Nanotechnology in the Treatment of Inflammatory Bowel Disease

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Due to the lack of cure for inflammatory bowel disease (IBD) and failure of current medical therapies in many patients with IBD, a need exists in finding novel ways to treat inflammation with a high benefit and the lowest risk possible. With current medical therapies, adverse events or risks of cancer/lymphoma and infections prevent patients—and sometimes providers—in using effective therapies for treatment. Some patients develop systemic side effects that preclude them from continuing a therapy that may have been efficacious, or in other cases, current medical therapies are not adequate to control disease.

Nanotechnology is an emerging field where particles, in the size of nanometers, can be used to deliver medications directly to the area of inflammation thus avoiding drug-associated systemic side effects. When using nanoparticles (NPs), only a small amount of the drug is needed, and it can be delivered directly to the inflamed site without exposure to the rest of the body. Here we review conventional and unconventional therapies applied in the treatment of IBD underlying how the introduction of NPs has improved their safety and efficacy.

Key Words: inflammatory bowel disease, nanoparticles, leukosomes, drug delivery, treatment

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic, chronic, relapsing, and remitting disorder that affects the gastrointestinal (GI) tract. The 2 major forms of IBD are ulcerative colitis (UC) and Crohn’s disease (CD), both characterized by alternating periods of relapse and remission.1 Ulcerative colitis typically involves the rectum and may affect part of the colon or the entire colon in a contiguous pattern, with the inflammation confined to the mucosal layer. Conversely, CD can affect any part of the GI tract in a noncontiguous pattern but most commonly involves the ileum and perianal regions, causing a transmural inflammation.2 Even though the etiology of IBD is still unknown, data suggest that it can be multifactorial to include genetic susceptibility, immune dysfunction, environmental stress, and alterations in the microbiota.3,4,5

To date, there is no cure for IBD, and therapy is aimed to achieve and maintain remission from inflammatory episodes. Inflammatory bowel disease treatment currently consists of anti-inflammatory agents such as 5-aminosalicylates (5-ASAs), corticosteroids, immunosuppressants, and biologic agents such as tumor necrosis factor alpha (TNF-α) antagonists, anti-interleukins, and anti-integrins.6 These drugs can induce and maintain remission; however, they are endowed with serious side effects including increased risk of infections and certain cancers.7 In addition, patients must continue drug administration for the long-term to prevent relapse of the disease. Discovering a treatment that can be delivered and localized to the inflamed tissues, avoiding systemic side effects, would prove beneficial for IBD patients. Nanopharmacology represents an avenue to achieve selective delivery of therapeutics to diseased sites using nanocarriers or nanoparticles (NPs).8

This review summarizes conventional and unconventional therapies applied in the treatment of IBD underlying how the introduction of NPs has improved their safety and efficacy profiles by providing local drug delivery, increasing drug concentration, and avoiding systemic side effects. See Table 1.

NANOPARTICLE PROPERTIES

Nanoparticles are small particles, with a conventional size of less than 100 nm in diameter,9 usually comprising three layers: surface, shell, and core. Most NPs are biodegradable and nontoxic for the human body, thus avoiding side effects. Within the core, NPs can be loaded with the interested drugs, which will be locally released into a target cell thus allowing the administration of lower drug doses. In this manner, NP treatment maintains the same effectiveness as the conventional formulation but reduces the side effects of free drug administration. Nanoparticles can be natural (phospholipids, lipids, lactic acid, chitosan) or have chemical compounds (polymers,
carbon, silica and metals). Properly created NPs should remain present in the blood circulation and have reduced interaction with the reticuloendothelial system (RES). Indeed, sequestration by RES can affect NP blood circulation, thus limiting their capability to reach the target tissues. In addition, NPs have to avoid macrophage phagocytosis and sequestration mediated by filtering organs (ie, liver, spleen, and kidney). To develop the most effective NPs, researchers have proposed several adjustments to the canonical formulation of NPs, altering size, zeta potential, composition, shape, or surface. In particular in IBD-treatment application, the major challenge for NPs is to arrive at the gastrointestinal mucosa and penetrate it to target the underlying inflammatory cells (see Fig. 1). Due to the biochemical and physiological characteristics of the gastric mucosa, one of the most efficient modifications is the addition on the NP surface of poly-ethylene-glycol (PEG), a hydrophilic polymer. The presence of PEG reduces serum protein binding, limits macrophage uptake, and facilitates NP penetration through the mucus layer of the gastrointestinal mucosa. Other polymers are commonly used in the development of NPs. These can be of natural or synthetic origin; in both cases, they should be biodegradable, and their degradation products should be nontoxic for the human body. The natural polymers are proteins (gelatin, albumin) or polysaccharides (alginate, chitosan, pectin), the latter having the advantage of being degraded by colonic bacteria. Synthetic polymers, unlike natural polymers, are easily modifiable in size and weight and are poly(D,L-lactide) (PLA), poly(glycolide) (PGA), and poly(lactide-co-glycolide) (PLGA), which degrade into lactic acid and glycolic acid in the body or poly-ε-caprolactone (PCL), a synthetic polymer degraded by lipase.

