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Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability

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ABSTRACT

Cannabinoid receptor activation is protective in animal colitis models. We sought to investigate if cannabinoids attenuated colitis-like tissue damage in human colonic specimens, with the hypothesis that cannabinoids would be protective in a cytokine-driven model of human colonic mucosal damage. Healthy human colonic mucosa was incubated with pro-inflammatory cytokines TNF-α and IL-1β to elicit colitis-like tissue damage. The cytokine-driven increase in scored crypt and mucosal damage and lymphocyte density was attenuated with concomitant hydrocortisone pretreatment. The cannabinoid receptor 2 (CB2) receptor-selective agonist JWH-015 significantly reduced colitis scores following cytokine incubation, as evidenced by a reduction in mucosal crypt and luminal epithelial damage and lymphocyte density in the lamina propria. The effect of JWH-015 was reversed in the presence of the CB2 receptor inverse agonist JTE-907. Anandamide was also protective in the cytokine-incubated explant colitis model in a manner reversible with JTE-907, while CB1 receptor agonism with ACEA was without effect. TNF-α and IL-1β together evoked an increase in paracellular epithelial permeability in Caco-2 cell monolayers over 48 h of incubation. However, neither CB2 nor CB1 receptor activation altered the cytokine-evoked increase in permeability. These findings support a discrete role for CB2 receptors in the attenuation of detrimental pro-inflammatory cytokine-mediated mucosal damage in the human colon without directly affecting mucosal epithelial barrier function.

1. Introduction

Cannabinoids (CBs) have demonstrated anti-inflammatory effects in the gastrointestinal tract in animal models of colitis [1]. Cannabinoid receptor 1 (CB1) knockout mice develop a significantly worsened form of experimental colitis when compared to wild type mice, indicating an acute requirement of CB1 receptors for protection from colitis [2]. Activation of cannabinoid receptor 2 (CB2) also inhibits both the extent of inflammatory damage and the accelerated gastrointestinal transit that occurs in experimental colitis [3,4]. Endocannabinoids also reduce pro-inflammatory cytokine expression in immune cells and colonic biopsies from inflammatory bowel disease (IBD) patients [5], suggesting a direct immune-modulatory role in this anti-inflammatory effect. However, the cannabinoid pharmacology underlying such an anti-inflammatory action has yet to be clearly established in the human colon, which is relevant when considering the many possible non-cannabinoid receptor-mediated actions of endocannabinoids in the gut [6]. In addition, the role of endocannabinoids in modulating epithelial barrier function and mucosal integrity is uncertain, as endocannabinoids and other cannabinoid ligands may have opposing effects on epithelial permeability *in vitro* [7,8].

In this study we developed a colitis explant model using healthy human colonic mucosal tissue incubated with the major proinflammatory cytokines TNF-α and IL-1β, known to be elevated in IBD with a capacity to induce mucosal chemokine expression in human colonic mucosa [9]. In this setting we measured indices of epithelial and crypt damage and lymphocyte numbers and investigated the effects of anandamide (AEA) and cannabinoid receptor-selective ligands on cytokine-evoked damage. In addition, we investigated whether pro-inflammatory cytokines such as TNF-α and IL-1β may mediate direct effects on epithelial permeability and if so, whether cannabinoid ligands may alter such changes in epithelial barrier function.

2. Materials and methods

2.1. Human colonic tissue collection

Experiments were performed using human colonic tissue taken from patients admitted to the Royal Adelaide Hospital with

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colorectal carcinoma as the primary indication for surgery. Approval was provided by the Royal Adelaide Hospital Human Research Ethics Committee for the study and informed patient consent given in all cases. Full-thickness specimens from descending and sigmoid colon were taken from up to 23 consenting patients (14 male, 9 female). Resections were transferred to pathology services on ice with no more than 30 min elapsing between resection removal and specimen collection. Specimens were placed in ice-cold carbogenated (95% O_2, 5% CO_2) Krebs solution and rapidly transferred on ice to the laboratory for further dissection. Only specimens obtained from macroscopically healthy tissue within the colon were utilised and confirmed via subsequent histological examination as free from neoplasia, fibrosis or inflammation.

2.2. Explant colitis model

Full-thickness colonic specimens were pinned in Sylgard-coated Petri dishes containing carbogenated Krebs solution at 4 °C. Mucosa and submucosa was dissected away from the muscularis propria. Colonic mucosal specimens containing intact submucosa were placed into separate Petri dishes containing 5 mL cell culture medium (RPMI 1640), bovine serum albumin (BSA) (0.01%), penicillin (100 U/mL), and streptomycin (100 μg/mL). Media was perfused with carbogen (95% O_2, 5% CO_2) via manifold inlets into each well. Mucosal specimens were then incubated at 37 °C for 20 h on an orbital mixing platform in a temperature-controlled chamber (OM11, Ratek Instruments, Victoria, Australia), alone or with cytokine and/or drug interventions. At the completion of the incubation period, tissue was immediately placed into neutral buffered formalin for histological processing and assessment and scoring of mucosal damage and lymphocyte numbers. Media was also retained for subsequent measurement of nitrite concentration, a biochemical marker of nitric oxide production accompanying inflammatory states.

