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Colonic mucus, smoking and ulcerative colitis

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Human colonic mucosal protection is not fully understood but may in part rely on a layer of mucus gel adherent to the mucosa. Ulcerative colitis may occur if mucosal protection breaks down. Two studies are presented, both of which relate to the aetiology of ulcerative colitis. First, a layer of adherent mucus gel was demonstrated by a simple, reliable method. Measurements of mucus layer thickness were made in freshly resected colonic specimens and shown to increase from a mean of 107 μm on the right colon to 155 μm in the rectum. In ulcerative colitis the layer is significantly thinner or absent, whereas in Crohn's disease the colonic mucus layer is significantly thicker. Second, the relationship between smoking and colitis is explored by a double-blind, randomised and placebo-controlled trial of transdermal nicotine in active disease. Significant clinical benefit was seen, indicating nicotine may be both useful therapeutically and the component of tobacco smoke that acts to protect against colitis. Since smoking and nicotine have actions on mucosae and mucus in other organs, it is argued that there is a mucus deficiency in ulcerative colitis that smoking acts to reverse.

Ulcerative colitis (UC) is a non-specific mucosal inflammation of unknown aetiology. The pattern of inflammation is mucosal, non-granulomatous and continuous from the distal rectum. It affects more proximal colon to a variable extent, in marked contrast to Crohn's disease (CD). Any explanation of pathogenesis must account for these features. Many abnormalities of colonic mucosal immunity, inflammatory function, colonocyte nutrition and colonic mucus are known to be associated with UC, but the triggers of mucosal inflammation remain elusive. The hypothesis that colonic mucosal protection is deficient in UC is attractive since progressively higher concentrations of damaging intraluminal contents and bacteria occur as faeces pass through the colon. This paper examines two phenomena bearing directly on the aetiology of ulcerative colitis, namely the colonic adherent surface mucus layer and the singular relationship between smoking and inflammatory bowel disease (IBD). Each of two studies will be presented separately.

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Colonic mucus

Ulcerative colitis has been postulated to occur because of a breakdown in the physiological barrier separating mucosa from luminal contents. Elsewhere in the gastrointestinal tract—most notably the stomach—an adherent mucus gel layer has a key role in mucosal protection. Factors influencing integrity of this layer have identified mechanisms behind mucosal damage. Such a layer has been suggested to play a protective role in the colon, has been studied in animals, but has never previously been shown in man. Few observations have been made on the role of colonic mucus gel and its relevance to disease *(1)*. Mucus is a complex colloid whose physicochemical properties are determined by specific mucus glycoproteins (mucins). Abnormalities of mucin histochemistry and biochemistry are seen in ulcerative colitis but not Crohn's disease, although their functional relevance has not been established. Since the composition, chemical structure and hydration of mucins determine the structure of a mucus gel, any alteration in these features may impair formation and adherence of the gel layer to the mucosa. Abnormalities of this layer found in IBD may therefore explain the pathogenesis of inflammation and lead to more rational therapy *(2)*.

Standard histological methods destroy the gel structure

of mucus by dehydration, therefore a method must be used to examine tissues without fixation.

Method

Fresh surgically resected colonic specimens were examined for the presence of an adherent mucus layer, its thickness and properties in patients with and without IBD *(3)*. Colonic specimens were opened longitudinally within 20 min of resection and samples of mucosa 1 cm square dissected from areas at least 5 cm from pathological features and resection margins. These were placed mucus surface uppermost on filter paper and a 1.6 mm thick section cut vertically from the mucosa using a pair of parallel razor blades (Fig. 1). Each section was teased carefully from the blades and placed on its side on a slide, the mucosal surface at right-angles to the slide surface *(3,4)*. The section was bathed in saline and viewed with an inverted microscope and phase contrast illumination. An optically distinct layer of mucus could be seen (Fig. 2). The thickness of the mucus layer was measured with a calibrated eyepiece graticule. At least ten thickness measurements were taken along each section at 0.5 mm intervals. At least two samples were taken from each colonic specimen, with a minimum of two sections cut from each. In colonic specimens from IBD, multiple samples were taken from several sites to include macroscopically normal and inflamed areas.

