Randomized Controlled Treatment Trial of Irritable Bowel Syndrome with a Probiotic E.-coli Preparation (DSM17252) Compared to Placebo

Eine randomisierte, kontrollierte Behandlungsstudie beim Reizdarmsyndrom mit einer probiotischen E.-coli-Präparation (DSM17252) im Vergleich zu Placebo

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Schlüsselwörter
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- irritable bowel syndrome
- probiotics
- gastroenterology
- primary care

Zusammenfassung

Hintergrund: Therapiestudien mit bakteriellen Produkten (Probiotika) haben beim Reizdarmsyndrom (RDS) bislang widersprüchliche Ergebnisse erbracht, und E.-coli-Präparate wurden bislang nicht eingesetzt.

Methoden: Bei insgesamt 298 Patienten mit Symptomen im unteren Gastrointestinaltrakt, die als Reizdarmsyndrom diagnostiziert worden waren, wurden doppelblind und randomisiert für 8 Wochen mit dem Probiotikum Symbioflor®-2 (Symbiopharm GmbH, Herborn), einem Escherichia-coli-Produkt (N = 148) oder mit Placebo (n = 150) eingesetzt. Die Patienten wurden wöchentlich von ihrem Hausarzt gesehen, der das Vorhandensein zentraler RDS-Symptome überprüfte. Ein abdomineller Schmerzscore (APS) und eine allgemeiner Symptomscore (GSS) waren die primären Endpunkte der Studie und als Responder wurden diejenigen gewertet, die an mehr als einem Zeitpunkt keine Symptome mehr aufwiesen.

Ergebnisse: Die Response-Rate betrug 27/148 (18,2%) für das Medikament und 7/150 (4,67%) für Placebo im Hinblick auf den GSS (p = 0,000397); die entsprechende Effektivität für den APS war 28/148 (18,9%) für Symbioflor und 10/150 (6,67%) für Placebo (p = 0,001649). Post-hoc fanden sich keine Unterschiede in der Medikamentenwirksamkeit zwischen den Geschlechtern und in unterschiedlichen Altersgruppen.

Schlussfolgerung: Die Behandlung des RDS mit dem probiotischen Medikament Symbioflor-2 ist wirksam und einer Placebobehandlung überlegen; sie reduziert die typischen RDS-Symptome bei diesen Patienten, die von Gastroenterologen und Allgemeinärzten gesehen werden.

Abstract

Background: Therapy trials with bacterial compounds in irritable bowel syndrome (IBS) have produced conflicting results and, so far, an E.-coli preparation has not been used.

Methods: Two hundred and ninety-eight patients with lower abdominal symptoms diagnosed as IBS were treated for 8 weeks by the compound Symbioflor®-2 (Symbiopharm GmbH, Herborn, Germany), an Escherichia coli product (N = 148), or placebo (n = 150) in a double-blinded, randomized fashion. Patients were seen weekly by the physician, who assessed the presence of core IBS symptoms. Both an abdominal pain score (APS) as well as a general symptom score (GSS) were used as primary endpoints. Responders had to have complete absence of IBS core symptoms at ≥ 1 visit during treatment.

Results: The responder rate in GSS to the drug was 27/148 (18.2%) in comparison to placebo with 7/150 (4.67%) (p = 0.000397). The improvement in APS was 28/148 (18.9%) and 10/150 (6.67%) for placebo (p = 0.001649). The response was reached from visit 3 onwards with both medication and placebo. Post-hoc analysis revealed no significant differences in efficacy of the drug between the gender and different age groups.

Conclusion: Treatment of IBS with the probiotic Symbioflor-2 is effective and superior to placebo in reducing typical symptoms of IBS patients seen by general practitioners and by gastroenterologists.

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Introduction

Between 1989 and today, a number of randomized and placebo-controlled trials have established the efficacy and value of probiotics in the treatment of IBS but without being able to reliably identify the mechanisms of action [1]: it may be either via the direct action of the bacteria on the local immune system, via systemic immunologic actions, or indirectly via interfering with the local bacterial flora [2].

