

Inflammatory Bowel Disease

Chapter 1

Preclinical Models of Inflammatory Bowel Disease and Colonic Treatments

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Abstract

Inflammatory bowel disease (IBD) is a group of chronic disorders of the gastrointestinal (GI) tract, which two main types are Crohn's disease (CD) and ulcerative colitis (UC). The exact causes of IBD still remain unknown but are accepted to be multifactorial. For this reason, the use of animal models of IBD is gaining importance in preclinical research to clarify the pathogenic mechanisms of the disease and evaluate the therapeutic potential of new compounds. Non-invasive imaging techniques commonly used in clinical practice have also demonstrated their usefulness in the characterization of these animal models, since repeated evaluations can be carried out on each living animal over time. Furthermore, the advances in targeted therapies for IBD make it possible to deliver the drug at the site of inflammation, prolonging local drug availability and reducing adverse effects. In this chapter, a review of the combined use of animal models and imaging techniques is made. These approaches can greatly help to bring new effective drugs on the market with the final purpose of improving the lives of IBD patients.

1. Introduction

Inflammatory bowel disease (IBD) is the term used to describe a group of chronic relapsing inflammatory diseases of the gastrointestinal (GI) tract, which encompasses two major phenotypes: Crohn's disease (CD) and ulcerative colitis (UC). IBD is an idiopathic disease caused by an interplay of dysregulated immune responses to luminal microflora that together with environmental risk factors can trigger the onset of the illness in genetically predisposed individuals [1]. Although it remains unclear how many susceptibility genes underlie the IBD, some of them have been identified, being NOD2 the most important one. The NOD2 gene variants are only associated with CD and not with UC [2]. In genetically susceptible hosts, pathogenic bacteria or commensal microbes disrupt epithelial barrier function, triggering an aberrant inflammatory immune response. There is a strong evidence of a CD4 T helper cell type 1 (Th1) response in CD, and a CD4 T helper cell type 2 (Th2) response in UC, highlighting the fact that CD and UC represents two distinct immunological forms of inflammation [3]. Environmental factors such as smoking, diet, stress and sleep among others have been identified as risk factors for IBD. The alteration of the gut microbiota due to an improvement in personal hygiene, the use of antibiotics, dietary changes and vitamin D deficiency also influence microbial mediated mechanisms of immunological tolerance [4].

This multifactorial nature of IBD has highlighted the need for developing animal models to shed light on the pathogenic mechanisms of the disease and for their use in preclinical studies of new drugs development. They have also contributed to the validation of immunological

targets for IBD treatment. Although these experimental animal models only partially reflect the complexity of the human disease, they allow us to control and define each interaction implicated in the pathogenesis of the illness [5]. To accurately characterize IBD experimental models and assess the therapeutic activity of new drugs, imaging techniques commonly used in the clinical practice, can also be applied in preclinical studies. In particular, molecular imaging is especially promising in the characterization and quantification of biological processes at the cellular level detecting molecular alterations in living subjects, not limiting the investigations to the macroscopic field as it happens with diagnostic imaging [6].

To date there is no curative therapy for IBD, therefore patients require life-long medications that can lead to frequent dosing, loss of response and severe side effects that can negatively affect patients' adherence to medication. Apart from conventional topical and oral treatments, over the last years colon specific drug delivery systems have been developed for colon targeting, improving IBD treatment [7]. Furthermore, the innovative work in this field has allowed to overcome previous limitations, which makes possible to target drugs to the specific site of inflammation, prolonging local drug availability and reducing side effects [8].

The aim of this chapter is to provide an overview on the role of experimental animal models in preclinical IBD research and to describe the most commonly used imaging techniques for the assessment of IBD in both experimental animals and human patients, highlighting the potential beneficial impact that these techniques could offer in disease management. In addition, this chapter will also present the existing and emerging approaches in colonic drug delivery.

2. *In Vivo* Animal Models of Ibd

During the last years, a large number of animal models have been developed to provide a valuable insight into the different pathways involved in IBD pathogenesis. Moreover, these animal models enabled the preclinical development of a large number of new therapies. Depending on the mode of induction of intestinal inflammation, experimental IBD models can be divided into chemically induced models, genetically engineered models, spontaneous models and cell transfer models [9]. The aim of this section, rather than provide a comprehensive review of every model, is to create an overview of the existing animal models of IBD, highlighting the potential translational impact arising from their use in preclinical research and giving some examples of each category.

2.1. Chemically induced animal models

Chemically induced IBD animal models are commonly used because of the immediate inflammation, the high reproducibility and the simplicity of the induction process. Although they have limitations, they resemble in some histopathological and immunological aspects the

human IBD.

2.1.1. TNBS colitis

Colitis can be induced in rats, mice and rabbits by intrarectal administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) diluted in varying concentrations of ethanol. TNBS is believed to haptenize colonic proteins rendering them immunogenic to the host immune system, thereby triggering the host innate and adaptive immune responses. Ethanol is a prerequisite to break the mucosal barrier to allow penetration of TNBS in the lamina propria. The intrarectal administration of this reagent induces a transmural colitis driven by CD4⁺ T cells, which makes it a valuable model to study T helper cell-dependent mucosal immune responses [10]. In murine TNBS colitis, the genetic background is thought to influence the immunopathogenesis with a predominant Th2 mediated immune response in BALB/c mice and a more Th1 response in SJL/J mice. Moreover, rats develop a distal colitis while mice often show a pancolitis. TNBS colitis initially cause injury to the epithelial barrier with infiltrations of lymphocytes and macrophages. In addition, a thickening of the colon wall can be appreciated with visible ulcers at the site of injection of the enema [11].

