Received: 20 March 2023 | Accepted: 13 June 2023

DOI: 10.1111/fcp.12935

ORIGINAL ARTICLE

Fundamental & Clinical Pharmacology | WILEY

Heterogeneity in protocols and outcomes to study the effect of renin-angiotensin system blockers in inflammatory bowel disease: A systematic review

Mariana Ferreira-Duarte[1,2] | **Fernanda S. Tonin**[3,4] | **Margarida Duarte-Araújo**[2,5] | **Fernando Fernandez-Llimos**[1,6] | **Manuela Morato**[1,2]

[1]Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

[2]LAQV@REQUIMTE, University of Porto, Porto, Portugal

[3]ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Lisbon, Portugal

[4]H&TRC-Health and Technology Research Center, Instituto Politécnico de Lisboa, Lisbon, Portugal

[5]Department of Immuno-Physiology and Pharmacology, School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal

[6]UCIBIO i4HB - Applied Molecular Biosciences Unit, Faculty of Pharmacy, University of Porto, Porto, Portugal

Correspondence
Fernando Fernandez-Llimos, UCIBIO i4HB - Applied Molecular Biosciences Unit, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal.
Email: fllimos@ff.up.pt

Funding information
This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Partnership Agreement UIDB-50006/2020 and SFRH/D/145654/2019 to M.F.D.).

Abstract

Background: The renin-angiotensin system (RAS) has been associated with inflammatory bowel disease (IBD), supporting translational relevance of RAS blockers. Comparability of study design/outcomes is fundamental for data analysis/discussion.

Objectives: We aimed at evaluating the heterogeneity among protocols and outcomes to study the effect of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in IBD.

Methods: This study was performed and reported in accordance with the Cochrane recommendations and PRISMA (PROSPERO-CRD42022323853). Systematic searches were performed in PubMed, Scopus and Web of Science. Studies that met the inclusion criteria were selected. Quality assessment of the studies was done with the SYRCLES's risk of bias tools for animal studies.

Results: Thirty-five pre-clinical studies and six clinical studies were included. Chemical induction of colitis was the most used model, but variable doses of the induction agent were reported. All studies reported at least a disease activity index, a macroscopic score, or a histologic assessment, but these scores were methodologically heterogeneous and reported for different characteristics. Great heterogeneity was also found in drug interventions. Inflammatory markers assessed as outcomes were different across studies.

Conclusion: Lack of standardization of protocols and outcomes among studies threatens the evidence on how RAS blockers influence IBD outcomes.

KEYWORDS
angiotensin-converting enzyme, colitis, heterogeneity, inflammatory bowel disease, renin-angiotensin system blockers, variability

Abbreviations: ACE, angiotensin-converting enzyme; ACEi, angiotensin-converting enzyme inhibitor; Ang, angiotensin; ARBs, angiotensin receptor blockers; AT1, angiotensin type 1 (receptor); AT2, angiotensin type 2 (receptor); CD, Crohn's disease; CRP, C reactive protein; DAI, disease activity index; DSS, dextran sulfate sodium; GI, gastrointestinal; IBD, inflammatory bowel disease; IFN-γ, interferon gamma; IL, interleukin; MaS, macroscopic score; MMP, metalloproteinase; MPO, myeloperoxidase; RAS, renin-angiotensin system; TGF-β, transforming growth factor beta; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF-α, tumour necrose factor alpha; UC, ulcerative colitis.

1 | INTRODUCTION

The renin-angiotensin system (RAS) is a complex network of peptides, enzymes and receptors that are tightly regulated and have relevant physiological and pathophysiological effects [1]. Angiotensin-converting enzyme (ACE) converts angiotensin (Ang) I into Ang II, which is the main effector peptide of this system, mediating effects such as vasoconstriction or pro-inflammatory and pro-fibrotic effects via angiotensin type 1 (AT_1) receptors [1], while activation of angiotensin type 2 (AT_2) receptors usually counterbalances those effects [2]. Besides its classic view as an endocrine system with renal and cardiovascular relevance, the RAS has been found locally in several tissues such as the gastrointestinal (GI) tract [3–5], where Ang II mediates glucose absorption [4] and intestinal smooth muscle contraction [6], while ACE takes part in peptide digestion [3]. The RAS also plays a role in GI inflammation [7] and has been associated with GI diseases like inflammatory bowel disease (IBD).

IBD includes Crohn's disease (CD) and ulcerative colitis (UC), both conditions being characterized by a chronic inflammation of the intestine [8]. IBD is a systemic chronic condition that has several extraintestinal manifestations such as psoriasis, uveitis and arthritis [9]. IBD is also associated with several comorbidities such as osteoporosis, Parkinson's disease, atherosclerosis and cardiovascular disease [10]. In fact, it has been reported that IBD patients have an increased cardiovascular risk [11, 12], possibly because chronic systemic inflammation promotes atherosclerotic events [13]. Given the systemic inflammatory nature of IBD and the increased cardiovascular risk, many IBD patients are under concomitant anti-hypertensive therapy with ACE inhibitors (ACEi) or angiotensin receptor blockers (ARBs).

Alterations in the levels of RAS-related peptides and receptors during colonic inflammation have been reported in experimental and clinical studies by our group [6] and others [14]. Further, in a pilot study with patients with CD and UC, no differences were found in the levels of ACE and Ang II between groups, neither between patients and healthy individuals, but levels of ACE2 and Ang 1–7, peptides of the counterregulatory RAS, were upregulated [15]. Additionally, Ang-II mediated colonic contraction is decreased in rats with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced [6] and dextran sulphate sodium (DSS)-induced [16] colitis.

Given the emerging data on RAS contribution to colonic inflammation, several studies using experimental models of IBD assessed whether manipulation of this system could improve experimental disease outcomes. Also, a few clinical retrospective studies investigated the possible effect of antihypertensive medications on the course of IBD and its clinical consequences. However, there are many different animal models of IBD [17] that report a wide range of disease severities based on different induction methods, and retrospective clinical studies suffer from limitations when analysing data [18], making it hard to compare IBD outcomes between studies. Consequently, there is a lack of standardized disease reporting that could improve the quality of these studies and assure replicability between experiments and analysis. As so, the aim of this systematic review was to ascertain the heterogeneity in published experimental and clinical data about the use of ACEi or ARBs in IBD outcomes.

2 | MATERIALS AND METHODS

This study was performed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [19] and Cochrane Collaboration recommendations [20]. The full protocol is available at PROSPERO registration number CRD42022323853. One author performed all steps of the studies' selection and data extraction. The process was independently reviewed by a second author. When necessary, a third referee was consulted in case of discrepancies.

2.1 | Search strategy and eligibility criteria

The following electronic databases were systematically searched: PubMed, Scopus and Web of Science, with no data or language restrictions (search updated in April 2022). The complete search strategy is available in supplementary material S1. Titles and abstracts of the retrieved articles were screened for eligibility. Relevant records were then read in full, and studies that met the following inclusion criteria were included for data extraction and analyses: studies evaluating the use of pharmacological agents acting on ACE or AT_1 receptors, with or without comparator, on patients with IBD as classified by the International Classification of Diseases–version 11 (DD70 and DD71, regardless of the region of the intestine affected) or experimental animal models of related diseases (i.e., colitis or ileitis). Studies were excluded if (a) they did not report original results, (b) they were written in non-Roman characters, or (c) the full-text pdf file was unavailable. References of the included papers were scrutinized for potentially additional studies. The comprehensive assessment of the eligibility criteria is described in supplementary material S2.

2.2 | Data extraction and methodological quality assessment

A standardized form (Microsoft Excel, Redmond, WA; supplementary material S3) was used to extract information about type of study (human vs. experimental studies), type of IBD (IBD, UC or CD), active vs. quiescent IBD, strain, sex, type of experimental animal condition (colitis vs. ileitis), induction method, time of induction, which ACEi or ARBs and dosages, route of administration, disease activity index (DAI), histologic scores, macroscopic scores (MaS), inflammatory markers (myeloperoxidase, MPO; C-reactive protein, CRP; calprotectin and cytokines), AT_1 receptor expression, ACE levels and ACE activity levels.

Quality assessment of the included studies was assured by following the SYRCLE's risk of bias tools for animal studies (supplementary material S4) [21].

2.3 | Data synthesis

A narrative synthesis of the findings from the included studies, structured around the type of intervention, target population characteristics, experimental model used, method for evaluation of the damage induced, and type of outcome, is provided in tables and figures.

Methods to evaluate the damage induced in experimental models of IBD were classified as DAI, MaS or histologic assessment. The duration of each study protocol was calculated considering the initiation of damage induction as the time zero.

