Published in final edited form as:

Inflamm Bowel Dis. 2016 January ; 22(1): 241–247. doi:10.1097/MIB.0000000000000598.

Reversibility of Stricturing Crohn's Disease – Fact or Fiction?

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Abstract

Intestinal fibrosis is a common feature of Crohn's disease (CD) and may appear as a stricture, stenosis or intestinal obstruction. Fibrostenosing CD leads to a significantly impaired quality of life in affected patients and constitutes a challenging treatment situation. In the absence of specific medical anti-fibrotic treatment options endoscopic or surgical therapy approaches with their potential harmful side effects are frequently used. However, our understanding of mechanisms of fibrogenesis in general and specifically intestinal fibrosis has emerged. Progression of fibrosis in the liver, lung or skin can be halted or even reversed and possible treatment targets have been identified. In face of this observation and given the fact that fibrotic alterations in various organs of the human body share distinct core characteristics, this article aims to address, whether reversibility of intestinal fibrosis may be conceivable and to highlight promising research avenues and therapies.

Keywords

Inflammatory bowel disease; fibrosis; stricture; disease course; stenosis; reversibility

Introduction

Fibrosis is defined as the exaggerated accumulation of collagen-rich extracellular matrix (ECM) and expansion of mesenchymal cells and may affect various organs of the human body as a consequence of chronic inflammatory diseases.^{1, 2} With regard to Crohn's disease (CD) patients, epidemiological data indicate that more than 40% of CD patients with ileal disease manifestation will develop clinically apparent stricture formation³ and the vast majority of these patients will have to undergo surgery at least once during the course of

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Conflicts of interest: None

I declare the authors have no competing interests or other interests that might be perceived to influence the interpretation of the article.

their disease.⁴ While recent observations indicate that immunosuppressive therapy may lead to a delay in disease progression⁵ or reduction in the need for intestinal surgery in CD patients in the long-run,⁶ endoscopic balloon dilation as well as surgical approaches such as strictureplasty or bowel segment resection still remain major therapeutic approaches for CD patients with symptomatic strictures.⁷ Of note, a recent study from Sweden reports that 13% of CD patients presented with a stricturing CD phenotype already at diagnosis,⁸ highlighting that this complication is not exclusively a feature of the long-term disease course but may result in symptomatic strictures in the short-run that require early interventional therapy. This frequent clinical observation together with the chronic progressive nature of stricturing CD lead to the common belief that fibrosis is a one-way street from fibrosis to stricture formation with intestinal obstruction followed by the eventual need for surgical resection. This notion however has to be challenged. Recently, a large combined analysis including a total of 1.112 patients assessed the efficacy of strictureplasty in CD and found an overall symptomatic recurrence rate of 39% for jejunoileal strictures (161 out of 411 patients) and 36% for ileocolonic strictures (9 out of 25 patients). Strikingly, only 3% or 20%, respectively, of CD patients strictures were found at the previous site of strictureplasty.⁹ This finding indicates that surgical intervention has the potential to stop progressing or even to reverse intestinal fibrosis. Based on Cleveland Clinic data, following strictureplasty operations, patients have been encouraged to undergo follow-up small bowel series after an interval of at least six months. Of the 44 asymptomatic patients who complied, recurrence at the strictureplasty sites with narrowing of the caliber of the bowel was noted in only 11% of patients after a median interval of two years.¹⁰ Maconi *et al.* performed serial ultrasound examinations in patients after strictureplasty and found a reduced thickness of the intestinal wall, suggesting a possible mechanism and further fueling the promise of reversibility of intestinal strictures.¹¹

The concept of reversibility of intestinal fibrosis is in concordance with various observations from other organs such as the improvement of skin scarring¹² and reduced skin thickening in systemic sclerosis,¹³ decreased proteinuria in patients with renal interstitial fibrosis,¹⁴ the improvement of vital capacity in idiopathic pulmonary fibrosis^{15, 16} the successful therapeutic reduction of myocardial collagen content in hypertensive patients¹⁷ and reversibility of myocardial fibrosis after adrenalectomy.¹⁸ In addition, there is a growing body of evidence indicating that liver fibrosis is a potentially reversible and bidirectional process overcoming the former paradigm of liver cirrhosis being an irreversible process. Repetitive histological evaluation via liver biopsies could prove reduction of the fibrosis grade after removal of the liver injury-causing triggers in patients with hepatitis C,¹⁹ hepatitis B,²⁰ non-alcoholic steatohepatitis (NASH)²¹ or autoimmune hepatitis.²²

