The Use of Cannabinoids in Colitis: A Systematic Review and Meta-Analysis

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Background: Clinical trials investigating the use of cannabinoid drugs for the treatment of intestinal inflammation are anticipated secondary to preclinical literature demonstrating efficacy in reducing inflammation.

Methods: We systematically reviewed publications on the benefit of drugs targeting the endo-cannabinoid system in intestinal inflammation. We collated studies examining outcomes for meta-analysis from EMBASE, MEDLINE and Pubmed until March 2017. Quality was assessed according to mSTAIR and SRYCLE score.

Results: From 2008 papers, 51 publications examining the effect of cannabinoid compounds on murine colitis and 2 clinical studies were identified. Twenty-four compounds were assessed across 71 endpoints. Cannabidiol, a phytocannabinoid, was the most investigated drug. Macroscopic colitis severity (disease activity index [DAI]) and myeloperoxidase activity (MPO) were assessed throughout publications and were meta-analyzed using random effects models. Cannabinoids reduced DAI in comparison with the vehicle (standard mean difference [SMD] -1.36; 95% CI, -1.62 to-1.09; I^2 = 61%). FAAH inhibitor URB597 had the largest effect size (SMD -4.43; 95% CI, -6.32 to -2.55), followed by the synthetic drug AM1241 (SMD -3.11; 95% CI, -5.01 to -1.22) and the endocannabinoid anandamide (SMD -3.03; 95% CI, -4.89 to -1.17; I^2 not assessed). Cannabinoids reduced MPO in rodents compared to the vehicle; SMD -1.26; 95% CI, -1.54 to -0.97; I^2 = 48.1%. Cannabigerol had the largest effect size (SMD -6.20; 95% CI, -9.90 to -2.50), followed by the synthetic CB_1 agonist ACEA (SMD -3.15; 95% CI, -4.75 to -1.55) and synthetic $CB_{1/2}$ agonist WIN55,212-2 (SMD -1.74; 95% CI, -2.81 to -0.67; I^2 = 57%). We found no evidence of reporting bias. No significant difference was found between the prophylactic and therapeutic use of cannabinoid drugs.

Conclusions: There is abundant preclinical literature demonstrating the anti-inflammatory effects of cannabinoid drugs in inflammation of the gut. Larger randomised controlled-trials are warranted.

Key words: cannabinoid, inflammation, gut, intestine, colitis

INTRODUCTION

Inflammatory bowel disease (IBD) affects 200 per 100,000 adults in the United States and 400 per 100,000 in the United Kingdom.[1, 2] Major subtypes consist of Crohn's disease and ulcerative colitis. A definitive clinical treatment for these chronic relapsing diseases remains elusive, as currently no therapy exists to reverse the clinical pathology without a risk of significant side effects. Corticosteroids, 5-ASA agents, anti-TNFα antibodies, and other immunomodulatory drugs have all been shown to induce significant remission in IBD, but are associated with bone marrow suppression, opportunistic infection, infusion reactions, and malignancy secondary to immunosuppression.[3–5]

The endocannabinoid system (ECS), consisting of multiple receptors and endogenous ligands, controls multiple homeostatic processes including gastrointestinal motility, hunger, perception of pain, and immunity.[6–10] The targets of the ECS consist of the classical CB_1 and CB_2 receptors, but also the orphan GPR55 receptor, peroxisome proliferator-activated receptors (PPARs), and transient receptor potential vanilloid (TRPV) receptors. These targets are all found on the cells of gut mucosa, submucosa, the enteric nervous system and the immune system. Endocannabinoids, such as anandamide (AEA) and 2-arachiodoylglycerol (2-AG), are intercellular

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Abbreviations: 2-AG, 2-arachidonoyl glycerol; Ab-CBD, Abnormal cannabidiol; AEA, Anandamide; CBD, Cannabidiol; CBG, Cannabigerol; CBN, Cannabinol; CI, Confidence interval; CO, Croton oil; DAI, Disease activity index; DNBS, Dinitrobenzene sulphonic acid; DSS, Dextran sulphate sodium; IC, Intracolonic; IL-10, Interleukin-10; IV, Intravenous; MMJ, Medicinal cannabis; MPO, Myeloperoxidase; OM, Oil of mustard; PEA, Palmitoylethanolamide; PO, Oral; PPAR, Peroxisome Proliferator Activating Receptor; PR, Per rectum; SC, Subcutaneous; SMD, Standard mean difference; THC, Δ^9-Tetrahydrocannabinol; TNBS, Trinitrobenzene sulphonic acid; TRPV1, Transient receptor potential vanilloid 1

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lipid-signalling molecules derived on demand from membrane precursors.[11] They are metabolised by fatty acid amide hydrolase (FAAH), as well as N-acyl ethanolamine-hydrolysing acid amidase (NAAA) in the case of AEA, and monoacylglycerol lipase (MAGL) in the case of 2-AG.[12–14] Palmitoylethanolamide (PEA), also metabolised by NAAA, has been shown to activate PPARα and may increase local concentrations of AEA or the affinity of AEA to the CB_1 receptor and, therefore, is included as an atypical cannabinoid.[15, 16] Phytocannabinoids include Δ-9 tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannibichromene (CBC), and up to 60 others and are isolated from *Cannabis Sativa*.[11] THC and CBD have found place in clinical practice in the treatment of childhood epilepsy and muscular spasticity in multiple sclerosis.[17, 18] A growing collection of synthetic cannabinoid agonists have been derived possessing selective high affinity for the CB_1, CB_2, GPR55 and TRPV1 receptors, and have been investigated pre-clinically for roles in gut motility, satiety and immunity.[8]

Under inflammatory conditions CB_1, CB_2, and both PPARα and PPARγ expressions increase on the submucosa and on adjacent immune cells, whereas GPR55 and TRPV1 expression decreases on the mucosa, but increases on enteric nervous tissue.[19–21] Levels of AEA, 2-AG, and PEA are upregulated in vitro, and also in animal in vivo and human ex-vivo models of intestinal inflammation.[22–24] Early experimentation in murine models demonstrated that cannabinoids prevent the onset of experimental murine colitis or reduced its severity.[25] Since these initial findings, many reports—including clinical trials—have now investigated the effect of cannabinoid ligands, or the effect of blockade of their metabolising enzymes, on inflammation of the gut.

There is a significant amount of promising preclinical evidence for the use of cannabinoid agents in the treatment of colitis. Within this study we aimed to gather all preclinical and clinical evidence for the use of these drugs in colitis, and where possible, perform meta-analyses across studies to assess the efficacy of cannabinoids for further clinical trials. Where possible, clinically relevant experimental endpoints were assessed.

METHODS

Search Strategy

All studies evaluating the effect of cannabinoid drugs on inflammation of the colon were searched from March 1980 to March 2017 by 2 independent researchers in Medline, EMBASE, and Pubmed. Keywords included cannabidiol, tetrahydrocannabinol, anandamide, 2-AG, cannibichromene, cannabigerol, cannabinoid, cannabis sativa, colon, intestine, gut, inflammation, Crohn's, ulcerative, and colitis. Names of synthetic cannabinoid agents were also included. References from included studies were searched by hand. Prespecified inclusion and exclusion criteria were used to prevent bias. Experiments must have been performed in the context of administration of cannabinoid drugs to inflammatory states of the colon in humans or animals, either experimental or due to endogenous disease (Crohn's disease or ulcerative colitis). In vitro studies, studies not examining the effect of cannabinoids in intestinal inflammation specifically, or studies using cannabinoid antagonists as a primary agent were excluded. A PRISMA checklist is included in the appendix.

Data Acquisition

The mode of colitis induction in preclinical studies was recorded in addition to the timing of cannabinoid application. For the purposes of meta-analysis, data on the macroscopic or histological disease scores (as listed in the disease activity index [DAI]) and myeloperoxidase (MPO) activity were collected. If the exact number of animals was not available, the lowest number of animals within the range given was used for the experimental group, and the highest number used for the control/vehicle group. Where studies reported the effects of more than 1 cannabinoid sharing a single control group for comparison, control group numbers were equally distributed between comparisons to avoid unit of analysis issues. WebPlotDigitiser (version 3.11) was used to extract values from figures in published articles where no data values were given in the text.