Surface decoration can also be performed with methylcellulose, which, thanks to its muco-adhesive properties, increases the contact of NPs with the mucosa. Alternatively, pH-sensitive polymers (ie, Eudragit S100) have been exploited to induce the release of the NP cargo only when it has reached the alkaline pH in the colon, thus avoiding side effects of the drug. Hydrophobicity, porosity, and surface charge are other aspects that affect NP-based therapy success. For example, surface charge impairs NP aggregation and improves their absorption with different affinity between inflamed and healthy colonic mucosa.

**NANOPHARMACOLOGY FOR IBD THERAPIES**

*5-Aminosalicylic Acid (5-ASA)*

The 5-ASA drugs such as sulfasalazine, mesalamines, olsalazine, and balsalazide act as free radical scavengers, inhibitors of leukotriene production and leukocyte recruitment, and inhibitors of nuclear factor-κB (NF-κB) involving the stimulation of receptors that control inflammation and cell proliferation. These drugs are mostly applied in the treatment of mild to moderate disease and to maintain remission in UC. However, 5-ASAs are rapidly absorbed in the small intestine, and usually the amount of drug reaching the colon is minimum. To overcome this issue, nanotechnologists have developed different formulation of NPs loaded with 5-ASAs to confer to the
formulation a controlled and sustained release. Varshosaz et al demonstrated that 5-ASA chitosan NPs were able to deliver 80% of the drug to the colon in an in vitro cellular assay. In vivo studies on 2,4,6-trinitrobenzenesulfonic acid (TNBS)–treated mice revealed that 5-ASA-loaded NPs were mainly localized in the colon with low systemic bioavailability. In addition, these NPs demonstrated an improved efficacy in treating colitis and decreasing the levels of myeloperoxidase (MPO), a marker of leukocyte infiltration. In another study, 5-ASA was loaded into the biodegradable polymer PCL and tested on TNBS-treated mice. Results showed that the 5-ASA/PCL NPs were 60 times more efficient in reducing inflammation at much lower doses.
than free 5-ASA treatment, thus offering an alternative for the
dosing of IBD drugs to avoid medication side effects.19

Due to its safe pharmacological profile, silica represents
a good candidate for drug delivery application. The loading
of 5-ASA within silica NPs (SiNPs) allows the drug to re-
main encapsulated until its selective delivery in the inflamed
colonic tissue. Moulari et al demonstrated that 5-ASA/SiNPs
at 25 or 50 mg/kg induced a higher decrease of MPO activity
than 100 mg/kg of free 5-ASA. Overall, 5-ASA/SiNPs ameli-
orated inflammation at lower doses than what is usually re-
quired with the conventional 5-ASA formulations.20 Recently,
rectal mesalamine was incorporated into the coating agent
hydroxypropyl methylcellulose (HPMC K4M) to provide a
controlled release of the anti-inflammatory drug in the treat-
ment of distal forms of UC. This improved formulation was
tested in acetic acid–treated mice, which had significantly lower
Disease Activity Index scores after treatment compared with the
polymer-free formula-treated mice. Those receiving the HPMC
K4M mesalamine had an intact mucosal lining and mild cel-
lular inflammatory infiltrates in comparison with the polymer-
free formulation where the mucosal lining was eroded with a
moderate inflammatory infiltrate, as demonstrated by histopa-
thology. As a film-former, hydroxypropyl methylcellulose pro-
vided muco-adhesive properties to the formula, increasing the
contact time between mesalamine and the damaged mucosa,
increasing its therapeutic efficacy.21

Corticosteroids

Another class of drugs used in the treatment of IBD are
corticosteroids such as prednisolone, dexamethasone (DX),
and budesonide (BDS). Steroids can modulate the immune
response by binding to glucocorticoid receptors in the cells.
However, their application for long-term use is limited due to
global immunosuppression and other numerous systemic side
effects.

