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-1β 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-1β 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-1β treated explants compared to controls. These damage parameters were attenuated by treatment with CB2R agonists. We found that PGE_2 concentration was significantly decreased after TNF-α + IL-1β incubation suggesting reductions in PGE_2 may partially mediate mucosal damage.

IL-17A also induced a course of mucosal damage similar to that observed with TNF-α + IL-1β 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-1β 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-1β 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

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution in my name and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Benjamin Scott Harvey **Date**

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Statements of Authorship

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Author Contributions

By signing the statement of Authorship, each author certifies that their stated contribution to the publication is accurate and permission is granted for the publication to be included in the candidate's thesis.

Mr. Benjamin Harvey conducted experiments, interpreted and analysed data, prepared graphical representations of data and wrote the draft manuscript for each section.

Signature …… Date……

Ms. Lauren Nicotra conducted experiments, analysed data and assisted with manuscript evaluation.

Signature …… Date…14/03/14…

Ms. Megan Vu conducted experiments and assisted with manuscript evaluation.

Signature …… Date…14/3/14……

Dr. Scott Smid supervised the development of work and experimental design. Assisted in data interpretation and critical manuscript evaluation. Acted as the corresponding author.

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Mr. Benjamin Harvey performed all experiments and analysed and interpreted data. Wrote the draft manuscript for each section and assisted with preparation of the revised manuscript.

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Dr. David Wattchow performed the colorectal surgery to obtain mucosal samples and assisted with manuscript evaluation and feedback.

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Dr. Tiong Sia assisted with mucosal sample collection and assisted with manuscript evaluation and feedback.

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Dr. Scott Smid supervised development of work and experimental design, assisted in data interpretation and manuscript evaluation. Acted as the corresponding author

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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