Quality

Quality of included studies were assessed by 2 independent researchers to quantify risk of bias according to the 6-point criteria developed by the Cochrane Collaboration risk of bias tool.[26] To assess the quality of preclinical studies, the STAIR and Arrive preclinical assessment tools were adapted.[27, 28] Each of these items was awarded 1 point: randomization, assessor blinding, results replicated in a second species, dose-response experiments, results replicated in a second model of colitis, $n = 5$ or greater in each group, the use of clinically relevant endpoint to assess response of colitis, a definitive statement of animal numbers in each group, a statement regarding the housing of animals, and a statement describing the location and timing of animal experimentation (i.e. in animal housing or a separate cage, time of day etc). The highest possible score was 10 points.

Data Analysis

Where possible, data were grouped into DAI and MPO activity, and subdivided by species and compound. Data from each group were analysed as forest plots using Cochrane Review Manager Software (Review Manager 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), and as funnel plots using Stat (Stat Corp. 2009 Stat Statistical Software: Release 11. College Station, TX, USA). Funnel plot asymmetry was tested using the Egger linear regression test. A P value of <0.05 was considered statistically significant. As differing studies measured MPO activity and DAI using various scales, we present effect estimates as standardized mean

differences (SMD) with 95% confidence intervals (CI). We used the following SMD values to assess results for clinical significance: <-0.5 small clinical significance, -0.5 to -0.8 moderate clinical significance, and >-0.8 high clinical significance. Due to clinical heterogeneity between the various studies, a random-effects model was used. We assessed statistical heterogeneity using the I^2 statistic, with >50% regarded as evidence of statistical heterogeneity. We assessed the quality of evidence using the previously validated SYRCLE criteria, with studies graded out of 10.[29] Studies were weighted by sample size and statistical significance was set at a minimum of $P < 0.05$.

RESULTS

Search Results and Study Characteristics

The search strategy returned 2008 results from which 199 relevant publications were identified. From these, 53 publications comprising 106 experiments examining 35 compounds met the inclusion criteria (Fig. 1, Tables 1 and 2). Thirty-four studies were included in the meta-analysis.

Forty-three publications studied the effects of cannabinoids on experimental murine colitis, 5 in rats, and 3 in both mice and rats. Two clinical trials examined the effect of a cannabinoid (THC and CBD) in Crohn's disease. Within animal publications, 43 used caustic agents (Di-nitrobenzine sulphonic acid (DNBS), trinitrobenzene sulphonic acid (TNBS), oil of mustard (OM), dextran sulphate sodium (DSS), and croton oil (CO)) to induce colitis; 6 used intravenous or topical lipopolysaccharide; 2 induced colonic inflammation using surgical arterial ligation or puncture of the colon; and 1 induced colitis with interleukin-10 (IL-10) knock-down and DSS (Fig. 2A).

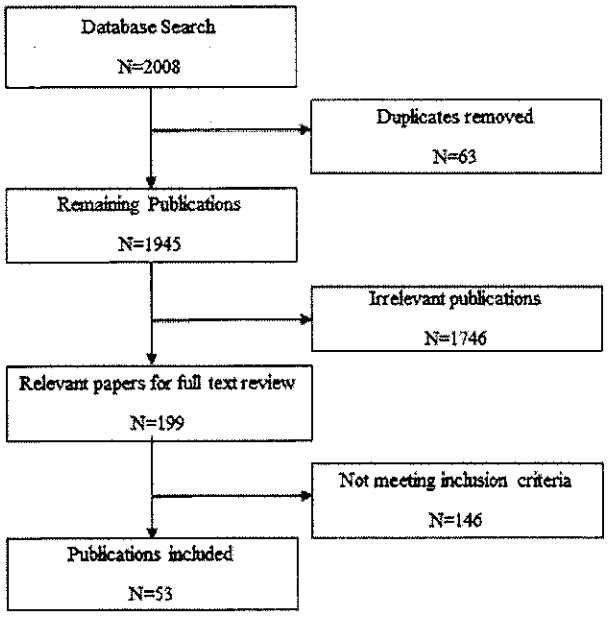

Figure 1. Record identification process.

Across all publications, including clinical trials, 71 endpoints were examined to evaluate the effect of cannabinoid drugs on colitis. Forty-nine publications (89 experiments) examined more than 1 endpoint. Of these endpoints, MPO and DAI were the most consistently used (34 and 26 studies, respectively) and were therefore selected for meta-analysis. Incidence of endpoints is given in Fig. 2B.

The effect of 7 phytocannabinoids were studied across 18 publications; cannabinol (CBN), CBD, THC, CBC, CBG, medicinal cannabis (MMJ), and abnormal CBD (Ab-CBD). Four endocannabinoids were studied across 11 publications (PEA, ultramicronized PEA [uPEA], Arachidonyl-2'-chloroethylamide [ACEA], and AEA); 15 synthetic cannabinoid agonists were studied across 22 publications (AM841, Adelmidrol, HU210, CP55940, WIN55,212-2, AM1241, JHW015, JWH133, βCaryophyllene, O-1602, HU308, αβ amyrin, CID16020046, compound 26, and SAB378); and 9 compounds targeting the catabolism or transport of endogenous cannabinoids were studied across 13 publications (ARN2508, PF-3845, compound 39, JZL184, AA5HT, VDM11, URB597, AM9053, and AM3506). These compounds are delineated by class in Table 1. The degrees of positivity or negativity of the outcomes of these studies are displayed in Fig. 2C. Twenty-three studies investigated underlying receptor mechanisms using knock-out (KO) animals or receptor antagonists.

Of the 105 experiments comparing cannabinoids with the vehicle or placebo, 67 (63.8%) favored cannabinoids, 34 (32.3%) reported no difference, and 4 (3.8%) favored vehicle. Mice were used in 89 experiments (68.5% of which favored cannabinoids), while rats were used in 14 (71.4% favored cannabinoids). In 4 experiments, both mice and rats were used showing no difference between cannabinoids and the vehicle. In the 2 clinical trials, no difference in primary outcome was found between the use of THC cigarettes or oral CBD and placebo. Eleven of 14 publications (78.6%) using synthetic CB_2 receptor agonists favored cannabinoid use over the vehicle, and a further 11 of 13 (84.6%) favored using FAAH inhibitors over the vehicle. The outcome of all cannabinoids across publications is given in Fig. 2C.

Two clinical trials examining the effect of CBD and THC in Crohn's disease were found. Naftali et al (2013) conducted a placebo-controlled study in Crohn's disease patients, comparing THC 115 mg inhaled alone with placebo. Disease activity was compared between groups by means of a validated questionnaire (Crohn's disease activity index [CDAI]) after 8 weeks of treatment. A nonsignificant reduction in clinical disease remission as defined by the authors was found at the end of the study period; however, a secondary endpoint of reduction in overall activity scores was found between groups ($P = 0.028$). In a second study, Naftali et al (2017) compared 10 mg of oral CBD twice daily with placebo in Crohn's disease, using CDAI in an identical fashion. No reduction in disease activity was detected