Although the gut comprises unique features compared to other organ fibroses, such as severity and chronicity of inflammation in the context of IBD, the quality and quantity of the commensal microbiota or environmental influences on the disease course, intestinal fibrosis shares essentially all core mechanistic features with fibrotic disease of the above-mentioned organs.^{1, 23-25} Therefore, it appears to be reasonable to consider these mechanisms and therapeutic approaches and apply them as promising approaches for the reversal of stricturing CD. In the following sections we will discuss putative mechanisms for the

reversal of intestinal fibrosis as seen in the phenomenon of intestinal strictureplasty, such as suppression of inflammation, the two forms of cellular transformation – epi- and endothelial to mesenchymal transition, macrophage subtype switch, fibroblast deactivation, and fibroblast apoptosis.

Review criteria

A comprehensive literature search was performed to assess all relevant citations found in Embase, Medline (service of the US National Library of Medicine (NLM) and the National Institutes of Health (NIH)) and the Cochrane Library for the following key words: ('Crohn's disease (CD') OR 'Crohn's' AND ('stricture' OR 'fibrosis'), ('kidney' OR 'liver' OR 'skin' OR 'lung' OR 'systemic nephrogenic' AND 'fibrosis' OR 'anti-fibrotic therapy'). Additionally, references of cited original articles and reviews were further assessed for relevant work. The search included studies between 1960 and 2014. These data together with the authors' personal experience in the field represent the basis of this review.

Mechanisms orchestrating reversibility of fibrosis

Multiple mechanisms that have been proven to be crucially involved in the reversal of fibrosis in organs other than the gut are candidates in the orchestration of resolution of intestinal fibrosis and could represent promising targets for anti-fibrotic therapy (Figure 1). To date only few of those mechanisms have been tested in the intestine.

Suppression of inflammation

Emerging data suggests a diminished degree of inflammation at the site of strictureplasty after surgical intervention.²⁶⁻²⁸ This observation could be confirmed by histological and molecular assessment and determination of pro-inflammatory cytokine production of the ileal mucosa.²⁹ While at the time of strictureplasty mucosal expression of Interleukin(IL)-1 β , IL-6, IL-8 or tumor necrosis factor (TNF)- α was markedly increased compared to expression levels in the non-inflamed mucosa, analysis 12 month after surgery revealed no difference in expression of these pro-inflammatory cytokines in the prior inflamed and non-inflamed areas.²⁹ It was hypothesized that the decrease in inflammation may be a result of the reduced mechanical and antigenic pressure after successful increase in luminal diameter leading to decreased microbial and food allergen contact with inflamed and permeable mucosal areas of the intestine.²⁹ As a consequence, at least in strictureplasties, a reduced inflammatory burden may be associated with a decrease in pro-fibrotic activity or even reversal due to a reduced fibrogenic pressure.

Over the recent years, the intestinal microbiome has been recognized to potentially modulate colitis and intestinal fibrogenesis due to its pleiotropic actions.³⁰ For example, isolated human colonic fibroblasts from CD patients that were stimulated via bacterial components and toll-like receptors (TLR) mediated pathways revealed a significantly enhanced proinflammatory and profibrotic cytokine production.³¹ Additionally, in experimental models of colitis, the absence of the microbiota protects animals from intestinal inflammation hinting at the regulatory role of the microbiota.³² In line with these findings, human IBD patients presenting with alterations in genes coding bacterial recognition and

processing were reported and antibodies against microbial components were shown to be associated with a severe disease phenotype and stricturing course of disease.³³⁻³⁵ In face of these observations, aside reduced inflammatory activity, an ameliorated antigenic pressure after stricture dilation may be one explanation for fibrosis reversal.

