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Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study

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SUMMARY

Background
Coeliac disease, an autoimmune disorder triggered by gluten ingestion, is managed by a gluten-free diet (GFD), which is difficult for many patients. Larazotide acetate is a first-in-class oral peptide that prevents tight junction opening, and may reduce gluten uptake and associated sequelae.

Aim
To evaluate the efficacy and tolerability of larazotide acetate during gluten challenge.

Methods
This exploratory, double-blind, randomised, placebo-controlled study included 184 patients maintaining a GFD before and during the study. After a GFD run-in, patients were randomised to larazotide acetate (1, 4, or 8 mg three times daily) or placebo and received 2.7 grams of gluten daily for 6 weeks. Outcomes included an experimental biomarker of intestinal permeability, the lactulose-to-mannitol (LAMA) ratio and clinical symptoms assessed by Gastrointestinal Symptom Rating Scale (GSRS) and anti-transglutaminase antibody levels.

Results
No significant differences in LAMA ratios were observed between larazotide acetate and placebo groups. Larazotide acetate 1-mg limited gluten-induced symptoms measured by GSRS ($P = 0.002$ vs. placebo). Mean ratio of anti-tissue transglutaminase IgA levels over baseline was 19.0 in the placebo group compared with 5.78 ($P = 0.010$), 3.88 ($P = 0.005$) and 7.72 ($P = 0.025$) in the larazotide acetate 1-, 4-, and 8-mg groups, respectively. Adverse event rates were similar between larazotide acetate and placebo groups.

Conclusions
Larazotide acetate reduced gluten-induced immune reactivity and symptoms in patients with coeliac disease undergoing gluten challenge and was generally well tolerated; however, no significant difference in LAMA ratios between larazotide acetate and placebo was observed. Results and design of this exploratory study can inform the design of future studies of pharmacological interventions in patients with coeliac disease.

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INTRODUCTION

Coeliac disease is an autoimmune disorder triggered by the ingestion of gluten by genetically susceptible individuals. Patients with coeliac disease frequently present with gastrointestinal symptoms such as diarrhoea, abdominal pain and bloating, and may also experience extra-intestinal signs such as iron deficiency anaemia, dermatitis, osteoporosis, infertility and neurological complications.[1–3] Serious complications include intestinal adenocarcinoma and non-Hodgkin's lymphoma, which may develop due to chronic inflammation and chronic stimulation of intestinal lymphocytes.[4, 5]

Currently, the only management option for coeliac disease is a gluten-free diet (GFD). Adherence to this highly restrictive diet is difficult due to the pervasiveness of gluten in foods. The estimated inadvertent exposure to gluten despite adherence to a GFD ranges from several milligrams up to 2 g per day.[6, 7] Exposure to even these small amounts can trigger signs and symptoms and cause histological changes including intestinal villous atrophy and increased intraepithelial lymphocytes.[8, 9] Consequently, many patients, even if they follow the GFD, do not attain full relief from symptoms, nor do they experience complete mucosal healing even after long-term maintenance of a GFD.[10, 11] Failure to heal is associated with excess morbidity and perhaps mortality.[12] A GFD alone, therefore, is not sufficient to fully control the disease in many patients.[13]

Gene pairs encoding the HLA molecules DQ2 or DQ8 and environmental exposure to the gluten antigen are required, but not sufficient for the development of coeliac disease.[14] In addition, tight junction opening may contribute to disease development and/or persistence.[15] Patients with coeliac disease have altered tight junction morphology and higher intestinal permeability compared with healthy controls.[16–18] Gluten triggers the opening of tight junctions, which may result in enhanced paracellular gluten transport and immunological exposure to luminal antigens in the lamina propria.[19] This is believed to be a factor in the increased secretion of inflammatory cytokines leading to prolonged opening of tight junctions, thus perpetuating a self-amplifying inflammation loop.[20, 21]

Larazotide acetate is a first-in-class, 8-amino acid, synthetic peptide that *in vitro* prevents the opening of tight junctions induced by multiple stimuli, including cytokines, bacterial antigens and gluten fragments.[22, 23] In a transgenic mouse model of gluten-sensitive enteropathy, larazotide acetate reduced the transport of a marker protein across the intestinal epithelium, preserved intestinal tight junction structure and reduced macrophage count in the lamina propria.[22] In a Phase 1 clinical trial of patients with coeliac disease, larazotide acetate blocked the gluten-induced increase in intestinal permeability and alleviated gastrointestinal symptoms.[24] In a dose-ranging, randomised, placebo-controlled study, some doses of larazotide acetate appeared to prevent the increase in gastrointestinal symptom severity during a gluten challenge.[25] Here, we report the results of a separate and larger, exploratory, dose-ranging study in which we evaluated the clinical efficacy and tolerability of larazotide acetate in patients with coeliac disease who have been observing a GFD. The study was also conducted to gain additional experience in the use of clinical outcome measures and gluten challenge in a randomised, placebo-controlled study of a pharmacological therapy in patients with coeliac disease.

