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TOPIC HIGHLIGHT

2015 Advances in inflammatory bowel disease

Nanomedicine and drug delivery strategies for treatment of inflammatory bowel disease

Hidetoshi Takedatsu, Keiichi Mitsuyama, Takuji Torimura

Hidetoshi Takedatsu, Keiichi Mitsuyama, Takuji Torimura, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka 830-0011, Japan

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Correspondence to: Hidetoshi Takedatsu, MD, PhD, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan. takedatsu_hidetoshi@kurume-u.ac.jp
Telephone: +81-942-353311
Fax: +81-942-342623

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Abstract

Crohn's disease and ulcerative colitis are two important categories of human inflammatory bowel disease (IBD). Because the precise mechanisms of the inflammation and immune responses in IBD have not been fully elucidated, the treatment of IBD primarily aims to inhibit the pathogenic factors of the inflammatory cascade. Inconsistencies exist regarding the response and side effects of the drugs that are currently used to treat IBD. Recent studies have suggested that the use of nanomedicine might be advantageous for the treatment of intestinal inflammation because nano-sized molecules can effectively penetrate epithelial and inflammatory cells. We reviewed nanomedicine treatments, such as the use of small interfering RNAs, antisense oligonucleotides, and anti-inflammatory molecules with delivery systems in experimental colitis models and clinical trials for IBD based on a systematic search. The efficacy and usefulness of the treatments reviewed in this manuscript have been demonstrated in experimental colitis models and clinical trials using various types of nanomedicine. Nanomedicine is expected to become a new therapeutic approach to the treatment of IBD.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Nanomedicine; Small interfering RNA; Antisense oligonucleotide

Core tip: Crohn's disease and ulcerative colitis are important categories of human inflammatory bowel disease (IBD). IBD treatment generally involves attempting to inhibit pathogenic factors of the inflammatory cascade. Recent studies suggest that nanomedicine provides advantages over conventional treatments for the treatment of intestinal inflammation because nano-size molecules can effectively penetrate epithelial and inflammatory cells. The efficacy and usefulness of the nanomedicine treatments reviewed

in this manuscript have been validated in experimental colitis models and clinical trials. Nanomedicine is therefore expected to become a new therapeutic approach to the treatment of IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD), which primarily refers to Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic inflammation of the gastrointestinal tract[1]. Although the etiology of these diseases remains unknown, several factors such as immune imbalance, dysregulation of the host-microbial interaction, and genetic susceptibility are involved in the pathogenesis of IBD[2]. IBD is treated using 5-aminosalicylic acid (5-ASA), corticosteroids, immunosuppressive drugs and anti-tumor necrosis factor α (TNF-α) antibodies (Abs). However, more than one-third of patients do not respond fully to these therapies. While the efficacy of these drugs decreases over time, the risks of infections and cancer associated with their use are increasing[3-5]. Seventy cases of mycobacterial infections were reported in patients receiving anti-TNF-α Abs by 2001, and the incident rate was more than 10 times the expected background rate[4]. Several studies have shown an association between anti-TNF-α Abs and cancers such as non-Hodgkin's lymphoma (NHL) and cutaneous malignancies. A standardized incidence rate of NHL in over 16000 IBD patients was reported to be 5.5 (95%CI: 4.4-6.6)[6], and the odds ratio of developing cutaneous malignancies was reported to be 2.07 (95%CI: 1.28-3.33)[7].

The medical applications of nanotechnology include the use of nano-particles (NPs) in imaging, pathological diagnosis, and drug delivery. Nanomedicine is a promising tool for the targeted delivery of drugs to specific tissues[8]. Several studies have shown that drugs that are delivered using NPs have advantages over conventional drugs, yielding more effective targeting, greater availability in diseased tissues, and fewer adverse effects. Thus, NPs represent an ideal drug delivery system for the treatment of IBD. NPs not only improve the efficacy of conventional drugs but also aid in the development of new therapeutic drugs. For example, 5-ASA, a conventional drug, is the drug most often studied when attempting to improve delivery systems because it acts only topically. Luminal pH and sustained release are important for delivery systems[9]. Recently, the use of NPs as delivery vehicles for 5-ASA, corticosteroids, and immunosuppressive drugs has been shown to result in greater therapeutic effects in experimental colitis models of IBD compared to standard formulations[10].

