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Double-blind comparison of an oral Escherichia coli *preparation and mesalazine in maintaining remission of ulcerative colitis*

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SUMMARY

Background: Aminosalicylates are used as standard treatment for maintaining remission in ulcerative colitis. As yet, there is no other existing alternative with proven efficacy. In light of the hypothesis that the intestinal environment may contribute to the pathophysiology of ulcerative colitis, a trial was conducted to test the effects of probiotic treatment with an oral preparation of non-pathogenic *E. coli*.

Methods: A total of 120 patients with inactive ulcerative colitis were included in a double-blind, double-dummy study comparing mesalazine 500 mg t.d.s. to an oral preparation of viable *E. coli* strain Nissle (Serotype 06: K5: H1) for 12 weeks with regard to their efficacy in preventing a relapse of the disease. Study objectives were to assess the equivalence of the clinical activity index (CAI) under the two treatment modalities and to compare relapse rates, relapse-free times and global assessment.

Results: The start and end scores of the CAI demonstrated no significant difference ($P = 0.12$) between the two treatment groups. Relapse rates were 11.3% under mesalazine and 16.0% under *E. coli* Nissle 1917 (N.S.). Life table analysis showed a relapse-free time of 103 ± 4 days for mesalazine and 106 ± 5 days for *E. coli* Nissle 1917 (N.S.). Global assessment was similar for both groups. Tolerability to the treatment was excellent and did not differ. No serious adverse events were reported.

Conclusions: From the results of this preliminary study, probiotic treatment appears to offer another option for maintenance therapy of ulcerative colitis. Additional support is provided for the hypothesis of a pathophysiological role for the intestinal environment in ulcerative colitis.

INTRODUCTION

Chronic treatment with aminosalicylates is well-established for maintaining remission in patients with ulcerative colitis (UC).[1, 2] However, there is considerable intolerance—not only to classic aminosalicylate sulphasalazine[3] but also to sulphur-free compounds such as mesalazine or olsalazine.[4] In such patients, there is no effective drug therapy for relapse prevention.

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Although the aetiology of UC is still unknown, it has been hypothesized that the intestinal environment plays a significant pathophysiological role.[5] High numbers of pathogenically adhesive and enterohaemorrhagic *Escherichia coli* (*E. coli*) have been reported for UC.[6, 7] The presence of pathogenic *E. coli* strains is reciprocally related to the number of non-pathogenic *E. coli* bacteria.[8] Thus, therapeutic colonization with non-pathogenic *E. coli* may be beneficial for the course of UC. Here, a trial is presented that investigates the therapeutic effects of oral administration with viable organisms of the non-pathogenic *E. coli* strain Nissle 1917,

which has been shown to act antagonistically towards different enteropathogenic bacteria and to colonize the intestine.[9, 10]

PATIENTS AND METHODS

A randomized, double-blind, double-dummy study was conducted on an out-patient basis in hospitals and private practice settings in Germany, the Czech Republic and Austria. A total of 120 patients were recruited. Eligibility criteria were: age older than 17 years, and the presence of chronic UC, previously diagnosed by endoscopic and histological criteria and now in remission as determined by the clinical activity index (CAI).[11] Exclusion criteria were: active UC, infectious colitis, existing or intended pregnancy, any other medication for UC besides the study drugs, antibiotics or sulphonamides, substantial cardiac, hepatic or renal disease, major operations on the bowels, and known intolerance to salicylates. The study was ethically approved by the Freiburger Ethik Kommission and written informed consent was obtained from all participants.

Patients received either 500 mg mesalazine t.d.s. (Salofalk, Dr Falk Pharma GmbH, Freiburg, Germany) 1 h before meals, plus a placebo indistinguishable from the *E. coli* preparation, or 200 mg/day (day 1–4, only 100 mg/day) of a preparation of viable *E. coli* strain Nissle 1917 (Serotype 06: K5: H1) (Mutaflor, Ardeypharm GmbH, Herdecke, Germany) taken as a single dose during breakfast, plus a mesalazine placebo. Mutaflor 100 mg contains 25×10^9 viable *E. coli* bacteria. The double-dummy technique warranted blindness despite the different administration of the two active drugs.

Patients remained in the study for 12 weeks with the exception of one centre (B.F.), where the total study period was 24 weeks.

