COLON CANCER

Potential role for peroxisome proliferator activated receptor (PPAR) in preventing colon cancer

L Jackson, W Wahli, L Michalik, S A Watson, T Morris, K Anderton, D R Bell, J A Smith, C J Hawkey, A J Bennett

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See end of article for authors' affiliations

Correspondence to: C J Hawkey, Department of Medicine, Division of Gastroenterology, University Hospital Nottingham, Nottingham NG7 2UH, UK; cj.hawkey@nottingham.ac.uk or Dr A Bennett, Biomedical Sciences, Nottingham University Medical School, University Hospital Nottingham, NG7 2HH, UK; andrew bennett@nottingham.ac.uk

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Background: Peroxisome proliferator activated receptors (PPARs) are nuclear hormone receptors involved in genetic control of many cellular processes. PPAR and PPAR have been implicated in colonic malignancy. Here we provide three lines of evidence suggesting an inhibitory role for PPAR in colorectal cancer development.
Methods: Levels of PPAR mRNA and protein in human colorectal cancers were compared with matched non-malignant mucosa using RNAse protection and western blotting. APC^{Min}/+ mice were randomised to receive the PPAR activator methylclofenapate 25 mg/kg or vehicle for up to 16 weeks, and small and large intestinal polyps were quantified by image analysis. The effect of methylclofenapate on serum stimulated mitogenesis (thymidine incorporation), linear cell growth, and annexin V and propidium iodide staining were assessed in human colonic epithelial cells.
Results: PPAR (mRNA and protein) expression levels were significantly depressed in colorectal cancer compared with matched non-malignant tissue. Methylclofenapate reduced polyp area in the small intestine from 18.7 mm^2 (median (interquartile range 11.1, 26.8)) to 9.90 (4.88, 13.21) mm^2 (p=0.003) and in the colon from 9.15 (6.31, 10.5) mm^2 to 3.71 (2.71, 5.99) mm^2 (p=0.009). Methylclofenapate significantly reduced thymidine incorporation and linear cell growth with no effect on annexin V or propidium iodide staining.
Conclusions: PPAR may inhibit colorectal tumour progression, possibly via inhibition of proliferation, and may be an important therapeutic target.

Colorectal cancer is the fourth commonest form of cancer occurring worldwide, with an estimated 783 000 new cases diagnosed in 1990,[1] and is responsible for 7.2% of all cancer deaths worldwide.[2] A moderately predictable evolution of oncogenic transformation is recognised[3 4] but as yet little current understanding has translated into therapeutic gain. Among the most promising approaches to chemoprevention are the use of aspirin or non- aspirin non-steroidal anti-inflammatory drugs (NSAIDs) or inducible cyclooxygenase (COX)- 2 inhibitors. However, toxicity and cost considerations may limit application at a population level. Moreover, how such chemopreventative strategies work, whether there are common or multiple mechanisms, and how interactions with oncogenic expression take place are all far from clear.

Peroxisome proliferator activated receptors (PPARs) are ligand activated nuclear transcription factors first recognised for their role in rodent livers[5] where they mediate the proliferative response of peroxisomes to various compounds. Since their identification, it has become clear that PPARs play a much wider and central role in orchestrating gene expression in response to exogenous ligands. In particular, they have an intimate two way relationship with NSAIDs. PPAR activation can lead to altered expression of COX-2,[6 7] while NSAIDs have been reported to be capable of activating PPARs.[8] In addition, NSAIDs, through effects on COX activity, can alter synthesis of eicosanoids that may bind to and act as ligands for PPARs.[9 10] In addition, NSAIDs have themselves been reported to inhibit DNA binding activity of PPARs with apparent functional consequences such as induction of apoptosis.[11]