Anti-TNF-α Abs, such as infliximab, adalimumab, certolizumab, and golimumab, have proven efficacious against IBD. However, anti-TNF-α Abs therapies require parenteral administration at relatively high doses to achieve their therapeutic effect in the inflamed intestine, increasing the risk of adverse effects, such as lymphoma, infections (especially tuberculosis reactivation), lupus-like syndrome and the generation of anti-infliximab Abs[11]. Strategies that blockade TNF-α effects are needed to improve the safety of these biological therapies. Small interfering RNA (siRNA) and antisense oligonucleotides (ASOs) are candidates for IBD treatment due to their ability to locally neutralize TNF-α.

Biological treatment strategies for IBD involve the neutralization of proinflammatory cytokines, the use of anti-inflammatory cytokines and the inhibition of neutrophil adhesion or T cell signaling. The biological delivery of drugs to inflamed intestines remains a crucial challenge in the current treatment of IBD; therefore, combining siRNA, ASO, and anti-inflammatory molecules with nanotechnology-based drug delivery methods represents a valuable therapeutic approach, and some ASO strategies are already undergoing clinical trials. In this review, we focus on novel therapeutic approaches using nanotechnological systems, such as those that combine siRNA, ASO, and anti-inflammatory molecules with a delivery system.

SIRNA THERAPIES

Gene silencing *via* RNA interference (RNAi) is a candidate treatment for IBD. siRNA, usually comprising 20-25 bp double-stranded nucleotides, is a powerful tool for post-transcriptionally silencing gene expression and interferes with the expression of specific genes. siRNA directed against proinflammatory cytokines might be useful in treating intestinal inflammation. However, the low penetration of siRNA across cell membranes is a major obstacle for siRNA therapy. To overcome this problem, various delivery systems have been developed to deliver siRNA to intestinal tissue (Table 1).

TNF-α siRNA therapies using a delivery system

Neutralization of TNF-α Abs was the first biological strategy used in clinical practice and was more effective at treating IBD than conventional therapies[12]. However, serious infections and side effects were reported, including infusion reactions and the formation of antibodies against TNF-α[13]. Recently, several groups have attempted to drive TNF-α gene silencing directly into inflammatory sites in experimental colitis models. Here, we describe six delivery systems that have been used with TNF-α siRNA for the treatment of

Table 1 Small interfering RNA therapies

Target gene	Delivery system	Administration	Ref.
TNF-α siRNA therapy with delivery system			
TNF-α	TKN	Oral	[14]
TNF-α	PEI-PVA	Oral	[15]
TNF-α	NiMOS	Oral	[16]
TNF-α	OMe-P	Oral	[17]
TNF-α	TPP-PPM	*Ex vivo*	[18]
TNF-α	Fab'-bearing PLA-PEG	Oral	[19]
siRNA therapy targeting other molecules with delivery system			
CyD1	Abs to β7 integrin	Intravenous	[22]
TNF-α/CyD1	NiMOS	Oral	[23]
Map4k4	β1,3-D-glucan shell	Oral	[24]
CD98	scCD98-functionalized	Oral	[25]

SiRNA: Small interfering RNA; TKN: Thioketal nanoparticle; PEI-PVA: Polyethyleneimine/polyvinyl alcohol; NiMOS: Nanoparticles-in-microspheres oral system; OMe-P: 2'-O-methyl and propanediol modification; TPP-PPM: Mannosylated bioreducible cationic polymer/sodium triphosphate; Fab'-bearing PLA-PEG: Polylactic acid-polyethylene glycol copolymer/Fab' portion of the F4/80 Ab; scCD98-functionalized: Chitosan-alginate hydrogel/single-chain CD98 Abs.

experimental colitis.

Thioketal nanoparticles (TKNs) were formulated from a poly-(1,4-phenyleneacetone dimethylene thioketal polymer and selectively degraded by reactive oxygen species (ROS). When TNF-α siRNA/TKN was delivered orally, siRNA was released from TKNs in response to abnormally high levels of specific ROS at sites of intestinal inflammation. Orally administered TNF-α siRNA/TKN protected against dextran sodium sulfate (DSS)-induced colitis and effectively decreased TNF-α mRNA levels at sites of intestinal inflammation[14].

