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ORIGINAL ARTICLE

Melatonin Regulation as a Possible Mechanism for Probiotic (VSL#3) in Irritable Bowel Syndrome: A Randomized Double-Blinded Placebo Study

Reuben K. Wong · Cao Yang · Guang-Hui Song · Jennie Wong · Khek-Yu Ho

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Abstract
Background Probiotics have treatment efficacy in irritable bowel syndrome (IBS), but the exact mechanism remains obscure. One hypothesis is the mediation of melatonin levels, leading to changes in IBS symptoms.
Aim The purpose of this study was to evaluate the effects of a probiotic, VSL#3, on symptoms, psychological and sleep parameters, and pain sensitivity in IBS, and relate these parameters to in vivo melatonin levels.
Methods Forty-two IBS patients were randomly assigned to receive VSL#3 or placebo for 6 weeks. Subjects completed bowel and psychological questionnaires, underwent rectal sensitivity testing and saliva melatonin assays.
Results Abdominal pain duration and distension intensity decreased significantly in the probiotic group, along with an increase in rectal distension pain thresholds. A correlation between increase in pain tolerance and improvement in abdominal pain scores ($r = 0.51$, $p = 0.02$) was seen with probiotic. There was an increase in salivary morning melatonin levels in males treated with VSL#3, which correlated ($r = 0.61$) with improved satisfaction in bowel habits. When grouped based on baseline diurnal melatonin levels, patients with normal diurnal fluctuations showed an increase in morning melatonin levels with VSL#3 treatment, which significantly correlated with improved satisfaction in bowel habits ($r = 0.68$). They also had reduced symptom severity scores and abdominal pain duration when treated with VSL#3, as well as satisfaction with bowel movements and quality-of-life.
Conclusions VSL#3 improved symptoms and increased rectal pain thresholds. Symptom improvement correlated with a rise in morning melatonin, significant in males and subjects with normal circadian rhythm. This suggests that probiotics may act by influencing melatonin production, hence modulating IBS symptoms, in individuals with a normal circadian rhythm.

Keywords Probiotic · Melatonin · Irritable bowel syndrome · Circadian rhythm · Rectal distension

R. K. Wong (✉) · K.-Y. Ho
Department of Gastroenterology and Hepatology, National University Health System, 1E Kent Ridge Road, NUHS Tower Block Level 10, Singapore 119228, Singapore
e-mail: reuben_wong@nuhs.edu.sg

C. Yang · G.-H. Song · J. Wong · K.-Y. Ho
Department of Medicine, Yong Loo Lin School of Medicine, Singapore, Singapore

Introduction

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder in the community. Epidemiological studies from the United States, the United Kingdom and China have shown that about 11–20 % of people in the community suffer from this condition [1], with a local population prevalence of 8.6 % in the Singaporean community [2]. The pathogenesis of IBS remains unclear. Besides the dysregulation of brain-gut axis, altered bowel motility, and visceral hypersensitivity, the imbalance in the intestinal microbiota is also considered to be one important etiologic factor for IBS [3, 4]. The findings of studies that some probiotics are effective in the prevention and treatment of various GI disturbances such as IBS [4–6] support this idea. Some studies suggest that probiotics may alter colonic fermentation causing increased formation of gas which induces propulsive contractions and accelerates transit [7]. Moreover, probiotics may also change the production of short-chain fatty acids which modulate the absorption of fluid and sodium in the colon [8]. Other studies also showed that

probiotics may exert their beneficial effects on GI disorders through a variety of mechanisms such as modulating of host immune response, enhancing epithelial barrier function, and anti-microbial effects [9, 10]. However, the pathophysiologic mechanisms by which probiotics work has not been fully explored.

