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Novel drug delivery strategies for the treatment of inflammatory bowel disease

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Inflammatory bowel disease (IBD) encompasses two idiopathic inflammatory diseases of the intestinal tract: Crohn's disease and ulcerative colitis. Existing therapy for IBD consists mainly of orally or rectally administered small drug molecules, such as 5-aminosalicylates and corticosteroids, or potent systemic immune suppressants. IBD presents a challenging target for drug delivery, particularly by the oral route, as, contrary to most therapeutic regimens, minimal systemic absorption and maximal intestinal wall drug levels are desired. Several delivery strategies are employed to achieve this goal, including the chemical modification of the drug molecules, the use of controlled- and delayed-release formulations and the use of bioadhesive particles. The goal of this review is to summarise existing IBD therapy and examine novel approaches in intestinal drug delivery.

Keywords: colon, Crohn's disease, drug delivery, drug design, drug therapy, gene therapy, inflammatory bowel disease, ulcerative colitis

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1. Introduction

Inflammatory bowel disease (IBD) refers to a chronic, relapsing, idiopathic inflammation of the gastrointestinal (GI) tract. It is traditionally divided into two major clinical entities: Crohn's disease (CD) and ulcerative colitis (UC), whose major pathological manifestation is the chronic, relapsing and remitting inflammation of the intestine. In the case of UC, inflammation is superficial, involving only the mucosal layer and is confined to the colon, whereas in the case of CD, inflammation is transmural, extending through the bowel wall to the serosal layer, and can be found throughout the large/small intestine [1,2]. Although both diseases have been well described for more than half a century, their aetiologies still remain largely unknown.

The goal of therapy for IBD is to reduce the extent and symptoms of the intestinal inflammation, rather than actually cure the disease. Furthermore, despite pharmacological therapy, 30 – 40% of UC patients and almost all CD patients will require surgery at least once during their life time [3]. Treatment selection is based on the type of disease and sites involved [1]. Aminosalicylates and corticosteroids are the traditional mainstays of IBD therapy, although recently corticosteroids have fallen out of favour due to long-term toxicity. Immunomodulators, such as 6-mercaptopurine or azathioprine, antibiotics and biological response modulators, such as infliximab, a high-affinity, chimeric anti-TNF-α monoclonal IgG1 antibody, are also commonly used.

IBD presents a challenging target for drug delivery. Because the original disease pathogenesis and some of the major pathological manifestations are confined to the intestinal tissue, the ideal delivery strategy for IBD would result in an elevated concentration of the therapeutic entity in the intestinal tissue with minimal systemic exposure. The oral route is the desired route of administration for most of the IBD medications in use. However, oral administration results in intestinal absorption of the

drug into the systemic circulation, with absorption typically occurring proximal to the area of inflammation. Thus, only a fraction of the dose reaches its intended target: the inflamed intestinal tissue. This review presents information on the delivery strategies used at present in promoting intestinal local drug delivery, and the novel delivery strategies undertaken towards improving on existing technologies.

2. Inflammatory bowel disease pathogenesis

Although several factors have been implicated with either CD or UC, no single agent or distinct mechanism can explain all aspects of IBD. Environmental factors, genetic factors, enteric microflora and the mucosal immune system appear to interact in a certain way, so that in genetically susceptible individuals, enteric bacteria (potentially including normal intestinal flora) cause a dysregulated mucosal immune response that leads to chronic mucosal inflammation [4-6].

The existence of a genetic linkage to both CD and UC has been hypothesised for several years, as several population studies have shown a familial tendency for both diseases [7-9]. Genome-wide linkage analysis has identified several susceptibility loci [7,9-12]. More recently, the first direct association between a gene and susceptibility to CD was identified [13-15]. *NOD2*, in *IBD1* loci, encodes a protein that acts as a NF-κB activator, an important transcription factor for bacterial recognition and induction of inflammatory response. Environmental and endogenous factors also seem to play a major role in the pathogenesis of IBD [7,8,16,17]. Increased intestinal permeability has been reported in IBD patients and their relatives [7,18-20], and is also common in experimental colitis animal models [21]. Loosening of the tight junctions [22] in the gut can lead to increased antigen absorption and, in turn, an exaggerated immune response. As far as microbial agents are concerned, no microbial causation has been proven yet, and so far evidence are against the existence of an infectious agent. The fact that pathogen-free animal models do not develop intestinal inflammation [2,23,24] suggests that normal intestinal microflora are somehow involved in IBD pathogenesis.