TNF-α siRNA/polyethyleneimine (PEI) was loaded into polylactide (PLA) (NP matrix) and then covered with polyvinyl alcohol (PVA) to form NPs, which were efficiently taken up by inflamed macrophages, thus inhibiting TNF-α secretion by the macrophages *in vitro*. The oral administration of TNF-α siRNA/PEI-PVA in lipopolysaccharide (LPS)-treated mouse models reduced the synthesis and secretion of TNF-α in the colon[15].

TNF-α siRNA was encapsulated in type B gelatin NPs and further entrapped in poly (epsilon-caprolactone) (PCL) microspheres to form a nanoparticles-in-microspheres oral system (NiMOS). This system, which exhibits particle sizes smaller than 5 μm, permitted localization in the colon by a controlled degradation of the outer layer and consequent release of the gelatin NPs to the site of inflammation. The oral administration of TNF-α siRNA/NiMOS attenuated DSS-induced colitis[16].

TNF-α siRNA involving 2'-O-methyl and propanediol modifications (TNF-α siRNA/OMe-P) was resistant to nuclease degradation and provided better silencing efficacy *in vitro* than unmodified siRNA. Intrarectally administered TNF-α siRNA/OMe-P significantly ameliorated DSS-induced colitis compared to unmodified and other chemically modified siRNAs[17].

TNF-α siRNA was formulated with mannosylated bioreducible cationic polymer (PPM) and sodium triphosphate (TPP). These NPs exhibited specific affinity to the mannose receptors that were exclusively expressed on the surfaces of the macrophages. TNF-α siRNA/TPP-PPM increased the efficiency of delivery by selectively targeting phagocytic cells at the inflammation site. These NPs reduced the TNF-α level in the intestine of DSS-induced colitis models in an ex vivo study[18].

TNF-α siRNA was loaded into polylactic acid-polyethylene glycol copolymer (PLA-PEG); then, the NPs were grafted to the Fab' portion of the F4/80 Ab (Fab'-bearing) on the surface of the NPs. Fab'-bearing PLA-PEG NPs exhibited improved macrophage-targeting kinetics *in vitro*. Orally administered TNF-α siRNA/Fab'-bearing PLA-PEG attenuated DSS-induced colitis more efficiently than uncovered NPs[19].

siRNA therapies targeting other molecules with delivery system

Other molecules, such as (1) Cyclin D1 (CyD1); (2) a combination of TNF-α and CyD1; (3) mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4); and (4) CD98, have been considered as novel targets for the treatment of IBD using siRNA delivery systems.

CyD1, a key cell cycle-regulating molecule, was upregulated in the epithelial and immune cells of IBD patients, which are implicated in promoting inflammation and epithelial colorectal dysplasia[20,21]. The liposome-based NPs used to target CyD1 siRNA were covered by Abs raised against β7 integrin, a receptor that is specifically present on leukocytes that are involved in intestinal inflammation. CyD1 siRNA/Abs raised against β7 integrin administered intravenously inhibited intestinal inflammatory responses in DSS-induced colitis. Silencing the CyD1 gene decreased the production of Th1 cytokines, such as TNF-α and IL-12[22].

Kriegel *et al*[23] targeted TNF-α and CyD1 using NiMOS[16]. CyD1 siRNA was combined with TNF-α siRNA/NiMOS. The dual silencing effect was more potent than the silencing of TNF-α siRNA alone. This study demonstrated the therapeutic potential of an oral NiMOS-based dual TNF-α and CyD1 gene silencing system for the treatment of IBD in a DSS-induced acute colitis model.

Map4k4 is a mediator of cytokine expression. Map4k4 siRNA was encapsulated in β1,3-D-glucan shells. Glucan has a specific affinity to glucan receptors that are present on macrophages and dendritic cells and is taken into targeted cells by phagocytosis. Orally administered NPs silenced Map4k4 expression in LPS-treated mice, thus protecting the mice from LPS-induced systemic inflammation by suppressing the production of TNF-α and IL-1β[24].

