Altered intestinal permeability in patients with primary fibromyalgia and in patients with complex regional pain syndrome

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Introduction

The diagnosis of FM is based on the reporting of widespread pain in a patient together with the demonstration of pain upon pressure at 11 or more of 18 specific tender points [1]. The aetiology of this pain syndrome remains uncertain [2]. Recently, small intestinal bacterial overgrowth (SIBO), defined as the inappropriate colonization of the distal small bowel with colonic bacteria, has been demonstrated in FM [3]. Pimentel et al. [3] demonstrated that the severity of SIBO correlated with FM patients’ pain intensity, suggestive of a pathophysiological relevance of SIBO in FM. But the mechanism behind the correlation between SIBO and pain intensity is unclear. In several disorders, SIBO is known to cause an increased intestinal permeability (IP) that is an increased degree of leakiness of the intestinal epithelial layer to luminal products [4, 5]. Such increased leakiness has direct pathophysiological relevance as luminal products passing through the epithelial layer gain abnormal access to both the intestinal and extraintestinal immune systems [6]. Upon contact these products may stimulate immunocompetent cells to play a role in causing the systemic disease such as in inflammatory bowel disease, allergies and arthritides [6–9]. It is currently unknown whether IP in patients with FM is altered.

This study was designed to measure IP in patients with FM compared with both control volunteers and patients suffering from an unrelated chronic pain syndrome, complex regional pain syndrome (CRPS). Our first hypothesis was that the IP in FM is increased, and might correlate with the patients’ pain intensity, but not in either control volunteers or patients with CRPS. In addition to SIBO’s effect on IP, the fermentation of luminal products leading to increased gas production may cause abdominal symptoms such as bloating, diarrhea and constipation [10]. We, therefore, also hypothesized that a subgroup of patients with both FM and abdominal symptoms [11] would be more likely to have increased IP [12]. Intestinal infections can increase IP [13]. FM has been reported to follow Campylobacter infection [14]. We therefore examined all participating patients for serological markers of recent or past intestinal infections for three common intestinal pathogens, Yersinia enterocolitica and Campylobacter jejuni, which are associated with pathology in the ileum and jejunum, and Helicobacter pylori, associated with gastric pathology. We hypothesized that an increased IP in FM may be associated with serological evidence of recent or past infection with these common intestinal pathogens [15].

Patients and methods

Following institutional review board approval patients with newly diagnosed primary FM syndrome who attended the outpatient Pain Clinic, Department of Anaesthesiology, University of Würzburg over a 12-month period were invited to participate. Included patients were between 18 and 65yrs of age. The diagnosis of FM was made in accordance with the ACR criteria [1]. The control group included age- and sex-matched healthy volunteers and patients with newly diagnosed CRPS. The diagnosis of CRPS was based on the classification of the International Association for the Study of Pain, IASP [16]. The presence of autonomic signs was required at presentation to increase diagnostic specificity [17]. Both pregnant and breastfeeding women were excluded. Patients taking drugs known to alter IP or kidney function, and those patients who had used antibiotics in other than local applications within the last 3-month period were also excluded. Patients with confirmed rheumatological disease and FM (secondary FM) were also excluded. The term ‘fibromyalgia’ in this article refers to primary FM syndrome.

Subject’s written consent was obtained according to the Declaration of Helsinki. All patients were asked to note their average pain over the 4-week period preceding the study on an 11-point numeric rating scale (NRS). In addition, patients received an adverse events diary to complete and return 1 week...
after the study. A careful drug history was taken with special emphasis on NSAID intake, as well as NSAIDs, with the exception of aspirin [18], alter IP [19]. Both abdominal symptoms and gastrointestinal disease were documented. Patients were prompted for the following symptoms: diarrhoea, constipation, abdominal distension/blouting, abdominal discomfort and abdominal pain, and they were prompted for the following intestinal diseases: gastritis, reflux disease, peptic ulcer disease, inflammatory bowel disease, sprue, lactose intolerance, other food intolerances or food allergies. A family history of intestinal disease was taken, as it is known that relatives of patients with inflammatory bowel disease and coeliac disease may have elevated IP [20]. All patients were asked to discontinue NSAID intake at least 4 weeks before the test. As this was not always possible, in some patients NSAID use was accepted up to 48 h before commencement of the test. All patients refrained from alcohol intake and dispensable medications for 48 h before the test.