MATERIALS AND METHODS

Patients

All patients provided written informed consent. Eligible patients were adults (aged 18–72 years) with coeliac disease diagnosed by duodenal/jejunal biopsy at least 6 months prior to study entry. Patients had to have been observing a GFD for $\geq$6 months, and anti-tTG antibody levels had to be $\leq$10 U/mL at study entry (normal range: 0–10 U/mL). Patients were ineligible for the study if they had refractory coeliac disease, severe complications of coeliac disease, or any other chronic active gastrointestinal disease, Type I or Type II diabetes, human immunodeficiency virus infection, hepatitis B or C infection, major active psychiatric or neurological disease, or a history of dermatitis herpetiformis. Patients were also excluded if they smoked or used nicotine products, had food allergies or any food intolerance, or were pregnant or lactating. Patients were not permitted to take medications known to affect their immune system or intestinal functioning or permeability, or to alter intestinal pH, including medications containing bismuth or diphenoxylate, immune suppressants, antibiotics, proton pump inhibitors, laxatives or colonic therapies, pancreatic enzyme replacement, corticosteroids, fibrates, herbal remedies, chewable antacids, probiotic medications, amphetamines, immune therapies and ezetimibe.

Procedures

This study was performed according to good clinical practice as described by the International Conference on Harmonization; the protocol was reviewed and approved by each site's institutional review board or ethics committee (Clinical Trials registration number:

NCT00492960). Patients were screened for eligibility up to 2 weeks before enrolment. On day 0, eligible patients began a 1-week single-blind (only the investigator was aware of the treatments) run-in period during which all patients received placebo drug capsules three times daily 15 min before each main meal and placebo gluten capsules three times daily with each main meal.

Patients were subsequently randomised in a double-blind fashion to 1 of 4 treatment groups in a 1:1:1:1 manner: placebo or 1-, 4- or 8-mg larazotide acetate to be administered orally three times daily 15 min before breakfast, lunch and dinner for a 42-day treatment period beginning on day 7 (Figure 1a). All patients also received 900 mg of gluten orally three times daily with each main meal for a total of 2.7 g of gluten (equivalent to approximately one slice of bread) per day. The purpose of this modified gluten challenge was to simulate the effect of inadvertent gluten ingestion by patients who intended to follow a GFD.

Drug capsules contained the active substance, larazotide acetate, in enteric-coated multi-particulate beads. Placebo drug was provided in identical capsules with similarly composed beads that lacked the active substance. The gluten challenge was provided as two 450-mg gluten capsules. Placebo gluten (100% cornstarch) was provided during the run-in period in two capsules identical in appearance to the gluten capsules.

A computer-generated randomisation schedule was prepared by an independent statistician and consisted of a permuted four-block scheme with no stratifications. Randomisation was allocated centrally using numbered containers and an interactive voice/Web response system through an independent contractor. All patients and study personnel remained unaware of treatment allocation throughout the study. Unblinding occurred after the data collection was complete and the database was locked.

The primary efficacy outcome measure was the lactulose-to-mannitol (LAMA) ratio, an experimental biomarker of intestinal permeability.[16, 26] The LAMA ratio is the ratio of a patient's fractional excretion of lactulose to the fractional excretion of mannitol in a urine sample. Mucosal injury in patients with coeliac disease leads to a reduction in the transmembrane absorption of monosaccharides (e.g. mannitol) and an increase in the paracellular absorption of disaccharides (e.g. lactulose), resulting in an increase in the LAMA ratio. Details of the LAMA test are provided in the supplemental material.

Serum was analysed for anti-tTG IgA and IgG antibodies at screening and on days 7, 49 and 56. Anti-tTG antibodies are an objective indicator of gluten exposure and autoimmune response in patients with coeliac disease. Because they are slower to respond to a gluten challenge than symptoms, anti-tTG levels were only measured before and after the gluten challenge. Anti-tTG antibodies were measured at a central laboratory using a commercially available enzyme-linked immunosorbent assay (ELISA) (The Binding Site Ltd., Birmingham, England). Anti-tTG IgA levels of >10 U/mL were designated as positive in accordance with the manufacturer's specifications.

Patients completed two questionnaires weekly, the Gastrointestinal Symptom Rating Scale (GSRS)[27] and the Psychological General Well-Being Index (PGWBI).[28] The Coeliac Disease GSRS (CeD-GSRS) was a composite of the 10 items in the diarrhoea, abdominal pain and indigestion sub-domains of the GSRS; these symptoms are generally more prominent than constipation and reflux in patients with coeliac disease. Further details and additional assessments are described in the supplemental material.