Clinical remission was defined according to the CAI (≤ 4 points) as published by Rachmilewitz[11] and listed in Table 1. In addition, the per protocol analysis required endoscopic and histological remission.[11] Each patient underwent colonoscopy and mucosal biopsy samples (rectum and sigmoid) were taken when entering and leaving the trial. At the start and end of the study and at each visit after 2, 4 and 8 weeks, a full blood count, CRP, ESR and liver function tests were carried out. The symptom score was rated according to the patient's diary. Compliance was checked by tablet counting and the diaries. Excellent compliance was defined as perfect diary records and no violation of the protocol with respect to the intake of the study medication. Severity of protocol violations was judged by the end-point committee of the study.

The data were evaluated by both per protocol (PP) and intention-to-treat (ITT) analyses. All patients who were randomized had a CAI ≤ 4 and who had started taking study medication were included in the ITT analysis ($n = 103$). Patients with a CAI ≤ 4, endoscopic and histological remission at the start and a complete study period or a relapse were assigned to the PP analysis ($n = 70$).

The main objective of the study was to prove equivalence of the CAI under *E. coli* Nissle 1917 and

Table 1. Clinical activity index of ulcerative colitis (according to Rachmilewitz[11])

	Item	Score
Number of stools weekly	<18	0
	18–35	1
	36–60	2
	>60	2
Blood in stools (based on weekly average)	none	0
	little	2
	a lot	4
Investigator's global assessment of symptomatic state	good	0
	average	1
	poor	2
	very poor	3
Abdominal pain/cramps	none	0
	mild	1
	moderate	2
	severe	3

Table 2. Demographic and clinical characteristics of the patients at entry

	E. coli Nissle $n = 50$	Mesalazine $n = 53$
Male/Female	29/21	26/27
Mean (range)		
Age (years)	43 (20–88)	44 (19–78)
Disease duration (months)	89 (6–276)	109 (1–516)
Time since last relapse (months)	14 (1–147)	12 (1–60)
No. (%) of patients with:		
Proctitis	10 (20.0)	18 (34.0)
Proctosigmoiditis	20 (40.0)	20 (37.7)
Left-sided colitis	13 (26.0)	6 (11.3)
Total/subtotal colitis	8 (16.0)	10 (18.9)
Pre-treatment		
Patients (%)	36 (72.0)	40 (75.5)
Drugs*		
Salicylates	41	41
Corticosteroids	9	14

* Multiple statements/patient.

mesalazine. Relapse rates were analysed according to changes in the CAI (exceeding 4).

Additional analyses were performed to assess the duration of relapse-free times (life table statistics), differences in global assessment, tolerance to the study medication and adverse events.

The sample size of the study was calculated with $n = 40$ patients per group in order to confirm equivalence between the treatment groups using Schuirmann's two one-sided test procedure[12] with an equivalence region of 4 points in change of the CAI. *P*-values (*t*-test, two-sided) are reported. The computer programme used for the evaluation was SPSS 4.0. Data are expressed as mean ± s.d..

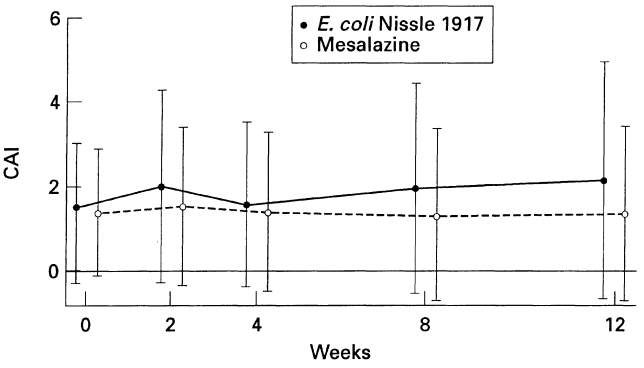

Fig. 1. CAI values.

RESULTS

A total of 120 patients were randomized. Since two patients had not started taking the study medication, 118 patients were eligible for safety analysis. In addition, 15 patients (eight patients randomized to *E. coli* Nissle 1917, seven to mesalazine) were excluded because they had a CAI of > 4. Thus, 103 patients (50 patients with *E. coli* Nissle 1917, 53 patients with mesalazine) were included in the ITT analysis. The demographic and clinical characteristics of the groups are listed in Table 2.