A series of validation experiments were undertaken to confirm the measured layer was mucus. These consisted of using carbon particles to define the interfaces, physical disruption of the layer and histochemical studies on fresh and snap-frozen material. Using the periodic acid-Schiff reaction and alcian blue techniques, both modified to prevent mucus disruption, it was shown that the surface layer stained in a manner consistent with mucus (Fig. 3*a–c*).

After measurement, each section was fixed and examined histologically for unsuspected pathology and inflammatory activity which was graded *(3)*.

A number of statistical analyses were used. Mucus thickness for each subject at each site was characterised by the mean of all replicate measurements available. Variation between sites in the control group was assessed by one-way analysis of variance (ANOVA). For the IBD group, non-orthogonal two-way ANOVA was used, many subjects contributing specimens from more than one site. Variation between diagnostic groups at each site was assessed by one-way ANOVA, with unpaired t tests for selected contrasts.

Patients

Specimens were obtained from three groups of patients undergoing colectomy—'controls', UC and CD. The control group consisted of 46 patients who did not have IBD; 25 were male with a mean age of 66 years (range 23–90 years); 42 had colonic carcinoma, two diverticular disease, one appendix mass and one submucosal lipoma. Specimens included 12 from the right colon, 17 left colon and 21 from the rectum. Seventeen patients had ulcerative colitis (10 males), with a mean age of 47 years (range 22–79 years). Bowel preparation was used in the majority of

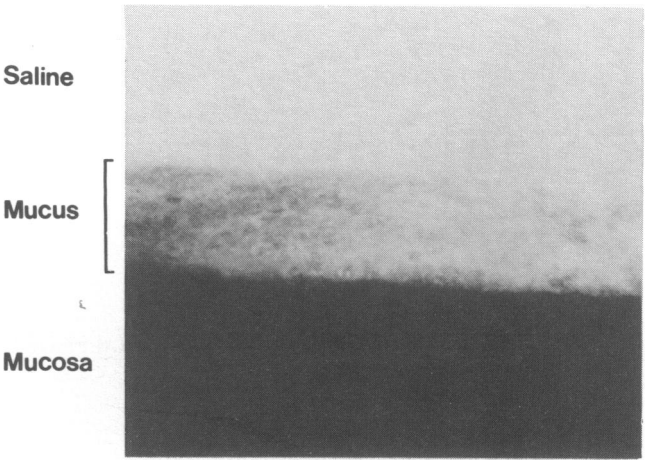


Figure 2. Photomicrograph of thick section of fresh colonic mucosa under phase-contrast illumination (magnification×100) with adherent mucus layer. The upper and lower interfaces, limits of the mucus layer, are saline–mucus and mucus–mucosa. The thickness shown (bar) is 150 μm.

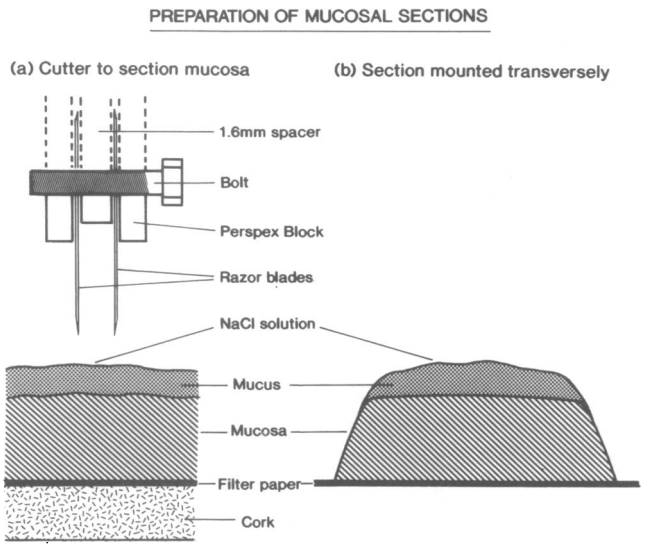
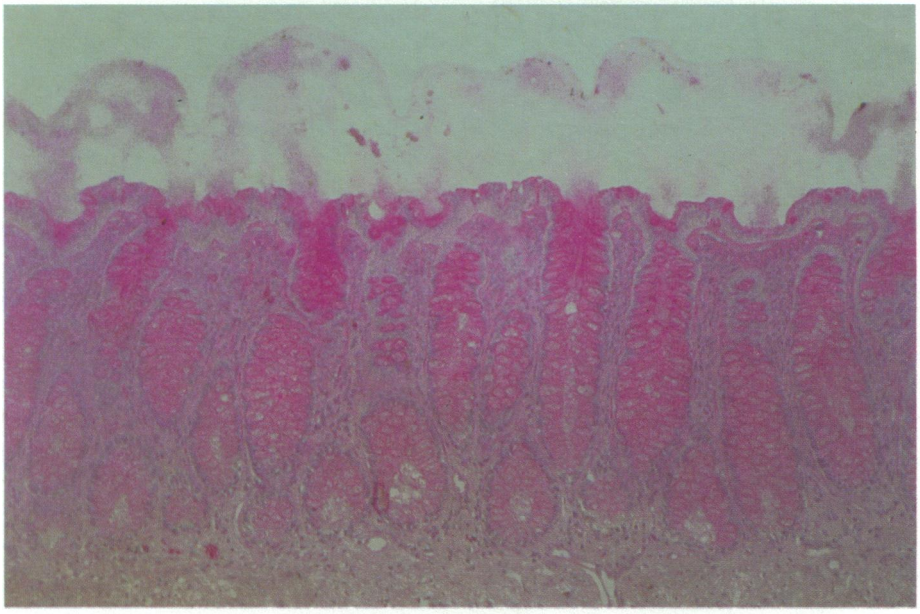