3 | RESULTS

3.1 | Literature search results

After the application of the search strategy, 282 different articles were retrieved. After title and abstract analysis, 204 articles were excluded for being out of the scope of this review. Application of the exclusion criteria led to a final number of 40 articles that were included in this review. Manual searches of references provided one additional article, leading to a final of 41 articles: 33 articles used experimental models of colitis, and one article used an experimental model of ileitis; six articles reported results in patients with IBD, but two of them were considered to be the same study. Finally, one article reported results from both patients with IBD and animals with experimental colitis (Figure 1). As the results of the two branches of the latter article (clinical and experimental) were so different, we decided to consider it as two independent studies (one clinical and one experimental).

3.2 | Quality of animal studies

The results of the quality assessment using SYRCLE's tool for assessing risk of bias in animal studies are presented in Figure 2. Our analysis showed that all studies failed to house the animals blindly and randomly during the experiments, as well as to select animals to assess the different outcomes (Figure 2). Regarding the allocation of animals to an experimental group, most of the studies did not randomize them or state that the animals were randomly assigned to an experimental group but do not describe the randomization procedure. However, although all studies presented flaws in describing several parameters, all of them reported the initially proposed outcomes.

3.3 | Experimental studies characteristics

All 35 experimental studies reported the condition studied, the induction methods and the species of rodent used, although only 23/35 reported the sex of the animals used, which were mostly males (Table 1). Regarding the experimental model, chemically induced colitis was the most widely used strategy: TNBS-induced model of colitis was used in 9/35 studies, the DSS-induced model of colitis was used in 16/35 studies and the acetic acid-induced colitis was reported in 6/35 studies. The TNBS-induced model of colitis was used more often in rats and the DSS model of colitis in mice (Table 1). Our search retrieved 1/35 study on ileitis, using the SAMP1/YitFc mice, a spontaneous model of CD-like ileitis (Table 1).

The three most reported chemical models for colitis induction presented an enormous variability in the induction protocol (Table 1). In the TNBS-induced model, TNBS dose ranged from 10 mg/rat to 30 mg/rat, with the percentage of ethanol varying from 40% to 50% (Table 1). One of the nine studies did not report sufficient information on the TNBS dose used. In the DSS-induced model, the DSS dose ranged from 4% to 5% in studies using rats and between 1% and 5% in studies using mice (Table 1).

3.4 | Evaluation of the experimental model

When using experimental models of colitis, it is essential to ensure that the clinical condition is mimicked, while maintaining animals' welfare. Only 23/35 experimental studies reported data on body weight changes throughout the protocol [14, 22–43], with 1/35 studies reporting body weight after animals' sacrifice [44]. Additionally, 2/35 studies reported both food and fluid intake [28, 44], while 1/35 reported only fluid intake [41]. Colon

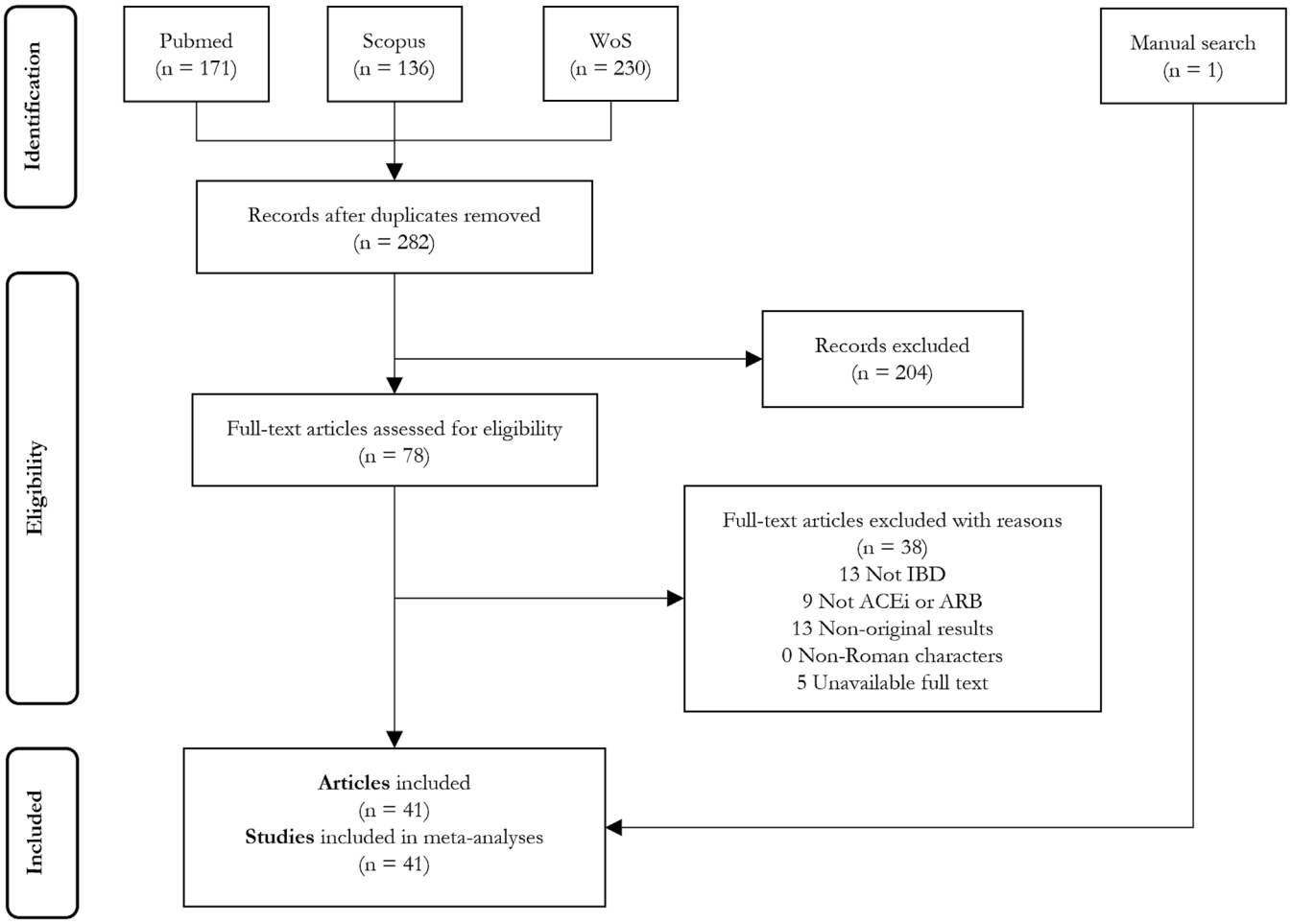

FIGURE 1 PRISMA flow-chart for literature selection.

weight and length were reported in 7/35 [28, 32, 42, 44–47] and 14/35 studies [14, 28, 31–33, 35, 38–40, 42–45, 48, 49], respectively, and colon weight/length ratio was reported in 8/35 studies [30, 32, 45, 48–52]. Stool characteristics were often included in disease scoring systems, but only 7/35 studies [14, 22, 24, 27, 41, 44, 53] gave information on stool characteristics (including the presence of diarrhoea or blood in the faeces or the rectum) independently from those scores. Only 4/35 studies reported the animals' survival [22, 26, 29] or mortality rate [22, 26, 28, 29].

All studies reported at least one of the following scoring systems to evaluate disease activity: DAI, MaS or histologic assessment. Seventeen out of 35 studies used DAI to evaluate the experimental model [14, 22, 23, 26–28, 30, 31, 38, 39, 42, 47–52], 17/35 studies used MaS [28, 30, 32, 34, 36, 37, 40, 41, 44–47, 50–54] and 34/35 studies used a histologic score [14, 22–26, 28–54]. Six out of the 35 studies used the three scoring systems [28, 30, 47, 50–52], 11/35 used MaS and histologic assessment [22, 32, 34, 36, 37, 40, 41, 44–46, 54] and 10/35 used DAI and histologic assessment [14, 22, 23, 26, 31, 38, 39, 42, 48, 49]. Interestingly, 7/35 studies used only histologic score [24, 25, 29, 33, 35, 43, 55], 1/35 used only DAI [27], but no study reported only MaS.

Regarding DAI, 14/17 studies based their DAIs in previously published studies without any modifications [14, 22, 26, 28, 30, 31, 38, 39, 47–52]. From those, 5/14 studies [14, 26, 30, 51, 52] used a DAI described by Cooper et al. [56], while the rest of the studies used DAIs referencing different authors. Also, 1/17 studies used a new DAI [23], but even though the authors explain which characteristics were assessed, they failed to report how to calculate it. Moreover, 2/17 studies only reported the final DAI [27, 42] but failed to describe the characteristics that determined it. For the studies that provided information on the characteristics assessed, 15/15 studies evaluated stool consistency [14, 22, 26, 28, 30, 31, 38, 39, 47–52], 13/15 studies considered body weight changes [14, 22, 26, 28, 30, 31, 38, 39, 47, 50–52], 12/15 studies assessed blood in the faeces [14, 22, 26, 28, 31, 47–52] and 4/15 rectal blood [30, 38, 39, 47], 2/15 studies report rectal prolapse [38, 48] and 1/15 studies reported changes in the animals' fur [48].