Kshirsagar et al loaded prednisolone with the
pH-sensitive polymer, Eudragit S100. Using this new formu-
lation, they showed both in vitro and in vivo that the NPs
degraded and released the drug only when reaching the alka-
line pH in the colon, thus avoiding side effects of the drug.22
In another study, DX was encapsulated in poly DL-lactic acid
(PDLLA) microspheres and orally administered to dextran sul-
fate sodium (DSS)–treated mice. As a result, the microspheres
specifically target the colon mucosa, thus reducing serum
levels of DX. Treatment with PDLLA-loaded NPs was associ-
ated with a significant reduction of histological score, MPO
activity, and nitric oxide (NO) production compared with the
untreated group and the group receiving DX alone. The ex-
pression of pro-inflammatory cytokines such as TNF-α, inter-
leukin-1 beta (IL-1β), and interferon gamma (IFN-γ) ceased
in the DX microsphere treated group; however, the group re-
ceiving DX alone still had some cytokine expression. In addi-
tion, the colitis score was significantly higher in the non-DX
treated groups than in the DX-treated groups.23 Similar results
were obtained when TNBS-treated mice received DX-PDLLA
NPs. This treatment was associated with a significant decrease
in inflammatory markers (MPO and NO production), histo-
logical scores, and pro-inflammatory cytokines in comparison
with mice receiving DX alone. The study also revealed the
ability of the DX microspheres to suppress NF-κB activation,
inducing a more potent suppression than the one exerted by
DX alone. Overall in TNBS-treated mice, DX microspheres dis-
played a stronger amelioration of mucosal damage than DX
alone formulations.24

One of the most recent studies used spherical polymeric
nanoconstructs (SPNs) to encapsulate DX. These DX-loaded
NPs, administered to DSS-treated mice, specifically accumu-
lated in the inflamed colonic sites and induced a higher de-
creased expression of pro-inflammatory cytokines compared
with free DX.25

To enhance inflamed tissue targeting, DX has been en-
capsulated in Rheum tanguticum polysaccharide (RTP), a
water-soluble fraction extracted from the Chinese remedy
Rhubarb. Rheum tanguticum polysaccharide has the ability to
target mannose receptors and improve the balance of Th1 and
Th2 cell polarization.26 27 The DX-loaded RTP microspheres
administered to TNBS-treated mice repaired the mucosal in-
jury induced by the colitis. These NPs directly targeted the
colon and maintained its inherited anti-inflammatory activity
by inhibiting the upregulation of CD4+ protein expression and
NF-κB activity, but avoided the severe immunosuppressive ef-
effects associated with the systemic administration of free DX.28

Dianzani et al instead took advantage of solid lipid
nanoparticles (SLNs) to enhance the anti-inflammatory ac-
tivity of some drugs. Loading DX in SLNs, the authors
conferred to the NPs a synergistic anti-inflammatory effect.
Indeed, DX-SLNs inhibited IL-1β and TNF-α both in vivo
and in vitro achieving its anti-inflammatory activity at much
lower doses than what is required for each compound sepa-
rately.29 Similar results were obtained encapsulating another
topical corticosteroid, budesonide (BDS), into nanostructured
lipid carriers (NLCs) and testing them on DSS-treated mice.
The BDS-NLC formulation decreased the levels of TNF-
α and interleukin-1 (IL-1), ameliorating inflammation and
maintaining the colonic mucosal architecture. Interestingly,
in LPS-inflamed cells, the levels of TNF-α were reduced when
treated with either empty or BDS-loaded NLCs; no change
was observed with free BDS alone. These results clearly in-
dicate inherited anti-inflammatory and immunomodulatory
properties of the lipid carriers.30

In another study, Leonard et al loaded BDS into PLGA
NPs and tested their effects in an in vitro model in comparison
with BDS-loaded liposomes. Using a 3D cell culture model of
chronic intestinal inflammation, they proved that BDS-PLGA
NPs were able to restore interleukin-8 (IL-8) levels to normal.
Notably, the cells treated with BDS-liposome worsened the
inflammation. Similar adverse outcomes of corticosteroid-loaded NPs were obtained when DX was coupled to liposomes and administered to DSS-treated mice. Even with this formulation, the colonic inflammation got worse, mainly due to an uptake by a different phenotype of macrophages.