CD98 overexpression on colonic epithelial cells

Table 2 Antisense oligonucleotide therapies

Target gene	Delivery system	Administration	Ref.
ASO			
TNF-α	No	Subcutaneous	[30]
CD40	No	Rectal	[33]
MAdCAM-1	No	Subcutaneous	[34]
STAT3	No	Rectal	[36]
NPY	No	Rectal	[38]
ASO with delivery system			
TNF-α	gal-LMWC	Rectal	[39]
NF-κB	CS-PLGA	Oral	[40]
MIF	SPG	Intraperitoneal	[42]
CD40	nov038	Intravenous	[43]
TNF-α	cKGM	Oral	[44]

ASO: Antisense oligonucleotide; gal-LMWC: Galactosylated low-molecular-weight chitosan; CS-PLGA: Chitosan-modified poly (D,L-lactide-co-glycolide); SPG: Schizophyllan; nov038: Amphoteric liposome; cKGM: Cationic konjac glucomannan phytagel.

and macrophages is involved in the development and progression of IBD[25]. CD98 siRNA was loaded into a chitosan/alginate hydrogel; then, NPs were grafted to single-chain CD98 Abs (scCD98) on the surface of NPs. The scCD98-functionalized CD98 siRNA-loaded NPs were approximately 200 nm in size and exhibited high affinity for CD98-overexpressing cells. These NPs significantly reduced CD98 levels in Colon-26 cells and RAW 264.7 macrophages. Orally administered NPs decreased the severity of colitis in both a T cell transfer mouse model and a DSS-induced colitis model[26].

ANTISENSE OLIGONUCLEOTIDE THERAPIES

Antisense oligonucleotide (ASO) are generally 13 to 25 bases in length; these oligomers are designed to hybridize to mRNA that codes for a targeted protein. ASOs can reduce the abundance of specific RNAs through multiple mechanisms, such as the RNase H-mediated degradation of target RNA, translational arrest, and altered RNA splicing[27]. However, ASOs have a short *in vivo* half-life and poor biological stability because they are rapidly degraded by intracellular endonucleases and exonucleases. Several studies have demonstrated that replacement of the native backbone phosphates with phosphorothioates diminishes the degradation of ASOs by nucleases, thus increasing their stability[28]. Moreover, phosphorothioate oligodeoxynucleotides (ODNs) are highly soluble, easily administered and capable of activating RNase H activity[29]. Phosphorothioate ASOs have been used to target: (1) TNF-α; (2) CD40; (3) mucosal addressing cell adhesion molecule (MAdCAM)-1; (4) signal transducers and activators of transcription 3 (STAT3); and (5) neuropeptide Y (NPY) (Table 2).

ISIS 25302, which is specific for murine TNF-α, is a phosphorothioate ODN that contains methoxyethyl-modified nucleosides on its 5′ and 3′ ends. The methoxyethyl modification increases the affinity of ASOs for targeted mRNA and nuclease resistance. In *in vitro* experiments, ISIS 25302 decreased TNF-α mRNA in a dose- and sequence-dependent manner in a mouse macrophage cell line. ISIS 25302 subcutaneous injection significantly decreased disease activity index scores in mice with both acute and chronic DSS-induced colitis and significantly improved histopathological scores in IL-10-deficient mice[30].

The involvement of CD40 and CD154 in the pathogenesis of IBD is apparent due to their increased expression in the inflamed mucosa of patients and based on the therapeutic effects of anti-CD154 Abs in experimental colitis[31]. Due to their adverse effects, the use of such Abs in patients with IBD might be limited[32]. The rectal administration of CD40 phosphorothioate ASO was used to block CD154/CD40 and effectively interfered with CD154/CD40 interactions and attenuated 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats[33].

The expression of MAdCAM-1 is restricted in gut-associated lymphoid tissues, and its expression is dramatically increased in IBD. MAdCAM-1 phosphorothioate ASOs were injected subcutaneously into TNBS-induced colitis model mice. MAdCAM-1 ASOs significantly suppressed the development of TNBS-induced colitis clinically and histopathologically compared with controls. MAdCAM-1 ASO also reduced the number of α4β7 lymphocytes in the inflamed colonic mucosa[34].

The expression levels of STAT3 are increased in IBD and colitis model mice[35]. STAT3 phosphorothioate ASO was administered by rectal enema during the early phase of TNBS-induced colitis. Administration of STAT3 ASO effectively inhibited STAT3 expression and phosphorylation in the inflamed colonic mucosa of the colitis models, and the rectal administration of STAT3 ASO significantly attenuated intestinal inflammation[36].

In the central nervous system, NPY regulates many physiological functions, including stress. NPY has been shown to play an important role in immune and inflammatory responses[37]. The rectal administration of a NPY phosphorothioate ASO ameliorated DSS-induced colitis in rats, suggesting that NPY plays an important role in modulating inflammation in colitis[38].