TABLE 1: Cannabinoid Drugs Found by Search Strategy

Cannabinoid Class		Drug Description
Endocannabinoids	AEA	Anandamide
	PEA	Palmitoylethanolamide
	uPEA	Ultramicronised PEA
Phytocannabinoids	Cannabis sativa	Multiple compounds
	CBC	Cannibichromene
	CBD	Cannabidiol
	CBG	Cannabigerol
	CBN	Cannabinol
	THC	Tetrahydrocannabinol
Cannabinomimetics	αβ Amyrin	CB_1 and CB_2 agonist
	ACEA	Arachidonyl-2'-chloroethylamide
	Adelmidrol	PEA analogue
	AM1241	CB_2 full agonist, partial CB_1 agonist
	AM841	Peripherally restricted CB_1 agonist
	βCaryophyllene	CB_2 agonist
	CID16020046	GPR55 inverse agonist
	Compound 26	CB_2 agonist
	CP55,940	CB_1 and CB_2 agonist
	HU210	THC analogue
	HU308	CB_2 agonist
	JWH015	CB_2 full agonist, weak CB_1 agonist
	JHW133	CB_2 full agonist, weak CB_1 agonist
	O-1602	GPR18 and GPR55 agonist
	SAB378	Peripherally restricted CB_1 and CB_2 agonist
	WIN55,212-2	CB_1 full agonist
Enzyme Inhibitors	AA5HT	FAAH inhibitor
	AM3506	FAAH inhibitor
	AM9053	NAAA inhibitor
	ARN2508	FAAH inhibitor
	compound 39	FAAH inhibitor
	JZL184	MAGL inhibitor
	PF-3845	FAAH inhibitor
	URB597	FAAH inhibitor
Reuptake inhibitors	VDM11	AEA reuptake inhibitor

between groups. In both studies, the authors measured changes in serum C-reactive protein (CRP). Within both experimental and placebo groups, CRP levels were below 5 units per milliliter at the end of the study periods. Clinically, CRP levels greater than 5 units per milliliter are considered indicative of inflammatory disease. Within both studies, the combination of CBD and THC within a single study were not assessed.

Of the 104 experiments where timing of drug administration was stated, 37 administered cannabinoids therapeutically, of which 62.2% favored cannabinoid treatment. Nineteen experiments administered cannabinoids prophylactically, of which 52.6% favored cannabinoid treatment. Forty-eight experiments administered cannabinoids both prophylactically and therapeutically, of which 75% favored cannabinoid treatment versus the vehicle.

Meta-analysis

Thirty-four studies reported the same endpoints of disease activity index or myeloperoxidase activity, allowing for meta-analysis. Of the remaining studies, heterogeneity of endpoints prevented further meta-analysis.

Crohn's Disease Activity Index (CDAI)

The use of 2 phytocannabinoids, THC or CBD, in 2 human studies were meta-analysed. Phytocannabinoid use decreased severity scores in comparison with placebo (mean difference [MD] -74.97; 95% CI, -229 to 0.79, I^2 = 75% Fig. 3). THC alone had a significant effect on reducing CDAI (MD -154.00; 95% CI, -2.68.57 to -44.43), whereas CBD alone did not (MD +4.00;, 95% CI -1.5.39 to +113.39).

TABLE 2: Characteristics of Studies Included for Systematic Review

Study	Species	Model	Compound	Route/dosage	Time of Administration Versus Inflammation	Time of Assessment Post Inflammation	Modified STAIR score	SRCYCLE Score
Capasso 2001[32]	ICR mice	CO	PEA	i.p 2.5–30 mg/kg	20 minutes pre	4 days	4	1
Izzo 2001[9]	ICR mice	CO	CP 55,940	i.p. 0.03–10 nmol/m	4 days post	20 minutes	3	0
			Cannabinol	i.p. 10–3000nmol/m				
Massa 2004[25]	C57BL/6N mice	DNBS	SR141716	i.p. 3 mg/kg	Pre, 24 and 48 hours post	3 & 7 days	4	2
			HU210	i.p. 0.05 mg/kg				
Mathison 2004[50]	Spr-Dawley rats	LPS	ACEA	i.p. 1mg/kg	70 minutes post	120 minutes	5	0
			JWH133	i.p. 1mg/kg				
D'Argenio 2006[22]	C57/BJ mice	DNBS	VDM11	SC 5 mg/kg	Post	3 & 7 days	6	0
	Wistar rats	TNBS	AA-5-HT	SC 10 mg/kg				
Kimball 2006[51]	CD-1 mice	OM	ACEA	i.p. 10 mg/kg	24 hours pre	3 days	3	1
			JWH133	i.p. 2.5 mg/kg				
Capasso 2008[52]	ICR mice	CO	CBD	i.p. 5 mg/kg	20 minutes pre Ach	4 days	5	0
			JWH015	i.p. 10 mg/kg				
Engel 2008[53]	AKR mice	TNBS	AEA	i.p. 5 mg/kg	30 minutes pre	3 days	3	1
Storr 2008[54]	C57/BL mice	TNBS	URB597	i.p. 5 mg/kg	30 minutes pre or 24 hours post	3 days	4	1
			VDM11	i.p.5 mg/kg				
Borelli 2009[46]	ICR mice	DNBS	CBD	i.p. 1, 2, 5, 10 mg/kg	24 hours post	3 days	3	0
Li 2009[55]	Rats Mice	LPS	HU210	100 μg.kg	5 minutes	30 minutes	8	1
			JWH133					
			AM630	100 μg.kg				
			AM251	3 mg/kg				
Storr 2009[56]	C57/BL mice	TNBS	JWH133	i.p. 20 mg/kg	30 minutes pre or 24 hours post	1, 3, 5, 7 days	7	1
		DSS	AM1241	i.p. 10–20 mg/kg				
			AM630	i.p. 10 mg/kg				
Cassol Jr 2010[47]	Wistar rats	CLP	CBD	i.p. 2.5, 4, 10 mg/kg	Simultaneous	9 days	8	2
Cluny 2010[57]	C57/BL mice	DSS	SAB378	i.p 0.1 or 1.0 mg/kg	4 days post	8 days	5	1
		TNBS	AM251	i.p 1.0 mg/kg				
			AM630	i.p 1.0 mg/kg				
			WIN55,212-2	i.p 1, 2 mg/kg				
Kimball 2010[58]	CD1 mice	OM	ACEA	i.p. 1 mg/kg	30 minutes pre	28 days	4	3
			JWH133					
				i.p. 1 mg/kg				
Jamontt 2010[45]	Wistar rats	TNBS	THC	i.p. 5–20 mg/kg	30 minutes pre	3 days	5	1
			CBD					
				i.p. 5–20 mg/kg				
Alhouayek 2011[59]	C57BL/6 mice	TNBS	JZL184	i.p. 16 mg/kg	Pre onset	3 days	2	1
Andrejak 2011[60]	C57/BL mice	TNBS	Compound 39	i.p. 5 mg/kg	3 days pre	3 days	6	1

(Continued)

