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EDITORIAL

Potential prospects of nanomedicine for targeted therapeutics in inflammatory bowel diseases

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Abstract

Inflammatory bowel diseases (IBDs) such as Crohn's disease are highly debilitating. There are inconsistencies in response to and side effects in the current conventional medications, failures in adequate drug delivery, and the lack of therapeutics to offer complete remission in the presently available treatments of IBD. This suggests the need to explore beyond the horizons of conventional approaches in IBD therapeutics. This review examines the arena of the evolving IBD nanomedicine, studied so far in animal and *in vitro* models, before comprehensive clinical testing in humans. The investigations carried out so far in IBD models have provided substantial evidence of the nanotherapeutic approach as having the potential to overcome some of the current drawbacks to conventional IBD therapy. We analyze the pros and cons of nanotechnology in IBD therapies studied in different models, aimed at different targets and mechanisms of IBD pathogenesis, in an attempt to predict its possible impact in humans.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Tumor necrosis factor-α; Nanomedicine

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) constitute the two principal components of inflammatory bowel diseases (IBDs), which occur as a result of dysregulated immune responses in genetically predisposed individuals due to various environmental conditions[1]. There are sufficient similarities in the pathological conditions in CD and UC that cause about 10% of IBD cases to be diagnosed as indeterminate IBD[2]. Nevertheless, CD and UC show discrete risk factors and dissimilar gene and protein expressions, which manifest distinctive pathophysiological mechanisms. CD exhibits a transmural inflammatory response and can be associated with granulomas, whereas UC usually shows mucosa-confined inflammation[2-8]. Genomic technologies are now being used to separate the effects of different susceptibility genes in the two diseases. For example, Wu *et al*[6] have studied 36 expression profiles of colonoscopic pinch biopsies from CD and UC patients. Affected genes, mostly related to interferon (IFN)-γ inducible T helper cell 1 (TH1) process and antigen presentation in CD patients, were differentially regulated, with the upregulation of 47 genes and downregulation of 30 genes. In contrast, the expression of genes from UC biopsies showed upregulation of 51 genes and downregulation of 81 other genes,

associated with biosynthesis, metabolism and electrolyte transport[6].

The common conventional medications currently in use to treat both CD and UC involve 5-aminosalicylic acid drugs, corticosteroids, immunosuppressive agents, biologic therapies and antibiotics[9], with a customary "step up" approach of starting with aminosalicylates and rising to corticosteroids and immunosuppressive agents in response to the persisting conditions of the disease. The more effective biological therapies are usually considered as a last option and only in case of refractory diseases, because their systemic action in the host often leads to adverse effects[10,11].

Nanomedicines are precise therapeutics established with the aid of nanotechnology to treat diseases at the molecular level[12]. The application of nanotechnology in medicine can be termed as nanomedicine. It is an evolving face of medicine that uses nanoparticulate carriers to deliver therapeutics targeted to specific cells, or constituents of cells or tissues. Studies have shown nanomedicines to be more beneficial than conventional medications, because their size leads to more effecting targeting, better availability at diseased tissues, and decreased adverse effects. Moreover, nanomedicines have been found to have similar or even better therapeutic impacts at lower drug concentrations than their conventional counterparts[12]. However, although the arena of nanomedicine appears to be encouraging for IBD therapy, concerns related to the impact of the nature of nanoparticles due to their size, shape, aggregation potential, and surface chemistry on the IBD gut need to be scrutinized[12,13], and investigations on the impact of nanomedicine in IBD therapy is currently in early stages. As targeted drug or biological delivery to sites of inflammation remains a crucial challenge in the current treatment of IBD[14], nanostrategies involving short interfering RNAs (siRNAs), antisense oligonucleotides, nanomedicines delivered to the sites of malfunction in IBD can be a valuable therapeutic approach.

