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Mesalazine treatment for Irritable Bowel Syndrome – effects on bowel habits and specific gastrointestinal symptoms

Degree Project in Medicine

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Abstract

Title: Mesalazine treatment for Irritable Bowel Syndrome – effects on bowel habits and specific gastrointestinal symptoms**.** Degree Project, Programme in Medicine, 2020, University of Gothenburg, Sweden

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Background: There is accumulating evidence for low-grade intestinal inflammation in the pathogenesis of Irritable Bowel Syndrome (IBS), which led to studies of mesalazine for IBS. These previous studies indicated that a subgroup of patients may benefit from this treatment. Warranting further study to characterize these patients.

Aims: This project investigated the effect of mesalazine treatment on individual IBS-symptoms as part of a randomised, double blind, placebo-controlled intervention trial of mesalazine treatment in patients with moderate to severe IBS.

Methods: Participants received 2400 mg mesalazine or placebo for 8 weeks. Bowel movements and specific IBS symptoms were registered daily in a validated IBS diary during 2 weeks prior to treatment, and 2 weeks at end of treatment. Data from the 2 IBS diaries were compared in a between-group analysis and in a within-group analysis. The efficacy endpoints were improvement in bowel movements, stool form, abdominal pain, nausea, and bloating.

Results: 45 participants were included in the analysis. In both the mesalazine group and the placebo group, there was a statistically significant reduction in abdominal pain and bloating on a within-group level. No significant changes were seen in the other endpoints, neither on a within-group level or in a between-group comparison. However, it is noteworthy that there was a larger numerical reduction of abdominal pain duration and bloating in the mesalazine group compared to the placebo group.

Conclusions: In this project, there was no benefit from treatment with mesalazine compared to placebo regarding bowel habits and specific IBS symptoms. However, results indicate that the limited sample size included in the analysis may have masked potential positive treatment effects. Data from another 113 participants was available but could not be included in the analysis due to time restraints. A next step would therefore be an analysis of the entire study database.

Key words: Irritable Bowel Syndrome. Inflammation. Mesalazine.

Background

Definition and classification

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by long-standing problems with recurring abdominal pain that is associated with altered bowel habits, such as constipation, diarrhea or both. Abdominal bloating or distension are also common symptoms. The onset of symptoms should be at least 6 months prior to diagnosis and the symptoms should be present during the last 3 months. [1]

The diagnostic criteria were developed by the Rome Foundation in order to facilitate diagnosis and recognition of the condition. The current criteria are labelled Rome IV and are presented for IBS in table 1.

Table 1. Rome IV-criteria for IBS

	Recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following criteria:
1)	Related to defecation
2)	Associated with a change in frequency of stool
3)	Associated with a change in form (appearance) of stool

The previous diagnostic criteria in use at the time of this study were the Rome III-criteria, which are presented for IBS in table 2.

Table 2. Rome III-criteria for IBS

	Recurrent abdominal pain or discomfort, i.e. uncomfortable sensation not described as pain, at least 3 days/month in the last 3 months associated with two or more of the following:
1)	Improvement with defecation
2)	Onset associated with a change in frequency of stool
3)	Onset associated with a change in form (appearance) of stool

IBS can be further specified into subtypes according to the criteria presented in table 3.

Table 3. IBS-subtypes and criteria according to Rome IV

IBS with predominant constipation (IBS-C): >25% of bowel movements with Bristol stool types 1 or 2 and < 25% of bowel movements with Bristol stool types 6 or 7.
IBS with predominant diarrhea (IBS-D): >25% of bowel movements with Bristol stool types 6 or 7 and <25% of bowel movements with Bristol types 1 or 2.
IBS with mixed bowel habits (IBS-M): >25% of bowel movements with Bristol stool types 1 or 2 and >25% of bowel movements with Bristol stool types 6 or 7.
IBS unclassified (IBS-U): Patients who meet diagnostic criteria for IBS but whose bowel habits cannot be accurately categorized into 1 of the 3 groups above should be categorized as having IBS-U.

The Rome IV-criteria differ from the Rome III-criteria in a number of ways. Abdominal discomfort has been removed from the definition because the term was considered imprecise, in combination with the fact that the word “discomfort” is not present in every language. The Rome IV-criteria requires abdominal pain to be present at least 1 day/week during the preceding 3 months. Another difference concerns the specification of subtypes, where in Rome IV, the predominant bowel habits are based on stool form on days with abnormal bowel

movements. As opposed to Rome III, where the predominant bowel habits included all bowel movements.

Epidemiology

The prevalence of IBS is reported to vary between different countries and regions. This is related to a number of different factors, where the changing definitions of IBS over time is important to note [1-6]. A meta-analysis including 80 studies estimated the world-wide prevalence to be 11.2% [7]. Recently, a population based Internet-survey reported a prevalence of 4.4-4.8% in three Western countries (United Stated, United Kingdom and Canada) when only the current Rome IV definition for IBS was used [8]. Two separate longitudinal population studies over 10 and 12 years, respectively, found an incidence of 1.5 and 1.35% [9, 10]. The prevalence is higher amongst women than men. Younger individuals are more likely acquire IBS than individuals over 50 years of age [7].