Figure 1. (a) The cutter with a sample of mucosa showing the vertical orientation of the razor blades to take 1.6 mm thick sections. Mucosa was placed on filter paper to allow easier manipulation. *(b)* Cut section, once teased from the blades of the cutter, placed on its side on a microscope slide for measurement of mucus thickness on an inverted microscope. Samples and sections of them were bathed in physiological saline throughout.

Figure 3. (a) Fresh, unfixed thick mucosal section stained with periodic acid–Schiff (PAS) reagents and viewed on an inverted microscope. The red layer is adherent mucus (magnification ×100). *(b)* Mucosal section stained with PAS with diastase after stabilising the mucus gel layer by cryostat and molten agar. Section 10 μm thick; magnification ×100. *(c)* As *(b)* stained with alcian blue and van Gieson counterstain, mucus staining greenish-blue.

(a)



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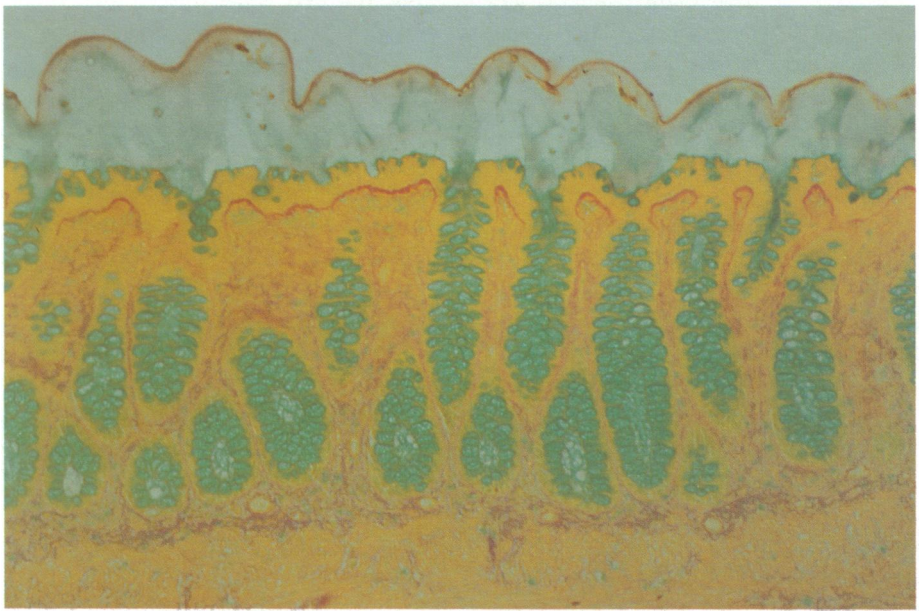