The RNA interference technique, notable for specificity, can be speculated to regulate the expression of proinflammatory cytokines and genes related to IBD at the mRNA level[15]. As the impact of noncoding RNAs and RNA silencing in gene modulation is known to be great[16], the use of siRNAs as drugs to silence proinflammatory genes is being scrutinized in various animal models of IBD. This strategy also reduces the chances of immune reactions usually associated with viral vectors[15,17,18]. Due to the potential importance of targeted therapy in IBD, this review is presented to explore the advancements in the prospects of nanomedicine in the modulation of gene expression and targeted therapeutics in IBD (Figure 1).

GENE AND PROTEIN MODULATIONS WITH NANO PROSPECTS

Amongst the key genes involved in IBD pathogenesis,

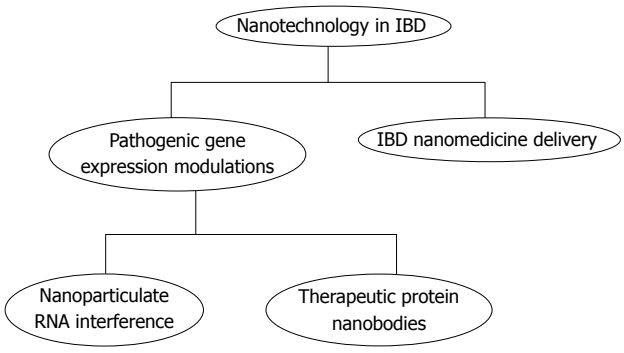

Figure 1 Graphical representation of nanoinvestigations in inflammatory bowel diseases models. IBD: Inflammatory bowel disease.

the function of tumor necrosis factor (TNF)-α in the mediation of inflammation in IBD is extensively acknowledged. Therefore, many biological therapies comprising monoclonal antibodies or soluble receptors are intended to reduce TNF-α activity, and have been extensively tested in many clinical trials[19-24]. However, there are adverse side effects due to the systemic depletion of TNF-α. These adverse effects involve amplified infusion reactions, immunosuppression, opportunistic infections and decreased efficacy of the biologics due to antibody formation against them[24-26].

The gene silencing nanostrategy, in which orally delivered TNF-α siRNA is encapsulated in thioketal nanoparticles (TKNs) made from the polymer poly-PPADT (1, 4-phenyleneacetone dimethylene thioketal), effectively decreases the levels of TNF-α mRNA levels at sites of intestinal inflammation in dextran sulfate sodium (DSS)-induced mouse models of UC. In this study, the site specific delivery of siRNA was made possible due to the ability of TKNs to degrade in the presence of higher levels of reactive oxygen species (ROS) present in regions of inflammation in the intestinal tissue[27]. In another study, TNF-α siRNA/polyethyleneimine (PEI) nanocomplex was shown to inhibit TNF-α secretion by macrophages *in vitro*, whereas the oral administration of TNF-α siRNA/PEI nanocomplexes in lipopolysaccharides (LPS)-treated mice models was found to reduce specifically the synthesis and secretion of TNF-α in the colon[28]. Nanoparticles in a microsphere oral system (Ni-MOS), comprised of TNF-α siRNA entrapped in type B gelatin enclosed in poly(ε-caprolactone) (PCL) microspheres, were found to exhibit favorable gene silencing in the colon tissues of DSS-treated murine models of UC. This treatment results in the suppression of proinflammatory cytokines such as interleukin (IL)-1β, IFN-γ, chemokine monocyte chemoattractant protein (MCP)-1, permitting an increase in body weight and diminished action of tissue myeloperoxidase in mouse models[29]. A protein modulation nanostrategy involving monovalent and bivalent murine TNF-α neutralizing nanobody proteins has been investigated in DSS-induced murine chronic colitis models. *Lactococcus lactis* engineered to produce the therapeutic nanobodies was orally administered, which