2.1.2. DSS colitis

The administration of dextran sulfate sodium (DSS) in drinking water for several days induces an acute colitis in mice, rats and hamsters characterized by bloody diarrhea, weight loss, ulcerations and neutrophilic infiltration. The mechanism by which DSS induces intestinal inflammation is unclear but it is believed to be toxic to epithelial cells of the basal crypts and to affect the integrity of the mucosal barrier [12]. Chronic colitis can be induced by the administration of multiple cycles of DSS to some strains of mice [13]. When administered to T- and B- cell deficient mice, they also developed acute intestinal inflammation, indicating that the adaptive immune system does not play an important role in this model, hence it is not well suited to address immunologic or therapeutic issues involving this immune response [14]. Otherwise, the DSS model is particularly useful to study the contribution of the innate immune system to the development of colitis.

2.1.3. Oxazolone colitis

The rectal administration of the contact sensitizing agent oxazolone dissolved in ethanol induces colitis in rats and mice, manifested by weight loss and diarrhea with high death rates [10]. In SJL/J mice, the inflammation affects only the distal colon with neutrophil/lymphocyte infiltration limited to the superficial layer of the mucosa. Examination of the cytokine profiles of T cells isolated from the lamina propria demonstrated an elevated production of Th2 cytokines (IL-4, IL-5 and IL-13) in CD3/CD28-stimulated T cell cultures. The combination of histological features and the cytokine profile of this animal model resembles to characteristics

that have been observed in human UC [15].

2.2. Genetically engineered animal models

More than 74 kinds of genetically engineered mouse strains have been developed since 1993 for studying IBD. Some of them carry the susceptibility genes identified in human IBD and other susceptibility genes have been discovered using these animal models. However, it is unlikely that these mutations represent the exact cause of human IBD due to the fact that IBD involves a complex interaction of factors. Genetically engineered mice can be classified in transgenic models (Tg) and knockout models (KO), which are genetically engineered to overexpress or lack a gene of interest in all cell types, respectively [16].

2.2.1. Conventional knockout (KO) models

2.2.1.1. Interleukin (IL-10) KO mice

The regulatory cytokine IL-10 is a susceptibility gene for IBD. IL-10 KO mice are genetically engineered to lack IL-10 gene, developing a spontaneous colitis after 3 months of age [17]. Development of colitis is inhibited if IL-10 deficient mice are raised under germ-free conditions [18] and the administration of probiotics and selective antibiotics can treat or prevent development of inflammation in mice colonized by bacteria [19]. The CD4⁺ T cells mediate pathogenesis of colitis in IL-10 mice. Such enhanced Th1 response leads to macrophage activation and overproduction of inflammatory cytokines [18]. Intestinal CX3CR1⁺ macrophages have been shown to be a major responder to IL-10 in the inflamed colon [20].

2.2.1.2. Interleukin (IL-2) KO mice

IL-2 deficient mice fail to maintain immune homeostasis and maintenance of self-tolerance, developing IBD after 4 weeks of age [21]. IL-2 KO mice spontaneously develop a systemic autoimmune disease characterized by hepatitis, gastritis, pancreatitis and haemolytic anaemia among others. In this KO model, an unremitting colitis develops spontaneously in germ-free condition [22] resulting in progressive weight loss and death, also displaying a large variety of immune abnormalities [23].

2.2.1.3. T cell receptor (TCR) α -chain-KO mice

The T cell receptor (TCR) is required for the recognition of antigens by T cells. Spontaneous development of colitis has been observed in both TCR α and TCR β -deficient mice, although only the 60 % of TCR α and none of TCR β -deficient mice consistently develop a spontaneous colitis mediated by Th2 [24]. By 4 months of age, unremitting chronic diarrhoea, consistent inflammation and rectal prolapse was observed in this model. Moreover, TCR α -deficient mice maintained in a germ-free environment do not develop colonic inflammation

[25]. In a B cell and TCR α -deficient mice, colitis is developed at an earlier age and of great severity than in mice B cell-competent and TCR α deficient, revealing a novel cell regulatory circuit in this model [26].

2.2.2. Conventional transgenic (Tg) models

2.2.2.1. Interleukin (IL-7) Tg mice

IL-7 is required for the development of mature T cells in the thymus and is implicated as a growth factor extrathymic T cell development in the intestine [27]. IL-7 Tg mice spontaneously develop colitis between 4 and 12 weeks of age, which is manifested by anal bleeding and infiltration of monocytes, decrease in goblet cells and an increase in crypt abscesses in the lamina propria. In this chronic colitis model, the lack of IL-7 results in the apoptosis of the activated lymphocytes, which is suggested to be the cause of colitis [28].

2.2.2.2. HLA-B27/ β 2M Tg rat

Transgenic rats for human HLA-B27 and β 2-microglobulin develop a spontaneous colitis manifested by chronic inflammation involving stomach, small and large intestine, being the colon the most affected site. It can be appreciated crypt hyperplasia and mucosal infiltration of mononuclear inflammatory cells [29] and CD4 T cells have been suggested to play a role in the pathogenesis of the disease [30]. This model has been used to study the effect of normal luminal bacteria in the acute and chronic stages of inflammation by the colonization of germ-free B27 rats with groups of defined bacteria, showing that some resident enteric bacteria are more active than others inducing inflammation in this rat model [31].

