Small therapeutic molecules for the treatment of inflammatory bowel disease

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New therapies for inflammatory bowel disease are needed, because standard therapies fail to induce remission in about 30% of patients, and because of the relative inefficacy of current maintenance therapies. This review summarises the current status of the development of small therapeutic molecules for inflammatory bowel disease.

Until the introduction of anti-inflammatory therapies in the midle of the last century, inflammatory bowel disease was a potentially lethal disorder that could only be treated by surgery. The discovery of the therapeutic efficacy of salazopyrine and corticosteroids for ulcerative colitis and subsequently Crohn’s disease has historically changed the prognosis of patients with inflammatory bowel disease, and the life expectancy of patients with ulcerative colitis and Crohn’s disease is now similar to healthy subjects. Immunosuppressive drugs, in particular azathioprine (6-mercaptopurine) and methotrexate are effective for remission induction and maintenance of Crohn’s disease; azathioprine is also used for remission maintenance of ulcerative colitis. With the exception of cyclosporine, which has no efficacy in Crohn’s disease, and limited efficacy in severe ulcerative colitis, with the exception of variations on the corticosteroid/salazopyrine theme, no effective small molecules have been developed for the treatment of inflammatory bowel disease in the past 50 years. Recently, “biologics” (monoclonal antibodies, therapeutic peptides, antisense oligonucleotides) have attracted significant interest as novel anti-inflammatory or immunomodulating approaches in inflammatory bowel disease, and at least one such approach (a tumour necrosis factor α (TNFα) binding monoclonal antibody) has been a breakthrough in the treatment of therapy refractory Crohn’s disease. However, compared to small molecules, “biologics” have certain disadvantages, including the restriction to non-oral routes of administration, immunogenicity, and high cost. Moreover, new therapies for inflammatory bowel disease are still needed, because standard therapies fail to induce remission in about 30% of patients, and because of the relative inefficacy of current maintenance therapies. In this paper, the current status of the development of small therapeutic molecules for inflammatory bowel disease is reviewed.

EICOSANOIDS

The search for new targets for anti-inflammatory therapies in inflammatory bowel disease was initiated by the characterisation of the production of specific eicosanoids in the inflamed mucosal in the late 1970s. It soon became apparent that certain prostaglandins produced by the inflamed mucosa, in particular prostaglandin E2, had anti-inflammatory activities; this explained the harmful effects of non-steroidal anti-inflammatory drugs (NSAIDs) in inflammatory bowel disease.

Inducible cyclooxygenase (COX) has been implicated in the maintenance of mucosal tolerance, which suggests that COX-2 specific NSAIDS may also be contraindicated in inflammatory bowel disease.

Using rectal dialysis as a tool to measure mucosal eicosanoid production, it was shown that administration of indomethacin and corticosteroids promptly reduced the production of prostaglandins, but only corticosteroid administration reduced the rectal dialysate leukotriene B4 concentration. Leukotriene B4 is a potent inflammatory mediator and activates neutrophils at low concentrations; this finding suggested that leukotrienes, but not prostaglandins, were proinflammatory in ulcerative colitis. This hypothesis received further support from studies which indicated that sulphasalazine and 5-aminosalicylic acid also inhibit leukotriene (LT) production. Indeed, a specific benzothiophene hydroxyurea 5-lipoxygenase inhibitor, zileuton, reduced LTβ4 production, neutrophil influx, and mucosal injury in several animal models of inflammatory bowel disease.

Zileuton also inhibited LTβ4 production in the human inflamed colon, and its ability to maintain remission (compared to placebo and mesalazine) in patients with ulcerative colitis was subsequently investigated. This study confirmed that mesalazine was superior to placebo in remission maintenance in ulcerative colitis, but failed to show that zileuton was better than placebo. As a result, further development of zileuton (or any other 5-lipoxygenase inhibitor) for inflammatory bowel disease was discontinued.

Ridogrel is an oral inhibitor of thromboxane synthase, as well as a thromboxane receptor blocker.
antagonist, that was initially developed as an antagonist of blood platelet aggregation. Treatment of ulcerative colitis patients with ridogrel resulted in a reduction of the mucosal production of thromboxane A2, but prostaglandin E release was not affected. Unfortunately, ridogrel did not decrease mucosal production of interleukin 6 (IL-6) and TNFα, and the disappointing results of (unpublished) controlled clinical trials led to the discontinuation of further development for treatment of inflammatory bowel diseases.

