Clinical and Experimental Pharmacology and Physiology (2009) **36**, 1170–1176 doi: 10.1111/j.1440-1681.2009.05211.x

EFFICACY OF BUDESONIDE THERAPY IN THE EARLY PHASE OF TREATMENT OF ADULT COELIAC DISEASE PATIENTS WITH MALABSORPTION: AN *IN VIVO*/*IN VITRO* PILOT STUDY

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SUMMARY

1. Budesonide is a glucocorticosteroid with a local anti-inflammatory effect. Coeliac disease is an immune-mediated disease caused by gluten ingestion in intolerant patients. The aim of the present study was to investigate the efficacy of budesonide in malabsorptive coeliac patients and its effect in an *in vitro* gliadin challenge.

2. Twenty coeliac patients with malabsorption were enrolled in the present study and were randomly assigned to one of two 4 week treatments: (i) a gluten-free diet alone; or (ii) a gluten-free diet plus 6 mg budesonide daily. At the end of 4 weeks treatment, all patients underwent clinical evaluation, laboratory tests and self-evaluation of well-being using a visual analogue scale. Intestinal biopsies from five coeliac patients (selected randomly) and four non-coeliac disease controls who underwent upper endoscopy for intestinal bleeding were challenged with gliadin (0.5 mg/mL) and budesonide (10–30 μg/mL) for 3 and 24 h. Biopsies were tested by immunohistochemistry and immunofluorescence for known markers of inflammation.

3. Treatment of patients with 6 mg budesonide daily for 4 weeks resulted in increased bodyweight, a decreased number of evacuations and decreased stool weight compared with patients on a gluten-free diet alone for 4 weeks. Well-being scores were higher in patients treated with both a gluten-free diet and budesonide compared with those receiving a gluten-free diet alone.

4. *In vitro* studies showed that budesonide reduced epithelial tyrosine phosphorylation and expression of histocompatibility leucocyte antigen complex DR (HLA-DR) elicited by gliadin-derived peptides. In addition, the expression of cyclo-oxygenase (COX)-2 and intercellular adhesion molecule (ICAM)-1 in the lamina propria was reduced in patients treated with both gliadin and budesonide compared with patients treated with gliadin alone. Budesonide alone decreased HLA-DR in crypt enterocytes, as well as ICAM-1 and COX-2 expression in the lamina propria of biopsy specimen of coeliac patients. Budesonide had no effect in control samples.

5. In conclusion, the results of the present study indicate that budesonide shows efficacy in the treatment of symptoms in adult coeliac patients with overt malabsorption. The mechanism underlying the effects of budesonide in reducing symptoms was elucidated by *in vitro* studies involving a gliadin challenge.

Key words: budesonide, coeliac disease, gastrointestinal symptoms, gluten-free diet, malabsorption.

INTRODUCTION

Budesonide, a glucocorticosteroid that has been used for many years in the treatment of asthma and rhinitis, has a marked local anti-inflammatory effect and, compared with prednisolone, has the advantage of low systemic effects due to extensive (85–90%) biotransformation to metabolites with minimal or no biological activity.[1]

Budesonide, given as oral controlled-release capsule, has been shown to be effective and safe for the acute and long-term treatment of active Crohn's disease localized to the ileum and/or the ascending colon.[2,3] Budesonide appears to be more effective and is at least as well tolerated as mesalazine, which is the current treatment of choice in mild-to-moderate Crohn's disease.[4]

Budesonide is absorbed rapidly by the gastrointestinal tract.[5] The controlled-release formulation tested in the present study (Entocir; SOFAR, Trezzano Rosa, Italy) consists of a gelatin capsule containing pellets that has been designed to release budesonide during passage through the intestine.[6] The primary target sites for the budesonide controlled-release formulation now available in most countries are the ileum and ascending colon.[7] This formulation is currently approved for the treatment of Crohn's disease,[8–10] but has also shown clear benefit in collagenous colitis.[11] Budesonide, however, appears to be a safe and effective drug and diseases affecting the upper segments of intestine, such as jejunal Crohn's and coeliac disease, could potentially benefit from its action.