2.2.2.3. STAT-4 Tg mice

Signal transducer and activator of transcription 4 (STAT-4) is a transcription factor that promotes Th1 development. The STAT-4 transgenic mice generated under the control of a cytomegalovirus (CMV) promoter overexpress this transcription factor developing a transmural colitis within 7-14 days after the activation of CMV promoter, also exhibiting a mediated CD4⁺ Th1 T cells inflammation [32].

2.3. Spontaneous animal models

Although uncommon, IBD has been reported to occur spontaneously in animals, especially in some mice strains and in the Cotton-top Tamarin. In the last one, the exact pathogenesis still remain unknown [33]. Disease in humans involves multiple pathways and the defects leading to IBD are more complex than those seen in genetically engineered animal models, therefore, spontaneous models may mimic better the complexity of human disease [18]. Furthermore, novel models still been generated to explore the axis between different potential

biomarkers and intestinal inflammation [34].

2.3.1. C3H/HeJBir mice

C3H/HeJBir mice develop spontaneous inflammation under certain housing conditions. Lesions occurred primarily in the cecum and proximal colon with acute and chronic inflammation, crypt abscesses and ulceration [35]. Disease appears early in life at 3-4 weeks and heals by 10-12 weeks. Moreover, C3H/HeJBir mice have no significant B or T cell reactivity to epithelial or food antigens whereas develop strong B cell and T cell responses to enteric bacterial antigen. This finding demonstrate that effector T cells reactive with conventional antigens of the enteric bacterial flora can mediate chronic IBD [36]. The innate immunity of C3H/HeJBir mice exhibit defects that translate to increased T-cell responses to bacterial antigens. This approach has proven to be particularly useful in the identification of bacterial antigens that are recognized by pathogenic T cells and thus induce IBD [37], being a valuable resource for genetic and immunologic studies of the disease.

2.3.2. SAMP1/Yit mice

The SAMP1/Yit mice develop spontaneous inflammation that closely resembles to Crohn's disease. In this model, the inflammation is primarily localized to the distal small intestine showing discontinuous lesions with transmural involvement, granulomas and alterations in the epithelial morphology [38]. The inflammatory infiltrate is composed of mononuclear cells and neutrophils. Germ-free SAMP1/Yit mice do not develop intestinal inflammation whereas in contact with intestinal microflora develop disease by 15 weeks of age [39]. Epithelial alterations may play a role in the pathogenesis in this model due to the activation of CD4⁺ T cells reactive to bacterial antigens, which mediate the inflammation [40,41].

2.4. Adoptive transfer animal models

Among the animal models available to assess the contribution of T cells to the pathogenesis of IBD, the T cell transfer model of colitis is the most widely used to study the initiation, induction and regulation of immunopathology in chronic colitis mediated by T cells [42]. Typically, naïve T-cells are transferred into immunocompromised mice and therefore develop intestinal inflammation because of inappropriate downregulation [43].

2.4.1. CD4⁺ /CD45RB high T-cell transfer colitis

Normal CD4⁺ T cells can be separated into naïve cells, which express high levels of CD45RB (CD4⁺CD45RB^{hi}), and memory cells, which express low levels of CD45RB (CD4⁺CD45RB^{lo}). The adoptive transfer of CD4⁺CD45RB^{hi} T cells collected from the spleen and lymph nodes of wild-type mice into SCID and RAG-2^{-/-} mice results in colitis and wasting [44,18]. However, CD4⁺CD45RB^{lo} T cells from germ-free mice are capable of suppressing

colitis induced by CD4⁺CD45RB^{hi} [45], suggesting that regulatory T cells are considered a member of the CD4⁺CD45RB^{lo} family and are believed to be involved in the regulation of inflammation [46]. Therefore, colitis in models with transferred CD4⁺CD45RB^{hi} can be caused by an impaired T-cell regulation [28]. In sum, normal T cells contain pathogenic cells that can cause intestinal inflammation, which is prevented by the action of regulatory cells.

2.4.2. Hsp60CD8⁺ T cells transfer colitis

In this mice model, intestinal inflammation is induced by the adoptive transfer of an hsp60-specific CD8⁺ T-lymphocyte clone. The inflammatory lesions are concentrated on the small bowel and it is also developed in germ-free animals, without depending on the presence of normal intestinal flora. Immunocompetent mice usually do not develop disease after transfer of hsp60-specific T cells. This finding highlights the pathogenic potential of such T cells in a state of immunodeficiency or immune dysregulation [47].

Table 1: Classification of animal models of IBD

Chemically induced	Genetically engineered		Adoptive transfer	Spontaneous
Enema	Knockout	Transgenic	CD45RB ^{high} cells	C3H/HeJBir
TNBS	IL-10	IL-7	CD62L ^{high} cells	SAMP1/Yit
DNBS	IL-2	HLA-B27	Hsp60CD8 ⁺ T cells	Cotton-top tamarin
Oxazolona	IL-2R	STAT-4		
Acetic acid	TCR α	TGF- β		
Oral	Gai2	NOD2		
DSS	STAT-3	NF- $\kappa\beta$		
Indomethacin				
Carragenan				

2.5. Lessons from the animal models

The knowledge obtained from the use of animal models in preclinical research is large, and there is no doubt about their contribution not only in defining the pathogenesis of IBD, but also in the inflammatory process itself. There has also been progress in understanding the complex interactions between the gut microbiota and the host, and how environmental and genetic factors could affect homeostasis.