Both CD and UC are characterised by a dysregulated/exacerbated immune response against intestinal pathogens, most likely enteric microflora. $CD4^+$ T cells appear to be the major effector cells in the pathogenesis of IBD [1,2,17,23], and large numbers of activated $CD4^+$ lymphocytes in the lamina propria of several experimental models and IBD patients have been reported [21,23]. Cytokines secreted mainly by these lymphocytes seem to play a central role in the pathogenesis of CD and UC, leading to a dysregulated immune response. In the case of CD, data from experimental models, as well as from IBD patients, suggest that cytokine balance is disturbed towards a T helper cell type 1 (T_H1) inflammatory response, leading to granulomatous inflammation characterised by elevated levels of proinflammatory cytokines such as IFN-γ, IL-2 and TNF-α [1,2,7,17,21,23,25]. Moreover, this exaggerated T_H1 response is coupled with a substantial decrease in the levels of suppressor cytokines (TGF-β, IL-10) normally produced by regulatory T cells (T_H3 or T_r1). This breakdown of oral tolerance may be even more important in the initiation of IBD than the T_H1 response itself [2,23]. In contrast to CD, the current consensus is that a T_H2 immune response is dominant in UC, although human data are not consistent in this regard [2,7,21], and is characterised by antibody production (possibly autoantibodies) and recruitment of acute inflammatory cells. A predominance of IL-5 has been shown in human UC [21]. The antigens that trigger the immune response remain unknown.

3. Pharmacological entities in inflammatory bowel disease therapy

3.1 Existing inflammatory bowel disease medications

Most of the existing IBD medications (**Table 1**) act by nonspecifically downregulating intestinal inflammation. 5-Aminosalicylates (5-ASAs) are being used as the primary therapy for both induction (mild-to-moderate CD or UC) and maintenance of remission [26,27]. Sulfasalazine is the prototype aminosalicylate formulation and has been used for ~ 50 years [27,28]. However, because 5-ASA was discovered as the active moiety, formulations with sulfa-free compounds (5-ASA, olsalazine, balsalazide and ipsalazide) have been developed [26-28]. These compounds are free from most of the side effects of sulfasalazine associated with the sulfapyridine moiety, and allow for the use of higher doses [3]. Aminosalicylates are administered orally in various delayed/controlled-release forms (Asacol®, P&G Pharmaceuticals; Pentasa®, Shire; Azulfidine EN®, Pfizer) or rectally (Rowasa®, Solvay Pharmaceuticals; Canasa®, Axcan Scandipharm). Corticosteroids are used as the initial therapy for moderate-to-severe active UC or CD, yet they are ineffective for the maintenance of remission [4]. They are administered orally, rectally or intravenously in severe disease. Several immunosuppressive agents also show efficacy in the treatment of IBD. Orally administered 6-mercaptopurine (Purinethol®, GlaxoSmithKline) and its prodrug azathioprine (Imuran®, GlaxoSmithKline) are used interchangeably in CD and UC for steroid-resistant or -dependent patients [27]. Due to severe side effects such as bone marrow suppression, close monitoring of the patients is required. Cyclosporin is administered intravenously for the treatment of severe UC. Methotrexate, an inhibitor of dihydrofolate reductase, also exhibits anti-inflammatory properties and has been shown to be effective in the treatment of CD [3]. Newer immunosuppressive agents such as mycophenolate and tacrolimus (FK-506) are also starting to attract attention [4,29]. Taking into account the hypothesised role of bacteria in IBD, antibiotics have also been used for the treatment of IBD. However, satisfactory results only exist in the case of CD. Metronidazole and ciprofloxacin are the most commonly used [27]. As far as biological agents are concerned, in October 1998, infliximab (Remicade®, Centocor) became the first drug of this category approved by the FDA for the treatment

Table 1. Drugs currently used in inflammatory bowel disease therapy.

Drug category	Active drug substance	Sample trade names
Aminosalicylates	Sulfasalazine	Azulfidine®, Azulfidine EN®
	5-Aminosalicylic acid	Asacol®, Pentasa®, Rowasa®, Canasa®
	Balsalazide	Colazal®
	Olsalazine	Dipentum®
Immunomodulators	6-Merpaptopurine	Purinethol®
	Azathioprine	Imuran®
	Cyclosporin	Sandimmune®, Neoral®, Gengraf®
	Methotrexate	Rheumatrex®, Trexall®
	Tacrolimus	Prograf®
	Mycophenolate	CellCept®
Corticosteroids	Prednisone	Deltasone®
	Methylprednisolone	Medrol®
	Hydrocortisone	Cortenema®, Hydrocortone®, Cortifoam®
	Budesonide	Entocort EC®
Antibiotics	Ciprofloxacin	Cipro®
	Metronidazole	Flagyl®
Biological therapy	Infliximab	Remicade®

of CD. Infliximab is a high-affinity, chimeric anti-TNF-α monoclonal IgG1 antibody that neutralises and effectively clears TNF-α. Infliximab is administered by infusion and appears to be suitable for both induction and maintenance of remission [4,27].

