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RAPID COMMUNICATION

N-acetyl-L-cysteine combined with mesalamine in the treatment of ulcerative colitis: Randomized, placebo-controlled pilot study

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Abstract

AIM: To evaluate the effectiveness and safety of oral N-acetyl-L-cysteine (NAC) co-administration with mesalamine in ulcerative colitis (UC) patients.

METHODS: Thirty seven patients with mild to moderate UC were randomized to receive a four-wk course of oral mesalamine (2.4 g/d) plus N-acetyl-L-cysteine (0.8 g/d) (group A) or mesalamine plus placebo (group B). Patients were monitored using the Modified Truelove-Witts Severity Index (MTWSI). The primary endpoint was clinical remission (MTWSI $\leqslant$ 2) at 4 wk. Secondary endpoints were clinical response (defined as a reduction from baseline in the MTWSI of $\geqslant$ 2 points) and drug safety. The serum TNF-α, interleukin-6, interleukin-8 and MCP-1 were evaluated at baseline and at 4 wk of treatment.

RESULTS: Analysis per-protocol criteria showed clinical remission rates of 63% and 50% after 4 wk treatment with mesalamine plus N-acetyl-L-cysteine (group A) and mesalamine plus placebo (group B) respectively (OR = 1.71; 95% CI: 0.46 to 6.36; P = 0.19; NNT = 7.7). Analysis of variance (ANOVA) of data indicated a significant reduction of MTWSI in group A (P = 0.046) with respect to basal condition without significant changes in the group B (P = 0.735) during treatment. Clinical responses were 66% (group A) *vs* 44% (group B) after 4 wk of treatment (OR = 2.5; 95% CI: 0.64 to 9.65; P = 0.11; NNT = 4.5). Clinical improvement in group A correlated with a decrease of IL-8 and MCP-1. Rates of adverse events did not differ significantly between both groups.

CONCLUSION: In group A (oral NAC combined with mesalamine) contrarily to group B (mesalamine alone), the clinical improvement correlates with a decrease of chemokines such as MCP-1 and IL-8. NAC addition not produced any side effects.

Key words: Ulcerative colitis; Interleukin; Mesalamine; N-acetyl-L-cysteine

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INTRODUCTION

Ulcerative colitis (UC) is a chronic idiopathic inflammatory disease of the colon characterized by rectal bleeding, diarrhea, abdominal pain, weight loss and fever[1]. Histological examination of biopsy specimens reveals the presence of infiltrated white blood cells such as neutrophils, monocytes and lymphocytes in the colonic interstitium[2]. In the recruitment and activation of white blood cells, participate cytokines [interleukin (IL)-1β, IL-2, IL-6, IL-12 and tumour necrosis factor-alpha][3], chemokines [IL-8, monocyte chemoattractant protein (MCP)-1 and MCP-3][4], cell adhesion molecules[5] as well as the inducible isoforms of nitric oxide synthase[6] and cyclooxygenase[7]. Moreover, the infiltration of phagocytes results in the stimulation of the latent plasma membrane associated NADPH oxidase, which release large amounts of superoxide (O_2) and H_2O_2, producing hydroxyl radicals[8].

Recently, a new hypothesis termed "Radical Induction Theory" has been proposed to explain the etiology of UC[9]. The excess of un-neutralized hydrogen peroxide (H_2O_2), produced within colonic epithelial cells as a result of aberrant cellular metabolism, diffuses through cell membranes to the extracellular space, where it is converted to the highly damaging hydroxyl radical (OH) resulting in oxidative damage to structures comprising the colonic epithelial barrier. Once damaged, the barrier is unable to exclude highly immunogenic fecal bacteria invading the normally sterile submucosa. This antigen exposure provokes an initial immune response of white blood cell infiltration into colonic mucosa. The injurious "reactive oxygen species" would be inactivated by protective enzymes (superoxide dismutase, catalase and glutathione peroxidase) and by non-enzymatic antioxidants (glutathione, ascorbate and α-tocopherol)[10,11]. A common feature of patients with inflammatory bowel disease is a depletion of endogenous oxidant defenses such as ascorbate, β-carotene, α-tocopherol and glutathione[12,13]. This last compound is a naturally occurring tripeptide (γ-Glu-Cys-Gly) found in high concentrations within tissues. In experimental acute colitis, cellular glutathione levels decreased significantly and the administration of the antioxidant N-acetyl-L-cysteine (NAC) restored glutathione level and decreased colonic inflammation[14]. More recently it has been showed that the combination of NAC and mesalamine accelerates mucosal healing in a rodent model of colitis[15]. Taken together, these results suggest an imbalance and inefficient endogenous antioxidant response in the intestinal mucosa of UC patients, which may contribute to the etiology, the pathogenesis and the perpetuation of the inflammatory processes[10]. The aim of the present pilot study was to evaluate the possible effectiveness and safety of NAC associated with mesalamine in mild to moderate UC.

