The Effect of Cannabinoids on Cytokine Evoked Human Colonic Mucosal Damage and Caco-2 Epithelial Permeability

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Abstract

Inflammatory bowel disease (IBD) is a disease characterised by two subtypes, ulcerative colitis (UC) and Crohn's disease (CD). Both conditions can lead to inflammation and ulceration of the gastrointestinal mucosa. Treatments are available for IBD, however they can cause severe adverse effects and may not be useful in all patients. As a result, there is still an unmet need for novel IBD treatments. In animal models of colitis, cannabinoid (CB) agonists have shown efficacy in reducing inflammation. To further investigate this, we used a human colonic mucosal explant model to determine if CB agonists could attenuate mucosal damage. To induce damage in colonic mucosa, pro-inflammatory cytokines (that are elevated in IBD patients) were used. These included a combination of TNF- α + IL-1 β and in other studies, IL-17A. Furthermore, we also tested if these cytokines modulated biochemical markers of inflammation. Immunohistochemistry was used to determine the identity of immune cells in the lamina propria of the mucosa and also localisation of IL-17A.

Treatment of colonic mucosa with TNF- α + IL-1 β induced damage characterised by luminal epithelial loss, crypt destruction and increased lymphocyte density. In addition, elevations in nitrite levels were found in TNF- α + IL-1 β treated explants compared to controls. These damage parameters were attenuated by treatment with CB2R agonists. We found that PGE₂ concentration was significantly decreased after TNF- α + IL-1 β incubation suggesting reductions in PGE₂ may partially mediate mucosal damage.

IL-17A also induced a course of mucosal damage similar to that observed with TNF- α + IL-1 β treatment, however no increase in lymphocyte density occurred. In this study, damage was reduced by the endocannabinoid anandamide as well as cannabidiol. We did not determine whether this effect was CB1R or CB2R mediated. Nitrite concentrations

were not elevated after IL-17A treatment, however increased matrix metalloprotease activity was detected, suggesting this may mediate IL-17A induced mucosal damage. ELISA and western blotting was used to determine if the TNF- α + IL-1 β combination we previously studied could influence IL-17A levels. There was no significant change in IL-17A expression, however basal expression of IL-17A was found in human colonic mucosa. This was confirmed by immunohistochemistry, showing extensive expression of IL-17A, particularly at the edge of the lumen. Therefore, IL-17A may also play a homeostatic or protective role against micro-organisms in the human colon.

Cell culture studies examined the effects of cytokines and cannabinoids on Caco-2 epithelial permeability. In IBD, it has been established that increased mucosal permeability contributes to inflammation. TNF- α + IL-1 β increased epithelial permeability; however this was not attenuated by CB ligands. IL-17A did not induce any significant increases in permeability.

In conclusion, this thesis demonstrates that CB2R agonists may be useful in attenuating damage in human colonic mucosa induced by cytokines. Therefore, CB2R agonists may have utility as novel therapeutics in IBD. In addition, IL-17A which can be damaging in this model is also expressed in healthy human colonic mucosa, suggesting a homeostatic or protective role. It may be the case that excessive expression of IL-17A in IBD contributes to inflammation.

Declaration

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Date

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Author Contributions

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Author Contributions

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Abbreviations

ABC avidin biotin complex

AEA anandamide

ACEA arachidonoyl 2'-chloroethylamide

AIN-457 secukinumab

2-AG 2- arachidonoylglycerol

5-ASA 5-aminosalicyclic acid (mesalazine)

BCA bicinchoninic acid

BrdU 5-bromo-2'-deoxyuridine

BSA bovine serum albumin

CAC colitis associated cancer

CD Crohn's Disease

CBD cannabidiol

COX cyclooxygenase

CB1R cannabinoid 1 receptor
CB2R cannabinoid 2 receptor

CNS central nervous system

DAB 3,3-diaminobenzidine tetrachloride

DAN 2,3-diaminonapthaline

DNBS 2,4 dinitrobenzene sulphonic acid

DSS dextran sodium sulphate

EC enterochromaffin

EFS electrical field stimulation

ELISA enzyme-linked immunosorbent assay

ENS enteric nervous system

EGC enteric glial cell

FAAH fatty acid amide hydrolase

FCS foetal calf serum

GALT gut associated lymphoid tissue

GI gastrointestinal

GPR G-protein coupled receptor

GWAS Genome wide association studies

H&E haematoxylin and eosin

HBD-1 human beta defensin 1

HCAEC human coronary artery endothelial cells

5-HT 5-hydroxytryptamine, serotonin ICAM-1 intracellular adhesion molecule 1

IBD inflammatory bowel disease

IL-1β interleukin 1 beta

IL interleukin

IELs intraepithelial lymphocytes

IFN-γ interferon gamma

IHC immunohistochemistry

iNOS inducible nitric oxide synthase

LPLs lamina propria lymphocytes

LPS lipopolysaccharide

MMP matrix metalloprotease

mRNA messenger ribonucleic acid

MLCK myosin light chain kinase

MPO myeloperoxidase

mAb monoclonal antibody

MAGL monoacylglycerol lipase

NO nitric oxide

NOD2 nucleotide-binding oligomerisation domain 2

NF-κB nuclear factor kappa B

NSAID non-steroidal anti-inflammatory drug

OM oil of mustard

PBS phosphate buffered saline PCR polymerase chain reaction

PPAR-γ peroxisome proliferator-activated receptor gamma

PMA phorbol 12-myristate 13-acetate

PMN polymorphonuclear neutrophil granulocytes

PMSF phenylmethylsulfonyl fluoride

PVDF polyvinylidene fluoride

RA rheumatoid arthritis

SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis

TLR-4 toll like receptor 4

 Δ^9 -THC Δ^9 -tetrahydrocannabinol

TNF-α tumour necrosis factor alpha

Th T-helper cell

TNBS 2,4,6 trinitrobenzine sulphonic acid

TEER trans epithelial electrical resistance

TGF transforming growth factor

TBST tris buffered saline with Tween- 20

TLC thin layer chromatography

UC ulcerative colitis

ZO-1 Zonula occludens 1