ASO DELIVERY SYSTEMS

Naked ASOs are unable to cross cellular membranes and are rapidly degraded *in vivo*. Specialized delivery systems are necessary for the delivery of ASOs to target tissues for therapeutic efficacy. Delivery systems have been reported for various targets, including (1) TNF-α; (2) NF-κB; (3) macrophage-migration inhibitor factor (MIF); (4) CD40; and (5) TNF-α for use in treating IBD (Table 2).

A nano-complex based on galactosylated low-molecular-weight chitosan (gal-LMWC) and TNF-α ASO was developed to target activated macrophages

for use in treating intestinal inflammation. Rectal administration of a TNF-α ASO/gal-LMWC complex resulted in the successful delivery of ASO into activated colonic macrophages and a significant reduction of colonic TNF-α in TNBS-induced colitis. A single injection of TNF-α ASO/gal-LMWC was used to treat TNBS-induced colitis and repeated injections were used to treat T cell-transfer colitis; both treatments significantly ameliorated colitis[39].

Chitosan (CS)-modified poly(D,L-lactide-co-glycolide) (PLGA) NPs were developed and evaluated for use with a NF-κB decoy ODN oral delivery system to treat DSS-induced colitis. NF-κB decoy ODN uptake studies using Caco-2 cells and confocal laser scanning microscopy indicated that CS-PLGA NPs were more effectively taken up by the cells than unmodified PLGA. NF-κB decoy ODN/CS-PLGA improved the stability of ODN against DNase Ⅰ and acidic media, such as gastric juices. Orally administered NF-κB decoy ODN/CS-PLGA significantly attenuated colitis[40].

MIF, which is mainly produced by macrophages, has been shown to have a pathogenic role in IBD[41]. A delivery system for ASO using schizophyllan (SPG), a polysaccharide that belongs to the β-(1-3) glucan family, has been developed. This system has several advantages, enabling the effective suppression of targeted RNA or DNA; the SPG complex is stable *in vivo*, and the SPG complex is effectively taken up into macrophages by phagocytosis through Dectin-1. The intraperitoneal injection of MIF ASO/SPG complex effectively suppressed MIF production and significantly ameliorated intestinal inflammation[42].

CD40-CD40L interactions appear to play an important role in the pathogenesis of experimental colitis. CD40 ASO was formulated in amphoteric liposomes (nov038/CD40 ASO). The charge characteristics of amphoteric liposomes facilitate the efficient sequestration of ASO inside the liposomes at low pH and direct the carriers to macrophages and dendritic cells. Delivery of nov038/CD40 ASO is highly cell-specific because it selectively suppresses CD40 on macrophages but not on B-cells. Systemic administration of nov038/CD40 ASO effectively treated TNBS-induced colitis and prevented its development[43].

TNF-α ASO NPs were constructed using cationic konjac glucomannan (cKGM), phytagel and TNF-α ASO. This DDS enabled the spontaneous release of an ASO/cKGM nano-complex from the phytagel scaffold into the colon lumen, where the ASO was transferred into colonic macrophages *via* receptor-mediated phagocytosis. Orally administered TNF-α ASO NPs significantly attenuated DSS-induced colitis[44].

ASO THERAPIES IN CLINICAL TRIAL

Accumulating evidence has suggested that ASOs can be used to inhibit specific targets, such as (1) NFκB-p65; (2) intercellular adhesion molecule (ICAM)-1; and (3) Smad7 in experimental colitis models; this research has led to clinical trials in IBD patients.

NF-κBp65 ASO

NF-κB is a member of a family of transcription factors that regulate the promoters of several genes, the products of which are involved in many biological processes[45,46]. In TNBS-induced colitis and IL-10-deficient mice (two murine models of colitis), the p65 subunit of NF-κB was strongly activated and played a role in the up-regulation of pro-inflammatory cytokines[47]. Targeting NF-κBp65 was also effective in treating DSS-induced colitis and TNBS-induced colitis[48,49]. Clinical trials for NF-κBp65 ASO are underway.

Alicaforsen

ICAM-1 is constitutively expressed at low levels in leukocytes and vascular endothelial cells. ICAM-1 was shown to be upregulated in the inflamed colon of IBD patients[50], and neutralizing ICAM-1 Abs and ICAM-1 ASOs attenuated colitis in mice[51,52]. Alicaforsen (ISIS 2302), an RNase H-dependent, 20-base-long phosphorothioate ASO that was designed to inhibit human ICAM-1, was the first ASO used to treat IBD. In a phase Ⅰ clinical trial, intravenous alicaforsen was well tolerated[53]. In 20 active CD patients, alicaforsen was superior to placebo in inducing clinical remission[54]. However, the efficacy of alicaforsen was not confirmed in two double-blind, placebo-controlled, multicenter clinical trials[55,56].