IP was measured using an established three-sugar test performed as described previously [21]. This involved drinking a sugar solution that contained sucrose, lactulose and mannitol. Lactulose and sucrose are both disaccharides that are absorbed via a paracellular epithelial route. The degree of the intestinal absorption of these disaccharides correlates with the intestinal epithelial tight junction permeability, IP [22]. Sucrose, in contrast to lactulose, is rapidly split by the enzyme sucrase-isomaltase that is present in the small bowel, but not in stomach or duodenal mucosa. The degree of sucrose absorption, therefore, has been shown to be sensitive to gastroduodenal damage [23]. In contrast to these disaccharides, smaller molecules, such as mannitol, pass the mucosal epithelial barrier mainly by unmediated diffusion, a process which is not modified by tight junction function. Small bowel permeability in the sugar test is expressed as a lactulose/ mannitol (L/M) ratio [24]. Since test variables such as gastric emptying, intraluminal dilution, intestinal transit time, systemic distribution and renal clearance affect both molecules, lactulose and mannitol, their effects are cancelled out [25]. This test is, therefore, specific for paracellular small bowel permeability. Patients were advised to fast and refrain from smoking for at least 9 h before the test. Water and tea intake was permitted. The normal morning urine was sampled in a container. Thereafter, patients drank 100 ml of a solution containing 20 g sucrose, 10 g lactulose and 5 g mannitol. This was followed by a fasting period of 5 h, during which all urine passed was sampled in a second container, with sodium acid as a preservative. At 2 h into the test patients were allowed to drink water ad libitum. Aliquots (10 ml) of the urine from both containers were then stored frozen at −20 °C for later analysis of sugar content. Sample preparation and HPLC were performed as previously described by Buhner et al. [15, 21]. Percentage recovery of ingested sucrose measured gastroduodenal permeability, while the L/M ratio reflected small bowel permeability. The cut-off for increased IP in the sucrose test was defined as 0.23%, and for the L/M ratio it was defined as 0.03, in keeping with earlier results in our laboratory [15].

Serum samples were taken on enrolment. Immunoblot testing for serum IgA, as an indicator of recent infection, and IgG, indicative of past infection, antibody response to C. jejuni, Y. enterocolitica and H. pylori was performed with the technician blinded to the patient diagnosis, as previously described [26–28]. The patients were defined seropositive if either the IgA or the IgG immunoblot or both blots were positive.

Statistical analysis was performed with GraphPad Prism 4® (GraphPad Software, San Diego, CA). The frequency of independent events was compared using the chi-square test or Fisher’s test for small expected numbers. The t-test was used to compare normally distributed data between patient and control groups and between male and female patients. The permeability values for the three groups were compared with the one-way analysis of variance (ANOVA). Correlation between sets of parametric data was calculated with the Pearson’s correlation coefficient. The influence of age on permeability in all tested subjects was tested using linear regression analysis.

Results
A total of 40 patients with FM, 8 males and 32 females, were enrolled into this study. Their mean age (±s.d.) was 48 ± 11 yrs, and the median disease duration was 60 months. Their mean number of specific tender points was 15 and the average NRS pain intensity over the 4 weeks preceding the study was 6 ± 1.9. Twenty-five patients with FM (62.5%) described gastrointestinal symptoms (Table 1). Three patients with FM also had a diagnosis of lactose intolerance. Three additional patients had described gastritis, although this was not confirmed endoscopically. No patient had a previous diagnosis of either inflammatory bowel disease or coeliac disease. Both ulcerative colitis and Crohn’s disease were noted in one first-degree relative. No family history of coeliac disease was reported. Seventeen patients with CRPS were enrolled, 4 males and 13 females. CRPS I, without nerve injury, was diagnosed in 11 patients and CRPS II, with nerve injury, in four patients. In two patients, the CRPS class was left undetermined. The upper extremity was affected in 15 and the lower extremity in two patients. Three of these 17 patients also fulfilled the criteria for a diagnosis of FM [1]. The CRPS group had a mean age (±s.d.) of 43 ± 13 yrs and a median disease duration of 12 months. The average pain intensity over the 4 weeks prior to study in this group was 6.1 ± 2.2. Only four CRPS patients (24%) reported intestinal symptoms (P < 0.0001 vs the FM group). This included those three patients who concomitantly fulfilled the diagnostic criteria for FM. One patient described past episodes of gastritis. In this CRPS group, no inflammatory bowel disease or other intestinal diseases were noted either for the patient or their relatives. Fifty-seven healthy volunteers were recruited from university staff. They were chosen to match the sex and the approximate age (±10 yrs) of the patient groups. The volunteer group had a younger mean age (40 ± 13 yrs, P < 0.004) than the patient groups.