Much attention has focused on the role of the different PPARs in the human intestine, in particular on their importance in neoplastic transformation. PPARβ and to a lesser degree PPARγ have been proposed as possible promoters of colorectal carcinoma.[11–13] Less attention has been paid to PPARα, probably because of a relatively lower baseline expression level of this isotype in non-malignant human colonic mucosa.[14] Although PPARα ligands appear to promote expression of COX-2 in animals,[6] human studies suggest that PPARα may act conversely and inhibit COX-2 expression.[7] PPARα is activated by the omega-3 fatty acid constituents of fish oil diet which have been demonstrated both to reduce colorectal cancer progression in various animal models[15–17] and to reduce cellular proliferation rates in colorectal mucosa of patients with familial adenomatous polyposis.[18] PPARα activation may thus be potentially beneficial in preventing colon cancer. This hypothesis is supported by the more recent study of Tanaka *et al* which demonstrated that bezafibrate, a PPARα ligand, inhibited azoxymethane induced aberrant crypt foci formation in rats.[19]

In order to understand further the biology of PPARα in human colorectal function and its possible role in malignant pathology, we compared expression of PPARα in non-malignant and malignant colonic mucosa, activation by ligands, and the potential value of these ligands in preventing colorectal cancer in the APC^{Min}/+ mouse model of familial adenomatous polyposis.

MATERIALS AND METHODS

Chemicals

Methylclofenapate (synthesised by Lancaster Synthesis Ltd, Lancashire, UK) was a kind gift from Dr CR Elcombe. We purchased Wy 14643 from Biomol (Affinity Research Products

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; PPAR, peroxisome proliferator activated receptor; IQR, interquartile range.

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Ltd, Mamhead, Exeter, UK). We obtained radiolabelled chemicals from Amersham International, UK. Unless otherwise stated, we obtained other compounds from Sigma (UK).

Plasmids

The reporter plasmid PPRE-tk Luc, a kind gift from Dr R Evans (Salk Institute), has previously been described, and contains three copies of the acyl-coA oxidase PPRE upstream of the herpes virus thymidine promoter.[8] We obtained human PPARα in pBK-CMV (1731 bp), used for RNAse protection assays, from the Image Consortium Library. pSG5-xPPARβ and pSG5-xPPARγ expression plasmids constructed by insertion of the entire xPPARβ and xPPARγ DNAs as EcoRI fragments into pSG5[20] have previously been described and were used for transfection studies, as was pSG5-mPPARα expression plasmid, a kind gift from Dr S Green.[21] pSG5 empty vector and pRL-CMV were purchased from Promega (Southampton, UK).

Comparison of PPAR expression in non-malignant and neoplastic mucosa

Tissue sampling

We obtained mucosal biopsies samples from tumour, and from macroscopically non- malignant mucosa >3 cm from the tumour, in 26 randomly selected patients undergoing resection. Biopsy samples were immediately frozen in liquid nitrogen and stored at −70°C until RNA preparation and/or protein extraction. We confirmed the benign or malignant status of all samples histologically.

RNA quantification

We extracted RNA from frozen tissue samples using standard techniques[22] and quantification was performed using a ribonuclease (RNAse) protection assay. Using this technique, labelled RNA probe complementary to target RNA is mixed with sample RNA, and the complementary transcripts hybridise and unhybridised probe is then degraded by the addition of ribonuclease. The protected probe is then run on a gel and visualised by autoradiography.

Construction of PPARα probe for RNAse protection assay

Human PPARα 263 corresponding to nucleotides 721–983 of the cDNA sequence was excised using restriction enzymes EcoRI and PstI, inserted into pBluescriptllKS (Stratagene, La Jolla, California, USA) with the 5′ end adjacent to the T3 promoter, and sequenced to confirm identity.

RNAse protection assay

We synthesised labelled RNA transcripts from the inserts with [α^{32}-P CTP] as the limiting nucleotide using the Stratagene RNA Transcription Kit. We linearised PPARα 263 with PstI and prepared antisense cRNA using T3 polymerase. We performed the RNAse protection assay using the Ambion Ribonuclease Protection Assay kit (RPA11). We hybridised 40 μg of total RNA (tumour and matching non-malignant colonic mucosa) with cRNA (100 000 cpm) overnight at 42°C. We digested the unhybridised probe with RNAse A/T1, as described in the manufacturer's instructions, and resolved the protected fragments on a 6% urea/acrylamide/TBE gel. Bands were imaged using a Fujix Bas 2000 phosphoimager (Fuji, Japan), and quantitated using AIDA software. Results were normalised using oligo dT_{18} hybridisation, as previously described.[23]

Western blot analysis of PPARα

We extracted protein from non-malignant or malignant mucosa, as previously described.[24] We separated 90 μg of protein by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred it to a Hybond ECL nitrocellulose membrane (Amersham, UK). We incubated the membrane with polyclonal rabbit anti-PPARα (A/B domain) IgG (diluted 1:500) using horseradish peroxidase conjugated goat anti-rabbit IgG (1:400) secondary antibody (Sigma) and visualised antigen/antibody complexes using the enhanced chemiluminescence detection system (ECL; Amersham). The membrane was blotted with β- actin to confirm consistent loading and sample quality.

