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Rifaximin Reduces Number and Severity of Intestinal Lesions Associated With use of Non-steroidal Anti-inflammatory Drugs in Humans

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Professor of Pharmacology & Therapeutics Associate Professor of Gastroenterology *Head*, Clinical Pharmacology & Digestive Pathophysiology Unit Department of Clinical & Experimental Medicine, University of Parma Maggiore University Hospital, Cattani Pavillon, I-43125 Parma, Italy E-mail: <u>scarpi@tin.it -</u> e-Fax: +1-603-843-5621 **Author Contributions** [according to the International Committee of Medical Journal Editors (JCMJE)]

Werner Dolak and Peter Matzneller had full access to all of the data in the study and take the responsibility for the integrity of the data

- Study concept: Carmelo Scarpignato
- *Study design:* Carmelo Scarpignato, Ingvar Bjarnason, Angel Lanas, Maria Grimaldi, Werner Dolak, Markus Zeitlinger
- Analysis and interpretation of data: Werner Dolak, Ingvar Bjarnason, Angel Lanas, Carmelo Scarpignato
- Drafting of the manuscript: Carmelo Scarpignato, Werner Dolak, Markus Zeitlinger
- Critical revision of the manuscript for important intellectual content: Ingvar Bjarnason, Angel Lanas
- Statistical analysis: CROss Alliance people, Cecilia Renzulli
- Obtained funding: Carmelo Scarpignato
- Administrative, technical, or material support: CROss Alliance people
- Study supervision: Maria Grimaldi

Author Conflicts of Interest

Carmelo Scarpignato is member of the Speakers' Bureau and of the Scientific Advisory Board of Alfa Wassermann.

Angel Lanas and Ingvar Bjarnason are members of the Scientific Advisory Board of Alfa Wassermann.

Maria Grimaldi and Cecilia Renzulli are employees of Alfa Wassermann

Markus Zeitlinger, Werner Dolak, Markus Matzneller have no conflicts of interest to disclose

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Abstract: The intestinal microbiota might contribute to enteropathy associated with use of non-steroidal anti-inflammatory drugs (NSAIDs), but there have been few human studies of this association. We performed a placebo-controlled study to determine whether a delayed release antibiotic formulation (rifaximin-EIR) prevented development of intestinal lesions in persons taking daily NSAIDs. Sixty healthy volunteers (median age 26 years, 42% female) were given the NSAID diclofenac (75 mg twice daily) plus omeprazole (20 mg once daily), and either rifaximin-EIR (400 mg) or placebo, twice daily for 14 days. Subjects were assessed by video-capsule endoscopy at baseline and after 2 weeks of treatment. The primary endpoint was the proportion of subjects developing at least 1 small bowel mucosal break at week 2. Secondary endpoints were the change in mean number of mucosal lesions and number of subjects with large erosions and/or ulcers after 14 days of exposure. We detected mucosal breaks in 20% of subjects given rifaximin and 43% of subjects given placebo (P=.05. in the post hoc sensitivity analysis). None of the subjects in the rifaximin group developed large lesions, compared with 9 subjects in the placebo group (P<.001). Our findings indicate that the intestinal bacteria contribute to development of NSAIDassociated enteropathy in humans. Clinical trial no: EudraCT 2013-000730-36.

KEY WORDS: controlled trial, microbiome, gastrointestinal adverse event, prevention

Short Title: Rifaximin for NSAID-enteropathy

Text

Non-steroidal anti-inflammatory drugs (NSAIDs) are very effective medications, but their use is associated with a broad spectrum of adverse reactions involving the liver, kidney, cardiovascular (CV) system, skin and gut. Gastrointestinal (GI) adverse effects are the most common and cover a wide clinical spectrum, ranging from dyspepsia, heartburn and abdominal discomfort to more serious events, such as peptic ulcer with life-threatening ulcer complications of bleeding and perforation [1].