Gluten ingestion in coeliac patients causes a variety of gastrointestinal and non-gastrointestinal symptoms and biochemical abnormalities,[12,13] which are usually ameliorated by gluten withdrawal. Diagnosis is often made in adulthood in patients with a long history of the disease, which can be misdiagnosed for years.[14] Gluten-induced lesions are localized mostly in the upper part of the small intestine.[15] Occasionally, gastrointestinal symptoms may cause severe illness and become life-threatening. It is important that symptomatic patients are offered a faster, more effective treatment than a gluten-free diet alone. In cases in which the malabsorptive component is predominant, treatment with glucocorticosteroids (prednisone) together with a gluten-free diet used empirically may accelerate the improvement of patient symptoms. The newly available oral budesonide formulation

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Received 7 January 2009; revision 20 April 2009; accepted 22 April 2009.

has fewer side-effects than the previously available glucorticosteroids. However, its use in controlled trials in coeliac disease has not been validated. One problem is that there is no rationale for using controlled release budesonide, which targets the distal small intestine/colon, in a disease that is predominant in the proximal small intestine. The aim of the present study was to evaluate the anti-inflammatory properties of the drug in coeliac disease in an *in vivo* and *in vitro* pilot study. The *in vivo* study was designed to test the effectiveness of budesonide treatment in accelerating improvement of gastrointestinal symptoms in adult coeliac patients with overt malabsorption. The *in vitro* study was designed to investigate the biological mechanism underlying the anti-inflammatory effect of budesonide.

METHODS

In vivo study

The *in vivo* study was planned as a pilot prospective randomized study. The study was approved as an open trial by the Ethical Committee of University Federico II of Naples (diagnosis and follow-up of coeliac patients), which allowed us to use some biopsies (apart from those needed for diagnosis) for organ culture. Twenty consecutive adult patients with newly diagnosed coeliac disease were enrolled in the study. The inclusion criteria included age between 18 and 65 years and the presence of overt malabsorption. Malabsorption, defined as an inability to absorb nutrients from food, was diagnosed in cases of diarrhoea (i.e. more than three bowel movements per day), associated with one or more of the following symptoms and laboratory findings: (i) weight loss (i.e. loss of > 10% bodyweight in past 6 months); (ii) glossitis; (iii) cutaneous bruising; (iv) flatulence; (v) abdominal distension; (vi) bloating; (vii) discomfort resulting from increased intestinal bulk and gas production; (viii) iron deficiency (serum iron level < 55 μg/dL); (ix) low plasma cholesterol (< 160 mg/dL); (x) low serum proteins (< 6.5 g/dL); and (xi) low albumin (< 3.6 g/dL). Patients with malabsorption were tested for the presence of steatorrhoea (fecal fat excretion > 6 g/24 h).[16] The exclusion criteria were age < 18 years and > 65 years, the absence of malabsorption (i.e. patients with no gastrointestinal symptoms or abnormal laboratory finding related to malabsorption), the presence of major associated diseases (neurological, cardiac or psychiatric disorders, diabetes or pregnancy). All patients enrolled in the study provided written informed consent and all underwent clinical evaluation, laboratory tests, upper and lower endoscopy with jejunal and rectal biopsy, respectively, and self-evaluation of well-being using a visual analogue scale.

A trained dietician explained the low-fibre, low-lactose, gluten-free diet to each patient. All patients were entitled to obtain gluten-free food under the National Health system. Patients were asked to record the number of bowel movements per day (i.e. the number of times stools were evacuated per day) and to weigh stools evacuated over a 24 h period once a week on the day before the visit to the clinic (patients were asked to place their stools from each evacuation into a plastic bag or paper dish and to weigh them on a small balance provided by the investigators; the weight of all stools was summed over the 24 h period). At the visit, patients were asked to indicate their well-being on a visual analogue scale, which consisted of a 10 cm line ranging from 'no well-being at all' at the far left to 'absolute well-being' at the far right.[17]

Patients were randomly assigned (by means of a computer software package) to one of two 4 week treatments, namely a gluten-free diet alone or a gluten-free diet plus 3 mg budesonide twice daily. In the absence of any similar experience, we chose a dose of 6 mg/day budesonide because we wanted the drug to work mainly in the first part of the intestine, which is damaged by gluten more severely in uncomplicated coeliac disease. The administration of microspheres free of any coating ensured a maximum concentration immediately after the pylorus and thus the dilution effect was, in part, avoided. At the end of the treatment period, patients underwent clinical evaluation, laboratory tests and marked a visual analogue scale for well-being.

