

## INFLAMMATORY BOWEL DISEASE

## Retarded release phosphatidylcholine benefits patients with chronic active ulcerative colitis

W Stremmel, U Merle, A Zahn, F Autschbach, U Hinz, R Ehehalt

Gut 2005;54:966–971. doi: 10.1136/gut.2004.052316

See end of article for authors' affiliations

Correspondence to:  
Dr W Stremmel,  
Department of  
Gastroenterology,  
University Hospital  
Heidelberg, Im  
Neuenheimer Feld 410,  
69120 Heidelberg,  
Germany;  
wolfgang\_stremmel@  
med.uni-heidelberg.de

Revised version received  
17 October 2004  
Accepted for publication  
20 October 2004

**Background and aims:** We examined the hypothesis of an anti-inflammatory effect of phosphatidylcholine in ulcerative colitis.

**Methods:** A phase IIA, double blind, randomised, placebo controlled study was performed in 60 patients with chronic active, non steroid dependent, ulcerative colitis, with a clinical activity index (CAI) of  $\geq 4$ . Retarded release phosphatidylcholine rich phospholipids and placebo were administered at a dose of 6 g daily over three months. The primary end point was a change in CAI towards clinical remission (CAI  $\leq 3$ ) or CAI improvement by  $\geq 50\%$ . Secondary end points included  $\geq 50\%$  changes in endoscopic activity index (EAI), histology, and quality of life scores.

**Results:** Induction of clinical remission (CAI  $\leq 3$ ) as the primary outcome variable was attained by 16 (53%) patients in the phosphatidylcholine treated group compared with three (10%) in the placebo group ( $p < 0.00001$ ). The rate of clinical remission and CAI improvement was 90% in the phosphatidylcholine group and only 10% in the placebo group. A median drop of seven points in the CAI score (70% improvement) was recorded in the phosphatidylcholine group compared with no change in the placebo group. Secondary end point analysis revealed concomitant drops in EAI and histology scores ( $p = 0.00016$  and  $p = 0.0067$  compared with placebo, respectively). Improvement in quality of life was reported by 16 of 29 evaluated patients in the phosphatidylcholine group compared with two of 30 in the placebo group ( $p = 0.00005$ ).

**Conclusion:** Retarded release oral phosphatidylcholine is effective in alleviating inflammatory activity caused by ulcerative colitis.

Ulcerative colitis (UC) is a chronic inflammatory condition of the colonic mucosa that, depending on the individual, can extend from the rectum to the caecum. The aetiology of the disease is unknown. One of the proposed hypotheses is that a disturbed mucosal barrier is an initiating factor, and subsequent attacks from colonic commensal bacterial flora lead to inflammation of the mucosa.<sup>1–4</sup> Intestinal mucosal cells are protected against the attacks of luminal bacteria by a continuous, hydrophobic, and adherent mucus layer.<sup>5–6</sup> Phospholipids are one of the components of mucus, consisting of up to 90% phosphatidylcholine (PC) and lysophosphatidylcholine (LPC).<sup>7–10</sup> They are found as a continuous layer at the luminal and mucosal cell side of the mucus gel and within the mucus as liposome-like aggregates.<sup>11–12</sup> PC is largely responsible for establishing a protective hydrophobic surface and therefore plays a key role in mucosal defence. A defective PC layer may contribute to the development of inflammation and ulceration, as previously shown in humans with human immunodeficiency virus and *Helicobacter pylori* infection. In these conditions the pathogenesis involves impairment of phospholipid barrier function as a result of high phospholipase activity.<sup>12–15</sup> It has been reported that PC, when topically applied to the colon, protects laboratory animals against colitis induced by acetic or trinitrobenzenesulphonic acid.<sup>16–17</sup> In addition, PC and other selected lipids have been shown to inhibit proinflammatory signalling in a phagosome model system derived from macrophages.<sup>18</sup>

Recent analysis of rectoscopically acquired mucus aliquots revealed a significant decrease in PC and LPC content in patients with UC compared with healthy controls or patients with Crohn's disease.<sup>10</sup> Moreover, we have shown in rat intestinal perfusion studies that PC was indeed actively

secreted by jejunum and ileum whereas secretion in the colon was only marginal.<sup>19</sup> We therefore hypothesised that PC integrates into the distal small intestinal mucus and then moves downwards to the colon. Thus deficiency of small intestinal PC secretion in UC would be consistent with a low colonic mucus PC content and with an increase in inflammatory activity from the rectum to the caecum.