In all these studies, diffferent probiotic compounds, but mostly various strains of lactobacillae [3–7], bifidobacteria [8, 9] or a mixture of thereof [2, 10–13] have been utilized; a single study [14] used inactivated Streptococcus faecalis bacteria. In contrast, an E. coli Nissle-type probiotic was used in chronic constipation and IBS mostly in uncontrolled studies [15, 16] while only one study used a placebo control group to verify its efficacy [17].

As we could show recently [18], a mixture of E. coli (DSM17252) and Enterococcus faecalis (DSM16440) was highly effective in the treatment of the irritable bowel syndrome as compared to placebo. In this case, however, the bacterial lysate used contained 1.5 to 4.5×10^7 CFU per mL of living bacteria at the time of their mixture only. After mixing both strains, the compound was immediately exposed to heat and pressure (via autoclave), and was sterile thereafter. This raised an issue that cannot be answered currently, related to the potential mechanism of action: preliminary unpublished data indicate that in vitro the cytokine expression of both strains differ from the cytokine expression of the mixed compound.

Most controlled clinical trials in IBS have been performed in gastroenterological outpatient settings. It has, however, been noted [19–21] that knowledge and acceptance of these gastroenterological criteria for functional bowel disorders such as the Rome criteria for IBS [22, 23] are not well developed among primary care physicians. On the other hand, family physicians have established their own criteria for the diagnosis of IBS over the past 20 years, that differ to some degree from those of gastroenterologists: These so-called WONCA criteria were established in 1983 [24] in parallel to the Rome consensus process [25], and they were last updated in 1998 [26]. These criteria are less restrictive and easier to handle in everyday practice.

The study we report here was conducted in primary care in 1988 and 1989, i. e., after the Manning criteria had been published [27] but before the first version of the Rome criteria for the diagnosis of IBS [28] was available. It was re-analyzed based on more restricting criteria that were released by FDA and EMEA [29, 30] to test whether the initially recorded efficacy [31] that had initiated registration of the compound (approved in Austria on November 10th, 2000, Reg. No. 1–23846 and Switzerland by SWISSMEDIC on October 4th, 2005, Reg. No 00675, still pending in Germany) [32] would be preserved under these circumstances.

Different from the Rome II responder definition in current GI pharmacological testing [33], responders had to report none of the core IBS symptoms to be present at the study termination.

The bacterial strain used here was an Escherichia coli (DSM 17252) that has been demonstrated to survive the gastric passage and was identified in respective stool samples [34]. To the best of our knowledge this is the first report of an E. coli probiotic in the treatment of IBS.

Patients and Methods

Two hundred and ninety-eight patients with the diagnosis of IBS according to the criteria of primary care physicians [19] were recruited in 12 primary care centres between August 1988 and February 1989. The study was conducted according to German national legal requirements (§41 [3], Arzneimittelgesetz, as of July 20th, 1988), the protocol had been approved by the local ethical committee of the physician organisation (Landesärztekammer

	symptoms	ICHPPC-2 (WONCA)[1]	Manning	Rome I/II/III
		Primary Care	Gastroenterology	
1	lower GI pain, spontaneous	yes	yes	yes
2	pain, spontaneous, diffuse	yes	yes	yes
3	lower GI ain, w/palpation	yes	no	no
4	altered stool consistency	yes	yes	yes
5	altered stool frequency	yes	yes	yes
6	palpable, tender sigma	yes	no	no
7	bloating	yes	yes	yes
8	upper abdominal pain, spontaneous	yes	yes	yes
9	gall bladder pain w/palpation	no	no	no
10	pain prior to a meal	no	no	no
11	pain after a meal	no	no	no
12	pain at night	no	no	no
13	upper abdominal pain w/palpation	no	no	no
14	heartburn	no	no	no
15	belching	no	no	no
16	nausea	no	no	no
17	vomiting	no	no	no
18	bowel sounds	no	no	no
19	meteorism	no	no	no
20	headache	no	no	no
21	depression	no	no	no
22	sleeplessness	no	no	no

Table 1 Symptoms evaluated that matched or did not match IBS criteria in primary care and in gastroenterology.