Over the past decade, there has been a progressive change in the overall pattern of GI events leading to hospitalization, with a clear decreasing trend in upper GI events and a slight, but significant, increase in lower GI events [2]. Indeed, available studies [3, 4] have shown that about 75% of NSAID users display intestinal mucosal injury, ranging from denuded areas (seen mainly in the proximal small bowel) to the so-called mucosal breaks (erosions and ulcers), observed in its distal part. While the incidence of upper GI injury can be reduced by proton pump inhibitors (PPIs), this is not the case for NSAID-associated intestinal lesions, which may actually be aggravated by acid suppression [5].

The pathogenesis of small intestinal damage is complex and still not completely understood. Several lines of experimental evidence implicate commensal enterobacteria in the pathogenesis of NSAID-enteropathy and suggest that luminal bacterial may represent a potential target for prevention/or treatment [6]. In this context, the efficacy of a delayed release formulation of rifaximin, a poorly absorbed antibiotic [7], in the prevention of NSAID-associated lesions was evaluated in man, by means of video-capsule endoscopy.

Sixty healthy volunteers (median age 26 years, 42% female) were randomized to receive diclofenac SR 75 mg BID plus omeprazole 20 mg OD and either rifaximin-EIR 400 mg or rifaximin-EIR matching placebo BID for 14 days (**Figure 1**). The *primary endpoint* was the proportion of subjects developing at least one small bowel mucosal break at final VCE. *Secondary endpoints* were the change at VCE in the mean number of mucosal lesions and the number of subjects with large erosions and/or ulcers at the end of treatment (*see* Supplementary Material and Supplementary Table).

Six patients in the rifaximin group and 13 patients in the placebo group (12 of per protocol set) developed at least one mucosal lesion in the small bowel (primary end-point), which gives an odds ratio (OR) of 0.33 [95% CI 0.10, 1.03] and 0.35 [95% CI 0.11, 1.12] in the modified full analysis (mFA) and PP sets, respectively, for subjects in the rifaximin group to develop at least one mucosal lesion in the small bowel at logistic regression analysis.

The efficacy on primary end-point was not significant. However, when *post-hoc* sensitivity analysis, using age as covariate, was performed, the difference between rifaximin and placebo group became significant (**Table 1**). The average mucosal score (mFA set) at the end of the treatment was 0.87±0.13 *versus* 1.83±0.28 for rifaximin and placebo, respectively (p=0.021).

Both secondary end-points were successfully reached in the study (**Table 1**). Subjects in the placebo group developed a higher number of mucosal lesions compared to those in the rifaximin group $(1.2\pm2.3 \text{ vs. } 0.3\pm0.7 \text{ in the mFA set})$. Negative binomial regression analysis proved a protective effect of rifaximin on mean changes from baseline in total number of lesions (treatment effect point estimate [PE] -1.41 [95% CI - 2.49, -0.34]; p-value=0.010). A similar result was detected for the change from baseline in the number of lesions without hemorrhage (PE -1.44 [95% CI -2.53, -0.35; p-value=0.009).

Both treatments were well tolerated and no serious adverse events were recorded.

This is the first randomized, controlled trial, investigating the effect of an antibiotic on NSAID-induced intestinal mucosal injury in human beings. This study shows that fewer rifaximin-treated volunteers developed small bowel lesions compared to placebo-treated subjects. The antibiotic also reduced the mean number of lesions and appeared to abolish the larger lesions, with a tolerability profile, overlapping that of placebo. Although short-term studies, such as this one, and mucosal breaks may not have straightforward clinical implications [8], the results of this *proof-of-concept* study strongly suggest the role of enteric bacteria in the pathogenesis of NSAID-enteropathy. and call for a prospective trial in patients on long-term NSAID therapy.

The entero-protective effect of rifaximin most likely depends on its broad spectrum of antibacterial activity [7]. Experimental studies [9-11] not only show that this antibiotic reduces the total bacterial load, but also modulates bacterial community composition, an effect associated with a reduction of intestinal inflammation [10, 11] and improvement of gut barrier function [10].

Recent evidence points out that – besides non-antimicrobial activities – rifaximin may also displays "eubiotic" properties. Indeed, in patients with inflammatory conditions (like inflammatory bowel disease, colonic diverticular disease or hepatic encephalopathy), the drug - while not altering the overall structure of human colonic microbiota - increased the relative abundance of *Bifidobacteria* and *Lactobacilli* [12, 13].