Based on the above information, the hypothesis which forms the foundation for the current study is that a local increase in PC within the colonic mucus may improve intestinal barrier function and decrease inflammatory activity in UC. We therefore supplemented the colonic mucosa of UC patients with a PC rich phospholipid mixture. In order to avoid early reabsorption in the upper small intestine, an oral retarded release PC rich preparation was developed. By encapsulation with Eudragit S 100, pH dependent release into the distal ileum is permitted which allows integration of PC into the colonic mucus. Retarded release preparations of this type have been described previously (for example, for preparation of 5-aminosalicylate and budenoside).<sup>20</sup> To evaluate the clinical effectiveness of this novel PC rich preparation, we performed a prospective, randomised, placebo controlled, double blind study in 60 patients with long term, non-steroid dependent, chronic, active UC. Our results argue strongly that oral administration of PC has considerable therapeutic potential against UC.

**Abbreviations:** CAI, clinical activity index; EAI, endoscopic activity index; IBD, inflammatory bowel disease; IBDQ-D, inflammatory bowel disease questionnaire-Deutschland; IQR, interquartile range; LPC, lysophosphatidylcholine; LQI, life quality index; PC, phosphatidylcholine; UC, ulcerative colitis

## METHODS

### Patients and study medication

Patients were considered eligible for this phase IIA study if they were 18 years of age and presented with long term UC (duration >2 years). They were characterised by a chronic active course of  $\geq 4$  months with a clinical activity index (CAI) of  $\geq 4$ . The clinical course was followed for a three month period. The main criterion for exclusion was treatment with steroids and/or immunosuppressive agents within the three month period before entering the study. Therefore, only patients who refused to take steroids and/or immunosuppressives, or who had a history of severe side effects with these compounds, were recruited. None of the patients had a surgical history.

Eligible patients were randomly assigned to one of two parallel groups (PC or placebo) with 30 patients each. All had been given continuous standard oral therapy with 3–4 g aminosalicylates daily for at least four months. To avoid interference of endoscopic and histological evaluation of the rectal mucosa, local application of aminosalicylates was omitted. Treatment with steroids and/or immunosuppressives was not allowed during the study. Patients were free to withdraw from the study at any time during its course. The primary efficacy end point was defined at the end of the study at three months, on patient withdrawal, or after deterioration in clinical course. The final examinations were then performed. Fifty eight patients agreed to a final colonoscopy and two did not.

Due to the difficulty in meeting the selection criteria, patients from all over Germany had to be recruited. Forty eight patients were examined at the Department of Gastroenterology, Heidelberg University Hospital, by the principal investigator or a panel of five experienced endoscopists using the standard endoscopic activity index (EAI).<sup>21</sup> For patients from other areas of Germany (12 patients) who were unable to visit our department, the local physician/endoscopist who cared for the patient performed the required clinical and endoscopic examinations according to the study protocol (CAI and EAI criteria). Site differences in examinations by local physicians/endoscopists and the university were not observed. According to the design as a single centre study, all patients were instructed and guided through the study by the responsible principal investigator in close cooperation with the other physicians involved.

The study was approved by the institutional ethics committee of the University of Heidelberg. Written informed consent was obtained from each patient before enrolment in the study.