3.2 Evolving inflammatory bowel disease medications

Several newer biological agents are under investigation for the treatment of IBD and have been extensively reviewed elsewhere [3,4,29-32]. Anti-TNF factors such as infliximab have gained significant attention [32]. Examples are CDP-571, a humanised (95% human, 5% murine) IgG4 anti-TNF-α antibody [33,34]; adalimumab (Humira®, Abbott; already approved by the FDA for the treatment of rheumatoid arthritis), a new-generation completely human IgG1 TNF-α neutralising antibody; onercept, a human soluble receptor TNF (p75); and CDP-870, an anti-TVF human antibody Fab′ fragment–PEG conjugate [35]. Etanercept (Enbrel®), a recombinant human TNF-R p75-Fc fusion protein currently used for the treatment of rheumatoid arthritis, has proven ineffective in the management of CD [36]. Etanercept binds to soluble TNF-α, but does not bind to transmembrane TNF-α. Thus, unlike infiximab, etanercept fails to promote T-cell apoptosis [37]. Small molecules such as thalidomide and CNI-I493 also exhibit similar anti-TNF-α activity. Because cytokine imbalance seems to play a role in the pathogenesis of the disease, several anti-inflammatory cytokines such as IL-10, -11, and anti-IL-12 antibodies, have been shown effective in experimental models and/or clinical studies [31]. Other approaches include NF-κB inhibitors and antisense oligonucleotides [38] or targeting of adhesion molecules (ICAM-1, α_4-integrins) as in the case of alicaforsen (ISIS-2302), an antisense oligonucleotide to ICAM-1 mRNA [39]. Based on the observation that UC flares in recent former smokers, nicotine has been studied for UC therapy [17]. However, although statistically effective it is not well tolerated by patients. A new therapeutic strategy that has been gaining attention is the manipulation of the intestinal microflora with the use of probiotics. Probiotics are live microbial food ingredients that, when ingested, confer a health benefit by altering the intestinal microflora [40]. The most commonly used organisms are lactobacilli and bifidobacteria [40]. Although their exact mechanism of action is not known, competitive metabolic interactions with existing microflora, production of antimicrobial metabolites or immune modulation are probable contributory effects in IBD treatment [6,40]. Probiotic therapy has shown potential in experimental models, although human data are limited [6,41]. A more recent intriguing twist in probiotic therapy is the generation of genetically modified food-grade organisms [42]. For example, intragastric administration of *Lactococcus lactis* secreting IL-10 showed efficacy in reducing dextran sodium sulfate (DSS)-induced colitis in Balb/c mice or preventing colitis in IL-$10^{-/-}$ mice [43]. Finally, a completely different approach is leukocytapheresis based on the selective removal of granulocytes and monocytes from the peripheral blood with the use of special filtration devices [44,45].

4. Delivery strategies in inflammatory bowel disease

Because most of the oral medications used today are intended to act systemically, little attention has been given to the local delivery of drug to the GI tissue. However, the later would be particularly useful in the management of diseases such as IBD, in which the original pathogenesis and some of the major pathological manifestations are confined to the intestinal tissue. Furthermore, in the case of IBD there is evidence that, at least for some drugs, therapeutic effect directly correlates with

the intestinal mucosa drug concentrations [46]. Most of the delivery strategies used at present are based on modifying formulations traditionally used to control oral absorption of drugs towards promoting drug absorption in the diseased areas of the intestine. These approaches include the chemical modification of the drug to allow for absorption only in the colon in the case of colonic disease; the use of delayed-/controlled-release formulations to deliver the drug to lower segments of the GI tract where inflammation usually occurs; and the use of enemas in the case of inflammation in the distal colon and rectum. Another approach that is tailored towards reducing systemic drug absorption is the promotion of first-pass metabolism by chemical modification of the drug. Some of these drug delivery approaches are summarised here, in addition to a limited number of studies utilising more specific strategies to local drug delivery to the intestine. Although strategies are presented in three different categories, in several cases these categories overlap.

4.1 Oral drug delivery strategies to reduce systemic absorption

Several of the commonly used IBD drugs, such as corticosteroids and immunosuppressants, are associated with severe side effects that limit their clinical use or require constant patient monitoring. Most of the side effects are associated with the systemic distribution of the drug after oral administration, and their action in nontarget tissues. The lifelong nature of the disease requires frequent administration and often high doses, which leads to drug accumulation. As a result, a major goal for drug delivery scientists in IBD is to limit systemic drug absorption. This is usually achieved either by chemical modification of the drug molecule or by the use of a delayed-release formulation that bypasses absorption in the upper small intestine.