Patients with FM had a significantly higher IP than healthy volunteers. Indeed, both FM and CRPS groups had significantly higher IP values than healthy volunteers for both gastroduodenal permeability (FM 0.22 ± 0.2% vs CRPS 0.29 ± 0.27% vs volunteers 0.19 ± 0.075%, P < 0.0001) and small bowel permeability (FM 0.025 ± 0.012% vs CRPS 0.026 ± 0.020% vs volunteers 0.0155 ± 0.006%, P < 0.0002). The difference in gastroduodenal permeability between the two patient groups did not reach significance (P < 0.26). Gastroduodenal permeability, as measured with the sucrose test, was increased in 13 patients with FM (32.5%) and in six patients with CRPS (35.3%) and in one healthy volunteer. The small bowel permeability index was increased in 15 patients with FM (37.5%), three patients with CRPS (17.6%) and in none of the volunteers (P < 0.0001 for both permeability

<table>
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<tr>
<th>Table 1. Abdominal symptoms</th>
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<td>Heartburn</td>
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<td>Sensitive stomach</td>
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<td>Gastritis</td>
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<td>Abdominal cramps</td>
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<td>Abdominal pain, (non-crampy)</td>
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<td>Abdominal bloating</td>
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<tr>
<td>Pathological lactose intolerance test</td>
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<td>Chronic nausea</td>
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Patients with fibromyalgia reported significantly more abdominal symptoms than patients with CRPS (P < 0.0001). The total number of patients with symptoms (first row%) is lower than the number of noted symptoms because multiple symptoms were reported by some patients. This had been diagnosed but was not confirmed by endoscopy.
tests, Fig. 1A and B). Overall, there was a strong correlation between the two tests in both the FM and CRPS groups (FM: Pearson 0.68, $P < 0.0001$; CRPS: Pearson 0.88, $P < 0.0001$, data not shown). This was not noted in the healthy volunteer group (Pearson 0.25, $P < 0.061$). Only five patients (all with FM) had marked discrepancies between the results of both IP tests with a normal saccharose recovery in percentage (S%) test and an abnormal L/M ratio in these cases. The male patients with FM had a significantly increased small bowel permeability ($n = 8$, mean ± s.d. L/M ratio: $0.033 ± 0.008$) when compared with the female patients ($n = 34$, mean L/M ratio: $0.022 ± 0.012$, $P < 0.022$). No such correlation was demonstrated in the CRPS group, and there was no correlation between the male sex and altered gastroduodenal permeability in either patient group (data not shown). There was no correlation between either the number of specific FM tender points or the FM/CRPS disease duration and either the S% or L/M values (data not shown). An additional analysis was performed to test whether age influenced permeability. This was not the case ($r^2$ gastric permeability: $< 0.04$, small bowel permeability: $< 0.005$).

We demonstrated no correlation between the IP and pain intensity in the FM group. In addition, there was also no correlation between the IP and pain intensity in the CRPS patient group. In the FM group, the correlation between IP and NRS pain was $−0.3$ for both the S% and L/M tests, while in the CRPS group it was $0.23$ for the S% test and 0.19 for the L/M test.

Of the three patients with lactose intolerance one patient had both increased gastric permeability and small bowel permeability (data not shown).