The study medication was provided by Allphamed Pharmed Arzneimittel GmbH (Göttingen, Germany). The PC rich phospholipid mixture (Sterpur P-30 Granulat; Stern-Lecithin and Soja GmbH, Hamburg, Germany) contained 30% PC, 21% phosphatidylethanolamine, and 8% phosphatidylinositol. Verum containing PC rich phospholipids and placebo containing microcrystalline cellulose (Avicel PH102; FMC Company, USA) were both encapsulated with Eudragit S 100 (1:1 wt/wt for each preparation). According to good manufacturing practice, both study medications were manufactured as indistinguishable granular preparations and packed into numbered boxes using random permuted blocks of six patients. Thus randomisation was done independently of the investigating team. The study medication was administered four times daily (1.5 g /dose) after meals and before retiring. Compliance was measured by obtaining a detailed study history in a personal interview at the end of the study, return of empty medication boxes, and control of daily medication recorded on a diary card. Every participant was requested to complete these diary cards daily which also included a performance report on CAI parameters. At the end

of the study after all data had been collected and documented, randomisation was decoded by the manufacturer.

Earlier experiments were conducted to document that the retarded release PC preparations actually reached the colonic mucus. Seven representative UC patients on continuous standard oral therapy with 3–4 g aminosalicylates daily were chosen to potentially utilise the 5-ASA antioxidant effect in protecting PC from peroxidation. These patients, not included in the efficacy population of the main study, were treated with PC rich phospholipids according to the above regimen for one week. In these UC patients prior to and after PC administration and in seven untreated healthy individuals, the rectal mucosa was exposed by proctoscopy and specimens of rectal mucus removed by gentle swabbing of the rectal wall (10–20 cm ab ano) with a cotton wool sponge. After determination of mucus dry weight, PC species were quantified by mass spectroscopy, as previously described.<sup>10</sup> In UC patients, baseline total PC content was 0.53 (0.14) nmol PC/mg dry wt, which was significantly lower than that obtained from seven healthy controls (1.17 (0.72) nmol PC/mg dry wt) ( $p = 0.0133$ ). This finding is consistent with data from our previous study.<sup>10</sup> After one week of PC rich phospholipid administration to these seven patients with UC, PC levels were increased by 4.5-fold to 3.23 (0.72) nmol PC/mg dry wt ( $p < 0.00145$ ). This concentration was even higher than that obtained from untreated controls ( $p = 0.0280$ ). These data demonstrated that PC indeed reached the colonic mucus.

### Efficacy and safety analysis

CAI includes number of bowel movements, presence of blood in the stool, general well being, abdominal pain, extraintestinal manifestations, and fever (all of which were recorded in a patient diary card) as well as erythrocyte sedimentation rate and haemoglobin values.<sup>21</sup> Further assessment criteria included EAI according to Rachmilewitz<sup>21</sup> and life quality index (LQI), as measured using the inflammatory bowel disease questionnaire-Deutschland (IBDQ-D).<sup>22</sup> Histology scores for biopsies at entry and at the end of the study were evaluated by an independent pathologist, blinded to the clinical information, using index parameters described by Truelove and Richards.<sup>23</sup> Histological appearance in a given biopsy was scored from 0–4 as follows: 0 = no; 1 = mild inactive; 2 = mild active; 3 = moderate active, and 4 = severe active inflammation. The score taken into account for evaluation was the value of the scored rectal/sigmoidal mucosal biopsies. Patients were further evaluated with regard to extension of disease.

Patients were observed and questioned regarding adverse events and were instructed to report any symptoms. All adverse events were recorded during the three month study period. As additional safety parameters, white blood cell count, creatinine, urea, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, lipase, amylase, and cholesterol were determined after 2, 6, and 12 weeks or when clinical conditions required control.

### Statistical analysis

Quantitative results of PC determination during the preceding experiments were analysed using the *t* test for comparison between UC patients and healthy subjects, and the *t* test for paired groups of UC patients before and after PC rich phospholipid administration.

For the main study, the change in CAI was considered as the primary end point. Cut off values for the primary end point evaluation were: (a) number of patients with a reduction in CAI of  $\geq 50\%$  from baseline<sup>24</sup> and (b) achievement of

**Table 1** Patient characteristics at entry into the study for the phosphatidylcholine (PC) and placebo groups

Treatment group	PC group (n = 30)	Placebo group (n = 30)	p Value
Sex			0.793*
Male	19	17	
Female	11	13	
Age (median (IQR))	35.5 (24–58)	36.5 (28–46)	0.773†
Extension			0.606*‡
Pancolitis	13	16	
To right flexure	2	1	
To transversum	4	1	
To left flexure	8	2	
To sigma	2	9	
To rectum	1	1	
CAI (median (IQR))	10 (7–12)	7 (5–9)	0.0071†
EAI (median (IQR))	7 (6–8)	6.5 (6–8)	0.725†
Histology score			0.483*
No/mild inactive	2	5	
Mild/moderate active	15	11	
Severe active	8	7	
Not determined	5	7	
LQI (median (IQR))	3.0 (2.3–3.5)	3.3 (2.8–3.8)	0.170†
Not determined	1	0	

IQR, interquartile range; CAI, clinical activity index; EAI, endoscopic activity index; LQI, life quality index.