Clinical presentation and management

The defining symptoms of IBS according to the Rome IV criteria should be the ones guiding the clinical suspicion of the diagnosis. Other intestinal symptoms associated with IBS are mucus in faeces, urgency, straining, and a feeling of incomplete evacuation. IBS may overlap with other functional gastrointestinal disorders as well [11]. This explains symptoms from the upper gastrointestinal tract, such as heartburn, epigastric pain, early satiety, postprandial fulness and nausea. A number of extraintestinal symptoms are commonly associated with IBS. These include fatigue, headache, respiratory tract symptoms, lower urinary tract

symptoms, joint pain, muscle pain, as well as psychiatric symptoms such as anxiety and depressed mood. The common feature of these widespread symptoms is that they lack objective findings on clinical examination and testing [12, 13].

The diagnostic workup is based on clinical history, physical examination, appropriate laboratory tests and other tests, such as colonoscopy when clinically indicated. The need for diagnostic testing can be limited in the majority of patients where diagnostic criteria are fulfilled and alarm features are absent. A positive diagnosis based on a characteristic clinical picture is encouraged, as opposed to a diagnosis purely based on exclusion. Based on patient characteristics and pre-test probability, targeted diagnostics for a number of diseases that may mimic IBS, such as inflammatory bowel disease, celiac disease, lactose intolerance and microscopic colitis, may be indicated. Alarm features mainly include unintended weight loss (>10% in 3 months), blood in stools not caused by haemorrhoids or anal fissures, nocturnal diarrhoea, fever, family history of colorectal cancer (or polyposis syndromes), inflammatory bowel disease or celiac disease [12, 13].

The general aspects guiding treatment of patients with IBS include a number of components. Initially focusing on reassurance and patient education, identifying type and severity of predominant symptoms, and during this process initiating a positive patient-clinician relationship. An assessment is made regarding the impact on quality of life and level of daily functioning. This includes taking personality, recent life stress, anxiety, and depression into account. A number of directed interventions may be considered. Dietary and lifestyle modification may be helpful for some, including physical exercise and individualized dietary advice including traditional IBS recommendations according to the National Institute for

Health and Care Excellence (NICE)-guideline, a low fermentable oligo-, di-, monosaccharides and polyols (FODMAP) diet, a gluten-free diet, amongst others [14].

Based on the predominant symptoms, different pharmacological strategies may be selected. Those with predominant constipation may benefit from osmotic laxative agents, prosecretory agents, and 5-hydroxytryptamine 4 (5-HT_4) receptor agonists. Patients with predominant diarrhea can be treated with antidiarrheal agents, such as the mu-opioid agonist loperamide. Some patients with may experience relief by bile acid sequestrants such as cholestyramine. In some cases, treatment with a 5-hydroxytryptamine 3 (5-HT_3) receptor antagonist can be tried due to their effect on prolonging gastrointestinal transit time, despite their main use in Sweden as an antiemetic. In patients with predominant pain, there are a number of treatment approaches. Antispasmodics such as hyoscyamine have been shown to provide short term relief [15]. Central neuromodulators, especially low-dose tricyclic antidepressants, mediate their effects through multiple mechanisms independent of their mood improving effects. Peripherally they slow intestinal transit time by their anticholinergic properties. Centrally they modulate pain through enhancing descending serotonergic and noradrenergic pathways [16]**.** The pharmacological treatment should be tailored to fit individual needs [12, 13]. Psychological therapies have been shown to exert central effects on mood, as well as peripheral effects on pain perception, visceral hypersensitivity, and gastrointestinal motility. In particular cognitive behavioural therapy and hypnotherapy [17-20].

Pathophysiology

The pathophysiology of IBS is complex and multifactorial. Certain factors representing vulnerability to develop IBS has been identified, as well as factors associated with symptom generation and exacerbation. The common theme for these factors is that they lead to dysregulation of the gut-brain axis.

Enhanced visceral perception is a central pathophysiologic mechanism in IBS recognized long ago [21]. It involves increased sensitivity of visceral afferent pathways and/or central amplification of visceral afferent input. It includes hyperalgesia, a painful sensation from non-painful stimuli, and hypervigilance, in this context defined as heightened awareness and anticipation of stimuli [22-25]. Psychological components, such as mood and cognition, are known to influence visceral perception through central modulating pathways, and vice versa, with psychiatric symptoms such as depressed mood or anxiety occurring after onset of IBS symptoms. Exemplifying a bidirectional dysfunctional gut-brain interaction as a central component in the pathophysiology of IBS [26]. Furthermore, based on clinical findings, it has been postulated that there is a process of upregulation in central stress and arousal circuits [27].

