the placebo group (0.6% and 58.4% of patients, respectively) (p<0.05). The mean average of abdominal pain score was 1.79 (CI95%: 1.38 to 2.12 score), being this score higher in patients treated with reticulated protein and oligo- and poly-saccharides, with a mean (± SD) score of 4.92 (± 0.86), than in patients treated with placebo, with a mean (± SD) score of 3.13 (±1.36) (Figure 3a). The mean difference was statistically significant (p<0.0001).

Regarding flatulence, treatment with oligo- and poly-saccharides and reticulated protein produced a mean reduction of 0.43 (CI95%: 0.13 to 0.728) in the score reported by the patients at the end of the study, being this reduction was statistically significant (paired-t test, p=0.005). At the end of the study, the mean average of flatulence score was a 1.98 (CI95%: 1.58 to 2.38 score), significantly higher in patients treated with oligo- and poly-saccharides and reticulated protein than in patients treated with placebo (p<0.0001) (figure 3b). At the end of the study, the percentage of patients with flatulence was significantly lower than the percentage in the active group than in

patients treated with placebo (0.8% and 63.1% of patients, respectively) (Fisher's Exact test, p<0.05).

Regarding the punctuation in the IBS QoL questionnaire, we detected a statistically significant increase in the quality of life of patients receiving oligo- and poly-saccharides and reticulated protein from baseline to visit 3 (99.59±23.17 at baseline to 69.22±24.79 at visit 3, p<0.0001, Mann Whitney test) (Figure 4), while a lower improvement in quality of life was detected in the placebo group (94.95±25.62 at baseline to 76.94±27.12 at visit 3, p<0.0001, unpaired t test) (Figure 4). In the comparison of scores obtained between groups, we detected statistically a significant higher improvement in the active group than in the placebo group (p=0.0053, Mann-Whitney U test).

Finally, treatment compliance was high in the whole sample (100% at visit 2 and 99.16% at visit 3).

Discussion

Nowadays, in a context in which IBS is described as multifactorial with a strong psychosocial component, in which many different drugs have been proposed but with debatable real benefits,[1,12] the use of non-pharmacological strategies, as prebiotics and/or film-forming agents is receiving increasing attention.[4,16,23,24,25,26,27]

In our study, for the first time, we have demonstrated that the combination of a mixture of oligo- and poly-saccharides and a reticulated protein is a safe and feasible treatment to reduce main symptoms and to improve quality of life in patients with IBS. Both ingredients are not absorbed, being effect local on the intestinal mucosa. We consider that, since IBS is a multifactorial disease, in which dietary habits and gastrointestinal microbiota has a role, we consider that the favorable effects observed are due to the synergistic action of both components, acting on the intestinal mucosa (reticulated protein) and increasing bifidobacteria counts (mixture of oligo- and poly-saccharides). These two effects have been synergistically produced the observed decrease of IBS symptoms, such as diarrhea, abdominal pain and flatulence, and the increase of quality of life.

These results support the use of the combination versus the use of the components alone (reticulated protein and oligo- and poly-saccharides), as reported in previous studies where no clear benefits were observed with these or similar compounds.[15,28,29]

In a multicenter, randomized, double-blind, placebo-controlled parallel group study consisting of a 2-week, in 96 patients with IBS, although symptoms worsened in patients with IBS at the onset of treatment with 20 g fructo-oligosaccharides/day, continuous treatment for 12 weeks resulted in no worsening of symptoms.[29]

Better results were obtained in another comparative, randomized, double-blind study, in which participants with IBS symptoms were randomized to receive either 5 g of short-chain fructo-oligosaccharides or placebo daily in divided doses. Following six weeks of supplementation a statistically significant decrease in symptom scores was observed in the group taking fructo-oligosaccharides compared to placebo.[15,28]

In our study, the use of a combination of a prebiotic and a film-forming agent can provide synergistic effects to regulate the two main conditions of IBS, visceral hypersensitivity (which leads to abdominal discomfort or pain) and gastrointestinal motor disturbances (leading to diarrhea or constipation),[17] by influencing the gut microbiota balance (mixture) and the micro-inflammation state of the intestine (protein) present in patients with IBS.

