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ORIGINAL ARTICLE

Effects of rifaximin-resistant *Bifidobacterium longum* W11 in subjects with symptomatic uncomplicated diverticular disease treated with rifaximin

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ABSTRACT

BACKGROUND: In medical practice, the use of rifaximin and a probiotic is quite common in patients with a diagnosis of symptomatic uncomplicated diverticular disease (SUDD), with the latter being administered at the end of the rifaximin cycle. The opportunity of having a probiotic strain (*Bifidobacterium longum* W11) described as being resistant to rifaximin has prompted us to use it routinely in subjects with SUDD, administering it concomitantly with rifaximin.
METHODS: Retrospectively, we have analyzed whether our approach conferred a real clinical advantage to patients. The results seem to confirm the logic of our approach.
RESULTS: Patients treated with rifaximin concomitantly receiving strain W11 demonstrated better clinical outcomes than subjects treated with rifaximin followed by strain W11. Moreover, we have observed that the concomitant use of a rifaximin-resistant probiotic has improved the stool consistency of most patients. Finally, the adherence to the given therapy was very different, being very high in subjects undergoing concomitant use of the W11 strain and rifaximin, and being low in the other group. This is probably because of the different duration of therapy (7 days *versus* 14 days) and due to the fact that after 7 days of rifaximin treatment, patients felt better and decided not to proceed with the probiotic administration.
CONCLUSIONS: Despite the many biases that our retrospective analysis presents, we believe that a probiotic strain demonstrating a strong non-transferable resistance to a particular antibiotic should be used along with that specific antibiotic, at least in cases of SUDD diagnosis.

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KEY WORDS: Microbiota; Diverticulum; Probiotics; Intestinal diseases; Anti-bacterial agents.

Diverticulosis prevalence increases with age and diverticula can be found in more than half of people aged 60 years or older.[1] Some of these individuals develop mainly mild to moderate symptoms, such as abdominal pain, bloating, and changing bowel habits, without objective evidence of inflammation. This condition affects one out of four patients with diverticulosis and is defined as symptomatic uncomplicated diverticular disease (SUDD)[2] while acute diverticulitis is diagnosed in less than 10% of patients with diverticulosis.[3] The pathogenesis of symptomatic diverticular disease is still being debated within the scientific community. Recently, many researchers have pointed out the possible role played by the gut microbiota, the alteration of

which could be necessary for the occurrence and persistence of symptoms.[4, 5] In fact, changes in the gut microbiota phyla and taxa and reductions in the rarefaction curve between patients without diverticula and those with SUDD have been observed.[6] These findings are quite important and suggest a possible role for probiotics too. In clinical practice, and in accordance with most international guidelines, physicians propose the use of probiotics to patients with SUDD after rifaximin treatment. However, patient adherence to the probiotic therapy is generally low, probably because of the beneficial effect the antibiotic has already had on symptoms.[7] To improve patient adherence, the two treatments, the antibiotic and probiotic, should be concomitantly administered but the antibiotic sensitivity profile of probiotics make this strategy illogical.[8] European Food Safety Authority (EFSA) rules do not prohibit the development of antibiotic-resistant strains, but consider the non-transferability of the antibiotic resistance pattern of these potential strains mandatory.[9] *Bifidobacterium longum* W11 (LMG P-21586) is a probiotic strain which has been demonstrated to be rifaximin-resistant due to a mutation in the *rpoB* (β-subunit of DNA-dependent RNA polymerase) gene.[10] As its resistance profile is not transferable, the strain is allowed to be traded and used for medical purposes. We have therefore retrospectively analyzed the role of this rifaximin-resistant strain as an adjuvant and concomitant therapy to rifaximin, comparing this regime with a more conventional approach where the same probiotic was administered after rifaximin treatment in patients with a diagnosis of SUDD.

Materials and methods

Study design and aim

Our study encompassed a retrospective analysis of the data obtained from routine procedures conducted in the Digestive Endoscopy Department at Ceva Hospital (Italy) between June 2018 and May 2019. The aim of the study was to compare the concomitant administration of a rifaximin-resistant probiotic strain and rifaximin to administration of the same probiotic after completion of the rifaximin cycle in patients with a diagnosis of SUDD. All patient data were completely anonymized and the study was performed in accordance with the ethical standards established by the local (CN) institutional committee. Despite the retrospective and anonymized features of the study, all patients provided signed informed consent to publish the results.