\*Fisher's exact test; †Mann-Whitney U test; ‡p value is given for differences between rates of patients with pancolitis versus patients with partial colitis.

clinical remission (CAI  $\leq 3$ ).<sup>24–25</sup> Baseline CAI was defined as the mean activity in the week before the start of the study and was compared with the CAI at the end of the study. Secondary end points included the number of patients with  $\geq 50\%$  improvement in the following variables over the evaluation period: EAI, histology score of rectal/sigmoidal mucosal biopsies, and IBDQ-D. Life quality was determined by the mean value of the 32 items of the IBDQ-D and by subgroup analysis. Furthermore, disease extension during the study period was evaluated. The study analysis was by intention to treat.

Statistical analysis was performed using SAS software (release 8.02; SAS Institute, Inc., Cary, North Carolina, USA). Non-parametric statistical methods were used to analyse study end points in both treatment groups, judged by the Shapiro-Wilk test. Comparison of changes in primary and secondary study end points between the two treatment groups was performed using Fisher's exact test. The distribution of changes in scores obtained from the PC and placebo groups were analysed using the Mann-Whitney U test. Differences between scores obtained at entry and at the end of the study for each individual patient were compared within both treatment groups using Wilcoxon's signed rank test. Study entry parameters were compared between the PC rich phospholipid group and the placebo group using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical data. Continuous variables were expressed as median with interquartile range (IQR). The distribution of changes in CAI, EAI, and LQI at entry and at the end of the study was presented by box and whisker plots. Two sided p values were reported in all cases and an effect was considered statistically significant at a p value of  $\leq 0.05$ .

## RESULTS

### Patient characteristics

Between April 2000 and October 2002, 60 patients were recruited into the study and randomly assigned to one of the two groups of 30 patients each. Patients were comparable in age, sex, extension of disease, EAI, histology, and life quality scores (table 1). For CAI, the PC group had a significantly higher score compared with the placebo group ( $p = 0.0071$ ). This was due to a higher blood in stool content in the PC

group (median score 2 (IQR 2–3) v 2 (IQR 1–2) in the placebo group;  $p = 0.0003$ ). For all other CAI parameters there were no significant differences between the two groups.

### Treatment efficacy

#### Primary end point analysis

##### Clinical activity index

Two cut off values for primary end point analysis were chosen: (1) the absolute threshold value of CAI  $\leq 3$ , defining clinical remission<sup>24–25</sup> and (2) the relative improvement in CAI  $\geq 50\%$  (table 2). Clinical remission was observed in 16 PC treated patients (53%) but only in three (10%) patients in the placebo group ( $p = 0.00063$ ). Improvement in CAI  $\geq 50\%$  was recorded in 27 of 30 PC patients but only in three of 30 placebo patients (all three also achieved clinical remission) ( $p < 0.0001$ ). With regard to the magnitude of change over the study period, median CAI in the PC group decreased from 10 (IQR 7–12) to 3 (1–5) ( $p < 0.0001$ ). In the placebo group, CAI increased from 7 (IQR 5–9) to 9 (5–11) ( $p = 0.139$ ) (see also fig 1A).