Furthermore, the efficacy of alicaforsen was investigated by administering this drug by rectal enema to patients with mild to moderate left-sided UC[57]. Alicaforsen enema showed promising acute and long-term benefits in UC patients. Individual patient data in a meta-analysis of 200 patients from four phase Ⅱ clinical trials confirmed the efficacy of alicaforsen enema in patients with active UC[58].

Mongersen

The cytokine transforming growth factor (TGF)-β1, which is produced by many mucosal cell types, is able to negatively regulate the activation and function of several immune cell types[59]. The immunoregulatory properties of TGF-β1 are mainly mediated by the Smad pathway[60]. Smad7, an inhibitor of TGF-β1 signaling, is overexpressed in IBD mucosa and purified mucosal T cells. Smad7, which is also inhibited by Smad7 ASO in cells isolated from IBD patients, restored TGF-β1 signaling and enabled TGF-β1 to inhibit cytokine production[61]. Smad7 ASO (mongersen), an RNase H-dependent, 21-base phosphorothioate ASO, has been formulated as a solid oral dose. This formulation is protected by an external tablet coating made of pH (6.6-7.2)-dependent metacrylic acid polymers, enabling the antisense to be released only in the lumen of the terminal ileum and right colon. In a phase Ⅰ study, mongersen was demonstrated

Table 3 Administration of anti-inflammatory mediators

Mediator	Delivery system	Administration	Ref.
IL-10			
IL-10	*L. lactis*	Oral	[65]
IL-10	Gelatin microspheres	Rectal	[66]
IL-10	NiMOS	Oral	[67]
Other anti-inflammatory molecules			
TNF-neutralization	*L. lactis*	Oral	[68]
PHB1	PEI-PVA	Oral	[70]
TFF	*L. lactis*	Oral	[72]
KPV	PLA	Oral	[74]
IL-27	*L. lactis*	Oral	[77]

L. lactis: *Lactococcus lactis*; NiMOS: Nanoparticles-in-microspheres oral system; PEI-PVA: Polyethyleneimine/polyvinyl alcohol; PLA: Polylactide.

to be safe and well tolerated in active CD patients. Mongersen treatment produced a significant decrease in CDAI scores[62]. Furthermore, the efficacy of mongersen for the treatment of active CD patients was evaluated in a double-blind, placebo-controlled, phase Ⅱ trial. This study demonstrated that the treatment of active CD patients with mongersen resulted in significantly higher rates of remission and clinical response compared to placebo[63].

ADMINISTRATION OF ANTI-INFLAMMATORY MEDIATORS

The administration of anti-inflammatory mediators, especially IL-10, represents another biologic strategy for IBD. Several anti-inflammatory mediator candidates have been investigated using experimental colitis models (Table 3).

IL-10 NPs

IL-10 is an anti-inflammatory cytokine that suppresses the T helper 1 immune response and down-regulates macrophages and monocytes. The therapeutic effect of the systemic administration of IL-10 to IBD patients has not been satisfactory[55]. This failure is thought to be due to the delivery of only low concentrations of IL-10 to the intestinal tissues. Moreover, higher doses of systemically administered IL-10 caused adverse effects[64]. Topical therapy using nanotechnology, such as oral and rectal administration, might improve efficacy and safety by localizing the effect of IL-10 to the inflammation site, thus preventing side effects.

The oral administration of genetically engineered IL-10-secreting *Lactococcus lactis* (*L. lactis*) provided in situ synthesis of IL-10, which resulted in a 50% reduction of inflammation in DSS-induced colitis mice and prevented the onset of colitis in IL-10-deficient mice[65].

Recombinant IL-10 was loaded into gelatin microspheres (GMs). Rectal administration of these GMs (GM-IL-10) attenuated colitis in IL-10-deficient mice[66].

NiMOS was formulated with IL-10-expressing plasmid DNA in type-B gelatin NPs. These NPs directed the local transfection of IL-10 plasmid in inflamed intestinal tissues and enhanced IL-10 expression. Orally administered plasmid DNA encoding IL-10/NiMOS suppressed proinflammatory cytokines, consequently attenuating TNBS-induced acute colitis[67].