Statistical evaluation

The 'fold ratio' is the ratio of the LAMA ratio on a given day to that at baseline (day 7). The primary efficacy outcome measure of this study was the mean fold ratio at the last double-blind treatment period visit. The relative efficacy of each treatment group was calculated by dividing the geometric mean of the fold ratio of the placebo group by that of the dose group, and was analysed using an analysis of covariance (ANCOVA) model, with the baseline log LAMA ratio value used as a covariate. Secondary analyses tested response by age, sex and disease presentation. Preplanned secondary outcomes derived from the GSRS and PGWBI were investigated using the same ANCOVA approach as described above. The mean total GSRS and PGWBI scores were assessed for all questions in the indices, and sub-scores were calculated similarly for each of the dimensions. The CeD-GSRS was analysed similarly to the total GSRS. All statistical analyses were conducted using SAS version 9.1 (SAS Inc., Cary, NC, USA).

The safety analysis set included all randomised patients who received 1 or more doses of larazotide acetate, placebo drug or gluten capsules during the double-blind treatment period. The full efficacy analysis set included those patients in the safety analysis set who had at least 1 efficacy outcome value during the double-blind treatment period, who fulfilled the major entrance criterion, biopsy-confirmed coeliac disease, and who were not taking a medication that was excluded by the protocol.

A planned blinded interim analysis was conducted after 74 patients completed the study to evaluate data

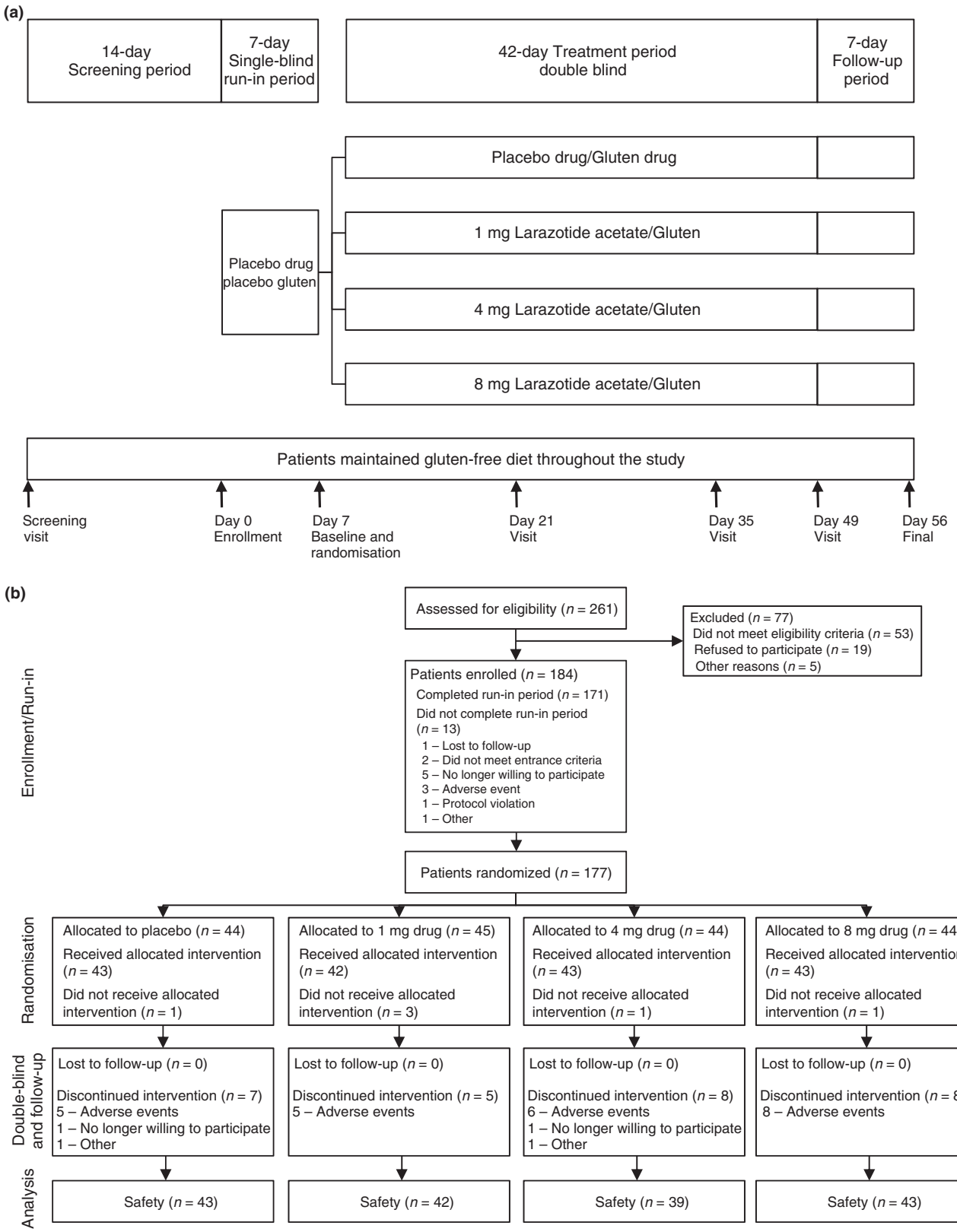

Figure 1 | Study design (a) and disposition of patients (b).

variability to determine if the sample size needed to be re-estimated and to evaluate adverse events and clinical laboratory data for safety review. An independent statistician not otherwise associated with the study performed the analyses. Variability was in the expected range, so the sample size was not changed.