Chemoprevention of polyps in APC^{Min}/+ mice

We commenced C57BL/6J APC^{Min}/+ male and female mice, once weaned, on methylclofenapate 25 mg/kg/day (made up in safflower oil) (n=13) or safflower oil alone (n=14), which was administered by oral gavage. Animal experiments were performed according to EU regulations. C57BL/6J APC^{Min}/+ mice were group housed, tap water was available ad libitum, and they were fed standard chow (Beekay, Humberside, UK) throughout the study. We determined time of sacrifice (14.3±0.4 weeks for treated and 13.3±0.3 weeks for control mice; NS) by onset of clinically detected anaemia or >20% body weight. At time of sacrifice, we flushed the entire intestine with saline and opened it lengthways. After fixing the tissue in formol calcium, we immersed it in thiazine dye (Baxter, UK) for five minutes before returning it to 70% C_2H_5OH for 24 hours. Using this methodology, normal mucosa is stained blue allowing good differentiation from polyps (which remain unstained). We scanned each full length intestine on a 1 mm^2 grid and, with specimens for comparison, highlighted the polyps manually and calculated their number. Finally, using the image analysis programme Qwinstandard (Leica, Milton Keynes, UK), we calculated the individual area of the large and small intestinal polyps, and the total area of abnormal and normal intestinal mucosa in each mouse.

In vitro effects of PPARα ligands

Cell culture

We cultured human colonic adenocarcinoma (HCA7) cells (a kind gift from Dr S Kirkland, London)[25] in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum, glutamine (2 mmol/l), penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (0.05 mg/ml) in a 5% CO_2 atmosphere at 37°C.

Transfection studies

Using the cationic lipid transfection reagent Transfast (Promega) in a charge ratio of 1:2, we transfected HCA7 cells, which were 70–80% confluent, in serum free medium with a mix containing PPRE-tk-luc (1.1 μg), pRL-CMV (0.33 μg, as internal transfection control), and PPAR expression vector or empty pSG5 (2 μg), where indicated. After a 60 minute incubation period, we supplemented the medium with 0.1% fetal calf serum and added pharmacological ligands for individual PPARs four hours later if appropriate. We calculated luciferase and renilla activity 40 hours after transfection using the Dual-Luciferase Reporter Assay System (Promega). We determined normalised luciferase activity which we plotted as fold activation relative to untreated cells. We performed all points in triplicate in at least two independent experiments.

Measurement of linear cell growth and DNA synthesis

We added methylclofenapate in DMSO, or DMSO alone, to subconfluent cultures of (70%) HCA7 cells maintained in serum free medium for 24 hours. Two hours later, we stimulated mitogenesis by addition of 10% v/v fetal calf serum. We cultured the cells continuously in the presence of methylclofenapate (100 μM) and assessed cell number at one, two, and five days.

We assessed DNA synthesis after 24 hours of exposure to ligand by measuring tritiated thymidine incorporation at 24 hours after a three hour pulse exposure to [H^3] thymidine (1 μCi/ml). At the end of this period, the supernatant was aspirated and cells washed twice with 1×phosphate buffered saline (Invitogen, Paisley, UK) before fixing with methanol-glacial acetic acid (3:1)(v/v) for at least one hour at room temperature. Two further washes with 80% methanol were

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performed before lysing the cells with 1 ml of 1 M NaOH. Lysate (900 µl) was then added to 10 ml of scintillation fluid (Packard, Meriden, Connecticut, USA) and disintegrations per minute counted by liquid scintillation spectrometry (Wallace, Milton Keyes, UK).

