Effect of prednisolone on prostaglandin synthesis by rectal mucosa in ulcerative colitis: investigation by laminar flow bioassay and radioimmunoassay

C J HAWKEY AND S C TRUELOVE

From the Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford

SUMMARY The effect of two concentrations of prednisolone on synthesis of prostaglandin E2 (PGE2) by 40 rectal biopsies in organ culture was investigated using both laminar flow bioassay and radioimmunoassay (RIA). Prednisolone (concentration $8.33 \times 10^{-7}$M) reduced mean synthesis of PGE2 to 36.4% of control values (measured by bioassay) or 26.2% of control values (measured by RIA). With prednisolone (concentration $5.66 \times 10^{-4}$M) synthesis of PGE2 was 7.7% of control values (RIA). The two concentrations are similar respectively to those achieved in plasma after oral prednisolone and delivered topically by prednisolone enema. Inhibition of PG synthesis may thus explain prednisolone’s anti-inflammatory action in the treatment of ulcerative colitis.

Accumulating evidence points to a role for PGs in the mediation of inflammation in ulcerative colitis. Both sulphasalazine and its active moiety 5-aminosalicylic acid (5-ASA) can inhibit PG synthesis by rectal mucosa in vitro. By contrast, corticosteroids have no effect on PG synthesis by homogenates of rectal mucosa or other tissues and only inhibit PG synthesis in intact cells. We decided therefore to examine the effect of prednisolone on basal PGE2 synthesis by whole rectal biopsies in organ culture. This method enabled us to show that prednisolone clearly inhibits basal PGE2 synthesis.

Methods

PATIENTS Rectal biopsies weighing 7.0–34.2 mg (mean 19.3 mg) were taken 6–10 cm proximal to the anus from 40 patients suffering from ulcerative colitis or the irritable colon syndrome (ICS). None was receiving systemic or topical corticosteroid treatment and all but two of the patients with ulcerative colitis were taking sulphasalazine 1–2 g daily.

Biopsies from 26 patients were used to study the effects of prednisolone $8.33 \times 10^{-7}$M (0.3 µg/ml) using a laminar flow bioassay method. The biopsies from the other 14 patients were used to confirm the effects of prednisolone $8.33 \times 10^{-7}$M (0.3 µg/ml), n=8, and study those of prednisolone $5.66 \times 10^{-4}$M (200 µg/ml), n=6 by RIA. Details of the patients studied by each method are shown in Table 1.

ORGAN CULTURE RPMI 1640 containing 25 mM HEPES buffer, 10% fetal calf serum, gentamicin $7.37 \times 10^{-4}$M (40 µg/ml), and amphotericin B $3.13 \times 10^{-7}$M (0.5 µg/ml) was used as culture medium. After excision, rectal biopsies were washed in normal saline at 4°C and a portion removed for histology. The rest of each biopsy was bisected and each half was put into organ culture for up to 40 hours.

The culture medium for one half of each biopsy contained prednisolone (as prednisolone phosphate, pure powder, Organon Laboratories) at a concentration of $8.33 \times 10^{-7}$M (0.3 µg/ml) or $5.66 \times 10^{-4}$M (200 µg/ml). The other half was treated identically except that prednisolone was excluded from the culture medium. Addition of prednisolone did not alter the pH of the medium.

PROCEDURE WITH BIOASSAY After 40 hours, biopsies were removed and homogenised for protein estimation. The supernatant was extracted into chloroform and PGE2 was separated by ascending thin layer chromatography, using LQD6 plates (Whatman) with ethyl acetate: acetic acid, 99:1, as solvent system, and resuspended for...
bioassay in Tris HC1, 0-15M, pH 7-4. Recovery was estimated using (H3)PGE2 (Radiochemical Centre, Amersham).