[1] International Classification of Health Problems in Primary Care (ICHPPC) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians (WONCA).

Hessen, Germany), and patients had given written informed consent prior to participation.

Initial entry criteria were patients of both genders aged 18 to 70 years, who had been treated for IBS symptoms during the past year, who presented with abdominal pain, had a minimum score of 44 points on the Kruis scale [34], and who were willing to refrain from any spasmolytics medication during the course of the study. Exclusion criteria were the absence of abdominal pain, the presence of an organic origin of the symptoms, acute cholecystitis or post-cholecystectomy symptoms, acute pancreatic inflammation, a medical history in liver damage, ileus, severe chronic diseases of any kind, acute fever, cachexy, patients who had taken in spasmolytics during the past 7 days, pregnancy, a Kruis score less that 44 points, patients who were not fully legal competent, and patients evidently unable to cooperate in the trial.

They were assessed during an initial doctor's visit of the patients. Patients identified as having IBS were then randomized to receive either Symbioflor®2 (10 drops = 0.75 mL t.i.d. as an oral liquid during the first week, 20 drops t.i.d. for weeks 2 to 8) or placebo, identical in taste and texture in a double-blinded fashion for 8 weeks. Symbioflor-2 is an Escherichia coli (DSM 17252) preparation, 1 mL containing 1.5 to 4.5×10^7 CFU of living bacteria.

Symptoms were assessed at days 0, 7, 14, 21, 28, 35, 42, 49 and 56 – by interview during a doctor's office visit – to verify improvement or not. The initial evaluation assessed the presence of the core IBS criteria and a number of other symptoms (**Table 1**), but was based on the criteria of the International Classification of Health Problems in Primary Care (ICHPPC) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians (WONCA) [24, 26] and not the gastroenterological set of symptoms proposed by Manning et al. [27] or by the first version of the Rome criteria [28].

Patients had to have abdominal pain, and altered bowel habits (diarrhoea, constipation, or both alternating) as well as bloating from the list of symptoms recorded. To comply with the EMEA request [30] for two primary endpoints (global assessment and pain), a general symptom score (GSS) as well as an abdominal pain score (APS) was computed. A responder was defined as no longer having IBS symptoms for GSS and APS separately (dichotomous score).

Responders and non-responders were compared for drug efficacy by conventional statistical measures, including Fisher's exact test (chi-square test) for overall efficacy. The analysis was repeated for secondary endpoints of the study: these included the individual symptoms that contribute to the IBS diagnosis with current diagnostic standards, and the symptoms that are not core symptoms of the IBS definition but were recorded as well during the study.

Data analysis is based on the intent-to-treat (ITT) population, with the last value carried forward in case of drop-outs. Assuming a placebo responder rate of 50%, 125 patients per group would have been necessary to detect an increase of the responder rate by 20% with an alpha of 0.05 and a power of 0.85.

Post-hoc analyses were carried out to estimate the importance of the following factors for the overall assessment of drug efficacy: centre effects, gender effects, and age.

All data are given as mean ± SEM. For all tests, a level of 5% was set to indicate significance.

Results

From the initially seen 318 patients, 298 were recruited while 20 were excluded as non-IBS patients. Seven of the 298 patients (4 in the drug and 3 in the placebo arm) dropped out during the course of the study; In 76 cases, incomplete data were available, mostly due to missing reports on the primary endpoint variables so that 214 completed the trial (per protocol sample, PP) (**Fig. 1**). Patients in both arms of the study were comparable with respect to basic demographic data (**Table 2**).