MATERIALS AND METHODS

Patient's selection criteria and study protocol

Thirty seven patients with mild to moderate UC according to the Modified Truelove and Witts Severity Index (MTWSI)[16] were randomized to receive a 4-wk course of oral mesalamine (2.4 g/d) plus N-acetyl-L-cysteine (NAC) (0.8 g/d) (group A) or mesalamine plus placebo (group B). Exclusion criteria were age over 70 or less 18 years, pregnancy, serious underlying systemic diseases and MTWSI > 10. Treatment with oral or topical steroids, topical mesalamine, immunosuppressors (azathioprine, 6-mercaptopurine, *etc.*) or antioxidants was discontinued at least three weeks before commencement of the trial. The protocol was approved by the Ethical Investigation Committee of the corresponding Institutions, and written informed consent was obtained from all the patients. Diagnosis of UC was established by standard clinical, radiological, histological, and endoscopic criteria[1]. The patient's characteristics are described in Table 1. Patients were allocated to one of two treatment groups according to a centrally computer-assisted randomization list and received oral mesalamine at a dose of 2.4 g/d combined with NAC at a dose of 0.8 g/d (group A) or oral mesalamine at a dose of 2.4 g/d and placebo (group B) during four wk. NAC was provided as 200 mg sacks of water-soluble powder identical in taste, bulk and appearance to those of placebo. Mesalamine was provided as an 800 mg tablets coated with 1:2 copolymer of metacrylic acid and methylmethacrylate (Eudragit-S) used for colonic delivery of mesalamine[17].

Measurement of disease activity

Clinical and biochemical findings were assessed by the gastroenterologist at intervals of two and four weeks respectively. All patients were asked to record stool frequency (number of daily stools) and consistency, nocturnal stools, visible blood in stool, fecal incontinence, abdominal pain, abdominal tenderness, need for antidiarrheals and a patient self-rating evaluation based upon the impact of symptoms on normal life activities. For stool frequency and abdominal pain, a scale from 0 (normal) to 4 (markedly abnormal) was used. For use of antidiarrheal medication, a scale from 0 (no) to 1 (yes) was used. For the other parameters, the scale ranged from 0 (normal) to 3 (markedly abnormal). The Modified Truelove-Witts Severity Index, which has been considered useful in therapeutic trials[16], was calculated from these data. The primary endpoint was clinical remission (MTWSI $\leqslant$ 2) at 4 wk. Secondary endpoints were clinical response (defined as a reduction from baseline in the MTWSI of $\geqslant$ 2 points) and drug safety.

Assessment of safety

The hematological and biochemical studies were performed at regular intervals by the analytical laboratory services of the corresponding hospitals: complete blood count, hepatic enzymes, bilirubin, erythrocyte sedimentation rate and C-reactive protein were measured between other biochemical parameters.

Table 1 Basal and demographic data

Data	Group A Mesalamine + NAC (n = 19)	Group B Mesalamine + placebo (n = 18)	Total (n = 37)
Age (mean ± SD)	51.4 ± 14	42.2 ± 13	46.9 ± 14
White race, n (%)	11 (57.8)	13 (72.2)	24 (64.8)
Smoker, n (%)	2 (10.5)	3 (16.7)	5 (13.5)
Male, n (%)	8 (42.1)	5 (27.8)	13 (35.1)
Basal modified Truelove-Witts severity index (mean ± SD)	5.95 ± 2.22	4.61 ± 2.09	5.30 ± 2.20

NAC: N-acetyl-L-cysteine.

Table 2 Changes in disease activity assessed using modified Truelove-Witts Severity Index (mean ± SD) in subjects who received the drugs for 2 wk and 4 wk

Week of treatment	0	2	4	ANOVA statistical test (number of patients)
Group A mesalamine plus NAC	5.95 ± 2.22	4.68 ± 3.40	3.32 ± 3.71[a]	P = 0.046 (n = 19)
Group B mesalamine plus placebo	4.61 ± 2.09	4.17 ± 3.85	3.72 ± 3.91	P = 0.735 (n = 18)

[a]$P < 0.05$ represents the significance levels compared with the corresponding basal values using the ANOVA statistical test.