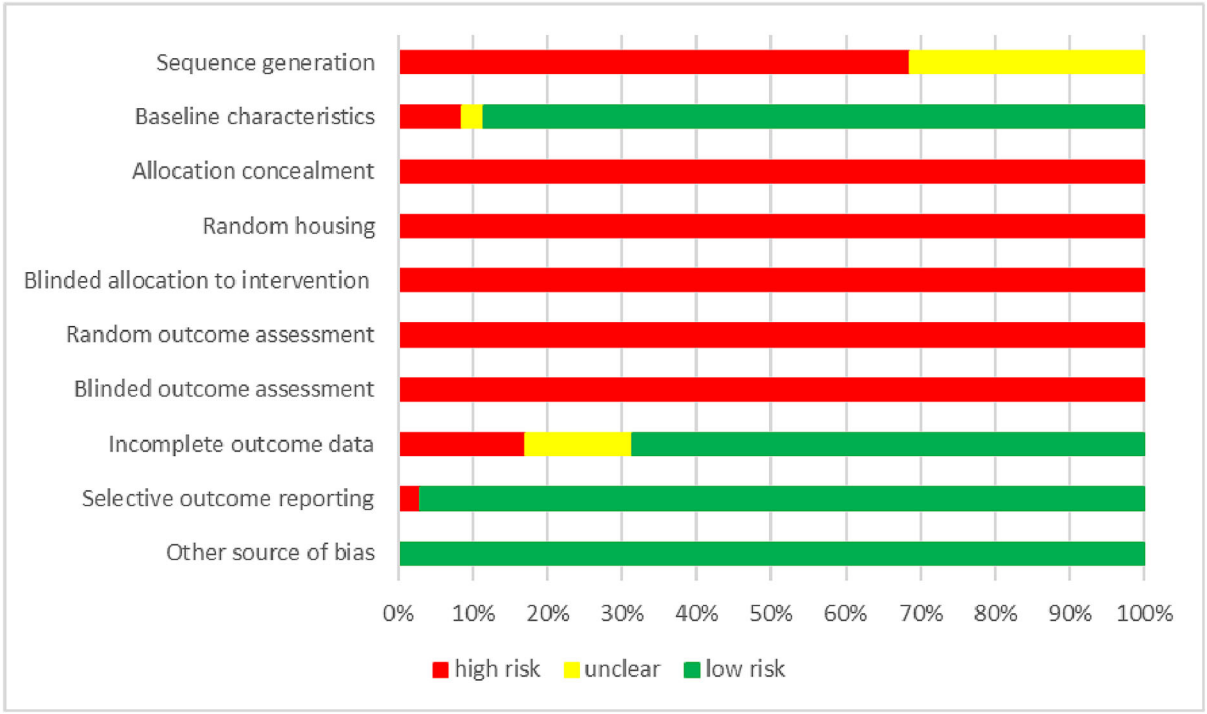

FIGURE 2 Assessment of the risk of bias (%) in the animal studies included in this review. Red, green and yellow lines indicate high, low or unclear risk of bias, respectively.

Fourteen out of 17 studies used an already published MaS [30, 34, 37, 40, 41, 44–47, 50–54] and, from those, 4/14 studies [34, 37, 41, 53] used a MaS presented by Appleyard CB et al. [57], while the others cited different authors. Slight modifications were introduced to previously published MaS in 2/14 studies [30, 40], whereas 3/17 studies reported the use of a new MaS [28, 32, 36]. All studies provided the characteristics included in the correspondent MaS: ulceration (12/17 studies [28, 30, 32, 34, 37, 41, 44–46, 50, 53, 54]), wall thickness (6/17 studies [34, 37, 41, 44, 46, 53]), diarrhoea (6/17 studies [34, 37, 41, 51–53]), edema (5/17 studies [28, 36, 40, 44, 50]), hyperaemia (5/17 studies [30, 44–47]), presence of blood (5/17 studies [36, 40, 50–52]), adhesions (5/17 studies [30, 34, 37, 41, 53]), degree of inflammation (4/17 studies [28, 32, 45, 54]), extent of damage (4/17 studies [30, 44–46]), necrosis (3/17 studies [32, 50, 54]) and cell depletion, mucosal atrophy, cell infiltration and vascular dilation (1/17 study [28]). Although several studies reported a MaS that evaluated at least two of the same characteristics, the quantitative scale was highly variable.

Globally, histologic assessment included a quantitative score and/or a qualitative description of the microscopic alterations observed, often associated with representative images of the colon. Twenty-three out of 35 studies [14, 23–26, 30, 33–37, 39–41, 44–48, 50, 51, 53, 54] used a histologic score that was previously reported by other authors, but two of them added minor alterations [26, 40]. The most frequently cited histologic scores were Cooper et al. [56] (3/23 studies [25, 37, 47]), Appleyard et al. [57] (3/23 studies [34, 41, 53]) and Spencer et al. [25] (3/23 studies [22, 24, 35]), although Spencer et al. [25] used the score defined by Cooper and colleagues [56]. Five out of 35 studies presented only a quantitative score of histologic alterations [26, 32, 33, 42, 55], while 28/35 studies presented a quantitative score associated with a qualitative description and representative images of the damaged colon [14, 23–26, 30, 31, 33–41, 44–54]. Also, 4/35 studies failed to describe the microscopic characteristics assessed in those scores [14, 27, 31, 49]. In the 27/35 studies that describe those characteristics [22–26, 28, 30, 32–41, 44–48, 50–54], the most prevalent were related to alterations in the (i) intestinal wall architecture—crypt damage (14/27 studies [22–25, 33–35, 37, 38, 40, 41, 47, 52, 53]), ulceration (7/27 studies [26, 28, 30, 32, 44, 45, 54]), thickening of different intestinal layers (5/27 studies [28, 34, 41, 51, 53]), edema (4/27 studies [28, 46, 47, 52]), goblet cells depletion (4/27 studies [34, 41, 47, 53]) and necrosis (2/27 studies [46, 52])—and (ii) inflammatory status—inflammatory cell infiltration (17/27 studies [22, 24–26, 32, 34–37, 40, 41, 46, 47, 51–54]) and degree of inflammation (10/27 studies [23, 30, 33, 38, 39, 44, 45, 48, 50, 51]). The histologic score was highly variable among studies, albeit being the most reported score to evaluate the induction of this experimental model.

TABLE 1 General characteristics of the 35 experimental studies included in this review.

		Rat, *n*	Mouse, *n*
Species		14	21
Sex		14	11
	Male	12	8
	Female	1	2
	Both	1	1
Experimental model			
Chemical	TNBS	7	2
	2.5 mg/animal; 50% ethanol	0	1
	10 mg/animal; 50% ethanol	1	0
	15 mg/animal; 50% ethanol	2	0
	25 mg/animal; 50% ethanol	1	0
	30 mg/animal; 40% ethanol	1	0
	30 mg/animal; 50% ethanol	2	0
	DSS	4	12
	1%	0	1
	2%	0	1
	2.5%	0	4
	3%	0	4
	4%	1	1
	5%	3	1
	AOM/DSS	0	1
	Acetic Acid	5	1
	1.05 mL 4% (v/v)	1	0
	0.5 mL 10% v/v in saline 0.9%	1	0
	2 mL 3% v/v in saline 0.9%	2	0
	2 mL 4% v/v in saline 0.9%	1	0
Spontaneous	SAMP1/YitFc	0	1
Genetic	IL-10 knock-out	0	1
Mixed	Piroxicam in IL-10−/− mice	0	5

3.5 | ARBs and ACEi in experimental models of IBD

Among the 35 studies using experimental models of IBD, 21 reported the effect of ARBs [22–24, 27, 30–32, 34, 35, 37, 38, 40, 41, 44, 49–52, 54, 55] and 12 reported the effect of ACEi in experimental disease outcomes [22, 25, 26, 28, 29, 33, 36, 40, 42, 43, 46, 47]. Additionally, two studies used AT_1 receptor knock-out models induced with colitis [14, 23], to assess the same effects.

A great heterogeneity in the drug intervention was found among these studies, namely concerning the variability of the drug tested (six different ARBs and six different ACEi), the dose used of each drug and the route of administration (Table 2).