Thus, it is important to remember that nanopharmacology is not always foolproof or beneficial. Careful consideration should be taken to find what in fact may be harmful in inflammatory states. These negative findings may even help provide insight in the pathophysiology of inflammation in IBD.

**Immunomodulators**

Immunomodulators have been used as one of the earliest medications for long-term maintenance treatment of IBD. Thanks to their immunosuppression abilities (exploited in transplant patients), these medications provided a longer-term treatment option for corticosteroid-refractory or corticosteroid-dependent IBD patients. However, even for this class of drug, its application is associated with side effects. Finding ways to improve their onset of action, reduce the need for long-term monitoring with frequent laboratory evaluation, and minimize side effects could improve their popularity in IBD treatment for patients.

Tacrolimus (FK506) is an inhibitor of calcineurin, a protein that plays a key role in the regulation of interleukin 2 (IL-2), interleukin 4 (IL-4), and IFN-γ expression and in the modulation of NF-κB activity. As an immunosuppressive drug, it offers promising results in the treatment of IBD, but its numerous systemic side effects such as hypertension, nausea, diarrhea, hematologic abnormalities, and renal impairment significantly limit its application. To avoid these adverse reactions and to ensure a localized colonic delivery of the drug, Lamprecht et al developed Eudragit microspheres loaded with tacrolimus and tested their efficacy on TNBS-treated mice. Microspheres, orally administered, were able to prevent early absorption of the drug, thus releasing the cargo in the upper GI tract to the distal ileum and colon. This specific delivery to the colon mucosa decreased the drug’s systemic absorption, thereby reducing nephrotoxicity.

In a similar study by Lamprecht et al, tacrolimus was entrapped in the polymer PLGA (FK506-NPs) and tested on TNBS and Oxazolone colitis mice. Although it has been demonstrated that the rectal administration of the NPs was better than the oral route in both models, either oral or rectal formulations had a curentine clearance similar to the control group, implying a reduction in nephrotoxicity. In addition, with both administration routes, the drug concentration within the inflamed tissues was 3-fold higher than in healthy tissues, demonstrating the selective targeting of the FK506-NPs for the inflamed areas of the colon.

Azathioprine (AZA), another immunomodulator used in IBD treatment, is a purine synthesis inhibitor that is transformed in the body to its active metabolites 6-mercaptopurine and 6-thioguanosine acid. Despite its immunosuppressive activity against activated T-lymphocytes, AZA treatment is associated with a slow onset of action (often taking 2 to 3 months to reach therapeutic efficacy), the need for frequent medication monitoring with laboratory evaluations, and side effects such as hepatotoxicity, bone marrow suppression, severe allergic reactions such as pancreatitis, and several cancer risks, especially lymphoma. To overcome these limitations, Helmy et al loaded AZA into chitosan beads and administered them to an acetic acid–induced colitis rabbit model. In the free AZA-treated group, the levels of TNF-α and MPO were significantly higher than the control groups, probably due to a delay in the action of the systemic AZA. In contrast, in the AZA/chitosan-treated group, MPO and TNF-α levels decreased to the levels of the healthy group, suggesting a local release of the drug to the inflamed colon, a rapid onset of its immunosuppressant activity, and the restoration of the normal microscopic colonic architectural structure.

Methotrexate (MTX) is another antiproliferative and anti-inflammatory drug that interferes with DNA synthesis inhibiting important enzymes required for the synthesis of purines and pyrimidines, such as dihydrofolate reductase. However, MTX treatment is associated with severe side effects such as leukopenia and hepatotoxicity. To overcome these effects, targeted drug delivery strategy was achieved by coupling MTX to G5 PAMAM dendrimer NPs. The G5-MTX NPs protected mice against DSS-colitis by increasing induced regulatory T cells (iTreg) in mesenteric lymph nodes that control the immune system and maintain tolerance to self-antigens. These results suggest that the G5-MTX NP–induced resistance in DSS-colitis mice is secondary to the increased activity of iTreg compared with naïve mice.

Another recent study incorporated MTX into grapefruit-derived nanovesicles (GDNs), combining the effects of DTX with the antioxidant and anti-inflammatory effects of GDN component (ie, naringenin). The MTX-GDNs were administered to DSS-treated mice. The authors showed a selective intestinal macrophage uptake of the nanovesicle resulting in an induction of heme oxygenase-1 (HO-1) expression and a suppression of IL-1β and TNF-α expression. As proved by histologic analysis, MTX-GDNs significantly decreased MTX toxicity and severity of colitis compared with the group receiving free MTX. The anti-inflammatory effect of MTX was enhanced by GDNs, and MTX side effects were decreased when administered with these nanovesicles. These formulations can constitute a future approach for the safe administration of MTX.