In terms of modifying drug molecules to reduce systemic availability, most of the studies have focused on glucocorticosteroids, due to their potency in treating IBD and the severe side effects, such as adrenal suppression and osteoporosis, associated with drug distribution to the systemic circulation. The chemical modification typically results in increased first-pass, mainly hepatic, metabolism; thus effectively reducing drug systemic levels. This is the case for budesonide [47-51] and beclomethasone propionate [52,53]. Another approach is to synthesise a derivative to decrease enterocyte absorption, such as in the case of prednisolone metasulfobenzoate [54,55]. Fluticasone propionate [56] and tixocortol pivalate [57,58] appear to benefit from both these aspects. Several of these topically active steroids have been used for the treatment of asthma and have shown minimal systemic side effects [59]. Budesonide, is the most commonly used topically active glucocorticosteroid and is the primary alternative to prednisolone and hydrocortisone: the most commonly used oral and topical steroids in IBD therapy, respectively. Budesonide exhibits high first-pass metabolism by hepatic cytochrome P450 (CYP) 3A4 [47]. Of an oral budesonide dose, ~ 90% is metabolised in the liver, leading to an oral availability of 10 – 15% [60]. The metabolites are much less potent than the parent drug; thus, no systemic activity is observed. A controlled ileal release formulation, Entocort EC™ (AstraZeneca) [49], is available in the US, and an enema form is also available elsewhere. Topical corticosteroids used in IBD therapy have been reviewed elsewhere [59,61-63].

Another approach to decrease systemic drug absorption following oral administration is the use of formulations that delay the release of the drug in the intestinal lumen in order to bypass intestinal regions of high absorption. This is typically achieved with the use of polymer coatings. This strategy has been particularly effective for 5-ASAs [64]. For example, Eudragit S coating of tablets, used in Asacol®, results in drug release at pH > 7, which is found in the ileum. Ethylcellulose-coating (Pentasa®) results in a continuous release over a period of several hours. The success of oral delayed- and controlled-release formulation for 5-ASAs relies on the region dependent intestinal absorption of 5-ASA. Detailed preclinical absorption studies in the authors' laboratory had shown that 5-ASA exhibits much higher intestinal permeability in the jejunum compared with the lower intestine [65,66]. As a result, delayed-release formulations that release the drug in the lower bowel, typically the ileum, significantly reduce systemic availability of 5-ASA, while at the same time deliver the drug to the inflamed intestinal areas. Colonic delivery systems and topical formulations such as suppositories, foams and liquid enemas offer a similar advantage in the case of UC treatment [67]. A systematic review of the pharmacokinetic parameters of delayed-/controlled-release and colonic formulations of 5-ASAs has recently been published [68].

4.2 Colonic drug delivery

Colonic drug delivery is desirable for UC and many cases of Crohn's disease where the colon is the primary pathological site. Colon-specific oral drug delivery can be utilised to achieve several other goals. Colonic delivery systems can also be used to increase the bioavailability of intact peptides and proteins due to the less 'hostile' enzymatic environment of the colon, compared with the small intestine. In addition, drug release in the colon can delay systemic absorption of drugs if such a delay is therapeutically desirable. Furthermore, due to the longer retention time, colonic drug targeting can also be utilised to increase absorption of poorly absorbed drug molecules. Finally, the colon is rich in lymphoid tissue and, therefore, represents a desirable target for vaccine delivery. Colonic drug targeting is usually achieved by pH-dependent release, time-dependent release and bacterial degradation strategies. Detailed reviews of colon-specific drug delivery strategies can be found elsewhere [69-71]. This review concentrates on colon-specific delivery attempts for IBD drugs.

One of the most common approaches is the use of pH-sensitive polymers. In that direction, Leopold *et al.* proposed the use of basic polymer coatings such as the aminoalkyl

