Stimulation of colonic mucin synthesis by corticosteroids and nicotine

Ian A. FINNIE, Barry J. CAMPBELL, Barry A. TAYLOR*, Jeremy D. MILTON, Sherif K. SADEK, Lu-Gang YU and Jonathan M. RHODES
Departments of Medicine and *Surgery, University of Liverpool, Liverpool, U.K.

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INTRODUCTION

Mucus depletion is a typical histological feature of ulcerative colitis (UC), and it has been suggested that defects in mucus synthesis or structure could have a causal role in pathogenesis [1]. Although many of the described alterations in mucus [2–6] may be secondary to the disease, they could still contribute to reduced mucosal protection. Stimulation of mucus synthesis could therefore be beneficial therapeutically.

In previous studies we have established that sodium butyrate stimulates colonic mucin synthesis, at concentrations that are therapeutically and physiologically relevant [7]. There have been surprisingly few other studies of the effects of drugs on mucus synthesis although the striking association between non-smoking and UC [8] has led to speculation that nicotine could have a therapeutic effect mediated by increased mucin synthesis [9]. Studies to address this (reviewed below) have been inconclusive and have not previously assessed the effect of nicotine on mucin synthesis in vitro by human colonic explants.

Corticosteroids have a wide range of anti-inflammatory and immunomodulatory properties but it is not clear which of these primarily account for their therapeutic effect in colitis [10]. Carbenoxolone, the liquorice derivative which is itself a corticosteroid, is known to stimulate mucin synthesis in the gastric epithelium [11], and further studies have therefore been performed to assess the effects of corticosteroids including carbenoxolone, and nicotine on colonic mucin synthesis.

MATERIALS AND METHODS

Tissue source

Mucosal biopsies were dissected by a combination of scalpel and blunt dissection from operative resection specimens as soon as possible after their removal. Patients were undergoing elective resection for colonic malignancy (n = 12), UC that was unresponsive to medical treatment (n = 2), or colonic inertia (n = 2). Some of these patients have previously been included in a study into the effect of butyrate on colonic mucin synthesis [7]. In the patients with malignancy, studies were performed on mucosa obtained from the macroscopically normal
areas of colon, at least 10 cm distant to any lesion. In the patients with UC, the relatively less involved right colon was studied in each case. In the experiments assessing the effects of nicotine, only non-smoking patients were studied. The studies were conducted with the approval of the Royal Liverpool University Hospital ethical committee.

**Biopsy culture**

The culture method used was that of MCDermott et al. [12]. Briefly, each biopsy weighing approximately 20 mg was placed, mucosal surface uppermost, on alloy wire mesh floating in 1 ml of culture medium in a 60 x 15 mm organ culture dish (Becton Dickinson, NJ, U.S.A.). The culture medium was 90% RPMI 1640 with 300 mg/l glutamine (Gibco, Paisley, U.K.) and 10% foetal calf serum (Gibco), to which were added 100 µg/ml gentamicin, 60 units/ml nystatin, 1.5 µCi of N-[3H]acettylglicosamine (specific activity 10 Ci/mmol) (Amersham, Little Chalfont, U.K.), and 50 µl of various concentrations of the test drugs dissolved in PBS. Control biopsies had 50 µl of PBS added instead of drug solution. Culture was for 16 h in 95% O2/5% CO2 at 37°C. A previous study has shown a linear rate of incorporation of N-[3H]acettylglicosamine over 16 h with this system [7].

**Mucin extraction and purification**

Mucin was extracted and purified using methods outlined previously [7]. Biopsies were sonicated in PBS (over ice) for 2 min or until the biopsy had been disrupted sufficiently to leave a white ‘ghost’, centrifuged for 15 min at 15,000 g, and the supernatant lyophilized after removal of an aliquot for protein estimation. The culture medium was lyophilized separately. The lyophilate was resuspended in 200 µl of Tris–HCl (pH 8) and subjected to gel filtration using PD10 mini-columns packed with approximately 10 ml of Sepharose CL-2B (Pharmacia, Uppsala, Sweden). The mucin-containing fractions (void volume) were mixed with 5 ml of scintillant (Optiphase Safe, Pharmacia) and counted using an LKB β-counter. Previous studies have shown no significant contamination of the mucin by radiolabelled non-mucin glycoproteins or proteoglycans using this technique [7].