**Biologics & Other Novel Agents**

To date, biologic therapies have changed the course of treating IBD as they have been found to be the most effective available treatment, especially for patients with moderate to severe IBD. For example, TNF-α inhibitors are currently used...
in IBD patients unable to maintain remission of their disease with conventional treatments or for patients that are refractory to or dependent on corticosteroids to control IBD-associated symptoms. However, biologics are also associated with significant systemic adverse effects such as infusion reactions, immunosuppression, opportunistic infections, and antibody formation against themselves that reduce their efficacy.\(^4\) To overcome these side effects, NPs represent a potent tool permitting smaller drug doses to reach the same therapeutic effects by delivering the drugs directly to the inflamed tissue, bypassing systemic toxicity and avoiding adverse reactions.

In a recent study, Vandenbroucke et al engineered *Lactococcus lactis* (L. lactis) to induce the local secretion of monovalent and bivalent murine (m) “nanobodies” (term used by the authors for their nanoparticles) against TNF-α. The authors demonstrated that oral administration of L. lactis to DSS-induced colitis mice was associated with anti-mTNF nanobodies secretion and a significant reduction of inflammation. In addition, results exhibited a 25% of improvement in the mean histological score of the distal colon and a significant decrease in MPO levels of at least 70% when compared with controls. Intriguingly, no measurable levels of the nanobodies in the systemic circulation have been recorded, suggesting this therapy is a promising alternative to systemic biologics, combining the same efficacy as conventional anti-TNF-α therapies with no systemic side effects.\(^3\) To locally neutralize pro-inflammatory cytokines (ie, TNF-α) in IBD treatment, the use of small interfering RNA (siRNA) has been evaluated. Although this technique permits gene expression silencing, it is associated with low penetration across the cell membrane. To overcome this problem, several researchers have developed novel formulation of nanocarriers.\(^6\) Laroui et al encapsulated TNF-α siRNA/polyethyleneimine (PEI) into a PLA nanoparticle further coated with polyvinyl alcohol (PVA). These NPs, orally administered to lipopolysaccharides (LPS)-treated mice, were efficiently phagocytosed by macrophages, resulting in a reduced expression of TNF-α in the colonic tissue and in an amelioration of inflammation. Most importantly, this therapy did not affect the liver levels of TNF-α, suggesting a localized efficacy of the drug in the inflamed colonic mucosa.\(^4\)

Recently, a novel drug delivery system for nucleic acid transportation, called NPs in microsphere oral system (NiMOS), has been developed for the delivery of RNA. It consists of a multicompartmental system of a solid internal phase covered by a solid external layer.\(^4\) To prove the efficacy of this technique, TNF-α-siRNA was encapsulated into gelatin NPs, covered by PCL microspheres and administered to DSS-treated mice. Interestingly, NiMOS treatment was associated with a significant reduction in the expression levels of TNF-α and other pro-inflammatory cytokines (ie, IL-1α, IL-1β, and IFN-γ) and the restoration of healthy epithelium. These results demonstrate the ability of the therapy to successfully ameliorate inflammation.\(^5\) In another study Laroui et al developed a delivery system that avoids the insults of the GI tract and specifically targets inflamed colonic mucosa. In brief, TNF-α siRNA was loaded into PLA/PEG NPs that were grafted to the Fab position of a macrophage-specific ligand (F4/80 Ab) on the surface of the NPs. Compared with the uncovered NPs, in DSS-treated mice the delivery of TNF-α siRNA to macrophages in inflamed sites was more efficient when the NPs were covered with Fab. In addition, treatment with Fab-covered NPs was associated with reduced weight loss and MPO activity.\(^3\)