2.3. Epithelial permeability measurements

Caco-2 cells (passage 40–70) derived from human colorectal carcinoma were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were maintained in 75 cm^2 tissue culture flasks (Corning Life Sciences, Lowell, MA, USA) at 37 °C with 5% CO_2. Cell culture media was DMEM (Invitrogen, Mulgrave, VIC, Australia) supplemented with 10% foetal calf serum (Sigma Aldrich, Castle Hill, NSW, Australia), 1% penicillin/streptomycin solution, 1% non-essential amino acids solution and 1% sodium pyruvate (complete DMEM) (Invitrogen, Mulgrave, VIC, Australia).

When cells reached 80% confluence cells were washed in phosphate buffered saline (PBS) pH 7.4 and trypsin EDTA (Invitrogen, Mulgrave, VIC, Australia) was added and incubated with the cells for 5 min to detach cells. Cells were then centrifuged and resuspended in complete DMEM. Experiments were carried out using Corning Costar Transwell Inserts (6.5 mm diameter, 0.4 μm pore size, tissue culture treated polyester membrane) (Corning Life Sciences, Lowell, MA, USA). The basolateral compartment was filled with 0.6 mL of complete DMEM containing 1% amphotericin B (Sigma Aldrich, Castle Hill, NSW, Australia). All subsequent media used in the Transwell plate was supplemented with 1% amphotericin B to avoid fungal contamination. Cells were seeded at a density of 16500 cells per insert in 0.1 mL of media in the apical compartment. An insert on the plate was left blank and contained media only. The Transwell plate was then incubated at 37 °C and 5% CO_2 for 6 h before having the media in the apical compartment removed and replaced with 0.1 mL of fresh media, to prevent excessive clumping of cells [10]. Media in the apical and basolateral well was replaced every 2–3 days for up to 25 days as the cells reached confluence and differentiated. During the period of cell growth the trans-epithelial electrical resistance (TEER) was measured using the EVOM2 epithelial voltohmeter with chopstick electrode (World Precision Instruments, Sarasota, FL, USA) to determine the integrity of the monolayer. The formula: *TEER Monolayer (cm^2) = [TEER total (Ω) − TEER blank (Ω)] × 0.33 (cm^2)* was used to calculate TEER. Inserts with TEER values over 500 Ω/cm^2 were used in experiments. Before TEER measurements, media was replaced with complete DMEM without amphotericin B. TEER was measured to obtain baseline TEER values after allowing the plate to equilibrate to room temperature for 5 min.

2.4. Cytokine and cannabinoid treatments

Human colonic mucosal explant samples were incubated with pro-inflammatory cytokines TNF-α and IL-1β (both at 10 ng/mL) for 20 h. ELISA assays for both TNF-α and IL-1β in media following incubation indicated less than 20% loss of cytokines over 20 h. Cytokine-incubated samples were compared against incubation controls consisting of adjacent tissue sections minus cytokine and drug incubations and including vehicle (0.1% ethanol) where appropriate. Incubation controls were also compared with non-incubated mucosal specimens retained in Krebs solution at 4 °C for 20 h, to indicate overall integrity of mucosal sections as they were obtained from resective procedures. Colonic mucosal explant specimens were removed from the incubation media at 20 h post-incubation and then fixed in 10% neutral-buffered formalin solution overnight prior to processing for histological assessment.

Mucosal specimens incubated with cytokines (TNF-α and IL-1β (10 ng/mL), 20 h) were compared with cytokine-incubated mucosa concomitantly treated with the following cannabinoid ligands: the endocannabinoid anandamide (10^{-6} M) or JWH-015 (CB2 receptor-selective agonist, 10^{-7} M) ± JTE-907 (CB2 receptor-selective inverse agonist, 10^{-7} M), or arachidonoyl chloroethylamide (ACEA, CB1 selective agonist, 10^{-7} M). Drug concentrations were chosen based on previous functional studies using human colonic tissue [11]. Additional studies using lipopolysaccharide (LPS, 1 μg/mL) or hydrocortisone (10^{-7} M) were performed to investigate additional potential inflammatory and anti-inflammatory stimuli on cytokine-treated mucosal samples. Appropriate incubation controls (minus cytokines) and drug controls were also performed.

Confluent Caco-2 cells were treated with both human recombinant TNF-α and IL-1β each at a concentration of 100 ng/mL, which produced optimal and significant TEER reductions in preliminary studies when compared to 10 ng/mL cytokine concentrations. All cytokine treatments and vehicle (PBS) were applied to the basolateral compartment of the Transwell. TEER measurements were taken at the time points of 5, 24 and 48 h. The TEER of each well was measured after allowing the temperature of the Transwell plate to equilibrate to room temperature for 5 min. For cannabinoid drug interventions, Caco-2 cells were treated basolaterally with either anandamide (AEA; 10^{-5} M), ACEA (10^{-6} M), JWH-015 (10^{-6} M) or the phytocannabinoid cannabidiol (CBD; 10^{-5} M), utilising concentrations based on previous studies in either colonic tissue [12] or Caco-2 cells [7]. Caco-2 cells were pretreated with CB ligands for 15 min prior to cytokine additions to allow for drug equilibration. Each CB ligand was also tested without cytokines to control for any direct effects on epithelial permeability.

2.5. Assessment of mucosal damage and inflammation

Mucosal damage and lymphocyte numbers in the lamina propria were determined via histological analysis following haematoxylin and eosin staining of paraffin-embedded sections. Scoring was performed by the same assessor in order to maintain consistency and the investigator was blinded to the sample groups.