TABLE 2: Continued

Study	Species	Model	Compound	Route/dosage	Time of Administration Versus Inflammation	Time of Assessment Post Inflammation	Modified STAIR score	SRCYCLE Score
Bento 2011[61]	CD1 mice	DSS	βCaryophyllene	i.p. 12.5, 25, 50 mg/kg	3 -7 days post	7 days	4	1
Defilipis 2011[49]	OF1 mice	LPS	CBD	i.p. 10 mg/kg	6 hours post	120 minutes	6	1
Lin 2011[43]	C57/BL mice	LPS	CBD O-1602	i.p. 10 mg/kg	30 minutes pre	20 minutes	5	1
	Spr-Dawley rats			I.p. 1 mg/kg				
Schicho 2011[62]	C57/BL mice	DSS	O-1602	i.p. 5 mg/kg	30 minutes pre	7 days	3	3
		TNBS						
Bashashati 2012[63]	CD1 mice	LPS	AM3506	i.p. 100 ug.kg	20 minutes pre	120 minutes	3	0
Izzo 2012[64]	ICR mice	CO	CBC	i.p. 15 mg/kg	20 minutes pre exam	4 days	5	2
Lehmann 2012[65]	Lewis rats	LPS	HU308	2.5 mg/kg	15 minutes post	2–16 hours	4	0
		CASP						
Schicho 2012[42]	C57/BL mice	TNBS	CBD	i.p. 10 mg/kg	30 minutes pre onset	7 days	4	0
				PO 20 mg/kg				
				PR 20 mg/kg				
Singh 2012[66]	C57/BL mice	IL-10 -/-DSS	JWH133	i.p. 2.5 mg/kg	Simultaneous	7–14 days	5	1
Borrelli 2013[67]	ICR mice	DNBS	CBG	i.p. 30 mg/kg	3 days pre	3 days	5	1
Esposito 2014[33]	CD-1 mice	DSS	PEA	i.p. 10 mg/kg	2 days post	7 days	5	2
Li 2013[68]	C57/BL mice	DSS	WIN55,212-2	i.p. 5 mg/kg	Simultaneous	7 days	4	1
Matos 2013[69]	CD1 mice	DSS	αβ Amyrin	PO 1, 3, 10 mg/kg	Pre and 3 days post	7 days	6	1
Naftali 2013[70]	Clinical trial	Crohn's	Cannabis sativa extract (THC)	115 mg inhaled	N/A	8 weeks	NA	NA
Romano 2013[71]	ICR mice	DNBS	CBC	i.p 0.1–1.0 mg/kg	24 hours post	3 days	6	0
Wallace 2013[72]	Wistar rats	DNBS	C. sativa (MMJ)	IC 6 mg/kg	30 minutes pre and 24 hours post	7 days	4	1
			AM630	PO 10 mg/kg				
Borelli 2015[73]	ICR mice	DNBS	PEA	i.p 1 mg/kg	3 days pre	3 days	5	1
				PO 1 mg/kg				
Capasso 2014[20]	ICR mice	OM	PEA	i.p. 10 mg/kg	30 minutes	3 and 7 days	6	2
Fichna 2014[74]	CD1 mice	DSS	AM841	i.p. 0.01, 0.1,1 mg/kg	15 minutes pre	3 and 7 days	4	0
		DNBS	CB13	i.p. 0.1 mg/kg				
Salaga 2014[75]	C57/BL mice	TNBS	PF3845	i.p. 10 mg/kg	30 minutes	3 and 7 days	2	0
		DSS		PO 5 mg/kg				
				IC 5mg/kg				
Sardinha 2014[76]	C57/BL mice	LPS	HU308	IV 2.5mg/kg	15 minutes pre	Simultaneous	6	0
			AM630	IV 2.5 mg/kg				
			URB597	i.p. 0.6 mg/kg				
			JZL184	i.p. 16 mg/kg				
Alhouayek 2015[77]	CD57/BL mice	TNBS	PEA	i.p. 10 mg/kg	Simultaneous and 5 days post	7 days	4	1
		DSS	PF-3845	i.p. 10 mg/kg				
			AM9503	i.p. 10 mg/kg				

TABLE 2: Continued

Study	Species	Model	Compound	Route/dosage	Time of Administration Versus Inflammation	Time of Assessment Post Inflammation	Modified STAIR score	SRCYCLE Score
El bakali 2015[78]	C57/BL mice	TNBS	Compound 26	PO 10 mg/kg	2 days pre	7 days	6	0
Impellizzeri 2015[79]	CD1 mice	DNBS	uPEA	i.p. 10 mg/kg	1 hour post	4 days	9	2
Sasso 2015[80]	CD1 mice	TNBS DSS	ARN2508	PO 5 mg/kg	Simultaneous	7 days	8	3
Stančić 2015[81]	C57/BL mice	DSS TNBS	CID16020046	SC 20 mg/kg	30 minutes	7 days	6	1
Cordaro 2016[82]	CD1 mice	DNBS	Adelmidrol	PO 10 mg/kg	60 minutes post	4 days	4	1
Feng 2016[83]	C57/BL mice	DSS	WIN55,212-2	i.p. 5 mg/kg	Simultaneous and 60 hours post	7 days	5	1
Ke 2016[84]	C57/BL mice	DSS	HU308	i.p. 1 mg/kg	Simultaneous and daily	8 days	4	2
Krohn 2016[40]	CD1 mice	TNBS	Ab-CBD	i.p. 5 mg/kg	45 minutes pre	4 days	6	1
			O-1918	i.p. 5 mg/kg				
			AM251	i.p. 5 mg/kg				
			AM630	i.p. 5 mg/kg				
Pagano 2016[39]	ICR mice	DNBS	CBD	i.p. 30 mg/kg	24 hours post	3 days	3	0
		CO	Pure CBD	PO 60 mg/kg				
Sarnelli 2016[85]	CD1 mice	DSS	PEA	i.p. 2, 10 mg/kg	2 days post	7 days	6	1
Lin 2017[86]	C57/BL mice	DSS	HU210	i.p. 0.05 mg/kg	30 minutes pre	7 days	5	1
Shamran 2017[87]	C57/BL mice	DSS	FAAH-II	i.p. 5–40 mg/kg	24 hours post	7 days	6	1
Naftali 2017[35]	Clinical trial	Crohn's	CBD	10 mg PO BD	N/A	8 weeks	NA	NA

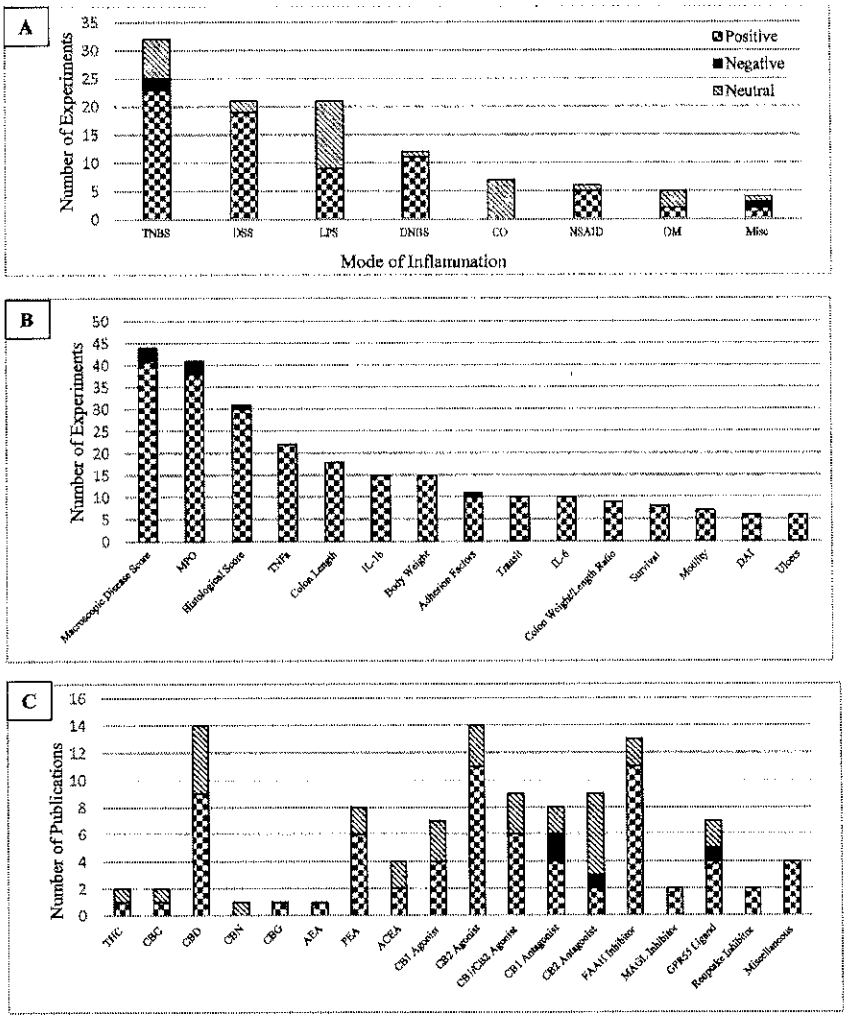

FIGURE 2. Positive, negative, and neutral outcomes of cannabinoid treatment across modes of inflammation (A). Incidence of endpoints across all experiments comparing cannabinoid treatment with control (B). The effect of cannabinoid drugs compared with control across all endpoints expressed as primary drug investigated (C).

Disease Activity Index (DAI)

Thirty-four publications examined the effects of 25 cannabinoid drugs across 68 experiments, within mouse and rat models (total n = 948; n = 519 experimental vs 429 in control groups). Cannabinoid drugs reduced DAI in comparison with the vehicle; SMD -1.36; 95% CI, -1.62 to -1.09; I^2 = 61% (Fig. 4, Table 3). On subgroup analysis, there was a significant difference between drug subtypes ($P < 0.001$). DAI was significantly reduced in mice (SMD -1.49; 95% CI, -1.77 to -1.22; I^2 = 61%). Seven experiments within one publication examined the effects of cannabinoids on rat colitis (THC and CBD, both conducted in a dose response manner), but did not reach significance at any concentration (SMD -0.29; 95% CI, -0.77 to 0.20; I^2 = 0%). SMD and confidence intervals for individual drugs on DAI are given in Table 3.