All but two patients in the drug group reported abdominal pain and diarrhoea or constipation. A differentiation between diarrhoea-predominant and constipation-predominant was not done, but n = 113 patients in the medication group (76.4%) and n = 112 patients in the placebo group (74.7%) reported alternat-

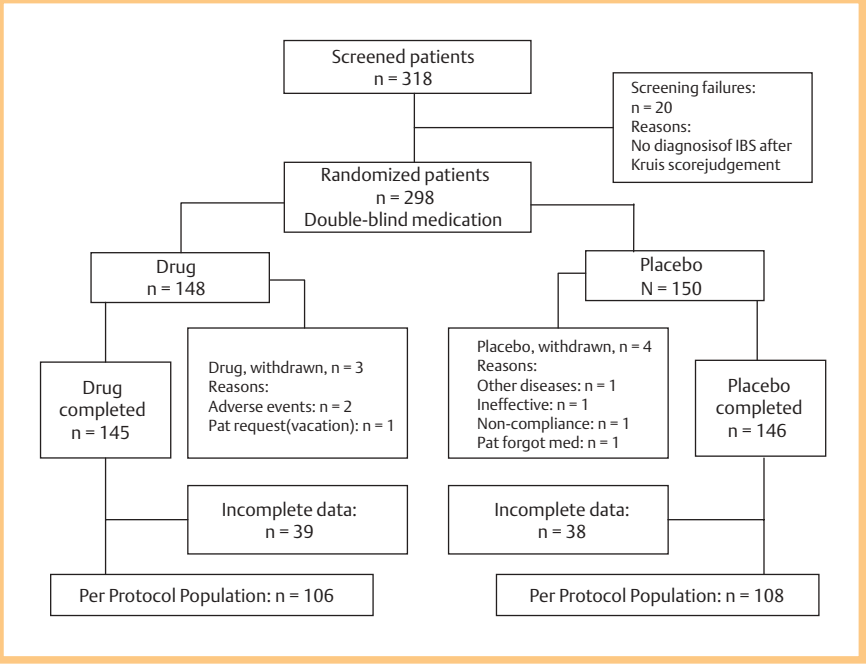

Fig. 1 CONSORT scheme of patient selection and distribution.

ing symptoms of constipation and diarrhoea. Median symptom duration was 3 years in both groups (95% CI: 2 – 4 years) but was maximal 22 years in the medication group, and 20 years in the placebo group.

Chronic comorbid conditions included hypertension, diabetes, low back pain and others. They were equally present in the drug and the placebo group (**Table 3**). Approx. 34% of all patients in the drug arm and 39% of patients in the placebo arm had at least one comorbid condition.

Adverse events (AE) that occurred during the study are listed in **Table 4**. The most frequent AE were fatique (n = 28), pruritus (n = 12), and diarrhoea (n = 9), that all occurred equally in the drug and placebo arms of the study. Intestinal symptoms occurring may be a flare of the IBS symptoms rather than a true AE. In one case a moderate gastrointestinal adverse event (nausea) and in another case a severe skin response (exanthema) occurred leading to trial discontinuation, both in the drug arm of the study. The reasons for discontinuation of the trial in all 7 cases (4 with the drug and 3 with placebo) are given in **Fig. 1**.

Drug efficacy in the ITT population

Based on the responder definition, 27/148 (18.2%) patients were symptom-free at visit 9, while the response rate in the placebo arm was 7/150 (4.67%) (p = 0.000397) for the GSS. For APS, the response rate was 28/148 (18.9%) for the drug and 10/150 (6.67%) for placebo (p = 0.001649). The response was reached from visit 3 onwards with both medication and placebo, but did not improve any further with placebo after visit 7 (**Fig. 2**).

The individual symptoms improved in parallel to GSS and APS, and superiority of the drug over placebo for all but one typical IBS symptom, while some non-IBS symptoms did not respond to drug treatment (**Table 5**).

Post-hoc analysis revealed no significant differences in efficacy of the drug between the gender and age (**Table 6**), but responder rates tended to be higher in males and with younger age.