RESULTS

Patients' disposition and baseline characteristics

The study was conducted at 20 sites in the United States and Canada. Patients were recruited between August 2007 and August 2008, and the last patient visit occurred in October 2008. Of 261 patients screened for eligibility in the study, 184 were enrolled into the single-blind run-in period during which all patients took placebo drug and placebo gluten. Of these, 177 were randomly assigned to study medication. Six of these randomised patients never received study medication or gluten challenge. The other 171 patients received at least one dose of study medication and gluten and were included in the safety analysis set. Four of the 171 patients who received the assigned intervention had a major protocol violation, leaving 167 patients in the efficacy analysis set (Figure 1b).

The randomised study population had a mean age of 49 years and comprised 72% women (Table 1), a percentage similar to that of the diagnosed coeliac population.[29] The only significant imbalance in the study was more women in the 1-mg treatment group (80%) compared with the placebo group (66%, $P = 0.028$). All participants were Caucasian, except one who was of Hispanic origin. Of the 132 patients who consented to de-identified genotyping, 131 were either HLA DQ2 or DQ8 positive. The clinical presentations were similarly distributed across groups (not shown), with a trend ($P = 0.064$) towards reduced history of diarrhoea at diagnosis in the 1-mg treatment group.

Efficacy

The LAMA fold-ratio increased over time in the placebo group and reached a plateau of 2.3–2.4 approximately 4 weeks after starting the gluten challenge (Figure 2). Although the LAMA fold-ratio trended lower than placebo in the 1-mg group, there was no significant effect of any dose of larazotide acetate on the LAMA fold-ratio or on the fractional excretion of either lactulose or mannitol individually, and there was no effect of age, gender or gastrointestinal presentation on the LAMA ratio.

Table 1 | Baseline demographic and clinical characteristics

	Larazotide acetate				
	Placebo *n* = 44	1 mg t.d.s. *n* = 45	4 mg t.d.s. *n* = 44	8 mg t.d.s. *n* = 44	Total *n* = 177
General characteristics					
Female	29 (66%)	36 (80%)*	31 (70%)	31 (70%)	127 (72%)
Age, years	50.3 (10.24)	50.3 (10.09)	49.5 (12.42)	47.4 (13.61)	49.4 (11.64)
White	44 (100%)	45 (100%)	44 (100%)	44 (100%)	177 (100%)
Weight, kg	74.0 (15.25)	70.5 (12.72)	75.5 (17.07)	73.3 (13.83)	73.3 (14.78)
Height, cm	170.9 (9.20)	166.8 (9.51)	168.1 (8.84)	169.1 (7.60)	168.7 (8.88)
Body mass index, kg/m^2	25.2 (4.02)	25.3 (3.80)	26.7 (5.43)	25.6 (4.16)	25.7 (4.40)
Clinical characteristics					
Time since onset of coeliac disease, months†	143.2 (162.47)	139.8 (144.61)	155.0 (190.10)	132.1 (138.25)	142.5 (158.69)
Time since diagnosis of coeliac disease, months†	63.4 (52.08)	66.3 (52.53)	60.0 (65.54)	54.0 (43.52)	61.0 (53.72)
Time since start of a GFD, months†	68.7 (57.44)	68.4 (58.80)	81.0 (116.21)	63.3 (53.62)	70.3 (75.60)
Presentation at diagnosis					
Gastrointestinal features	43 (98%)	38 (84%)	39 (89%)	38 (86%)	158 (89%)
Diarrhoea	29 (66%)	21 (47%)	28 (64%)	28 (64%)	106 (60%)
Extra-intestinal features	32 (73%)	32 (71%)	29 (66%)	29 (66%)	122 (69%)
General features (short stature, failure to thrive, other)	7 (16%)	11 (24%)	14 (32%)	12 (27%)	44 (25%)

GFD, gluten-free diet.

Data are numbers (%) or mean (s.d.).

* *P*-value = 0.028 for 1-mg larazotide acetate group compared to placebo using Pearson's chi-square analysis.

† As assessed at screening visit.

