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

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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 $8{\cdot}33\times10^{-7}$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 $5{\cdot}66\times10^{-4}$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 enemata. 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.[1–7] Both sulphasalazine and its active moiety 5-aminosalicylic acid (5-ASA)[8] can inhibit PG synthesis by rectal mucosa *in vitro*.[5 7] By contrast, corticosteroids have no effect on PG synthesis by homogenates of rectal mucosa[7] or other tissues[9] and only inhibit PG synthesis in intact cells.[10 11] We decided therefore to examine the effect of prednisolone on basal PGE2 synthesis by whole rectal biopsies in organ culture.[12] 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 $8{\cdot}33\times10^{-7}$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 $8{\cdot}33\times10^{-7}$M (0·3 μg/ml), n=8, and study those of prednisolone $5{\cdot}66\times10^{-4}$M (200 μg/ml), n=6 by RIA. Details of the patients studied by each method are shown in Table 1.

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ORGAN CULTURE[12]

RPMI 1640 containing 25 mM HEPES buffer, 10% fetal calf serum, gentamicin $7{\cdot}37\times10^{-5}$M (40 μg/ml), and amphotericin B $3{\cdot}13\times10^{-7}$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 $8{\cdot}33\times10^{-7}$M (0·3 μg/ml) or $5{\cdot}66\times10^{-4}$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.[13] The supernatant was extracted into chloroform[14] 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

Table 1 *Distribution of patients by disease and sigmoidoscopic severity**

	ICS	GRADE		
		0	1	2–3
Bioassay n=	6	2	12	6
RIA n=	0	6	5	

*In all patients the diagnosis was confirmed histologically.[20]

bioassay in Tris HCl, 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.[15] The Krebs solution contained indomethacin ($1{\cdot}14\times10^{-5}$M, 4 μg/ml), phenoxybenzamine hydrochloride ($2{\cdot}94\times10^{-7}$M, 0·1 μg/ml), propranolol hydrochloride ($7{\cdot}71\times10^{-6}$M, 2 μg/ml), hyoscine hydrobromide ($3{\cdot}30\times10^{-7}$M, 0·1 μg/ml), mepyramine dimaleate ($3{\cdot}51\times10^{-7}$M, 0·1 μg/ml), and methysergide dimaleate ($5{\cdot}67\times10^{-7}$M, 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.).[16] 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, PGF1α, PGF2α, 6 keto PGF1α thromboxane 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 ($P<0{\cdot}001$, 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 $2{\cdot}8\times10^{-7}$M, 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-

Table 2 *Synthesis of PGE2 in organ culture: effect of prednisolone*

Assay method	Prednisolone concentration	Time in organ culture (hr)	Patients	Synthesis of PGE2			P
				Untreated (pmol/mg)	Prednisolone treated		
					(pmol/mg)	Untreated (%)	
Bioassay	$8{\cdot}33\times10^{-7}$M	40	26 (pooled data)*	16·64 (11·18–24·78)	6·05 (4·0–9·15)	36·4	<0·001
			18 (inflamed)	22·83 (14·30–36·45)	7·35 (4·17–12·95)	32·2	<0·001
			8 (uninflamed)†	8·17 (5·09–12·34)	3·90 (2·54–6·00)	47·7	0·05–0·1
RIA	$8{\cdot}33\times10^{-7}$M	16	8 (pooled data)‡	9·35 (6·67–13·11)	1·89 (0·66–5·48)	26·2	<0·01
	$5{\cdot}66\times10^{-4}$M	16	6 (pooled data)‡	5·10 (2·55–10·19)	0·39 (0·23–0·67)	7·7	<0·001

Mean values (with 95% confidence limits) are shown. 1 pmol=352·5 pg.
*Pooled bioassay data from the 18 inflamed and eight uninflamed specimens (shown separately below).
†'Uninflamed' includes specimens from ICS patients and colitics in remission.
‡Pooled RIA data; the numbers are two small to show inflamed and uninflamed data separately.