Other anti-inflammatory molecules delivered using NPs

Other anti-inflammatory molecules, such as (1) TNF-neutralizing nanobodies; (2) prohibitin 1 (PHB); (3) trefoil factors (TFF); (4) the tripeptide Lys-Pro-Val (KPV); and (5) IL-27, were investigated in experimental colitis models and might represent novel candidate therapeutics for the treatment of human IBD.

L. lactis was engineered to secrete monovalent and bivalent murine TNF-neutralizing nanobodies as therapeutic proteins. These therapeutic proteins are derived from fragments of heavy-chain camelid antibodies and are more stable than conventional antibodies. Orally administered nanobody-secreting *L. lactis* significantly reduced inflammation in DSS-induced chronic colitis mice and in IL-10-deficient mice[68].

Genetic restoration of intestinal epithelial PHB1 levels during experimental colitis reduced the severity of the disease by sustaining epithelial antioxidant expression and reducing NF-κB activation[69]. Recombinant PHB/polyethyleneimine (PEI) was loaded into polylactide (PLA) NPs and then covered with polyvinyl alcohol (PVA). The therapeutic potential of this system for restoring epithelial PHB was then examined in a DSS-induced colitis model. The oral administration of PHB/PEI-PVA resulted in increased levels of PHB in colonic epithelial cells and decreased severity of colitis[70].

TFFs are cytoprotective and promote epithelial wound healing and reconstitution of the gastrointestinal tract; thus, TFFs are good candidate therapeutics for use in treating acute colitis[71]. The food-grade bacterium *L. lactis* was engineered to secrete bioactive murine TFF. Oral administration of TFF-secreting *L. lactis* led to the active delivery of TFF at the mucosa of the colon and proved very effective in the prevention and healing of acute DSS-induced colitis and in improving established chronic colitis in IL-10 deficient mice[72].

The anti-inflammatory tripeptide Lys-Pro-Val (KPV)[73] was loaded into polylactide (PLA) nanoparticles and encapsulated into a polysaccharide gel containing alginate and chitosan polymers. NP-KPV was much more effective than free KPV in reducing the inflammatory response induced by LPS in the intestinal epithelia of mice. The effective dose of NP-KPV was 12000 times lower than that of KPV in free solution. Furthermore, NP-KPV demonstrated therapeutic efficiency in treating DSS-induced colitis models[74].

IL-27 has an immunosuppressive role[75,76]. A localized IL-27 delivery system was synthesized in *L. lactis* by incorporating a linker between the two chains

of IL-27; codons and a secretory signal sequence preferred by *L. lactis* (LL-IL-27) were used. LL-IL-27 administration protected against colitis in a T cell transfer model by increasing the production of IL-10. The oral administration of LL-IL-27 might be a more effective and safe therapy for IBD[77].

CONCLUSION

In this review, we provide novel insights into the role of nanomedicine in IBD treatment. ASO, siRNA and anti-inflammatory molecules with drug delivery vehicles generally undergo cellular internalization by paracellular transport or endocytosis into intestinal epithelial cells. Specialized differentiated epithelial cells called M cells are involved in the predominant uptake of nanoparticles in healthy intestinal mucosa. In intestinal inflammation, a loss of mucous-gel layers and the epithelial barrier through enterocyte damage and increased delivery of immune cells to the mucosal tissue have been shown to lead to the preferential accumulation and uptake of nanomedicines by both enterocytes and macrophages[78]. Therefore, the topical therapy of nanomedicine by oral and rectal administration can be effective in treating the inflammation site.

Important factors in targeting the intestine are not only the use of nano-size molecules but also the implementation of additional strategies to enhance drug delivery to inflamed intestinal mucosa and achieve maximal retention time in tissues. As summarized in this review, nanomedicine strategies for IBD treatment have proven effective for the treatment of experimental colitis models; however, further studies on the effects of nanomedicine in human IBD are warranted. Specifically, there is a need for further investigation of the safety and efficacy of nanomedicine in human IBD. Recently, the efficacy of phosphorothioate ASOs was demonstrated in patients with IBD and various types of cancer[79,80]. By accumulating further evidence, clinical applications of nanomedicine will be realized. In the future, locally targeted nanomedicine may provide a tailored treatment for the control of the immune response and the inhibition of inflammation in individual IBD patients.

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