Measurement of apoptosis and necrosis using flow cytometry: annexin V and propidium iodide staining

We assessed rates of apoptosis and necrosis of HCA7 cells using flow cytometry, as described previously.[26] After 12 hours of incubation with methylclofenapate, we treated cells with trypsin/EDTA, washed the resultant cell pellet in ice cold phosphate buffered saline, and resuspended cells in ice cold diluted binding buffer to concentrations of $10^5/10^6$ cells/ml. We next added 25 ng/ml of FITC conjugated annexin V and 10 µg/ml of propidium iodide to cell suspension and, after 10 minutes of incubation in the dark, analysed cell staining using a fluorescence activated cell sorter (Becton Dickinson, USA)

Cell cycle analysis

We determined the proportion of nuclei in each phase of the cell cycle after exposure to methylclofenapate (100 µM) using flow cytometry. Treated and control HCA7 cells were stained with propidium iodide (0.5 µg/ml) and fixed in 70% ethanol. We measured the relative DNA content of nuclei using a fluorescence activated cell sorter (Becton Dickinson). The proportion of nuclei in each phase of the cell cycle was determined using Cylchred analysis software.

Statistics employed

Expression levels of RNA and protein in tumour and normal mucosa were not normally distributed and therefore the Mann-Whitney U test was used. ANOVA and *t* testing were used for other parameters.

RESULTS

PPAR expression in non-malignant and neoplastic mucosa

RNA expression

Using quantitative RNAse protection assays, we measured levels of PPARα mRNA in non-malignant human colonic mucosal biopsy samples as 1.38 (0.30) attamoles/µg total RNA. Comparable values for PPARγ were 15.6 (10.4) attamoles/µg. When mRNA levels in neoplastic colonic tissues were compared with matched non-malignant mucosa, PPARα was significantly decreased by 38.8% (median (interquartile range (IQR) 11.7–62.1%); p=0.05) (fig 1A).

Protein expression

Using western blot analysis, we showed expression of both PPARα and PPARγ in non-malignant human colonic mucosa. In malignant tissue there was a highly significant reduction in PPARα protein levels (median reduction of 67.0% (IQR 55.2, 80.3%); p=0.001) (fig 1B). There were no differences in β-actin levels between normal and malignant tissues.

Activation of colonic epithelial PPARs

In order to identify ligands capable of selectively activating PPARα in colonocytes, PPRE- tk-luc (reporter gene) and individual PPARs were cotransfected into the human colon adenocarcinoma cell line HCA7 (fig 2A). Over expression of PPARα in HCA7 cells increased PPRE-tk-luc activation significantly compared with the reporter gene activity of empty pSG5 vector (p<0.001). Over expression of PPARγ or PPARβ alone did not result in significant PPRE-tk-luc activation in the absence of ligand (fig 2A).

Effect of ligands

The known PPARα ligand methylclofenapate further enhanced PPRE-tk-luc activation in cells cotransfected with PPARα, over the concentration range 1–100 µM. Methylclofenapate 10 µM stimulated 2.50 (0.22)-fold activation relative to no ligand (fig 3A). The better known PPARα ligand Wy14643 was less effective: Wy14643 10 µM increased PPRE-tk-luc activation 1.4 (0.12)-fold when PPARα was transfected (fig 2B). Although significant activation of PPARβ and PPARγ was achieved with PPARβ and PPARγ ligands, as appropriate (data not shown), methylclofenapate 10 µM had no significant effect on PPARβ or PPARγ activity (activation of 0.92 (0.07) and 0.91 (0.12) relative to no ligand) (fig 2A), confirming the potency and selectivity for PPARα at this concentration.