2.5.1. Advantages and limitations of animal models

Nowadays, there are many animal models available to study IBD. Each model has specific advantages over other models. For instance, the use of CD4⁺/CD45RB model has brought several information about the adaptive immune mechanism involved in the IBD pathogenesis. TNBS model has allowed the development of the monoclonal anti-IL-12p40 antibody that has demonstrated efficacy in the human CD treatment [48] and the mechanism of inflammation

associated with epithelial homeostasis has been analysed using the DSS model. Furthermore, TCR α model shares etiological characteristics (smoking) with human UC [49]. In addition, IL-10 KO model has contributed to the understanding of the probiotics mechanism in IBD [19,23].

Moreover, the possibility of being developed under certain conditions with a controlled microbial environment and with minimal differences in gut microbiota provides reproducible results. Also, the ability to control each stage of the inflammatory process and analyse the mechanisms from the beginning to the late events of the disease is an advantage of experimental models which cannot be done in patient studies. This allows to examine separately the events of the acute phase of the disease and those of the chronic phase, also distinguishing the underlying pathways of inflammation from the clinical features of the illness [5,50].

Nevertheless, these animal models can present some limitations. When interpreting data from experimental colitis, it must be taken into account the environmental influences that affect the pathophysiology of the intestinal mucosa in human IBD, which are not the same that in experimental colitis. This could be solved using specific microbial communities to evaluate their impact in the development of experimental colitis or even transplanting human faecal microbiota into germ-free mice for better simulate human IBD [51,52]. Another disadvantage that could be mentioned is the different indexes usually used for evaluate the therapeutic efficacy of drugs between animal models and patients. The last ones are usually evaluated by activity scores of the disease, while IBD studies in animal models are normally assessed by histopathological scores of colonic tissue damage in combination with differences in the rate of weight loss between treated and non-treated animals [53].

Finally, when interpreting the treatment efficacy, it has to be taken into consideration the time of intervention. In experimental IBD, onset of inflammation occurs over defined time courses that enable a therapeutic intervention at specific time points. If a treatment is able to prevent the development of inflammation in an experimental model may not be equally effective in resolving stablished disease, it only proof the existence of a novel pathway of inflammation and will not be useful in patients with an established disease [9]. Therefore, the development of new models that could better resemble human IBD and a more thorough interpretation of data obtained from them is still necessary.

2.5.2. Considerations for choosing a preclinical model

When choosing a preclinical IBD model, it is important to determine the model of greatest relevance to the particular scientific question to be addressed. For example, as said in the introduction, Crohn's disease and ulcerative colitis usually occur in different anatomic regions of the TGI. For this reason, to test a therapeutic candidate it must be taken into account if the inflammation will affect mainly the colon or the small intestine, since both differ in anatomical

and cellular structure as well as bacterial diversity.

On the other hand, IBD models differ in the nature of the response that is evoked. Since pathological responses are different among animal models, it is necessary to take into consideration whether the therapeutic compound will target prevention of epithelium damage, oxidative stress, inflammation mediated by an innate immune response or by an adaptive immune response, for example [54].

For instance, if research is focus on exploring therapeutics that may have protective properties for the intestinal epithelium, a chemically induced model such as the TNBS model could be more useful due to the acute damage it causes to the epithelial cells. One other example is the altered mucosal permeability that causes DSS or is present in mice with genetic deficiencies in permeability. Therapeutic compounds that maintain mucosal permeability will be protective in those types of colitis [55].

2.5.3. Relevance of animal models of IBD

Although none of the existent models are exact replicates of the human disease, the different subgroups of patients with IBD may be possibly reflected in the differences between animal models [56]. With the development of personalized therapies, animal models will continue to play an important role in IBD research. In future studies, a specific model targeting a defined phenotype could provide a more relevant data. Furthermore, the identification of novel diagnostic biomarkers could be particularly beneficial, leading to a better classification of the illness and subsequent monitoring of the effectiveness of therapies [57].

In addition, to accurately characterize IBD models and evaluate the therapeutic efficacy of new compounds in preclinical studies, some diagnostic techniques such as diagnostic imaging have been applied in the preclinical field with a high translational potential to clinical practice. These innovative approaches may be of particular relevance in the management of IBD patients in combination with current diagnostic tools.

3. Diagnostic Imaging Techniques in Ibd

In clinical practice, the standard diagnosis of IBD involves endoscopy and biopsy. Colonoscopy is the type of endoscopy most often performed to both monitor and diagnose the illness. Moreover, the biopsy tissue obtained during the colonoscopy is examined for specific features to help in the diagnosis [58]. Nevertheless, this technique is very invasive and it is not able to evaluate the upper part of the small bowel as well as other areas outside the bowel. Furthermore, endoscopic imaging is restricted to the superficial mucosal layers of the intestine, so they do not allow a complete transmural view of the entire intestinal wall and thus do not reveal the extent and severity of the inflammatory process [59]. For this reason, less inva-

sive diagnostic imaging technologies such as barium X-rays, ultrasound, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) and single photon emission computed tomography (SPECT), can play multiple roles not only in managing IBD in clinical patients, but also in preclinical research monitoring disease progression and treatment efficacy from its early stages.