Autonomic nervous system imbalance with increased sympathetic tone has been implicated as a contributing factor in subsets of IBS patients based on studies in which this was observed in comparison with healthy controls. Activation of central and peripheral pathways involving corticotropin-releasing factor and its regulation of the hypothalamic-pituitary-adrenal axis have been implicated in changes in gastrointestinal motility, permeability, and stress-induced

visceral hyperalgesia in IBS. Which altogether supports the role of stress as a pathophysiological mechanism [28-30].

A number of abnormalities of gastrointestinal motility in IBS has been observed. Increased frequency and irregularity of luminal contractions of small intestine and colon as well as exaggerated motor response to meal ingestion were seen in some patients with diarrhea-prominent IBS [31-33]. Accelerated colonic transit time was seen in approximately 1/3 of patients with IBS-D [34]. IBS-C have been associated with prolonged intestinal transit time [35], with approximately 1/10 of these patients showing a delayed colonic transit time [34]. These differences were associated with stool form, but not other IBS symptoms [34].

The gut microbiome is large and complex, serving a number of different roles, including its importance in the intestinal immune system and aiding in digestion. Changes in gastrointestinal tract microbiota have been described in IBS patients, with data suggesting an altered diversity of gut microbiota [36-38] as well as possible differences in microbiota composition between healthy controls and IBS patients [38-43]. The relationship between gut microbiota and host immunity is widely established [44, 45], with studies suggesting that changes in microbiota possibly leading to an altered immune activity [46], which in turn may contribute to low-grade inflammation in IBS [47, 48]. However, further studies are needed before clear conclusions can be drawn from this, including its influence on IBS symptoms.

Diet is considered an important component in the pathophysiology of IBS. A number of mechanisms have been proposed, including osmotic, chemical, mechanical, neuroendocrine, and gut microbiota [14]. Some patients seem to respond to restricting fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) in their diet, which are poorly absorbed in small

intestine. Due to their osmotically active properties, they induce fecal water retention and accelerate gut transit, providing large boluses of water and rapidly fermentable substrates to colonic bacteria. This leads to production of intestinal gas and short-chain fatty acids, which stimulate colonic contractions and bowel distension. In individuals with visceral hypersensitivity, this is associated with abdominal pain and frequent defecation. Another implicated factor are dietary fibres, where insoluble fibres have been associated with symptom exacerbation, as opposed to soluble fibres which may alleviate symptoms in some patients [13, 14, 49].

Abnormalities in serotonin metabolism has been suggested as a pathophysiological mechanism, especially in IBS-D. Studies have suggested reduced re-uptake by the serotonin reuptake transporter (SERT) in patients with IBS with diarrhoea. Uptake of serotonin by platelets was reduced in patients with IBS with diarrhoea. Expression of SERT mRNA in duodenal mucosa was also reduced, which seemed to be associated with duodenal immune activation. Various genetic influences in serotonin metabolism have been demonstrated, as well as a possible association with other pathophysiological mechanisms in IBS. However, the clinical implications of the role of serotonin metabolism in IBS remain inconclusive [49].

Genome-wide studies have shown associations with variants on chromosome 9 (9q31.2 locus) linked to the functions of ion channels and autonomic dysfunction [50] as well as mutations in the sucrase-isomaltase gene [51, 52]. One study showed that 2.2% of patients with IBS carry mutations in SCN5A, resulting in altered function of voltage-gated sodium ion channels, as well as affecting smooth muscle function and mechanical sensitivity [53]. Twin studies show higher concordance in monozygotic twins than in dizygotic twins [54]. However, it was found

that there is a higher probability of having a parent with IBS than a co-twin with IBS, suggesting that environmental factors are of great importance in relation to genetic factor [55]

Evidence of low-grade inflammation

An important observation when it comes to the role of immunologic factors and the risk of developing IBS is the association between onset of IBS and infectious gastroenteritis – post-infection (PI-) IBS [56]. Several studies show that gastroenteritis is the strongest known risk factor for developing IBS. The severity of tissue damage and immune activation seems important, and bacterial infections are more commonly implicated. As opposed to viral infections, which produce less tissue damage and appears to be associated with lower risk of PI-IBS in comparison with bacterial infections. The IBS-D subtype is most common, but all subtypes may result [12].

A large number of studies have now demonstrated some discrete immunologic abnormalities consistent with low-grade inflammation and/or abnormal immune function. An increased number of mast cells in the gut mucosa have been demonstrated, as well as the presence of increased levels of mast cell mediators, mainly tryptase, trypsin and histamine, are reported [57-59]. Interestingly, increased levels of local [60] and systemic pro-inflammatory cytokines [61] and an increased number of mast cells in close proximity to enteric nerve fibres has been correlated to the intensity of abdominal pain [62].