**Expression of results**

Protein content of each biopsy homogenate was assessed using the bichoninic acid method as supplied in kit form by Sigma Chemicals, St Louis, MO, U.S.A. Total mucin synthesis was expressed as d.p.m. N-[3H]acettylglicosamine incorporated in 16 h into purified mucin (biopsy plus culture medium) per µg of biopsy protein. The results for each patient were expressed as percentage change in mucin synthesis by drug-treated biopsies compared with control biopsies from the same patient. Five biopsies were studied at each concentration and site studied. Results from the left colon (descending or sigmoid colon or rectum) are generally presented separately from those obtained in the right colon (caecum or ascending colon).

**Drugs studied**

The effects of the following agents on colonic mucin synthesis were studied: hydrocortisone, prednisolone, carbemoxolone, fludrocortisone, aldosterone and nicotine (all obtained from Sigma).

**Viability of colon in culture**

Colonic biopsies were cultured in identical conditions to those described above, but in the absence of N-[3H]acettylglicosamine, and stained with Haematoxylin and Eosin or Periodic Acid–Schiff and Alcian Blue (pH 2.5). In addition, an experiment was undertaken to check for any toxic effects of nicotine on HT29 human colorectal cancer cells (European Cell Culture Collection; Public Health Laboratory Service, Porton Down, U.K.) in culture. The cells were precultured in 24-well plates in Dulbecco’s modified Eagle’s medium with 2% foetal calf serum at 37°C in 95% air/5% CO2 for 48 h. Varying concentrations of nicotine (final concentrations 0–6.25 µmol/l) dissolved in PBS were added and further culture was undertaken for 16 h. After this, cells were stained with Trypan Blue to assess viability.

**Statistical analysis**

For comparison of the varying concentrations of drugs on mucin synthesis, results were analysed using non-parametric analysis of variance (Kruskal–Wallis) followed by multiple paired analysis. Comparison between the effects of different drugs and their inhibitors was also by non-parametric analysis (Mann–Whitney U-test). Comparison of the effects of different drugs on biopsies from different sites in individual patients was by paired Student’s t-test. The statistical analysis was performed using Arcus Pro II software (Medical Computing, Aughton, Lancs, U.K.).

**RESULTS**

The rate of mucin synthesis [mean (SD)] in histologically normal control biopsies from the right colon [29.3 (12.4) d.p.m. h−1 µg−1 protein] did not differ significantly from that in the left colon [23.4 (10.2) d.p.m. h−1 µg−1 protein] (P = 0.18, Mann–Whitney U-test).

A summary of the effects on mucin synthesis of the various drugs tested is given in Table 1. Prednisolone caused a marked, dose-dependent increase in mucin synthesis by histologically normal
Table 1. Effect of drugs on mucin incorporation of precursor. The number of patients studied is given in parentheses.

<table>
<thead>
<tr>
<th>Diagnosis...</th>
<th>Colorectal cancer</th>
<th>Colonic inertia</th>
<th>UC</th>
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<tbody>
<tr>
<td></td>
<td>Left colon</td>
<td>Right colon</td>
<td>Left colon</td>
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<tr>
<td>Hydrocortisone</td>
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<td>0 (2)</td>
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<tr>
<td>Prednisolone</td>
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<tr>
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<td>0 (2)</td>
<td></td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Carbenoxolone</td>
<td>1 (3)</td>
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![Graphs](image)

**Fig. 1.** Effect of prednisolone on mucin synthesis by histologically normal biopsies from the left and right colon. Mucin synthesis was assessed by incorporation of N-[3H]acetylglucosamine into mucin by colonic biopsies in vitro. Total (biopsy homogenate and culture medium) mucin synthesis (mean ± SD) is expressed as a percentage compared with synthesis in the absence of drug. *P* values relate to comparisons with control. Number of biopsies (patients) studied from left colon for controls = 35 (7), 0.015 μmol/l = 30 (6), 0.15 μmol/l = 30 (6), 1.5 μmol/l = 35 (7), 15 μmol/l = 35 (7). Number of biopsies (patients) studied from right colon for controls = 20 (4), 0.015 μmol/l = 15 (3), 0.15 μmol/l = 15 (3), 1.5 μmol/l = 20 (4), 15 μmol/l = 20 (4).