A dysregulation of the brain-gut axis has been evidenced in a number of subjects suffering from IBS. These individuals suffer from pain and altered bowel motility in a more severe manner when they are under stress or experience situations affecting their coping strategies or personality traits [11]. The hormone corticotrophin releasing factor (CRF) links the central nervous system to the gut, as witnessed by the increased salivary levels of cortisol in patients with IBS, compared to healthy controls [12]. This "stress hormone" is antagonized by the pineal gland. When the subject is under over-stimulated cortisol secretion, the production of melatonin by the pineal gland undergoes significant suppressing [13]. Melatonin (5-methoxy-*N*-acetyltryptamine), a close derivative of serotonin (5-HT), is a hormone not only implicated in the control of the sleep-wake cycle but also in the modulation of bowel function. Our previous two randomized double blind placebo controlled studies showed that administration of melatonin 3 mg nocte for 2 weeks significantly attenuated abdominal pain, while reducing rectal pain sensitivity. Furthermore, it also had a tendency to alleviate bowel distension [14, 15].

Besides production in the pineal gland, high amounts of melatonin were found in bowel [16]. Thus we hypothesize that the function of probiotics in treating IBS may be due to its influence on the levels of melatonin in the gut, and this in turn had an impact on the symptomatology of patients with IBS. In order to prove this hypothesis, we designed a randomized double blind placebo controlled study using VSL#3, a probiotic containing eight different strains of beneficial bacteria, to investigate the effects of this probiotic in treating IBS bowel symptoms with changes of salivary melatonin. Specifically, this study aimed to (1) assess the effectiveness of VSL#3 in treating IBS symptoms and to explore the possible changes in rectal sensitivity and motility; (2) to investigate the melatonin concentration changes in vivo through measuring saliva melatonin before and after VSL#3 treatment; (3) to explore the mechanism underlying the possible changes of in vivo melatonin and bowel symptoms; and (4) to evaluate the effectiveness of VSL#3 in improving psychological and sleep parameters of IBS subjects.

Materials and Methods

Subjects

Forty-two unselected IBS patients aged 20–76 years, were recruited from the gastroenterology clinic or via an advertisement posted on the university campus. Patients had to satisfy the inclusion criteria of a diagnosis of IBS based on the Rome III criteria [17]. There was a wash-out period of 1 month, whereby patients had to abstain from any medication known to alter gastrointestinal function within a month before and during the study, and their medications history was recorded. Subjects were excluded if their stool culture was positive for bacterial pathogens (Salmonella and Shigella); parasites (Giardia) and ova/cysts on microscopy; if they had a positive faecal occult blood test; if they were pregnant or breast-feeding females; if they had organic gastrointestinal, anal, hepatic, or other systemic disorders; if they had previous gastrointestinal surgery history except appendectomy; or if they gave a history of cerebral disease or surgery.

Ethics Approval

The study was approved by the Institutional Review Board and Domain Specific Review Board of the National Healthcare Group. As with other probiotics, VSL#3 is not a regulated pharmaceutical agent, and no regulatory approval was required by the Singapore Health Sciences Authority (HSA) at the time this study was performed. Written informed consent was obtained from the subjects prior to commencement of the study, and subjects could withdraw at any point during the study if they chose to do so.

Methods

Experiment Design

This trial was a parallel-group, randomized, double blind, placebo controlled design. The qualified participants were randomized into two groups receiving either VSL#3 ($n = 20$) or identically appearing placebo ($n = 22$). Randomization was performed using a computer generated randomisation list. The VSL#3 was a composite probiotic in a powder form, contained in a soluble capsule. Each study capsule contained 112.5 billion viable lyophilized bacteria: *Bifidobacterium* (*B. longum, B. infantis* and *B. breve*); *Lactobacillus* (*L. acidophilus, L. casei, L. delbrueckii* ssp. *bulgaricus* and *L. plantarum*); and *Streptococcus salivarius* ssp. *thermophilus*. VSL#3 and placebo were identical with respect to preparation and packaging and were distinguishable only by batch number held by the manufacturer. After randomization, each participant ingested either four VSL#3 capsules or four identical placebo capsules, twice daily, for 6 weeks. Participants had to avoid drinking of any hot beverage like tea/coffee for at least 30 min after having VSL#3 or placebo. Both VSL#3 and placebo were supplied by VSL Pharmaceuticals, Inc. (Gaithersburg, MD, USA).