PGE2-like activity was measured using rat or hamster stomach strips superfused with gassed Krebs solution in a laminar flow bioassay system. The Krebs solution contained indomethacin (1-14× 10⁻⁶M, 4 μg/ml), phenoxazepam hydrochloride (2-94× 10⁻⁷M, 0-1 μg/ml), propranolol hydrochloride (7-71× 10⁻⁶M, 2 μg/ml), hyoscine hydrobromide (3-30× 10⁻⁷M, 0-1 μg/ml), mepryzine dimaleate (3-51× 10⁻⁷M, 0-1 μg/ml), and methysesaride dimaleate (5-67× 10⁻⁷M, 0-2 μg/ml) to render the preparation more specific for PGs. The PGE2 content of each sample was estimated by reference to two closely related standards of authentic PGE2 (bracketing). The coefficient of variation of the assay in this series was 20-6% (intra-assay) and 29-0% (interassay).

**PROCEDURE WITH RADIOIMMUNOASSAY**

Fourteen pairs of biopsies were removed from organ culture after 16 hours, and PGE2 was measured in the unextracted supernatant by RIA using PGE2 antiserum (Sigma Chemical Co.). The threshold of detection was less than 2-84 fmol (1 pg) and the coefficient of variation of the assay in this series was 7-2% (intra-assay) and 12-2% (interassay). Cross-reactions with PGA2, PGD2, PGE1, PGI1α, PGF2α, 6 keto PGF1α thomboxane B2, arachidonic acid, and prednisolone were less than 0-1%. Authentic PGE2 added to samples was measured accurately (correlation coefficient between added PGE2 and measured increment = 0-99, n = 54) and precisely (p < 0-001, for increments of 5-67 fmol, 2 pg, n = 6).

**STATISTICAL METHODS**

All data were logarithmically transformed to obtain a normal distribution for analysis. T tests (paired where appropriate) were used, and two-tailed values for significance are quoted except for the comparison of doses where one-tailed values were used. Average data are expressed as mean (with 95% confidence limits) derived from the transformed data.

**Results**

In preliminary experiments PGE2 was shown to accumulate linearly over at least 40 hours; there was a mean inhibition of 90-8% by indomethacin 2-8× 10⁻⁷M, 0-1 μg/ml (n = 6) suggesting that PGE2 was being synthesised enzymatically. Biopsy specimens boiled for two minutes before organ culture (n = 6) did not synthesise PGE2 detectable by bioassay, nor did biopsies placed in normal saline, and these were histologically disorganised within eight hours. By contrast, biopsies in organ culture had intact epithelium on light and electron microscopy, and could incorporate (H3) thymidine after 40 hours in culture.

**BIOASSAY RESULTS**

Synthesis of PGE2 over 40 hours measured by bioassay of extracted PGE2-like activity was signi-
Results

Fig. 1  Mean synthesis of PGE2 in organ culture by inflamed and uninfammed biopsies. Left hand panel related to wet weight. Right hand panel related to protein content. Bars show 95% confidence limits. 1 pmol PGE2 = 352.5 pg.

Significantly higher in inflamed (mean 22.83 pmol/mg 7.99 ng/mg) than in uninfammed biopsies (mean 8.17 pmol/mg, 2.86 ng/mg, p < 0.05; Fig. 1, Table 2). There was no obvious difference in PGE2 synthesis between different sigmoidoscopic grades of inflammation.

Addition of prednisolone $8.33 \times 10^{-7}$M (0.3 $\mu$g/ml) reduced mean synthesis of PGE2 to 36.4% (p < 0.001) of that seen in untreated biopsies (wet weight) or to 37.2% (p < 0.001) expressed in terms of protein content. For inflammed biopsies, synthesis of PGE2 was significantly reduced by prednisolone to 32.2% of control values (p < 0.001, wet weight) or 33.7% (p < 0.001), protein content). The effect of prednisolone in uninfammed biopsies was not statistically significant whether expressed in terms of wet weight (47.7% of untreated, 0.05 < p < 0.1) or protein content (46.2% of untreated, 0.05 < p < 0.1) (Fig. 2, Table 2).