These inulin-type prebiotics are oligo- or polysaccharide long chains comprised primarily of linked fructose molecules that are considered to be bifidogenic (stimulating the growth of Bifidobacteria and Lactobacilli species), thus promoting specific changes in the composition of the gastrointestinal microbiota.[15,30-32]

Since the role of the gastrointestinal microbiota in pathogenesis of IBS, and in particular the relative lower numbers of bifidobacteria in diarrhoea-predominant IBS, has been demonstrated,[2,4] the results of our study support the use of the mixture in IBS at the studied low doses (1.2 g/day). Our results are in line with other studies,[2,33] indicating that the dose of the prebiotic is important in determining the clinical benefit

in IBS, with some evidence that higher doses may have a negative impact on symptoms.[2]

Our results support the previous knowledge according which a less diverse and unstable community of bacteria is present in IBS and that manipulation of microbiota can influence the key symptoms, including abdominal pain and bowel habit and other prominent features of IBS.[4]

In addition, the favourable results obtained in our study should also be attributed to the effect of the reticulated protein, belonging to a new class of agents, which may be defined as "film-forming agents" or "mucosal protectors" (also including gelatin tannate, gelatin or xyloglucan), which are able to form a protective mucoadhesive film in the intestine, reducing inflammation of the wall[13,23] (Bueno et al, in preparation). In previous *in vitro* and *in vivo* studies, we have demonstrated that this type of proteins, linked to tannins´ molecules, are able to prevent gut leakiness and subsequent inflammation by creating a consistent biobarrier in the intestine[13,14] (Bueno et al, in preparation).

We consider that the reticulated protein, acting as intestinal film-forming and protective agent, can also contribute to the reduction of characteristic symptoms in patients with diarrhea-predominant IBS, thus leading to an improvement of quality of life during the treatment.

Since in diarrhea-predominant IBS, altered intestinal barrier is present and it is associated with immune activation and clinical symptoms,[34] the use of mucosal protectors constitutes new alternatives for a more efficient control of symptoms in diarrhea-predominant IBS.

Of note, for all efficacy variables, we obtained better outcomes at the end of the treatment, after 56 days of treatment, than at visit 2, after 28 days. We consider that these results support the long term use of the product to produce those intestinal changes necessary to decrease IBS symptoms and to increase quality of life of patients.

Overall, the combination of oligo- and poly-saccharides and reticulated protein is a safe and feasible option, able to reduce IBS symptoms and increase quality of life in patients, thus supporting the inclusion of oligo- and poly-saccharides and reticulated protein for the management of IBS in the current clinical practice.

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Tables

Table 1. Baseline characteristics, anthropometric data, analytical parameters and concomitant and previous diseases.

	Statistic variable	Active Group	Placebo Group
Gender (F / M)	n (%)	43 (68.25) / 20 (31.75)	45 (69.23) / 20 (30.77)
Age (years)	Mean (SD)	48.29 (13.95)	47.72 (14.21)
Weight (kg)	Mean (SD)	69.91 (13.48)	72.27(13.70)
Height (cm)	Mean (SD)	166.00 (7.34)	167 (8.92)
Temperature (ºC)	Mean (SD)	36.50 (0.41)	36.49(0.30)
Systolic blood pressure (mmHg)	Mean (SD)	124.1 (15.16)	124.9 (15.99)
Diastolic blood pressure (mmHg)	Mean (SD)	76.77 (9.85)	76.58 (10.51)
Heart rate (beats/min)	Mean (SD)	71.6 (7.68)	71.8 (8.61)
Analytical parameters	Mean (SD)		
Haemoglobin (g/dl)		13.68 (1.31)	13.80 (1.42)

Glucose (mg/dl)	96.66 (26.93)	95.33 (17.07)
Creatinine (mg/dl)	0.87 (0.18)	0.86 (0.22)
AST (U/l)	24.48 (10)	22.40 (7.71)
ALT (U/l)	28.26 (13.89)	25.75 (14.01)
Gamma-GT (U/l)	32.66 (25.46)	32.20 (20.62)
Alkaline phosphatase (U/l)	115.9 (62.56)	113.70 (55.75)
Erythrocyte sedimentation rate (mm/h)	13.13 (10.20)	16.67 (17.06)
Concomitant and previous diseases n (%)		
Cardiovascular diseases	7 (11.11)	6 (9.23)
Endocrine diseases	4 (6.35)	2 (3.07)
Gastrointestinal diseases	3 (4.76)	7 (10.67)
Hepatic diseases	2 (3.17)	3 (4.61)
Musculoskeletal diseases	1 (1.59)	2 (3.07)
Dermatological diseases	1 (1.59)	--
Respiratory diseases	1 (1.59)	--
Urinary disorders	1 (1.59)	2 (3.07)
Arteriosclerosis	--	1 (1.54)