Macrophage subtypes

Besides antigenic pressure and cellular transition, activation of inflammatory cells such as monocytes and macrophages is a crucial step in fibrogenesis. Interestingly, these cells may facilitate pleiotropic and divergent actions depending on the stage of fibrosis development. In the early stages of liver fibrosis, macrophages may enable pro-fibrotic actions through production of transforming growth factor-beta (TGF- β) and platelet-derived-growth-factor (PDGF)-mediated hepatic stellate cell (HSC) activation. Thus, targeted macrophage depletion during the injury phase of experimental liver fibrosis resulted in decreased scarring and reduced numbers of myofibroblasts.³⁶ In contrast, their depletion during the recovery phase of experimental liver fibrosis culminated in impaired scar resolution³⁶ indicating that this cell type has not only a role in initiation, but also in resolution of fibrosis depending on the timing of action. These ambiguous properties could be explained by: (1) macrophages are a source of matrix metalloproteinases (MMPs). More specifically, MMP-2, -9 and 13 digest ECM compounds and therefore contribute to ECM degradation;^{36, 37} (2) macrophages mediate elimination of apoptotic cells resulting in clearance of pro-fibrotic triggers;^{38, 39} (3) anti-inflammatory IL-10 is secreted by macrophages and can limit TGF- β -induced collagen production in fibroblast;⁴⁰ (4) macrophages can express HSC-specific death ligands.^{41, 42} In particular, M2 macrophages, a specific subset of macrophages, that is activated by IL-4 or IL-13, are crucially involved in fibrogenesis.⁴³ The phenotype of these cells is defined by distinct products such as the mannose receptor, chitinase-3-like-protein-3 and the enzyme arginase-1 (Arg1), which regulates l-proline production, a prerequisite for collagen production^{2, 44}. Additionally, it is known that M2 cells compete with T-helper 2 cells and fibroblasts for l-arginine, which is required for l-proline synthesis.⁴⁵ M2 cells link fibrosis and inflammation due to their capability to induce T reg cells.⁴⁶ In vivo, M2 macrophages were shown to decelerate fibrosis development upon helminth infection⁴⁷. A more detailed description of the role of various macrophage subtypes as well as other cell types relevant for fibrogenesis has been published elsewhere⁴⁸⁻⁵².

In addition to macrophages, natural killer (NK) cells have the ability to promote fibrosis resolution. In murine models of liver fibrosis, increased NKG2D receptor expression on NK cells resulted in enhanced HSC deletion through interaction with its ligand retinoic acid early inducible-1 on HSC and facilitated amelioration of experimental fibrosis.⁵³ Interestingly, NK cells could be activated by the pro-inflammatory, but anti-fibrotic cytokine interferon-gamma (IFN- γ).⁵³

The above-mentioned findings emphasize the potential pro- as well as anti-fibrotic actions of inflammatory cells, depending on the quality, quantity and timing of their response. Accordingly, targeted intervention during fibrosis development by the use of anti-inflammatory agents or cells may imply a high therapeutic potential. With regard to

intestinal stenosis, it is well likely that a change in macrophage polarization or activation of NK cells contribute to the reversal of strictureplasty associated fibrosis in IBD.