#### Secondary end point analysis

##### Endoscopic activity index and histology score

In parallel with the clinical results there was an improvement in EAI in 11 of 29 evaluated PC patients compared with none of 29 placebo patients (table 2). This corresponded to a median reduction in score from 7 (IQR 6–8) to 4 (3–5) ( $p < 0.0001$ ) compared with no difference in the placebo group (6.5 (IQR 6–8) v 7 (6–9);  $p = 0.144$ ) (see also fig 1B). An improvement in histology score was also noted in 13 of 25 evaluated patients in the PC group versus three of 23 of the placebo group ( $p = 0.0067$ ) (table 2). An initial median score of 3 (IQR 2–4) decreased to 2 (1–2) ( $p < 0.0001$ ) in the PC group (median drop of 1 point;  $p = 0.0056$ ) while with placebo unchanged median values of 2 (IQR 2–4) and 2 (1–4) ( $p = 0.406$ ) were obtained.

##### Disease extension

With regard to extension of disease, 19 of 30 patients (63.3%) in the PC group showed a median reduction in the length of the affected area of 40 cm. In the placebo group, this parameter remained unchanged in the majority of patients.

**Table 2** Rates of response over the study period in the phosphatidylcholine (PC) and placebo groups

Treatment group	PC group (n = 30)	Placebo group (n = 30)	p Value*
Primary end point analysis			
Clinical remission/improvement ( $\geq 50\%$ )			$<0.0001$
Yes	27	3	
No	3	27	
Clinical remission (CAI $\leq 3$ )			0.00063
Yes	16	3	
No	14	27	
$\Delta$ CAI ( $\geq 50\%$ )			$<0.0001$
Decreased	27	3	
Unchanged	3	23	
Increased	0	4	
Secondary end point analysis			
$\Delta$ EAI ( $\geq 50\%$ )			0.00016
Decreased	11	0	
Unchanged	18	27	
Increased	0	2	
Not determined	1	1	
$\Delta$ Histology score ( $\geq 50\%$ )			0.0067
Decreased	13	3	
Unchanged	11	19	
Increased	1	1	
Not determined	5	7	
$\Delta$ Life quality index ( $\geq 50\%$ )			$<0.0001$
Increased	16	2	
Unchanged	13	28	
Decreased	0	0	
Not determined	1	0	

CAI, clinical activity index; EAI, endoscopic activity index.

\*Fisher's exact test.

Five of 29 placebo patients (17.2%) demonstrated a marginal increase in this parameter (that is, from 5 to 10 cm).

### Quality of life

Treatment efficacy was reflected in all subjective quality of life parameters, including bowel symptoms, systemic symptoms, and emotional and social functions, each of which improved ( $p < 0.00001$ ) with PC treatment compared with placebo. A  $>50\%$  increase in total LQI was recorded by 16 of 29 evaluated patients receiving PC. Placebo treated patients continued to experience poor to moderate quality of life, with only two of 28 patients responding in a positive manner (table 2). In the PC group, median LQI score increased from 3.0 (IQR 2.3–3.5) to 5.1 (4.4–5.8) ( $p < 0.0001$ ) compared with no change in the placebo group (see also fig 1C).

### Study withdrawal

Only one patient in the PC group withdrew from the study prematurely due to psychological decompensation while in the placebo group nine patients withdrew due to deterioration in clinical condition ( $p = 0.0122$ ). The reasons for discontinuation varied among individual patients. In five placebo patients, although the overall CAI did not deteriorate, the incentive to complete the study was lacking as subjective expectations of improvement were not met. Additionally, four patients withdrew due to CAI deterioration. In these patients, CAI increased by 5–11 points compared with baseline values, suggesting acute exacerbation of the disease, which was not alleviated by standard therapy with aminosalicylates alone.

### Adverse events

During the study approximately 50% of patients experienced tolerable bloating, corresponding to grade 1 of the SAE grading (NCI common terminology criteria for adverse events). However, no significant differences were found between the incidence in the PC and placebo groups. No other major adverse or side effects were observed. Additional

biochemical and haematological safety tests also did not reveal significant changes.