The activation of the immune system has been reflected by increased concentrations of cytokines in colonic mucosa, as well as an increase in release of pro-inflammatory cytokines from isolated peripheral blood mononuclear cells, especially in patients with IBS with diarrhoea [63]. High concentrations of these cytokines were associated with anxiety and

depression [63, 64]. Interestingly, supernatants from cultured peripheral blood mononucleocytes from patients with diarrhoea caused mechanical hypersensitivity when applied to mouse colonic afferent nerve endings [65]. Analysis of these mononucleocytes showed increased levels of interleukin-1B, interleukin-10, tumor necrosis factor-alfa, and interleukin-6 among others. The concentration of these cytokines appeared to be associated with frequency and severity of pain. The same investigators concluded that peripheral blood mononucleocyte supernatants from healthy controls had greater inhibitory effects on colorectal sensory afferent nerve endings in mouse models of visceral hypersensitivity, in comparison with mononucleocyte supernatants from patients with IBS. The main factor associated with the reduced inhibitory effects of the cells from patients with IBS was lower concentrations of B-endorphin [66]. Taken together, a number of findings suggests immune dysfunction as a putative mechanism for visceral hypersensitivity in IBS. An increased intestinal permeability in patients with PI-IBS has been postulated to have a mechanistic role mediated by alterations in tight junction proteins caused by mast-cell degranulation [67].

Additionally, activation of the humoral immunity has been suggested to be gastrointestinal-specific. Proliferation and activation of B-lymphocytes and immunoglobulin production in the jejunal mucosa has been reported in patients with IBS with diarrhea. The humoral activation appeared to be positively associated with number of bowel movements, loose stool form, and depression [68]. However, it is somewhat unclear whether these findings genuinely represent pathophysiological mechanisms or non-specific associations [49].

Transient gut inflammation may enhance visceral sensitivity through release of inflammatory mediators which results in increased firing of primary sensory afferent nerves, which in turn mediates peripheral sensitization [22].

In conclusion, there is accumulating evidence to support a role for low-grade inflammation in the pathogenesis of IBS [69].

Low-grade inflammation as a therapeutic target

The evidence supporting an inflammatory component in IBS served as a basis for studies investigating the effects of anti-inflammatory treatment. Despite this, treatment with prednisolone in patients with PI-IBS did not result in any symptom-reducing benefit compared with placebo treatment [70]. Mesalazine, or 5-Aminosalicylic acid (5-ASA), is an anti-inflammatory drug typically used for inflammatory bowel disease (IBD). Mesalazine exerts its anti-inflammatory effect through binding the peroxisome proliferator-activated receptor-gamma (PPAR-gamma), which through intracellular processes modulates the expression of genes involved in the inflammation process and reduces production of pro-inflammatory cytokines, mainly interleukin-1, interleukin-2 and tumor necrosis factor alpha (TNF-alpha) [71, 72]. Providing a rationale for studying its possible immunomodulatory effects in IBS. Other studies have used treatment with mesalazine without showing any favourable symptomatic outcomes compared to placebo treatment. However, they indicated that a subgroup of IBS patients may benefit from treatment with mesalazine [73, 74], which warrants further study to characterize these patients.

Aim

This project investigates the effect of mesalazine treatment on individual IBS-symptoms, as a secondary objective in the Mesalazine treatment in IBS (MIBS) trial, a randomised, double blind, placebo-controlled intervention trial of mesalazine treatment in patients with moderate to severe IBS.

Method

The MIBS-trial

The MIBS-trial is a randomised, double blind, placebo-controlled, multi-centre, parallel group, interventional study of mesalazine (Asacol®) treatment in IBS, including the evaluation of rectal inflammatory status using the mucosal patch technique. Eligible subjects were identified and recruited from outpatient clinics and local registries.

For selection, participants were required to fulfil the following inclusion criteria: Age 18-70 years, already diagnosed with IBS according to the Rome III criteria, IBS-symptom severity scale (IBS-SSS) [75] of at least 175 at randomization, which is equivalent to at least moderate symptom severity. All IBS subtypes were included and classified as IBS with predominant diarrhoea (IBS-D), IBS with predominant constipation (IBS-C), and IBS- with mixed or unspecified bowel habits (IBS-nonCnonD).

None of the following exclusion criteria were allowed to be met in order to participate: presence of a systemic inflammatory disease, other gastrointestinal disease likely to explain IBS symptoms or other significant somatic diseases as evaluated by the investigator, among them renal disease, considering the potential risk of nephrotoxicity by mesalazine. Treatment with non-steroid anti-inflammatory drugs, opioid analgesics or acetylsalicylic acid within 7

days prior to screening. Treatment with antibiotics, immunosuppressing drugs or other significant medical treatment that in the opinion of the investigator could compromise the objectives of the study within 28 days prior to screening. Previously confirmed allergy towards mesalazine or acetylsalicylic acid. Current infection. Pregnancy or lactation. History of, or current drug substance use disorder, including alcohol. Female of childbearing potential unwilling to use adequate contraceptive measures throughout the duration of the study.