Epi- and endothelial to mesenchymal transition

Another mechanism crucially involved in fibrosis establishment is transition of epithelial and endothelial cells into mesenchymal cells and hence their contribution to the pool of fibroblasts in fibrogenesis. Evidence exists about epi- (EMT) and endothelial to mesenchymal (EndoMT) transition occurring in intestinal inflammation.^{54, 55} Of note, this process is believed to be reversible. Although there is no study available demonstrating reversibility of EMT or EndoMT within in the intestine, there is a growing body of evidence *in vitro* and *in vivo* suggesting a presence of this mechanism in organs other than the gut. This is promising since this represents a means to reduce the excessive number of fibrotic effector cells in strictured areas in CD. A wide variety of distinct pathways have been identified to reverse EMT. *In vitro* examples include activation of protein phosphatase 2a in prostate cancer cell lines⁵⁶, inhibition mTOR signaling pathway via rapamycin in gallbladder cancer cells.⁵⁷, combined treatment with a TGF- β receptor I (T β RI) inhibitor and rho-associated protein kinase (ROCK) inhibitor in murine renal tubular epithelial cells⁵⁸ or fibroblast growth factor (FGF)-1 and heparin treatment in TGF- β 1-stimulated murine and alveolar epithelial-like cells.⁵⁹ *In vivo* observations fuel the hope for induction of MET as a possible treatment approach to inhibit and reverse fibrotic diseases. In a murine model of renal fibrosis, MET resulted in accurate repair of injured epithelium.⁶⁰ Moreover, anti-fibrotic effects via MET were reported in experimental models of pulmonary fibrosis by FGF-1 through MAPK/ERK kinase signaling, ERK-1 phosphorylation and Smad2 dephosphorylation.⁶¹ Zeisberg and coworkers suggested that bone morphogenic protein (BMP)-7, a member of the TGF- β superfamily, may be a promising target to facilitate the reversal of TGF- β -induced EMT. More specifically, BMP-7 administration resulted in reversal of chronic kidney disease in experimental models⁶² and mediated anti-fibrotic effects through the inhibition of HSC activation leading to amelioration of CCL4-induced liver fibrosis.⁶³ While less information is available comparable factors have been identified promoting mesenchymal-to-endothelial transition (MEndoT), including rapamycin in pulmonary arterial hypertension,⁶⁴ or BMP-7 in cardiac fibrosis⁶⁵. Nevertheless, the ultimate proof of MET would include reversibility of EMT in primary cells from human fibrotic organs. Inflammatory pressure appears to be the main driver of cellular transformation and hence the reduced degree of inflammation after strictureplasty could inhibit or even reverse this process.

Fibroblast deactivation

Given the fact that myofibroblasts are a major player in intestinal fibrosis development due to massive production and secretion of multiple collagen types^{66, 67} and ECM compounds,^{25, 68} therapeutic manipulation of these cells appears promising to stop or reverse fibrosis. Similarly, in liver fibrogenesis, HSCs, which are considered to be the main precursor of liver myofibroblasts, are a main driver in fibrogenesis. Upon chronic injury, HSC not only get activated and change their phenotype adopting myofibroblast features, but also increase ECM deposition.⁶⁹ In experimental models, upon removal of the fibrosis-inducing agent, activated liver myofibroblasts were shown to reduce their numbers. This

effect is mediated by senescence or apoptosis, but activated hepatic myofibroblasts can also revert back to a quiescent phenotype, characterized by decreased expression of fibrogenic molecules, such as collagen I and α -smooth muscle actin (SMA).^{70, 71} Fibroblast deactivation and apoptosis appear to share common pathways including activation of cell-death mediated pathways and caspase activation,⁷² increase of pro-apoptotic stimuli (for example p53, caspase 9) and reduction of anti-apoptotic genes like Bcl-2 and Hspa1a/b.^{71, 72} as shown in hepatic fibroblast deactivation. In consequence, reversal of fibrotic scars in liver fibrosis was observed. Troeger *et al.* used a model of transgenic mice to study activated HSC in CCl₄-induced liver fibrosis. They found that activated HSCs persisted in the liver up to 45 days after CCl₄ challenge. Subsequently, the reversal of HSC activation resulted in inhibition of fibrogenesis during fibrosis resolution.⁷³

Fibroblast apoptosis

Aside deactivation or senescence, apoptosis could account for the reduced numbers of myofibroblasts in reversal of fibrosis. Evidence can be derived from multiple organs outside of the intestine.^{69, 74, 75} For example, TNF-related apoptosis-induced ligand receptor (TRAIL-R)2 is expressed on activated HSC⁷⁶ and was found to initiate apoptosis after its activation, which appears to be its critical function. Recently, a subset of CCR6 expressing $\gamma\delta$ T cells were shown to accumulate in fibrotic livers of mice and to ameliorate fibrosis development through Fas-mediated induction of apoptosis in HSC.⁷⁷ Additionally, immune cells, including natural killer cells and $\gamma\delta$ T cells, stimulated by the pro-inflammatory cytokine IFN- γ were shown to possess a high degree of cytotoxicity towards HSC, contributing to resolution of fibrosis.⁵³ The principle of apoptosis of pro-fibrotic cells during the recovery phase is observed in other organs as well. For example, apoptosis of renal mesangial cells occurs during mesangial proliferative nephritis.²⁴ Similarly, the apoptosis of dermal myofibroblasts is crucial in the context of cutaneous wound healing to facilitate remodeling of the collagen-rich scar tissue.⁷⁸ Fibroblast apoptosis has been described in the intestine of IBD patients as well. Proliferation rates of human intestinal fibroblasts isolated from ileal specimen of CD patients, which were *ex vivo* treated with TNF- α or IFN- γ alone were not significantly decreased as compared to untreated controls.⁷⁹ However, combined stimulation with TNF- α and IFN- γ lead to a 4-fold increased apoptosis rate of intestinal fibroblasts.⁷⁹ Data supporting this mechanism *in vivo* are missing.