RIA Results

Prednisolone $8.33 \times 10^{-7}$M (0.3 $\mu$g/ml) reduced mean synthesis of PGE2 to 26.2% of untreated values over 16 hours (p < 0.01). At the higher concentration of prednisolone ($5.66 \times 10^{-4}$M, 200 $\mu$g/ml) PGE2 synthesis was further reduced to 7.7% of control values (p < 0.001) Table 2). This is a significantly greater reduction than that achieved by prednisolone $8.33 \times 10^{-7}$M (0.3 $\mu$g/mg) (p < 0.05).

Discussion

These results demonstrate that rectal mucosa can synthesise PGE2 in organ culture, that inflamed tissue synthesises greater amounts than uninfammed tissue, and that this can be inhibited by prednisolone.

PGE2 is thought to be the main PG synthesised by rectal mucosa; it seems likely that its appearance in organ culture represents enzymatic synthesis and is not an artefact of tissue disintegration, as it occurs linearly, is inhibited by indomethacin, and does not occur in boilled or saline controls. We observed somewhat lower rates of synthesis of PGE2 in organ culture than previous workers, possibly because most of our patients were taking sulphasalazine. In these and earlier experiments, no apparent differences were seen between biopsies from patients with inactive colitis (on sulphasalazine) and those from patients with the irritable colon syndrome (not on sulphasalazine). Data from each group were pooled to form the uninfammed 'control' group. Although the mean synthesis of PGE2 (measured by bioassay) by inflammed mucosa is about three times higher than by uninfammed mucosa, there is a wide variation in rates of PGE2 synthesis within both groups, whether measured by bioassay or RIA. Hence the difference is significant at the 5% level only in the larger bioassay series, and differences in the smaller RIA series do not reach significance.

It seems clear that prednisolone has a pronounced inhibitory effect on synthesis of PGE2 by rectal mucosa. The two concentrations of prednisolone
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studied were chosen because they represent concentrations found in the plasma with systemic treatment (8.33 x 10^{-9} M; 0.3 µg/ml) or locally with topical treatment (5.66 x 10^{-8} M, 200 µg/ml). The inhibition certainly occurs in inflamed biopsies; it is probably dose-dependent and is seen at 16 hours, well within the time that biopsies are still synthesising DNA. It is not certain whether there is an effect on uninflamed biopsies, as the observed differences did not reach statistical significance.

Organ culture was chosen as an appropriate method to investigate the effects of corticosteroids on PG synthesis in the light of the work of Flower and Blackwell. They showed, with perfused lungs, that corticosteroids act on intact cells to cause elaboration of a peptide which inhibits phospholipase A2; they thereby reduce PG synthesis indirectly by limiting the availability of substrate. We have confirmed that prednisolone does not inhibit synthesis of PGE2 from exogenous arachidonic acid in homogenates (unpublished observations) and our results are consistent with the mechanism proposed by Flower and Blackwell. Other mechanisms are possible, however, and work in progress to define the mode of action and time course of the inhibition by prednisolone.

Although both systemic and topical corticosteroids have been clinically useful in ulcerative colitis for many years, their mode of action is uncertain. Increased PG synthesis is a feature of active ulcerative colitis. Raised concentrations of various PGs are found in faeces, urine, blood, and basal synthesis of PGE2 and PG12 (prostacyclin) is higher when the disease is active. Tissue concentrations of active PG synthetase are raised during active disease and fall with successful treatment using a regimen which includes corticosteroids. Thus PGs appear to be important mediators of inflammation in ulcerative colitis. Our demonstration that prednisolone, like sulphasalazine, can inhibit PGE2 synthesis by rectal mucosa in organ culture now offers a possible explanation for the anti-inflammatory actions of corticosteroids in ulcerative colitis.

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References