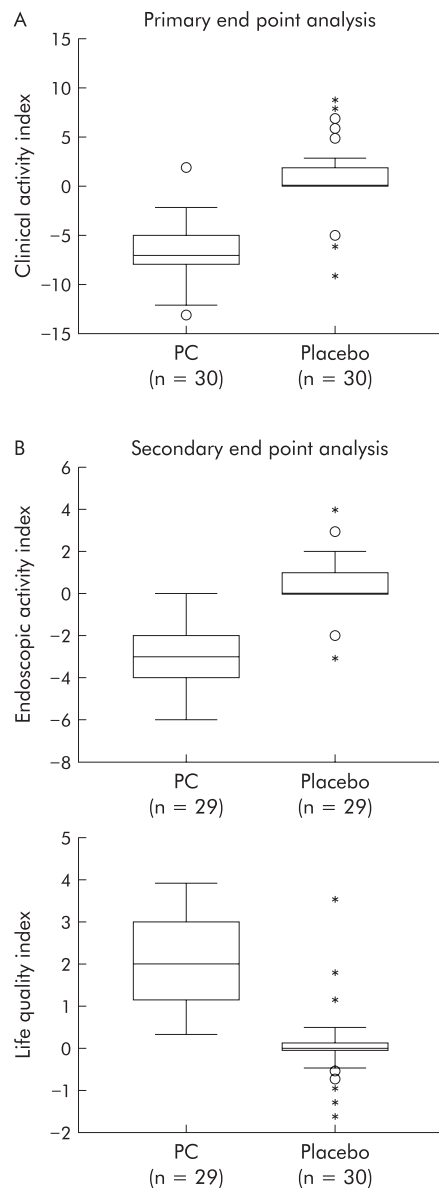
### DISCUSSION

The aim of the present study was to evaluate the efficacy of retarded release PC in inflammatory activity in UC. A retarded release preparation given orally is more suitable for integration into the colonic mucus compared with rectal instillation, in which superficial and short exposure of PC is expected.

The retarded release PC rich phospholipid preparation administered at a dose of 6 g/day reached the rectal mucus, resulting in higher PC concentrations (see methods). These levels of PC even surpassed concentrations seen in healthy individuals. Although this justifies the dose of PC administered in this phase IIA study, a formal dose finding study (phase IIB) is pending to determine the optimal dose for efficacy. Interestingly, PC concentrations measured in non-treated UC patients were lower than in healthy controls. This supports our earlier findings: in a larger cohort of inactive UC patients, a significantly lower PC concentration was detected in the rectal mucus compared with healthy controls and patients with Crohn's disease.<sup>10</sup> This may be of potentially pathophysiological significance. The underlying molecular mechanisms need to be explored.

For efficacy evaluation, it was essential to minimise spontaneous remission and maximise study drug related remission. Therefore, only patients with longstanding UC ( $>2$  years) and chronic active disease were included in the study. Patients receiving steroids and/or immunosuppressive agents were not included for similar reasons. However, standard therapy with aminosalicylates was provided to both groups to ensure some protective. To enable detection of significant clinical changes, only patients with a CAI  $\geq 4$  were included. Median baseline CAI scores in the PC and placebo groups were 10 and 7, respectively. This and the other patient characteristics indicate that moderate to severe disease activity was indeed present at entry to the study (table 1).





**Figure 1** Comparison of changes in disease activity. Distribution of data presented as a box and whisker plot: minimum, 25th percentile, median, 75th percentile, maximum, outliers (symbols), and extreme values (\*). (A) Primary end point analysis. Clinical activity index score showed a median decrease of 7 (interquartile range (IQR) –8 to –5) in the PC group compared with a median decrease of 0 (IQR 0–2) in the placebo group ( $p < 0.00001$ ). (B) Secondary end point analysis. Endoscopic activity index score showed a median reduction of 3 (IQR –4 to –2) in the PC group compared with a median reduction of 0 (IQR 0–1) in the placebo group ( $p < 0.00001$ ). Life quality index score showed a median increase of 2.06 (IQR 1.11–3.0) in the PC group compared with a median of 0 (IQR –0.11 to 0.14) in the placebo group ( $p < 0.00001$ ).