Subjects were then randomized into receiving either 2400 mg mesalazine daily or corresponding placebo for the subsequent 8-week treatment period. The time course and specific events during the study is described in figure 1. Several variables regarding IBS symptom scores, endoscopic data, biopsies, blood samples, fecal samples and other data from questionnaires regarding global functioning and psychiatric symptoms were collected, but was not used for this project.

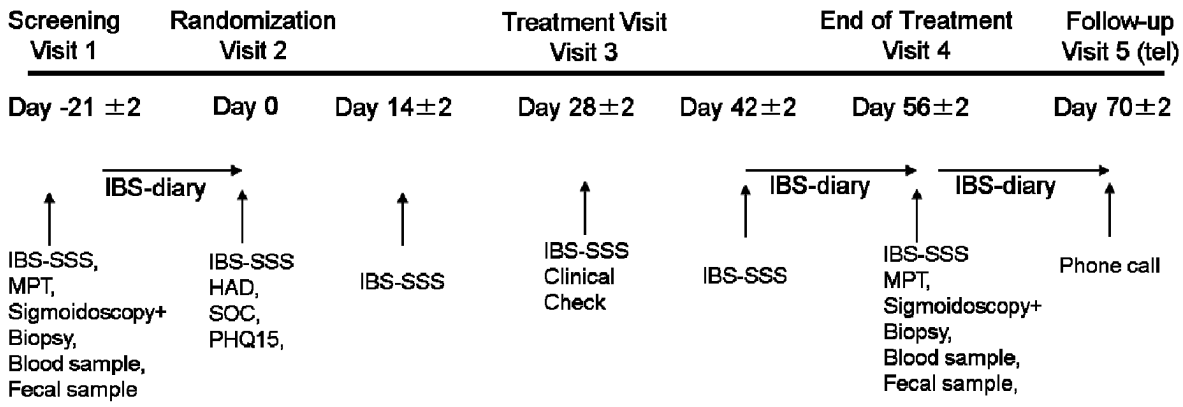

Figure 1. Time course for study participants

Effects on bowel habits and specific gastrointestinal symptoms

For the analysis in this degree project, IBS diaries from the first 45 patients were used. The first diary contains data from the 14 days prior to starting the intervention. The second diary contains data from the last 14 days of the intervention period. The third diary, with data from the 14 days post-intervention, was not used in this project. Instances of missing entries, incorrectly recorded entries, or unreadable entries were registered in the data file as missing data.

The IBS-diary was published in 1998 by Ragnarsson G and Bodemar G at Linköping University Hospital in Sweden. It is a validated method of recording symptoms related to IBS on a daily basis [76]. The variables that are registered by participants are: time of meals, nausea, abdominal pain, bloating, bowel movements, stool form, urgency, straining and whether complete bowel emptying was experienced or not. In addition to the number of events and their specific time of day, duration, intensity and localization of abdominal pain was recorded. Pain intensity was measured in a scale from one to three. With one being light tolerable pain, two being moderate pain, and three representing severe, unbearable pain. The duration of each event of nausea and bloating was also recorded. Stool form was recorded according to the Bristol stool form scale [77], a description of which was included on each page. In figure 2, a page of the IBS-diary is shown, which represents one day. The design also allows for registering the relationship between meals, symptoms and bowel habits.

Figure 2. The IBS diary

Data analysis and statistics

Demographic data is presented as means with standard deviations or proportions. Means were compared between two groups using the Students t-test, whereas nominal data were compared by use of the Pearson Chi-2 test. The following variables were calculated based on the 2-weeks of IBS-diary registrations before and at the end of mesalazine/placebo treatment: mean hours of nausea/day, mean hours of abdominal pain/day, mean number of abdominal pain episodes/day, mean daily stool form according to the Bristol Stool Form Scale, mean hours of

bloating/day. For each variable, the difference between diary 2 (end of treatment) and diary 1 (pre-treatment) was calculated, resulting in the delta value, followed by calculation of mean and median values. Days with missing data was taken into account when calculating the means. Statistical analysis was conducted using the software IBM SPSS, using comparison by non-parametric tests: Wilcoxon signed rank test for a within-group analysis and Mann-Whitney U test for a between-group analysis. The level of statistical significance was set at a P-value <0.05.

Ethical considerations

The study was approved by the Swedish Ethical Review Authority (reference number 2011/1793-31/2) as well as the Swedish Medical Products Agency (reference number 151:2011/91638).

Results

Subject characteristics

Baseline characteristics of the study participants are presented in table 4. There was a higher proportion of female than male participants enrolled in the study. Of notice is that the numerical difference regarding sex distribution in the mesalazine group and the placebo group was not statistically significant. No significant difference was seen in the IBS subtype distribution between the Mesalazine group and the placebo group. The symptom severity according to IBS-SSS was severe (>300) in both groups.