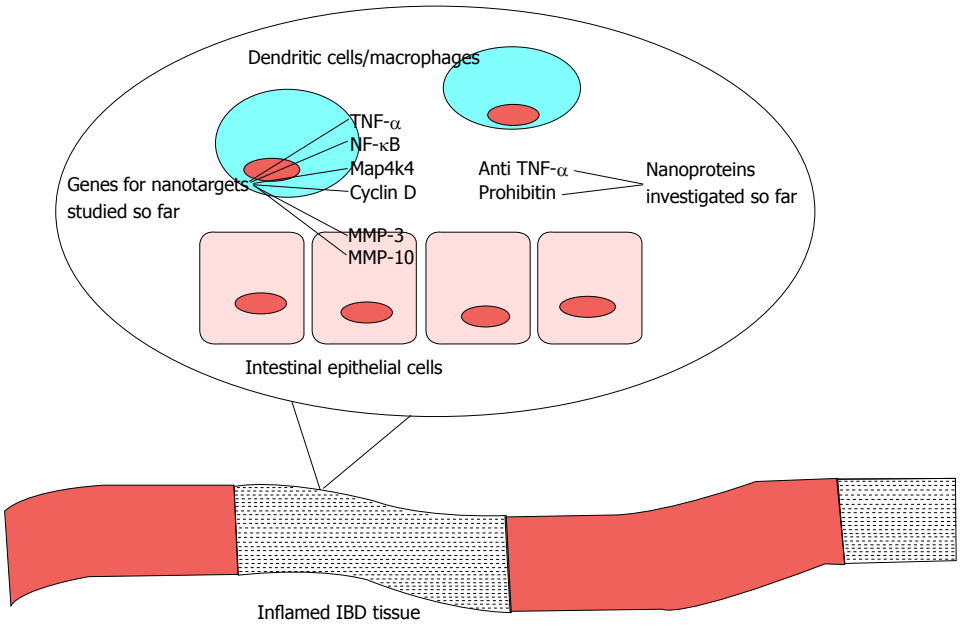

Figure 2 Nanomodulations whose efficacy has been validated in animal models of inflammatory bowel diseases. Genes regulated therapeutically by nano gene silencing in intestinal tissues and macrophages and protein nanobodies that have been investigated to have therapeutic impacts to help control inflammation and tissue destruction in animal models relevant to inflammatory bowel diseases (IBDs). TNF: Tumor necrosis factor; Map4k4: Mitogen-activated protein kinase kinase kinase kinase 4; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor kappa B.

resulted in a significant reduction in the TNF-α driven inflammation in the mucosa of the colon in mouse models, without affecting considerable TNF-α levels in the systemic circulation[30].

Increased TNF-α suppresses the expression of the anti-inflammatory protein prohibitin (PHB) in IBD[31,32], therefore, a study by Theiss *et al*[33] considered the oral delivery of PHB entrapped in poly (lactic acid) nanoparticles in mouse models of DSS-induced colitis. This strategy inhibited the TNF-α-induced nuclear factor (NF)-κB activation; consequently curtailing inflammatory reactions and reducing the severity of colitis. Double-stranded decoy oligonucleotides (ODNs) against the proinflammatory NF-κB gene were enclosed in chitosan-modified poly (D,L-lactide-co-glycolide) nanospheres (CS-PLGA NSs) and delivered orally to DSS-induced murine colitis models. This study showed the absorption of the ODN-CS-PLGA NSs in inflamed mucosal regions, producing considerable curative effects on DSS-induced diarrhea, bloody feces, shortening of colon length, and myeloperoxidase activity[34].

Besides directly inhibiting the TNF-α gene in macrophages, macrophages more generally play a role in inducing the pathogenic inflammatory reactions[35]. This study has revealed the importance of mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4) gene in macrophages in mediating the production of inflammatory cytokines. Map4k4 siRNA encapsulated in β1,3-D-glucan shells silenced Map4k4 expression *in vivo* in mice treated with LPS, protecting them from LPS-induced systemic inflammation by suppressing the production of TNF-α and IL-1β[35].