Primary end point analysis revealed induction of clinical remission in 16 (53%) PC treated patients compared with only three (10%) in the placebo group. Moreover, the rate of clinical remission/improvement ( $\geq 50\%$ ) was 90% in the PC group compared with 10% in placebo patients. While it is more difficult for the higher CAI group to reach the clinical remission margin ( $\text{CAI} \leq 3$ ), it is easier to achieve improvement of  $\geq 50\%$ , and vice versa for patients with a low CAI. The use of both of these cut off values counterbalanced differences in baseline CAIs in the PC and placebo group (see

table 1). Apart from the significant number of responders in the PC group, the magnitude of CAI improvement (70%) was impressive (fig 1). PC treated patients also demonstrated improvement in all other secondary end points assessed. This included EAI and histology score, reduction of disease extension, as well as LQI, with all of its subcategories. The only side effect noted was bloating which was observed in the PC and placebo groups. This may be due to Eudragit S 100 encapsulation.

In contrast with the rapid anti-inflammatory effects usually observed with steroids, improvement with retarded release PC rich phospholipids was gradual and was first seen after 2–4 weeks, as documented in the patient diary cards. The design of the study did not allow a more accurate time-response analysis. An ongoing dose finding study with a homogeneous study population with regard to disease extent and activity will provide data on the time course of improvement.

The anti-inflammatory effect of PC in UC could be attributed to the fact that it is lacking in colonic mucus.<sup>10</sup> Thus supplementation with PC may help to reconstitute the structure and density of the mucus to serve as a protective mechanical shield. In addition, PC as a hydrophobic lipid may exert a general defensive action by preventing attacks from commensal colonic flora. Alternatively, PC could also be incorporated into mucosal cell membranes where it influences signalling processes involved in inflammation. Recent studies using an in vitro phagosomal test model system support the notion that PC is involved in signalling networks that inhibit proinflammatory signalling “states” in membranes.<sup>18</sup> Accordingly, it would be most interesting to test purified phospholipids for their potential to inhibit mucosal inflammation which could be the basis for an effective and harmless lipid based therapy in IBD.

Although larger studies are needed for definitive confirmation, our results from the present proof of concept study (phase IIA) indicate that oral retarded release PC is safe and clinically useful in UC patients, as reflected by the decrease in overall inflammatory activity with an associated significant increase in quality of life. Long term PC application may be able to maintain clinical remission without the considerable adverse effects seen following steroid and immunosuppressive therapy.

## ACKNOWLEDGEMENTS

We thank Dr Wolf-Dieter Lehmann (DKFZ, Heidelberg, Germany) for providing technical support in mass spectrometry analyses and Jens Wagenblast for performing PC quantifications. This work was supported by a grant from the Dietmar Hopp Foundation.

## Authors' affiliations

**W Stremmel, R Ehehalt, A Zahn, U Merle**, Department of Gastroenterology, University Hospital Heidelberg, Heidelberg, Germany  
**F Autschbach**, Department of Pathology, University Hospital Heidelberg, Heidelberg, Germany  
**U Hinz**, Unit for Documentation and Statistics of the Department of Surgery, University of Heidelberg, Heidelberg, Germany

Conflict of interest: None declared.

## REFERENCES

- 1 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998;**115**:182–205.
- 2 **Podolsky D**. Inflammatory bowel disease. *N Engl J Med* 2002;**347**:417–29.
- 3 **Rath HC**, Schultz M, Freitag R, et al. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect Immun* 2001;**69**:2277–85.
- 4 **Velkamp C**, Tonkonogy SL, De Jong YP, et al. Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg(epsiln26) mice. *Gastroenterology* 2001;**120**:900–13.