Participants were required to pursue a baseline 2-week run-in period, recording daily symptoms by means of a diary and followed by a 6-week treatment period. In the middle of the treatment (week 3), the participants were assessed according to their symptoms and checked whether they took the medication according to the guideline. At the end of run-in and the end of the treatment phase, all patients were asked to complete several questionnaires, including standardized bowel disease questionnaire (SBDQ), Hospital Anxiety and Depression Scale, Pittsburgh sleep quality Index (PSQI) and Epworth sleepiness scale (ESS) for assessment of their bowel symptoms, psychological status and sleep disturbance [18–20]. During the run-in and treatment periods, subjects were asked to complete the Bowel Symptom Diary daily and record any adverse events. Patients were asked to report, on a weekly basis, the global satisfactory relief of bowel symptoms using a 7-point system [21].

Rectal Sensitivity Study

All patients were asked to undergo a rectal sensitivity study before and after the period of pharmacological treatment. Sensory and motor responses to rectal distension were assessed by a method employed in our previous study [22]; a rectal water enema (300 ml warm water at 36 °C) was applied before the study. After testing for leakage by inflating with air under water, a lubricated 400-ml polyethylene bag (Mui Scientific, Toronto, Canada) attached to the top of a flexible catheter was inserted 5 cm into the subject's rectum. It is then slowly inflated with air at low pressure (5 mmHg), ensuring contact between the bag and the distal rectal ampulla. The tubing was then taped to the subject's leg. The subject is positioned on the bed in a comfortable supine position with legs slightly apart, covered with a light sheet with the knees slightly flexed and supported on pillows. After a 5-min acclimatization period, first sensation, the sensation of defecation, first pain and pain tolerance thresholds were determined respectively, using an ascending method of limits protocol with incremental steps of 5 mmHg, 30 s distension and rest periods applied by a barostat (G&J Electronics Inc., Toronto, Canada) set at an inflation speed of 40 ml/s and a cut-off pressure of 60 mmHg. These thresholds were determined twice with a break of 30 s between the two runs. The average of the two runs was used for analysis. Subjects were asked to rate the rectal pain intensity after 30 s on a 100 mm VAS scale (0 = none, 100 = maximum bearable).

Saliva Melatonin Measurement

In order to observe the fluctuation of in vivo melatonin levels due to VSL#3 treatment, morning and night salivary melatonin levels were measured before and after the treatment period in all the subjects. A sample of 1 ml saliva was collected at 11 pm and upon awakening, at 7 am, before and after treatment. The patients were asked to put the samples into separate sterile containers and store in their home refrigerator at −25 °C before bringing them to the National University Hospital during the visits just before and after treatment. Then all the samples were stored at −80 °C until the measurement procedure was carried out; the samples were coded with numbers. Saliva melatonin was measured using the ELISA method (Salivary Melatonin Assay RE5404 kits; IBL International GmBH, Hamburg, Germany).

Statistical Analysis

All statistical analyses were performed using the standard SPSS package (version 17.0 for Windows). Continuous variables were expressed as the arithmetic mean ± standard error of the mean (SEM), and categorical variables were expressed as frequencies and percentages. Variables that were not normally distributed were transformed, as appropriate, by either logarithmic or square root transformations to satisfy normality assumptions. To compare the differences between the VSL#3 and placebo groups, the χ^2 test was used for categorical variables and the independent sample Student's t test for continuous variables. The paired-t test was used to compare differences in the VSL#3 group before and after treatment. A two-tailed p value less than 0.05 was considered statistically significant.

Results

Patients

Forty-two IBS patients were randomly assigned to receive either VSL#3 or placebo. However, the IBS patients who received VSL#3 happened to be older than the patients in the placebo arm. There were also differences between these two treatment groups in baseline psychological scores and baseline sleep disturbance scores (as illustrated in Table 1). As a result, subjects in the placebo arm had more psychological disturbance and worse sleep scores. On recruitment, all IBS patients had subjective sleep complaints as evidenced by global PSQI scores above 5. IBS patients assigned to receive placebo happened to have a higher anxiety ($p < 0.05$) with the mean value suggesting a borderline abnormality. However other psychological scores remained normal among all IBS before treatment, suggesting that there was no overall major difference in the psychological characteristics between the groups. All patients completed the 6-week study and reported no obvious side effects.