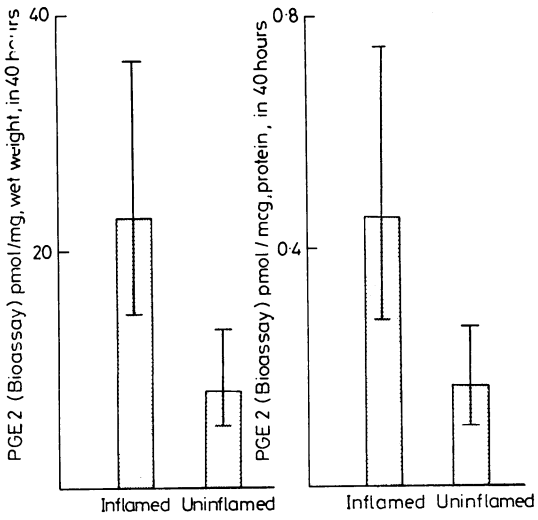

Fig. 1 *Mean synthesis of PGE2 in organ culture by inflamed and uninflamed biopsies. Left hand panel related to wet weight. Right hand panel related to protein content. Bars show 95% confidence limits. 1 pmol PGE2=352·5 pg.*

ficantly higher in inflamed (mean 22·83 pmol/mg 7·99 ng/mg) than in uninflamed 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 8.33×10^{-7}M (0·3 μg/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 inflamed 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 uninflamed 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 8.33×10^{-7}M (0·3 μg/ml) reduced mean synthesis of PGE2 to 26·2% of untreated values over 16 hours ($P<0.01$). At the higher concentration of prednisolone (5.66×10^{-4}M, 200 μg/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 8.33×10^{-7}M (0·3 μg/mg) ($P<0.05$).

Discussion

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

PGE2 is thought to be the main PG synthesised by rectal mucosa[8]; it seems likely that its appearance in organ culture represents enzymatic synthesis and is not an artefact of tissue disintegration,[17] as it occurs linearly, is inhibited by indomethacin, and does not occur in boiled or saline controls. We observed somewhat lower rates of synthesis of PGE2 in organ culture than previous workers,[5] 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 uninflamed 'control' group. Although the mean synthesis of PGE2 (measured by bioassay) by inflamed mucosa is about three times higher than by uninflamed 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

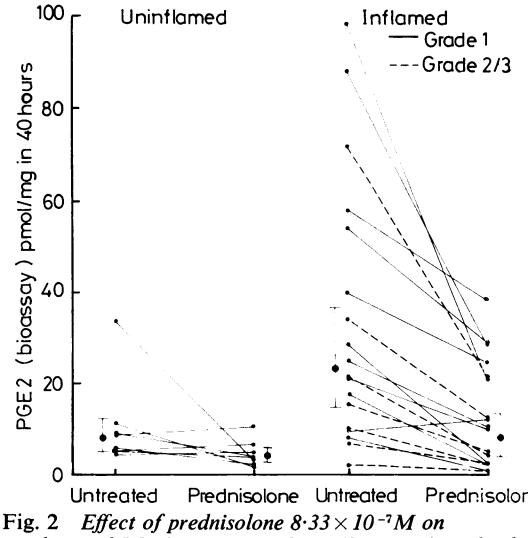

Fig. 2 *Effect of prednisolone 8.33×10^{-7}M on synthesis of PGE2 in organ culture (bioassay). Individual paired observations are shown, with means and 95% confidence limits to the side. 1 pmol PGE2=352·5 pg.*

studied were chosen because they represent concentrations found in the plasma with systemic treatment ($8{\cdot}33 \times 10^{-7}$M, 0·3 μg/ml)[18] or locally with topical treatment ($5{\cdot}66 \times 10^{-4}$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.[11] 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 is 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,[19] 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,[1] urine,[2] blood,[4] and basal synthesis of PGE2[5] and PGI2 (prostacyclin)[6] is higher when the disease is active. Tissue concentrations of active PG synthetase are raised during active disease[3] and fall with successful treatment using a regimen which includes corticosteroids.[7] 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

[1]Gould SR. Assay of prostaglandin-like substances in faeces and their measurements in ulcerative colitis. *Prostaglandins* 1976; **11:** 489–97.