Table 4. Baseline characteristics of study participants

Characteristics	Mesalazine (n=24)	Placebo (n=21)	p value
Age, years (±SD)	48.9 (12.9)	46.7 (16.1)	1.000
Female sex, n (%)	14 (58.3)	17 (81)	0.121
IBS subtype			1.000
IBS-D, n (%)	12 (50)	10 (47.6)	
IBS-C, n (%)	4 (16.7)	4 (19)	
IBS non C or D, n (%)	8 (33.3)	7 (33.3)	
IBS-SSS (±SD)*	323.5 (72.3)	340 (53.2)	0.948

Data reported as number of patients (n) or mean ±SD.
*Collected at baseline visit

Missing data

In the first diary, there were no instances of missing data. However, in the second diary, for three participants, there was one, two and five missing days, respectively. Where no data was registered for these days. There were no other instances of missing data among the analysed variables.

Mesalazine treatment effects

a. Abdominal pain

As seen in tables 5 and 6, there was a numerical reduction in number of abdominal pain episodes as well as duration of abdominal pain in both the mesalazine-group and the placebo-group. In the between-group analysis, the reduction was not statistically significant in either the mesalazine group nor the placebo group. In the within-group analysis, a statistically significant reduction in abdominal pain episodes was seen in both the mesalazine-group ($p=0.012$) and the placebo-group ($p=0.009$). The within-group analysis also showed a significant reduction of abdominal pain duration in both the mesalazine-group ($p=0.013$) and the placebo-group ($p=0.012$).

b. Nausea

As seen in table 6, on a between-group level, there was a larger numerical reduction in median duration per day in the intervention group compared to the reduction in the placebo group, although neither changes were statistically significant. In the within-group analysis, there was no statistically significant change in neither the mesalazine-group ($p=0.716$) or the placebo-group ($p=0.973$).

c. Stool frequency

No significant change was seen regarding average daily number of bowel movements on a between-group level, as shown in table 6. This was also the case in the within-group analysis where no change was seen in neither the mesalazine-group (p=0.714) or the placebo-group (p=0.199).

d. Stool form

Regarding stool form, as seen with other variables in table 6, there was no significant change between the groups. The same outcome was seen in the within-group analysis, with no significant change in either the mesalazine-group (p=0.8) or the placebo-group (p=0.349).

e. Bloating

A larger numerical reduction was seen in the mesalazine group compared to the placebo group in the between-group analysis, however, neither were significant. In the within-group analysis, there was a statistically significant reduction of bloating in both the mesalazine-group (p=0.007), and the placebo-group (p=0.009).

Table 5. Group mean and median values for specific gastrointestinal symptoms in the mesalazine group and the placebo group

		Pre-treatment		End of treatment	
		Mean (SD)	**Median**	**Mean (SD)**	**Median**
Abdominal pain episodes (n)	Mesalazine	1.363 (1.361)	1.070	1.051 (1.326)	0.570
	Placebo	1.155 (0.775)	0.930	0.894 (1.129)	0.570
Abdominal pain duration (hours/day)	Mesalazine	4.943 (4.635)	3.607	3.179 (4.503)	1.036
	Placebo	4.248 (3.053)	3.571	3.313 (3.471)	2.000
Nausea duration (hours/day)	Mesalazine	1.298 (1.639	0.643	1.484 (2.770)	0.107
	Placebo	0.803 (1.189)	0.286	0.867 (1.639)	0.214
Average stools per day (n)	Mesalazine	1.904 (1.023)	1.600	1.763 (0.888)	1.550
	Placebo	1.581 (0.876)	1.400	1.757 (0.913)	1.400
Average stool form*	Mesalazine	4.319 (1.110)	4.356	4.228 (1.017)	4.167
	Placebo	3.849 (1.271)	3.714	3.967 (1.098)	4.231
Duration of bloating (hours/day)	Mesalazine	8.336 (7.120)	6.500	5.353 (6.368)	2.286
	Placebo	7.027 (4.860)	6.357	5.622 (5.475)	4.214

Data presented as average number of episodes (n) or duration in hours per day (hours/day).
Mesalazine group n=25
Placebo group n=21
*As measured with the Bristol Stool form scale.

Table 6. Treatment effects of mesalazine and placebo on specific gastrointestinal symptoms

		Mean change between end of treatment and pre-treatment (SD)	Median change between end of treatment and pre-treatment (SD)	P value (within-group analysis)	P value (between-group analysis)
Abdominal pain episodes (n)	Mesalazine	- 0.312 (0.687)	- 0.395	0.012	0.723
	Placebo	- 0.261 (1.03)	- 0.210	0.009	
Abdominal pain duration (hours/day)	Mesalazine	- 1.764 (3.43)	- 1.557	0.013	0.207
	Placebo	- 0.935 (1.598)	- 0.500	0.012	
Nausea duration (hours/day)	Mesalazine	0.186 (1.867)	- 0.071	0.716	0.428
	Placebo	0.064 (0.700)	0.000	0.973	
Average stools per day (n)	Mesalazine	- 0.141 (0.814)	- 0.050	0.714	0.284
	Placebo	0.176 (0.585)	0.000	0.199	
Average stool form*	Mesalazine	- 0.092 (0.874)	0.017	0.8	0.436
	Placebo	0.117 (0.949)	0.228	0.349	
Duration of bloating (hours/day)	Mesalazine	- 2.983 (5.533)	- 1.892	0.007	0.915
	Placebo	- 1.405 (3.122)	- 1.928	0.009	