Serological results were available for 33 of 40 FM and for 15 of 17 CRPS patients. We had hypothesized that those patients testing positive for *H. pylori* may have increased gastroduodenal permeability when compared with *Helicobacter*-negative patients. Eleven of 33 patients (33%) in the FM group tested positive for *Helicobacter* and 9 of 33 (27%) tested positive for either *Yersinia* or *Campylobacter*. In keeping with our hypothesis, there was a trend for higher IP values in seropositive patients, but this trend did not reach significance (Table 3).

<table>
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<th>Table 3. Serology and medication with NSAIDs</th>
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<td><strong>FM L/M</strong></td>
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<tr>
<td>All patients ($n = 40$)</td>
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<tr>
<td>$HP$ pos. ($n = 18$)</td>
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<tr>
<td>$HP$ neg. ($n = 30$)</td>
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<tr>
<td>$Y$ or $C$ pos. ($n = 11$)</td>
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<tr>
<td>$Y$ and $C$ neg. ($n = 39$)</td>
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<tr>
<td>NSAID &lt; 4/52 ($n = 15$)</td>
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<tr>
<td>No NSAID or ≥ 4/52 ($n = 42$)</td>
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The intestinal permeability was compared between patients who were seropositive or seronegative for three common intestinal pathogens, *Helicobacter pylori* (HP, a pathogen of the gastric mucosa), *Campylobacter jejuni* (C) or *Yersinia enterocolitica* (Y). L/M: lactulose/mannitol ratio, reflecting the permeability of the small intestine; S%: percentage recovery in the saccharose test reflecting gastric and duodenal permeability. NSAID < 4/52: patients with intake of NSAIDs between 4 weeks and 2 days before the test.
a trend for higher gastroduodenal permeability, but not for higher IP values in those patients who had taken NSAIDs (Table 3). This trend was stronger in those five patients who had taken NSAIDs between 24 h and 1 week before the test (data not shown). Only two patients in the CRPS group (12%) had taken NSAIDs.

Adverse event reports for the week following the test were available from 31 patients in the FM group. Thirteen patients reported moderate or severe diarrhoea for up to 2 days after test. In contrast, in the CRPS group, only one of the 13 patients with available reports reported moderate diarrhoea (7.7%, P < 0.04). No adverse events were reported in the volunteer group (P < 0.0001).

Discussion

This is the first published study that has demonstrated increased IP in patients with FM and CRPS. The pathophysiology of FM is unknown. Several aetiological mechanisms have been suggested including genetic disposition, trigger events such as infection, neurotransmitter abnormalities, immune dysregulation and central sensitization [29]. A recent study by Pimentel et al. [3] reported an increased SIBO in patients with FM and correlated this with pain intensity suggesting a pathophysiological role. As SIBO can alter IP, we hypothesized that FM may be associated with a leaky gut barrier. We measured both gastroduodenal and small bowel IP in patients with FM. In our 40 patients with FM, we found increased gastroduodenal permeability in 13 and small bowel permeability in 15 patients. Overall, FM had significantly higher IP values for both gastroduodenal and small bowel permeability when compared with healthy volunteers. Unexpectedly, patients with CRPS also had an altered IP. This increased IP that we observed may have pathophysiological relevance in both FM and CRPS. Investigations in other medical disorders, such as enteropathic arthritis and allergies and in chronic fatigue syndrome suggest that increased IP may allow luminal antigens inappropriate access to immunocompetent cells, which are consequently modulated to cause extraintestinal manifestation of the disease [6, 7, 30]. Immune cell function in FM and CRPS is defective and it has been proposed that this defect contributes to the pathophysiology of these pain syndromes [31–33]. Peripheral blood mononuclear cells (PBMCs) both from patients with FM and CRPS had reduced mRNA expression for anti-inflammatory cytokines suggestive of the contribution of these PBMCs to an increased inflammatory response [31, 33]. One may speculate that a leaky gut barrier in both patients with FM and CRPS may facilitate specific immunological responses to cause and or sustain these syndromes. There is evidence that the restoration of normal IP may improve disease activity in certain human conditions. For example, in children with atopic dermatitis, probiotic treatment normalizes IP and reduces eczema severity [34]. In addition, there is laboratory evidence to support the benefit of the restoration of normal IP. In a model of irritable bowel syndrome, a stress-induced visceral hypersensitivity model, stress causes both increased colonic IP and visceral hypersensitivity. In this model, local treatment with a tight junction chemical blocker reduces both IP and visceral hypersensitivity in the face of ongoing stress [35].