NSAID-enteropathy is associated with significant GI complications [14, 15], but there are no proven strategies for the prevention or healing of this subtle clinical condition. However, there is now good evidence that intestinal bacteria play a pathogenic role in NSAID-enteropathy in man. Treatments aiming at correcting the shift of intestinal microbiota towards pro-inflammatory Gram-negative bacteria [6] are therefore potential avenues to explore in the prevention and treatment of NSAID-enteropathy. After almost 40 years, with the advancement of knowledge on the pathogenic role of gut microbiota in NSAID-enteropathy [6], the time seems now ripe to study in well-designed, large, randomized, clinical trials microbiota-directed interventions to protect the small bowel from NSAID injury and to allow safer anti-inflammatory therapy.

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Table 1 - Primary and Secondary Endpoints of the Study

Primary End-point	Rifaximin	Placebo	p value	
No of subjects developing at least one mucosal lesion	6/30 (20%)	13/30 (43%)	0.0566 ¹	
Sensitivity Analysis (treatment and sex as fixed effect and age as covariate)			0.0490	
Secondary End-points				
Change in the mean number (± SEM) of mucosal lesions	0.3±0.7	1.2±2.3	0.0103 ²	
Number of subjects with large erosions and/or ulcers at the end of treatment	0	9	0.001 ³	
¹ Logistic Regression Analysis ² Negative Binomial Regression Analysis ³ Chi- square Test				

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Figure 1 - CONSORT 2010 Flow Diagram



mFA =modified Full Analysis

PP = Per Protocol

Subjects and Methods

This double-blind, randomized, placebo-controlled, phase IIa trial was conducted according to the CONSORT guidelines [16] at the Department of Clinical Pharmacology at the General Hospital of Vienna, Austria. The study protocol was approved by the internal review board of the Medical University of Vienna (EK 1244/2013) and the national competent authority (Ref N. 718031). The study was registered at EudraCT (2013-000730-36).

The trial was performed according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), guidelines for Good Clinical Practice (GCP) [17] and the Declaration of Helsinki (1996 version, amended October 2000) [18].

Study Subjects and Baseline Evaluations

Healthy male and female volunteers (18-64 years) gave their availability and willingness to participate in the study. Sixty subjects met all the *inclusion criteria*, i.e. unremarkable physical examination and medical history, normal vital signs, and all laboratory test values (including hematology, blood chemistry, virology, urinalysis and screened for drug of abuse) within the reference ranges. Female subjects with child bearing potential were required to have negative urine pregnancy test. *Exclusion criteria* included any serious neurologic, psychiatric, cardiovascular, respiratory, gastrointestinal, renal or musculoskeletal disease, drug misuse including alcohol, pregnancy and age outside the 18-64 year range. Subjects having used steroids, non-steroidal anti-inflammatory drugs, biphosphonates, sulphasalazine, biological drugs, treatments targeting gut microbiota (antibiotics, prebiotics or probiotics) as well as gastrointestinal prokinetic drugs within the previous 30 days were also excluded. The use of these drugs, even occasionally, was prohibited during the study.

All participants were admitted to the Investigational Unit. After overnight stay at the department with appropriate preparation (*see below*) eligible subjects underwent VCE of the small bowel (baseline VCE). To be included in the study, video capsule endoscopy should have shown no abnormality, but a maximum of one mucosal break, (erosion or ulcer) in the small bowel was allowed.

Study treatment

After re-assessment of inclusion/exclusion criteria subjects were randomized according to a computer-generated list on a 1:1 basis to receive diclofenac SR 75 mg BID (Voltaren, Novartis Farma S.p.A., Italy) plus omeprazole 20 mg OD (Losec, AstraZeneca AB, Sweden) plus either rifaximin-EIR 400 mg (Alfa Wasserman S.p.A., Italy) or rifaximin-EIR matching placebo BID (Alfa Wasserman S.p.A., Italy) for a total of 14 days. Amongst different NSAIDs, diclofenac was selected because its administration is associated with a definite risk small intestinal injury [19] and because it has been used in almost all the video capsule studies, carried out on the topic with VCE [for review see 20]. In a previous study [4], we found that its slow-release formulation (combined with omeprazole) was associated – after 14 days – with intestinal mucosal lesions in 68% of healthy subjects.