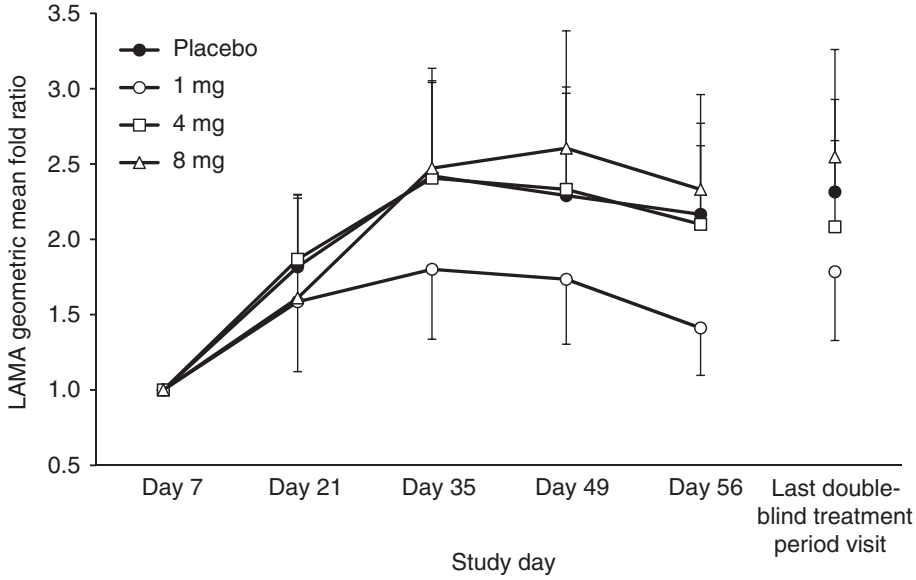

Figure 2 | Mean change from baseline in the urinary lactulose-to-mannitol (LAMA) ratio. Values are the geometric mean fold ratio on days 21, 35, 49 and 56 over baseline (day 7). Vertical bars = 95% confidence interval.

The mean total GSRS scores increased slightly during the run-in period when all patients were receiving gluten placebo, possibly because patients thought they were receiving gluten. Baseline (day 7) scores indicated that this patient population had few symptoms on their GFDs. The mean GSRS scores increased in the placebo group during the first 3 weeks of gluten challenge and then reached a plateau with a mean increase relative to baseline of 0.3–0.4 units during the last 3 weeks of the 6-week gluten challenge (Figure 3a). Scores decreased once the gluten was withdrawn. In the 1-mg larazotide acetate group, the mean GSRS score remained near baseline throughout the 6-week gluten challenge period, suggesting that there was no increase in gluten-induced gastrointestinal symptoms in this group (Table S1). The total GSRS change from baseline score in the 1-mg group was significantly lower than that of placebo at the end of the treatment period ($P = 0.017$) and when averaged over the treatment period ($P = 0.002$) (Table S1). The 4- and 8-mg larazotide acetate groups had GSRS reductions that were intermediate between the placebo and the 1-mg group and that were not significantly different from placebo.

The strongest difference between the treatment groups and placebo group occurred in the diarrhoea sub-domain of the GSRS. The average change from baseline over the treatment period in the 1-mg group was significantly lower than that in the placebo group (−0.2 vs. 0.3, respectively, $P = 0.001$). Scores in all other sub-domains of the GSRS in the 1-mg larazotide acetate group were generally lower than placebo, with significantly lower average scores in the indigestion and abdominal pain sub-domains over the treatment period ($P = 0.002$ and 0.042 respectively). Results of the CeD-GSRS were similar to those of the total GSRS (Figure 3b).

Mean anti-tTG IgA antibody levels at baseline ranged between 1 and 2 U/mL, indicating that the study population was adhering to a GFD. Anti-tTG IgA levels increased after the 6-week gluten challenge, with the largest response in the placebo group (Table 2), in which 30% of patients seroconverted. The mean antibody level in the 1- and 4-mg drug groups remained below 10 U/mL, the cut-off for a positive test result. The relative fold increase in anti-tTG levels in all three drug treatment groups was significantly lower than in the placebo group (Table 2).

To better understand how patients responded to the gluten challenge and the effect larazotide acetate had on that response, an exploratory binary analysis was performed (Figure S1).

Patients in the placebo group tended to have lower (poorer) PGWBI scores during the last 2 weeks of the gluten challenge, whereas the drug-treatment groups tended to remain near or above baseline (no change or improved) (Figure S2). These nonsignificant trends persisted throughout all sub-domains of the PGWBI, especially in the vitality sub-domain. Similarly, in the clinician global assessment, there were trends towards bet-