For the specific purpose of increasing the bioavailability of the drug in the upper intestinal segments, patients were asked to open the capsules and to dip the pellets in a small quantity of water and to take 20 mg omeprazole daily 30 min before breakfast and at least 2 h before taking the first dose of budesonide (at approximately 1000 hours). The second dose of budesonide was taken 2 h after lunch (at approximately 1500 hours). Pharmacokinetic studies indicate that budesonide is released in a non-acidic environment.[18] There is no evidence that omeprazole increases the bioavailability of budesonide in the colon,[19] but we needed to achieve a basic pH in the stomach. Therefore, the administration of omeprazole 2 h before the administration of the budesonide suspension ensured stomach pH that was favourable for pellet activation.[20] Patients on the gluten-free diet alone were asked to take omeprazole at the same time and at the same dose. The two groups were started on omeprazole therapy 4 days before initiating budesonide treatment.

The present study is a pilot study and we overcame the necessity of blinding patients to the treatment as follows. Both groups knew that they were participating in an open trial in which one group was receiving therapy, although no other clue was given about the therapy or the final outcomes of the study. Therefore, because patients in the control group were receiving omeprazole, they were blinded to the fact that they were not receiving budesonide.

Patients attended the outpatient clinic every week for 4 weeks. At each visit, patients were asked about the frequency and intensity of their gastrointestinal symptoms and were asked to mark their well-being on the visual analogue scale. A checklist for adverse reactions during the course of the therapy, currently used in the Unit of Gastroenterology, Department of Clinical and Experimental Medicine, Frederico II University for clinical trials, was administered at each visit. The list included gastrointestinal, neurological, cutaneous, urinary, psychological and other more general symptoms, such as fever, discomfort and malaise.

In vitro study

Patients

Duodenal biopsies of five patients with active coeliac disease (mean age 25.4 years, range 21–30 years) and from four non-coeliac disease controls (mean age 24.7 years, range 22–27 years) affected by intestinal bleeding were used for both diagnosis and *in vitro* studies. Informed consent was given by all patients before these procedures. All specimens were washed in 0.15 mol/L sodium chloride and examined under a dissecting microscope. One specimen from each patient was orientated and embedded in OCT compound (Tissue Tek; Miles Laboratories, Elkhart, IN, USA), snap-frozen in isopenthane cooled in liquid nitrogen and stored at –70°C until cryosectioning into 5 μm sections that were stained with haematoxylin and used for diagnosis. The histological grading of mucosal lesion was performed using the modified Marsh scale[21,22] The remaining samples were cultured *in vitro* as described below.

Peptide preparation

Peptic-tryptic (PT) gliadin digest from bread wheat was prepared as described previously and used at a concentration of 0.5 mg/mL.[23] Gliadin-derived peptides pα-9(57–68) and p31–43, which is the non-immunodominant epitope of gliadin, were synthesized with 9-fluorenylmethoxycarnonyl (Fmoc) chemistry by the Advanced Biotechnology Centre (Imperial College, London, UK) and were used at concentrations of 20 μg/mL.[24,25] Purity (> 95%) was determined by HPLC and mass spectrometry.

In vitro *organ culture of biopsy specimens from patients with coeliac disease*

Immediately after removal, biopsies were cut under a stereomicroscope into several fragments of similar size and weight. Mucosal samples were placed on a stainless-steel mesh positioned over the central well of an organ culture dish with the epithelium of the biopsy sample facing up. The well of the culture dish was then filled with culture medium at 37°C so as to just reach the cut surface of the sample. In this way, the surface, which is normally exposed to the luminal contents, is fed by capillary action and retains its normal polarity, thus providing an appropriate physiological model. The *ex vivo* challenge took place as described previoulsy[26] using 10 mL culture medium consisting

of Trowell's T8 medium (6.5 mL), NCTC 135 medium (2 mL), fetal calf serum (1.5 mL), penicillin (50 000 IU) and streptomycin (5000 IU).

Duodenal biopsy samples from coeliac and control patients were cultured *in vitro* for 3 h with p31–43 or pα-9 and for 3 or 24 h in the presence or absence of a PT gliadin digest.

Budesonide (Astra-Zeneca, Lund, Sweden) was added to the incubation medium containing PT digest or the abovementioned peptides. Cultures with budesonide alone were used as an internal control. The budesonide was made up as a stock solution in 99% methanol, which was diluted to the desired concentration with culture medium immediately before use. The final concentration of methanol biopsy fragments were exposed to was 0.0099%. As a control, biopsy fragments were also cultured in the presence of 0.0099% methanol solution. Budesonide was used concentrations of 10–30 μg/mL with comparable results. After incubation, biopsy specimens were harvested, snap-frozen in isopenthane cooled in liquid nitrogen and prepared for cryosectioning.