The duration of the experimental protocols was also greatly variable (Figure 3). Most studies started administrating the drug prior to or on the day of colitis induction, assuming a preventive strategy, while only 3/35 studies reported that drugs were administered 3 or 7 days after colitis induction, adopting a therapeutic approach (Figure 3). For the sake of ensuring comparability, studies using SAMP1/YitFc mice with spontaneous ileitis [55], IL-10 knock-out mice with spontaneous colitis [48] and mice induced with AOM/DSS [39] were not included in Figure 3. The study using SAMP1/YitFc mice [55] had a therapeutic approach, since animals were treated with dexamethasone (to induce remission) for 7 days, after which they were then treated with losartan for 2 or 5 weeks. In the study using IL-10 knockout mice with colitis [48], telmisartan was administered on the day of induction and for 12 weeks, assuming a preventive approach. Finally, in mice treated with AOM followed by DSS treatment for three cycles, Hachiya et al. [39] used a mixed approach, with

TABLE 2 Description of the ARBs and ACEi (drug, dose and administration route) and type of genetic manipulation used in experimental studies, and outcomes assessed.

	Experimental studies				
				Outcomes	
	n	Dose range	Administration route	Cytokines	Unspecific
ARBs					
Candesartan	3	0.4 mg/kg/day	IP[a]	TNF-α [23]	MPO, AT_1R [27]
		10 mg/kg/day	Transanal	IL-1β, IL-6, IL-10, TNF-α [24]	None [24]
Valsartan	3	160 mg/kg/day	Oral	None [38]	None [38]
		160 mg/L/day	Oral	IL-10, IL-18, TNF-α, TGF-β [41, 53]	MPO [41, 53]
Losartan	7	7 or 10 mg/kg/day	Oral	IL-1β [34, 40]; IL-6, IL-23, IL-17, IL-12, IL-2 [34]; TNF-α [34, 40]; TGF-β [44]; INF-γ [34]	ACE levels [40]
		100 mg/kg/day	Transanal	IL-1β, IL-6; IL-10, TNF-α [24, 35]; INF-γ [35]	AT_1R [35]
		0.6-10 mg/mL/day	Oral	IL-33, TGF-β [55]	AT_1R [37]
Irbesartan	1	30 mg/kg/day	Oral	IL-6, IL-10, TNF-α, TGF-β [39]	None [39]
Telmisartan	5	1–10 mg/kg/day	Oral	IL-1β [51]; IL-6 [48, 51]; IL-10 [30, 45, 51]; IL-17 [48]; TNF-α [30, 45, 48, 51]; IFN-γ [51]	MPO [30, 45, 48, 51]; PCR [51]
		0.01 and 5 mg/kg/day	Transanal	IL-1β, IL-6; TNF-α [31]	None [31]
Olmesartan	5	1–10 mg/kg/day	Oral	IL-1β [52]; IL-6 [50, 52]; IL-10 [52]; TNF-α [49, 50, 52]; TGF-β [52]; None [32, 54]	MP [49, 50, 52]; PCR [52]; None [32, 54]
ACEi					
Enalapril	6	1–5 mg/kg/day	Oral	IL-1β [40]; IL-6 [43]; IL-12 [33, 43]; TNF-α [33, 40, 43]	ACE levels [40]; None [33, 43]
		6.25–25 mg/kg/day	Transanal	IL-1β, IL-6, IL-8, INF-γ [26]; TNF-α [26, 29]	None [26, 29]
		0.03 mg/mL/day	IP[a]	TNF-α [25]	ACE levels [25]
Enalaprilat suspended in PEG-1500	1	14.5–145 μg/250 μL PEG	Transanal	IL-1β, TNF-α, TGF-β [22]	None [22]
Captopril	4	30 or 50 mg/kg/day	Oral	IL-6, TNF-α [47]; TGF-β [28]	MPO [47]; Ang II levels [28]
		0.1 mg/kg/day	IP[a]	TNF-α [46]	MPO [46]
		0.11 mg/day	Oral	IL-1β, IL-6, TNF-α [36]	ACE levels [36]
Lisinopril	1	1 mg/kg/day	IP[a]	TNF-α [46]	MPO [46]
Zofenopril	1	1.5 mg/kg	Not stated	None [42]	None [42]
ILE-PRO-PRO	1	5 mg/day	Oral	IL-1β, IL-6, TNF-α [36]	ACE levels [36]
Genetic manipulation					
AT_1 receptor knock-out	2	N/A[b]	N/A[b]	TNF-α [14, 23]	MPO, AT_1e [14]; None [23]

[a]IP, intraperitoneal.
[b]N/A, not applicable.

a subgroup of animals to whom irbesartan was administered from the beginning of the induction protocol (preventive approach) and another subgroup of animals that started treatment with irbesartan 10 weeks after induction of colitis (therapeutic approach).

3.6 | Experimental outcomes

From the 35 experimental studies, 11 assessed the levels of myeloperoxidase (MPO) [14, 27, 30, 41, 45–50, 53] and two of them assessed both MPO

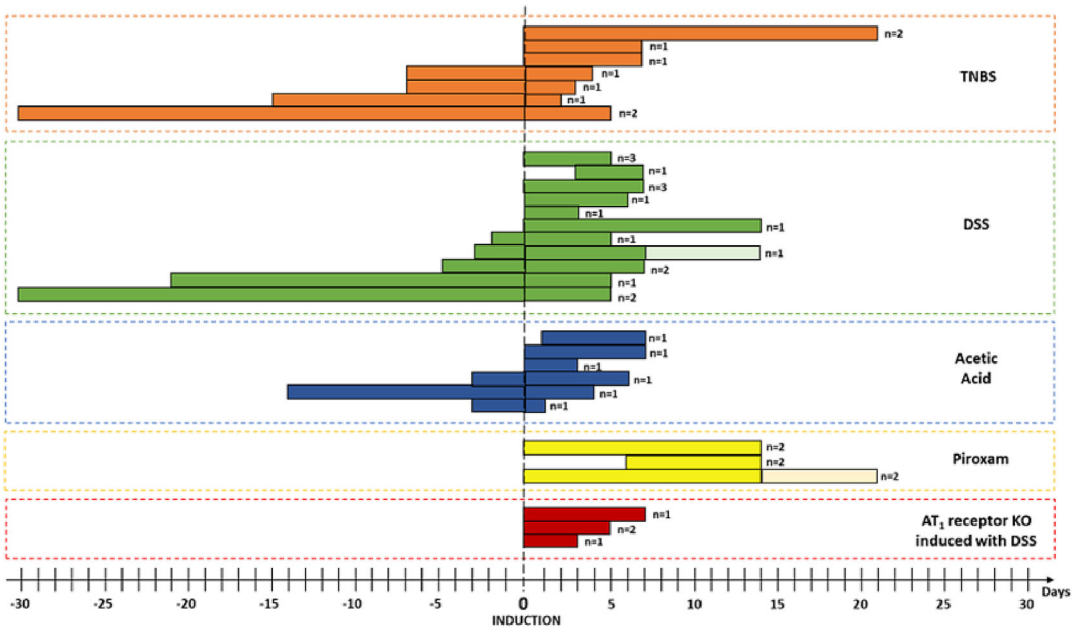

FIGURE 3 Variability of the experimental protocols using TNBS, DSS, acetic acid, piroxam and the AT_1 receptor knock-out (KO) model induced with DSS. Light green and light yellow refer to the timepoint where animals stopped receiving DSS or piroxam, respectively, but continued to receive the ARB or ACEi; *n* refers to the number of studies that used that experimental protocol.

and C-reactive protein (CRP) [51, 52], as unspecific biomarkers of inflammation (Table 2). None of the animal studies reported for faecal calprotectin quantification. Additionally, several different cytokines were quantified (Table 2). In 16 out of 35 studies, pro-inflammatory interleukins (ILs) were measured: IL-1β [22, 24, 26, 31, 35, 36, 40, 51, 52], IL-12 [33, 43], IL-17 [48], IL-8 [26] and IL-18 [41, 53], and one study quantified IL-1β, IL-12, IL-17 and IL-23 [34]. Also, 9/35 studies quantified IL-10 [24, 30, 35, 39, 41, 45, 51–53], an anti-inflammatory IL. Finally, 13/35 studies assessed ILs that can mediate pro- or anti-inflammatory effects: IL-6 [24, 26, 31, 35, 36, 43, 47, 48, 50–52] and IL-33 [55], and one study measured both IL-6 and IL-2 [34]. Most studies (26/35 in total [14, 22–26, 29, 30, 33–36, 39–41, 43, 45–53]) also quantified tumour necrosis factor-α (TNF-α), while 8/35 studies measured transforming growth factor beta (TGF-β) [22, 28, 39, 41, 44, 52, 53, 55] and 4/35 studies measured interferon gamma (IFN-γ) [26, 34, 35, 48].