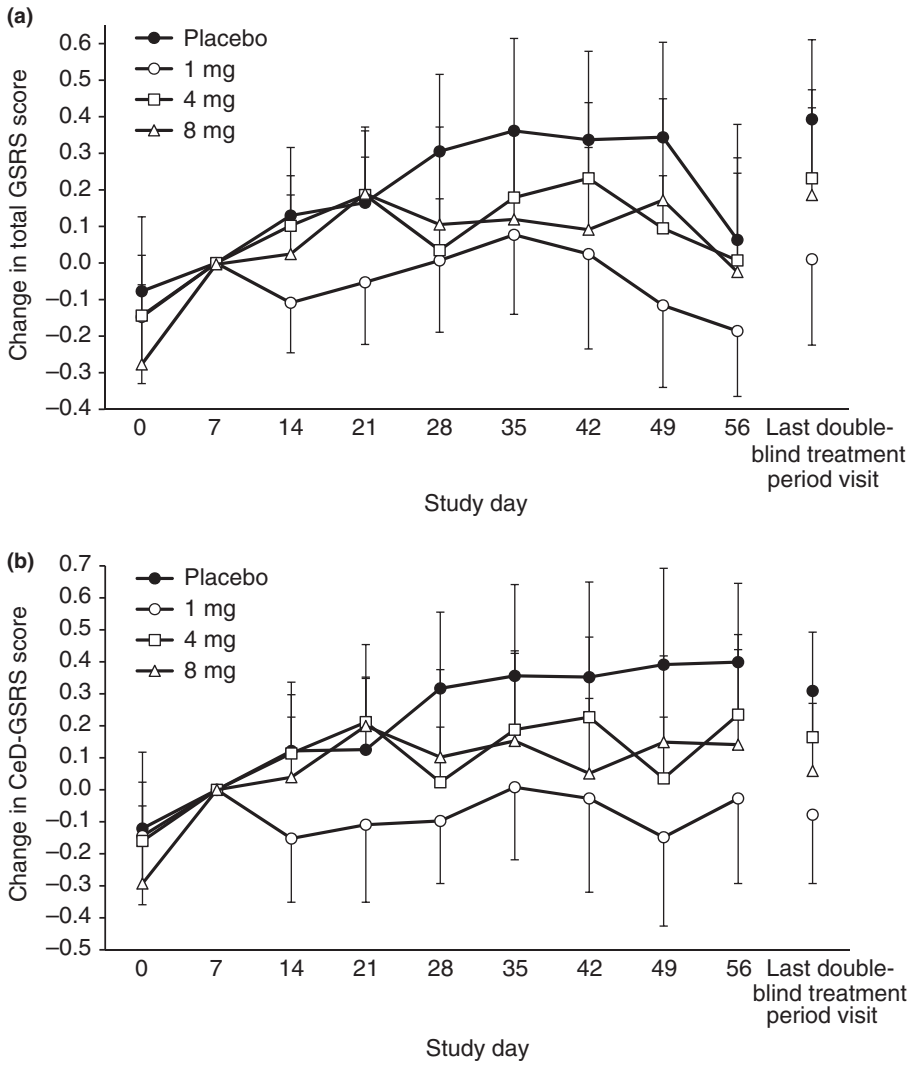

Figure 3 | Weekly change from baseline in gastrointestinal symptoms as rated by the total Gastrointestinal Symptom Rating Scale (a) and the coeliac disease-specific Gastrointestinal Symptom Rating Scale (b). Analysis for the LDBTP (last double-blind treatment period) visit uses the last nonmissing observations after baseline (day 7) during the double-blind treatment period. GSRS, Gastrointestinal Symptom Rating Scale. *P*-values shown are for the 1-mg group at the LDBTP visit and the mean over the double-blind treatment period (in parentheses).

ter scores in the active groups compared with the placebo group at the last visit of the double-blind treatment period (1-mg group, $P = 0.075$) (Figure S3).

Safety and tolerability

The incidence of adverse events in the placebo, 4-mg and 8-mg groups ranged from 77% to 81% with a slightly lower percentage (69%) in the 1-mg drug group (Table 3). Adverse events were clustered in gastrointestinal disorders; 58% of patients expressed gastrointestinal-related adverse events, which were distributed evenly among the groups. The incidences of gastrointestinal disorders in the active groups were similar to or lower than the placebo group, with the exception of abdominal pain in the 8-mg group. In addition, 12% and 8% of patients experienced fatigue or headaches, respectively, with no evidence of drug-relatedness.

Investigators indicated whether they believed that adverse events were related to the gluten challenge. Gluten-related adverse events were clustered in the gastrointestinal disorders; approximately 40% of patients in the placebo, 4-mg and 8-mg groups, and 26% in the 1-mg group had gluten-related gastrointestinal adverse events (Table 3). The lower percentage in the 1-mg

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Table 2 | Mean (standard deviation) anti-transglutaminase (tTG) IgA levels

	Larazotide acetate			
	Placebo (n = 43)	1 mg t.d.s. (n = 42)	4 mg t.d.s. (n = 39)	8 mg t.d.s. (n = 43)
Baseline anti-tTG antibody level (U/mL)	1.89 (3.05)	1.33 (1.44)	2.02 (1.84)	1.60 (1.34)
Anti- tTG antibody level at LDBTP visit (U/mL)	18.8 (29.9)	7.12 (17.1)	6.56 (7.18)	15.0 (28.8)
Ratio over baseline	19.0 (42.1)	5.78 (13.4)	3.88 (5.27)	7.72 (14.3)
P-value	–	0.010	0.005	0.025

LDBTP, last double-blind treatment period.