Drug intake was performed and witnessed at the study site at 8 A.M. and 8 P.M. (+/- 1 hour). Within 36 hours after the last study drug intake, the subjects underwent a second VCE assessment (final VCE), vital signs check, physical examination and safety laboratory tests (hematology, blood chemistry and urinalysis). In case of study withdrawal, an early termination visit including final VCE was performed. All patients were followed up using a telephone interview one week after the final VCE visit to assure capsule excretion.

Video Capsule Endoscopy

Baseline and post-treatment VCE were performed on an inpatient basis at the study site. On the day prior to VCE subjects were allowed to eat solid food until 1:00 p.m. and to drink clear liquids until 10:00 p.m. In the evening they received two liters of a polyethylene glycol (PEG) washout solution (Klean-Prep, Norgine, Germany), according to the current guidelines [21] and on-site protocol [22]. The next morning VCE was performed after attachment of the sensor belt and data recorder. Before swallowing the capsule (PillCam SB2, Given Imaging, Yokneam, Israel) all subjects received 70 mg of simethicone (Sab-Simplex, Pfizer, Austria) to avoid visual interference by intestinal air bubbles [23, 24]. Subjects were allowed to drink clear liquids two hours after capsule intake and to eat a light meal two hours later. Sensor belt and data recorder were detached after a total capsule operation time of 8 hours. All capsule videos were pre-analyzed on site by a gastrointestinal endoscopist (WD) with experience in VCE, who had no knowledge of the treatment regimen. VCE results were re-evaluated blindly by a panel of three independent VCE-experts (IB, AL, CS). In case of disagreement between initial evaluation and re-assessment, consensus was achieved

on the final diagnosis between WD and the three VCE-experts. Patient's data remained blinded until final consensus was achieved. All findings were classified following the "five-point scoring system for endoscopic lesions with capsule endoscopy" (Supplementary **Table 1**) [3].

Study endpoints

The primary endpoint of the study was the proportion of subjects developing at least one mucosal break, defined as erosion or ulcer, in the small bowel at final VCE. Secondary endpoints were the change from baseline to final VCE in the number of mucosal breaks, those with and those without hemorrhage, as well as factors reflecting safety and tolerability of the study treatment like the distribution of treatment-emergent adverse events (TEAEs, defined as all adverse events occurring or worsening after the first dose of the study medication), overall adverse events (AEs) or a change in clinical laboratory parameters and vital signs.

Statistical Analyses

Based on an estimated rate of mucosal lesions of 58% in the control group, a sample size of 60 subjects (30 subjects per group) was calculated to detect a reduction of approximately 33% in the primary endpoint for subjects in the active group, considering a one-sided significance level of 0.05, a power of 80% and a 10% dropout rate. Descriptive statistics were used to report baseline characteristics and final results. Statistical analyses were performed for the following datasets: modified Full Analysis set (mFA), consisting of all randomized subjects who received at least one dose of study medication and who successfully completed the final VCE; per Protocol set (PP), consisting of all randomized subjects included in the mFA without any major protocol deviation; safety set (SA), consisting of all randomized subjects who received at least one dose of study medication. Logistic regression analysis and McNemard Chi-Square test were used for comparison of the primary endpoint between groups. A negative binomial model with the change from baseline as dependent variable and treatment as fixed effect was used to compare the secondary efficacy endpoints between groups. Safety parameters were only reported descriptively. SAS version 9.1.3 Service Pack 4 for Windows[®] was used for all statistical analyses in the context of this study. As customary, codes were broken only after the statistical analysis of primary and secondary end-points.

All authors had access to the study data, checked the statistical analyses and reviewed

and approved the final manuscript.

Supplementary Table 1 - Five-Point Scoring System for Endoscopic Lesions

With Capsule Endoscopy

Category	Score		
Normal	0		
Petechiae/red spot (demarcated, usually circular, area of crimson mucosa with preservation of villi)	1		
Small number of erosions (1-4 erosions)	2		
High number of erosion (>4 erosions)	3		
Mucosal breaks (large erosion and/or ulcer)	4		

4

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