Matrix metalloproteinases (MMPs) play a vital role in tissue remodeling by regulating the intestinal tissue architecture during the inflammatory reactions and wound healing in IBD[36,37]. Studies have indicated the increased expression of MMP-3 (stromelysin-1) and MMP-10 (stromelysin-2) in causing enhanced tissue injury in DSS-induced murine colitis[38,39]. Furthermore, IBD patients have shown increased MMP-3 and MMP-10 expression in the gut and intestinal ulcer tissues[39-42]. Polymorphisms in various MMP genes may be susceptibility factors for IBD risk, at least in some populations[43]. A study by Kobayashi *et al*[39] demonstrated the specific inhibition of MMP-3 and MMP-10 by siRNA targeted against MMP-3 and MMP-10, having a therapeutic benefit in protecting the colon tissue and reducing the severity of colitis in DSS-treated murine models, which could therefore be a valuable gene silencing option to prevent intestinal damage in IBD (Figure 2).

Cyclin D1 (CyD1) is a cell cycle regulatory protein that is upregulated in IBD in both epithelial and immune cells[44]. A leukocyte-directed siRNA against CyD1 mRNA inhibits the intestinal inflammatory responses in murine models of DSS-induced colitis. Silencing the CyD1 gene decreases the induction of TH1 cell inflammatory cytokines TNF-α and IL-12, but has no impact on the production of TH2 cell cytokine IL-10[45]. Therapeutic efforts to enhance the action of the anti-inflammatory cytokine IL-10, which is known to be critically involved in maintaining mucosal immune balance due to its potent impact on immunosuppression[46] and involvement in CD pathogenesis[47,48], have been largely unsuccessful to date. This is thought to be due to the adverse side effects caused by systemic action of the IL-10 therapies, and the low concentrations of IL-10 delivered to the intestinal tissues[49]. Therefore, biologics intend-

ing to enhance cytokine IL-10 action have been dropped from the current IBD therapies[50]. However, because the involvement of IL-10 and its genetic variations in IBD is great[47,48,51], a consideration of the targeted study by Bhavsar *et al*[52], which involved the nanodelivery of IL-10-producing plasmid to the mucosa in murine models mimicking IBD intestinal epithelial pathogenesis[53] can be scrutinized. According to this study, trinitrobenzene sulfonic acid (TNBS)-induced acute colitis models in Balb/c mice were treated with NiMOS intended for oral gene therapy. This comprised the pORF5-mIL-10 plasmid DNA encapsulated in type B gelatin nanoparticles in PCL. This strategy directed the local transfection of IL-10 plasmid in inflamed intestinal tissues and caused its enhanced expression, which led to suppression of predominant proinflammatory cytokines such as IFN-γ, TNF-α, IL-1α, IL-1β and IL-12, consequently causing the therapeutic benefits of restored colon length and weight, increased body weight, and beneficial clinical activity score[52].

IMPLICATIONS OF IBD DRUGS GOING NANO

Nanomedicines comprising IBD drugs loaded onto nanoparticles, designed to cope and act in accordance with the pathophysiological changes in the intestinal tissues of IBD, can be an intelligent mode of targeted drug delivery. This approach offers the possibility of eliminating undesirable side effects usually caused by systemic action of the drugs[14]. The usual pathophysiological conditions related to inflamed intestinal tissues in IBD predominantly involve abnormal intestinal permeability, increased presence of immune cells, and higher levels of mucus production[54-56].