Table 2. Concomitant medications taken during the study period

Group	Medication (active substance)	Duration of treatment during the study period
Active group	metoprolol/enalapril	56 days
	sucralfate	1 day
	oral combined contraceptives	56 days
	perindopril/indapamide	56 days
	amlopidine/metoprolol/ginko biloba/diosmine	56 days
	atenolol/glycazide/insulin	56 days
	pantoprazole	29 days
Placebo group	levothyroxine/indapamide/enalapril	56 days
	omeprazol/ramipril	56 days
	metoprolol/enalapril	56 days
	oral combined contraceptives	56 days
	perindropil/metoprolol/leflunomide/AAS/metformine	56 days

Metoprolol/quinapril/fenofibrate/esomeprazol/nicergoline/AAS

AAS: acetylsalicylic acid

Figure Legends

Figure1. Study chronogram (a) and study workflow (b).

a)

Activities	Study day -14/0	Study day 1-28	Study day 29	Study day 30-56	Study day 57-62
Informed consent	X				
Demographic data	X				
Anamnesis	X				
Physical examination	X				X
Vital signs	X				X
Clinical laboratory test	X				X
Pregnancy test	X				
Inclusion/exclusion criteria	X				
Study treatments administration		X[1]	X[1]	X[1]	
Safety evaluation by primary endpoints	X[3]	X[2]	X[2]	X[2]	X[3]
Efficacy evaluation by secondary endpoints	X[6]	X[4]	X[5], X[4]	X[4]	X[5],[6]
Use of rescue medication		X	X	X	

[1] Treatment administration: 2 tablets before breakfast and 2 tablets before dinner.

[2] AE monitoring by answering to a standardized questionnaire reported onto Daily Diary by patients.

[3] Results of clinical laboratory parameter and vital signs monitoring between pre- and post-study visit.

[4] Patient assessment of stools emissions on Daily Diary.

[5] Patient assessment of subjective parameters (abdominal pain, bloating)

[6] IBS QoL questionnaire.

b)

	Study day -14/-1
Informed consent	
Demographic data	
Anamnesis	
Physical examination	
Vital signs	
Clinical laboratory test	
Pregnancy test	
Inclusion/exclusion criteria	

⇩

Randomization and delivery of assigned treatment	**Study day 0**

⇩

Laboratory parameter and vital signs monitoring
Patient assessment of subjective parameters (quality of life)

Study day 1-28

⇩

Study treatments administration
AE monitoring
Patient assessment of stool emissions
Use of rescue medication

Study day 29

⇩

Routine control at investigational site
Study treatments administration
AE monitoring
Patient assessment of stool emissions
Patient assessment of subjective parameters (abdominal pain, bloating)
Use of rescue medication

Study day 30-56

⇩

Study treatments administration
AE monitoring
Patient assessment of stool emissions
Use of rescue medication

