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Title	Inhibition of protein kinase C (PKC) induces apoptosis in COLO 205 human colon carcinoma cells
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NUCLEAR FACTOR KAPPA B (NFκB) AND DIFFERENTIATION OF HUMAN INTESTINAL EPITHELIUM.

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Regulation of cytodifferentiation is crucial to healing in inflammatory bowel disease (IBD), but mechanisms are unclear. NFκB is a therapeutic target in IBD and is a key regulatory transcription factor of diverse genes. We test the hypothesis that NFκB regulates human intestinal cytodifferentiation.

Methods. Normal human fetal intestinal epithelium (HFIE) was cultured then transfected with a NFκB luciferase reporter construct (3enh-kb-CONA-Luc) incorporating 3 copies of a NFκB dependent promoter or control empty vector (CONA-Luc). NF-κB activation was stimulated by tumour necrosis factor alpha (TNFα) while inhibition was achieved by caffeic acid phenethyl ester (CAPE), a potent and specific inhibitor. NF-κB activation was assessed by luciferase activity. Effects on cytodifferentiation were assessed by reverse transcriptase polymerase chain reaction assay (RT-PCR) of HFIE for the cystic fibrosis transporter (CFTR) and alkaline phosphatase (Alk Phos), markers of crypt and villus epithelium respectively. An amino acid transporter (hATB°) common to both cell types was also assessed.

Results TNFα stimulated NF-κB activation in HFIE. CAPE (20μg/ml) inhibited NF-κB activation by approximately 70% against control and gave a dose dependent inhibition of CFTR and Alk Phos expression. CAPE had no effect on hATB°. CAPE also promoted apoptosis in intestinal epithelium.

Conclusions. NFκB exerts an important regulatory role in intestinal epithelial differentiation and apoptosis. Anti NFκB therapy in IBD may influence cytodifferentiation.

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THE IMPORTANCE OF APOPTOSIS AND p53 EXPRESSION UPON THE INTESTINAL TOXICITY INDUCED BY TOMUDEX IN BALB/C AND DBA/2 MICE.

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Background: Tomudex (ZD1694) is a novel quinazoline pure thymidylate synthase (TS) inhibitor that has been assessed in phase III trials in colorectal cancer. Tomudex induces much more intestinal toxicity, manifested as diarrhoea and weight loss in one inbred mouse strain, Balb/c than another, DBA/2. No pharmacokinetic or pharmacodynamic reason for this has yet been established. We have previously investigated the importance of acute induction of apoptosis upon the histopathological gut damage induced by another TS inhibitor, 5-fluorouracil (5-FU) and have shown that this response was strongly dependent upon p53 expression.
Aims: (1) To establish whether the different sensitivities of Balb/c and DBA/2 mouse intestines to Tomudex are related to the susceptibilities of their intestinal mucosae to undergo apoptosis. (2) To determine whether p53 expression, a critical factor in 5FU-induced intestinal apoptosis and toxicity also modulates the response of intestinal epithelium to Tomudex.
Methods: 10mg/kg or 100mg/kg Tomudex was administered as single or double intraperitoneal injections 24h apart to groups of four Balb/c, DBA/2 and *p53*-/- mice. Mouse survival and weights were recorded and groups of mice were sacrificed at times ranging from 12h to 10 days. H & E stained sections of small intestine and colon were analysed for apoptosis and mitosis on a cell positional basis. Crypt and villus cell counts were used to indicate histological gut damage and hence drug toxicity.
Results: Balb/c mice developed diarrhoea and weight loss following 100mg/kg x2 Tomudex, which was absent in DBA/2 mice. Balb/c mice were also more sensitive than DBA/2 to induction of small intestinal and colonic apoptosis 24h following 100mg/kg Tomudex. Both strains showed histopathological damage to the small intestine after 100mg/kg x2 Tomudex, but only Balb/c mice demonstrated colonic damage. *p53*-null (-/-) mice showed the same level of intestinal apoptosis 24h following 100mg/kg Tomudex and also the same levels of intestinal toxicity 3, 5 and 7 days after 100mg/kg x2 Tomudex, as their wild-type counterparts.
Conclusions: Following Tomudex administration, Balb/c mice were more susceptible to induction of intestinal apoptosis than DBA/2 and also demonstrated more histopathological damage in the colon, correlating with induction of diarrhoea and weight loss. In contrast to 5-FU, the apoptosis and toxicity induced by Tomudex was p53-independent.