We demonstrated no correlation between IP values and either the pain intensity or the presence of abdominal symptoms in our FM patient study group. This is contrary to Pimentel et al.’s [3] report of a correlation between SIBO and pain intensity in patients with FM. However, in other clinical conditions, which are known to be associated with increased IP, and where IP is considered of pathophysiological relevance, such as AS, no correlation between the degree of IP and disease activity is observed [36]. In these cases, an altered IP may be a necessary but not a sufficient factor alone for an abnormal mucosal immune response. Additional factors interacting with an increased IP may be important, and these may include abnormalities in the mucosal immune system. One example where this has been established is in a mouse model of diabetes type 1, in which a subset of natural T killer lymphocytes suppress the intestinal immune response to luminal antigens, and when IP is disturbed, a defect in this T-cell population is necessary to cause this extraintestinal disease [37, 38].

Altered IP may be caused by several factors including SIBO, gut infection, gut inflammation, medications, stress and trauma [6, 39, 40]. An episode of intestinal infection can lastingly increase IP [13, 41]. Since many patients with FM report that their symptoms start following an intestinal infection [14], we hypothesized that current or past infection with common intestinal pathogens may account for a raised IP in some patients. Patients were tested for seropositivity to H. pylori, C. jejuni and Y. enterocolitica. In the FM group, we demonstrated seropositivity for Helicobacter in 11 patients and in 9 for either Campylobacter or Yersinia. In keeping with our hypothesis in both patient groups we identified a trend whereby those patients who had serological evidence for current or prior infections also had increased IP (Table 3). One cannot comment on the significance of this finding, unless one examines the serology of the healthy volunteers. It is possible that other intestinal bacterial or viral infections may play a role [42]. NSAIDs may alter IP. No patient took NSAIDs in the 48-h period prior to this study. Thirteen patients with FM took NSAIDs in the preceding month. We observed a minimal, non-significant, increase in gastroduodenal IP in this patient group while the small intestinal IP was lower than in those who did not take NSAIDs (Table 3). The increase in IP that we observed in the FM group is, therefore, independent of NSAID medication. Another alternative explanation for the raised IP in both the FM and the CRPS patient groups may be pain-related distress. It is well-established that pain causes stress, although with no direct correlation between pain intensity and distress level [43]. There is clear evidence both from animal models and in vitro human mucosal investigations supporting a role for stress in altering IP [44, 45]. One may hypothesize that pain-related distress can independently contribute to alter IP in our patient population. In our study, we observed a trend towards a greater increase in the gastroduodenal IP in patients with CRPS when compared with those with FM. This may suggest that the combination of factors which affect gastroduodenal IP may vary between these two pain syndromes.

This study has several limitations. First, as our study was designed to test whether patients with FM have increased IP, we chose not to measure SIBO and the question of a potential association between SIBO and IP in patients with FM remains open. Future studies on IP in FM should include the lactulose breath testing, a standard test of SIBO, to further clarify the association between SIBO and IP [10]. Second, we did not require that the patients document whether the condition may have evolved after an intestinal infection. Therefore, we cannot comment on whether patients with reported post-infectious FM have an increased IP above others. Third, our questionnaire did not enquire as to the patients’ perceived level of distress and we are unable to directly assess whether an association exists, possibly independent from the pain intensity, between stress and IP in these patients.

In summary, we present for the first time data that demonstrate significantly increased gastroduodenal and small bowel permeability in two chronic pain syndromes, FM and CRPS. Both the pathophysiological and therapeutic implication of such a markedly increased IP await further study.

Rheumatology key messages

- The permeability of the small intestine is increased in patients with primary FM and CRPS.
- This finding may have both pathogenetic and therapeutic implications awaiting further study.
Acknowledgements

The authors are grateful to the patients who participated in the study. They would also like to thank Nesrin Halicgil, Brigitte Trochner-Klinger, Karin Herzele and Guido Klueber for invaluable organizational and technical support and Martina Werich for technical expertise.

Funding: This study was funded from internal departmental funds.

Disclosure statement: The authors have declared no conflicts of interest.

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