Study day 57-62

⇩

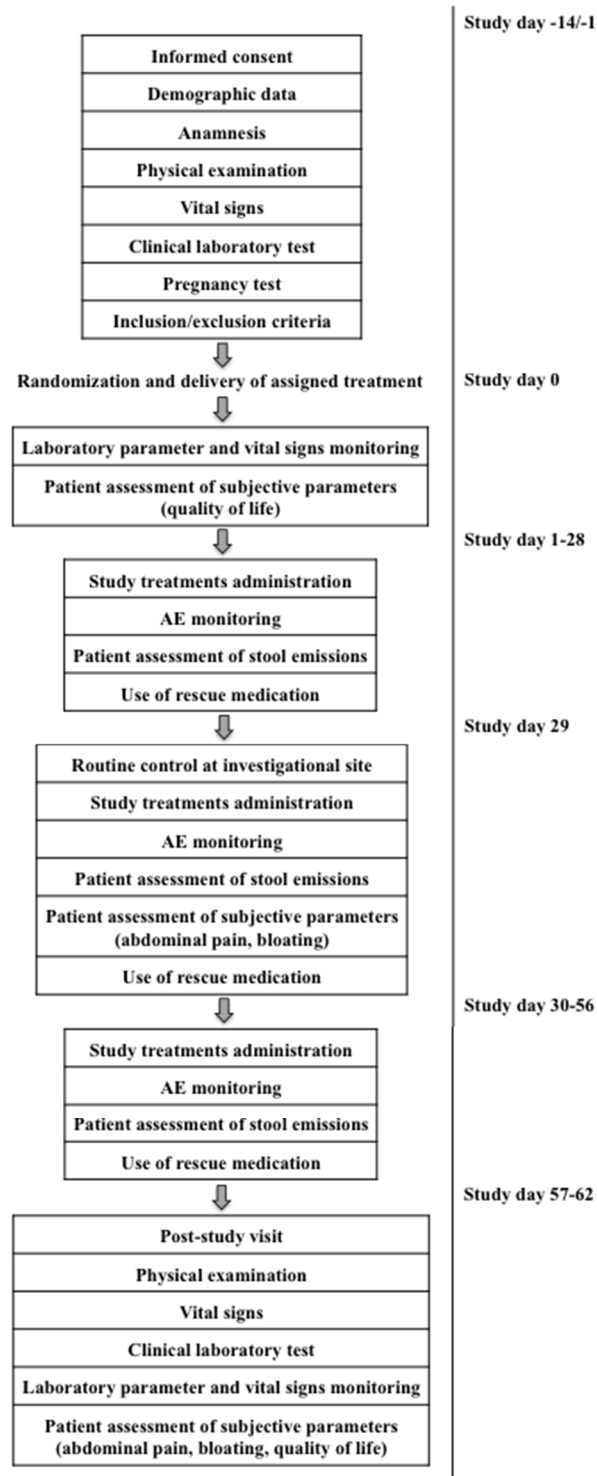| **Post-study visit** |
|---|
| **Physical examination** |
| **Vital signs** |
| **Clinical laboratory test** |
| **Laboratory parameter and vital signs monitoring** |
| **Patient assessment of subjective parameters (abdominal pain, bloating, quality of life)** |