Evaluation of reduced glutathione, TNF-α, IL-6, IL-8 and MCP-1 circulating levels

Blood samples were obtained by venipuncture and placed into tubes containing lithium heparin as anticoagulant. For the measurement of GSH in whole-blood samples, 0.5 mL of blood was treated immediately with 0.25 mL of trichloroacetic acid (12%) on ice. After 5 min tubes were centrifuged at 13 000 g during 10 min at 4℃ and the acidic supernatants were immediately used for GSH measure. GSH determinations were performed as described previously[18] with some modifications. Briefly, the amount of lactoyl-glutathione formed between methylglyoxal (110 mmol/L) and GSH in presence of glyoxalase-I (lactoyl-glutathione lyase) at pH 7.0 buffered with 0.1mmol/L sodium phosphate was measured spectro-photometrically at 240 nm.

The concentration of IL-8, MCP-1, TNF-α and IL-6 present in plasma was determined by using specific sandwich ELISA following manufacturer protocol. Briefly plates (Costar) were coated overnight at 4℃ with specific mouse anti-human monoclonal antibody (Becton Dickinson) in 0.1 mol/L Na_2HPO_4 (pH 9) (dilution 1:200). After washing with PBS containing 0.5% Tween 20 unspecific sites were blocked with PBS containing 3% BSA. Plasma was added to each well and incubated for 12 h at 4℃. Unbound material was discarded and biotinylated mouse anti-human monoclonal antibody (Becton Dickinson) was incubated during 1 h at room temperature. After washing bound antibodies were detected by incubation with avidin-peroxidase (Sigma) for 30 min in presence of the 2,2 azinobis (3-ethybenzthiazolinesulfonic acid) (ABTS of Sigma) as substrate. Absorbance was measured at 405 nm. A Standard curve was constructed for each cytokine or/and chemokines by using recombinant human molecules (Becton Dickinson) in PBS containing 3% BSA.

Statistical analysis

For quantitative variables, mean and standard deviation were calculated. Statistical analysis was performed with the SAS program. For the comparison of treatment effect on Modified Truelove-Witts Severity Index between both groups of patients, the analysis of covariance (ANCOVA) was used after adjusting for baseline values. To study the evolution of disease score for each group, the analysis of variance (ANOVA) with Bonferroni corrected *post hoc* comparisons was used to determine significant treatment effects. $P < 0.05$ was considered statistically significant.

RESULTS

Thirty seven patients were included in the trial. All patients had mild to moderate UC according to the criteria of Modified Truelove-Witts Severity Index[16]. Treatment groups were comparable at randomization for baseline demographic characteristics (Table 1). All patients completed the one month study (19 treated with mesalamine plus NAC and 18 treated with mesalamine plus placebo) following the correct protocol.

Treatment effect on Modified Truelove-Witts Severity Index

The disease score was calculated by "per-protocol" criteria, the data of all randomized patients at 0, 2 and 4 wk of treatment were included. Twelve of 19 patients (63%) in the combination treatment group (A) underwent remission (score ⩽ 2) at 4 wk of treatment. At the same period of time nine of 18 patients (50%) in the mesalamine plus placebo group (B) underwent clinical remission (score ⩽ 2). However, statistically not-significant differences were observed between both groups in this clinical parameter (OR = 1.71; 95% CI: 0.46 to 6.36; P = 0.19; NNT = 7.7). During 4 wk of treatment, clinical response measured by MTWSI improved in group A compared with the corresponding baseline values (P = 0.046, ANOVA test) (Table 2). Whereas in the group B no statistical improvement were reached (P = 0.735, ANOVA test) at the same period of time. Although a better favorable trend was observed with the combined therapy (group A) as compared to that observed in the mesalamine plus placebo arm (group B), the significance threshold was not reached (ANCOVA test). The disease score decreased in both groups of patients at 4 wk of treatment with respect to the corresponding baseline values, however, the group B was not statistically significant; the MTWSI change was 2.63 ± 0.82 (P = 0.004) and 0.89 ± 0.9 (P = 0.33) (paired student' t-test) at 4 wk of treatment for A and B groups, respectively.