Immunolocalization on tissue sections

Frozen tissue sections (4 μm) of biopsy samples from each patient after *in vitro* culture were fixed in acetone for 10 min. Sections were incubated individually for 2 h at room temperature with the following antibodies: anti-phospho-tyrosine PY-99 monoclonal antibodies (1 : 80; mouse IgG2b; Santa Cruz Biotechnology, Santa Cruz, CA, USA); intercellular adhesion molecule (ICAM)-1 (1 : 800; mouse IgG; Ylem, Avezzano, Italy); histocompatibility leucocyte antigen complex DR (HLA-DR; 1 : 10; mouse IgG; BD Pharmigen, Franklin Lakes, NJ USA); and cyclo-oxygenase (COX)-2 (1 : 200; mouse IgG; Cayman Chemical, Ann Arbor, MI, USA). Antigen expression and distribution were visualized by indirect immunofluorescence, as described previously.[24,25] The aforementioned antibodies were selected because they have been used in the same model and are known to be expressed when gluten is added to coeliac mucosa in culture.[24,25] Immunofluorescence was visualized by confocal microscopy (Zeiss LSM510 Pascal; Zeiss, Jena, Germany).

The number of cells expressing COX-2 per mm^2 lamina propria was counted as described previously.[25]

Statistical analysis

In vivo *study*

Descriptive statistics (mean±SD, minimum and maximum values) were computed for each variable. Statistics were reported for each specific lengths of time (i.e. 0, 1, 2, 3 and 4 weeks) for the two treatment groups.

For each variable measured at baseline, Student's *t*-test for independent samples between treated and control groups was used to verify that the two samples were homogeneous at the beginning of the study and at the beginning of the treatment. The Mann-Whitney *U*-test was used for analysis of non-parametric variables.

Analysis of variance (ANOVA) for repeated measures, with treatment and sex as the variables, was used to verify whether there were differences over time for well-being, as determined on the visual analogue scale, between treated and control groups, as well as between men and women.

In vitro *study*

Paired data analysis of the number of cells expressing COX-2 was used to compare samples cultured with medium and those cultured in the presence of gliadin peptides or budesonide plus gliadin peptides. A non-parametric test (i.e. Wilcoxon's test) was used because of the small sample size. Statistical analysis was performed using SPSS v. 11 software (SPSS, Chicago, IL, USA).

RESULTS

In vivo study

Patient characteristics prior to treatment are given in Table 1. There were no significant differences in terms of gender, anthropometry or the severity of gastrointestinal symptoms measured with the main nutritional parameters and/or gluten-induced auto-antibody levels between the two groups. In addition, there were no differences between the two groups in terms of the severity of jejunal biopsy damage or in terms of well-being, as determined using the visual analogue scale (data not shown).

Lower endoscopy showed normal morphology of the rectal mucosa and histology indicated normal mucosal architecture in all patients. There were no differences noted between men and women before treatment in either group (data not shown).

Table 2 gives a comparison of selected variables in the two groups. Bodyweight and plasma cholesterol levels were increased and stool weight was decreased in coeliac patients treated with both a gluten-free diet and budesonide compared with patients treated with a gluten-free diet alone. Figure 1 shows the number of bowel movements per day. There was a decrease in the number of bowel movements in both groups, although the decrease in the budesonide-treated group was significantly greater than that in the group treated with a gluten-free diet alone. Figure 2 shows the trend of well-being, as determined

Table 1 Patient characteristics before treatment

	Gluten-free diet ($n = 10$)	Gluten-free diet + budesonide ($n = 10$)
Gender (M/F)	2/8	3/7
Age (years)	27.5 ± 7.1	29.6 ± 6.0
Bodyweight (kg)	57.6 ± 8.1	60.8 ± 14.5
Body mass index (kg/m^2)	21.9 ± 2.5	23.1 ± 4.8
Anti-human tissue-transglutaminase antibodies (U/L)	58.4 ± 36.4	70.6 ± 47.5
Anti-endomysial antibodies (no. positive/total no. patients)	10/10	10/10
Haemoglobin (g/dL)	12.1 ± 1.2	11.9 ± 1.4
Ferritin (μg/dL)	17.5 ± 14.2	13.7 ± 16.0
Total serum proteins (g/dL)	6.2 ± 0.7	6.1 ± 0.9
Serum albumin (g/dL)	3.8 ± 1.1	3.9 ± 1.0
Plasma cholesterol (mg/dL)	138.5 ± 16.3	145.0 ± 37.3
No. bowel movements/day	3.3 ± 0.9	4.5 ± 1.6
Stool weight/day (g)	398.0 ± 148.1	409.0 ± 133.0

Where appropriate, data are given as the mean±SD. No significant differences were noted between the two groups before treatment for any of the variables examined ($P > 0.05$ for all).