Only 2/35 studies quantified Ang II [14, 28], and 4/35 studies assessed the expression of the AT_1 receptor in the intestine [14, 27, 35, 37] (Table 2). Just 3/35 studies reported for the ACE level [25, 36, 40], and all failed to report ACE activity levels (Table 2).

Other parameters were assessed throughout the different studies, like apoptosis, lipid peroxidation biomarkers, oxidative stress markers or other proteins that were specific to the aim of each study, but irrelevant for an overall analysis of disease outcomes.

4 | CLINICAL STUDIES

The clinical studies included in this systematic review comprised a total of 1841 IBD patients, from which 539 patients were exposed to RAS blockers. Additionally, Efsen et al. [58] used fistula specimens from 22 CD patients to assess the in vitro effect of ramiprilate on the activity of matrix metalloproteinases (MMPs). Only three out of the six studies reported the sex of the IBD patients, and these three included both male and female patients [58–60]. All human studies reported the disease diagnosis [37, 58–63]. Although 3/6 studies reported outcomes within a population of IBD patients [37, 61, 62], other 3/6 studies reported outcomes within a population of IBD patients plus a sub-analysis on UC or CD patients [59, 60, 63]; 1/6 studies only included CD patients [58]. Only 1/6 studies differentiated between active and quiescent disease [37].

Five out of six were retrospective studies, meaning that they reported data on ARBs or ACEi usage in IBD patients that were under that medication prior to being recruited for the study [37, 59–63]. Also, because these were retrospective studies, none of them reported the dose of the ARB or ACEi used. Five out of six studies assessed the effect of ARBs on disease outcomes [37, 59–63], while 5/6 studies reported results on ACEi [58–60, 62, 63]. Only one study described in more detail which ARBs (candesartan, irbesartan and telmisartan) or ACEi (perindopril and ramipril) were assessed [63]. Another study conducted a sub-analysis

on the effect of olmesartan in disease outcomes in a subset of IBD patients under therapy with ARBs [60].

No differences were found between the demographics of IBD patients included in studies using and not using antihypertensive drugs. All the human studies evaluated some type of outcomes, whether they were IBD-related characteristics, severity or clinical activity, or inflammatory biomarkers. Clinical outcomes reported are summarized in Table 3. Only one study evaluated the inflammatory biomarkers profile between IBD

TABLE 3 ARBs or ACEi treatment effect on inflammatory bowel disease outcomes.

Study	Study design	Effect of ARBs or ACEi treatment on disease outcomes
Shi Y et al. [37]	Retrospective study; 11 quiescent and 7 active UC and CD patients who were on ARB vs. 24 quiescent and 14 active UC and CD patients who were not on ARB.	Reduced pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IL-17A, IL-17F, IL-23, TNF-α) in IBD patients on ARB therapy, especially for the active patients.
Mantaka A et al. [60]	Retrospective study; 150 IBD patients who were on antihypertensive drugs vs. 150 IBD patients who were not on antihypertensive drugs.	IBD patients on ARBs were less frequently on steroid (58.6% vs. 71.7% non-ARBs, $p = 0.041$) and immunomodulators (31.4% vs. 45.7% non-ARBs, $p = 0.039$); Rates of lifetime steroid use were statistically significantly lower among patients with ACEi or ARBs use (61.4% vs. 73.1%, $p = 0.035$, OR = 1.191, 95% CI, 1.005–1.411); Fewer hospitalizations for flare in olmesartan users (47.4% vs. 63.7%, $p = 0.025$); IBD patients that use ARBs associated with milder disease course (aOR (95% CI) = 3.913 (1.084–14.119), $p = 0.037$), lower rates of immunomodulators use (aOR (95% CI) = 0.369 (0.145–0.944), $p = 0.038$), lower rates of ileocolonic CD location (aOR (95% CI) 0.098 (0.015–0.658), $p = 0.017$), lower rates of penetrating CD behaviour (aOR (95% CI) = 0.113 (0.013–1.006), $p = 0.051$).
Fairbrass KM et al. [61, 62]	Retrospective study; 104 IBD patients on RAS blockers (out of 764 IBD patients (UC = 321, CD = 443).	Decreased likelihood of intestinal resection (1.9% vs. 7.6% non-ACEi or ARBs, $p = 0.03$); A trend towards fewer flares (25.6% vs. 35.7% non-ACEi or ARBs, $p = 0.06$) and hospitalizations (9.6% vs. 16.5% non-ACEi or ARBs, $p = 0.07$).
Garg M et al. [63]	Retrospective study; 26 IBD patients on RAS blockers (out of a total of 296 IBD patients)	Lower disease activity scores (0:1:2:3:4 [%]) in IBD patients using RAS blockers (50:23:12:12:4 vs. 24:32:15:21:7 non-RAS blockers use, $p = 0.016$); Lower requirement for hospitalization not requiring surgery in IBD patients using RAS blockers (0% vs. 15% non-RAS blockers; $p = 0.033$); Lower concentration of faecal calprotectin in 8 IBD patients using RAS blockers (log faecal calprotectin (mean, CI) = 1.66(1.37–1.95) vs. 2.09(1.96–2.21 non-RAS blocker use, $p = 0.046$);
	First nested case–control study; 39 IBD patients that required surgery vs. 75 IBD controls	Lower requirements for surgery in patients (all with CD) using RAS blockers (16% vs. 3% IBD patients not requiring surgery, $p = 0.034$).
	Second nested case–control study; 34 IBD patients that were hospitalized vs. 68 IBD controls	Patients not requiring hospitalization were more likely to be treated with RAS blockers (12% vs. 0% IBD patients hospitalized, $p = 0.049$).
Jacobs JD et al. [59]	Retrospective study; 111 IBD patients using ACEi or ARBs vs. 111 IBD controls	Fewer hospitalizations (OR 0.26, $p < 0.01$), fewer operations (OR 0.08, $p = 0.02$), and fewer corticosteroid prescriptions (OR 0.5, $p = 0.01$) in patients with ACEI or ARB exposure.
	Retrospective study; 76 IBD patients using ACEi + 54 IBD patients using ARBs—before and during use	Fewer hospitalizations (OR 0.08 vs. 0.16, $p = 0.03$) and more corticosteroid use (OR 0.24 vs. 0.12, $p < 0.01$) prior to than during ACEI/ARB use, respectively, in UC patients.

patients in the presence and absence of antihypertensive drugs (Table 3), while Efsen et al. [58] assessed differences in MMP activity, as it was the main aim of their work. Only one study assessed the levels of faecal calprotectin between IBD patients using and not using antihypertensive drugs (Table 3).

5 | DISCUSSION

To our knowledge, this is the first study to synthesize published data on the use of ACEi or ARBs in IBD outcomes. Furthermore, our results demonstrate that there is a lack of standardization in the experimental models of colitis, as well as in the disease severity scores and experimental outcomes used for assessing the efficiency of disease induction, compromising replicability and comparability across studies.

There are several animal models used for inducing experimental colitis [64], but the chemically induced models are the most used because they are easy and cost-effective procedures that mimic immunological and histological features of human IBD [65]. Our results showed that in studies aiming to assess the role of ARBs and ACEi in IBD outcomes, the TNBS- and the DSS-induced models of colitis were the most frequently used. This is in accordance with the literature that reports both models as the most widely used, regardless of the specific aim being studied [66]. However, differences between both models may resemble subgroups of IBD patients. While the TNBS-induced model promotes a T_h1-mediated inflammatory response resembling CD in humans, the DSS-induced model promotes a T_h2-mediated inflammatory response resembling human UC [66]. Despite this difference, we found that in studies using chemical models of IBD, authors do not discriminate whether they are aiming to study CD or UC, use the term 'colitis' as a generic description of the experimental disease and focus mainly on the colon.

Literature demonstrated that the report of the methods in studies using these animal models of colitis presented low quality, compromising the translational value to clinical research of human IBD [67]. In addition to the generic guidelines to report animal studies [68], Bramhall et al. [67] even created a checklist to ensure quality when reporting experiments with animal models of colitis. Good reporting practice is a crucial requirement, especially when models present the enormous variability as identified in our study. The three most commonly used models to induce colitis (TNBS-, DSS- and acetic acid-induced colitis) varied in the dosage of the damage-inducing agent, the time the animals remain induced, the method to assess colitis severity/extent, the evaluation of animal welfare and the outcomes measured to assess the intervention.