Figure 1 depicts the CAI values during the study. The difference in the start and end scores showed no significant difference ($P = 0.12$) between the two treatment groups. The 95% confidence interval (CI) for the difference between the two treatment groups concerning the change in CAI ranged from −0.21 (difference in favour of *E. coli* Nissle 1917) to 1.81 (difference in favour of mesalazine). The trend to a higher CAI in the *E. coli* Nissle 1917 treated group throughout the study failed to demonstrate significance. Detailed data of the respective activity scores are summarized in Table 3. The total relapse rate was 14/103 patients (13.6%), in the *E. coli* Nissle 1917 group it was 8/50 patients (16.0%), and in the mesalazine group it was 6/53 patients (11.3%). The 95% CI for the difference between the relapse rates of the two treatment groups was −8.6% and 17.9%, respectively. Mean

Table 3. CAI during the trial (mean scores and 95% confidence intervals of the mean scores)

Time	*E. coli* Nissle ($n = 50$) Mean	95% CI	Mesalazine ($n = 53$) Mean	95% CI
At start	1.5	(1.0; 1.9)	1.3	(0.9; 1.7)
After:				
−2 weeks	2.0	(1.3; 2.6)	1.5	(0.9; 2.0)
−4 weeks	1.5	(0.9; 2.1)	1.3	(0.8; 1.9)
−8 weeks	1.9	(1.1; 2.7)	1.2	(0.6; 1.8)
−12 weeks	2.1	(1.2; 3.0)	1.3	(0.7; 1.9)
Last observation	2.5	(1.6; 3.3)	1.5	(0.8; 2.2)
Difference between last observation and start	1.0	(0.1; 1.8)	0.1	(−0.3; 0.7)

time until the first relapse was 41 ± 30 days under *E. coli* Nissle 1917 and 42 ± 28 days under mesalazine. By Kaplan–Meier life table analysis (Figure 2) a mean relapse-free time of 106 ± 5 days (s.d.) was calculated for *E. coli* Nissle 1917 (95% CI: 97; 115 days) and of 103 ± 4 days for mesalazine (95% CI: 95; 110 days).

The histological findings at the end of the study revealed no significantly different results. Sigmoidal biopsies were free of inflammation in 18/50 (36.0%) patients with *E. coli* Nissle 1917 and in 29/53 (54.7%) patients with mesalazine, whereas signs of active disease were present in 6/50 (12.0%) patients (*E. coli* Nissle 1917) and 2/53 (3.8%) patients (mesalazine), respectively. Altogether, 50% of the *E. coli* Nissle 1917 patients rated the efficacy of treatment as very good, 20% as good and 12% as satisfactory. The corresponding figures associated with mesalazine treatment were 62%, 17% and 4%.

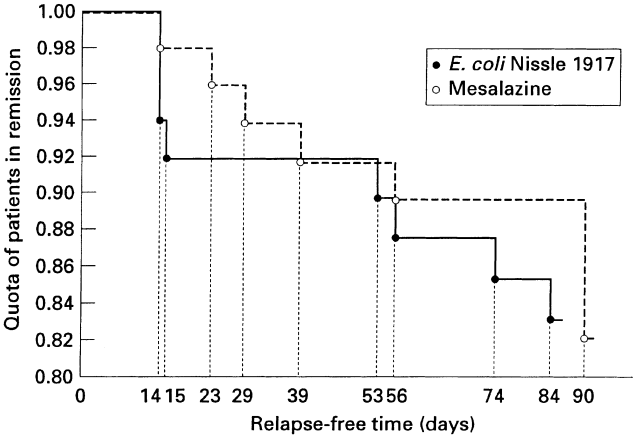

Fig. 2. Kaplan–Meier life table analysis.

In one centre (B.F.), 15 patients were treated for a total of 24 weeks. Neither under *E. coli* Nissle 1917 ($n = 7$) nor under mesalazine ($n = 8$) did any additional relapses occur between weeks 12 and 24. The difference between the CAI scores at the start and end of the treatment was −1.14 (95% CI: −1.98; −0.31) in the *E. coli* Nissle 1917 group and −0.50 (95% CI: −1.39; 0.39) in the mesalazine group.