Table 2 Patient characteristics at the end of the 4 week treatment period

	Gluten-free diet ($n = 10$)	Gluten-free diet + budesonide ($n = 10$)	*P*
Bodyweight (kg)	57.8 ± 7.7	64.5 ± 12.9	NS
Body mass index (kg/m^2)	22.3 ± 2.5	23.1 ± 3.6	NS
Anti-human tissue-transglutaminase antibodies (U/L)	48.4 ± 46.6	55.8 ± 42.0	NS
Total serum proteins (g/dL)	6.73 ± 0.65	6.72 ± 0.56	NS
Serum albumin (g/dL)	4.9 ± 1.2	3.7 ± 0.9	NS
Plasma cholesterol (mg/dL)	140.5 ± 14.3	170.3 ± 39.0	0.042
Stool weight/day (g)	270.0 ± 77.8	191.6 ± 53.6	0.016

Data are given as the mean±SD.

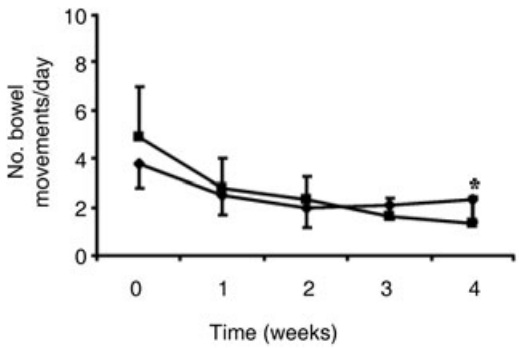

Fig. 1 Progressive reduction over time of the number of bowel movements per day in patients on 6 mg budesonide daily plus a gluten-free diet for 4 weeks (■) and in patients on a gluten-free diet alone (◆). At the end of the treatment period, the number of bowel movements in the budesonide-treated group was significantly less than that in the group treated with a gluten-free diet alone. Data are the mean±SD. *$P < 0.036$ (ANOVA).

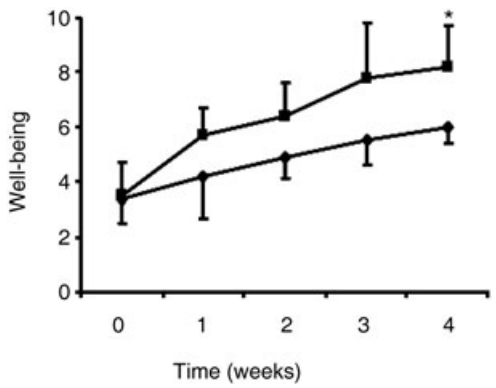

Fig. 2 Progressive increase over time in perceived well-being, as measured on a visual analogue scale, in patients on 6 mg budesonide daily plus a gluten-free diet for 4 weeks (■) and in patients on a gluten-free diet alone (◆). At 3 weeks and at the end of the treatment period, there was a significant increase in the perceived well-being score in the budesonide-treated group compared with the group of patients treated with a gluten-free diet alone. Data are the mean±SD. *$P < 0.005$ (ANOVA).

using the visual analogue scale, over time. At 3 weeks and again at the end of the treatment, patients treated with budesonide indicated that their perceived well-being had improved more than did patients on the gluten-free diet alone ($P < 0.005$ for both 3 and 4 weeks).