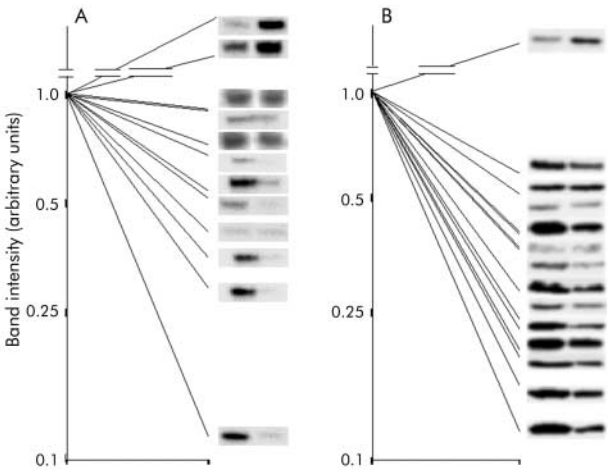

Figure 1 Peroxisome proliferator activated receptor α (PPARα) mRNA and protein expression in non-involved and neoplastic human colon. (A) RNAse protection analysis of PPARα mRNA expression in tumour samples and matched non-malignant colonic mucosa. RNA was hybridised with cRNA PPARα probe, unhybridised RNA was digested with RNAse solution 1:70, and the protected fragments were resolved on a 6% urea/acrylamide/TBE gel. Data are shown as band intensity (arbitrary units) on a logarithmic scale, with individual bands (normal:tumour) to the side. (B) Western blot analysis of PPARα protein expression in tumour samples and matched non-malignant colonic mucosa using rabbit antimouse PPARα IgG antibody. Data are shown as band intensity (arbitrary units) on a logarithmic scale, with individual bands (normal:tumour) to the side.

Functional responsiveness of endogenous PPARs in HCA7 cells

We confirmed the functional responsiveness of endogenous PPARs in HCA7 cells by observing the ability of PPAR pharmacological ligands to activate a PPRE driven reporter gene (PPRE-tk-luc) transfected into these cells. Methylclofenapate significantly induced reporter gene activity at concentrations ⩾10 µM, indicating the presence of functionally active endogenous PPARα in these cells (fig 3).

Effect of methylclofenapate treatment on small and large intestinal polyp formation in APC^{Min}/+ mice

We used methylclofenapate as the most potent and specific PPARα activator from our transfection experiments to evaluate its effects on polyp formation in APC^{Min}/+ mice. Fifteen mice (eight males, seven females) received methylclofenapate 25 mg/kg daily in safflower oil, and 14 (eight males, six females) received safflower oil alone. At sacrifice, there was a highly significant reduction in the total polyp burden in both the small intestine (median 18.67 (IQR 11.13, 26.84) mm^2 to 9.9 (IQR 4.88,13.21) mm^2; p=0.003) and the large intestine (9.15 (IQR 6.31,10.51) mm^2 to 3.71 (IQR 2.71,5.99) mm^2; p=0.009) (fig 4). These differences were seen in both male and female mice. Tumour burden/time also revealed significant differences for both the small and large intestine (p=0.006 small intestine, p=0.008 large intestine).

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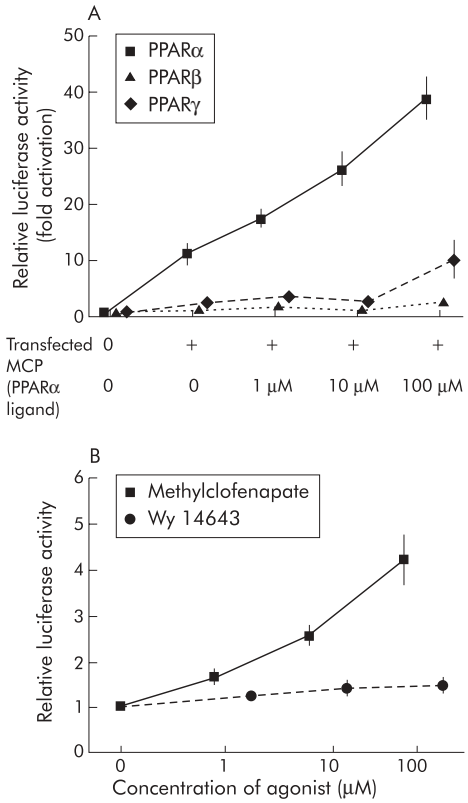