[2]Gould SR, Brash AR, Connoly ME. Increased prostaglandin production in ulcerative colitis. *Lancet* 1977; **2:** 98.

[3]Harris DW, Smith PR, Swan CHJ. Determination of prostaglandin synthetase activity in rectal biopsy material and its significance in colonic disease. *Gut* 1978; **19:** 875–7.

[4]Harris DW, Swan CHJ, Smith PR. Venous prostaglandin-like activity in diarrhoeal states. *Gut* 1978; **19:** 1057–8.

[5]Sharon P, Ligumsky M, Rachmilewitz D, Zor U. Role of prostaglandins in ulceretive colitis; enhanced production during active disease and inhibition by sulfasalazine. *Gastroenterology* 1978; **75:** 638–40.

[6]Sinzinger H, Silberbaver K, Seyfriend H. Rectal mucosal prostacyclin formation in ulcerative colitis. *Lancet* 1979; **1:** 44.

[7]Smith PR, Dawson DJ, Swan CHJ. Prostaglandin synthetase activity in acute ulcerative colitis: effects of treatment with sulphasalazine, codeine phosphate, and prednisolone. *Gut* 1979; **20:** 802–5.

[8]Azad Khan AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* 1977; **2:** 892–5.

[9]Flower R, Gryglewski R, Herbaczynska-Cedro K, Vane JR. Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature. New Bio* 1972; **238:** 104–6.

[10]Blackwell GJ, Flower RJ, Nijkamp FP, Vane JR. Phospholipase A2 activity of guinea pig isolated perfused lungs: stimulation and inhibition by anti-inflammatory steriods. *Br J Pharmac* 1978; **62:** 79–89.

[11]Flower RJ, Blackwell GJ. Anti-inflammatory steriods induce biosynthesis of a phospholipase A2 inhibitor which prevents prostaglandin generation. *Nature* 1979; **278:** 456–9.

[12]Eastwood GL, Trier JS. Organ culture of human rectal mucosa. *Gastroenterology* 1973; **64:** 375–82.

[13]Lowry O, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193:** 265–75.

[14]Unger WG, Stamford IF, Bennett A. Extraction of prostaglandins from human blood. *Nature* 1971; **233:** 336–7.

[15]Ferreira SH, De Souza Costa F. A laminar flow superfusion technique with much increased sensitivity for the detection of smooth muscle-stimulating substances. *Eur J Pharmacol* 1976; **39:** 379–81.

[16]Dray F, Charbonnel B, Maclouf J. Radioimmunoassay of prostaglandins F, E1 and E2 in human plasma. *Eur J Clin Invest* 1975; **5:** 311–8.

[17]Nugteren DH, Vonkeman H, Vandorp DA. Non-enzymatic conversion of all-cis 8, 11, 14 eicosatrienoic acid into prostaglandin E1. *Recl Trav Chim Pays-Bas* 1966; **86:** 1237–45.

[18]Elliott PR, Powell-Tuck J, Gillespie PC, Laidlow JM, Lennard-Jones JE, English J, Chakraborty J, Marks V. Prednisolone absorption in acute colitis. *Gut* 1980; **21:** 49–51.

[19]Truelove SC, Witts LJ. Cortisone in ulcerative colitis. Final report on a therapeutic trial. *Br Med J* 1955; **2:** 1041–8.

[20]Truelove SC, Richards WCD. Biopsy studies in ulcerative colitis. *Br Med J* 1956; **1:** 1315–8.