Effect of treatment on reduced glutathione, TNF-α, IL-6, IL-8 and MCP-1 circulating levels

Measurement of biochemical parameters was performed

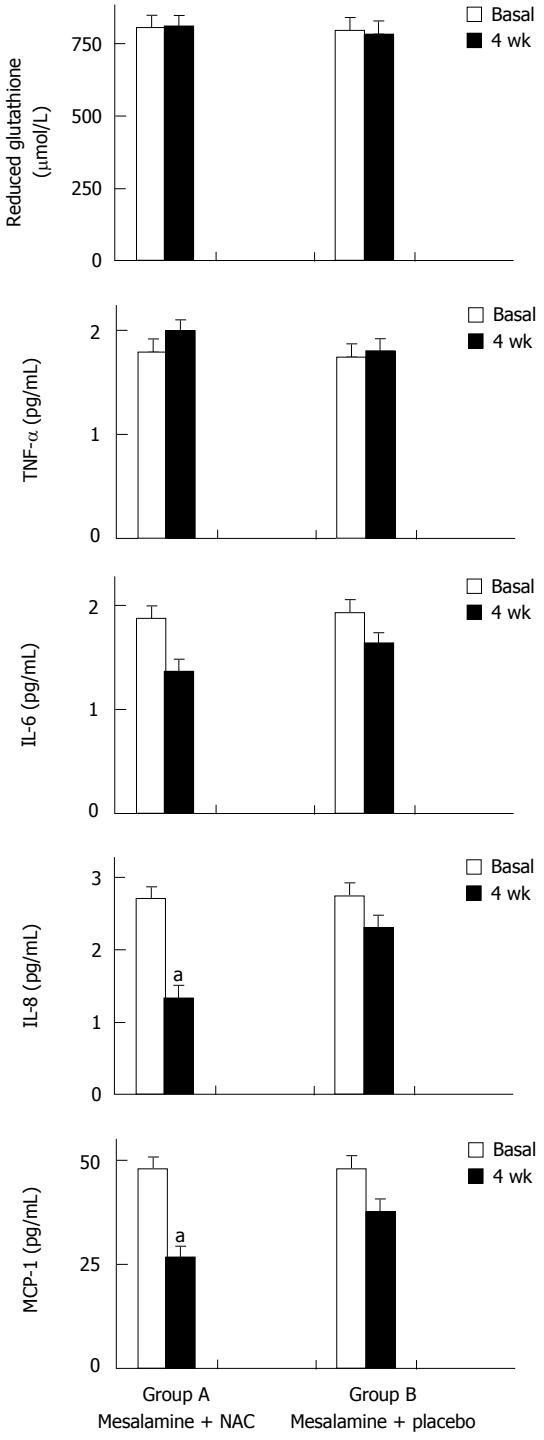

Figure 1 Reduced glutathione levels in whole blood and tumor necrosis factor alpha (TNF-α), interleukin IL-6, IL-8, and monocyte chemoattractant protein (MCP)-1 levels in plasma of subjects after 4 wk of treatment with mesalamine plus NAC (Group A) or mesalamine plus placebo (Group B). mean ± SD. [a]$P < 0.05$ represent the significance level compared with basal values using the Student' *t*-test.

at the beginning and at 4 wk of treatment (Figure 1). Blood levels of reduced glutathione (GSH) did not change significantly at 4 wk of treatment in group A (NAC plus mesalamine) with respect to control group (placebo plus mesalamine). TNF-α plasma levels remained constant and did not change along the period studied in either group A or B. IL-6 plasma levels decreased slightly, although not significantly, in group A, without changes in the group B. By contrast, circulating levels of IL-8 decreased significantly ($P < 0.05$), with respect to baseline values, at 4 wk of treatment in group A, without changes in the group B. The decrease in IL-8 coincided with the induction of remission of the disease in group A. Finally, the profile of MCP-1 circulating levels was similar to that found for IL-8. Thus, at 4 wk of treatment with NAC plus mesalamine (Group A), a significant ($P < 0.05$) decrease in MCP-1 with respect to the corresponding baseline values was demonstrated, while statistically non-significant changes were observed in group B.

Safety

No serious adverse effects, clinical or analytical, were observed in either treatment group. The hematological and biochemical results at baseline and 4 wk are summarized in Tables 3 and 4 respectively.