biopsies from the left colon (Fig. 1). The increase was not of equal proportions in all patients; mean increase in mucin synthesis ranged between 1.5-fold and 6-fold with a mean 3-fold increase at a prednisolone concentration of 1.5 μmol/l. It was not possible to identify specific factors that would explain the greater response in certain patients; there was no obvious correlation with age, sex, blood pressure or cigarette smoking. In contrast, only a small, non-significant increase in mucin synthesis was found in the right colon at prednisolone concentrations of 0.15–15 μmol/l (Fig. 1). In biopsies from the two patients with colonic inertia who underwent total colectomy, 1.5 μmol/l prednisolone caused a 362±55% increase (*P* < 0.005) and 15 μmol/l prednisolone caused a 412±65% increase (*P* < 0.005) in mucin synthesis in the left colon, but again had no significant effect on right colonic samples (*n* = 10 biopsies from two patients at each site and concentration).

Hydrocortisone caused a dose-dependent increase in mucin synthesis in the histologically normal left colon with a mean increase of six times control values at the highest concentration tested (6 μmol/l) (Fig. 2). Again, there was very little response in the right colon, and direct comparisons of the right and left colon from the patients undergoing total colectomy confirmed this. Hydrocortisone, 0.6 μmol/l, caused a 264±44% increase in left colon mucin synthesis (*P* < 0.01) and hydrocortisone, 6 μmol/l, caused a 324±44% increase in left colon mucin synthesis (*P* < 0.01) compared with control values; in the right colon, no significant effect was found at either concentration (*n* = 10 biopsies at each site and concentration).

In patients tested with both agents, the increase in mucin synthesis in the normal left colon was significantly greater with hydrocortisone, 6 μmol/l, than with prednisolone, 1.5 μmol/l (*P* < 0.05, Student’s *t*-test).

Fludrocortisone had no effect on mucin synthesis (Fig. 3); biopsies from the same patients cultured with hydrocortisone, 6 μmol/l, increased mucin synthesis by 342±40% of control values. In a further single experiment, five histologically normal biopsies from the recto-sigmoid junction cultured in the presence of aldosterone (1, 10 and 100 μmol/l) had similar rates of mucin synthesis to controls (89±39, 94±32 and 121±28% of control values respectively); biopsies from the same patient cultured in hydrocortisone, 6 μmol/l, had a rate of synthesis 278±63% of control values.

Carbenoxolone, 0.17 mmol/l, increased mucin synthesis significantly in the left colon, but fludrocorti-
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Fig. 2. Effect of hydrocortisone on mucin synthesis in the histologically normal left and right colon. Number of biopsies (and patients) studied from left colon = 35 (7) at all concentrations; number of biopsies (patients) studied from right colon = 20 (4) at each concentration.

Fig. 3. Effects of carbenoxolone, 0.17 mmol/l, fludrocortisone, 2 and 20 μmol/l, on mucin synthesis in the left colon. Number of biopsies (patients) in all groups = 15 (3), except control and fludrocortisone, 2 and 20 μmol/l, where number of biopsies (patients) = 20 (4).

Fig. 4. Effect of nicotine on colonic mucin synthesis (right and left colonic biopsies combined). Although a direct comparison in the same colon was not possible, the increases in the left colon did not differ significantly from those in the right colon. Number of biopsies (and patients) studied were 35 (7) at all concentrations, except at 31.25 nmol/l and 62.5 nmol/l where number of biopsies (patients) = 20 (4).

Viability and reproducibility

Biopsies, whether cultured in the presence of drugs or as controls, were histologically normal after culture for 16 h.

HT29 cells remained >98% viable at all concentrations of nicotine tested.

The mean coefficient of variation for mucin synthesis (expressed as d.p.m./μg of protein) for different biopsies from the same site, same patient and same drug concentration was 18%. Mean coefficient of variation between patients at the same site was 36%, for controls, and 46% at maximal concentrations of hydrocortisone and prednisolone.

Mean incorporation of radiolabel into mucin...
The increase in mucin synthesis during 16 h culture was 4.6% of that present at the start of culture.