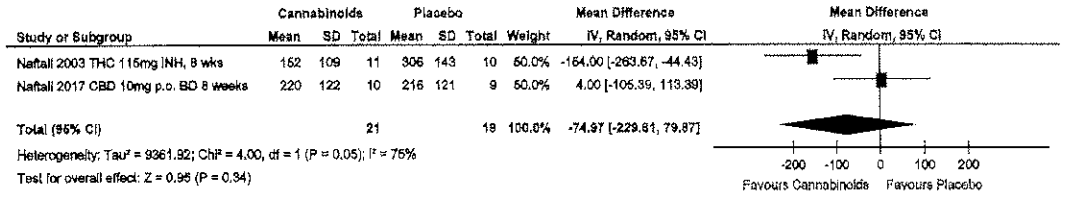

FIGURE 3. Forest plot of the effects of cannabinoid treatment on Crohn's Disease, assessed by reduction in CDAI in human studies.

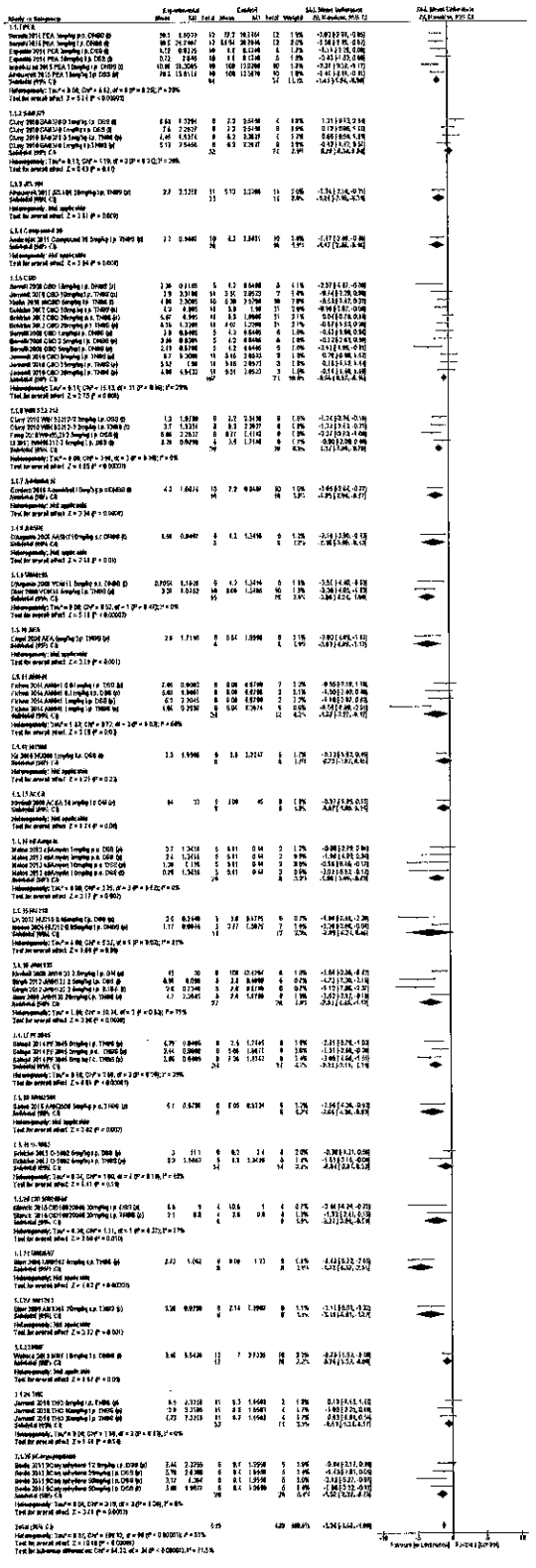

FIGURE 4. Forest plot of the effects of cannabinoid treatment on Disease Activity Score subdivided by drug type. Time of administration in relation to onset of colitis is given where "p" represents prophylactic administration, and "t" represents therapeutic administration.

The largest effect size in DAI reduction was caused by an enzyme inhibitor: the FAAH inhibitor URB597 (SMD -4.43; 95% CI, -6.32 to -2.55). The largest effect size of DAI reduction by an endocannabinoid was AEA (SMD -3.03; 95% CI, -4.89 to -1.17); the largest effect size of DAI reduction by a phytocannabinoid was CBD (SMD -0.56; 95% CI, -0.97 to -0.16; I^2 = 29%), and the largest synthetic cannabinoid effect size on DAI was AM1241 (SMD -3.11; 95% CI, -5.01 to -1.22). SMD and confidence intervals of individual drugs on DAI are given in Table 4. Eighteen of 25 drugs had a large effect size, 1 had a moderate effect size, and 6 had no significant effect on DAI.

Myeloperoxidase Activity (MPO)

Twenty-six publications investigated the effects of 21 cannabinoid drugs on MPO activity throughout 57 individual experiments (total n = 757, n = 419 in experimental vs 338 in control groups). Cannabinoid drugs reduced MPO in comparison with the vehicle (SMD -1.26; 95% CI, -1.54 to -0.97; I^2 = 48.1%, Fig. 5, Table 4). Overall, there was significant heterogeneity between studies, and there was significant subgroup difference (I^2 = 48.1%; $P < 0.008$). MPO was significantly reduced in mice and rats (SMD -1.28; 95% CI, -1.59 to -0.98; I^2 = 61%; and -1.06; 95% CI, -1.99 to -0.13; I^2 = 56%, respectively).

The largest effect size in MPO reduction was caused by the phytocannabinoid CBG (SMD -6.20; 95% CI, -9.90 to -2.50; I^2 not assessed). The largest effect size by an endocannabinoid was PEA (SMD -2.74; 95% CI, -4.42 to -1.06; I^2 = 85%). The largest synthetic cannabinoid effect size on MPO was caused by ACEA (SMD -3.15; 95% CI, -4.75 to -1.55; I^2 not assessed). And the largest effect size of any enzyme or transport inhibitor was AA5HT (SMD -2.27; 95% CI, -4.05 to -0.49; I^2 not assessed). SMD and confidence intervals of individual drugs on MPO activity are given in Table 4. Thirteen of 21 cannabinoid drugs had a large clinical effect, the remaining of which had no significant effect on MPO.

Time of Administration

From the 50 publications examining the effect of cannabinoids on murine colitis, 28 studies administered cannabinoid agents either simultaneously with colitis onset or prophylactically. Seventeen studies administered drugs between 15 minutes and 7 days after the onset of colitis. Additionally, 7 studies compared the benefit of prophylactic cannabinoid use to therapeutic, but did not find any difference in efficacy. To investigate if the timing of drug treatment affected DAI or MPO, we compared study size-weighted effect sizes (dependent variable) with time of administration (covariate) using meta-regression. We found that the timing of drug administration weakly predicted effect size in reducing DAI and MPO, although this was of borderline statistical significance (P = 0.09, R^2 = 11%, and P = 0.055, R^2 = 41%, respectively, Fig. 6A and B).