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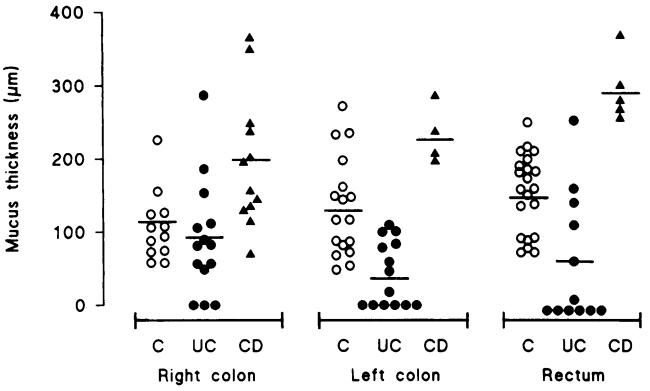

Figure 4. Thickness of adherent colonic mucus (μm) in controls (C) ○, ulcerative colitis (UC) ● and Crohn's disease (CD) ▲. Control specimens (50) were obtained from 46 patients, most of whom had colonic carcinoma. Forty UC specimens were from 17 patients and 21 Crohn's disease specimens from 15 patients. The number of measurements from each resected specimen was determined by the extent of the resection; many with inflammatory bowel disease with a proctocolectomy had samples from each of the three sites. Means are shown.

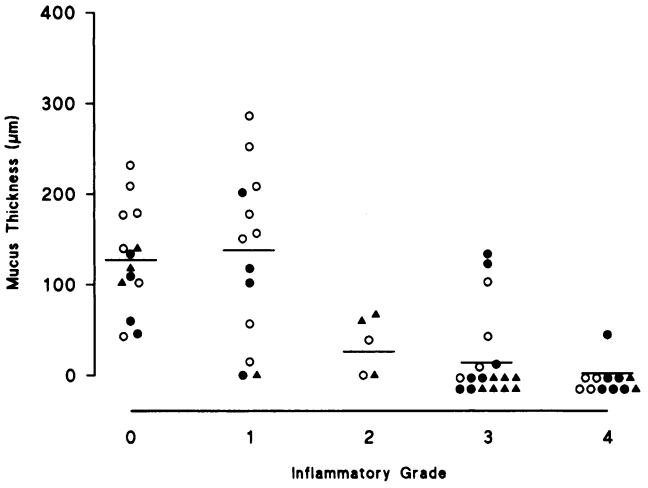

Figure 5. Relationship in ulcerative colitis of mucus thickness and inflammatory activity from right colon ○, left colon ● and rectum ▲. Inflammation was graded for each of 62 sections in 40 specimens from 17 patients. Grade 0 represented no acute inflammation; grade 4 is acute inflammation with loss of surface epithelium; grades 1, 2 and 3 have increasing numbers of neutrophils in the lamina propria, crypts and finally crypt abscesses, but with an intact epithelium. Inflammation graded 0 and 1 did not differ for mucus thickness, although this was significantly reduced for higher grades of inflammation.

cases; either Picolax® (Ferring, UK) or Kleanprep® (Nordica, UK) being used.

In all, 14 patients had a proctocolectomy as an elective procedure for persistent severe disease, one patient had a colectomy and ileorectal anastomosis and two patients had completion proctectomies. Fifteen patients had Crohn's disease (11 females) with a mean age of 38 years (range 18–64 years). Of these, ten had ileocaecal resections, two left colectomies, one proctectomy and two had proctocolectomies.

Results

A continuous adherent layer of mucus was seen in controls group (Figs 2, 3). The thickness of the layer varied according to site in the colon—thinner on the right (mean±standard deviation (SD); 107±48 μm) than the left colon (134±68 μm) or rectum (155±54 μm). The difference in mucus thickness between the right colon and rectum is significant ($P<0.05$). There was considerable intersubject variation (Fig. 4). Age, sex, bowel preparation and smoking status before resection had no influence on the thickness of the layer.