Inclusion and exclusion criteria

Forty-five consecutive outpatients (25 males, 20 females; mean age 68.4±8.5, range 52-75 years), affected by SUDD were involved in our retrospective analysis. The diagnosis of diverticulosis was established by means of a double-contrast barium enema or endoscopy and all patients presented symptoms such as upper and/or lower abdominal pain and/or discomfort, bloating, tenesmus, diarrhea, abdominal tenderness, fever, and dysuria. Exclusion criteria were a solitary diverticulum of the colon, diverticulitis, previous major colonic or abdominal surgery, colonic or extracolonic cancer, the use of antibiotics or mesalazine in the previous four weeks, chronic hematological and/or hepatic and/or renal diseases, an immunodeficiency, being pregnant or lactating, and proven intolerability to rifaximin. We considered patients using prokinetics, spasmolytics, and analgesics to be eligible.

Analyzed parameters

Patients considered eligible were all those who agreed to complete a very basic questionnaire at the beginning of treatment. The questionnaires reported primary and secondary endpoints. Primary endpoints concerned gut symptoms and stool consistency. Symptoms evaluated were abdominal pain, abdominal tenderness, diarrhea, constipation, changing bowel habits, and bloating, assessed on a 4-point scale (0: no symptoms, 1: mild, 2: moderate, 3: severe) at baseline and after 1, 2, and 3 months from the start of treatment. A total symptom score was calculated by summing up the individual symptoms at each timepoint (maximum value: 18). By using the self-reported value obtained following categorization by the Bristol Stool Scale we analyzed stool consistency at baseline and after three months of treatment. Secondary endpoints were tolerability (0-10), adherence (%) to the agreed

treatment, and side effects (the number of subjects reporting side effects throughout the period of treatment).

Treatment

Patients were asked to agree to one of two different protocols. One possibility was to be administered rifaximin at a dose of 400 mg every 12 hours for 7 consecutive days and to continue the therapy with a probiotic containing *B. longum* W11, a strain described to be rifaximin-resistant, for the following 7 days (group A; N.=22). The second was to be administered rifaximin (400 mg every 12 hours) over the same 7 days along with the same rifaximin-resistant probiotic (group B; N.=23). For both group A and group B, the probiotic was administered 30 minutes after breakfast.

Products

The rifaximin product was Normix® coated tablets containing 200 mg each of rifaximin (Alfa-Wassermann, Italy). The probiotic containing strain W11, which is rifaximin-resistant, was formulated as Bowell® sachets containing not less than 10 billion bacteria (ISO 19344:2015 IDF 232:2015 > 10 x 10^9 AFU) before the expiry date (manufactured by Probiotical, Novara, Italy; traded by Omeopiacenza/Pharmextracta group, Pontenure, Italy). Bowell® was notified to the Italian Health Authorities in April 2017; its notification number is: 92629.

Statistical analysis

The equivalence of the two groups was determined using Fisher's Exact Test and the two-tailed Wilcoxon-Mann-Whitney Test. The difference in terms of outcome was determined using the two-tailed Wilcoxon-Mann-Whitney Test. The statistical software used was JMP 10 for Mac OsX and the threshold for statistical significance was 95%.

Results

Our retrospective analysis constituted an attempt to evaluate the effects of a rifaximin-resistant probiotic administered along with rifaximin in patients with a diagnosis of SUDD, comparing this treatment with a conventional one where the probiotic is proposed for administration at the end of the rifaximin cycle. The two groups (A: rifaximin followed by the probiotic, N.=22; B: rifaximin along with the probiotic, N.=23) were analyzed at baseline in terms of age, sex, therapy before enrolment, white blood cell count, red cell sedimentation rate, C-reactive protein levels, and symptoms of SUDD, and were demonstrated to overlap with no statistical differences observed (data not shown). As reported in Table I, group B (rifaximin along with the probiotic) demonstrated somewhat more significant results with respect to all symptoms apart from changing bowel habits, *versus* group A (Table II, the probiotic after rifaximin). The global score for all symptoms (Table III) shows that the results become statistically different after 3 cycles of therapy with a greater reduction of approximately 20% more in the score for group B, when comparing the final timepoint values to baseline. As categorized by the Bristol Stool Scale (Table IV), the self-reported values demonstrate that stool consistency seems to be better in group B with 16 out of 23 subjects reporting stool types 3 and 4 *versus* 10 out of 22 in group A. In addition, 7 subjects in group A and 2 in group B report having type 2 stools (Table

