Rectal mucosal prostaglandin E₂ release and its relation to disease activity, electrical potential difference, and treatment in ulcerative colitis

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SUMMARY In vivo rectal dialysis was used to study rectal mucosal release of immunoreactive prostaglandin E₂-like material and its relation to disease activity, rectal electrical potential difference (PD), and treatment in 24 patients with ulcerative colitis. In untreated colitics in remission and in relapse, median values for apparent mucosal prostaglandin E₂ (PGE₂) release were increased three-fold (p < 0.05) and 13-fold (p < 0.002) respectively over that found in control subjects. In patients in remission during treatment with sulphasalazine and/or corticosteroids, median apparent PGE₂ release was similar to that of controls, but in colitics in relapse, despite treatment, it was greatly increased (p < 0.002). Ulcerative colitis in relapse was associated with a significant reduction in rectal PD (p < 0.002); in patients with quiescent ulcerative colitis, a smaller reduction was found (p < 0.05). In nine patients studied serially before and during treatment, there were associations between changes in disease activity assessed sigmoidoscopically, in PD and in apparent mucosal PGE₂ release. Furthermore, rectal mucosal PGE₂ release and PD were linearly correlated (p < 0.01). These findings indicate that mucosal PGE₂ release is markedly enhanced in active ulcerative colitis, and they confirm the value of rectal PD as a guide to disease activity. In addition, they suggest that rectal dialysis may be a useful way of studying rectal prostaglandin metabolism in man.

The enhancement of prostaglandin (PG) synthesis found in rectal mucosal biopsies taken from patients with active untreated ulcerative colitis¹ ³ is of uncertain pathogenetic significance. Increased amounts of PG-like material have also been found in the faeces of patients with ulcerative colitis,⁴ presumably as a consequence of release into the lumen of PGs formed within the colorectal mucosa. Unfortunately, mucosal PG production may be stimulated both by rectal biopsy itself and by subsequent processing of tissue obtained.⁵ In addition, the traumatic nature of the procedure precludes its too frequent repetition in individual patients.

We have therefore applied the technique of in vivo rectal dialysis⁶ to investigate luminal accumulation of PGE₂-like material, measured by radioimmunoassay of the dialysate. PGE₂ was selected for study because it appears to be the predominant stable PG in normal human colon.⁶ We describe here our observations of the inter-relationships between apparent rectal mucosal PGE₂ release, disease activity, and treatment in patients with ulcerative colitis. Disease activity was appraised sigmoidoscopically⁷ and by measurement of rectal transmucosal electrical potential difference (PD) which previous reports indicate is a useful means of assessing mucosal functional integrity in ulcerative colitis.⁶ ¹⁰ Part of this work has been presented elsewhere in preliminary form.¹¹

Methods

PATIENTS

We studied 24 patients in whom ulcerative colitis had been diagnosed by conventional clinical, radiological, and histological criteria. Activity of disease in the rectum was scored according to the sigmoidoscopic criteria of Baron et al.⁷; patients with grades 0 (normal appearance) and 1 (loss of the normal vascular pattern) were judged to be in remission, and those with grades 2 (contact bleeding)
and 3 (spontaneous bleeding) to be in relapse. Fifteen patients, of whom nine were in remission, were studied while on no treatment; nine had never had therapy for colitis, and the remaining six were known colitics who had been off all treatment for at least four weeks before the study. Sixteen patients were studied during treatment of at least two weeks' duration; seven of these had been studied once previously while untreated. Of the nine patients assessed while in treated remission, eight were taking oral SASP (3–4 g daily) and one was using prednisolone retention enema (20 mg daily). Of the seven patients in active relapse despite treatment, five were taking prednisolone orally (5–40 mg daily) and/or topically (40 mg daily); in two this was supplemented with SASP 4 g daily and in one with azathioprine 100 mg daily. The two remaining patients in this group were taking SASP (4 g) alone.

Eighteen patients with the irritable bowel syndrome served as controls. These had various bowel symptoms for which thorough investigation had revealed no organic cause, and for at least two weeks before the study had been on no treatment.

All patients gave informed consent to the study, approval for which was obtained from the Guy’s Hospital Ethical Committee.