Figure 2 Selective activation of peroxisome proliferator activated receptor α (PPARα) by methylclofenapate. (A) Differential effects on transfected PPARα, PPARβ, and PPARγ. HCA7 cells were transfected with 2 μg of PPARα, PPARγ, or PPARβ, expression vector or empty vector PSG5, PPRE-tk-luc (1.1 μg), and pRL-CMV (0.33 μg, as internal control), and exposed to the PPARα ligand methylclofen apate (MCP) for 36 hours. Normalised luciferase activity is plotted as mean (SEM) fold activation relative to untreated cells transfected with empty vector pSG5. Over expression of PPARα, in HCA7 cells, in the absence of ligand, increased PPRE-tk-luc activation (23.2 (3.9)-fold) compared with reporter gene activation by pSG5 vector (p=0.001). Methylclofenapate caused a further significant dose dependent increase in reporter gene activity. Over expression of PPARγ or PPARβ alone did not result in significant PPRE-tk-luc activation. Methylclofenapate had no significant effect in the presence of transfected PPARβ at all concentrations tested. It had no significant effect in the presence of transfected PPARγ at ≤10 μM, but caused a small increase in activity at 100 μM. (B) Comparative potency of PPARα ligands as activators of PPARα. HCA7 cells were transfected with PPARα expression vector (2 μg), PPRE-tk-luc (1.1 μg), and pRL-CMV (0.33 μg, as internal control), and exposed to the PPARα ligands methylclofenapate and Wy14643 (1–100 μM) for 36 hours. In contrast with (A), normalised luciferase activity is plotted as mean (SEM) fold activation relative to cells that were transfected with PPARα expression vector but not exposed to ligand. Methylclofenapate activated PPRE-tk-luc more effectively than Wy 14643 at all concentrations used.

The reduction in tumour burden appeared mainly to be attributable to a reduction in individual polyp size in the large intestine (table 1) and to a reduction in polyp number in the small intestine (table 1).

Effect of PPARα on proliferation, apoptosis, and necrosis

Fetal calf serum stimulated HCA7 cell proliferation, measured by [^{3}H]thymidine incorporation, by approximately threefold. Methylclofenapate inhibited this serum stimulated thymidine incorporation in a dose dependent manner (fig 5A). Methylclofenapate caused a time and concentration dependent reduction in cell number (fig 5B), amounting to 39.7 (4.1)% with 100 μM by day 5 ($p<0.001$). The proportion of cells in the

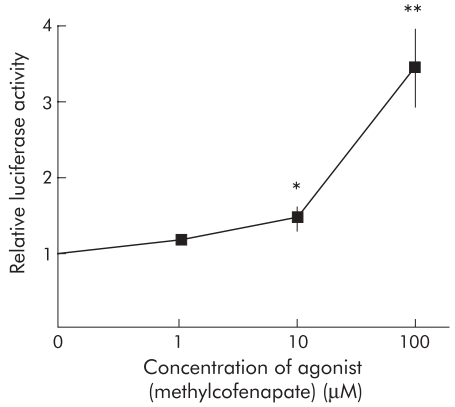

Figure 3 HCA7 cells were transfected with reporter plasmid PPRE-tk-luc (1.1 μg) and pRL-CMV (0.33 μg, as internal control) and exposed to the peroxisome proliferator activated receptor α (PPARα) ligand methylclofenapate or Wy14643 for 48 hours. Normalised luciferase activity is plotted as mean (SEM) fold activation relative to untreated cells. Methylclofenapate and Wy14643 significantly induced reporter gene activity at concentrations ≥10 μM, indicating the presence of functionally active endogenous PPARα. (*p<0.05, ** p<0.001).

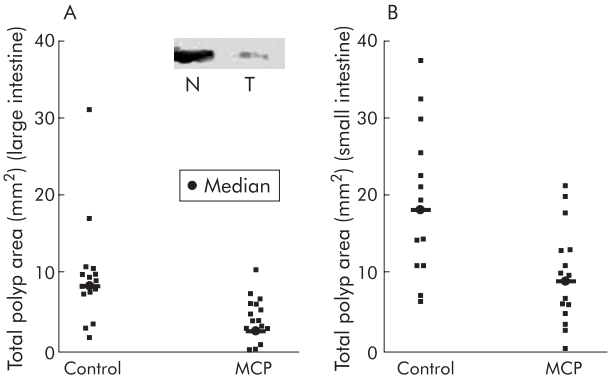

Figure 4 Peroxisome proliferator activated receptor α (PPARα) expression and reduction in total polyp area in APC^{Min}/+ mice by treatment with the PPARα activator methylclofenapate (MCP 25 mg/kg/day). (A) Large intestine; (B) small intestine. Each point represents polyp area in an individual mouse. Median values are also shown. N, normal; T, tumour.