3.1. *In vivo* imaging in preclinical research

In vivo imaging of small animals is increasingly being developed for the assessment of disease-specific animal models. These models are mainly rodents (mice and rats) that are too small to be managed accurately by the devices used in clinical practice, which raises problems such as low spatial resolution and low sensitivity. To overcome these limitations, miniaturized versions of clinical devices are currently available for small animal imaging in preclinical research [60].

3.2. Structural imaging techniques

3.2.1. Micro-computed tomography (μ CT)

Computed tomography is an application of X-ray imaging that provides detailed images of organs, blood vessels, bones and soft tissue. A detector situated in opposition to the X-rays source sense those X-rays that are not absorbed by the tissues, which is inversely related to the density of the structure. The CT scan generate a 3D anatomical image, from which sectional or spatial images can be reconstructed [61]. Computed tomography enterography (CTE) is a variation of standard abdominal CT scan that involves the use of small bowel distension in combination with the administration of an oral contrast agent [62]. This technique is highly sensitive and specific for active small intestinal inflammation and therefore it is considered as an useful tool to assess Crohn's disease patients [63,64].

In small animal studies, μ CT has been used for the assessment of bone and soft-tissue tumour animal models, demonstrating the potential usefulness of μ CT in cancer research [65]. Furthermore, this technique allow the detection of colon wall thickening in DSS-induced colitis model in mice, thus serving as a non-invasive tool for monitoring colitis [66].

3.2.2. Micro-magnetic resonance imaging (μ MRI)

Magnetic resonance imaging (MRI) is based on the interaction of nuclear spin with an external magnetic field. MRI produces spatial maps based on the properties of hydrogen nuclei (protons) contained in water molecules. This technique portrays anatomic details with high resolution in three dimensions (3D) and is especially useful for imaging soft tissues [61]. MRI presents several advantages over CT for evaluating IBD, for example the lack of ionizing radiation exposure in patients, which allows the realization of numerous studies throughout their

lives, and the better soft tissue contrast without the need for intravenous contrast [67]. Furthermore, MRI offers a great opportunity to study the changes in the small intestine wall in patients with Crohn's disease in combination with endoscopy, both in adults and paediatric patients [68,69]. In particular, taking into consideration just the small bowel, MRI can be performed using other approaches such as MR-enterography and MR-enteroclysis [70,64]. Although in UC patients the extent of inflammation can be assessed by endoscopy since the extent of lesions are limited to the inner wall layers, MRI could also be important when endoscopy is not feasible [71].

In small animals, μ MRI is frequently used to provide anatomic images and delineate tumours or areas of necrosis [72]. Furthermore, MRI has been used to characterize potential disease biomarkers in DSS mice p [73] and to perform a longitudinal study to investigate the evolution of IBD also in a DSS animal model [74], demonstrating the viability of this technique to monitor the development of colitis.

3.2.3. Micro-ultrasound (μ US)

Ultrasound represents an accurate and reproducible modality to assess disease activity in IBD patients since it can detect disease complications such as strictures and abscesses [75]. This technique has been applied extensively in the follow-up of Crohn's disease patients, providing prognostic information to the clinician [76]. Ultrasound imaging is based on the differing acoustic properties of tissue and present some advantages such as high spatial and temporal resolutions without ionizing radiation [60]. In preclinical research, micro-ultrasound represents a rapid, cost-efficient and non-invasive imaging tool for studying the development of model animals of human disease where high spatial resolution is needed. In mice cancer models, 3D high-frequency micro-ultrasound (3D- μ US) was used to perform measurements of colonic tumours [77].

A variant of ultrasound is the ultrasound molecular imaging that utilizes micron-sized, gas filled contrast microbubbles that are able to bind proteins on endothelial cells that are upregulated in the inflammation process. This modality was used for the assessment of acute inflammation in DSS-induced colitis model in mice [78]. Another method is the ultrasound elasticity imaging, which allows to evaluate the changes in mechanical properties of the intestine due development of fibrosis after repeated cycles of inflammation. This technique was used in TNBS induced colitis in rats since it is known that this model develops fibrosis [79].

3.3. Functional imaging techniques

3.3.1. Micro-single photon emission computed tomography (μ SPECT)

Single photon emission computed tomography is cross-sectional nuclear imaging us-

ing single photon emitting radionuclides. The most common radionuclides used in clinical practice are ^{99m}Tc , ^{67}Ga , ^{201}Tl , ^{111}In or 123 , 125 , ^{131}I , which have to be injected in the patient [80]. ^{99m}Tc -labeled CXCL8 (interleukin-8) has used in IBD patients to detect an localize disease activity with good diagnostic accuracy [81]. Also, ^{99m}Tc HMPAO-labelled white blood cell (WBC) has been found to be useful in the assessment of disease extent without the need for colonoscopy [82]. Nevertheless, SPECT alone is limited by low spatial resolution and it is not able to provide anatomical details, therefore this technique is usually used in combination with CT scan for an accurate lesion localization, avoiding erroneous interpretations [80]. Although the molecular sensitivity of PET is higher than that of SPECT, it continues to be widely used in both preclinical research and clinical practice due to its lower cost and the longer half-life of its radionuclides that allow in vivo evaluations for a longer time [61].