Table 1 Characteristics of study subjects

Parameter	VSL	Placebo	*p* value
n	20	22	–
Age (year) mean (SEM)	53.35 (4.15)	40.86 (3.51)	<0.03
Sex (M/F)	12/8	11/11	0.37
Anxiety score	5.9 (0.86)	8.6 (0.76)	<0.03
Depression score	3.52 (0.43)	5.5 (0.84)	<0.05
Sleep quality			
PSQI	5.2 (0.66)	7.18 (0.70)	<0.03
EPWORTH	4.6 (0.82)	8.91 (1.22)	<0.01

Anxiety score: 0–7 = normal; 8–10 = borderline abnormal; 11–21 = abnormal

Depression score: 8 and above = abnormal

PSQI: Above 5 = abnormal

EPWORTH: 1–6 = normal; 7–8 = borderline abnormal; 9 and above = abnormal

Table 2 IBS symptoms before and after treatment

Parameter	Before treatment		After treatment	
	VSL#3	Placebo	VSL#3	Placebo
IBS-SSS	224.5	226.36	158.00	183.46
Abdominal pain intensity	18.75	27.73	15	21.82
Abdominal pain duration	37.50*	30.91	19*	23.63
Abdominal distension intensity	34.50*	39.55	20*	27.27
Satisfaction with bowel movement	70.09	68.64	55.26	55.68
Quality of life	63.75	59.55	52.5	50.23
Frequency of defecation	1.9	1.91	1.55	1.5
Stool type	4.20	4.19	3.9	3.89

* Significant difference at $p < 0.05$

Bowel Parameters

Bowel Symptoms

IBS bowel symptom parameters (that is, abdominal pain, abdominal distension, frequency of defecation, stool type, abnormal sensation of defecation, quality of life, and total bowel symptom score), as obtained from the IBS symptom severity score [23], were evaluated. Differences in these parameters before and after 6 weeks of treatment for the VSL#3 and placebo-treated groups were compared. As shown in Table 2, abdominal pain duration score and the abdominal distension severity scores were significantly decreased in the VSL#3 treated group after 6 weeks of treatment ($p < 0.05$), as compared to no difference in the placebo group. Both VSL#3 and placebo reduced total bowel symptom score, abdominal pain intensity and improved satisfaction with bowel habit and quality of life,