DISCUSSION

The idea that NAC treatment could be useful in UC emerged from previous studies in animal models of that disease[14]. Very recently it has been showed that antioxidant therapy with NAC plus mesalamine accelerates mucosal healing in rodent model of colitis[15]. Mucosal biopsies taken from active gut inflammation sites from UC patients produced significantly more reactive oxygen species (H_2O_2 and superoxide) compared to either uninvolved or healthy tissue[10,19]. Glutathione and glutathione peroxidase are the most important system for elimination of H_2O_2. Consequently, the reduced glutathione levels in the inflamed mucosa from patients with UC could be decreased[12,13]. The oral treatment with NAC (a precursor of glutathione) of UC patients could restore the circulating and local levels of reduced glutathione to respond to the excess of reactive oxygen species. Recently, the effectiveness of antioxidant therapy in pediatric Cohn's disease has been reported in an open-label pilot study[20].

Monitoring of standard laboratory data of our patients demonstrated no worsening of the values with respect to baseline data during treatment (with or without NAC). Moreover, no significant differences in standard laboratory data were observed between the values of the two treatment groups (Tables 3 and 4). Finally, no significant adverse events related with the NAC treatment occurred in any of the patients.

Treatment with mesalamine was maintained constant throughout the study for all patients, with improvement of the disease measured by MTWSI. However in the group receiving a supplement of NAC (0.8 g/d), a more favorable trend was seen with respect to mesalamine treatment alone. The mesalamine plus NAC group underwent a significant clinical response with respect to basal state measured by MTWSI at 4 wk of treatment. However, in the control group (mesalamine plus placebo) no significant improvement was observed in the course of the disease at that time. These results suggest that NAC could accelerate the healing process in UC. However, the conclusion could be affected as consequence of non-homogeneity of both

Table 3 Hematological parameters

	Group A		Group B	
	Basal	4 wk	Basal	4 wk
Haemoglobin (g/100 mL)	14.28	13.98	13.81	13.79
Haematocrit (g/100 mL)	42.55	42.01	40.50	40.61
Erythrocytes ($\times 10^6$/mm^3)	4.81	4.69	4.60	4.58
Platelet count ($\times 10^3$/mm^3)	232.95	249.84	263.22	260.50
VSG (mm/h)	17.13	15.00	19.60	17.38
Leukocytes ($\times 10^3$/mm^3)	7.14	7.06	7.86	7.90
Neutrophils (%)	62.63	61.45	63.75	58.61
Eosinophils (%)	2.56	3.34	3.81	3.22
Basophils (%)	0.32	0.55	0.44	0.22
Lymphocytes (%)	25.89	28.03	27.82	27.74
Monocytes (%)	6.97	7.20	7.14	7.88

Group A: Mesalamine + NAC; Group B: Mesalamine + placebo.

Table 4 Biochemical parameters

	Group A		Group B	
	Basal	4 wk	Basal	4 wk
SGOT (units/L)	16.89	16.47	20.47	25.06
SGPT (units/L)	15.58	17.53	36.94	43.59
Phosphatase alkaline (units/L)	93.56	86.61	93.59	86.71
Bilirubin (mg/100 mL)	0.68	0.67	0.50	0.51
LDH (units/L)	258.22	265.45	307.63	299.30
Serum creatinine (mg/100 mL)	0.95	0.95	0.90	0.93
Urea/BUN (mg/100 mL)	35.23	34.64	35.90	33.75
Total Proteins (g/100 mL)	7.17	7.06	7.18	7.08
Albumin (g/100 mL)	4.16	4.11	4.13	4.12
Na^+ (mmol/L	138.10	139.20	138.11	139.10
K^+ (mmol/L)	4.17	4.24	4.34	4.28
Glucose (mg/100 mL)	91.53	89.54	83.53	85.88
Iron (μg/dL)	70.27	69.00	78.56	84.82
Orosomucoid (mg/100 mL)	85.19	90.98	98.06	89.98
CRP (mg/L)	5.37	4.27	5.20	4.85

Group A: Mesalamine + NAC; Group B: Mesalamine + placebo.

groups according to the age; a definite limitation of this pilot trial is the small size of the groups. This fact must be kept in mind for planning further studies (with higher doses of NAC, route of administration or larger sample size).