G1 phase of the cell cycle (62.2 (2.6)% control; 63.5 (1.6)% with methylclofenapate 100 μM) and in G2 (11.8 (0.4)% control; 13.8 (3.0)% with methylclofenapate 100 μM) did not differ with treatment. Methylclofenapate had no significant effect on annexin V (137.3 (31.6)% of control values with methylclofenapate 100 μM) or propidium iodide (137.3 (32.3)%) staining.

DISCUSSION

In this study, we have shown a reduction in PPARα expression in 89% of 26 colorectal cancers (two of 12 in which mRNA was studied and one of 14 in which protein was studied). Methylclofenapate had a potent and relatively selective effect on epithelial PPARα activity and reduced the polyp burden in both the small and large intestine of APC^{Min}/+ mice. Functional studies suggested that PPARα activation reduced epithelial proliferation with no effect on apoptosis or necrosis. Our results as a whole strongly support a central role for PPARα activation in preventing neoplastic transformation or growth.

The role of PPARα in non-malignant colon is uncertain. It is present in normal human colonic mucosa from an early stage of development, suggesting its involvement in the establishment of lipid metabolism.[27] Its pattern of expression changes

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Table 1 Differences in individual polyp area and polyp number in treated and control $APC^{Min}/+$ mice at the time of sacrifice

	Large intestine		Small intestine	
	Control	Treated	Control	Treated
Individual polyp area (mm^2)	1.123 (0.690, 1.786)	0.848 (0.570, 1.265)**	0.710 (0.518, 1.050)	0.701 (0.496, 0.879)
Individual polyp area (mm^2) (corrected for time of death)†	0.085 (0.046, 0.121)	0.065 (0.042, 0.089)*	0.049 (0.037, 0.071)	0.051 (0.037, 0.069
Polyp No.	6.5 (4.1, 10.0)	4.0 (1.0, 7.0)*	24.0 (15.8, 30.3)	13.0 (5.0, 7.0)**
Polyp No (corrected for time of death)†	0.425 (0.308, 0.630)	0.308 (0.077, 0.540)	1.60 (1.080, 2.070)	0.920 (0.539, 1.167)**

*p<0.05, **p<0.01.
†Values at the time of death were divided by the weeks of life.

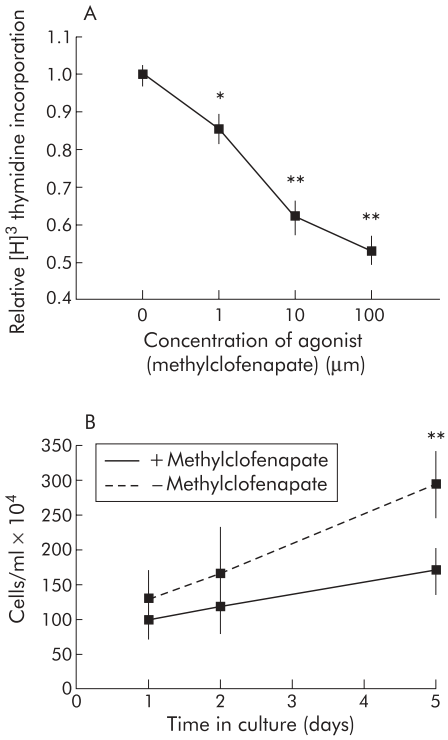

Figure 5 Effect of methylclofenapate on: (A) serum stimulated [^{3}H]thymidine incorporation; (B) HCA7 linear cell growth in the presence or absence of methylclofenapate 100μm. There was a significant reduction in thymidine incorporation after 24 hours of exposure to methylclofenapate, indicating an effect on DNA synthesis, and a significant reduction in cell number by day 5 (*p<0.05, **p<0.001).

with progression through embryogenesis, becoming increasingly confined to the nuclei of epithelial cells on the surface of villi, with adult mucosa exhibiting expression only in these cells.[27] In this study, we have shown that PPARα mRNA and protein expression become substantially depressed during carcinogenesis. The previous demonstration of reduced peroxisomal oxidative activity in neoplastic colonic mucosa[28] suggested the functional significance of reduction in PPARα expression levels that we observed. There was a substantially greater reduction in protein relative to mRNA expression, suggesting that repression of PPARα expression in human colorectal cancer may possibly occur at both the transcriptional and post-transcriptional levels. A reduction in PPARα protein expression was also observed in tumour tissue in $APC^{Min}/+$ mice.