RECTAL DIALYSIS

After a 10 hour fast, a gentle sigmoidoscopic examination to 12–15 cm was performed in order to assess disease activity. After measurement of rectal mucosal potential difference, rectal dialysis was initiated by insertion into the rectum of a PVC cannula on which was mounted a knotted Visking 8/32 dialysis tube (diameter 6 mm, length 8 cm) containing about 2-5 ml dialysate (Na 120 mmol/l, K 30, Cl 120, HCO₃₈ pH 8.1); the tip of the dialysis bag was 12 cm from the anus. After one hour, the dialysis bag was removed, and the dialysate volume measured by weighing. Dialysates discoloured by contact of the bag with faeces were discarded. The rate of flux of PGE₂ (pg/cm²/h) into the dialysis was calculated from the product of the PGE₂ concentration and the volume of the dialysate at the end of the dialysis period, divided by the product of the surface area of the bag and the duration of dialysis.

PGE₂ ASSAY

PGE₂-like material in duplicate aliquots of the dialysate was measured by radioimmunoassay, using an antiserum with a cross-reactivity of 3-2% with PGE₁ and of <0.5% with other tested prostaglandin derivatives. The lower limit of detection was 2 pg/tube, the coefficient of variation (CV) of nine replicate estimations was 2.9%, and recovery of PGE₂ added to dialysate 93.4±5.4% (mean±SD) (n=6). Assay of serial dilutions of the dialysate gave a curve parallel to that of the standards.

VALIDITY OF THE METHOD

The use of rectal dialysis as a measure of rectal mucosal solute transport rests on assumptions most of which apply as well to PGE₂ as to sodium, for which it has been tested and validated by Edmonds. One of the requirements of the method is that the dialysis membrane is very permeable to the solute under consideration. Experiments in which dialysis bags containing PGE₂-free dialysate were placed in a large stirred volume of the same fluid containing PGE₂ showed that, at 37°C, the PGE₂ concentration within the bag after one hour was 0.51±0.03 (seven) (mean±SD) of that outside. Similar experiments using sodium as the test solute gave a concentration ratio of 0.96±0.01 (four) after one hour.

Because the dialysis membrane is not freely permeable to PGE₂, PGE₂ flux rate into the dialysis bag in vivo should be regarded as a reflection of both the PGE₂ content of the intermediate fluid layer and rectal mucosal PGE₂ release (to which the former itself is related), rather than as an absolute measure of the rate of rectal mucosal release of PGE₂. For convenience, we use the term 'apparent mucosal release of PGE₂' to indicate the original source of the PGE₂-like material in the dialysis bag after one hour's dialysis.

To test the reproducibility of the method in vivo, apparent rectal mucosal PGE₂ release was measured in two consecutive one-hour dialysis periods in 10 control subjects; the CV of these duplicate estimates, of which the mean was 30 pg/cm²/h, was 37%. Similar duplicate estimates of PGE₂ release in three patients with active ulcerative colitis gave a mean of 742 pg/cm²/h and a CV of 9%.

RECTAL POTENTIAL DIFFERENCE

Potential difference was measured immediately before and after rectal dialysis using electrodes incorporating silver-silver chloride junctions in a solution of NaCl 150 mmol/l in agar 4%, and connected to a high impedance millivoltmeter (Levell). The reference electrode was placed on the thigh in contact with an area of skin the PD of which had been abolished by an intradermal injection of saline.

The probe electrode made contact with the rectal mucosa, at 6–9 cm from the anus, through a dialysis bag (length 3 cm) containing dialysate (composition as for rectal dialysis). Preliminary experiments in control subjects showed that the PD obtained in this way was more reproducible in individual subjects.
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(CV of duplicate measurements 7% (n=7)) than when the probe electrode was applied to the mucosa under direct vision through a sigmoidoscope (CV 13% (n=7)). The former value (−52 (−45 −60) mV) was also higher than the latter (−38 (−32 −51) mV) (p<0.05).

For each patient the quoted value of PD is the mean of the figures obtained immediately before and after rectal dialysis; the CV of these duplicate measurements was 5% (n=18) in the control subjects and 6% (n=30) in patients with ulcerative colitis. The polarity of the potential difference refers to the luminal side of the mucosa. In each experiment, PD was corrected for asymmetry potential, which never exceeded 2 mV. No correction was made for the junction potential between NaCl 150 mmol/l and the dialysate; this was small (1 mV) and similar in every subject.

Statistical Methods

Results are shown as median (range) (n) unless otherwise stated. Paired and unpaired data were compared using Wilcoxon's signed ranks and sum of ranks tests (two-tailed), respectively. Regressions were calculated by the method of least squares, and correlations determined by Spearman's rank correlation test.

Results

Mucosal PGE₂ Release

In patients with untreated ulcerative colitis in remission, median apparent mucosal PGE₂ release was nearly three times as high as in controls (p<0.05), and in relapse it was increased about 13 times over control values (p<0.002) (Table).