methacrylate copolymer Eudragit E and the polyvinylacetal diethylaminoacetate polymer AEA, and studied the *in vitro* release of dexamethasone from the formulations [72]. Lamprecht *et al.* designed a tacrolimus microsphere formulation using another pH-sensitive polymer, Eudragit P-4125F, and demonstrated desirable *in vitro* release profiles [73]. Cheng *et al.* utilised a combination of Eudragit L100 and S100 [74] and Khan *et al.* utilised Eudragit L100-55 and Eudragit S100 combinations for the colonic delivery of 5-ASA [75]. Rudolph *et al.* utilised Eudragit FS-30D-coated pellets to achieve release of 5-ASA close to the ileocecal valve [76]. Rodriguez *et al.* proposed the use a multiparticulate system consisting of drug-loaded cellulose acetate butyrate coated by the enteric polymer Eudragit S to combine pH-dependent and controlled-release profiles. The authors demonstrated successful controlled release of budesonide *in vitro* at pH > 7 [77]. After oral administration to TNBS-colitic rats, the authors demonstrated a significant advantage of this new formulation compared with budesonide suspension in reducing intestinal inflammation and an improvement over budesonide-loaded Eudragit S microparticles [78]. Similarly, Bott *et al.* demonstrated desirable *in vivo* release properties of a pH- and time-based multiunit delivery system composed of an inner layer of the pH-independent polymers Eudragit RL and RS and an outer layer of the pH-dependent polymer Eudragit FS-30D [79-81]. As in the case of the system designed by Rodriguez *et al.*, the outer layer delays release until the formulation reaches the colon, whereas the inner layer provides a sustained release of the drug. Brunner *et al.* recently reported two Phase I clinical studies, monitoring the 5-ASA release profiles from a patented extended-release gastro-resistant tablet [201]. Drug release was sustained throughout the colon, whereas systemic absorption was decreased. Single- and multiple-dose administration was well tolerated [82].

Other approaches utilise polymer coatings that are degraded by microorganisms in the colon [83,84]. Tozaki *et al.* utilised azo-containing polyurethane-coated tables to deliver budesonide to the inflamed colon of TNBS-colitic rats. After oral administration of the formulation, the authors were able to reduce the budesonide dose required to produce a therapeutic effect to a fifth of the solution dose [85]. Orally administered chitosan capsules containing 5-ASA released the drug exclusively in the colon, thus reducing absorption and increasing local drug levels, while at the same time producing a stronger therapeutic effect compared with a 5-ASA suspension [86]. Tugcu-Demiroz *et al.* recently reported the use of guar gum matrix tablets for the colonic delivery of 5-ASA [87]. Similary, Krishnaiah *et al.* utilised guar gum-based tablets to deliver metronidazole specifically to the colon and delay absorption [88]. Pectin has also been studied *in vitro* for delivery of 5-ASA [89]. Sriamornsak *et al.*, proposed the addition of polygalacturonic acid to Eudragit RS to control the release of colonic delivery systems [90].

Timed-release systems are based on the relatively constant transit time in the small intestine. Several different formulations have been developed for colonic drug delivery. Alvarez-Fuentes *et al.* prepared matrix tablets by the compression of mixtures of hydroxyethylcellulose with ethylcellulose or microcrystalline cellulose polymers, and using Eudragit S100 as a pH-sensitive coating [91]. Fukui *et al.* used tablets press-coated with an outer shell of hydroxypropylcellulose [92]. Sangalli *et al.* developed an oral and/or site-specific delivery system (chronotopic) consisting of a drug-containing core coated by a hydrophilic swellable polymer [93]. In all three cases, however, the model compound was not an IBD medication. In some of the few studies utilising IBD drugs, Muraoka *et al.* prepared a pressure-controlled system by coating the inner surface of a gelatin capsule with ethylcellulose to achieve colon delivery of 5-ASA [94,95]. Similarly, colonic delivery of 5-ASA was also achieved with the use of the Timeclock® system comprising a tablet core coated with a mixture of hydrophobic polymer and surfactant [96,97].

Chemical modification of the drug molecule to achieve colonic drug delivery is also a common approach and has been used extensively in the case of aminosalicylates, with several prodrugs of 5-ASA available on the market. The original 5-ASA, sulfasalazine, consisted of a 5-ASA molecule linked to sulfapyridine [98,99]. Due to sulfapyridine toxicity, it has been replaced with other, less toxic carrier molecules. These include 4-amino benzoyl-β-alanine in balsaladine (Colazal®, Salix Pharmaceuticals) and *p*-aminohippurate in ipsalazine [100]. Olsalazine (Dipentum®, Celltech) is a dimer of two 5-ASA molecules linked via an azo bond [98]. A systematic review of the pharmacokinetic parameters of 5-ASA prodrugs used in IBD therapy has recently been published [68]. Other prodrugs utilise carriers such as amino acids [101-104], dextran [105], polymers such as polyanhydrides [106] and methacrylates [107], polyamidoamine dendrimers [108] or newer azo-derivatives [109,110]. Release is typically dependent on pH changes or degradation by bacterial enzymes. Less attention has been given to colon-specific prodrugs of corticosteroids. Glucuronide prodrugs of dexamethasone [111-113], methylprednisolone [114] and budesonide [115] were demonstrated to deliver the drug in the colon while reducing systemic steroid levels; more recently, Yano *et al.* proposed the use of α-cyclodextrin conjugate of prednisolone [116,117], and Doh *et al.* exhibited colonic targeting properties for prednisolone 21-sulfate sodium [118].