P-values comparing treatment group to placebo were calculated using an ANCOVA model with the corresponding day 7 baseline value as a covariate.

group was largely attributable to lower incidences of diarrhoea, nausea, constipation and abdominal pain. Nine per cent of patients had gluten-related general disorders, which were highest in the placebo group (14%) and lowest in the 1-mg group (5%), largely due to differences in fatigue. Fewer patients in the larazotide acetate groups had severe gluten-related adverse events compared with the placebo group, especially the 1-mg group in which no patients had severe gluten-related adverse events (Table 3).

No clinically significant changes in serum chemistry, haematology, urinalyses or EKG parameters were identified. There was a trend (P = 0.07) towards lower ferritin in the 1-mg group at the end of the study, possibly because of the greater percentage of women in this group. Antibodies to larazotide acetate were not detected in the serum of any patients in the study.

DISCUSSION

In this exploratory study, we evaluated the effect of larazotide acetate on intestinal permeability, development of antibodies to tTG and symptoms of coeliac disease during gluten challenge equivalent to the inadvertent ingestion of gluten in patients whose disease was well controlled on a GFD. We observed a reduction in an experimental biomarker for intestinal permeability, the LAMA ratio, in the larazotide acetate 1-mg group, but the difference was not statistically significant compared with placebo. However, results of prespecified secondary endpoints suggest that larazotide acetate reduced antigen exposure as manifested by reduced production of anti-tTG antibodies. Larazotide acetate also reduced gastrointestinal symptoms upon gluten challenge.

Gluten was administered with each meal, ingested within the time frame that larazotide acetate is expected to be present in the proximal small intestine: approximately 2–3 h. However, the LAMA assay was performed at least 4 h after drug administration, which may have been after the drug was present and active. LAMA results were also highly variable, perhaps because the test was performed in an out-patient setting. This may explain why results of this study differ from those of an earlier in-patient study in which larazotide acetate was taken 60 min before the LAMA test, and a statistically significant difference between active treatment and placebo was reported.[24]

While all three doses of larazotide acetate showed a benefit in the anti-tTG and gluten responder analyses, only the 1-mg dose provided statistically significant protection from gluten-induced gastrointestinal symptoms as measured by the GSRS score. Unlike systemically absorbed small molecule drugs, a 'classic' dose–response relationship may not apply to minimally or non-absorbed oral peptides, which can exhibit better benefit–risk ratios at lower doses. For example, linaclotide was evaluated for treatment of irritable bowel syndrome with constipation and chronic constipation in dose-finding phase II studies at doses up to 1000 μg/day.[30] Much lower doses (145 μg/day and 290 μg/day) were found to be optimal, moved forward to phase III studies and were subsequently approved.[31, 32]

The magnitude of the difference in GSRS scores between the placebo and 1-mg groups was approximately 0.4 units. Other studies have shown that the GSRS scores of patients with coeliac disease improved approximately 0.4–0.7 units after following a GFD for 1 year, and patients on a GFD had scores that were 0.3 units worse than those of healthy subjects,[8, 33] suggesting that the magnitude of the response in this study is clinically meaningful and that the GSRS instrument is appropriate for assessing symptoms in coeliac disease. GSRS scores were corroborated by adverse events; the 1-mg group

Table 3 | Treatment-emergent adverse events

All-cause adverse events	Larazotide acetate				
	Placebo *n* = 43	1 mg t.d.s. *n* = 42	4 mg t.d.s. *n* = 43	8 mg t.d.s. *n* = 43	Total *n* = 171
Subjects with 1 or more adverse event	35 (81%)	29 (69%)	34 (79%)	33 (77%)	131 (77%)
Gastrointestinal disorders	26 (60%)	24 (57%)	24 (56%)	26 (60%)	100 (58%)
Diarrhoea	12 (28%)	7 (17%)	12 (28%)	8 (19%)	39 (23%)
Abdominal distention	8 (19%)	6 (14%)	5 (12%)	6 (14%)	25 (15%)
Flatulence	6 (14%)	7 (17%)	6 (14%)	5 (12%)	24 (14%)
Nausea	8 (19%)	4 (10%)	6 (14%)	4 (9%)	22 (13%)
Constipation	5 (12%)	5 (12%)	1 (2%)	7 (16%)	18 (11%)
Abdominal pain	2 (5%)	2 (5%)	2 (5%)	6 (14%)	12 (7%)
General disorders	11 (26%)	4 (10%)	5 (12%)	6 (14%)	26 (15%)
Fatigue	8 (19%)	4 (10%)	4 (9%)	4 (9%)	20 (12%)
Nervous system disorders	6 (14%)	7 (17%)	5 (12%)	6 (14%)	24 (14%)
Headache	3 (7%)	3 (7%)	2 (5%)	5 (12%)	13 (8%)
Subjects who withdrew due to 1 or more adverse events	5 (12%)	5 (12%)	3 (7%)	6 (14%)	19 (11%)
Subjects with 1 or more severe adverse events	5 (12%)	0	1 (2%)	6 (14%)	12 (7%)
Gluten-related adverse events as assessed by the investigator					
Subjects with 1 or more gluten-related adverse event	20 (47%)	12 (29%)	22 (51%)	18 (42%)	72 (42%)
All gluten-related gastrointestinal disorders	19 (44%)	11 (26%)	17 (40%)	16 (37%)	63 (37%)
Diarrhoea	10 (23%)	2 (5%)	8 (19%)	4 (9%)	24 (14%)
Abdominal distention	6 (14%)	5 (12%)	4 (9%)	2 (5%)	17 (10%)
Flatulence	5 (12%)	4 (10%)	4 (9%)	3 (7%)	16 (9%)
Nausea	5 (12%)	2 (5%)	4 (9%)	2 (5%)	13 (8%)
Constipation	3 (7%)	1 (2%)	1 (2%)	6 (14%)	11 (6%)
All gluten-related general disorders	6 (14%)	2 (5%)	4 (9%)	4 (9%)	16 (9%)
Fatigue	5 (12%)	2 (5%)	4 (9%)	3 (7%)	14 (8%)
Subjects who withdrew due to 1 or more gluten-related adverse event	5 (12%)	2 (5%)	2 (5%)	3 (7%)	12 (7%)
Subjects with 1 or more severe gluten-related adverse events	5 (12%)	0	1 (2%)	4 (9%)	10 (6%)