Discussion

Recently published trials [5 – 7] have demonstrated the overall superiority of probiotic compounds in comparison to placebo in the treatment of IBS, while studies published before 2005 usually were unable to verify this [1]. This was in part due to the fact that small sample sizes usually result in high variabil-

Table 2 Demographic data of patients in the placebo and the drug arm of the study.

	drug	placebo
no	148	150
females	72	75
mean age (range), years	49.8 (19 – 70)	49.4 (18 – 76)
mean weight (range), kg	69.3 (46– 100)	68.9 (44 – 109)
BMI (range), kg/m^2	24.4 (17.2 – 37.6)	24.0 (17.1 – 33.2)
medication during study	–	n = 1

Table 3 Comorbidity in the drug and placebo arms of the study Note that multiple comorbidities were possible in individual cases. Approx. 34% of all patients in the drug arm and 39% of patients in the placebo arm had at least one comorbid condition.

	number of cases of comorbid diseases		
organ Class	drug	placebo	total
cardiovascular	15	27	42
musculosceletal	6	15	21
allergic	2	0	2
skin responses	0	2	2
endocrine	8	10	18
psychiatric/neurological	8	3	11
respiratory	4	4	8
intestinal	2	1	3
metabolic/nutritional	8	4	12
urogenital	6	5	11
missing	3	1	4
total	62	72	134

Table 4 Adverse events (AE) occurring in the drug and placebo arms of the study. Note that intestinal symptoms occurring may be a flare of the IBS symptoms rather than true AE.

	number of adverse events		
organ Class	drug	placebo	total
general dysfunction	15	16	31
skin responses	12	11	23
psychiatric/neurological	6	4	10
digestive system	15	9	21
total	48	40	88

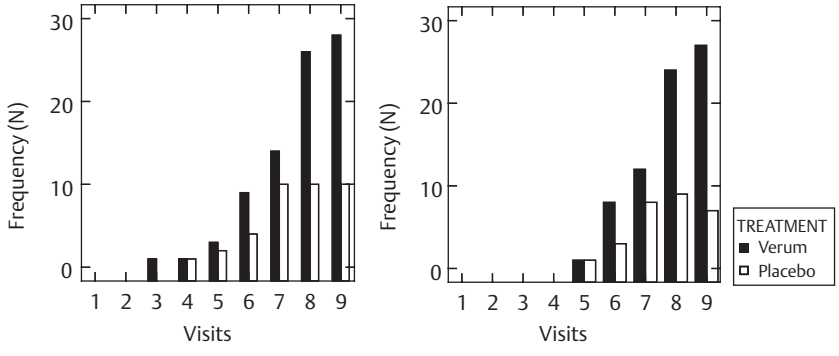

Fig. 2 Number of patients responding (= symptom-free) with drug and placebo for the primary endpoint global symptom score (GSS) (left panel) and for the abdominal pain score (APS) (right panel) at the different clinical visits.

Table 5 Response (% change of symptom, dichotomous rating) with drug or placebo for IBS typical and non-typical symptoms (Fisher's exact test).

IBS symptoms	drug (n = 148)	placebo (n = 150)	statistics
lower abdominal pain, spontaneous	42	26	p = 0.013345
diffuse pain, spontaneous	30	20	p = 0.007207
pain during palpation, gall bladder	51	23	p = 0.000059
pain during palpation, colon	32	19	p = 0.000059
stool consistency	43	28	p = 0.001671
stool frequency	6	3	n. s.
palpable colon	25	4	p = 0.000145
bloating	23	9	p = 0.000815
Non-IBS symptoms			
upper abdominal pain, spontaneous	46	21	p = 0.000008
pain prior to a meal	46	31	p = 0.012330
pain after a meal	39	24	p = 0.008542
pain at night	44	31	n. s.
upper abdominal pain with palpation	42	25	p = 0.002156
heartburn	34	31	n. s.
belching	40	30	n. s.
nausea	52	42	p = 0.0334808
vomiting	26	14	p = 0.0092114
bowel sounds	35	20	p = 0.0028793
meteorism	29	18	p = 0.0207328
headache	51	29	p = 0.0002324
depression	32	24	n. s.
loss of appetite	35	20	p = 0.0028793
sleep disturbances	37	20	p = 0.0012882