Tumor necrosis factor alpha inhibition can be achieved also using antisense oligonucleotide therapies (ASOs). These therapies are based on polymers that block the translation of TNF-α into protein.\(^6\) One issue with ASO therapy is their poor stability and low cellular uptake, thus safe and effective carriers are required to improve their delivery. Zuo et al developed a system in which TNF-α ASOs were attached to galactosylated low molecular weight chitosan (gal-LMWC). This new formulation protects DNA from degradation and increases the affinity of galactose for the macrophage receptor MGL, responsible for receptor mediated endocytosis. When administered to TNBS-induced colitic mice, the TNF-α ASO/gal-LMWC successfully delivered the ASOs to the macrophages in the inflamed colonic tissue and induced a higher decrease of TNF-α levels compared with naked ASOs.\(^2\) Antisense oligonucleotide therapies can also be delivered by vectors. The swelling properties of cationic konjac glucomannan (cKGM) transported ASOs against TNF-α. The cKGM swelling released the NPs in the colonic lumen where they were preferentially absorbed by macrophages through phagocytosis, yielding a significant decrease in the expression levels of TNF-α and alleviating colitis in DSS-treated mice.\(^3\)

In the treatment of IBD, a gene silencing technique was also exploited to reduce Cyclin D1 (CyD1) expression. Cyclin D1 is an important cell cycle regulating molecule found to be upregulated in epithelial cells and inflammatory cells in the colon in an experimental model of colitis.\(^8\) Peer et al developed a liposome NP (β, I-tsNPs) loaded with CyD1 siRNA and coated with antibodies directed against β, integrin receptors. The administration of this NP complex to DSS-treated mice decreased CyD1 levels to those of the uninfamed gut, reduced colonic damage, suppressed leukocyte infiltration, and decreased the expression of TNF-α and interleukin 12 (IL-12) without affecting interleukin 10 (IL-10) levels.\(^5\) In another study, the inhibition of CyD1 was coupled with TNF-α inhibition. The siRNA directed against both molecules was encapsulated in NiMOS and administered to DSS-treated mice. Results indicated that the mice receiving the dual siRNA treatment alleviated inflammation and decreased the levels of TNF-α and CyD1.\(^5\)

Interleukin 10 (IL-10) has a key anti-inflammatory role in that it downregulates the expression of pro-inflammatory cytokines in IBD.\(^7\) However, long-term treatment is associated with enormous adverse effects that lead patients to abandon
the therapy. To overcome these issues, Steidler et al used L. lactis as a vector for murine IL-10 and delivered it to DSS-treated mice. The results proved an amelioration of inflammation and prevention of the onset of colitis in IL-10 knockout mice (IL-10−/−). In another study, Nakase et al treated IL-10−/− mice with recombinant mouse IL-10 loaded into gelatin microspheres (GM-IL-10). The study showed that the rectal administration of GM-IL-10 was more efficient in ameliorating colonic inflammation than treatment with IL-10 alone. The GM-IL-10 formulation remained in the colon for a longer period than the other formulations (GM alone, IL-10 alone), providing a sustained release of IL-10 to the inflamed mucosa. Macroscopic evaluation of the colonic tissue in the group treated with GM-IL-10 revealed no thickening of the colonic wall compared with marked wall thickening in the untreated, GM alone, or IL-10 alone groups. Histologic analysis further revealed lower scores in the group treated with GM-IL-10 compared with the other treatment groups. The mRNA expression of IL-12 was upregulated in the colon of nontreated, GM alone and IL-10 alone groups, but IL-12 levels were significantly lower in the group treated with GM-IL-10. In another study, NiMOS formulated using mIL-10 expressing plasmid DNA were encapsulated in gelatin NPs and further entrapped in PCL microspheres. After oral administration to TNBS-treated mice, the local expression if IL-10 suppressed the expression of IFN-γ, TNF-α, IL-1α, IL-1β and IL-12 and certain chemokines. This study also demonstrated the immunomodulatory activities of IL-10.

Nuclear factor-κB is a transcription factor involved in the activation of pro-inflammatory cytokines (ie, IL-1, IL-2, IL-6, IL-8, and TNF-α) and adhesion molecule genes (ie, vascular cell adhesion molecule [VCAM-1], E-selectin, intercellular adhesion molecule-1 [ICAM-1], and mucosal vascular addressin cell adhesion molecule [MadCAM-1]). A recent study developed a decoy therapeutic system encapsulating an oligonucleotides (ODN) against NF-κB in chitosan-PLGA NPs to inhibit the binding of NF-κB to its target genes. The NF-κB decoy ODN/Chitosan-PLGA orally administered to DSS-treated mice showed stability and interaction with the inflamed mucosa leading to a significant attenuation of colitis.