TABLE 3: The Effects of Cannabinoids on Disease Activity Score Caused by Experimental Colitis Grouped by Drug

	No. of Studies	No. of animals	SMD [95% CI]	*P*	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	6	118	-1.45 [-1.94, -0.96]	<0.00001	25	High
AEA	1	12	-3.03 [-4.89, -1.17]	0.001	N/A	High
Phytocannabinoids						
CBD	12	181	-0.56 [-0.97, -0.16]	0.006	29	NS
THC	3	44	-0.53 [-1.24, 0.17]	0.14	0	NS
MMJ	1	30	-0.76 [-1.52, -0.00]	0.05	N/A	Moderate
Cannabinomimetics						
αβ Amyrin	4	28	-1.88 [-3.05, -0.72]	0.002	0	High
AM841	4	36	-1.87 [-3.57, -0.17]	0.03	66	High
βCaryophyllene	4	40	-1.52 [-2.32, -0.72]	0.0002	6	High
SAB378	4	56	0.28 [-0.38, 0.94]	0.41	28	NS
WIN55,212-2	4	60	-1.37 [-1.96, -0.78]	<0.00001	0	High
CID16020046	2	16	-2.24 [-3.94, -0.54]	0.01	17	High
HU210	2	24	-2.89 [-6.24, 0.46]	0.09	81	NS
O-1602	2	28	-0.84 [-2.01, 0.33]	0.16	45	NS
ACEA	1	18	-0.87 [-1.85, 0.11]	0.08	N/A	High
Adelmidrol	1	20	-1.85 [-2.94, -0.77]	0.0008	N/A	High
AM1241	1	12	-3.11 [-5.01, -1.22]	0.001	N/A	High
HU308	1	12	-0.73 [-1.92, 0.45]	0.23	N/A	NS
Enzyme inhibitors						
JWH133	4	53	-2.81 [-4.45, -1.17]	0.0008	71	High
PF3845	3	48	-2.21 [-3.11, -1.31]	<0.00001	25	High
AA5HT	1	10	-2.16 [-3.90, -0.43]	0.01	N/A	High
ARN2508	1	12	-2.66 [-4.38, -0.93]	0.002	N/A	High
Compound 39	1	20	-1.47 [-2.48, -0.46]	0.004	N/A	High
JZL184	1	22	-1.24 [-2.16, -0.31]	0.009	N/A	High
URB597	1	18	-4.43 [-6.32, -2.55]	<0.00001	N/A	High
Transport inhibitors						
VDM115	2	30	-3.06 [-4.21, -1.90]	<0.00001	0	High
Total	**68**	**948**	**-1.36 [-1.62, -1.09]**	**<0.00001**	**61**	**High**

Quality and Risk of Bias

Of the 53 papers, 21 used randomization in their design, 7 reported blinding of assessment, 5 replicated their results in a second species, and 14 replicated their findings in a second model of colitis. Fifty reported n ≥ 5 in control and experimental groups. Fifteen publications reported specific numbers within groups. All papers reported a clinically relevant endpoint. The median study quality modified STAIR score was 5 out of 10 (mean 4.9, SD 2.29). Using meta-regression, higher quality scores predicted greater reductions in MPO activity ($P = 0.043$, $R^2 = 65\%$, Fig. 6D), but not in DAI ($P = 0.98$, $R^2 = 35\%$, Fig. 6C).

The SYRCLE risk-of-bias score for each endpoint showed a trend to larger reduction in DAI in studies with a larger risk of bias ($P = 0.084$, $R^2 = 69\%$, Fig. 6E), but not MPO ($P = 0.345$, $R^2 = 8\%$, Fig. 6F).

Publication Bias

Funnel plots comparing MPO activity and DAI were constructed and analysed statistically for bias. The presence of publication bias was not found in either group (MPO: Egger's statistic $P = 0.570$, Fig. 7A; DAI: Egger's statistic $P = 0.274$, Fig. 7B).

DISCUSSION

The aim of this study was to determine the efficacy of cannabinoid drugs in reducing gut inflammation to aid the design of further clinical studies. We found 53 studies that examined this effect using endocannabinoids, phytocannabinoids, synthetic cannabinoids, and enzyme and reuptake inhibitors across multiple models of murine and human colitis. In both qualitative assessment and meta-analysis, these controlled studies

TABLE 4: The Effects of Cannabinoids on Mpo Activity Caused by Experimental Colitis Grouped by Drug

	No. of Studies	No. of animals	SMD [95% CI]	*P*	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	7	94	-2.74 [-4.42, -1.06]	0.001	85	High
Phytocannabinoids						
CBD	10	157	-1.03 [-1.40, -0.66]	<0.00001	0	High
THC	3	29	-1.40 [-3.97, 1.17]	0.28	80	NS
CBC	1	10	-2.97 [-5.05, -0.89]	0.005	N/A	High
CBG	1	10	-6.20 [-9.90, -2.50]	0.01	N/A	High
Cannabinomimetics						
βCaryophyllene	4	40	-1.26 [-2.48, -0.05]	0.04	60	High
AM841	4	48	-1.56 [-2.71, -0.41]	0.008	54	High
SAB378	4	42	-0.23 [-0.86, 0.39]	0.46	0	NS
WIN55,212-2	4	52	-1.74 [-2.81, -0.67]	0.001	57	High
αβ Amyrin	2	15	-0.38 [-1.48, 0.71]	0.5	0	NS
CID16020046	2	56	-1.04 [-1.61, -0.48]	0.0003	0	High
HU210	2	24	-0.63 [-1.48, 0.23]	0.15	2	NS
O-1602	2	20	-1.70 [-2.81, -0.60]	0.003	0	High
ACEA	1	16	-3.15 [-4.75, -1.55]	0.0001	N/A	High
AM1241	1	10	-0.96 [-2.31, 0.39]	0.16	N/A	NS
JWH133	1	16	-0.98 [-2.04, 0.07]	0.09	N/A	NS
Ademidrol	1	20	-1.33 [-2.31, -0.34]	0.009	N/A	High
Enzyme inhibitors						
PF3745	3	46	-0.12 [-1.56, 1.32]	0.81	81	NS
AA5HT	1	10	-2.27 [-4.05, -0.49]	0.01	N/A	High
URB597	1	16	-1.00 [-2.06, 0.06]	0.06	N/A	NS
Transport inhibitors						
VDM115	2	26	-1.91 [-3.72, -0.10]	0.04	59	High
Total	**57**	**757**	**-1.26 [-1.54, -0.97]**	**<0.00001**	**48.1**	**High**

demonstrate that the use of cannabinoid drugs are beneficial in reducing colonic inflammation in rats and mice, with unclear effects in human subjects.

In animal studies, cannabinoids were shown to reduce inflammation both qualitatively and at meta-analysis. Across experiments included in this review, CB_2 agonists, FAAH inhibitors, and CBD were the most widely studied and showed the greatest therapeutic benefit across all endpoints. Subgroup analyses suggested that CBG caused the greatest reduction in MPO activity scores, followed by synthetic CB_1 agonist ACEA. However, both agents were only studied within a single publication. In the MPO analysis, the most studied drug was CBD, with 157 animals across 7 publications, demonstrating a significant effect on MPO activity reduction. Similarly, within DAI analysis, CBD was again the most-studied single drug, including 181 animals across 6 publications. Although CBD demonstrated a significant effect on DAI reduction, the largest reduction in DAI was caused by the FAAH antagonist URB597, studied in 1 publication. There was statistical heterogeneity in both MPO and DAI analyses, which was partially accounted for by subgroup differences. At meta-regression, factors leading to subgroup differences were quality, timing, and risk of bias.