Apparent rectal mucosal release of PGE₂ in treated colitics with quiescent disease was similar to that of the control group, and not significantly lower than that of the untreated patients with ulcerative colitis in remission (Table). In patients in relapse despite treatment, apparent PGE₂ release was significantly greater than in controls (p<0.002) and than in patients in remission (p<0.002), but was not significantly different from that in patients with untreated relapse (Table).

Sixteen paired observations were made on nine of the above patients, the second and any additional tests being performed either after initiation of treatment or after modification of the treatment they were taking at the time of their first rectal dialysis (Fig. 1). In seven instances, improvement in sigmoidoscopic appearances was associated with a reduction in apparent mucosal release of PGE₂ (p<0.05), while in the two in which the sigmoidoscopic appearance deteriorated despite treatment, an increase in apparent PGE₂ release was found; on the other hand, when disease activity was unchanged at the time of the second study, no consistent change in apparent PGE₂ release occurred.

Table Apparent rectal mucosal release of PGE₂ and rectal potential difference in control subjects, in untreated and treated colitis in remission, and in untreated and treated colitis in relapse

<table>
<thead>
<tr>
<th>Patients</th>
<th>PGE₂ release (pg/cm²/h)*</th>
<th>PD (mV)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>34 (5−86) (13)</td>
<td>−50 (−45−65) (18)</td>
</tr>
<tr>
<td>UC in remission</td>
<td>91 (29−152) (9)†</td>
<td>−40 (−35−60) (8)†</td>
</tr>
<tr>
<td>Treated</td>
<td>32 (14−132) (9)</td>
<td>−48 (−37−60) (9)†</td>
</tr>
<tr>
<td>UC in relapse</td>
<td>454 (258−870) (6)§</td>
<td>−36 (−23−40) (6)§</td>
</tr>
<tr>
<td>Treated</td>
<td>802 (346−1500) (7)§‡</td>
<td>−27 (−7−48) (7)‡</td>
</tr>
</tbody>
</table>

*Results are shown as median (range) (n)
†p<0.05 from control.
‡p<0.05 from UC in remission.
§p<0.002 from UC in remission.

Fig. 1 Apparent rectal mucosal PGE₂ release (above) and PD (below) related to the change in sigmoidoscopic score found at the second of each of 16 pairs of observations made in nine patients. (A fall in sigmoidoscopic score indicates a reduction in disease activity.)

Rectal PD

Transmucosal potential difference in 18 control subjects was −50 (−45 −65) mV (Table). In patients
with active ulcerative colitis, whether treated or untreated, rectal PD was reduced (p<0.002 from control); in both groups of colitics in remission, the change in PD was similar in direction but smaller in magnitude (p<0.05 from control) (Table).

In the nine patients studied more than once (Fig. 1), improvement in sigmoidoscopic appearance was always associated with an increase in rectal PD (p<0.05) and clinical deterioration with a reduction in PD. In the seven instances in which disease activity was unaltered at the second study, no significant change in potential difference was found.

**Relation between PGE$_2$ release and PD**

Rectal potential difference showed a significant linear correlation with apparent mucosal PGE$_2$ release in the untreated colitics (Fig. 2); a similar relationship was found in the treated group (y = 0.021x - 45.1, Spearman’s r = 0.69, p < 0.01).

In the patients studied on more than one occasion (Fig. 1), there were 13 instances in which a fall in PGE$_2$ release was found at the second of each pair of observations; in 11 of these PD rose and two it was unchanged (p<0.002). In the three cases in which PGE$_2$ release was increased at the second study, a reduction in PD was found.

**Serial studies of individual patient**

Figure 3 shows how serial assessments of mucosal PGE$_2$ release, rectal PD, and sigmoidoscopic appearance can be made in individual patients with ulcerative colitis. This 32 year old man was studied five times over a four-week period during which he was treated first with oral SASP (4 g daily) and then prednisolone enemata (40 g daily) additionally. Before he started treatment, apparent mucosal PGE$_2$ release was high, and this was associated with a slightly reduced and PD spontaneous bleeding on sigmoidoscopy. Although after five days of treatment, PGE$_2$ release PD and showed some improvement, on the third occasion that he was tested, PGE$_2$ release had again increased, with a parallel fall in rectal PD. Thereafter, PGE$_2$ release, potential difference, and sigmoidoscopic appearance all returned towards normal.

**Discussion**

Release of PGE$_2$ from the rectal mucosa is probably determined mainly by mucosal PGE$_2$ synthesis, which is enhanced in ulcerative colitis, by mucosal PGE$_2$ content, which is increased two-fold in ulcerative colitis, and by epithelial permeability, which is also abnormal in ulcerative colitis.