**DISCUSSION**

This study has demonstrated that mucin synthesis by colonic biopsies in vitro is markedly increased by prednisolone and hydrocortisone, and to a lesser extent by nicotine, at concentrations of the drugs that are therapeutically relevant. It is striking that corticosteroids have a much greater effect on the left than the right colon, although the number of patients studied at both sites is relatively small, and it is interesting that there was increased mucin synthesis in response to corticosteroids in the proximal colon biopsies from two patients with UC. There were no significant alterations in incorporation of the radioa$\beta$el when biopsies were cultured in the presence of aldosterone or fludrocortisone, implying that the effect is not mediated via the mineralocorticosteroid actions of the steroids.

The increase in mucin synthesis in the presence of glucocorticosteroids was sufficiently marked to suggest that this may be a potential mode of action. The maximal effect of prednisolone was seen at a concentration of 1.5 $\mu$mol/l, which is similar to the peak concentration in the serum of patients taking 40 mg of prednisolone per day [13]. It is interesting that a further 10-fold increase in drug concentration had no incremental effect on mucin synthesis, in keeping with the lack of additional effect of doses of prednisolone higher than 40 mg/day in UC [14].

Hydrocortisone is approximately four times weaker than prednisolone in anti-inflammatory properties on a milligram for milligram basis [15], and given that the molecular masses are similar, 1.5 $\mu$mol/l prednisolone was taken as the equivalent of 6 $\mu$mol/l hydrocortisone. At these concentrations, hydrocortisone had a greater effect on mucin synthesis, and direct comparisons between biopsies from the same patients studied with both drugs confirmed this. It is unclear why hydrocortisone should stimulate mucin synthesis more than prednisolone, but it is interesting in view of anecdotal reports that some patients with UC do seem to respond better to hydrocortisone or adrenocorticotropic hormone [16] than prednisolone. In UC the response to corticosteroids depends largely on factors that have so far not been characterized, and there is, in general, a poor correlation between serum levels of glucocorticoids and clinical response in UC [16–18]. Neither fludrocortisone, at concentrations greater than those found in patients receiving replacement therapy [19], nor aldosterone, at concentrations that affect short-circuit current in the rabbit colon [20], had any effect on colonic mucin synthesis.

The fact that corticosteroids had a greater effect on the left colon may be a further reflection of the functional heterogeneity between proximal and distal colon, as illustrated by the differences in salt and water transport [21, 22] and substrate metabolism [23, 24] in the two areas.

Smoking seems to protect against the development of UC. It has been speculated that some of the protective effect of smoking may be related to nicotine, and nicotine patches have been used, with some benefit, to treat UC [25, 26]. Previous work has suggested that mucosal biopsies from smokers with UC have higher rates of mucin synthesis when cultured in vitro than biopsies from non-smokers [27], although studies on mucosal biopsies in vitro from smoking and non-smoking control subjects have failed to show any such effect [28]. Furthermore, nicotine infusions in rabbits have an unusual effect on mucus thickness in the colon; causing a reduction in the thickness at very low concentrations, and an increase at higher concentrations [29]. Biopsies taken from the rabbits in the latter study showed no significant difference in rates of mucin synthesis when cultured in vitro (in the absence of nicotine), but no previous studies have examined the effects of nicotine in vitro on mucin synthesis. The increased colonic mucin synthesis in vitro demonstrated in the present study in response to nicotine gives some support to the hypothesis that nicotine protects against colitis by enhancing mucus synthesis, but the magnitude of the effect does not seem to be very great, and there was no effect in the right colonic biopsies from the patients with UC. The increase in mucin synthesis reached a plateau above 62.5 nmol/l; concentrations that are typically found in smokers are in the range of 90–350 nmol/l [30]. The increase in mucin synthesis was maintained in vitro at very high concentrations of nicotine, even at concentrations which, if present in the serum of laboratory animals, would result in lethal convulsions [31]. We did not find any evidence of toxicity on histological examination of biopsies cultured in the presence of nicotine, or in
the HT29 cells cultured in nicotine at concentrations up to 6.25 µmol/l.

These results suggest that the therapeutic effects of corticosteroids and possibly nicotine in UC could in part be explained by increased colonic mucin synthesis.

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REFERENCES