4.3 Oral particulate systems for delivery of inflammatory bowel disease drugs

A limited number of studies have been published on more specific approaches to deliver the drug locally to the intestine. These studies usually involve the use of polymeric particulate systems, such as microspheres. These particulate systems have been reported to exhibit increased binding to the intestinal wall in healthy intestinal tissue [119,120], while Lamprecht *et al.* reported that gut wall attachment of poly-DL-lactide-co-glycolide (PLGA) microspheres and nanospheres was significantly

increased in inflamed intestinal segments of colitic rats [121]. This study concluded that similarly to healthy tissue, adherence was size dependent, with smaller particles exhibiting greater adherence. Most particles were associated with the mucus layer. The rationale behind using particulate systems for drug delivery in IBD is that adhesion to the intestinal wall will significantly increase retention time at the tissue of interest, providing for a possible accumulation of the dosage form in the intestinal tissue either from nonspecific uptake due to loss of intestinal epithelial integrity or due to uptake by macrophages and other immune system cells. Increased local drug load may also allow for dose reduction.

Microspheres or nanospheres composed of biocompatible polymers have attracted most of the attention as delivery systems for the local disposition of drugs to treat intestinal inflammation. Nakase *et al.* utilised orally administered poly-DL-lactic acid (PDLLA) microspheres loaded with dexamethasone to treat dextran sodium sulfate colitic BALB/c mice [122]. The microspheres were predominantly taken up by the inflamed colon. Compared with solution administration, microspheres achieved better therapeutic effect and reduced systemic absorption of dexamethasone. The authors attributed the therapeutic effect to microsphere uptake by macrophages present in the inflamed tissue. The same authors were able to utilise the same formulation to treat TNBS-induce colitis in rats, which resembles human CD, whereas DSS-colitis mostly resembles human UC. Again, microspheres showed a better therapeutic effect, assessed by markers of intestinal inflammation, compared with solution administration [123]. More recently, the group reported that PDLLA microspheres containing dichloromethylene diphosphonate (DMDP) and gelatin microsperes containing IL-10 were successful in inhibiting inflammation in IL-10-deficient colitic mice after rectal administration [124,125]. According to the authors, DMDP acted by reducing the number of active macrophages in the colon without affecting systemic macrophages, whereas the therapeutic effects of GM-IL-10 were associated with decreased expression of IL-12 mRNA and downregulation of CD40 expression in Mac-1-positive cells due to sustained IL-10 release from the formulation. Utilising PLGA nanospheres containing rolipram, phosphodiesterase inhibitor with anti-inflammatory properties, Lambrecht *et al.* successfully treated TNBS-induced colitis in rats, after oral administration of the formulation [126]. Animals were treated with rolipram nanosphere formulation or solution for 5 days after establishment of colitis. Compared with solution, nanosphere-treated animals did not exhibit a strong relapse of inflammation, assessed 5 days after therapy had ended.

Liposomes have also attracted attention as oral drug delivery vehicles [127] and there are evidence supporting a potential role of liposomes in local drug delivery to the intestinal tissue. Studies in the authors' laboratory have shown interaction of liposomes with intestinal cells in rats as well as in Caco-2 cell monolayers *in vitro.* Furthermore, intralumenal administration of 5-ASA encapsulated in phospholipid liposomes composed of phosphatidyl choline, cholesterol and phosphatidyl glycerol, resulted in decrease in blood levels of 5-ASA and its major metabolite *N*-acetyl-5-ASA compared with a solution of the same concentration. Tissue levels of 5-ASA were also increased after liposomal administration compared with solution [128-130]. More recently, it was reported that the charge and size of liposomes dictates their adherence properties as well as the tissue pathological state. Comparing cationic, anionic and neutral liposomes in a colonic sac experimental setup, the authors observed that although cationic liposomes exhibited increased adsorption to the healthy epithelium of rat colon, anionic ones achieved the better adherence on tissue from DNBS-induced colitic rats [131]. Although these studies support the use of liposomes in IBD drug delivery, further studies are required to understand the exact interactions of liposomes with the diseased intestinal tissue, how attachment affects the stability of liposomes and drug release rate and ultimately the mechanism by which liposomes affect oral drug delivery. Recent studies in the authors' laboratory suggest that physicochemical properties and absorption characteristics of the encapsulated drug significantly affect the utility of liposomes in topical intestinal drug delivery via the oral route. Another concern is the stability of liposomes at the low pH of the stomach and in the GI tract where they are subject to the action of pancreatic enzymes that digest phospholipids or bile salts that disrupt the lipid bilayer [132]. Several strategies have been developed to improve stability, including optimisation of lipid composition and the use of surface-coated [133-136] or polymerised liposomes [137,138]. The utility of these strategies in delivery of IBD therapies has yet to be evaluated. Non-phospholipid-based liposomes have been suggested to exhibit increased stability in the intestinal environment [139-141] and may also prove useful for oral drug delivery in IBD. Studies in the authors' laboratory suggest that like their phospholipid counterparts, non-phospholipid liposomes attach to intestinal membranes. Use in enema formulations where attachment to the intestinal wall and drug release is more immediate or encapsulation in delayed-release capsules are possible solutions to extending *in vivo* liposomal stability. Finally, it is worth mentioning that Awasthi *et al.* reported increased intestinal accumulation of phospholipid PEG-liposomes administered intravenously to TNBS-colitic rats [142], suggesting an alternative route to administration of liposomal formulations for the treatment of IBD.