We have used in vivo rectal dialysis to obtain an estimate of mucosal release of PGE$_2$ to which we have applied the term ‘apparent rectal mucosal release of PGE$_2$. If mucosal PGE synthesis is indeed a major determinant of its release into the rectal lumen, support for the assumption that flux of PGE$_2$ into the dialysate reflects its mucosal release can be obtained from the results of Harris et al., who measured PGE synthetase activity in rectal biopsy.
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specimens using a radiometric technique. They found that mucosal PG synthetase activity in untreated patients with active ulcerative colitis was about 11 times that of patients with the irritable bowel syndrome, in whom it was similar to synthetase activity in treated colitics with quiescent disease. The differences in synthetase activity between these three groups of subjects are very similar to the differences in apparent mucosal PGE₂ release that we measured in three comparable groups (Table).

The uncertainty about the precise relationship between PGE₂ flux into the dialysis bag and its rate of release from the mucosa is, we believe, outweighed by the advantages conferred by the technique on the study of conditions in which rectal prostaglandin metabolism is abnormal. Firstly, rectal dialysis is safe and can, therefore, be performed repeatedly, without risk of haemorrhage or perforation, in individual patients before and during treatment. Secondly, it allows simultaneous assessment of mucosal water and electrolyte transport as well as of PGE₂ release.¹¹

In our patients with ulcerative colitis, transmucosal rectal PD was reduced in relation to activity of disease assessed sigmoidoscopically. Our results in each group of subjects are qualitatively similar to but somewhat higher than those of earlier workers⁸ ¹⁰ who applied probe electrodes to the rectal mucosa under direct vision through a sigmoidoscope. Our preliminary studies (see Methods section) indicate that this discrepancy is due to our use of a dialysis bag system to establish contact with the mucosa. Mohamed et al.¹₈ found a similar discrepancy between the PD measured through an intrarectal pool of saline and that obtained by direct application of an electrode to the mucosa itself; they suggested that the difference in PD recorded might result from electrical contact being made in the former instance over a wide area of mucosa rather than, as in the latter case, at a single point at which the mucosa could be damaged by the electrode.

Our results confirm the value of rectal potential difference as a simple objective measure of disease activity in ulcerative colitis. The reduction in PD found in patients with ulcerative colitis is probably a consequence of impaired sodium pump activity and of increased epithelial permeability.⁸⁻¹⁰ The correlation we observed between mucosal PD and PGE₂ release does not of course, suggest a causal relationship between them, and indeed these variables could well be related through some third factor, such as inflammatory tissue damage. Whether it indicates an influence of prostaglandins upon colorectal sodium transport or permeability is not yet clear, but, in this context, mention should be made of the recent direct demonstration that PGs are capable of interfering with sodium absorption in the colon,¹⁷ ¹₈ as well as in the small intestine.¹⁷

It has been suggested that PGs may have a cytoprotective effect in the colon, similar to that described in the stomach and small intestine,¹⁹ and, furthermore, that SASP may reduce the frequency of relapses in patients with ulcerative colitis by reducing degradation of PGs, thereby preventing any relative mucosal PG deficiency.²⁰ Our results seem to conflict with these proposals. We found that rectal mucosal release of PGE₂ was directly related to disease activity in both treated and untreated patients (Table, Fig. 1), and that in no case was active disease associated with low levels of PGE₂ release. Furthermore, PGE₂ release in colitics with quiescent disease during treatment with SASP was similar to, and not higher than, that of untreated patients in remission (Table).

Although Hoults and Moore reported that SASP, unlike its metabolite 5-aminosalicylic acid, inhibited degradation of PGF₂α,²⁰ other studies have shown that both agents can reduce production of PGs, probably by inhibition of the microsomal PG synthetase system.¹³ ²¹⁻²³ Corticosteroids also decrease PG production, apparently by limiting availability of substrate for PG synthesis through interference with phospholipase A₂ activation.²₅ Our findings are consistent with the hypothesis that increased mucosal prostaglandin synthesis plays a pathogenetic role in active ulcerative colitis and that successful treatment of acute attacks with steroids and/or SASP is related to their inhibition of PG production. However, it is also possible that increased PG synthesis in active disease is a secondary phenomenon, and that the beneficial effect of steroids and SASP in vivo is independent of their inhibitory effect on PG production in vitro. Studies with stronger inhibitors of prostaglandin synthesis may shed additional light on this problem and are in progress.

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References