Table 6 Post-hoc analysis of subgroups of the ITT population on the GSS responder rate (%) for drug and placebo.

	drug	placebo	statistics
gender			
males	22.6	7.2	p = 0.0233741
females	15.5	2.5	p = 0.0054129
age			
< 40 years	28.1	13.9	n. s.
40 to 60 years	16.9	2.4	p = 0.0015779
> 60 years	11.1	0	n. s.

ity of the placebo response [35] and carry the risk of failure. This is well in line with the large-scale study reported here, that was conducted between August 1988 and February 1989. When analyzed according to current standards of IBS definitions [36] and trial requirements [37], but with a more restricted responder definition to account for a potential post-hoc analysis bias, it showed high efficacy of the compound, a living E. coli preparation [38] in comparison to placebo.

In vitro immunological and in vivo functional properties of bacterial products have been shown [38, 39] also in IBS patients [2]. To the best of our knowledge this is the first double-blinded, randomized and placebo-controlled study in a large population of IBS patients using an E. coli strain. Since different bacteria may exhibit different functional consequences [38], one cannot conclude from the present data on the mechanisms of action, but mediation through the intestinal immune system as well as direct effects on gut function seem possible [39], but direct interactions with the stationary colonic bacteria cannot be excluded.

However, re-analyses such as ours have methodological limitations that need to be acknowledged. One is that data that are required for IBS studies nowadays and that have not been recording previously are limiting the comparability of results to recently analysed and published data. This refers, e. g., to IBS symptom duration and severity that are prerequisites of current IBS studies but are missing in our data set. Others are assessment of symptoms by physicians rather than the currently used "subjective global assessment" of symptoms and symptom changes by the patient [35]. To compensate for such limitations, the threshold for being a responder was elevated: compared to currently performed trials where patients have to show "at least 50% symptom improvement" [35], the analysis used the criterion "symptom-free" to define a responder. In consequence of this, the response rates were substantially lower both in the drug and the placebo arm of this study but the gain above placebo [14%] was significant and in the range of what has been reported for other compounds in IBS [40, 41]. In a second study reported recently [18] we could demonstrate that analysing the data according to current responder definition would not corroborate this advantage of the drug above placebo.

Other limitations of our evaluation are related to diagnostic differentiating that is done nowadays but was not routine at the time of the study performance: symptomatic differentiation between diarrhoea- and constipation-predominant IBS, diagnostic validation of small intestine bacterial overgrowth and lactose and fructose malabsorption, and assessment of a history of intestinal infection (to verify post-infectious IBS) may have improved the overall outcome from currently 18 to 50% and more, at least in selected IBS subgroups.

While more and more probiotic compounds become available on the market or are seeking approval, their specific mechanisms of action in various clinical conditions remain obscure. Based on the reviewed and meta-analysed data, bifido bacteria, lactobacillus, and a mixture of different bacterial strains have been shown to be effective in clinical conditions such as IBS; E. coli preparations, in contrast, have so far only been evaluated in non-controlled trials. This casts some doubts on the overall rationale for their use, unless a specific

mechanism of action – via the intestinal immune system, the enteric nervous system, or otherwise – has been demonstrated to operate. A recent IBS trial [42] that attempted to do this was unable to identify a mechanism (e.g., via short-chain fatty acid modulation) but speculated that efficacy must be due to factors other than the presence of induced microbiota itself; this is further supported by another study [18] in which a sterile bacterial lysate was used that may have elicited its mechanisms of action via direct interaction with the immune system rather than via interaction with the local bacterial colony. However, their low profile for adverse events and their high acceptance in patients may justify their clinical use even though the basic scientific knowledge of their mode of operation is still lacking [43].

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