Use of mucoadhesive coatings may further improve specificity of the oral particulate systems described above and lead to extended interactions with the target intestinal tissue. Several polymers/copolymers have been shown to exhibit mucoadhesive properties such as *N*-(2-hydroxypropyl)methacrylamide [143,144], polycarbophil [145,146] and polysaccharides such as chitosan [86]. Further specificity in the bioadhesive properties is commonly sought by the incorporation of lectins: proteins of nonimmune origin that recognise and bind to glycoproteins expressed on cell surfaces [147-149]. Plant lectins such as wheat

germ agglutinin and Ulex europaeus isoagglutinin have been shown to bind to intestinal cell membranes [150,151]. For a lectin to be used as a successful targeting ligand in drug delivery for IBD, selectivity for the affected regions of the GI tract will be necessary. Species differences in ligand expression should also be taken into consideration during the design and study of the delivery system. A small number of examples exist for the use of bioadhesive systems in the delivery of IBD drugs. In one such example discussed earlier, Tozaki *et al.* utilised chitosan capsules to successfully deliver 5-ASA to the colon of TNBS-colitic rats. When compared with a carboxymethylcellulose suspension of 5-ASA, the chitosan capsules showed a more favourable therapeutic profile [86].

5. Gene therapy for inflammatory bowel disease

IBD pathogenesis is characterised by a cytokine imbalance with increased levels of pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-1, and decreased levels of regulatory cytokines such as TGF-β and IL-10. Delivery of the appropriate cytokines or inhibition of cytokine production has potential application for the treatment of IBD and has shown promising results in experimental models and clinical trials [152-154]. For this purpose, gene therapy appears to be a promising approach to restore the cytokine balance and downregulate the inflammation. Local or targeted delivery of viral vectors to the intestine will produce an enhanced local therapeutic effect with fewer systemic complications. Despite the difficulties associated with GI gene transfers, mainly the low transfectability of the intestinal epithelial cells by the commonly used viral vectors especially after intralumenal administration, several preclinical studies have been published describing promising results for therapeutic gene delivery to treat intestinal inflammation in animal IBD models.

Most studies have utilised adenoviral vectors, although other vectors have also shown promise in transduction of intestinal mucosa. Ad5 vectors administered by enema expressing the IL-18 antisense RNA significantly reduced IL-18 expression and improved colitis in SCID mice with chronic colitis [155]. Hogaboam *et al.* reported that intraperitoneal administration to TNBS colitic rats of an Ad5 vector carrying the IL-4 gene resulted in significant downregulation of inflammation [156]. Because administration of IL-10 significantly reduces inflammation in experimental models of IBD and has shown promising results in clinical trials [152-154], several groups have focused on IL-10-based gene therapy. Barbara *et al.* reported that intraperitoneal pretreatment of colitic rats with Ad5 expressing IL-10 prevents colitis [157]. Intravenous administration of Ad5 expressing IL-10 to TNBS colitic mice [158] or IL-10-deficient colitic mice [159] also showed significant therapeutic effect. More recently, intrarectal delivery of Ad5-expressing IL-10 vectors in colitic mice exhibited a local therapeutic effect without the complications associated with systemic administration [160]. In other gene delivery systems, Kitani *et al.* reported therapeutic and preventive effect in TNBS colitis after intranasal administration of a plasmid DNA encoding active TGF-β1 [161]. Finally, van Montfrans *et al.* reported therapeutic benefit of $CD4^+$ cells transduced *ex vivo* by retroviral vectors with IL-10. Gene therapy for IBD has recently been reviewed in detail elsewhere [162,163].