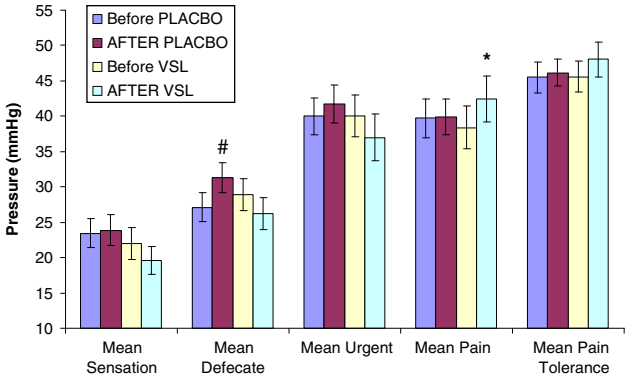

Fig. 1 Rectal sensory results before and after VSL treatment

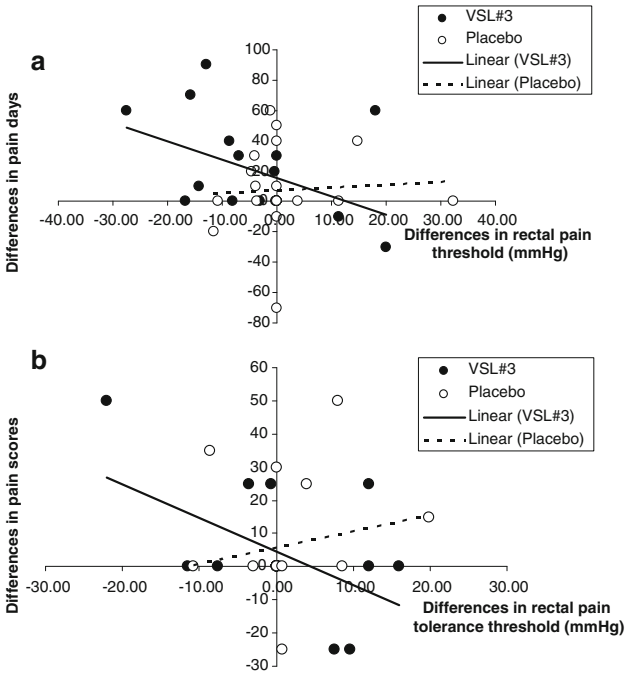

Fig. 2 **a** Correlation between changes in the distension pressure threshold of pain sensation and the change in pain duration (pre-treatment minus post-treatment). **b** Correlation between changes in the distension pressure threshold of pain tolerance sensation and the change in pain scores (pre-treatment minus post-treatment)

and there were no differences in post-treatment changes in stool type or abnormal sensation of defecation between the VSL#3 and placebo groups.

Rectal Sensory Thresholds

As shown in Fig. 1, among patients who were treated with VSL#3 for 6 weeks, rectal distension pressure threshold that was required to induce the pain sensations was significantly increased from 38.38 (3.02) to 42.47 (3.25) mm Hg ($p < 0.05$). Moreover, the increase in the distension

Table 3 Psychological and sleep quality parameters before and after treatment

Parameter	Before treatment		After treatment	
	VSL#3	Placebo	VSL#3	Placebo
Total anxiety score (HAD)	5.90	8.63	5.5	8.09
Total depression score (HAD)	3.5	5.5	2.75	4.10
Perceived Stress Scale-10 Item	14.95	17.73	15.65	16.45
PSQI	5.2	7.18	4.35	6.77
The Epworth sleepiness scale	4.6	8.91	5.25	8.22

pressure threshold of pain sensation was marginally correlated with the reduction of pain duration ($r = 0.45$, $p = 0.052$) (Fig. 2a). A increasing trend in the mean pain tolerance threshold was observed in VSL#3 treated groups [from 41.98 (2.53) to 45.87 (3.26) mmHg], which correlated with an improvement in the abdominal pain scores ($r = 0.51$, $p = 0.02$) (Fig. 2b). In contrast, sensory thresholds for pain and pain tolerance did not change at the end of the 6 weeks of placebo treatment. No correlations between improvement of abdominal pain by placebo and the changes of rectal sensory thresholds for pain or pain tolerance were seen. Both VSL#3 and placebo given for 6 weeks did not change distension pressure thresholds for the first sensation of distension and urgency.

Psychological and Sleep Quality Parameters

Changes in psychological parameters as shown in total anxiety, total depression scores and Perceived Stress Scale-10 Item scores, as well as changes in sleep quality parameters after treatment in the VSL#3 group were similar to those in the placebo group (Table 3).

Salivary Melatonin Parameters

In the present study, no detectable changes of either morning or night salivary melatonin were found among IBS patients grouped by treatment of VSL#3 or placebo. However, when patients were sub-grouped by gender within each treatment arm, a significant increase of the morning melatonin (from 5.43 to 9.74 pg/ml) was observed in male patients but not females with post-treatment of VSL#3 ($p = 0.03$) (Fig. 3). Moreover, the increase of their morning melatonin was marginally correlated with the increase of satisfaction in bowel habits among males treated with VSL#3 ($r = 0.61$, $p = 0.058$) (Fig. 4). Interestingly, although there was no significant difference in change of IBS symptoms between males and females in each treatment group; VSL#3 treated males significantly