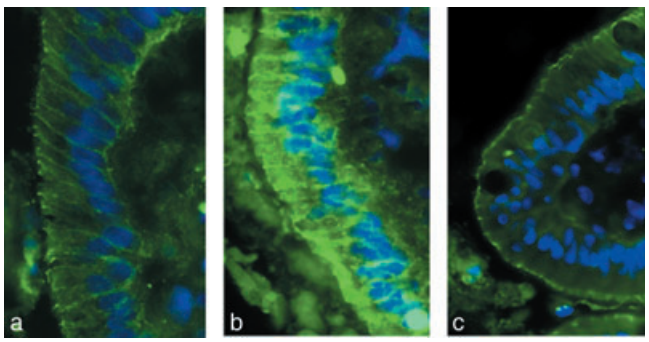

Fig. 3 Effect of budesonide on the expression of PY-99 by epithelial cells following incubation for 3 h with gliadin-derived p31–43 peptide in intestinal biopsies from coeliac disease patients. (a) Faint PY-99 epithelial expression is observed in most epithelial cells after incubation with medium alone. (b) Gliadin-derived p31–43 challenge led to a marked increase in the number of epithelial cells with intense PY-99 expression in the cytoplasm, as well as on the cell membranes, whereas (c) treatment with budesonide prevented PY-99 upregulation induced by gliadin-derived p31–43 challenge. Similar results were seen for peptic-tryptic (PT) gliadin digest. However, the expression pattern observed after incubation with pα-9 was similar to that observed after incubation with medium alone. Indirect immunofluorescence (original magnification ×600).

There were no side-effects reported during or immediately after budesonide treatment, therefore the drug appears to be safe for coeliac disease patients.

In vitro study

Both the PT gliadin digest and p31–43, which is the toxic fraction of gliadin, induced an early (within 3 h of challenge) increase in tyrosine phosphorylation (Fig. 3b) in the epithelial compartment and enhanced HLA-DR expression in villus enterocytes (Fig. 4b) compared with the pattern observed after incubation with medium alone. The PT gliadin digest and p31–43 produced similar results. In contrast, pα-9 was devoid of biological activity. Treatment with budesonide significantly inhibited epithelial phosphotyrosine induction (Fig. 3c) and HLA-DR expression (Fig. 4c) observed after incubation for 3 h with PT gliadin digest or p31–43.

After incubation for 24 h with PT gliadin digest, upregulation of HLA-DR expression by crypt enterocytes, as well as an increase in ICAM-1 (Fig. 5b) and COX-2 (Fig. 6) expression by lamina propria mononuclear cells (LPMNC), was observed compared with incubation with medium alone. Budesonide was effective in controlling the

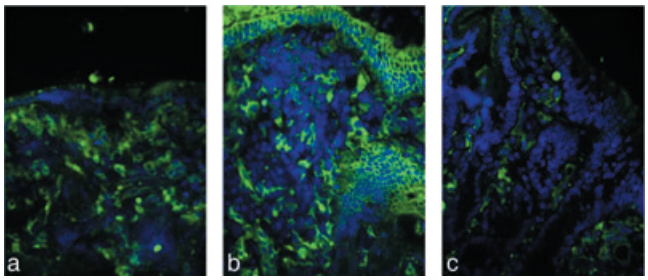

Fig. 4 Effect of budesonide on the expression of human leucocyte antigen complex DR (HLA-DR) by epithelial cells following 3 h of gliadin-derived p31–43 challenge in intestinal biopsies from coeliac disease patients. (a) No overexpression of HLA-DR was observed in most epithelial cells after incubation with medium alone. (b) Gliadin-derived p31–43 challenge led to marked expression of HLA-DR in the basal cytoplasmatic compartment, as well as on the brush border and basolateral membranes, whereas (c) treatment with budesonide prevented HLA-DR overexpression induced by gliadin-derived p31–43 challenge. Similar results were seen for peptic-tryptic (PT) gliadin digest. However, the expression pattern observed after incubation with pα-9 was similar to that observed after incubation with medium alone. Indirect immunofluorescence (original magnification ×200).

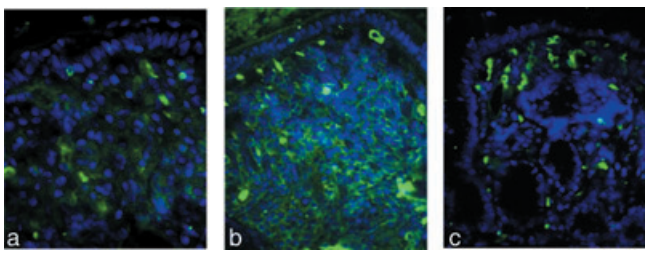

Fig. 5 Effect of budesonide on intercellular adhesion molecule (ICAM)-1 expression induced by incubation for 24 h with peptic-tryptic (PT) gliadin digest. In coeliac biopsies, PT gliadin digest induced a significant increase in the number of positive cells expressing ICAM-1 (b) compared with the pattern observed after incubation with medium alone (a). (c) Budesonide was effective in controlling gliadin-induced ICAM-1 overexpression. Indirect immunofluorescence (original magnification ×200).