Figure 2. Rate of clinical remission during the study period (defined as defined as the disappearance of diarrhea, i.e. 2 or less nonwatery stools emissions per day (stool of type 5 or less on the Bristol scale)
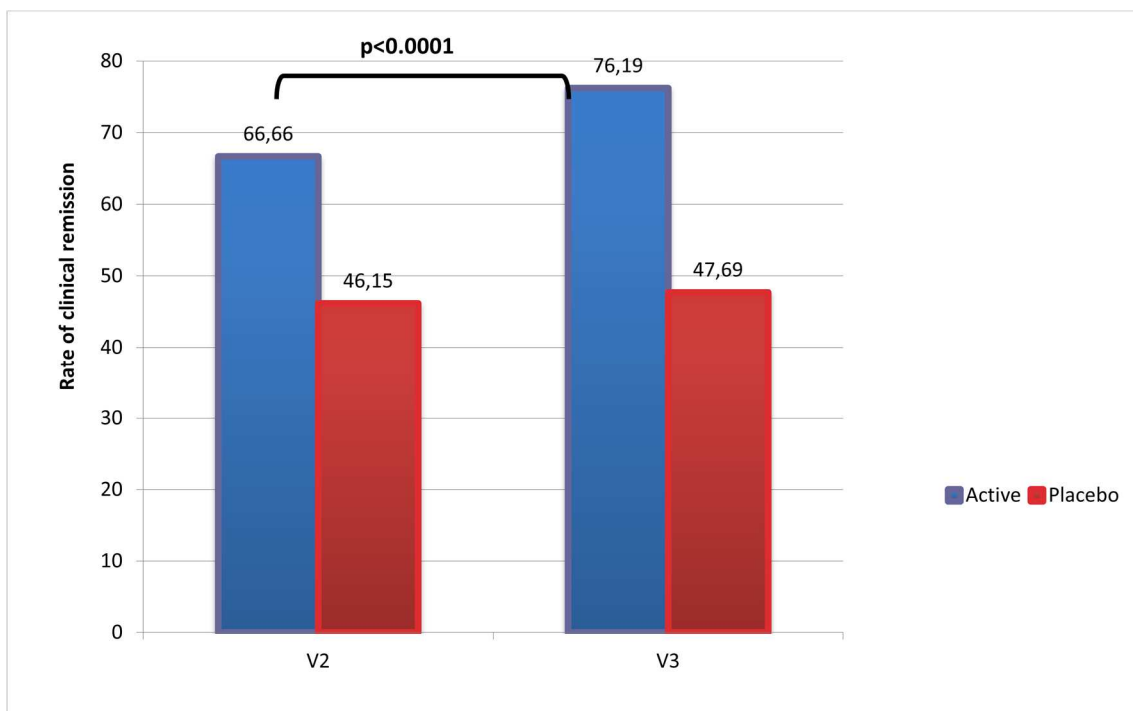
Figure 3. Evolution of abdominal pain and flatulence according to a 7-point Likert scale. a) Evolution of abdominal pain in active and placebo groups from baseline to visit 3 based on a 7-point Likert scale (7 = very much better, 6 = much better, 5 = somewhat better, 4 = same, 3 = somewhat worse, 2 = much worse, 1 = very much worse). b) Evolution of flatulence in active and placebo groups from baseline to visit 3 based on a 7-point Likert scale (7 = very much better, 6 = much better, 5 = somewhat better, 4 = same, 3 = somewhat worse, 2 = much worse, 1 = very much worse).
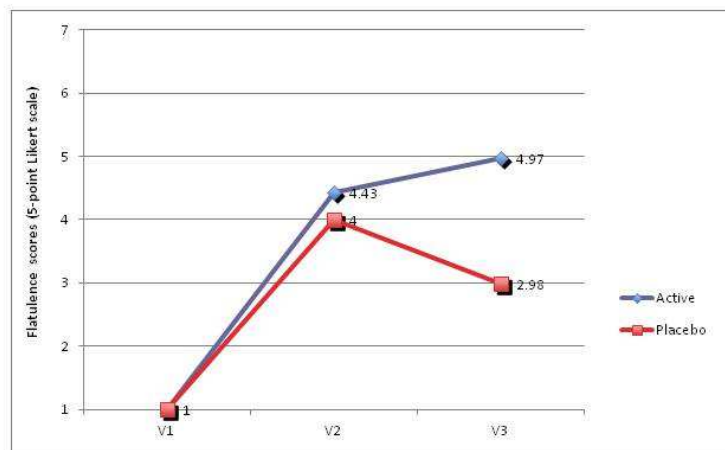
A)
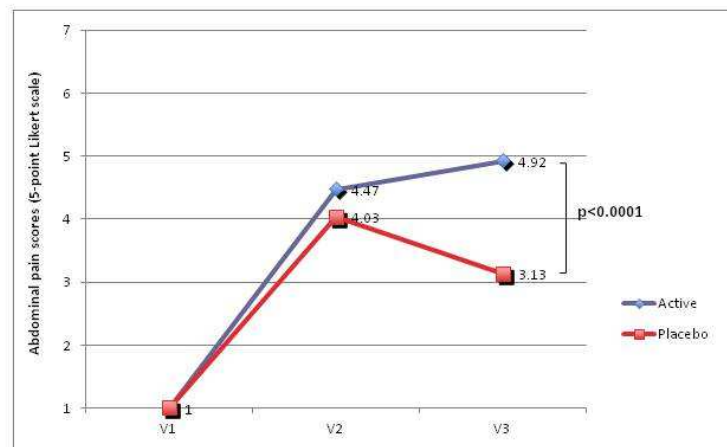
b)

Figure 4. Scores obtained in the IBS QoL questionnaire from baseline to visit 3. The IBS QoL questionnaire is a 34-item measure constructed specifically to assess the subjective well-being of patients with IBS, including eight dimensions (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual dysfunction, and relationships). Each item is scored on a five-point scale (1 = not at all, 5 = a great deal). To facilitate score interpretation, the summed total score is transformed to a zero to 100 scale ranging from zero (maximum quality of life) to 100 (minimum quality of life).