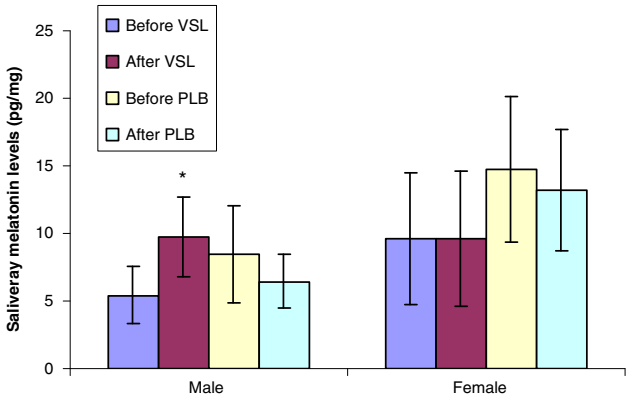

Fig. 3 The change of morning melatonin in patients sub-grouped by gender

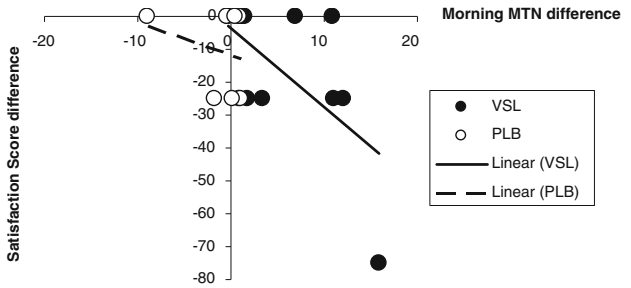

Fig. 4 Correlation between satisfaction scores on bowel habit difference (0 = very satisfied, 100 = extremely unsatisfied) and change of morning melatonin in male patients (post-treatment minus pre-treatment)

Table 4 IBS symptoms before and after treatment in male patients

Parameter	Before treatment		After treatment	
	VSL#3	Placebo	VSL#3	Placebo
IBS-SSS	193.33*	237.73	132.08*	205.9
Abdominal pain intensity	12.5	31.82	6.25	28.18
Abdominal pain duration	27.5*	38.18	13.3*	34.55
Abdominal distension intensity	30.42*	34.27	12.5*	31.82
Satisfaction with bowel movement	64.58	71.36	54.55	56.82
Quality of life	58.33	59.09	50.00	54.55

* Significant differences at $p < 0.05$

reduced their IBS severity scores in contrast to placebo treated males, which was not observed in female patients (Table 4).

Besides gender differences, whether patients had normal melatonin circadian rhythm or not upon recruitment also affects melatonin levels after treatment. A normal

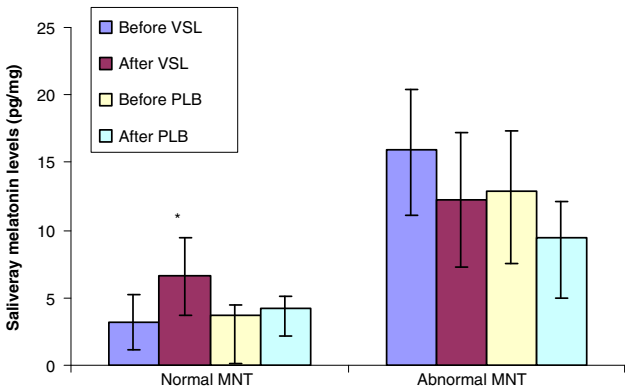

Fig. 5 The change of morning melatonin in patients sub-grouped by patients with a normal versus abnormal melatonin circadian rhythm

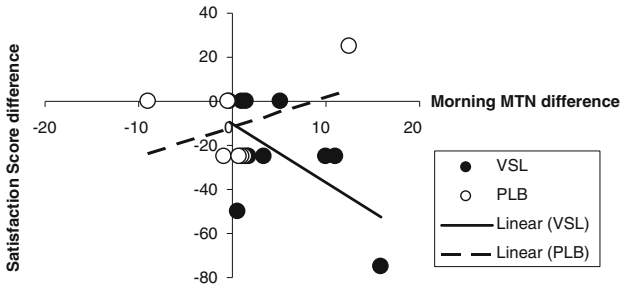

Fig. 6 The correlation between satisfaction scores on bowl habit difference (0 = very satisfied, 100 = extremely unsatisfied) and change of morning melatonin in patients who had normal melatonin circadian rhythm (post-treatment minus pre-treatment)