Receptor targets were explored in 23 publications using receptor-specific agonists or antagonists and receptor knockdown. In murine colitis, agonism of the CB_1 or CB_2 receptor brought about reduction in inflammation, and at subgroup analysis, the use of the synthetic CB_1/CB_2 agonists acting demonstrated the greatest reduction in disease scores and MPO activity. In addition, agonism of the PPARα, GPR55, and GPR18 receptors also reduced inflammation of the colon. The wide variation in the measured inflammatory endpoints across these studies prevented further meta-analysis. Interestingly, the use of the peripherally restricted synthetic agonist SAB378, which agonises both CB_1 and CB_2 receptors, had no significant effect on either MPO activity or DAI. This is in contrast to ex vivo explant human colonic data, which demonstrated that cannabinoid agonism with AEA or CBD was beneficial in colonic mucosal inflammation, which were peripherally restricted by

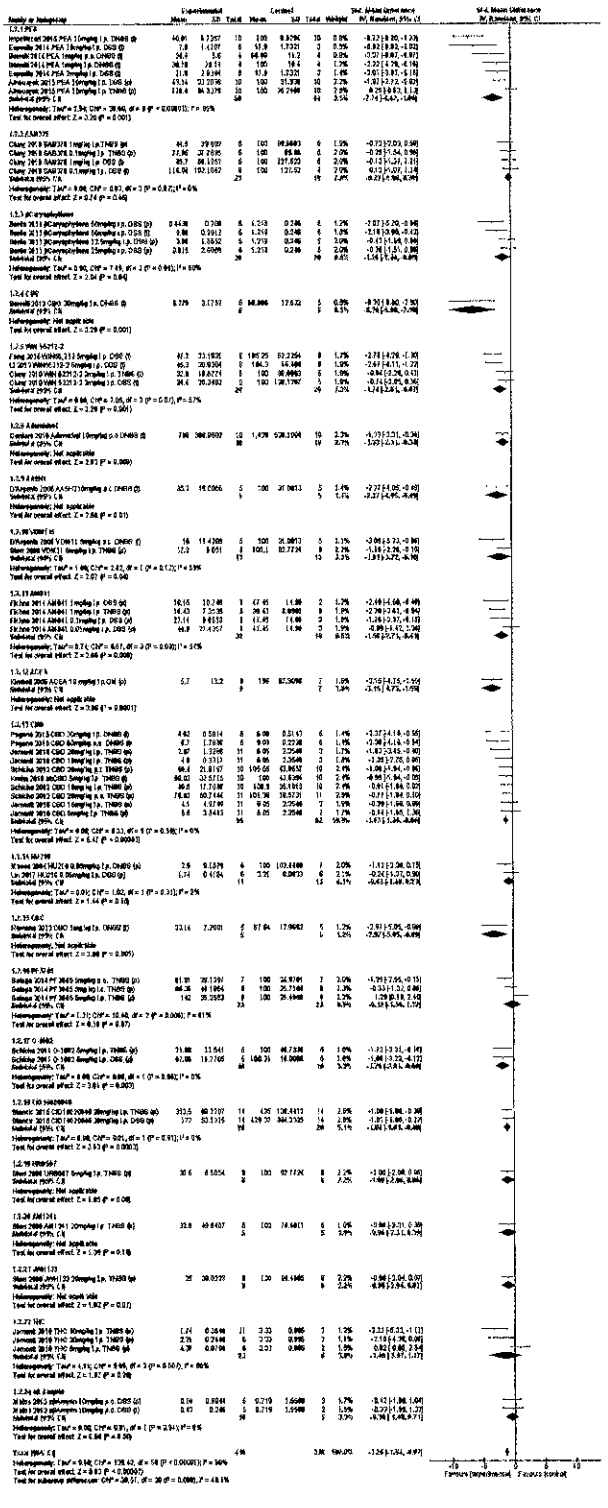

FIGURE 5. Forest plot of the effects of cannabinoid treatment on MPO activity subdivided by drug type. Time of administration in relation to onset of colitis is given, where "p" represents prophylactic administration and "t" represents therapeutic administration.

definition of the explant model.[30, 31] Izzo et al[9] found through receptor antagonism that the effect of CBN in preventing hypermobility caused by croton oil was mediated by CB_1, but not CB_2. PEA was investigated by Capasso et al[20, 32] using two models of inflammation-induced hypermotility. Using receptor antagonists in both experiments, Capasso et al found that PEA, in an OM model, acted through CB_1, but not CB_2 or PPARα. In a CO model, PEA was still effective, but did not act through CB_1 or CB_2. This suggests that the mechanism by which PEA acts as an anti-inflammatory agent was not mediated by a single receptor, but by receptor codependence. ACEA was investigated for receptor mechanism in 2 publications, both of which found ACEA dependent on CB_1. None of the reviewed studies investigated a mechanism of action for AEA in gut inflammation, however 1 ex vivo human study from Harvey et al found that AEA prevented increased cytokine production in experimentally inflamed human mucosa and was dependent on CB_2, although the authors did not report antagonism of any other receptor.[31]

The specific mechanism by which manipulation of the cannabinoid system affects inflammation is not clear. Esposito et al[33] demonstrated that PEA brought about anti-inflammatory effects on enteric glial cells acting at toll-like receptor 4, suggesting that rather than acting at an epithelial mucosal level, it acts at either an innate immune colonies or the enteric nervous system. This hypothesis has recently been evidenced by a study demonstrating that both CBD and PEA do not act on the immune response of epithelial cells, but are likely to require the presence of other cell types, acting through down regulation of NF-κβ.[34] This is challenged by Cluny et al, who demonstrate that peripherally restricted cannabinoids have a diminished effect on inflammation. Nevertheless, it is clear that the mechanism of action of cannabinoids does not simply lie at the epithelial level, but is likely to reside within the gut-brain axis.

From the clinical literature, we found 2 randomized placebo-controlled studies examining the effect of phytocannabinoids in humans. Our analysis found no overall effect of THC or CBD on disease scores; however; there was large statistical and clinical heterogeneity between these studies. We found from meta-analysis that inhaled THC did have a beneficial effect on CDAI at 8 weeks, whereas CBD did not. There may be several reasons for this heterogeneity: Firstly in all groups, small cohort sizes were used which may have overestimated positive or negative effects in both studies, making meaningful conclusions difficult regarding the use of CBD or THC in inflammatory bowel disease. Secondly, within the Naftali et al (2017) study, very low doses of CBD were utilized compared to the use of CBD in other clinical trials, which commonly used 600 mg twice daily.[35] A recent trial in drug-resistant epilepsy used 20mg.kg^{-1} daily for 4 weeks, with a small number of participants experiencing side effects such as vomiting and diarrhea.[36] It is likely that in adult males such