SPECT imaging is highly valuable for study dynamic biological processes at the cellular and subcellular levels. Among other applications [83], μSPECT is useful to monitor disease activity in animal models of IBD. The monoclonal antibody Infliximab radiolabelled with ^{99m}Tc has been able to detect inflammation in TNBS-induced colitis model in rats [84], demonstrating the target specificity of a clinical drug currently used in clinical practice. Other study injected superparamagnetic iron oxide (SPIO) nanoparticles and ^{111}In oxine-labelled macrophages in mice with DSS colitis. The SPECT/CT data provided information of pharmacokinetics and biodistribution of the macrophages in the IBD mice [85].

3.3.2. Micro-positron emission tomography (μPET)

Positron emission tomography is based on the detection of γ -photons emitted from a radiolabelled molecule with a positron-emitting radioisotope, mainly ^{11}C , ^{18}F and ^{64}Cu . A frequently used radiotracer is [^{18}F]Fluoro-2-deoxy-2-D-Glucose ([^{18}F]FDG), which is uptaken by tissues and emits positrons that react with electrons in the body. As a result, there is a production of photons that are detected by a PET scanner, which creates 3D images that show the distribution of the [^{18}F]FDG in the body. Areas with higher [^{18}F]FDG uptake appear more intense indicating a high level of metabolism occurring there due to inflammation or infection [86]. PET is usually used in combination with CT, offering an anatomical reference to the physiological data obtained by PET scan with high resolution and sensitivity [87].

In clinical practice, [^{18}F]FDG PET/CT allows to visualize not only the mucosal layer, but also to get a full transmural view of the entire intestinal wall, revealing the extent and severity of the inflammatory process. [^{18}F]FDG PET/CT may be of particular value in the evaluation of a treatment effectiveness in a patient with Crohn's disease, for whom a therapeutic decision has to be made on the basis of an objective assessment of the severity and extent of the inflammation. Pilot studies have shown good specificity and sensitivity for [^{18}F]FDG PET/CT in detecting and localize moderate to severe lesions in the bowel wall of patients with Crohn's

disease [88,89]. Therefore, it is likely that PET/CT scan can be used as a complementary diagnostic tool and in the follow-up of IBD patients, especially in those in whom an invasive colonoscopy, such as children, is not desirable.

Nevertheless, the significant patient irradiation associated with PET/CT scan will impede frequent use [90]. Moreover, the high cost of PET scanning will likely be a barrier for its implementation in the clinical practice. Unless the cost of this technique decrease, PET/CT probably will not be a major or common tool for monitoring IBD. Due to the increasing demand for this technique, it is expected that in the coming years it will be available at more affordable prices.

In preclinical research, several works have been carried out to study its feasibility in the assessment intestinal inflammation in IBD animal models. This technique has been used to evaluate the disease activity in mice with DSS induced colitis [91] demonstrating its potential translational value. Moreover, longitudinal quantifications of inflammation have been carried out in both DSS [92] and TNBS [93] animal models showing that [18F]FDG PET/CT is a reliable tool to assess disease activity and remission over time, therefore, this technique could have a major impact on preclinical *in vivo* imaging aiming to monitor the efficacy of new therapeutic compounds. Furthermore, white blood cells (WBC) radiolabelled with [18F]FDG have been proposed to serve as a quantitative marker of intestinal inflammation [94]. This method could be useful to distinguish between non-malignant and malignant tissues where the inflammatory process could interfere in the accuracy of PET imaging. PET/CT technique has also demonstrated its usefulness to evaluate the *in vivo* behaviour of 3D printed devices, allowing the location and tracking of the device in the GI tract of the animal [95].

Currently, other radiotracers are available, such as the [18F]DPA-714, which is a radioligand of the translocator protein 18 kDa (TSPO) that has been used to study inflammation in IBD models. However, it has not yet been implemented in the clinical practice [96].

3.4. Imaging IBD: remarks and considerations

Nowadays in clinical practice, endoscopy and biopsy are the gold standards for diagnosis and management of IBD patients. These two procedures are highly invasive and uncomfortable for patients, especially in those cases where evaluations over time are required. Moreover, it is not possible to evaluate the upper part of the small bowel as well as other areas outside the bowel [59].

Classical imaging modalities such as ultrasound, CT enterography and MRI enterography, have been implemented over the last few years as complementary tools that aim to get a solid diagnosis of IBD, especially Crohn's disease. In addition, these techniques also help in the assessment of disease extent, activity and severity, influencing management decisions such

as medication selection or the most appropriate type of surgery [97]. Nevertheless, these imaging tools have some limitations. For instance, the difficulties encountered in distinguishing between active inflammatory disease and chronic fibrostenotic disease and the ionizing radiation to which patients are exposed with CT [98]. Finally, there is still a need for technologies that provide more cellular level information to better understand the etiology of IBD that could lead to improve therapies aimed at specific biological targets.

Molecular imaging techniques are gaining a role of primary importance in preclinical research since it allows to characterize and quantify biological processes at the molecular and cellular level in living animals, and also to detect molecular alterations at the basis of the pathological pathway. Another asset of these techniques is that repeated evaluations can be carried out on each living animal, thus limiting the number of animals needed for individual measures of intestinal inflammation during the follow-up of the disease [99]. Thereby, molecular imaging could help in the identification of biomarkers that will permit to more accurately assess the efficacy and mechanism of action of a new drug in preclinical models [6].