Data are numbers of subjects (%). Adverse events occurring after baseline (day 7) with >10% frequency in any treatment group, as well as withdrawals and severe adverse events are reported. The gluten-related adverse events are a subset of all-cause adverse events.

had a lower incidence of gluten-related gastrointestinal symptoms, particularly diarrhoea, compared with the placebo group.

In a previous, smaller, study of larazotide acetate during gluten challenge, 0.25- and 4-mg doses of larazotide acetate resulted in significantly lower changes from baseline to day 14 in GSRS scores compared with placebo.[25] Lower changes were also observed in patients who received 1-mg larazotide acetate, but the difference from placebo (approximately 0.5 points) did not reach statistical significance ($P = 0.067$). In this study, we did not evaluate the 0.25-mg dose, but the magnitude of the change in the 1-mg group was consistent with the results of the previous study, and did reach statistical significance vs. placebo, perhaps because of the larger sample size.

The reduction in anti-tTG across all larazotide acetate dose groups provides an objective measure of drug effect and suggests that the drug may reduce the autoimmune response, presumably due to reduced exposure of the gluten antigen to the immune system.

This study also supports findings of a previous study showing larazotide acetate safety and tolerability.[24] No indications of drug-related adverse events or safety signals were observed. No patient developed antibodies to larazotide acetate as the drug is not systemically available in humans.[24] Gluten-related adverse events resulted in early withdrawal of some patients. Nonetheless, symptoms generally resolved during the 1-week follow-up period, indicating that a gluten challenge design utilising 2.7 g of gluten per day is feasible for future studies.

In conclusion, results of this exploratory study suggest that larazotide acetate reduced immunological activation by gluten and mitigated gluten-related signs and symptoms. The design and results of this study can be used to inform the design of future studies of pharmacological interventions for coeliac disease.

AUTHORSHIP

Guarantor of the article: C. P. Kelly.

Author contributions: CPK, JAM, RNF and BMP designed the study. CPK, PHRG, JAM, ADM, AC, DAL, TA, RA and RNF collected the data. JGJ, FL and LAA analysed the data. CPK, FL, LAA, BMP and RNF interpreted the data. All authors contributed to the development of the manuscript. CPK made the final decision to submit the manuscript. All authors approved the final version of the manuscript.

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Declaration of personal interests: Drs Leon, Jiang and Arterburn were employed by Alba Therapeutics and owned stock or stock options in the company at the time of the study. Drs Kelly, Green, DiMarino, Leffler and Fedorak have served as principal investigators for clinical trials in coeliac disease and have received advisory board honoraria and/or consultation honoraria from Alba Therapeutics. Drs Murray, Colatrella, Alexander, and Arsenescu have served as principal investigators for clinical studies in coeliac disease sponsored by Alba Therapeutics, and Dr Arsenescu has received a grant for a coeliac disease screening programme from Alba. Dr Paterson is co-founder and former CEO of Alba Therapeutics and owns stock in the company.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean change from baseline in gastrointestinal symptoms as rated by the Gastrointestinal Symptoms Rating Scale (GSRS).

Figure S1. Exploratory binary analysis. Proportions of patients who responded to the gluten challenge, as indicated by an anti-tTG score ≥15 U/mL or an increase of ≥0.3 units in the CeD-GSRS symptom score (or both) at the end of the treatment period in the placebo and active treatment groups.

Figure S2. Psychological General Well-Being Index scores for anxiety (a), depressed mood (b), positive well-being (c), self-control (d), general health (e), and vitality (f) domains and the global score (g).

Figure S3. Changes from baseline (day 7) to the last double-blind treatment period visit in clinician global assessment scores.

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