Platelet activating factor (PAF) is a potent stimulator of neutrophils and endothelial cells (being intimately involved in neutrophil migration through endothelial monolayers), and PAF and TNFα reciprocally control leukocyte migration across intestinal epithelial barriers. The impaired motility and dilatation that is the hallmark of muscularis propria has been implicated as a mechanism for increased. A potential role of PAF as mediator of mucosal inflammation was suggested by studies that showed increased mucosal PAF production in experimental inflammatory bowel disease and in ulcerative colitis. Subsequently, several PAF antagonists were shown to prevent mucosal damage in various animal models of mucosal inflammatory disease. However, an (unpublished) controlled clinical trial failed to show a therapeutic effect of intravenous administration of BB 882 (a potent PAF antagonist) in fulminant ulcerative colitis. A molecule that is composed of a PAF antagonist linked to 5-acetylsalicylic acid is currently being developed for inflammatory bowel disease.

Taken together, these data may be interpreted to indicate that eicosanoids (leukotrienes, PAF, thromboxane A2) do not represent useful targets for therapies of inflammatory bowel disease. This conclusion should be made with some caution, because several of the negative studies have not been published in detail, and because some of the inhibitors used (zileuton, ridogrel) incompletely inhibited the production of the target eicosanoid.

**NITRIC OXIDE**

In active ulcerative colitis and Crohn’s disease the intestinal mucosal production of nitric oxide (NO) is greatly increased. NO production in the inflamed mucosa has several cellular sources, and increased NO production in the muscularis propria has been implicated as a mechanism for mucosal re-epithelialization. Excessive production of NO may cause inflammation through formation of intermediaries such as peroxynitrite, and in rats intrarectal instillation of peroxynitrite caused mucosal inflammation. Trinitro-benzene-sulphonic acid (TNBS) induced colitis in rats is characterised by greatly increased NO production, and in this model interference with NO production by oral administration of L-NAME had protective effects. However, it well known that NO also has protective activities in TNBS induced colitis, and pretreatment with L-NAME before induction of colitis, increased mucosal damage. Similar observations were reported in HLA-B27/human β₂ microglobulin transgenic rats, and conflicting data have been published concerning efficacy of the NO blocker aminoguanidine in TNBS induced colitis. An NO releasing preparation of mesalazine was superior to mesalazine in a rat model of colitis, and inhibited neutrophil adherence and the production of IL-1 and interferon γ (IFNγ). In spontaneously occuring colitis in Rhesus macaques, administration of various blockers of inducible NO synthase did not alter the clinical severity.

In conclusion, NO has many physiological functions in the human gut, and the production of NO by intestinal epithelial cells via inducible NO synthase is often observed in the non-diseased bowel. At high concentrations, NO may cause damage to the intestinal mucosal, through the formation of intermediaries such as peroxynitrite. It is extremely difficult to design a therapeutic strategy that would target only excessive NO production, and both protective and harmful effects of NO blockade have been observed in animal models. For this reason, it is not likely that currently available NO blockers will be effective in inflammatory bowel disease, but no clinical data have been reported.

**PPARα**

Peroxisome proliferator activated receptor γ (PPARγ) is a nuclear receptor that was initially identified as a major regulator of adipose differentiation and metabolism, and later as a pivotal receptor for immunity and inflammation. Stimulation of PPARγ in macrophages results in inhibition of nuclear factor κB (NFκB), which is associated with induction of apoptosis. In addition, activation of PPARγ causes inhibition of the STAT and AP-1 signalling pathways, and together this explains the PPARγ mediated reduction of IL-2, IL-6, IL-8, TNFα, IL-12, and metalloproteinase release. These results are likely to be important for inflammatory bowel disease, because the colon mucosa expresses high concentrations of PPARγ. Mice that are deficient for PPARγ, or the RXR retinoid receptor, which is a heterodimeric PPARγ partner, display an increased sensitivity to hapten induced colitis. In addition, administration of agonistic ligands of the two receptors (synergistically) attenuated the severity of TNBS colitis in mice; this was shown to be associated with a reduction of activation of the NFκB, colonic Map p38 activity, and JNK (c-JUN NH₂ terminal kinase) activation, resulting in reduced production of proinflammatory cytokines. These results are of great importance in view of the fact that PPARγ activating small molecules have been developed as antidiabetic and antiatherogenic drugs, and are currently available.