The presence and type of IBD did influence the layer. In UC, mucus thickness was highly variable yet significantly thinner than controls in the left colon (mean 43±45 μm; $P=0.0001$) and rectum (mean 60±86 μm; $P<0.01$), but no difference was seen in the right colon. The mucosa was denuded of the adherent layer in many specimens. Where inflammation was more severe, the layer tended to be thinner, but this was not simply a consequence of ulceration since mucus was absent on areas which were severely inflamed yet not ulcerated (Fig. 5). In those areas with little or no inflammation (grades 0 and 1) the mucus thickness was similar to the controls, but where severe inflammation occurred—grades 2 and 3—most sections had little adherent mucus.

In Crohn's disease, the mucus layer tended to be thicker than controls at each site (Fig. 4); right colon (mean 190±83 μm; $P<0.01$), left colon (mean 232±40 μm; $P<0.01$) and rectum (mean 294±45 μm; $P<0.001$). Caution should be exercised in interpreting this data in the small number of specimens distal to the splenic flexure.

Discussion

For the first time, this simple, repeatable technique has identified a continuous layer of adherent mucus gel coating the human colon to a depth of 100–150 μm in 'controls'. The mucus layer is thinner on the right side of the colon, but becomes thicker more distally. This may reflect a simple requirement for lubrication; right colonic contents being more liquid and those on the left progressively more solid. Alternatively, the physiological demands for mucosal protection by mucus may be greater as colonic effluent becomes more concentrated in its transit through the colon.

The limitations of the control group are acknowledged, as colorectal neoplasia is known to be associated with abnormal mucins. Attempts were made to reduce this problem by avoiding the transitional zones around neoplastic features.

Corroboration of these findings requires measurements from patients without colonic disease. Development of a colonoscopic biopsy device able to sample mucosa without disruption of the surface mucus is required since standard methods are unsuitable.

The thickness of the physiological layer of mucus gel should depend on a dynamic equilibrium between mucus glycoprotein synthesis, secretion and hydration and loss by shear, degradation by luminal enzymes and digestion by colonic microflora. The layer may be thinner or denuded in ulcerative colitis. It is known that goblet cells and mucin depletion are characteristic features of UC. Histochemical studies of mucus glycoproteins have shown abnormal sulphation and sialation patterns, truncated oligosaccharide chains and altered lectin binding *(5)*. Specific mucin subtypes may be depleted *(2)*. Colonic explant cultures show mucin synthesis to be abnormal. Colonic mucus breakdown is accelerated in colitis, with abnormally high levels of faecal proteinase and sulphatase activities which degrade and solubilise the mucus gel *(1)*.

The observations on Crohn's disease require further investigation, especially as few specimens were studied. Both mucus composition and synthesis appear normal in Crohn's disease. It may be that patchy involvement of Crohn's mucosa is responsible, with high levels of mucosal inflammatory mediators driving release of mucin from adjacent normal goblet cells.

Adherent mucus gel thickness is not, according to this study, a sufficiently discriminating method to aid classification of indeterminate colitis, as might be hoped since there is considerable variability and overlap among diagnostic groups.

The mucus layer and its abnormalities in IBD require further study since agents maintaining or enhancing a healthy layer of gel could have therapeutic value *(6)*.

Smoking and ulcerative colitis

Epidemiological evidence shows a reliable relationship between IBD and smoking. UC is predominantly a disease of non-smokers and ex-smokers with some patients finding smoking improves their disease. By contrast, Crohn's disease is more common in smokers. The association with UC is a unique 'experiment of nature' since smoking has, with few exceptions, been shown to be injurious to health. It may both illuminate mechanisms of disease and indicate potential new therapeutic avenues.

Epidemiology

A chance observation during a nutritional study of IBD showed that of 230 UC patients only 8% were current smokers compared with 44% of matched controls *(7)*. This observation has been corroborated by workers in many countries using various designs. A rigorous meta-analysis scrutinised nine studies showing a pooled odds ratio for UC in smokers to be 0.41—significantly fewer smokers develop UC compared with controls. With non-smoking as a risk factor for UC, the lifetime risk to non-smokers becomes 2.9. Former smokers have an increased risk of developing UC when compared with non-smokers, the pooled odds ratio being 1.64. The contrary association is seen for Crohn's disease, with current smokers having a pooled odds ratio of 2.0 compared with non-smokers and 1.8 for ex-smokers *(8)*. Over 60% of ex-smoking colitics developed their disease after cessation of smoking, most within 4 years of quitting *(9)*. How this reciprocal association operates is unclear. Possibly both diseases arise in a subpopulation genetically predisposed to IBD. If smoking protects against UC then smokers would be more likely to develop CD. Cessation of smoking removes this protection, accounting for the higher prevalence of UC among ex-smokers.