4. Pharmaceutical Approaches in Drug Therapy of Ibd

The therapeutic management of IBD constitutes a special situation in drug delivery since most of its pathological manifestations are located in the gastrointestinal (GI) tract. Therefore, the main approaches have been focused on target formulations to deliver the drug directly in the intestine and retaining it in the intestinal lumen. This strategies are expected to reduce the adverse reactions derived from its systemic absorption and improve the pharmaceutical effect, mainly due to an increased contact time with the inflamed tissue [100].

IBD patients require lifelong treatment, whose two main goals are the induction and maintenance of remission and the prevention of disease flares. Conventional treatments are based on anti-inflammatory drugs (5-aminosalicylic acid and corticosteroids), immunosuppressives (azathioprine, 6-mercaptopurine), antibiotics and biologic agents (Infliximab and other anti-TNF agents). Because of the aim of this section is to review the main dosage forms from the point of view of novel drug delivery strategies, the reader is referred to other works to obtain more information about pharmacological treatments of IBD [101-103].

Current drug-delivery strategies are focused on targeting the drug to the small intestine and colon. This goal can be achieved mainly through oral, rectal and parenteral administration approaches [104].

4.1. Oral route of administration

Oral drug-delivery systems for the treatment of IBD have been developed to achieve a more effective drug delivery to the site of the disease. In UC, inflammation appears in a

continuous manner in the large intestine, so most of oral dosage forms have been designed to target the colon. In CD, the inflammation is discontinuous and could appear in the entire GI tract, which makes its treatment by oral dosage forms more difficult. This fact has encouraged the research on pharmaceutical technology to develop innovative drug carrier systems, which are intended to deliver the drugs specifically to the inflamed intestinal areas [104].

Targeting drugs to the colon could improve pharmacological effects and reduce adverse reactions. However, several limitations related to biochemical, physical and environmental barriers must be taken into account when a new drug formulation is being developed, such as the different pH values and enzymatic barriers, mucus layer, epithelium, P-glycoprotein efflux pump and microbiota [105].

For instance, the solubility of the drug is affected by the low colonic fluid volume, the pH and the higher viscosity, which may represent a limiting factor for the colonic absorption. The colonic content and the colonic bacterial enzymes could also affect the stability of the drug, rendering it ineffective [7]. The existing oral drug-delivery strategies can be divided based on the novelty of its approach.

4.1.1. Conventional pharmaceutical strategies

Several mechanisms have been developed to trigger drug release in the intestine, in particular from the distal ileum to the sigmoid colon. These mechanisms generally respond to variables, mainly time, pH, enzymatic activity and intraluminal pressure.

These conventional strategies have been extensively reviewed elsewhere [106,104]. Although its use has been successful in IBD therapy, they may also have some disadvantages. The transit time of the drug through the GI tract is altered in IBD patients compared to the healthy population due to the typical symptoms, such as diarrhoea, which accelerate it. The colonic pH also differs from the normal value, which compromises the accuracy of pH-dependent formulations. In addition, in IBD patients the contractility is decreased, which reduces colonic pressure. The colonic flora is also altered, and in fact, variations in bacterial concentrations were observed, particularly in Crohn's disease patients [107].

4.1.2. Novel pharmaceutical strategies

To overcome the limitations derived from the use of conventional drug release systems, micro- and nano- sized carriers have been developed to increase colonic residence time and provide additional benefits for IBD therapy. Of particular importance is their high surface-to-mass ratio, which make these carriers capable to bind to the mucous surface and carry many compounds [108].

4.1.2.1. Micro- and nanoparticles

A size dependent accumulation of micro- and nanoparticles (MPs and NPs) can be observed in the inflamed intestinal tissues, particularly of those smaller than 1000 and 100 nm [109]. These smaller drug carriers more effectively pass the mucus barrier due to the average pore size of the mucus, which is below 100 nm, in such a way that smaller particles can diffuse smoothly through it. The transport through the inflamed mucosa depends on particle size. MPs are retained in the superficial layers while NPs penetrate to greater depths. In Crohn's disease patients, there is an increase in mucus production leading to thicker mucus layer, particularly in ulcerated areas. Therefore, strategies that modify the particle surface such as muco-adhesion, bio-adhesion and muco-penetration could improve targeting and retention of drug delivery systems [110].

The deposition of NPs in the inflamed tissue was evaluated in a TNBS-induced colitis animal model. NPs loaded with an anti-inflammatory were administered to the animals and compared with a control group to which the drug was administered in solution. When the animals were kept without treatment, the group that received NPs continued to show reduced inflammation levels whereas the other group displayed a strong relapse, demonstrating that the nanosized carrier provides a sustained drug release due to the retention of NPs in the inflamed area [111]. Several studies such as the one mentioned above have been carried out in animal models of IBD with promising results and translational potential to the treatment of IBD patients [109].

4.1.2.2. Liposomes

Liposomes are double-layer and sphere-shaped vesicle structures based on phospholipids that are enclosed in aqueous volumes. Due to their amphoteric properties, they are capable of carrying both hydrophilic and lipophilic compounds [112].

The activity of antioxidants encapsulated into negatively charged liposomes was tested in a DNBS-induced colitis animal model. Liposomal preparations were more effective than free molecules in the treatment of colitis, probably due to the binding of negatively charged liposomes to the inflamed mucosa, leading to the accumulation of the antioxidants in the target area [113]. Likewise, budesonide loaded liposomes showed a higher accumulation in the inflamed area due to its lipoidal nature compared to the free drug [114].