**PHOSPHODIESTERASE INHIBITORS**

Cyclic AMP (cAMP) is involved in regulation of the expression of several proinflammatory cytokine genes via phosphorylation of cAMP response element binding protein (CREB). Phosphodiesterase 4 (PDE4) is an important regulator of the intracellular cAMP concentration, and several PDE4 inhibitors have anti-inflammatory and immunomodulating effects. PDE4 inhibitors, at relatively high concentrations, inhibit the production of proinflammatory cytokines, including TNFα, in vitro as well as in vivo, most likely by reducing transcription of the gene. Hence, the mechanism of action differs from the effects of corticosteroids, that also inhibit post-transcriptional events, in particular translational efficacy. It should be noted that the first generation PDE4 inhibitors, including pentoxifylline, amrinone, and rolipram, were not primarily designed to inhibit cytokine production. Indeed, these drugs have many other effects, including attenuation of activation of neutrophils, endothelial cells, and blood platelets. Apart from inhibiting inflammation that results from activation of the innate immune response, inhibition of PDE4 also has an effect on T lymphocyte mediated inflammation. For example, rolipram inhibited IL-15 induced expression of cell adhesion molecules and interfered with PAF and IL-8 directed T lymphocyte chemotaxis.

PDE4 inhibitors have been reported to have protective effects in many animal models of (intestinal) inflammation, including inflammatory bowel disease. However, the reported data are inconsistent, and several investigators have commented that they have been unable to find an association between the protective effects and mucosal production of TNFα. Moreover, at the relatively high doses required to achieve a TNFα inhibitory
effect in primates, including humans, first generation PDE4 inhibitors cause gastrointestinal side effects that are in part mediated by a stimulation of gastric acid production. A second generation PDE4 inhibitor has been synthesised that apparently lacks this side effect, but has not been tested in inflammatory bowel disease.

Only one PDE4 inhibitor (expentifylline, a pentoxifylline analogue) has been systematically studied in Crohn’s disease, and no clinical efficacy was found despite a reduction of the capacity of peripheral blood mononuclear cells to produce TNF-α.

In conclusion, phosphodiesterase inhibitors, in particular PDE4 specific compounds, have multiple anti-inflammatory effects and, at high concentrations, interfere with the production of proinflammatory cytokines. First generation PDE4 inhibitors are relatively weak inhibitors of TNF transcription and cause gastrointestinal side effects. A single study using expentifylline in Crohn’s disease did not show clinical efficacy.