Frequent monitoring of animals (i.e., body weight and food/fluid intake assessment or implementation of disease activity scores) is essential to maintain animal welfare during the experimental protocol. A decrease of 20% of the initial body weight of the animal is a strong indicator of decreased animal welfare and a humane endpoint to consider the removal of that animal from the study [69]. Only half of the studies included in this systematic review reported body weight along the protocol, while one reported the animals' body weight just after sacrifice. Since these are experimental models of colitis, all studies should report stool consistency, as well as the presence of diarrhoea or obstipation. However, although all studies include stool consistency in their DAIs, they fail to report stool characteristics, hiding relevant information such as the presence of diarrhoea or constipation. Colonic weight, length and weight/length ratio are important indicators of colonic damage, because they correlate with intestinal inflammation [70]. Our analysis revealed that only a few studies specifically reported these characteristics, with most studies using DAI to monitor disease activity after animals' sacrifice.

The severity of the damage induced in the animals depends on many different factors: the species strain [65, 71], the dose and the characteristics of the chemical agent used [72], the environment [73] and the time course of the experiment. Since these factors contribute to the variability of the studies, assessing the severity of induced colitis is crucial to ensure the comparability of the outcomes obtained. DAI, MaS and histologic assessment were almost ubiquitous among the included studies. Although DAIs assessed similar characteristics, the quantitative value associated with each qualitative assessment was highly variable between studies. Besides DAIs, macroscopic and microscopic scoring systems are useful tools to assess disease induction. Only half of the studies reported histologic evaluation, which included several different intestinal inflammation characteristics, such as ulceration, edema, hyperaemia, crypt damage, wall thickness or inflammatory cell infiltration [74–76]. As with DAIs, there was high variability in the constitution of the different MaS, which weakens the comparability of results between studies. Most studies that were included reported the use of a microscopic score for histologic assessment, often associated with images or a qualitative description. Histopathology analysis provides a more accurate and comprehensive description of the disease since it preserves the tissue architecture [77]. As in previous tools, albeit characteristics evaluated were common between scoring systems, reported scores were highly variable across studies. The variability identified in the components and the weight of each component among the different DAI, MaS and microscopic scores raises doubts about the similarity of the colitis induction between two animals with identical scores.

The beginning of pharmacological intervention after colitis induction is another variable to be considered as

a factor that produces experimental heterogeneity. In models with lower inductive strength, induced colitis tends to spontaneously disappear after some days [78]. In our study, the time that animals remain induced varied from 1 to 21 days. In addition, some studies administered the ARB or ACEi before induction (varying from 30 to 2 days before induction), while others administered the drugs after induction with different delays.

While all the studies aimed at evaluating the use of ARBs or ACEi on IBD, similar outcomes could be expected bearing in mind the differences between the different conditions included under IBD. The T_h1-mediated inflammatory response produced in CD is usually associated with an increase in IL-2, IL-12, TNF-α and IFN-γ, while the T_h2-mediated inflammatory response of UCs is associated with an increase in IL-4, IL-5, IL-10 and IL-13 [79–83]. Subsequently, depending on the induction method, primarily mimicking of UC or CD should be monitored. We found that these inflammatory mediators were not always assessed, and some studies did not assess any of these cytokines. Surprisingly, no evaluation of ACE activity was reported to prove that the ACEi used was effective, and only two studies quantified Ang II.

The few clinical studies were retrospective and designed to study the effect of antihypertensive medication on IBD outcomes, and not particularly the effect of ACEi or ARBs on disease outcomes. Although retrospective studies can provide valuable information on the effect of RAS blockers in disease outcomes, they lack detailed information that would be crucial for a robust evaluation; in this review, they did not discriminate neither the drug that the patients were taking nor its dose. Only one of these studies reported the inflammatory profile of IBD patients included, and no study evaluated ACE activity levels or faecal calprotectin (a standard faecal biomarker of IBD in clinical practice [84].

6 | CONCLUSION

Current research using animal models to assess the effects of RAS-acting agents (i.e., ACEi or ARBs) on IBD lacks standardization, preventing comparisons between studies and drugs. Researchers, but also journal editors and peer reviewers, should commit to reducing variability in the induction protocol, disease activity scores, macro- and microscopic evaluation, time that animals remain induced, drug initiation time, mediators involved and outcomes.

AUTHORS CONTRIBUTIONS

Mariana Ferreira-Duarte: Data curation; Formal analysis; Investigation; Methodology; Writing—original draft; **Fernanda S. Tonin:** Data curation; Methodology; Supervision; Validation; Writing—review and editing; **Margarida Duarte-Araújo:** Formal analysis; Writing—review and editing; **Fernando Fernandez-Llimos:** Data curation; Methodology; Writing—review and editing **Manuela Morato:** Conceptualization; Project administration; Supervision; Writing—review and editing.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material.

ORCID

Fernando Fernandez-Llimos https://orcid.org/0000-0002-8529-9595

REFERENCES

1. Fyhrquist F, Saijonmaa O. Renin-angiotensin system revisited. *J Intern Med*. 2008;264(3):224-236. doi:10.1111/j.1365-2796.2008.01981.x
2. Ewert S, Spak E, Olbers T, Johnsson E, Edebo A, Fandriks L. Angiotensin II induced contraction of rat and human small intestinal wall musculature in vitro. *Acta Physiol (Oxf)*. 2006;188(1):33-40. doi:10.1111/j.1748-1716.2006.01600.x
3. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev*. 2006;86(3):747-803. doi:10.1152/physrev.00036.2005
4. Garg M, Angus PW, Burrell LM, Herath C, Gibson PR, Lubel JS. Review article: the pathophysiological roles of the renin-angiotensin system in the gastrointestinal tract. *Aliment Pharmacol Ther*. 2012;35(4):414-428. doi:10.1111/j.1365-2036.2011.04971.x
5. Fandriks L. The renin-angiotensin system and the gastrointestinal mucosa. *Acta Physiol (Oxf)*. 2011;201(1):157-167. doi:10.1111/j.1748-1716.2010.02165.x
6. Ferreira-Duarte M, Rodrigues-Pinto T, Sousa T, et al. Interaction between the renin-angiotensin system and enteric neurotransmission contributes to colonic dysmotility in the TNBS-induced model of colitis. *Int J Mol Sci*. 2021;22(9):4836. doi:10.3390/ijms22094836
7. Capettini LS, Montecucco F, Mach F, Stergiopulos N, Santos RA, da Silva RF. Role of renin-angiotensin system in inflammation, immunity and aging. *Curr Pharm des*. 2012;18(7):963-970. doi:10.2174/138161212799436593
8. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol*. 2010;28(1):573-621. doi:10.1146/annurev-immunol-030409-101225
9. Rogler G, Singh A, Kavanaugh A, Rubin DT. Extraintestinal manifestations of inflammatory bowel disease: current concepts, treatment, and implications for disease management. *Gastroenterology*. 2021;161(4):1118-1132. doi:10.1053/j.gastro.2021.07.042
10. Argollo M, Gilardi D, Peyrin-Biroulet C, Chabot JF, Peyrin-Biroulet L, Danese S. Comorbidities in inflammatory bowel disease: a call for action. *Lancet Gastroenterol Hepatol*. 2019;4(8):643-654. doi:10.1016/S2468-1253(19)30173-6
11. Yarur AJ, Deshpande AR, Pechman DM, Tamariz L, Abreu MT, Sussman DA. Inflammatory bowel disease is associated with an increased incidence of cardiovascular events. *Am J Gastroenterol*. 2011;106(4):741-747. doi:10.1038/ajg.2011.63