Data presented as average number of episodes (n) or duration in hours per day (hours/day).
Mesalazine group n=24
Placebo group n=21
*As measured with the Bristol Stool form scale.

Discussion

This degree project assessed the effect of mesalazine on specific IBS symptoms, compared to placebo, after 8 weeks of treatment. This was done as part of a double-blinded, randomized, placebo-controlled trial. The symptoms included in the analysis were abdominal pain frequency and duration, duration of nausea, stool frequency and stool form, and bloating. Comparison was made both in a within-group analysis as well as on a between-group level. Within-group analysis in both the placebo group and the mesalazine group showed a significant symptom reduction in frequency and duration of abdominal pain, and bloating, but not regarding duration of nausea, stool frequency, and stool form. In the between-group comparison, there was no significant change in any symptom domain. Therefore, based on the results from this degree project, there was no significant benefit from treatment with mesalazine compared with placebo.

A previous randomized control trial of 185 patients with IBS according to the Rome III criteria, published in 2016 by Barbara et al.[73], used satisfactory relief of abdominal pain/discomfort for at least 50% of the 12-week treatment as the primary endpoint. Their secondary endpoint was satisfactory relief of overall IBS symptoms. They concluded that in a subgroup of patients, there was a statistically significant improvement, however, they were not able to further characterize this subgroup. The use of more precise outcome measures in this degree project allows for assessment of the treatment effect on specific IBS symptoms, which in the case of a statistically significant improvement would allow for further demographic and symptomatic characterization of these patients. In 2016, another randomized control trial was published by Lam C. et al.[74] on 136 patients with IBS-D according to

Rome III criteria. They used daily average stool frequency as their primary outcome measure. Their secondary outcome measures were abdominal pain, stool consistency, urgency and satisfactory relief of IBS symptoms, they found no significant change in either of the symptom categories compared to placebo. But looking at the 13 patients with PI-IBS, they found a significant reduction in abdominal pain severity, urgency and stool consistency. Out of the patients included in our project, only 5 patients had an onset consistent with PI-IBS. Therefore, a subgroup analysis of these would not be meaningful without adding data from a larger part of the study material. However, considering previous studies, it would be of interest to study the effects on patients with PI-IBS, both compared to treatment response in other IBS subtypes, as well as to placebo treatment. Overall, our results seem to be in line with the abovementioned previous studies on mesalazine treatment for IBS.

Methodological considerations

Interestingly, looking at our between-group analysis, specifically regarding duration of abdominal pain and duration of bloating, a larger numerical reduction can be seen in the mesalazine group compared to the placebo group. This may indicate positive effects that did not reach statistical significance, suggesting a type 2 error. One possibility is that this is due to the small sample size (n=45), which was limited by the time frame allowed for this degree project. However, data from another 113 participants is available, including IBS diaries from the 2 weeks following end of treatment. Noteworthy is that we have not yet studied the effects of mesalazine on abdominal pain intensity due to the time restraints. With future use of this data, it would also be worth exploring whether a reduction in pain intensity was seen.

The sample size (n=45) is considerably smaller in comparison with the previous studies by Barbara G. et al[73] (n=185) and by Lam C. et al.[74] (n=136). However, there is little missing data, with missing days for only three participants. The data in the IBS-diary is registered daily and requires relatively short recall, which in part mitigates recall bias. However, this cannot be completely eliminated due to the nature of this method. In general, a strong placebo response was seen, which, in addition to administration of the placebo-medication, may also be attributable to the physician visits, extensive investigations and frequent follow-ups. Similarly, a strong placebo effect was also noted by Lam C. et al. [74]. This aspect may be taken into consideration when designing future studies.

There was a higher proportion of females to males, consistent with epidemiological data [7]. This seems to be a recurring phenomenon in studies on IBS, including the abovementioned intervention studies. The implications of which is that there may be slightly less knowledge about IBS in males.

Is there a future for treatment with mesalazine in IBS?