TABLE I.—*Symptoms (mean±standard deviation) at baseline (T0) and after 1, 2, and 3 months of treatment in the group receiving the probiotic concomitant with the rifaximin cycle (group B).*

Symptom	T0	T1	T2	T3	P (T3 *vs.* TO)
Abdominal pain	2.6±0.3	2.0±0.4	1.8±0.4	0.8±0.2	<0.001
Abdominal tenderness	2.5±0.4	1.9±0.3	1.2±0.3	0.9±0.2	<0.01
Diarrhea	1.4±0.3	1.0±0.4	0.6±0.4	0.3±0.3	<0.01
Constipation	1.6±0.5	1.2±0.1	1.0±0.2	0.5±0.4	<0.01
Changing bowel habits	1.0±0.2	0.8±0.3	0.7±0.3	0.6±0.5	NS
Bloating	2.6±0.2	2.2±0.6	1.6±0.5	1.4±0.5	<0.01

NS: not significant.

TABLE II.—*Symptoms (mean±standard deviation) at baseline (T0) and after 1, 2, and 3 months of treatment in the group receiving the probiotic at the end of the rifaximin cycle (group A).*

Symptom	T0	T1	T2	T3	P (T3 *vs.* TO)
Abdominal pain	2.5±0.2	2.2±0.5	1.9±0.4	1.4±0.3	<0.01
Abdominal tenderness	2.4±0.4	2.0±0.4	1.6±0.4	1.2±0.6	<0.05
Diarrhea	1.2±0.3	0.9±0.5	0.8±0.4	0.6±0.3	<0.05
Constipation	1.8±0.6	1.3±0.5	1.2±0.6	1.0±0.5	<0.05
Changing bowel habits	1.0±0.3	0.8±0.4	0.7±0.5	0.5±0.5	NS
Bloating	2.5±0.4	2.2±0.5	1.8±0.5	1.7±0.6	<0.05

NS: not significant.

TABLE III.—*Total symptom score (mean±standard deviation) in group A (probiotic after rifaximin) and group B (probiotic along with rifaximin) at baseline (T0) and after 1, 2, and 3 months of treatment.*

Group	T0	T1	T2	T3	Δ (T3 *vs.* TO)
A	11.4±0.8	9.4±0.7	8.0±0.5°	6.4±0.6°	-43.9%
B	11.7±0.9	9.1±0.8	6.9±0.4°	4.5±0.4°°^	-62.5%

°P<0.05 *vs.* T0; °°P<0.01 *vs.* T0; ^P<0.05 *vs.* group A.

TABLE IV.—*Self-reported Bristol Stool Scale (BSS) categorization by subjects of study group A (probiotic after rifaximin) and group B (probiotic along with rifaximin) evaluated at baseline (T0) and 3 months (T3) after the beginning of therapy.*

BSS	A (N.=22)		B (N.=23)		P	P
	T0	T3	T0	T3	T0 *vs.* T0	T3 *vs.* T3
1	4	2	4	2	NS	NS
2	8	7	9	2	NS	<0.01
3	3	5	4	8	NS	<0.05
4	2	5	1	8	NS	<0.05
5	2	3	1	3	NS	NS
6	2	0	2	0	NS	NS
7	1	0	2	0	NS	NS

NS: not significant.

IV). Tolerability and side effects (mainly nausea, vomiting, and headache) seem to be overlapping in terms of values (Table V). In contrast, the values regarding adherence to therapy are completely different, calculated as the average value of the single percentages reported by each subject, where the two groups show a significant difference in favor of group B (Table V).

TABLE V.—*Tolerability, side effects and global adherence to therapy in study groups A (probiotic after rifaximin) and B (probiotic along with rifaximin) evaluated 3 months after the beginning of therapy.*

Parameter	A	B	P
Tolerability	8.5±1.2	8.0±0.9	NS
Side effects°	12	14	NS
Adherence	65%	95%	<0.01

NS: not significant.
°Expressed as number of subjects reporting side effects.

