Calcipotriol inhibits rectal epithelial cell proliferation in ulcerative proctocolitis

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Abstract

Vitamin D₃ reduces human rectal crypt cell production rate (CCPR) and may thereby protect against colorectal cancer. Cell turnover is increased in ulcerative proctocolitis, which might therefore respond to vitamin D₃ metabolites. This study investigated the effect of calcipotriol, a synthetic vitamin D₃ analogue that avoids hypercalcaemia, on human rectal CCPR in ulcerative proctocolitis. Paired rectal biopsy specimens from seven patients with severe disease were established in organ culture with or without calcipotriol (1×10^{-6} M). After 15 hours, vincristine (0.6 µg/ml) was added to induce metaphase arrest, and CCPR was determined by linear regression analysis of accumulated metaphases. Compared with values in 17 controls with incidental anal conditions, median rectal CCPR was 28% higher in ulcerative proctocolitis: 5.90 (5.00-9.50) v 4.80 (2.85-7.07) cells/crypt/hour ($p < 0.01$). Calcipotriol reduced CCPR by 62% in patients with ulcerative proctocolitis, from 5.90 (5.00-9.50) to 2.21 (0.81-3.22) cells/crypt/hour (median with range) $p < 0.01$. Thus calcipotriol can dampen the hyperproliferative state in ulcerative proctocolitis and could have a therapeutic role in the control of this inflammatory condition.

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In normal rectal epithelium static measurements of cell proliferation suggest that the proliferative compartment is confined to the lower third of the crypt, but in ulcerative proctocolitis there is a two to threefold expansion of this compartment.¹⁻⁴ A more dynamic method using the stathmokinetic technique of metaphase arrest shows that cell birth (crypt cell production rate, CCPR) is increased in both active and quiescent ulcerative proctocolitis.⁵ There is increasing evidence that accelerated cell proliferation in the large bowel predisposes to malignancy, and this may help to explain the correlation between the extent and chronicity of colitis and the subsequent risk of developing carcinoma.⁵⁻⁹

Epidemiological evidence shows that vitamin D₃ can protect against colorectal cancer.¹⁰ The vitamin D receptor is expressed in both normal¹¹ and malignant colorectal epithelial cells.¹²⁻¹⁴ The active form of the vitamin, 1,25 (OH)₂ D₃, also reduces in vitro CCPR in colorectal cell lines, normal rectal tissue, and

tissue from familial adenomatous polyposis patients.¹⁵ Although the clinical use of 1,25 (OH)₂ D₃ is severely limited by its profound effects on calcium metabolism, synthetic vitamin D₃ metabolites can avoid these hypercalcaemic and hypercalciuric effects.^{15 16} One such metabolite is calcipotriol,^{16 17} which also reduces in vitro epithelial cell proliferation (CCPR) in normal human rectal tissue and malignant colorectal cells.¹⁵ We have therefore investigated the effect of calcipotriol on in vitro rectal epithelial cell proliferation in ulcerative proctocolitis using a stathmokinetic technique to measure CCPR.

Methods

CHEMICALS

The secosterol calcipotriol (MC-903) was supplied by Leo Pharmaceutical Products (Ballerup, Denmark) and stored in isopropyl alcohol at -20°C until use. Control medium was prepared with a similar dilution of alcohol, which had no detectable effect on proliferation.¹⁵ Vincristine (Oncovin) was supplied by Eli Lilly (Basingstoke, UK). The standard culture medium consisted of CMRL 1066 (GIBCO, Paisley, UK), 5% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin, 2% L-glutamine (200 mM) and 1% HEPES buffer 1 M (all from Sero Labs, Crawley, UK). Glucose 20 mg, hydrocortisone 21-hemisuccinate 1 mg, and insulin 1 mg (Sero Labs, Crawley, UK) were added to each 20 ml of culture medium immediately before each experiment.^{15 18}

CLINICAL MATERIAL

Paired rectal biopsy specimens were taken from seven patients with longstanding ulcerative proctocolitis of moderate to severe activity at routine follow up proctosigmoidoscopy. There were five men and two women, with a median age of 43 years (range 34-80); all the patients were being treated with corticosteroid enemas and an oral 5-aminosalicylate. Two patients had extensive colitis and five had more distal disease. One specimen from each patient was examined histologically (as part of their routine treatment) while the other was set up in organ culture. Local ethical committee approval was obtained, and all patients gave fully informed consent.

ORGAN CULTURE

The rectal biopsy specimens were divided into numerous explants and orientated with the

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Crypt cell production rate (cells/crypt/hour)

	Control	Calcipotriol (1×10 ⁻⁶ M)
	5.42 (0.76)	1.11 (0.54)
	9.50 (1.27)	3.19 (0.98)
	5.00 (0.56)	3.22 (0.31)
	5.90 (0.56)	2.21 (0.28)
	6.10 (1.34)	1.63 (0.04)
	9.16 (0.67)	2.25 (0.86)
	5.00 (0.32)	0.81 (0.94)
Mean	6.58 (0.73)	2.06 (0.94)
Median (range)	5.90 (5.00–9.50)	2.21 (0.81–3.22)*

*Experiment v control p<0.01. Values are mean (SEM).