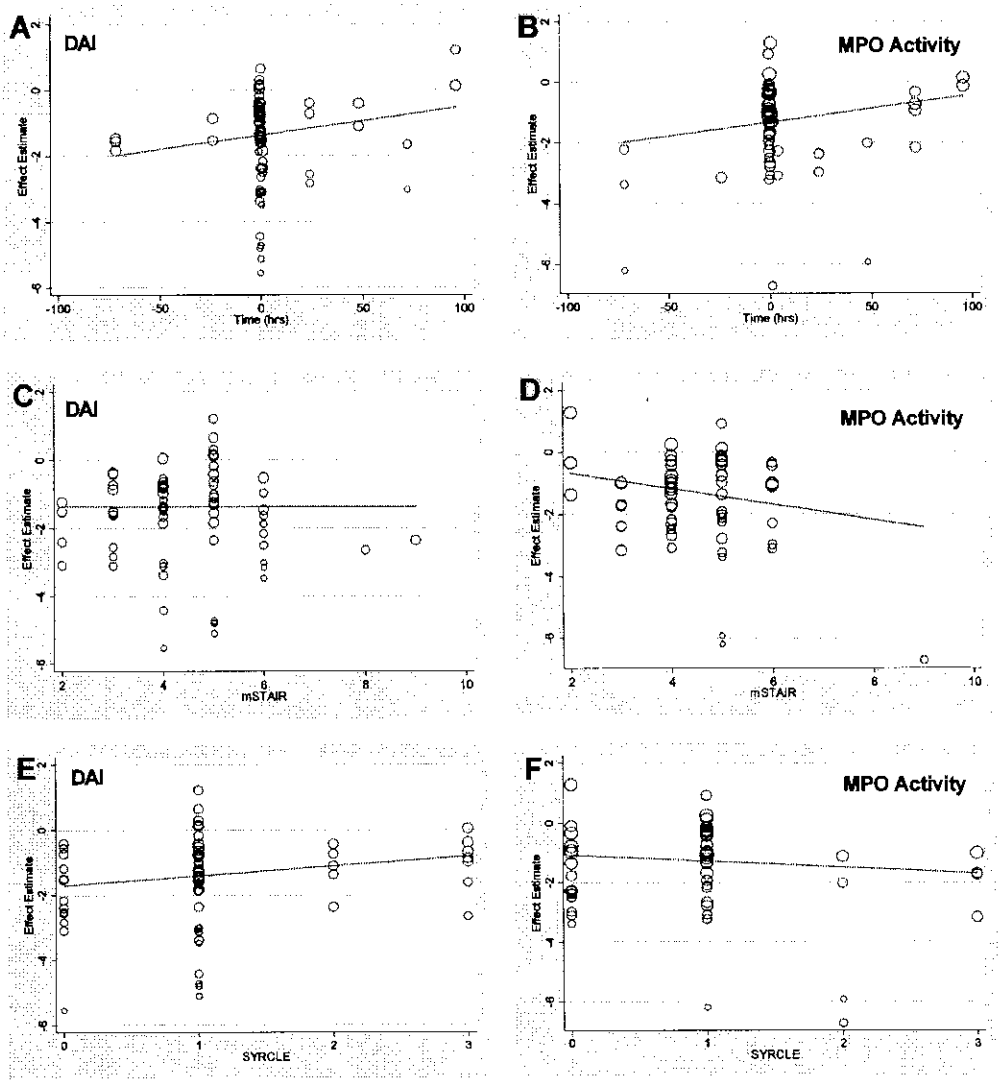

FIGURE 6. The effect of cannabinoid treatment on experimentally induced colitis determined by DAI (A) and MPO (B) predicted by timing of drug administration in relation to colitis onset. The effect of study quality, determined by mSTAIR score, and SYRCLE score, on effect size in DAI (C, E) and MPO (D, F). Study weights are represented by the diameter of the circle, with larger circles representing studies with largest weight in the analysis.

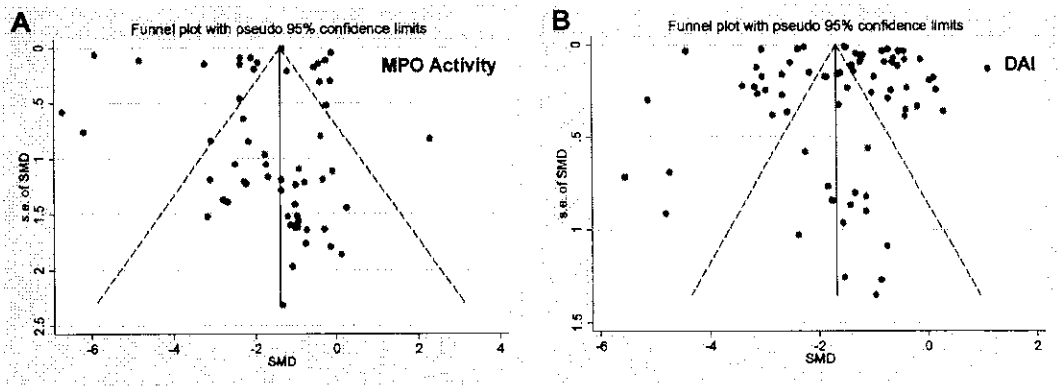

FIGURE 7. Funnel plots evaluating for publication bias in (A) MPO activity and (B) DAI. Standard error of the standardized mean difference (SE [SMD], *y* axes) for each study is plotted against its effect size (SMD, *x* axes).

10 mg doses had no clinical effect on Crohn's disease, as insufficient plasma concentrations may have been reached due to the poor bioavailability of oral CBD. A major flaw within the Naftali et al 2013 trial is that sham cigarettes contained cannabis sativa flowers in which active cannabinoids had been removed. However, it is unlikely that other compounds present in cannabis (such as terpenes), which are known to have an anti-inflammatory effect, had also been removed, which may have introduced positive bias into the study.[37] However, despite these drawbacks, the Naftali et al 2013 trial demonstrated a significant reduction in pain and the use of steroid therapy, with increased sleep and satisfaction levels with THC use compared with placebo. Although not included in this analysis, a study from Storr et al[38] demonstrated that although cannabis use provided symptomatic relief from Crohn's disease, the risk of salvage surgery was increased within 6 months of use (OR: 5.03; 95% CI, 1.45–17.46). However, these findings have not yet been supported from randomized, blinding controlled trials. We may suggest, therefore, that phytocannabinoid use may be a future therapy in intestinal inflammation. Although before firm conclusions are drawn, further clinical studies examining their effects should be conducted at higher, therapeutic dosages with adequately powered cohort sizes. As MMJ use in inflammatory bowel disease has been justified because of its effects on appetite and diarrhea, studies may be designed to examine these quality-of-life–affecting endpoints directly.

We found that most of the existing cannabinoid-gut research focuses on the therapeutic potential of CBD. This is unsurprising as CBD is currently used clinically, is well tolerated, and has shown consistently positive results. Nine studies found a positive, dose-dependent effect on local inflammatory cytokine expression, COX2 activation, MPO activity, enteric glial cell activation, and caspase-3 production, with associated improvements in macroscopic and histologic grades of inflammation.[39–46] One study also showed that intraperitoneal CBD administration decreased oxidative-stress scores of peripheral lung and brain tissue following intestinal inflammation,[47] adding to the existing evidence that CBD maintains the gut barrier during inflammation.[48] Despite being the most-studied drug, the mechanism by which CBD acts was not made clear by this review. One study by De Fillipis et al[44] found that hypermotility caused by LPS administration in mice was reduced by CBD through a CB_1 dependent mechanism. Similarly, Capasso et al (2008) found that CBD prevented croton oil-induced hypermotility via CB_1. de Fillipis et al (2011) demonstrated that in human explant tissue S100B levels, as a marker of glial cell activation, in vitro was decreased by CBD in a PPARγ dependent mechanism (although other antagonists were not investigated).[49]

The timing of cannabinoid administration correlated with reduction in effect on colitis activity, although this did not reach statistical significance. There was a correlation between the time of drug administration and effect size in both DAI and MPO reduction, with earlier administration of cannabinoid drugs producing a greater effect size. This suggests that in clinical trials, cannabinoids may be used prophylactically and therapeutically. There is promise, therefore, that compounds targeting the endocannabinoid system may be able to not only prevent colonic inflammation, but also treat established intestinal inflammatory conditions. Because it is not clear if cannabinoids are more effective when treating new-onset or established intestinal inflammation, further study designs should investigate this endpoint specifically.

One important potential area for research is the combination of cannabinoid drugs with existing treatments for inflammatory bowel disease. In clinical practice, it is common to treat patients with acute severe Crohn's disease and ulcerative colitis with a combination of agents, such as antibiotic, anti-TNFα, and corticosteroid therapy. One study compared the efficacy of CBD and THC with that of sulphasalazine, a 5-ASA, a drug commonly used in clinical practice.[45] Although in this study CBD and THC efficacy were comparable to that of sulphasalazine, the authors did not examine for the potential additive or subtractive effect of these agents in the context of colitis.

The findings of this study are limited by several factors typically seen in meta-analyses and systematic reviews. We found significant heterogeneity between subgroups in both DAI and MPO analyses, which suggested that 11% and 41% of this was due to the difference in time of administration in terms of changes in DAI and MPO, respectively. Additionally, we found risk-of-bias study design to be high and median study quality to be relatively low. Meta-regression demonstrated that these factors significantly correlated with study outcomes. Although we did not analyze for differences between scoring systems and mode of colitis, these factors may have also contributed to heterogeneity and influenced outcome. We sought to overcome this variability between scoring systems with random effects analysis. Additionally, within this review we have examined the effect of cannabinoid drugs en mass, which may have affected the overall outcome of meta-analyses. It is possible that some articles may not have been identified in initial searches, or conference abstracts may have been missed from the search period. Lastly, where control groups were compared to multiple experimental groups within the same set of experiments, variance and SMD may be exaggerated, leading to further bias.

In conclusion, we have shown in this systematic review and meta-analysis that cannabinoid drugs are beneficial in treating experimentally-induced murine models of colitis. These positive findings support the development of further human clinical trials. Current literature converges on CBD, and to avoid research bias, the effect of all cannabinoid drugs, including the large number of phytocannabinoid drugs not yet investigated, should also be investigated.

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APPENDIX: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6-7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8 + 19
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-11 + 28-29
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	30-31
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11

APPENDIX: Continued

Section/topic	#	Checklist item	Reported on page #
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

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