THALIDOMIDE

Thalidomide was first synthesised in 1954 and soon marketed as a sedative. Because of the induction of severe birth defects thalidomide was withdrawn from the market in the early 1960s. Meanwhile, it had been serendipitously found that patients with erythema nodosum leprosum responded well to thalidomide therapy; this finding was confirmed in a controlled clinical trial. 75–76 For many years the mechanism of action remained unknown, but in 1991 it was reported that thalidomide reduced the production of TNFα from lipopolysaccharide (LPS) stimulated monocytes. 77 Thalidomide does not affect signal transduction pathways (for example, activation of NfκB) that induce TNFα transcription, but increases TNFα mRNA degradation. 78–79 Thalidomide has efficacy in several TNFα mediated diseases, but it is uncertain whether the mechanism of action is a result of interference with the production of TNFα. It should be noted that thalidomide is a relatively weak TNFα inhibitor, and even at very high drug concentrations, inhibition of TNFα production by peripheral blood mononuclear cells and T lymphocytes is incomplete. 80 Recently, several thalidomide derivatives have been synthesised that are reported to have a much increased TNFα inhibiting effect. 81–83 These compounds do not affect TNFα mRNA degradation and do not interfere with NfκB activation, but have been shown to be PDE4 inhibitors. 84–85 This class of thalidomide derivatives mainly affects macrophages and monocytes, reducing TNFα and stimulating IL-10 production, but does not affect T lymphocyte activation. 86 A second class of thalidomide derivatives strongly boosts T lymphocyte activation, and production of IL-2 and IFNγ, and therefore is considered to be immunostimulatory. 87 The parent compound, thalidomide, has well known T lymphocyte co-stimulatory effects, and in healthy volunteers, thalidomide strongly boosts T lymphocyte proliferation and IFNγ production, while having a weak inhibitory effect on LPS induced TNFα production. 88–90 In thalidomide treated patients with pulmonary tuberculosis, TNFα production was reported to be reduced, but in HIV positive patients with tuberculosis, thalidomide treatment either increased or did not affect TNFα production. 91–92 The latter study did show an increase of soluble IL-2 receptor, IFNγ, and PPD dependent T lymphocyte proliferation, following thalidomide therapy, indicative of immunostimulatory effects. 93 Finally, in a prophylactic study, thalidomide therapy increased the incidence of graft versus host disease and decreased survival. 94 Hence, in healthy volunteers, as well as in immunosuppressed patients, the therapeutic profile of thalidomide is characterised by immunostimulatory effects rather than by suppression of TNFα production.

Thalidomide has been reported to have beneficial effects in erythema nodosum leprosum, in complications of HIV (tuberculosis, aphthous stomatitis), in Behcet’s syndrome, and in pyoderma gangrenosum, but its efficacy in rheumatoid arthritis is equivocal. 95–99 Following reports of healing of oral ulcers in Crohn’s disease by thalidomide treatment, the efficacy of thalidomide in active (steroid dependent) Crohn’s disease was investigated in two small uncontrolled studies; both suggested therapeutic efficacy. 100–104 In the first study, 12 patients with active Crohn’s disease, despite treatment with at least 20 mg prednisone, were included. The first six patients received 50 mg, the last six patients 100 mg thalidomide daily for 12 weeks. In seven patients clinical improvement was observed at week 4, and two patients had a complete clinical remission. After the fourth week of treatment steroids were tapered, and could be completely discontinued in almost half of the patients. 105 A second study included 22 patients with active Crohn’s disease, who were treated with either 200 mg (18 patients) or 300 mg (two patients) of thalidomide at bedtime. Of the 22 included patients, 14 were still in the study at 12 weeks, and nine were in clinical remission (six with fistulas, three with luminal disease). 106 Another open label follow up study in five children with Crohn’s disease reported a response in four, that was maintained for a period of 19–24 months; steroids were discontinued in all four responders. 107 Thalidomide has several side effects. Clearly, the well known teratogenicity precludes its usage in pregnant women, and mandates the use of adequate birth control. However, the efficacy of birth control is not complete, and even in phase II drug development, some women who have been adequately advised to use birth control nonetheless become pregnant. Other thalidomide related side effects include neuropathy, rash, and drowsiness. It seems that such side effects are of minor importance in life threatening diseases, such as tuberculosis in HIV infected patients, or when therapeutic alternatives are not available, such as in therapy refractory pyoderma gangrenosum. The results from the small clinical trials in chronic inflammatory disease (rheumatoid arthritis, Crohn’s disease) seem to indicate that a relatively large proportion (up to 30%) of the enrolled patients fail to complete a three month thalidomide course because of side effects. 108

Although there is no published evidence that the efficacy of thalidomide is related to a reduction of (mucosal) TNFα production, a controlled clinical study with one of the PDE4 specific thalidomide derivatives has been initiated targeting patients with active Crohn’s disease. Until the results of controlled clinical trials become available, the use of thalidomide should be restricted to severe therapy refractory complications of inflammatory bowel disease, including pyoderma gangrenosum and extensive oral ulceration.