The results of the PP analysis were similar to those of the ITT analysis. Treatment with both *E. coli* Nissle 1917 ($n = 32$) and mesalazine ($n = 38$) showed no significant difference with respect to the course of the CAI. The total relapse rate was 11/70 patients (15.7%); in the *E. coli* Nissle 1917 group it was 6/32 patients (18.8%), and in the mesalazine group it was 5/38 patients (13.2%). The 95% CI of the difference between the two study groups was −11.7% and 22.9%. The respective relapse-free times were 80 ± 23 days (*E. coli* Nissle 1917) and 83 ± 19 days (mesalazine).

Both *E. coli* Nissle 1917 and mesalazine were well tolerated, and there was no significant difference in the frequency of adverse events. A total of 13/118 patients (11.0%) reported adverse events, 5/58 patients (8.6%) in the *E. coli* Nissle 1917 group and 8/60 patients (13.3%) in the mesalazine group. Diarrhoea developed in four patients receiving *E. coli* Nissle 1917 and in one patient with mesalazine. Other reported adverse events were flatulence/distension (one with *E. coli* Nissle 1917, three with mesalazine), nausea/vomiting (1;3), headache (0;1), varia (3;2). These adverse events caused withdrawal from the study for two patients in the *E. coli* Nissle 1917 group and one patient in the mesalazine group. There were no relevant changes to any of the haematological or biochemical variables monitored during the study period.

Compliance with the study medication was excellent in 78.0% of the patients in the *E. coli* Nissle 1917 group and in 79.2% of the patients in the mesalazine group.

DISCUSSION

Studies of the therapeutic efficacy of antibiotics in UC report inconsistent results and few adequate trials exist. In a recent controlled trial[13] ciprofloxacin was shown to be effective in complicated UC. Oral tobramycin was shown to eliminate pathogenic *E. coli* strains; this was related to significant clinical and histological improvement of UC.[14] When tobramycin was stopped, however, pathogenic adhesive *E. coli* recolonized and relapses occurred in some patients.

The therapeutic benefit of enemas consisting of intestinal contents from healthy donors in refractory UC[15] indicates another possible way of treating inflammatory bowel disease, i.e. by changing the colonic flora. Substituting normal flora in antibiotic-associated colitis with an overgrowth of *Clostridium difficile* was reported to be successful.[16–18] In 1930 Nissle[19] observed alterations to the pattern of the aerobic intestinal flora and a significant decrease of non-pathogenic *E. coli* in patients with non-infectious bowel disorders. Administration of the non-pathogenic *E. coli* strain Nissle 1917 (serotype 06: K5: H1) resulted in a significant improvement in the patients' symptoms.[20]

The *E. coli* strain Nissle 1917 as applied in this trial has been demonstrated to colonize the intestines within a few days,[9, 10] and to remain a constituent of the colonic flora even for months after oral administration has been stopped.[10, 21] Oral ingestion of *E. coli* Nissle 1917 leads to a serologic antibody response.[9] Immunological competence was demonstrated further by immunomodulating effects on macrophages,[22] such as an increase in phagocytic capacity, secretion of tumour necrosis factor and production of spontaneous oxygen radicals.

In contrast to these microbiological and immunological investigations the trial presented here is the first to study therapeutic effects in inflammatory bowel disease. By defining equivalence of both treatment groups with a range of 4 in the CAI, no significant difference was observed, although a trend towards a slightly higher CAI in the *E. coli* Nissle 1917 group existed. Treatment of UC with non-pathogenic *E. coli* is of experimental character and, as far as effective standard therapy is concerned, involves some ethical objections. Therefore, a study duration of only 3 months with careful patient examination was chosen. The relapse rate of our investigation is similar when compared to life table data for the number of patients in remission reported in other relapse prevention trials.[23–26] Moreover, the data from the centre with a patient follow-up of 6 months indicate sustained efficacy of the study treatment.

Of particular interest are the results concerning safety and tolerance. There were no serious adverse events either in the group treated with *E. coli* Nissle 1917 or among the control patients. Overall, the frequency of minor side-effects was similar in both patient groups.

In conclusion, treatment with an oral preparation of viable *E. coli* bacteria of the non-pathogenic strain Nissle 1917 (serotype 06: K5: H1) showed in this short-term pilot study similar efficacy in maintaining remission of UC as standard treatment with mesalazine. These preliminary results and the excellent tolerability demonstrated should give rise to further trials. Furthermore, the effects of alteration to the bacterial flora gives support to the hypothesis of a pathophysiological role for the intestinal environment in UC.

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