12. Nunez P, Garcia Mateo S, Quera R, Gomollon F. Inflammatory bowel disease and the risk of cardiovascular diseases. *Gastroenterol Hepatol*. 2021;44(3):236-242. doi:10.1016/j.gastrohep.2020.09.002
13. Sinh P, Cross R. Cardiovascular risk assessment and impact of medications on cardiovascular disease in inflammatory bowel disease. *Inflamm Bowel Dis*. 2021;27(7):1107-1115. doi:10.1093/ibd/izaa258
14. Katada K, Yoshida N, Suzuki T, et al. Dextran sulfate sodium-induced acute colonic inflammation in angiotensin II type 1a receptor deficient mice. *Inflamm Res*. 2008;57(2):84-91. doi:10.1007/s00011-007-7098-y
15. Garg M, Burrell LM, Velkoska E, et al. Upregulation of circulating components of the alternative renin-angiotensin system in inflammatory bowel disease: a pilot study. *J Renin Angiotensin Aldosterone Syst*. 2015;16(3):559-569. doi:10.1177/1470320314521086
16. Zizzo MG, Auteri M, Amato A, et al. Angiotensin II type II receptors and colonic dysmotility in 2,4-dinitrofluorobenzenesulfonic acid-induced colitis in rats. *Neurogastroenterol Motil*. 2017;29(6):e13019. doi:10.1111/nmo.13019
17. Kiesler P, Fuss IJ, Strober W. Experimental models of inflammatory bowel diseases. *Cell Mol Gastroenterol Hepatol*. 2015;1(2):154-170. doi:10.1016/j.jcmgh.2015.01.006
18. Talari K, Goyal M. Retrospective studies—utility and caveats. *J R Coll Physicians Edinb*. 2020;50(4):398-402. doi:10.4997/jrcpe.2020.409
19. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
20. Higgins JPT, Thomas J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (editors). *Cochrane Handbook for Systematic Reviews of Interventions, version 6.3 (updated 2022)* 2022.
21. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14(1):43. doi:10.1186/1471-2288-14-43
22. Koga H, Yang H, Adler J, Zimmermann EM, Teitelbaum DH. Transanal delivery of angiotensin converting enzyme inhibitor prevents colonic fibrosis in a mouse colitis model: development of a unique mode of treatment. *Surgery*. 2008;144(2):259-268. doi:10.1016/j.surg.2008.03.043
23. Mizushima T, Sasaki M, Ando T, et al. Blockage of angiotensin II type 1 receptor regulates TNF-alpha-induced MAdCAM-1 expression via inhibition of NF-kappaB translocation to the nucleus and ameliorates colitis. *Am J Physiol Gastrointest Liver Physiol*. 2010;298(2):G255-G266. doi:10.1152/ajpgi.00264.2009
24. Okawada M, Koga H, Larsen SD, et al. Use of enterally delivered angiotensin II type Ia receptor antagonists to reduce the severity of colitis. *Dig Dis Sci*. 2011;56(9):2553-2565. doi:10.1007/s10620-011-1651-9
25. Spencer AU, Yang H, Haxhija EQ, Wildhaber BE, Greenson JK, Teitelbaum DH. Reduced severity of a mouse colitis model with angiotensin converting enzyme inhibition. *Dig Dis Sci*. 2007;52(4):1060-1070. doi:10.1007/s10620-006-9124-2
26. Sueyoshi R, Ignatoski KM, Daignault S, Okawada M, Teitelbaum DH. Angiotensin converting enzyme-inhibitor reduces colitis severity in an IL-10 knockout model. *Dig Dis Sci*. 2013;58(11):3165-3177. doi:10.1007/s10620-013-2825-4
27. Wada T, Sasaki M, Oshima T, et al. Angiotensin II type 1 receptor blocker, candesartan attenuated colonic inflammation in dextran-sulfate induced colitis via inhibition of madcam-1 expression (abstracts of the AGA Institute). *Gastroenterology*. 2006;130(4):A-344-A-345.
28. Wengrower D, Zanninelli G, Pappo O, et al. Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. *Inflamm Bowel Dis*. 2004;10(5):536-545. doi:10.1097/00054725-200409000-00007
29. Sueyoshi RIK, Ignatoski K, Okawada M, Teitelbaum DH. Blockade of angiotensin converting enzyme-inhibitor (ACE-I) prevents in immunologically relevant colitis model. *J am Coll Surg*. 2012;215(3):S11-S12. doi:10.1016/j.jamcollsurg.2012.06.055
30. Arab HH, Al-Shorbagy MY, Abdallah DM, Nassar NN. Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. *PLoS ONE*. 2014;9(5):e97193. doi:10.1371/journal.pone.0097193
31. Arumugam S, Sreedhar R, Thandavarayan RA, et al. Telmisartan treatment targets inflammatory cytokines to suppress the pathogenesis of acute colitis induced by dextran sulphate sodium. *Cytokine*. 2015;74(2):305-312. doi:10.1016/j.cyto.2015.03.017
32. Fatima DSA. Therapeutic effect of olmesartan medoxomile alone and in combination with sulfasalazine in experimentally ulcerative colitis model in rats. *Int J Toxicol Pharmacol Res*. 2016;8(2):97-106.
33. Lee C, Chun J, Im JP, Jung HC, Kim JS. Tu1636 immunomodulatory effect of enalapril on peritoneal macrophage and chronic colitis in interleukin-10-deficient mice. *Gastroenterology*. 2013;144(5):S-811. doi:10.1016/S0016-5085(13)63005-X
34. Liu TJ, Shi YY, Wang EB, Zhu T, Zhao Q. AT1R blocker losartan attenuates intestinal epithelial cell apoptosis in a mouse model of Crohn's disease. *Mol Med Rep*. 2016;13(2):1156-1162. doi:10.3892/mmr.2015.4686
35. Okawada M, Wilson MW, Larsen SD, Lipka E, Hillfinger J, Teitelbaum DH. Blockade of the renin-angiotensin system prevents acute and immunologically relevant colitis in murine models. *Pediatr Surg Int*. 2016;32(12):1103-1114. doi:10.1007/s00383-016-3965-3
36. Salmenkari HHM, Holappa M, Forsgard RA, Korpela R, Vapaatalo H. Orally administered angiotensin-converting enzyme-inhibitors captopril and isoleucine-proline-proline have distinct effects on local renin-angiotensin system and corticosterone synthesis in dextran sulfate sodium-induced colitis in mice. *J Physiol Pharmacol*. 2017;68(3):355-362.
37. Shi Y, Liu T, He L, et al. Activation of the renin-angiotensin system promotes colitis development. *Sci Rep*. 2016;6(1):27552. doi:10.1038/srep27552
38. Asgharzadeh F, Yaghoubi A, Nazari SE, et al. The beneficial effect of combination therapy with sulfasalazine and valsartan in the treatment of ulcerative colitis. *EXCLI j*. 2021;20:236-247. doi:10.17179/excli2021-3370
39. Hachiya K, Masuya M, Kuroda N, et al. Irbesartan, an angiotensin II type 1 receptor blocker, inhibits colitis-associated tumourigenesis by blocking the MCP-1/CCR2 pathway. *Sci Rep*. 2021;11(1):19943. doi:10.1038/s41598-021-99412-8
40. Salmenkari H, Pasanen L, Linden J, Korpela R, Vapaatalo H. Beneficial anti-inflammatory effect of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker in the treatment of dextran sulfate sodium-induced colitis in mice. *J Physiol Pharmacol*. 2018;69(4):07. doi:10.26402/jpp.2018.4.07
41. Santiago OI, Rivera E, Ferder L, Appleyard C, B. An angiotensin II receptor antagonist alters the expression of inflammatory cytokines in acute experimental colitis. *FASEB j*. 2007;21(6):A1322-A1322. doi:10.1096/fasebj.21.6.A1322-a
42. Zhang L, Dai X, Zhao M, Sha L. Su1824—effect of zofenopril on acetic acid-induced ulcerative colitis. *Gastroenterology*. 2019;156(6):S-625. doi:10.1016/S0016-5085(19)38461-6
43. Lee C, Chun J, Hwang SW, Kang SJ, Im JP, Kim JS. Enalapril inhibits nuclear factor-kappaB signaling in intestinal epithelial cells and peritoneal macrophages and attenuates experimental colitis in mice. *Life Sci*. 2014;95(1):29-39. doi:10.1016/j.lfs.2013.11.005