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ALLELE-SPECIFIC mRNA ASSESSMENT IN COLONIC MUCOSA IN ULCERATIVE COLITIS: ASSOCIATION OF THE INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE ALLELE 2 WITH REDUCED STEADY-STATE mRNA LEVELS

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Background: Association studies have identified the allele 2 of the Interleukin-1 receptor antagonist (IL-1ra) gene (IL-1RN) variable number of tandem repeats (VNTR) polymorphism as a marker of genetic susceptibility and disease extent in ulcerative colitis (UC). The allele has been correlated with reduced expression of IL-1ra in the colonic mucosa.

Aims: To analyse allele-specific IL-1ra mRNA accumulation in the colonic mucosa of patients with UC.

Methods: Rectal mucosal biopsies were obtained from 7 patients with UC who were heterozygous for the IL-1RN (VNTR) polymorphism and for the single base polymorphism (C/T at +2018) in exon 2 of the IL-1RN gene. These markers are 100% in linkage disequilibrium. We used the +2018 polymorphism as an exonic marker to recognise mRNA copies which had been transcribed from either allele of the IL-1RN gene. After RNA extraction allele-specific mRNA accumulation was performed by reverse transcription followed by PCR with excess ^{32}P-end-labelled reverse primer being added for the final cycle. Allele-specific restriction digested products were electrophoresed on 9% polyacrylamide gels which were dried and analysed on a Biorad G-250 molecular imager. Data was expressed as the ratio of allele 1 / allele 2 mRNA and statistically compared using the Wilcoxon signed rank test.

Results:

Patient Number	1	2	3	4	5	6	7
mRNA: Allele 1 /Allele 2	1.65	1.37	3.01	1.25	1.20	1.39	1.17

The ratio of allele 1 mRNA / allele 2 mRNA was greater than 1 in all cases and results were significant (Z-value = -2.366; P = 0.018).

Conclusions: This is the first study to show that the allele 2 of the IL-1RN VNTR polymorphism is associated with reduced accumulation of mRNA for the anti-inflammatory cytokine IL-1ra in the colonic mucosa.

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INHIBITION OF PROTEIN KINASE C (PKC) INDUCES APOPTOSIS IN COLO 205 HUMAN COLON CARCINOMA CELLS

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In order to elucidate the reasons for discrepant results in studies of growth inhibition and apoptosis with PKC and in particular PKC-δ, we have used a human colon carcinoma cell line, COLO 205, to compare the actions of a pan-PKC activator (TPA) and pan-PKC inhibitor (chelerythrine chloride), and of PKC-δ selective activator (bistratene A, BisA) and inhibitor (rottlerin), upon apoptosis. Apoptosis was assayed by 1) DNA fragmentation of floating cells (apoptotic fraction) and 2) cell cycle analysis using fluorescence activated cell sorting (FACS) on both attached and floating cells stained with propidium iodide (PI). Regulation of PKC–δ protein expression was assessed by western immunoblotting. Immunoprecipitated PKC–δ was used in kinase assays with [γ^{33}P] labelled ATP and histone H1 as substrate. Both TPA and chelerythrine chloride induced DNA fragmentation and increased the percentage of PI-stained cells in the sub-G1 peak, with 23.7% at 50nM and 11% increase at 10μM respectively. BisA and rottlerin, also induced apoptosis, 9.1% at 140nM and 17.4% at 1μM respectively. Additive effects were also seen with a combination of TPA with rottlerin. Pan-activators and inhibitors induced DNA fragmentation within 2-3h, compared with 9h for the PKC-δ-specific activator and inhibitor. None of the treatments depleted PKC–δ in the attached cells at any time from 3 to 24h but an inactive fragment of 46kDa appeared in the apoptotic fraction and correlated with the onset of apoptosis induced by all substances. Increased PKC–δ activity was observed 15 min following treatment with BisA or TPA, and then inhibition at 1h and 2h respectively. Caspase-3 inhibitors Ac-DEVD-CMK or Ac-DEVD-CHO did not block TPA- or chelerythrine-induced apoptosis and did not prevent PKC–δ proteolysis. Since all treatments induced apoptosis and their effects were additive, PKC–δ was not activated but inhibited during apoptosis. Caspase-3 inhibitors did not block either apoptosis or PKC–δ proteolysis. We conclude that PKC-δ inhibition rather than activation is associated with apoptosis in COLO 205 cells. Furthermore since TPA and chelerythrine are more rapid in their effects than specific PKC-δ inhibition we conclude that PKC-δ inhibition in isolation is not sufficient to induce apoptosis. Other PKC isoenzyme(s) may be involved in triggering apoptosis.