The beneficial effect of NAC at 4 wk did not correlate with changes in reduced glutathione in blood, which suggests that the mechanism of action of NAC is local, and not systemic, at that time. Similar pharmacokinetic results were observed in the treatment with 0.6 g/d of NAC of HIV infected patients, resulting in a glutathione increase at 8 wk of treatment[21]. Although oral doses of 1.8 g/d of NAC increased the percentage of CD4 lymphocyte counts in HIV infected patients, it did not produce changes in plasma glutathione levels[22], only high oral doses (4-8 g/d of NAC) of this antioxidant could replenished glutathione levels and improved survival rates of HIV infected patients[23]. Treatment with comparables doses (100 mg/kg NAC) of acetic acid-induced experimental colitis substantially reduced the degree of colonic injury. Lower doses (20 mg/kg) had no protective effect[24]. Moreover, clinical studies suggest liver absorption of NAC when it is administered orally because hepatic clearance of NAC decreased significantly during cirrhosis[25] and its metabolism improves hepatosplanchnic flow and liver function in septic shock patients[26]. In experimental colitis the beneficial effects of NAC were observed by intrarectal and intraperitoneal administration[27]. For all these reasons, clinical trials have used higher doses of NAC bypassing digestive tract without serious adverse events[28,29].

Our study also suggests that the beneficial effect of NAC in UC is related to the down-regulation of chemokines such as MCP-1 and IL-8. At 4 wk of treatment, both molecules decreased in plasma of patients treated with NAC plus mesalamine, whereas in the mesalamine plus placebo group this change was not statistically significant. The induction of the remission in the group A coincided with the decrease in IL-8 and MCP-1 plasma levels, suggesting that the effect of NAC is related to the decrease of molecules that activates the recruitment and activation of neutrophils and monocytes to the inflamed mucosa. Our data are in concordance with previous studies performed in alcoholic hepatitis, where MCP-1 production stimulated by lipopolysaccharides decreased with NAC treatment, suggesting that the antioxidant act directly on target cells[30]. Previously, it was observed that the percentage of cells expressing IL-8 and MCP-1 was significantly enhanced in UC samples as compared to controls[31]. Furthermore, the local overexpression of MCP-1 and IL-8 was associated with inflammation in UC[32]. A close correlation has been demonstrated between mucosa IL-8 mRNA expression and the colonic inflammation in UC patients, and a decrease of local IL-8 expression has been reported during UC improvement after granulocytapheresis[33]. Local changes in IL-8 expression correlates with urinary IL-8, and this chemokine increases around ten fold in the urine during active UC compared with control subjects[34], suggesting that circulating IL-8 is a good indirect marker for the assessment of active inflammation in the colonic mucosa. Other pro-inflammatory cytokines such as TNF-α and IL-6, previously implicated in inflammatory bowel diseases, did not change significantly in plasma with the treatment prescribed in our study. However we can not rule out the hypothesis that down-regulation of TNF-α and IL-6 in the mucosa could explain the improvement observed.

In conclusion, the results of the present pilot study suggest that combined therapy (NAC and mesalamine) produces a clinical improvement of UC patients which correlates with a decrease of MCP-1 and IL8. However, the difference in clinical effect with respect to the control group (mesalamine alone) is not conclusive. NAC is safe and well tolerated.

COMMENTS

Background

A new hypothesis termed "Radical Induction Theory" has been proposed to explain the etiology of ulcerative colitis (UC) (J Pravda. *World J Gastroenterol* 2005; 11: 2371). The excess of hydrogen peroxide (H_2O_2) could damage the colonic epithelial barrier. In experimental acute colitis, cellular glutathione levels decreased and the administration of their precursor (N-acetyl-L-cysteine) (NAC) restore the endogenous level and decreased colonic inflammation. Recently, it has been showed that the combination of NAC and mesalamine accelerates mucosal healing in a rodent model of colitis (Siddiqui *et al*, *Dig Dis Sci* 2006; 51: 698).

Innovations and breakthroughs

Local overexpression of MCP-1 and IL-8 was associated with inflammation in UC and a decrease of colonic IL-8 has been reported during active UC. Our results show that antioxidant therapy with NAC decreases the levels of IL-8 that activates the recruitment and activation of neutrophils to the inflamed mucosa. It is suggested that higher dose or targeted NAC can alleviate colonic inflammation and hopefully become a novel agent for the treatment of UC.

Peer review

This is an excellent research paper which highlights the dual role antioxidants play in the pathogenesis of ulcerative colitis. And this is the first paper to cite radical induction theory which logically explains why the addition of NAC to conventional 5-ASA therapy significantly increases the remission rate observed in ulcerative colitis. The findings are potentially important for planning of furthers studies (e.g. with higher dose of N-acetyl-L-cysteine; more homogenous groups of comparison, larger sample size, et cetera).

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