Cellular interaction of nanoparticles in the IBD gut

Nanomedicines in IBD can potentially be more efficient in their mechanism due to the cellular intake of the nanoparticles by the cells at the targeted sites of delivery. This means that they are not eliminated from the intestinal tract by diarrhea, as are many current conventional medications. This is an important IBD symptom[57,58]. Nanoparticles in the gastrointestinal tract are usually found to be adsorbed either by paracellular transport or endocytosis by regular epithelial cells[59]. Specialized differentiated epithelial cells called M cells, which form major populations of Peyer's patches are involved in the predominant uptake of nanoparticles through transcytosis[60,61]. Predominant CD mutations such as R702W, G908R and 3020insC have been associated with ileal-specific disease[62,63], which show an enhanced presence in Peyer's patches and M cells, which may cause an increase in the uptake of dietary and nanoparticulate substances[13]. In addition to these, translocation of nanoparticles in the intestinal tract can also occur by persorption through gaps or holes at the villous tips[64,65]. Cells involve the autophagic mechanism to cause the clearance of nanoparticles[66], and Powell *et al*[13] have indicated that mutations in the autophagy gene *Atg16L1* in IBD subjects can be susceptible to possible alterations in the clearance of nanoparticles.

Investigations of IBD drugs in nanomodes

Furthermore, IBD drugs delivered in nanomodes have been shown to have greater therapeutic impacts as compared to their conventional delivery studied in animal models, For example, the anti-inflammatory IBD drug mesalamine (5-ASA) covalently linked to the PCL nanoparticles was found to be 60 times more efficient as a nanomedicine at much lower doses (0.5 mg/kg) than the free solution of 5-ASA (30 mg/kg) in treating TNBS-induced colitis in BALB/c murine models[67]. Moulari *et al*[68] have established that silicon nanoparticles have a sixfold increased ability to adhere to inflamed tissues when compared to tissues in healthy controls. In this study, 5-ASA loaded in its methylated form in silicon nanoparticles was shown to collect in inflamed regions in TNBS-induced murine colitis models, inducing a positive impact on clinical activity score and myeloperoxidase activity at reduced drug doses, as compared to conventional delivery[68]. An immunosuppressive drug tacrolimus, used to treat UC, was encapsulated in polylactic-co-glycolic acid (PLGA) nanoparticles and used to treat murine models of TNBS- and oxazolone-induced colitis. This study showed that nanomedicine had an augmented and specific action with a threefold increased penetration in inflamed tissues when compared to healthy tissues[69]. Also, tacrolimus-loaded PLGA nanoparticles and tacrolimus-loaded pH-sensitive Eudragit P-4135F nanoparticles showed diminished side effects in DSS-induced murine colitis models when compared to the free tacrolimus which causes nephrotoxicity in traditional delivery[70]. An anti-inflammatory tripeptide Lys-Pro-Val (KPV) loaded into polylactide (PLA) nanoparticles delivered in combination with a polysaccharide hydrogel had a similar anti-inflammatory effect at 12 000-fold lower doses (25.2 ng/d) to that of KPV in free solution (200 μg/d), thus demonstrating the greater therapeutic efficiency of the nanomode of the drug in treating DSS-induced colitis in murine models[71]. Nakase *et al*[72] demonstrated that dexamethasone, encapsulated in poly-DL-lactic acid (PDLLA) microspheres was more effective in ameliorating DSS-induced murine colitis when compared to the free solution of the same drug, because the microsphere form was engulfed by the immune cells in the inflamed colonic tissue, which resulted in increased efficiency of the drug in mouse models. An *ex vivo* study by Serpe *et al*[73] showed solid lipid nanoparticles (SLNs) comprising the anti-inflammatory molecule cholesteryl butyrate (chol-but) showed a greater impact than butyrate alone in significantly reducing proinflammatory cytokines such as IL-1β and TNF-α and increasing IL-10 production in whole blood *ex vivo* models of peripheral blood mononuclear cells (PBMCs) obtained from IBD patients taking no anti-inflammatory medications. Furthermore, this study demonstrated SLNs, consisting of the immunosuppres-