TNFα CONVERTING ENZYME INHIBITORS

The post-translational processing of TNFα includes cleavage of the membrane bound TNF precursor molecule by a metalloproteinase. 109–111 The responsible enzyme, which acts at the cell membrane, has been identified as TNFα converting enzyme (TACE; ADAM17), and is a member of the extensive ADAM (a disintegrin and metalloproteinase) family of proteases. Apart from TNFα, TACE cleaves several other membrane bound proteins, including CD16, CD27, CD30, the two TNF receptors, and itself. 106–107 TACE is an interesting target for therapy of chronic inflammatory diseases, because structure–function relations are well known and have allowed the development of (hydroxamate based) small molecular inhibitors. Indeed, in a phase II clinical trial in low dose endotoxaemia in volunteers, TACE inhibition dramatically reduced the amount of LPS induced circulating TNFα. 108 The TACE inhibitors that are currently available for use in clinical trials are not very specific and also inhibited other ADAM family members. In view of the known pathogenic importance of metalloproteinases for the induction of damage to the inflamed intestinal mucosa, this might be a desired effect. 109–111 Indeed, the efficacy of the metalloproteinase marimastat in experimental colitis

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was suggested to be dependent on metalloproteinase inhibi-
tion rather than a reduction of TNFα production. Unfortunately, non-specific metalloproteinase inhibitors either cause prohibitive side effects, or have been ineffective in chronic inflammatory disease. Another point of concern has been the prevention of TNF receptor shedding by TACE inhibition. Following activation of target cells by TNFα, both TNFα receptors are rapidly shed by activation of TACE, and as a consequence these cells become TNFα unresponsive. In addition, soluble TNF receptors retain the ability to bind TNFα; this is considered to be a natural TNFα scavenging principle. In rheumatoid synovial membrane cell cultures, treatment with a TACE inhibitor reduced TNFα production, but paradoxically increased the release of IL-1β, IL-6, and IL-8, which was suggested to be related to a reduction of the release of both TNF receptors. Similar, albeit relatively minor, effects have been observed in low dose endotoxaemia.

In conclusion, TACE remains an interesting target for the development of anti-inflammatory small molecules. However, further development requires generation of molecules with much greater TACE specificity than those that have been studied to date. It should be noted that the effects of specific TACE inhibitors are not restricted to membrane bound TNFα, because several other membrane expressed molecules are cleaved by TACE.

**SIGNAL TRANSDUCTION INHIBITORS**

Several interacting cascades of signalling molecules regulate cellular death and survival. The importance of these signal transduction pathways for cytokine production and inflammation became apparent through two independent lines of research that led to the identification of MAP kinases as regulators of the transcription and translation of TNFα. The first line of research aimed at the identification of intracellular targets for a class of pyridyl-imidazole compounds that inhibited the production of TNFα and IL-1β; the second line investigated the nature of the proteins that were tyrosine phosphorylated following cellular stress. The results of these studies converged on the notion that the 38 kD mitogen activated protein kinase (MAP p38) is a key enzyme, that regulates cell responses to cytokine stimulation, osmotic stress, and radiation injury. It is now known that at least three tightly linked signal transduction pathways regulate the production of proinflammatory cytokines—that is, the NFκB, MAPK p38, and JNK pathways. Not only are these pathways regulators of cytokine production, all three pathways also act downstream of several receptors of proinflammatory cytokines. There is now evidence that activation of all these pathways occurs in inflammatory bowel disease, and with the exception of JNK, more or less specific inhibitors are available.