upregulation of HLA-DR expression by crypt enterocytes, as well as that of ICAM-1 and COX-2 expression by LPMNC (Figs 5c,6). Analysis of tissue sections of the mucosa of the four non-coeliac patients showed that incubation with gliadin digest and peptide p31–43 increased HLA-DR, ICAM-1 and COX2 expression, as expected.[23]

DISCUSSION

There is some evidence supporting the use of locally acting controlled-released corticosteroids in coeliac disease. First, coeliac disease is a chronic inflammatory disease of the small intestine that primarily involves the proximal small intestine, but can involve the entire small intestine in some patients.[27,28] Second, this class of drug has been demonstrated to be efficacious in coeliac disease. Bramble *et al.*[29] and Mitchison *et al.*[30] used topically active corticosteroids in patients with coeliac disease that were on a regular diet and noted improvements in both histology and parameters of absorption. Budesonide is currently used in the treatment of refractory coeliac disease and in enteropathy associated T cell lymphoma.[31,32]

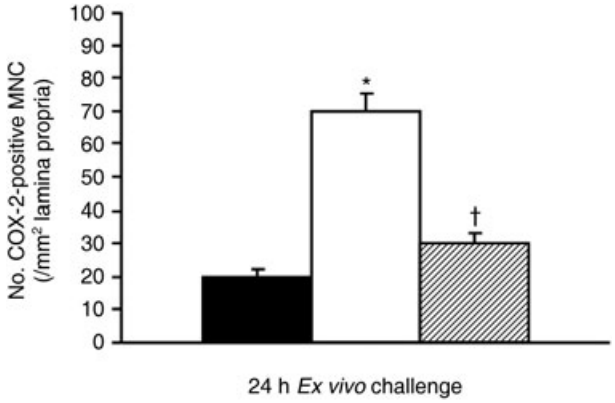

Fig. 6 Effect of budesonide on the cyclo-oxygenase (COX) 2 expression induced by incubation for 24 h with peptic-tryptic (PT) gliadin digest. In coeliac biopsies, PT gliadin digest (□) induced a significant increase in the number of positive cells expressing COX-2 compared with the pattern observed after incubation with medium alone (■) for 24 h. Budesonide (▨) was effective in controlling gliadin-induced COX-2 overexpression. Data are the mean±SD. *$P < 0.001$ compared with medium alone; †$P < 0.005$ compared with culture with PT gliadin digest. MNC, mononuclear cells.

There is no published tool for assessing the severity of the illness in patients with coeliac disease. The assessment in the present study was based on patient claims of an improvement, with loss of systemic symptoms such as fatigue and an increase in well-being, in addition to a physician's assessment of both laboratory and clinical data. In order to assess the response objectively, the number of bowel movements for subjects with classical presentation and the body mass index (BMI) of all subjects were recorded before and after treatment with budesonide.

The findings of the present study indicate that budesonide, together with a gluten-free diet, may be effective and safe in the early phase of treatment of symptomatic adult coeliac disease with malabsorption. In fact, treatment with budesonide induced a faster improvement of gastrointestinal symptoms, evaluated as a decreased number of evacuations per day and of 24 h stool weight.

The decrease in the number of evacuations is a rough measure of slower transit time and/or improved absorption of nutrients. We believe that it may be related mostly to mucosal recovery and improved absorption. The reduced stool weight should be interpreted as the result of improved cholesterol absorption, as shown by the significant increase in plasma cholesterol in the budesonide-treated group. The increase in bodyweight was greater in budesonide-treated coeliac patients than in patients on a gluten free diet alone, although the difference did not reach statistical significance ($P = 0.058$). Patient scores on a visual analogue scale of well-being indicate that patients on both budesonide and a gluten-free diet felt better than those on a gluten-free diet alone. This is unlikely to reflect any systemic effect of budesonide but simply, in our opinion, a faster recovery of general health status.

None of the side-effects frequently reported during clinical trials was noted in the present study. However, the number of patients treated in this pilot study was too small to allow us to make a definitive statement regarding the use of budesonide for the treatment of coeliac disease.

The present study was designed to maximize the bioavailability of budesonide in the upper tract of the small intestine by both

pretreatment with a proton pump inhibitor and dissolving the pellets in water before administration. It is known that the target sites for the only oral pharmaceutical formula existing in Europe are the distal ileum and colon. The capsules are gastroresistant and the inner pellets are soluble at neutral to alkaline pH. The absorption of budesonide in the distal tract of the ileum may explain the increased level of plasma cholesterol in the budesonide-treated coeliac patients. In fact, cholesterol is absorbed along the entire small intestine and its absorption may be strongly influenced by reduced inflammation of the intestinal surface. It can be hypothesised that a different formulation may increase the efficacy of budesonide in coeliac disease and in other inflammatory diseases localized to the upper gastrointestinal tract, such as Crohn's disease.