Another molecule studied for its anti-inflammatory potential is prohibitin (PHB), a protein whose production is decreased in IBD patients. Prohibitin regulates colonic epithelial integrity by modulating antioxidants and NF-κB pathway. To evaluate the efficacy of this protein, DSS-treated mice receiving the recombinant prohibitin cDNA encapsulated in hydrogel PLA NPs and then covered with PVA presented higher levels of PHB, which correlated with an attenuation in the severity of colonic inflammation.

Lys-Pro-Val (KPV), a tripeptide that originates from the α-melanocyte–stimulating hormone, has been exploited in IBD treatment for its anti-inflammatory activity. Bound to PLA NPs and encapsulated into a polysaccharide hydrogel vector containing alginate and chitosan, the efficiency of the KPV nanocomplex was evaluated on LPS-treated mice. Under the protection conferred by the capsule, the NPs passed through the upper GI tract without being degraded until reaching the colon, where they proved to be effective in ameliorating inflammation, reducing TNF-α and IL-1β levels with a 12,000-times lower dose than the one used in free KPV solution.

Due to its long-term safety at high doses, curcumin (CC) is one of the most studied natural compounds in medicine. Curcumin is a natural polyphenol extracted from turmeric, endowed with anti-inflammatory, antioxidative, anticancer, and antiangiogenic effects. Indeed, CC has the ability to inhibit NF-κB activation, thus downregulating COX-2 expression along with other pro-inflammatory cytokines such as IL-1β, interleukin 6 (IL-6), and TNF-α, and it has been demonstrated to improve colitis in animal models. Taking advantage of these properties, CC and the anti-inflammatory drug celecoxib—a COX-2 inhibitor—have been combined and encapsulated in Eudragit NPs to evaluate their effectiveness in TNBS-induced colitis mice. Results showed that these NPs resisted gastric acid degradation and localized their delivery to the colon, improving colitis symptoms (diarrhea and body weight loss) at much lower doses than the ones required for each component administered separately. In another study, CC was loaded to PLGA/Eudragit NPs. This formulation increased the release of the compound to the colonic mucosa up to 80%, thus leading to a rapid decrease on the inflammatory cytokine levels and the restoration of the normal colonic architecture.

Another compound recently investigated for the treatment of IBD is raloxifene (Ral), a selective estrogen receptor modulator (SERM) capable of suppressing NF-κB activation. To improve raloxifene’s bioavailability and enhance its anti-inflammatory effect, Greish et al coupled raloxifene with poly (styreneco-maleic acid [SMA]) nanomicelles. In DSS-induced colitis mice treated with either free Ral or SMA-Ral, those on the NP treatment improved animal weight, prevented DSS-induced diarrhea, stimulated colonic tissue recovery after the treatment, and had lower pro-inflammatory cytokines (TNF-α and IL-6) levels compared with the mice treated with free Ral formulation.

Similar results were obtained for vasoactive intestinal peptide (VIP), an endogenous neuropeptide endowed with anti-inflammatory and immunomodulatory properties recently applied as a therapeutic option for IBD patients. However, when systemically administered, VIP can be rapidly degraded, rendering it ineffective. To prevent early degradation and improve VIP bioavailability, Jayawardena et al loaded the peptide into a sterically stabilized micelles (SSMs), a lipid-based nanocarrier. The VIP-SSMs tested on DSS-induced colitis mice had ameliorated inflammation and diarrhea, along with significantly reduced expression of proinflammatory cytokines. This study provides tools necessary to formulate VIP as a possible safe biodegradable therapeutic option for IBD if coupled with SSMs.
Glucagon like peptide-1 (GLP-1), a hormone secreted by enteroendocrine L-cells after food intake, is endowed with immunomodulatory effects such as reduction of the levels of pro-inflammatory cytokines (ie, C-X-C motif chemokine 10 [CXCL-10], signal transducer and activator of transcription 3 [STAT-3], monocyte chemoattractant protein-1 [MCP-1], and TNF-α), inhibition of NF-κB activation, and modulation of natural killer cells in the pancreas, central nervous system, and endothelial cells. A recent study has demonstrated some of the anti-inflammatory properties that GLP-1 exerts over the GI tract by coupling GLP-1 with sterically stabilized phospholipid micelles (GLP-1-SSMs). The GLP-1-SSMs were administered to DSS-treated mice obtaining an amelioration in colonic inflammation and associated diarrhea and a decreased expression of the pro-inflammatory cytokine (IL-1β) without altering blood glucose levels. These results suggest that GLP-1-SSMs localize to the inflamed mucosa without causing any adverse systemic effects and suggest GLP-1 as a possible therapeutic option for patients suffering from IBD-associated diarrhea.