44. Wengrower DZG, Zanninelli G, Latella G, et al. Losartan reduces trinitrobenzene sulphonic acid-induced colorectal fibrosis in rats. *Can J Gastroenterol*. 2012;26(1):33-39. doi:10.1155/2012/628268
45. Guerra GC, Araujo AA, Lira GA, et al. Telmisartan decreases inflammation by modulating TNF-alpha, IL-10, and RANK/RANKL in a rat model of ulcerative colitis. *Pharmacol Rep*. 2015;67(3):520-526. doi:10.1016/j.pharep.2014.12.011
46. Jahovic N, Ercan F, Gedik N, Yuksel M, Sener G, Alican I. The effect of angiotensin-converting enzyme inhibitors on experimental colitis in rats. *Regul Pept*. 2005;130(1–2):67-74. doi:10.1016/j.regpep.2005.03.009
47. Manna MJ, Abu-Raghif A, Abbood MS. Effect of captopril on inflammatory biomarkers, oxidative stress parameters. *J Pharm Sci Res*. 2017;9(9):1629-1636.
48. Li Y, Zuo L, Zhu W, et al. Telmisartan attenuates the inflamed mesenteric adipose tissue in spontaneous colitis by mechanisms involving regulation of neurotensin/microRNA-155 pathway. *Biochem Pharmacol*. 2015;93(4):461-469. doi:10.1016/j.bcp.2014.12.020
49. Nagib MM, Tadros MG, ElSayed MI, Khalifa AE. Anti-inflammatory and anti-oxidant activities of olmesartan medoxomil ameliorate experimental colitis in rats. *Toxicol Appl Pharmacol*. 2013;271(1):106-113. doi:10.1016/j.taap.2013.04.026
50. Murad H, Ahmed O, Alqurashi T, Hussien M. Olmesartan medoxomil self-microemulsifying drug delivery system reverses apoptosis and improves cell adhesion in trinitrobenzene sulfonic acid-induced colitis in rats. *Drug Deliv*. 2022;29(1):2017-2028. doi:10.1080/10717544.2022.2086939
51. Saber S, Basuony M, Eldin AS. Telmisartan ameliorates dextran sodium sulfate-induced colitis in rats by modulating NF-kappaB signalling in the context of PPARgamma agonistic activity. *Arch Biochem Biophys*. 2019;671:185-195. doi:10.1016/j.abb.2019.07.014
52. Saber S, Khalil RM, Abdo WS, Nassif D, El-Ahwany E. Olmesartan ameliorates chemically-induced ulcerative colitis in rats via modulating NFkappaB and Nrf-2/HO-1 signaling crosstalk. *Toxicol Appl Pharmacol*. 2019;364:120-132. doi:10.1016/j.taap.2018.12.020
53. Santiago OI, Rivera E, Ferder L, Appleyard CB. An angiotensin II receptor antagonist reduces inflammatory parameters in two models of colitis. *Regul Pept*. 2008;146(1–3):250-259. doi:10.1016/j.regpep.2007.10.004
54. Fatima DSA, Ahmad AM. Ameliorative effect of olmesartan medoxomil on acetic acid-induced colitis in rats. *Int J Pharmaceut Clin Res*. 2016;8(6):604-609.
55. Ray S, De Salvo C, Lopetuso LR, et al. Losartan abrogates intestinal fibrosis after steroid induced remission in experimental ileitis. *Gastroenterology*. 2015;148(4):S-26. doi:10.1016/S0016-5085(15)30091-3
56. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest*. 1993;69(2):238-249.
57. Appleyard CB, Wallace JL. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. *Am J Physiol*. 1995;269(1 Pt 1):G119-G125. doi:10.1152/ajpgi.1995.269.1.G119
58. Efsen E, Saermark T, Hansen A, Bruun E, Brynskov J. Ramiprilate inhibits functional matrix metalloproteinase activity in Crohn's disease fistulas. *Basic Clin Pharmacol Toxicol*. 2011;109(3):208-216. doi:10.1111/j.1742-7843.2011.00713.x
59. Jacobs JD, Wagner T, Gulotta G, et al. Impact of angiotensin II signaling blockade on clinical outcomes in patients with inflammatory bowel disease. *Dig Dis Sci*. 2019;64(7):1938-1944. doi:10.1007/s10620-019-5474-4
60. Mantaka A, Tsoukali E, Fragkaki M, et al. Is there any role of renin-angiotensin system inhibitors in modulating inflammatory bowel disease outcome? *Eur J Gastroenterol Hepatol*. 2021;33(3):364-371. doi:10.1097/MEG.0000000000001912
61. Fairbrass KM, Hoshen D, Ford A, et al. Do ACE inhibitors and angiotensin II receptor blockers improve disease outcomes in inflammatory bowel disease? *J Crohns Colitis*. 2020;14-(Supplement_1):S380-S381. doi:10.1093/ecco-jcc/jjz203.546
62. Fairbrass KM, Hoshen D, Gracie DJ, Ford AC. Effect of ACE inhibitors and angiotensin II receptor blockers on disease outcomes in inflammatory bowel disease. *Gut*. 2021;70(1):218-219. doi:10.1136/gutjnl-2020-321186
63. Garg M, Royce SG, Tikellis C, et al. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? *Gut*. 2020;69(5):841-851. doi:10.1136/gutjnl-2019-318512
64. Jiminez JA, Uwiera TC, Douglas Inglis G, Uwiera RR. Animal models to study acute and chronic intestinal inflammation in mammals. *Gut Pathog*. 2015;7(1):29. doi:10.1186/s13099-015-0076-y
65. Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. *Nat Protoc*. 2007;2(3):541-546. doi:10.1038/nprot.2007.41
66. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. *Korean J Physiol Pharmacol*. 2014;18(4):279-288. doi:10.4196/kjpp.2014.18.4.279
67. Bramhall M, Florez-Vargas O, Stevens R, Brass A, Cruickshank S. Quality of methods reporting in animal models of colitis. *Inflamm Bowel Dis*. 2015;21(6):1248-1259. doi:10.1097/MIB.0000000000000369
68. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMJ Open Sci*. 2020;4(1):e100115. doi:10.1136/bmjos-2020-100115
69. Talbot SR, Biernot S, Bleich A, et al. Defining body-weight reduction as a humane endpoint: a critical appraisal. *Lab Anim*. 2020;54(1):99-110. doi:10.1177/0023677219883319
70. Soliman GA, Gabr GA, al-Saikhan FI, et al. Protective effects of two Astragalus species on ulcerative colitis in rats. *Trop J Pharmaceut Res*. 2016;15(10):2155. doi:10.4314/tjpr.v15i10.14
71. Ostanin DV, Bao J, Koboziev I, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*. 2009;296(2):G135-G146. doi:10.1152/ajpgi.90462.2008
72. Perse M, Cerar A. Dextran sodium sulphate colitis mouse model: traps and tricks. *J Biomed Biotechnol*. 2012;2012:1-13. doi:10.1155/2012/718617
73. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. *Drug des Devel Ther*. 2013;7:1341-1357. doi:10.2147/DDDT.S40107
74. Bressenot A, Geboes K, Vignaud JM, Gueant JL, Peyrin-Biroulet L. Microscopic features for initial diagnosis and disease activity evaluation in inflammatory bowel disease. *Inflamm Bowel Dis*. 2013;19(8):1745-1752. doi:10.1097/MIB.0b013e318281f2e8
75. Villanacci V, Reggiani-Bonetti L, Salviato T, et al. Histopathology of IBD colitis. A practical approach from the pathologists of the Italian Group for the study of the gastrointestinal tract (GIPAD). *Pathologica*. 2021;113(1):39-53. doi:10.32074/1591-951X-235
76. Vespa E, D'Amico F, Sollai M, et al. Histological scores in patients with inflammatory bowel diseases: the state of the art. *J Clin Med*. 2022;11(4):939. doi:10.3390/jcm11040939
77. Gurcan MN, Boucheron LE, Can A, Madabhushi A, Rajpoot NM, Yener B. Histopathological image analysis: a review. *IEEE Rev Biomed Eng*. 2009;2:147-171. doi:10.1109/RBME.2009.2034865
78. Bahrami G, Malekshahi H, Miraghaee S, Madani H, Babaei A. Improving animal model of induced colitis by acetic acid in terms of fibrosis and inflammation incidence in the colon. *J Invest*

Surg. 2022;35(1):214-222. doi:10.1080/08941939.2020.1821844
79. Fuss IJ, Neurath M, Boirivant M, et al. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol*. 1996;157(3):1261-1270. doi:10.4049/jimmunol.157.3.1261
80. Fuss IJ, Heller F, Boirivant M, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest*. 2004;113(10):1490-1497. doi:10.1172/JCI19836
81. Camoglio L, Te Velde AA, Tigges AJ, Das PK, Van Deventer SJ. Altered expression of interferon-gamma and interleukin-4 in inflammatory bowel disease. *Inflamm Bowel Dis*. 1998;4(4):285-290. doi:10.1097/00054725-199811000-00005
82. Melgar S, Yeung MM, Bas A, et al. Over-expression of interleukin 10 in mucosal T cells of patients with active ulcerative colitis. *Clin Exp Immunol*. 2003;134(1):127-137. doi:10.1046/j.1365-2249.2003.02268.x
83. Rana SV, Sharma S, Kaur J, et al. Relationship of cytokines, oxidative stress and GI motility with bacterial overgrowth in ulcerative colitis patients. *J Crohns Colitis*. 2014;8(8):859-865. doi:10.1016/j.crohns.2014.01.007
84. Brookes MJ, Whitehead S, Gaya DR, Hawthorne AB. Practical guidance on the use of faecal calprotectin. *Frontline Gastroenterol*. 2018;9(2):87-91. doi:10.1136/flgastro-2016-100762

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ferreira-Duarte M, Tonin FS, Duarte-Araújo M, Fernandez-Llimos F, Morato M. Heterogeneity in protocols and outcomes to study the effect of renin-angiotensin system blockers in inflammatory bowel disease: A systematic review. *Fundam Clin Pharmacol*. 2023;37(6):1139-1152. doi:10.1111/fcp.12935