mucosal surface uppermost on a metal grid within an organ culture dish (Lux Laboratories). Explants from each patient were cultured as paired samples (control and experimental) in standard culture medium (CMRL 1066, Gibco, Paisley, UK) or in standard culture medium to which 1×10⁻⁶ M calcipotriol had been added. This dose of calcipotriol has provisionally been shown to reduce CCPR in tissue taken from patients with familial adenomatous polyposis¹⁹ and is slightly higher than that which reduces CCPR in normal rectal tissue.¹⁵ The organ culture dishes were sealed in an atmosphere of 95% O₂ and 5% CO₂ at a temperature of 37°C and were rocked at five cycles per minute. After 15 hours, vincristine (0.6 µg/ml) was added to the culture medium to produce metaphase arrest within the colonic crypts. Explants were sequentially removed one, two, and three hours later, were fixed in Carnoy's fluid, and then stored in 70% alcohol. Explants were rehydrated in solutions of 50%, 25%, and 10% alcohol. After acid hydrolysis in 1 M HCl at 60°C for six minutes, the tissue was stained with Schiff's reagent. At least 20 crypts were microdissected from each explant, and the number of metaphases per crypt was counted. The mean number of metaphases was plotted against the time from vincristine administration, and the crypt cell production rate was determined by least squares linear regression analysis, giving a value for the crypt cell production rate in cells/crypt/hour.^{15 18 20 21} Explants showing evidence of infection or crypt necrosis were discarded.

Results

Cultured tissue showed a good preservation of architecture with an infection and crypt necrosis rate of 2%, in line with our previous results.^{15 21} There was no evidence of metaphase escape in any of the microdissected crypts, which implies that the dose of vincristine used was sufficient to produce complete metaphase arrest. In addition the crypts appeared intact. Histological examination confirmed the presence of active colitis in all samples with no evidence of dysplasia in any of the specimens. The overall median control CCPR was 5.90 cells/crypt/hour (range 5.00–9.50 cells/crypt/hour). This value is lower than that reported by Allan *et al.*,⁵ but when compared with our previous control values (mean (SEM) 4.74 (0.25), median 4.80 range 2.85–7.07 cells/crypt/hour)

in patients with incidental anal conditions,¹⁵ it represents a 28% increase in CCPR (p<0.01 Mann-Whitney U test).
CCPR was reduced in all explants cultured with calcipotriol when compared with their own control (Table). The overall median CCPR was reduced by 62% from 5.90 (5.00–9.50) to 2.21 (0.81–3.22) cells/crypt/hour (p<0.01 Mann-Whitney U test).

Discussion

We have shown, for the first time, that a synthetic vitamin D₃ metabolite (calcipotriol) reduces rectal epithelial cell proliferation in tissue taken from patients with active ulcerative proctocolitis. Calcipotriol reduced CCPR by about 60%, to a value well below that seen in our previously reported controls.¹⁵ These findings are in keeping with the well reported anti-proliferative effects of 1,25 (OH)₂ D₃^{12–15} and show that colorectal tissue in inflammatory bowel disease retains its ability to respond to vitamin D₃ metabolites. We have previously reported a similar effect of both 1,25 (OH)₂ D₃ and calcipotriol on normal rectal tissue,¹⁵ but we could not show a clear cut dose response effect in organ culture. There was no microscopic evidence to suggest that the observed effect in this experiment resulted from calcipotriol induced toxicity; indeed, the linear accumulation of metaphases seen in our experimental group suggests that the tissue was healthy and retained its ability to proliferate.
Ulcerative proctocolitis is associated with a hyperproliferative colorectal epithelium.^{1–5} There is an expansion or a major shift of the proliferative compartment in active and quiescent disease, and an altered cytokinetic state is retained after total abdominal colectomy with ileorectal anastomosis.^{5 22} The accelerated rate of cell proliferation might contribute to the observed increase in incidence of large bowel cancer in ulcerative proctocolitis. Indeed, the distribution of mutations in dysplastic colitic tissue suggests that dysplastic fields might originate by clonal proliferation from a single cell containing mutations.²³ A reduction in the rate of cell proliferation or an induction of differentiation in longstanding disease using synthetic vitamin D analogues might protect against the subsequent development of colorectal carcinoma. In addition, synthetic metabolites might offer a therapeutic option in the control of disease in those patients who relapse with corticosteroid and aminosalicylate treatment.
The topical administration of calcipotriol has a dramatic effect in psoriasis vulgaris,^{24–26} but it is not at present available for enteric use; its short half life may limit its oral or rectal therapeutic use.¹⁷ It is not yet known whether a reduction in colorectal epithelial cell proliferation would control symptoms in ulcerative proctocolitis or whether this would have a beneficial effect on the disease process.
We did not measure serum concentrations of calcium or vitamin D₃ metabolites or the

release of cytokines from the tissue. Vitamin D₃ metabolites can inhibit the proliferation of peripheral blood lymphocytes.²⁷ Cellular activation of peripheral blood T lymphocytes by antigen or lectin is accompanied by synthesis of the vitamin D receptor,^{28 29} and the reduction in proliferation of these cells by 1,25 (OH)₂ D₃ has been correlated with a decreased production of interleukin 2.^{30 31}

It is known that some of the differentiating effects of 1,25 (OH)₂ D₃ are associated with a modulation of vitamin D receptor expression,^{29 32} but, vitamin D receptor expression has not yet been reported in colorectal tissue from patients with ulcerative proctocolitis. Calcipotriol clearly reduces in vitro CCPR in ulcerative proctocolitis, but more sophisticated methods will be required to determine the intracellular events responsible for the effect of vitamin D₃ and its metabolites on human colorectal epithelial cell proliferation.

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