In evaluating the effect of budesonide, it is important to take into account the possibility that the drug has an effect in the upper tract of the ileum and makes only a very brief presence in the bloodstream.[7] In fact, the rheumatic joint pain of Crohn's disease patients is ameliorated by treatment with budesonide.[33]

The possibility that the effect of budesonide in coeliac disease is linked to the concomitant presence of collagenous or lymphocitic colitis, in which budesonide has been proven to be effective,[11,34] is ruled out by the fact that colitis was not present in our series of patients.

The choice of treating the patients with overt gluten-related malabsorption in the present protocol is limited to assessing the efficacy of budesonide treatment. In fact, malabsorption may interfere with budesonide absorption. The pharmacological effect of budesonide may be delayed until absorption improves and becomes noticeable (i.e. a decrease in the number of bowel movements per day and increased bodyweight) only at the end of the treatment period. It is possible that budesonide treatment could be even more effective in atypical coeliac disease, in particular in improving well-being and other non-gastrointestinal gluten-related problems such as anaemia or symptoms such as fatigue.

There are three important limitations of the present study. First, no sample power was calculated for assessing the number of patients to be treated because no data from similar studies in coeliac disease are available. Second, this is not a placebo-controlled trial. Conversely, the use of budesonide in coeliac disease is not supported by any clinical study, no drug company was asked to support the present study and a double-blind study would have been costly and hazardous without a simple pilot study. However, the patients who were asked to report their well-being (subjective) and the number of evacuations per day (objective) were not influenced strongly by the treatment they were receiving. The researchers analysing the data were aware of treatment group and, for this reason, were not asked for their opinion about patient health during treatment. Third, no schintigraphic study exists to fully support the modality of administration we propose. Nevertheless, the strength of the study is that it should be considered as a pilot study from which the number of patients to be enrolled in a placebo-controlled trial can be calculated and that the data presented here could move research forward on a drug that seems to be safe and effective in different inflammatory diseases.

In conclusion, a gluten-free diet is, in most cases, sufficient to obtain an improvement of symptoms in a few weeks in coeliac disease and this was true also in our patients. However, in selected cases, such as patients with diarrhoea, budesonide may help in obtaining faster and greater improvement of symptoms in addition to a gluten-free diet. The rapid, significant reduction of diarrhoea and the increased well-being may compensate for the often abrupt major change in lifestyle forced by the disease, which may adversely affect a patient's quality of life.[35,36]

In the second *in vitro* part of the study we investigated the biological effects of budesonide on the intestinal mucosa of coeliac patients. We used an established organ culture method that allowed us to determine that pretreatment with budesonide reduced the known effects of gliadin toxic peptides on the intestinal mucosa of coeliac patients.[21] In particular, PT gliadin and p31–43 induced an early increase of epithelial tyrosine and of HLA-DR expression in villus enterocytes compared with incubation with medium alone. Treatment with budesonide significantly inhibited the observed induction of epithelial phosphotyrosine and HLA-DR expression. Challenge with p31–43 induces PY-99 expression at the apical and basolateral membrane of the villus enterocytes after 3 h of challenge. No staining was evident at the enterocyte level after 3 h of challenge with pα-9. The latter pattern is similar to that observed after incubation with medium alone. After incubation for 24 h with PT gliadin digest, upregulation of HLA-DR expression by crypt enterocytes, as well as increases in ICAM-1 and COX-2 expression by LPMNC, were observed with respect to the pattern observed after incubation with medium alone, as expected.[24,25] Budesonide was effective in controlling the upregulation of HLA-DR expression by crypt enterocytes and of ICAM-1 and COX-2 by LPMNC, demonstrating a sustained anti-inflammatory efficacy in this experimental model.

In conclusion, our *in vitro* study indicates that the efficacy of budesonide in lowering the inflammatory response to gluten is evident in both early and late immunoresponses after gliadin challenge. Budesonide appears useful in accelerating the improvement of symptoms, but its use in coeliac disease is limited by the absence of jejunal activity of the formulation currently available commerically.

ACKNOWLEDGEMENT

IR was supported, in part, by a grant from the National Research Fund (PRIN 2006, #2006099155_004).

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