A number of anecdotal reports have indicated improvements in UC symptoms on restarting smoking or using nicotine substitutes such as gum or transdermal patches. Some patients smoke intermittently for this reason *(10)*. The possibility exists that nicotine, the principal pharmacologically active alkaloid in tobacco smoke, may be an active ingredient in 'protection' and therefore a potential therapeutic agent. Experimentally, nicotine has the advantages of an acceptable delivery system—transdermal patches—and of being readily measurable in blood.

Srivastava *et al. (11)* used transdermal nicotine in an open placebo crossover pilot study. Sixteen patients with active refractory ulcerative colitis were treated with 30 mg nicotine daily for 4 weeks. They were then switched to placebo patches without their knowledge. In the active treatment phase, 12 of 16 patients improved symptomatically, 12 improved sigmoidoscopically and 10 improved histologically. In the placebo period deterioration was seen clinically in seven patients, sigmoidoscopically in five and histologically in six. This early indication suggested a formal trial be undertaken.

Smoking and colitis: a therapeutic trial

A randomised, double-blind and placebo-controlled study was designed to test the twin hypotheses that nicotine can act therapeutically in active UC and is the active part of tobacco smoke responsible for the epidemiological association *(12)*.

Methods

Patients who relapsed with UC despite maintenance mesalazine were randomised to receive transdermal nicotine or identical placebo patches for 6 weeks with regular assessment of clinical features and sigmoidoscopic and histological indices of disease activity. In all, 72 patients were randomised to receive up to 25 mg nicotine daily or placebo. This dose was introduced as gradual increments to minimise side-effects. Patients were given 5 mg daily for 2 days, 10 mg daily for 2 days and then 15 mg for a further 10 days. In those able to tolerate or with no clinical benefit, the dose was increased to 25 mg after 2 weeks.

Statistical analyses used were χ^2 and Mantel–Haenszel methods where appropriate. Changes in clinical scores, sigmoidoscopic and histological indices were compared by analysis of covariance with the corresponding baseline value as the covariate.

Results

Seventeen of 35 patients in the nicotine group and nine of 37 in the placebo group had full clinical remission ($\chi^2 = 4.58$; $P < 0.05$). Further, comparisons of change between nicotine and placebo groups by analysis of variance showed significantly greater improvements in overall Truelove and Witts *(12)* clinical score ($P = 0.001$), histological grade ($P < 0.05$), stool frequency ($P < 0.01$), abdominal pain ($P = 0.05$) and urgency to stool ($P < 0.01$). The proportion of patients reporting no mucus in stool at the end of the trial was 57% (20 patients) in the nicotine group and 22% (eight patients) in the placebo group ($P = 0.002$ Mantel–Haenszel method). Bleeding per rectum and sigmoidoscopic scores were not significantly different.

In the nicotine group, plasma levels of nicotine were 9.6±8.8 ng/ml and of cotinine, the chief metabolite of nicotine, were 149±86 ng/ml. This is 30% to 40% of levels seen in smokers of 20 cigarettes a day. Withdrawals because of ineffective treatment were more common in the placebo group, but not significantly (three in the nicotine group and eight with placebo; $P = 0.12$).

Side-effects were a problem as all subjects were non- or ex-smokers and therefore nicotine naïve. Significant side-effects of dizziness, headache, nausea, vomiting, disturbed sleep and vivid dreaming were reported in 23 of 35 taking nicotine and 11 of 37 on placebo ($\chi^2 = 9.34$; $P = 0.0022$). Two patients on nicotine and one on placebo withdrew because of side-effects alone. No withdrawal syndromes indicating dependency were seen at the conclusion of the study.

Discussion

This study showed a modest but measurable beneficial effect of nicotine on active UC. Most patients were able to tolerate nicotine-related side-effects, which occurred in spite of gradual dose increases. It may be suggested that the high incidence of these symptoms influenced the subjective assessment of colitis. However, many patients did not have side-effects and the clinicians responsible for the assessments remained unaware of group assignments. The extent of nicotine exposure was limited as there was a modest rise in the plasma nicotine and cotinine levels. Higher doses, while possibly more effective, would be intolerable as nicotine has a narrow therapeutic index. This study is consistent with the epidemiological and anecdotal evidence about UC and smoking.