Discussion

We have attempted to retrospectively analyze the symptoms trend in two groups of subjects with a diagnosis of SUDD that have been treated conventionally (7 days of rifaximin administration followed by 7 days of probiotic therapy) or treated with rifaximin and the probiotic at the same time for 7 days, having had the opportunity to use a rifaximin-resistant strain (*B. longum* W11). There were two main reasons for such an investigation. First, we wanted to see if it would make sense, in terms of conferring a clinical advantage, to use an antibiotic along with a probiotic that is apparently resistant to that very antibiotic. Second, we wanted to check if the symptomatic relief afforded by any cycle of rifaximin in SUDD patients prompted avoidance of the probiotic cycle. The latter is believed, and therefore suggested to patients, to be beneficial due to its probable positive effects on the colon microbiota, but in our opinion, only some patients are truly adherent to the probiotic follow-up.

The results that we have obtained seem to be sufficiently clear to demonstrate that the use of a bacterial strain that is antibiotic-resistant along with the respective antibiotic, in this case, rifaximin, makes sense and seems to bring some clinical advantages in terms of the treatment of symptoms, especially pain, tenderness, diarrhea and bloating, at least after 3 cycles of therapy. In any case, even if our results do not seem to high-

light any advantage in terms of tolerability and side effects, it seems quite clear that reducing the global treatment from 14 to 7 days increased patient adherence. In our opinion, this is a very good result since it represents a practical way of limiting the gradual worsening, cycle by cycle, of the gut microbiota status of SUDD patients. It is likely that this is not just speculative thinking on our part, as demonstrated by the self-reported evaluation of stools by subjects within the two groups using the Bristol Stool Scale.[11]

Panda *et al.*[12] have reported that short antibiotic treatment could bring changes to the gut microbiota that are observable by three different parameters: first, a reduced richness and α-biodiversity with the loss of some low-expressed taxa; second, a probable increase in the absolute number of bacteria (this sounds perhaps counterintuitive); and third, an increase in the global gut Gram-negative bacteria. We can speculate that the W11 strain administered along with rifaximin somewhat improved the clinical outcome as it limited these factors, thereby preventing damage to the gut bacterial consortium. Indeed, we do not propose that the reported difference in adherence alone could explain the divergence observed in terms of fecal consistency.

Indeed, a comparison between group B and those subjects from group A declaring a complete adherence to the proposed therapy (N.=7) did not change the meaning of the results in terms of symptoms and Bristol Stool Scale scores (data not shown).

Limitations of the study

We are aware that our retrospective analysis has limitations. First of all, this has not been a prospective, randomized, double-blind, placebo-controlled clinical trial. Second, our analysis contains some important bias. For instance, a high-fiber diet was recommended to all patients. Fiber supplements have high heterogeneity regarding their various forms; they can be soluble, insoluble, viscous, non-viscous, prebiotic, and so on. We were unable to conduct our analysis taking the type of fiber used fully into consideration, and the effective adherence to this recommendation. Similarly, we have been unable to control any other aspect of diet, such as the use of yogurt (a probiotic food) during breakfast, or the overall consumption of fruit, vegetables and/or pectin- or cellulose-rich foods during the three months of treatment. Moreover, our observation lasted only three months. Pietrzak *et al.*[13] have reported highly significant results starting from six months of treatment, also by using the same retrospective approach. In addition, we have used one dose (400 mg × 2/day) of rifaximin and the treatment lasted 7 days. It could be that by using a different dosage, and with a different duration of treatment, results could be different. Finally, our observations took place with only 45 patients.

Conclusions

Despite all these, and perhaps more, limitations, we believe our study is the first, at least to our knowledge, to report the concomitant use of an antibiotic along with a probiotic strain that has been demonstrated to be strongly resistant to that particular antibiotic. Previously, another group of researchers[14] analyzed the effects of rifaximin and strain W11 administration in the same group of patients, who had a diagnosis of irritable bowel syndrome. In that study, the W11 strain was administered after completion of the rifaximin cycle. Those results demonstrated clinical advantages *versus* treatment with rifaximin and placebo. However, in our opinion, the two studies cannot be compared since we administered the two agents, rifaximin and strain W11, concomitantly.

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Conflicts of interest.—Francesco Di Pierro is part of the scientific board of Pharmextracta; Marco Pane is a scientist at Biolab. The other authors declare no conflict of interest.

Authors' contributions.—Conceptualization: Francesco Di Pierro, Alexander Bertuccioli, Leandro Ivaldi; methodology and formal analysis: Leandro Ivaldi; data curation: Leandro Ivaldi, Alexander Bertuccioli; writing and original draft preparation: Francesco Di Pierro.

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