In resting cells, NFκB is localised within the cytoplasm, being bound by IkB. Activation of IkB kinases (IKK) leads to phosphorylation of IkB and subsequent degradation in the proteasome, allowing NFκB to enter the nucleus and bind to NFκB specific DNA sequences that are found in the promoter of many proinflammatory cytokine genes. It should be noted that the NFκB family of proteins includes several members that are able to form various homo- and heterodimers that may have different effects. Activation of NFκB does occur in ulcerative colitis and Crohn’s disease, but the cells in which NFκB is translocated into the nucleus differ (lamina propria cells in Crohn’s disease and ulcerative colitis and in epithelial cells in ulcerative colitis). In intestinal epithelial cells, NFκB is an important regulator of chemokine (IL-8) production as well as ICAM-1 expression, and the pathogenicity of certain bacteria is in part a result of the induction of NFκB in epithelial cells. It has recently been found that certain non-pathogenic bacteria prevent the translocation of NFκB (by interfering with the degradation of IkB) in intestinal epithelial cells; this may be an explanation for the efficacy of certain “probiotics”. In both T lymphocyte mediated and epithelial cell dependent animal models of inflammatory bowel disease, interference with the NFκB pathway by administration of antisense p65 (an NFκB member) oligonucleotides had a protective effect. Several currently used drugs, including corticosteroids and aspirin, are known to target NFκB, and several redox sensitive molecular interactions that are necessary for NFκB translocation can be relatively easily targeted by small molecules. Because aspirin is not effective and many patients are refractory to corticosteroids, it is clear that not all these approaches will be effective in inflammatory bowel disease. Apart from mentioned potential (anti-inflammatory) regulation of NFκB in intestinal epithelial cells, food derived small molecules may also regulate intestinal NFκB translocation. An example is the inhibition of NFκB activation (by interfering with IkB kinase activation) in intestinal epithelial cells by the flavonoid curcumin, and by food derived butyrate. To date no controlled clinical trials using specific inhibitors of NFκB in inflammatory bowel disease have been reported.

Activation of MAPK as well as JNK in active inflammatory bowel disease has been recently reported. Several generations of MAP p38 targeting small molecules have been tested in animal models of inflammation, and efficacy has been shown in experimental arthritis. We have recently found that a first generation MAP p38 kinase inhibitor (SB 203580) did inhibit IFNγ, but not TNFα production in T lymphocyte and synovial cells of arthritis (unpublished results). At present it is unclear why this compound blocks activation by binding to the MAP p38 ATP binding pocket, but the drug does not prevent phosphorylation. In several experimental systems this paradoxically leads to a profound activation of upstream kinases that may cause inflammation through activation of parallel signal transduction pathways. If this hypothesis is correct, a better strategy would be the inhibition of MAP kinase activation at a more proximal level. Alternatively, as it is known that MAP p38 activation is a negative regulator of proliferation of certain cells, its inhibition may lead to uncontrolled proliferation. c-JUN NH2 terminal kinases (JNK, also known as stress activated MAP kinase, SAPK) constitute a family of MAP signal transduction proteins, that are involved in cell proliferation, apoptosis, morphogenesis, and tumour formation. A recent phase II study suggested that JNK inhibition may have protective effects in severe Crohn’s disease, reducing circulating C reactive protein concentrations, and causing mucosal healing. Several controlled clinical trials using blockers of MAP p38 and JNK in active inflammatory bowel disease have been planned.

**CONCLUSIONS**

Despite a clear unmet need for new effective and non-toxic therapies for induction and maintenance of remission, no therapeutic small molecules for the treatment of inflammatory bowel disease have been introduced in the past decades. Eicosanoids (thromboxane A2, leukotrienes, and PAF) are produced at an increased rate by the inflamed bowel mucosal, and are considered to have proinflammatory effects. In several animal models, compounds that inhibit these proinflammatory eicosanoids were protective, but clinical efficacy has been disappointing. PDE4 inhibitors have anti-inflammatory effects, but the inhibition of the production of proinflammatory cytokines is relatively weak, and gastrointestinal side effects are common. Thalidomide has complex biological effects, including the stimulation of (a subset of) T lymphocytes, and inhibition of IL-12 and TNFα; it remains to be seen which of these effects is responsible for the therapeutic efficacy in
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In addition, small molecules that directly interfere with the activation of T lymphocyte mediated diseases. Two uncontrolled studies have reported that thalidomide may be effective in steroid refractory Crohn’s disease; these data need to be confirmed.

Several T lymphocyte mediated diseases. One of these compounds may have important animal models. In addition, small molecules that directly interfere with the NFkB, JNK, and MAPK pathways have promising effects in animal models. One of these compounds may have important therapeutic efficacy in severe Crohn’s disease; this may herald an emerging class of new therapeutic small molecules for the treatment of inflammatory bowel disease.

REFERENCES


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139 Murakami-Mori K, Mori S, Nakamura S. p38MAP kinase is a negative regulator for ERK1/2-mediated growth of AIDS-associated Kaposi’s sarcoma cells. Biochem Biophys Res Commun 1999;264:676–82.

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