4.1.2.3. Self-microemulsifying drug delivery systems

Self-microemulsifying drug delivery systems (SMEDDS) are isotropic mixtures of oil, surfactants, co-surfactants and drug which in contact with aqueous media, spontaneously form oil-in-water (o/w) microemulsion under conditions of agitation. The agitation required for

the self-emulsification is provided by the digestive motility of stomach and intestine [115]. The nanosized droplets have thermodynamic stability and high surface area, which ensures an effective absorption and delivery of the drug. SMEDDS are particularly useful to enhance oral bioavailability of poorly water soluble drugs by keeping them solubilized in the small oil globules during their transit through the GI tract [116].

SMEDDS formulations containing prednisolone were developed for colon-specific delivery and tested in rats with acetic acid-induced colitis. The results showed a lower histological disease score and a decreased activity of myeloperoxidase (MPO) compared with the group that received standard prednisolone treatment due to the higher accumulation of prednisolone SMEDDS in the inflamed area [116]. Likewise, SMEDDS as a carrier of curcumin, a poorly water-soluble drug, were designed and its bioavailability was evaluated in mice, resulting in an increase in the drug dissolution *in vitro* and bioavailability *in vivo* [117].

4.1.2.4. Self-assembled polymer systems: hydrogels

Hydrogels are cross-linked networks of hydrophobic polymeric materials physically or chemically linked to each other. This structure is capable of holding large amounts of water in the 2D or 3D network and release the drug immediately or in a sustained way by mechanisms such as diffusion or erosion [118].

The efficacy of a hydrogel loaded with NPs containing an anti-inflammatory was evaluated in a DSS-induced colitis animal model. Due to the protection of the hydrogel, the NPs were able to pass through the stomach and small intestine without damage and being degraded in the inflamed colon. As a result, there was a reduction in the colitis symptoms accompanied by a reduction in MPO activity and a lower histological disease score [119].

4.2. Rectal route of administration

The main rectal formulations for drug delivery to the rectum and lower parts of the distal colon are enemas, foams and suppositories. Enemas and foams spread from rectum to the sigmoid colon and even the transverse colon, depending on the volume administered, whereas suppositories deliver drugs only in the rectum [120]. This route has several benefits, for example, the minimization of first-pass effect since portal circulation is avoided and a higher drug concentration is reached at the site of inflammation, leading to a decrease in systemic absorption and subsequently, to adverse drug reactions.

However, the rate of rectal transmucosal absorption is affected by several factors such as the type of formulation of suppositories, since the different polymers used may vary in the time to liquefaction, and the site of absorption. With regard to this last point, drugs administered in the upper part of the rectum are subjected to first-pass effect due to the superior rectal

veins draining this area, whereas drugs administered in the lower part of the rectum are firstly delivered into the venous circulation before their pass through the liver [121]. The most common drugs in rectal formulations are 5-aminosalicylic acid and corticosteroids [122].

4.3. Parenteral route of administration

The parenteral route is used for a systemic immune suppressive therapy in case of a severe exacerbation or severe extra-intestinal disease. Solutions, suspensions and emulsions mainly of corticosteroids and anti-TNF agents, are administered by an intravenous, subcutaneous or intramuscular injection. This route has some advantages, such as fast drug reaction and no first-pass effect, obtaining a bioavailability of almost 100%. Conversely, this kind of administration has the potential risk of adverse reactions [104].

4.4. Future directions in targeted therapies for IBD

Colon targeted drug delivery strategies have been used successfully in the treatment of IBD. The main objective of this targeting approach is to provide the maximum concentration of drug in the inflamed intestinal tissues, achieving therapeutic efficacy while reducing adverse effects. The next steps for optimizing the drug delivery may come from multiparticulate systems, such as MPs and NPs, whose size and structure could be modified according to the severity and characteristics of the disease and being targeted only where they are needed. In addition, achieving a longer lasting permanence of the drug within the mucosa could reduce the frequency of drug administration, thus improving the patient adherence to the treatment. Another innovative approach could be the oral administration of biologic compounds loaded in these carriers, which protect them through the GI tract until they reach the target site, improving both safety and efficacy [104,109].

Nevertheless, these multiparticulate systems, especially NPs, display altered physical and chemical properties with a potential risk of causing toxicity. For this reason, animal models of IBD are gaining importance in the study of multiparticulate systems effects in the GI tract during treatment. The data obtained from these *in vivo* studies in combination with the development of new carriers for targeted therapies will lead in the near future to achieve maximum drug efficacy at lower drug doses, finally offering to the patient prolonged remissions and fewer side effects, improving the patient's quality of life.

5. Conclusion

Animal models of IBD have contributed to an increased understanding of the underlying disease processes. In combination with imaging techniques, animal models have allowed the development of new therapies and the subsequently evaluation of their efficacy in achieving a remission of the disease. In particular, molecular imaging has demonstrated its potential

in the characterization of the disease at a cellular level in a non-invasive way, providing additional data that could help in the assessment of the disease activity. Furthermore, the advent of targeted therapies has improved the outlook for IBD patients, achieving longer remissions with lower drug doses and fewer side effects. The next step in IBD therapy could be the development of personalized therapies by obtaining knowledge about which active agents to use in which patients, targeting different sites and factors in the pathogenic cascade of IBD.

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