- 5 **Lichtenberger LM**. The hydrophobic barrier properties of gastrointestinal mucus. *Annu Rev Physiol* 1995;**57**:565–83.
- 6 **Wallace JL**, Granger DN. The cellular and molecular basis of gastric mucosal defense. *FASEB J* 1996;**10**:731–40.
- 7 **Bernhard W**, Postle AD, Linck M, *et al*. Composition of phospholipid classes and phosphatidylcholine molecular species of gastric mucosa and mucus. *Biochim Biophys Acta* 1995;**1255**:99–104.
- 8 **Butler BD**, Lichtenberger LM, Hills BA. Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate. *Am J Physiol* 1983;**244**:G645–51.
- 9 **DeSchryver-Kecskemeti K**, Eliakim R, Carroll S, *et al*. Intestinal surfactant-like material. A novel secretory product of the rat enterocyte. *J Clin Invest* 1989;**84**:1355–61.
- 10 **Ehehalt R**, Wagenblast J, Erben G, *et al*. Phosphatidylcholine and lysophosphatidylcholine in intestinal mucus of ulcerative colitis patients. A quantitative approach by electrospray-tandem mass spectroscopy. *Scand J Gastroent* 2004;**39**:737–42.
- 11 **Kao YC**, Lichtenberger LM. A method to preserve extracellular surfactant-like phospholipids on the luminal surface of rodent gastric mucosa. *J Histochem Cytochem* 1990;**38**:427–31.
- 12 **Bengmark S**, Jeppsson B. Gastrointestinal surface protection and mucosa reconditioning. *JPN J Parenter Enteral Nutr* 1995;**19**:410–15.
- 13 **Ottlecz A**, Romero JJ, Hazell SL, *et al*. Phospholipase activity of *Helicobacter pylori* and its inhibition by bismuth salts. Biochemical and biophysical studies. *Dig Dis Sci* 1993;**38**:2071–80.
- 14 **Weitkamp JH**, Perez-Perez GI, Bode G, *et al*. Identification and characterization of *Helicobacter pylori* phospholipase C activity. *Zentralbl Bakteriol* 1993;**280**:11–27.
- 15 **Foltz CJ**, Fox JG, Cahill R, *et al*. Spontaneous inflammatory bowel disease in multiple mutant mouse lines: association with colonization by *Helicobacter hepaticus*. *Helicobacter* 1998;**3**:69–78.
- 16 **Fabia R**, Ar'Rajab A, Willen R, *et al*. Effects of phosphatidylcholine and phosphatidylinositol on acetic-acid-induced colitis in the rat. *Digestion* 1992;**53**:35–44.
- 17 **Mourelle M**, Guarner F, Malagelada JR. Polyunsaturated phosphatidylcholine prevents stricture formation in a rat model of colitis. *Gastroenterology* 1996;**110**:1093–7.
- 18 **Anes E**, Kühnel M, Bos E, *et al*. Selected lipids can activate phagosome actin assembly and maturation leading to the killing of bpathogenic mycobacteria. *Nat Cell Biol* 2003;**5**:793–802.
- 19 **Ehehalt R**, Jochims C, Staffer S, *et al*. Evidence of luminal phosphatidylcholine secretion in rat ileum. *Biochim Biophys Acta* 2004;**1682**:63–71.
- 20 **Nugent SG**, Kumar D, Rampton DS, *et al*. Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosacilates and other drugs. *Gut* 2001;**48**:571–7.
- 21 **Rachmilewitz D**. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989;**298**:82–6.
- 22 **Guyatt G**, Mitchell A, Irvine EJ, *et al*. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology* 1989;**96**:804–10.
- 23 **Truelove SC**, Richards WD. Biopsy studies in ulcerative colitis. *BMJ* 1956;**1**:1315–18.
- 24 **Vecchi M**, Meucci G, Gionchetti P, *et al*. Oral versus combination mesalazine therapy in active ulcerative colitis: a double-blind, double-dummy, randomized multicentre study. *Aliment Pharmacol Ther* 2001;**15**:251–6.
- 25 **Kruis W**, Schreiber S, Theuer D, *et al*. Low dose balsalazide (1.5 g twice daily) and mesalazine (0.5 g three times daily) maintained remission of ulcerative colitis but high dose balsalazide (3.0 g twice daily) was superior in preventing relapses. *Gut* 2001;**49**:783–9.

### bmjupdates+

bmjupdates+ is a unique and free alerting service, designed to keep you up to date with the medical literature that is truly important to your practice. bmjupdates+ will alert you to important new research and will provide you with the best new evidence concerning important advances in health care, tailored to your medical interests and time demands.

#### Where does the information come from?

bmjupdates+ applies an expert critical appraisal filter to over 100 top medical journals. A panel of over 2000 physicians find the few 'must read' studies for each area of clinical interest.

Sign up to receive your tailored email alerts, searching access and more...

[www.bmjupdates.com](http://www.bmjupdates.com)