A recent study examined the effect of chronic transdermal nicotine on maintenance of remission. Eighty patients with quiescent colitis were randomised to receive either nicotine or placebo over 6 months in addition to mesalazine. No benefit was seen for nicotine use over placebo. Relapse and withdrawal rates were similar in both groups *(13)*. Nicotine shares this property with corticosteroid use in UC, as both are effective in treating active UC but ineffective in maintaining remission. Whether nicotine becomes an established treatment for active UC awaits further investigation. It cannot maintain remission and has a tarnished image in view of its known addictive qualities. Nicotine or agents that have similar effects on colonic mucosa may emerge as useful therapeutic agents for colitis. Better understanding of its mode of action could allow development of other therapeutic approaches more acceptable than nicotine.

Mechanism of interaction of smoking, nicotine and colitis

Tobacco smoke has profound effects on lung mucosae, mucus and pathophysiology, with bronchial mucus hypersecretion and mucus gland hyperplasia characteristic features of smoking-related diseases. These effects were thought to be solely local irritant actions, but there is also a pharmacological effect. Both smoke extracts and nicotine stimulate mucus production in isolated cat tracheal preparations; an action abolished by autonomic ganglion blockade *(14)*. Chronic tobacco exposure increases the secretion and alters the composition of human tracheobronchial mucus, increasing the ratio of sulphated to sialyated mucins *(15)*. There is evidence that local mucosal eicosanoids may be physiological mucus secretagogues *(2)*. In the stomach, topically applied prostaglandin dm-PGE2 increases the thickness of the adherent mucus gel layer *(4)*. The effects of smoking and nicotine on the colon are less well understood, but may be relevant to inflammation in colitis. Smoking is known to influence cellular and humoral immunity. Eicosanoids are reduced in rectal biopsies from smokers *(16)*. Rectal blood flow measured with a laser Doppler device was higher in quiescent colitics than controls, with smoking reducing blood flow, possibly via vasoconstriction *(17)*.

Rectal biopsies from non-smoking patients with UC were found to have significantly lower rates of colonic mucus glycoprotein synthesis than controls when cultured as explants. Smoking colitics had 'normal' rates similar to control subjects, suggesting smoking boosts a deficient mucus synthetic process *(18)*. In a rabbit model, Zijlstra *et al. (19)* infused subcutaneous nicotine by pump for 14 days using doses of 0, 0.5, 1 and 2 mg/kg/day. The thickness of adherent mucus in the rabbit rectum was significantly reduced by a low dose of nicotine but enhanced by a high dose. Mucin synthesis rates, measured by incorporation of tritiated glucosamine into mucins, was unchanged. Several mucosal eicosanoids were assayed and there were significant reductions in levels of 6-keto-PGF1α, PGF2α and hydroxy-5,8,10-heptadecatirenoic acid (HHT) at low but not at higher doses. This model shows some influences upon mucosal mucus and eicosanoids by nicotine, but the unusual inverse dose–response relationship leaves more questions than it answers. Incubation of normal colonic tissue in explant culture with different concentrations of nicotine showed that nicotine altered synthetic rate. Finnie *et al. (20)* used nicotine at levels seen in smokers. Mucus glycoprotein synthesis increased by up to 200% of controls when nicotine was present, but without a clear

dose–response relationship. Nicotine usually acts at autonomic ganglia. Since these are normally absent from colonic mucosal explants, this would indicate a direct action on colonocytes.

Whether any of these postulated mechanisms are relevant to the effect of smoking on UC is conjectural. Understanding them would provide considerable insight into the pathophysiology of ulcerative colitis.

Conclusions

Ulcerative colitis is a disease of non-smokers and ex-smokers. This study has shown that nicotine has a therapeutically beneficial effect in active disease. Our knowledge of colonic mucus has been augmented by observations on the adherent surface mucus layer in humans and its relative deficiency in UC. Given that both smoking and nicotine have effects on mucosae and mucus these two lines of evidence about UC may provide a fertile field for future studies.

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