physiologic circadian rhythm causes a ramping up of melatonin production by the pineal gland in preparation for sleep and a drop in the level (with a corresponding rise in cortisol and other arousal hormones) leading up to awakening. Salivary sampling closely mirrors actual serum levels, and so a baseline higher pre-sleep and lower post-awakening level would constitute normal physiologic levels. In the subgroup of patients who had normal melatonin circadian rhythm ($n = 15$), there was a tendency towards a greater increase in the morning melatonin (from 3.19 to 6.58 pg/ml) with VSL#3 treatment ($p = 0.07$) (Fig. 5). Moreover, their increase of morning melatonin was significantly correlated with the increase of satisfaction in bowel habits ($r = 0.68$, $p = 0.036$) (Fig. 6). Notably among patients with normal salivary baseline diurnal melatonin level, VSL#3 showed significantly superior therapy outcome than placebo in a number of the IBS symptoms, namely, pain days, satisfaction in bowel habits, quality of life as well as total IBS symptoms severity scores (Table 5).

Table 5 IBS symptoms before and after treatment in patients with a normal circadian rhythm

Parameter	Before treatment		After treatment	
	VSL#3	Placebo	VSL#3	Placebo
IBS-SSS	273.78*	198.57	150.00*	153.57
Abdominal pain intensity	16.67	32.14	13.89	25.71
Abdominal pain duration	50.00*	30.00	22.22*	17.14
Abdominal distension intensity	26.67	31.43	13.89	14.29
Satisfaction with bowel movement	72.22*	57.14	56.25*	53.57
Quality of life	72.22*	47.86	50.00*	42.86

* Significant difference at $p < 0.05$

Discussion

The promising efficacy of probiotics in the treatment of IBS has been established in the last decade. In the present randomized, double-blinded and placebo-controlled trial, although placebo was equally effective in some of the parameters such as total bowel symptom score, abdominal pain intensity, satisfaction on bowel habit and quality of life, VSL#3 was significantly superior to it in alleviating abdominal pain durations ($p < 0.05$) and the severity of abdominal discomfort ($p < 0.05$). No significant difference was found in the stool pattern and frequency of defecation. These findings are in line with observations in previous IBS trials using VSL#3 [5, 24].

The lack of any significant difference in psychological scores and sleep indices could be explained by the fact that the subjects in the placebo arm, by chance, had worse psychological and sleep indices to begin with. It is therefore possible, that if the two groups were equally balanced, a significant difference may have emerged in favour of the probiotic.

Although probiotics have already been used for IBS treatment in clinical practice, the mechanisms underlining its efficacy on IBS still remain unknown. Our data showed that therapeutic efficacy of VSL# 3 was associated with heightened pressure thresholds for pain sensations. It could be argued that the rectal distension assessment before treatment may have increased their vigilance and made them habituated with the same rectal distension procedure performed after treatment with the probiotic. However, this possibility seems unlikely as changes in distension thresholds occurred only in the VSL#3 group and not in the placebo treated group, who would have been expected to experience the same potentially biased effect. Significantly, the increase of pain sensation threshold was shown to be positively correlated with reduction of pain symptoms on both pain intensity and pain duration in VSL#3 treated

patients. In contrast, no such correlations were found amongst the placebo treated subjects. This suggests that visceral hypersensitivity plays an important role in the aetiology of IBS, and distinguished from placebo, the probiotic has a direct effect on gut visceral sensitivity which in turn ameliorates the IBS pain symptoms.

Interestingly, VSL#3 did not alter pressure or volume thresholds for those non-noxious sensations which were yet to reach the pain level, such as the first sensation of distension, desire to defecate and urgency. Although in the present study, the reasons why VSL#3 is only responsible for altering pain sensation threshold but not all of the sensations to balloon distension cannot be clearly elucidated; we postulate that it may be related to the effect of the probiotic on endogenous pain modulation in IBS. This was demonstrated in rats, where the probiotic *B. infantis* increased the threshold to pain and reduced the total number of pain related behaviours [25]. It is known that endogenous pain mechanisms are implicated in central sensitisation and are pivotal in regulating, fine-tuning and integrating pain perception and homeostatic responses. This modulation is initiated only when the brain receives the nociceptive signals from the peripheral system, but not the non-pain signals. A majority of IBS patients have been shown to exhibit abnormal endogenous pain modulation, as evidenced by lowered pain threshold for experimental painful stimulations as well as the facilitating pain effect during heterotopic stimulation [22, 26–30]. Therefore, it could be that VSL#3 may help to ameliorate the dysfunctional endogenous pain modulation, which results in reducing clinical pain symptoms as well as ameliorating rectal sensitivity to pain stimulation.