Despite these results, the beforementioned evidence of low-grade inflammation in IBS remains. The question is whether an anti-inflammatory therapeutic approach still is feasible. As previously mentioned, mesalazine exerts its anti-inflammatory effect through binding the peroxisome proliferator-activated receptor-gamma (PPAR-gamma), which through intracellular processes modulates the expression of genes involved in the inflammation process and reduces production of pro-inflammatory cytokines, mainly interleukin-1, interleukin-2 and tumor necrosis factor alfa [71, 72]. Since these increased levels of these cytokines have been demonstrated in IBS [61], particularly locally in PI-IBS [60]. It could

simply be that the treatment duration was not sufficient and that longer time is needed for remodulating gut-brain interaction. Questioning this hypothesis are the two previous studies with mesalazine in IBS [73, 74] which both had a longer treatment duration of 12 weeks. Another aspect is that mesalazine could be missing the target, e.g. the increased levels of mast cells and mast cell mediators that has been reported to correlate with abdominal pain [58, 62]. In a rat study, the mast cell stabiliser disodium cromoglycate reversed colonic hypersensitivity [78]. One study of 60 patients with IBS showed decreased visceral hypersensitivity with significantly decreased IBS symptoms, including abdominal pain, after 8 weeks of treatment with the mast cell stabiliser ketotifen [79]. Apart from further studies on mast cells stabilizers alone, one could consider the idea of treatment with both mesalazine and a mast cell stabiliser, studying the effect on combination treatment compared with each treatment alone to see if a synergistic effect would be achieved.

Other factors generating or maintaining inflammation in IBS may be alterations in gut microbiota [46-48], which would not be primarily affected by mesalazine. A recent study of fecal microbiota transplantation for patients with IBS showed promising results [80], providing further support for the role of gut microbiota in IBS.

Previous studies have indicated that female sex hormones may affect the regulatory mechanisms of the gut-brain axis, as well as immune activation and gut microbiota. In females, higher levels of tumor necrosis factor-alpha, increased number of colonic mucosal mast cells, and decreased number of T cells was seen compared to males. However, in males there was increased levels of interleukin-10 [81-83]. Further studies are needed to investigate whether treatment aimed at individualized cytokine profiles can provide new treatment opportunities.

Conclusions

In this degree project, there was no benefit from treatment with mesalazine compared to placebo regarding bowel habits and specific IBS symptoms in patients with moderate to severe IBS. It is noteworthy that the limited sample size may have masked potential positive treatment effects. Therefore, a next step to provide clarification into this would be an analysis of the entire study database of 158 participants.

Populärvetenskaplig sammanfattning

Irritable bowel syndrome (IBS) – irritabel tarm är en vanligt förekommande mag-tarmsjukdom. Den ger långvariga besvär med återkommande buksmärtor i kombination med avföringsbesvär som diarré, förstoppning eller både och. Andra vanliga symtom från buken är en känsla av uppblåsthet, besvärande gaser, slem i avföringen och en känsla av att inte kunna tömma tarmen helt. Det är också vanligt med besvär från övre mag-tarmkanalen så som illamående. Symtomen varierar mellan olika individer.

Orsaken till IBS är en kombination av flera olika faktorer som bidrar till ett dysfunktionellt samspel mellan hjärna och tarm. Det finns belägg för att en låggradig aktivering av immunförsvaret i tarmvävnaden är en av dessa bidragande faktorer. En av de viktigaste beläggen för detta är att IBS kan debutera efter en mag-tarminfektion. Varför det är av intresse att ta reda på om läkemedel riktade mot immunförsvaret, så kallade antiinflammatoriska läkemedel, har någon effekt mot IBS-symtom.

Detta projekt syftar till att undersöka just detta. Alltså om specifika symtom vid IBS kan lindras av behandling med mesalazin, ett antiinflammatoriskt läkemedel som vanligtvis används vid inflammatorisk tarmsjukdom. Projektet är del av en större studie där man också tittade på flera andra utfall. Deltagarna slumpades till att antingen erhålla behandling med mesalazin, eller verkningslösa tabletter, så kallat placebobehandling, utan att veta vilket av dessa de fick. Under två veckor före behandlingen samt under de två sista behandlingsveckorna fick deltagarna fylla i en särskild IBS-dagbok, där man för varje dag

registrerar symtom och tarmtömningar. De symtom som detta projekt avsåg var illamående, buksmärta, uppblåsthetskänsla i buken, tarmtömningar och avföringskonsistens. IBS-dagboken vid slutet av behandlingen jämfördes med dagboken innan behandlingen för att se om symtomen förbättrades i mesalazingruppen jämfört med placebogruppen.

Resultat från 45 deltagare analyserades och visade att det inte fanns någon signifikant skillnad mellan effekten av mesalazin jämfört med placebo avseende ovannämnda IBS-symtom. Dagböcker från fler deltagare finns tillgängliga, men alla dessa kunde inte analyseras på grund av den begränsade tidsrymden för detta projektarbete.

Således hittades i detta projekt inte någon betydande effekt av mesalazinbehandling mot specifika symtom vid IBS. Men resultaten avseende vissa av symtomen, som buksmärta och uppblåsthetskänsla indikerar att dagböcker från fler deltagare krävs för att klargöra om behandlingen faktiskt kan ha effekt.

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