Recently, Pabari et al used infliximab (INF) NPs combined with the biodegradable polymers of polyesterurethane (PU) and its PEGylated (PU-PEG) form to act as nanocarriers to treat inflammation in an epithelial Caco-2 cell monolayer model and in LPS-treated monocytes. It was found that the INF-NPs had an increased cellular uptake and increased permeability in the inflamed epithelium when compared with healthy epithelium. This increased uptake of INF-PU and INF-PU-PEG led to a rapid and complete recovery of the epithelial barrier function in a 24 to 48-hour period. Conversely, the INF treated epithelium had a slower recovery (85% at 24 hours). The authors believe that the rapid recovery in the epithelial barrier is due to the increased cellular uptake of the INF NPs across the inflamed epithelium. These findings were also seen on LPS-treated monocytes, where INF-PU and INF-PU-PEG NPs had higher uptake compared with PCL-PEG NPs. Notably, treated monocytes with INF NPs had a decrease in the secretion of inflammatory cytokines such as TNF-α and IL-8. This study indicates that PU-PEG NPs can function as carriers for INF due to their increased uptake across the inflamed tissue and can decrease secretion of proinflammatory cytokines thereby restoring epithelial barrier function, which can play a vital role in treating IBD.

NANOTECHNOLOGY LIMITATIONS

Nanotechnology offers innovative therapeutic options for IBD due to the increased effectiveness of the NPs and specificity accumulating in injured tissue. Despite its many benefits, none of the NPs have been approved for clinical use, and like other conventional treatments, NPs come with their limitations. Nanoparticles have a complex formulation. They require encapsulation and surface modifications for their functionality. As such their manufacturing process is highly complex. Moreover, NPs must be formulated in a way that they can be suitable for human administration. The studies performed here are in animals, and consequently the size and dosage of the NPs being administered may differ.

The side effects and toxicity caused by NPs in human cells have not been extensively evaluated. Nanoparticle stability and interaction with the GI tract has been tested in animals, but their interaction in the human gut of IBD patients is still to be evaluated.

Most of the studies mentioned use antigen presenting cells (APCs) as a target for NPs to deliver their cargo. This is explained by the natural biodistribution of the NPs and their interaction with cells from the reticuloendothelial system. The interaction between APCs and NPs can modify further immunological pathways and serve as a link between the innate and adaptive immune system. Therefore, APCs are considered excellent targets for NPs because they can trigger responses in both systems. Lymphocytes are also an important part of IBD pathophysiology, so it is expected to have NPs that can target them, as well. However, one of the major limitations with lymphocytes is that they are very difficult to target in vivo, making APCs a more attractive target. An important study evaluated the ability of T cells to directly induce apoptosis in IBD. Atreya et al revealed that anti-TNF antibodies can induce apoptosis of CD4 T cells in IBD patients, when the T cells were cocultured with CD14 macrophages. No induction of apoptosis was seen when the T cells were cultured alone. Authors believe this was likely due to the low expression of membrane-bound TNF (m-TNF) on CD4 T cells, which have higher m-TNF expression in IBD. Through this pathway, macrophages indirectly induce apoptosis of CD4 T cells. With this knowledge, it would be logical for NPs to target macrophages, as they are able to indirectly induce apoptosis efficiently.

Finally, we must consider the possibility of translating these studies performed on animals to humans. Animal models have been used to represent the pathophysiology of IBD, but there is no exact model that mimics UC or CD completely. When interpreting these results, one must consider the different models used that may represent different types, stages, and severity of IBD. For instance in one study, it was found that cyclosporine was able to ameliorate colitis in an acute DSS colitis model, whereas no effect was seen in a transfer colitis model. Therefore, the translation of findings from animal models to humans is very difficult and varies according on the animal model that was used.

CONCLUSION

Nanopharmacology offers a promising future for medicine, providing the opportunity to develop molecules or carriers that can target agents to their site of activity. This can help treat medical conditions such as IBD, where certain medications have limited applications due to inherent side effects. Because this is an evolving science, more studies are required to identify the NPs’ pharmacokinetics, therapeutic efficacy, and safety in
humans. The studies presented so far encourage researchers to continue to identify and experiment with possible drug alternatives. Moreover, these studies can serve as a tool for future studies in humans, with the potential to safely treat a broader range of diseases by targeting their exact location of disease and avoiding systemic toxicity and side effects.

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