6. Conclusions

IBD represents a major challenge for drug delivery. Oral administration of drugs is the most desired route of administration due to the ease of use, the reduced cost of medications and the increased patient compliance. After oral administration, the drug is absorbed by the intestinal epithelial cells into the systemic circulation. Although for most therapeutic regimens the goal is to achieve the highest absorption possible, this is not the case in IBD therapy. Because the pathogenesis of the disease as well as the major clinical manifestations are confined to the intestine, high drug concentration levels are desired within the intestinal wall. At the same time, systemic distribution of the drug is commonly associated with undesired side effects. As a result, the ideal IBD formulation will selectively deliver the drug to the inflamed intestine while minimising systemic absorption. This constitutes a major challenge for drug delivery scientists, as they would have to counter the physiological absorptive role of the intestine.

Several strategies have been undertaken to improve drug delivery for IBD. Drugs can be chemically modified to increase their clearance before they reach the systemic circulation. This strategy resulted in the synthesis of budesonide, one of the most commonly used steroids in IBD therapy. Drugs can also be chemically modified to control their absorption through the GI tract, allowing sufficient absorption only in the colon. This has led to the development of compounds such as balsalazide and olsalazine that are extensively used in the treatment of UC. Formulation approaches are also used extensively. Controlled- and delayed-release formulations can be prepared by the use of specific polymers that allow for pH- or time-dependent release of the drug, or that are degraded by microorganisms present in the colon, thus releasing their contents only in the large bowel. Several controlled-release formulations for IBD are available on the market. More recently, the use of particulate systems such as microspheres and liposomes have attracted attention. Due to their bioadhesive properties, these formulations provide for the possibility of sustained release in the vicinity of the inflamed intestine and local drug action.

Although a wide range of medications is currently used in IBD therapy, their main purpose is to nonspecifically reduce the extent of the intestinal inflammation, thus improving the quality of life of the patient, than provide an actual cure of the disease. With our knowledge of IBD pathogenesis increasing in recent years, gene therapy approaches have been attracting attention. Although gene therapy for IBD is still in its infancy, promising results for therapeutic gene delivery to treat intestinal inflammation have been described in animal IBD models.

7. Expert opinion

UC and CD are idiopathic inflammatory diseases of the intestine. Extraintestinal manifestations occur; however, most improve with treatment of the intestinal inflammation. The diseases are idiopathic: years of research into inflammatory mechanisms highlights the importance of the intestinal immune system and the interaction between the immune system and lumenal bacteria. Genetics are important and complex, such that no single mutation is responsible for the majority of IBD cases. Several disease-susceptibility genes have been indentified; most significantly, the gene for NOD2 encoding an important mediator of innate immunity. Nonfunction of NOD2 due to a genetic mutation results in inappropriate immune response to commonly encountered lumenal bacteria, leading to chronic inflammation.

Existing medications used to treat IBD include prodrugs and various formulations of 5-ASA, as well as oral and topical corticosteroids. Immunomodulators such as azathioprine, 6-MP, cyclosporin and methotrexate are widely used, but have potent systemic toxicities. Newer immunomodulators such as infliximab are systemically administered and because they are infused proteins, have allergic and immunological side effects, in addition to marked systemic immunosuppressive effects.

The primary intestinal site of pathology, together with the intimated association of the intestinal tract with the external environment, provides a strong case for local rather than systemic drug therapy for the treatment of IBD. The case for local delivery is strengthened by data demonstrating that efficacy of many IBD drugs including 5-ASA products improves with increasing local drug concentrations. Therefore, an ideal drug delivery system for IBD would be one that delivers high intestinal tissue levels, but low systemic blood levels of the drug. Therefore, all classes of commonly used drugs to treat IBD, particularly those with systemic toxicity, may be improved by strategies to increase local intestinal tissue concentrations and decrease systemic delivery.

The authors' laboratory has studied several strategies to deliver drugs and genes to the inflamed intestine through the lumenal route. Liposomal formulations of 5-ASA and 6-MP are being studied with the goal of increasing local and decreasing systemic delivery; thereby, limiting systemic toxicity. The mechanism of adenoviral and other gene vector delivery systems to deliver genes for anti-inflammatory proteins to the intestinal epithelium is being explored. Although years away as a potential therapy, gene delivery has the utility in studying the local effects of delivered genes on mucosal inflammation. Other strategies for targeting specific membrane receptors or transporters, with the goal of increasing local drug concentrations and decreasing systemic absorption, should be explored.

In search of the 'magic bullet', investigators at academic institutions and pharmaceutical companies have developed many new exciting anti-inflammatory agents for treating IBD and other inflammatory diseases. Even though the cure remains elusive, this effort has resulted in a solid pipeline of potent anti-inflammatory drugs currently under investigation. The intestinal tract has characteristics that make a strong case for targeted oral drug delivery. While we wait for the 'magic bullet' to emerge, a smart investment in time and resources will yield an effective means to aim the magic bullet at the inflamed intestine.

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Patent

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