We confirmed the presence of functionally active endogenous PPARα in colon cancer derived cell lines. The level of PPRE driven luciferase activation achieved by PPARα ligands was similar to that previously reported with PPARγ ligands in colon cancer cell lines.[29] The substantial stimulation of PPRE containing reporter gene expression achieved when PPARα expression vector was transfected in the absence of ligand suggests the presence of endogenous ligand(s), as has been determined previously for other cell lines.[30] Against this substantial background of endogenous activation, which has previously been reported,[30] methylclofenapate further increased transactivation by PPARα in a dose dependant fashion, confirming the potency of this isoform.

Although methylclofenapate may have pleiotrophic antitumour effects, this compound is a potent and selective PPARα agonist, and it is likely that the polyp reduction demonstrated in $APC^{Min}/+$ mice occurred, at least in part, due to PPARα activation. Methylclofenapate is clearly an efficacious activator of PPARα, although some loss of selectivity occurs at high concentrations. As PPARγ selective ligands have been shown in the $APC^{Min}/+$ mice model to have an opposite effect on colorectal carcinogenesis,[12 13] the effects seen in our study are more likely to be PPARα specific. This is supported by the earlier observation that bezafibrate (a less specific PPARα ligand) inhibits the formation of aberrant crypt foci, which are cited as precursor lesions for colon carcinoma, in rats.[19] Downstream effects of PPARα activation and their subsequent influence on tumour progression remain to be determined.

Our data suggest that PPARα activation by methylclofenapate significantly reduces serum stimulated mitogenesis, as measured by tritiated thymidine incorporation and linear cell growth. Preclinical toxicity studies suggest that the concentrations of methylclofenapate achieved in the animal study were equivalent to the higher concentrations employed in our in vitro work.[31] Methylclofenapate had no effect on cell death, as assessed by annexin V or propidium iodide staining or trypan blue exclusion. While annexin V staining may indicate processes other than apoptosis, the absence of any induction in annexin V or propidium iodide staining means that PPARα activation had no effect on any of the processes, including apoptosis, that are associated with such staining. One inference is that, while NSAIDs have been shown to compete with PPAR ligands,[11] it is likely that they increase apoptosis via effects on PPARα. How PPARα activation inhibits cellular proliferation is not clear. One possibility is that it occurs through effects on β oxidation[32] as PPARα is a key transcription factor in the regulation of both peroxisomal and mitochondrial β oxidation.[20] The fact that MCP treatment results in a reduction in polyp size rather than number in the colon is intriguing, particularly as it contrasts with effects seen in the small intestine (where polyp number is reduced). It suggests that in the colon, MCP reduces polyp cell proliferation whereas in the small intestine it may affect actual tumour initiation. These apparent tissue specific effects of MCP may be attributable to differences in PPARα expression, as demonstrated in the small and large intestine.

Although toxicity studies have led to termination of the development of methylclofenapate,[31] our studies suggest that

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identification and investigation of other PPARα ligands as possible anticancer agents would be justified. Moreover, in view of the importance of dietary fat in the development of colorectal cancer and the key role of PPARs in fat metabolism, our data may assist in both understanding the process of malignant transformation and highlighting potential therapeutic strategies.

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Authors' affiliations

L Jackson, S A Watson, T Morris, J A Smith, C J Hawkey Wolfon Digestive Diseases Centre, University of Nottingham, UK
W Wahli, L Michalik, Institut de Biologie Animale, Universite de Lausanne, Batiment de Biologie, Lausanne, Switzerland
K Anderton, A Bennett School of Biomedical Sciences, Queen's Medical Centre, Nottingham, UK
D R Bell School of Life Sciences, University of Nottingham, UK

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