Table 1 Comparison of therapeutic parameters

Experimental system	Drug in nano mode	Comparison of differences in certain distinct therapeutic parameters		Ref.
		Nano mode	Controls	
In vivo TNBS-induced murine colitis	5-ASA covalently linked to PCL nanoparticles	Myeloperoxidase (MPO) activity of 5-ASA-NP at 0.5 mg/kg: 15.2 ± 5.6 U/mg	MPO activity of 5-ASA free solution at 30 mg/kg: 16.2 ± 3.6 U/mg	[67]
In vivo TNBS-induced murine colitis	5-ASA in silicon nanoparticles	MPO activity of 5-ASA-Si NP at 25 mg/kg: 5.2 ± 2.4 U/mg	MPO activity of 5-ASA-free solution at 100 mg/kg: 8.2 ± 3.4 U/mg	[68]
In vivo TNBS-induced murine colitis/oxazolone-induced murine colitis	Tacrolimus in PLGA NPs	Enhanced penetration into the inflamed tissue-FK506-NP, 105 ± 24 nmol/cm^2	Healthy tissue penetration-FK506-NP, 51 ± 13 nmol/cm^2	[69]
In vivo DSS- induced murine colitis	Tacrolimus in PLGA/or pH sensitive Eudragit P-4135F NPs	Diminished side effects	Increased susceptibility to nephrotoxicity	[70]
In vivo DSS-induced murine colitis	Anti-inflammatory tripeptide KPV in PLA NPs	Nanomode with lowered doses at 25.2 ng/d	Free solution has the similar anti-inflammatory impact at 200 μg/d	[71]
Whole blood *ex vivo* models of PBMCs	Dexamethasone in SLNs	90% reduction in proinflammatory cytokines IL-1β and TNF-α	25% reduction in TNF-α by the free solution	[73]
Whole blood *ex vivo* models of PBMCs	chol-but in SLNs	Significant decrease in IL-1β, and TNF-α increase in IL-10	-	[73]

NP: Nanoparticle; TNBS: Trinitrobenzene sulfonic acid; DSS: Dextran sulfate sodium; PBMCs: Peripheral blood mononuclear cells; 5-ASA: Mesalamine; KPV: Lys-Pro-Val; PLA: Polylactide; SLNs: Solid lipid nanoparticles; IL: Interleukin; TNF: Tumor necrosis factor; MPO: Myeloperoxidase; PLGA: Polylactic-co-glycolic acid.

sive corticosteroid dexamethasone, suppressed TNF-α by 90% when the free solution of dexamethasone showed a TNF-α suppression of 25% at the highest concentrations in similar whole blood IBD *ex vivo* models. These studies provide preliminary support for the effects of SLNs chol-but and SLN dexamethasone in inducing an enhanced anti-inflammatory activity, due to the more effective cellular intake of the nanodrug forms, as compared to the free drugs in solution (Table 1).

LIKELY CONCERNS OF GOING NANO

The general concern associated with the nano approach is due to the fact that nano-sized materials display altered physicochemical properties[74] as compared to their larger counterparts, with chances of causing possible toxicity, since nonbiological nanoparticulate carriers above a particle size of 100-200 nm can alter normal cellular activity, because they can invoke cell membrane ruffling, cytoskeletal rearrangement and stimulate endocytic machinery causing their ingression in phagocytic cells[75]. However, reliable data on the adverse impacts of nanomedicine in IBD is unavailable, whereas the impact of nanoparticles themselves in the gastrointestinal tract might vary according to the nanoparticle polymer material and nanoparticle size, as surface interactions and surface chemistry differ for different nanoparticle sizes. Also, different nanoparticle sizes can cause different mechanisms of cellular uptake, due to which, nanoparticle sizes can be modulated to cause different intracellular effects[13,76,77]. The studies on the effects of nanoparticles themselves in the human gastrointestinal tract in IBD have been limited and need to be explored further.

Although nanostrategies for IBD therapeutics investigated in animal and *in vitro* models of IBD have shown promise, it is still only the dawn of the era of interest in IBD nanomedicine, and there is a definite need for further extensive investigations on many issues related to the safety and uptake of the different nanomedical therapeutics acting on various pathways and phases in the human gastrointestinal tract. It is essential to be confident of their consequent impact on immune responses and therapeutic effects in the different genotypic populations, before recommending the clinical use of nanomedicines to treat IBD in humans.

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