Besides heightened pressure thresholds for pain sensations, the therapeutic efficacy was associated with an increase of morning salivary melatonin, significantly so in male patients and patients who had normal melatonin circadian rhythm. The role of melatonin as regulator of circadian and seasonal rhythmicity has been established [16, 31, 32]. Patients with functional disorders of the gastrointestinal tract also had sleep disorders and some of them suffered from the increased neural excitability and anxiety [32, 33]. The involvement of melatonin in IBS was first established by our group, which showed that when it was administered orally in pharmacological doses it had the beneficial effect on abdominal pain in IBS patients without improving the sleep disturbances [14]. Later on, melatonin was also shown to be involved in the regulation of gastrointestinal motility, and different melatonin secretion in male and female patients were suggested to be involved in the pathogenesis of certain subtypes of IBS [15, 34]. A recent controlled human study showed there are gender differences in the circulating melatonin amplitude, and this was borne out in our findings, where females had a higher baseline early morning systemic melatonin level [35]. What is novel is the way probiotics potentially alter the metabolism and production of endogenous melatonin differently between the genders. Our study exemplified this, with the male subjects showing a significant increase in early morning systemic melatonin in response to the probiotic, which was not seen in the females. The mechanism could be due to the differences in how alterations in the gut microbioma affects the metabolism of tryptophan between the genders.

Melatonin is both an exogenous substance, present in certain foods, and an endogenous substance, produced by the pineal gland, retina and gut, using tryptophan as a substrate. There is an inverse relationship between melatonin synthesis and levels of serotonin. Serotonin or 5-Hydroxytryptamine (5-HT) is a monoamine neurotransmitter, biochemically derived from tryptophan, that is primarily found in the gastrointestinal (GI) tract, platelets, and central nervous system (CNS) of humans and animals. Approximately 80 % of the human body's total serotonin is located in the enterochromaffin cells in the gut, where it is used to regulate intestinal movements. VSL#3 is composed of strains that do not contain tryptophanase. We postulate that the administration of VSL#3 shifts the microbiota composition, resulting in an increase in gut tryptophan, which increases the bioavailability of the compound in the systemic circulation. Physiologically, the increased tryptophan is converted into melatonin by the pineal gland, with the net effect of increased melatonin levels and a concomitant reduction of serotonin. In the present study we observed patients in this trial can be divided into two groups, a first group with normal baseline diurnal melatonin level (normal circadian cycle) and the other group with a reversed or abnormal baseline diurnal melatonin level. A significant effect of VSL#3 on IBS symptoms was limited to patients with normal salivary baseline diurnal melatonin level, as evidenced by significant change of IBS symptoms compared to placebo and increase of morning melatonin levels. There are two possible mechanisms for this finding—first, it is possible that individuals with an aberrant diurnal melatonin level have an altered mechanism for metabolism and production of melatonin, and so attempts to change their endogenous melatonin level through the use of probiotics has little effect. Second, these individuals may have a disordered central regulatory system for their circadian rhythm, which reflects a greater underlying difference in brain structure and pain processing, hence the lack of a symptom response to the treatment with probiotic. These postulates would have to be proven by further mechanistic studies.

Conclusions

In summary, the present study showed that the probiotic, VSL#3, significantly improved abdominal pain duration and distension severity score, through a route independent

of its effects on psychological and sleep parameters. The function of probiotics in treating IBS may be due to its influence on reduction of visceral hypersensitivity through recovery of dysfunctional endogenous pain modulation. Mechanistically, the beneficial effect of the probiotic may be associated with the alteration in melatonin metabolism and secretion, especially in patients who have a normal melatonin circadian rhythm. There also existed gender differences in the response to probiotics, which could be related to an underlying difference in the metabolism and production of melatonin between males and females.

Conflict of interest None.

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