Furthermore, the matrix microenvironment also modulates myofibroblast viability. Enhanced ECM stiffness, which is present in scar tissue and fibrosis results in increased activation and survival of myofibroblasts through cell to matrix interactions.⁸⁰ For example, spatial changes in hepatic matrix mechanics may lead to the development of fibrous septa and reorganize the liver architecture.⁸¹ Additionally, stage and progression of liver fibrosis are associated with septal width since massive septae are characteristic for advanced stage liver cirrhosis. Mechanistic studies identified liver stiffness as a core feature of the ECM for the development of liver fibrosis. More specifically, it was demonstrated *in vitro*, that HSC differentiation into myofibroblasts requires a mechanically stiff substrate, with adhesion to matrix proteins and the generation of mechanical tension. *In vitro*, activation of rat HSC was found to be significantly influenced by the stiffness of coating matrices. While HSC exposed

to intermediate stiffness adopted stable intermediate phenotypes, cells challenged with stiff support differentiated into myofibroblasts.⁸²

Conceptually, inflammation is undoubtedly an initiator and likely driver of intestinal fibrosis, but it becomes apparent that the quality, quantity and the timing of the inflammatory infiltrate are critical in determining its pro- or anti-fibrotic properties. This fact is underlined by the observation that effective anti-inflammatory intervention does not necessarily result in reduced frequency of fibrostenotic complications in CD patients and fibrotic alterations in animal models^{5, 83}. Understanding the core mechanisms of fibroblast apoptosis could serve as a tool for novel anti-fibrotic therapies by limiting a main source of collagen-producing cells.

Conclusion and outlook

The ultimate goal to reverse fibrosis and to induce physiological tissue regeneration is challenging and will not readily be realized in CD patients in the near future. However, necessary steps on the way towards at least partial amelioration of intestinal fibrotic complications are anticipated. The balance of synthesis and degradation of various matrix protein compounds crucially influences the excessive ECM accumulation in CD-associated strictures. The commonly believed notion that scar tissue is a hypometabolic or even ametabolic compartment needs to be revisited. This could be shown by measuring collagen turnover rates by administration of heavy water.⁸⁴ In experimental pulmonary fibrosis, bleomycin-treatment resulted not only in increased matrix deposition, but also increased matrix turnover rates in the lung.⁸⁵ Hence even the most severe fibrotic areas, such as established strictures in CD, could be amendable to anti-fibrotic therapy leading to its complete reversal, given a continuous matrix turnover.

Multiple distinct signaling cascades and mechanisms have been identified to contribute to fibrosis. Therefore, potential therapeutic approaches should not only address a single but multiple targets in form of a combined approach. The complexity of their interactions is high. For example, cytokines such as IFN- γ or TNF- α may represent anti-fibrotic, but pro-inflammatory mediators at the same time. They did not lead to fibroblast apoptosis on their own, but only together. It is obvious that especially in CD patients the quality, quantity and timing of profibrotic mediators deserves thoughtful evaluation.

Targeting cell types over mediators could be a promising approach: stimulation of subpopulations of macrophages to increase MMP-expression and to induce apoptosis of myofibroblasts may be suitable to emerge the anti-fibrotic therapeutic armamentarium.² Another approach could be the stimulation of collagenase production or MMPs and has been successfully performed in animal studies.⁸⁶ Finally, gastroenterologists should be stimulated by fibrosis research in other organs. An example could be a knowledge transfer to the intestine from scleroderma research, like the anti-fibrotic protein melanocortin^{87, 88}.

From the clinical point of view, early risk stratification of individual CD patients for stricturing disease courses is paramount and may be evaluated by appropriate biomarkers, which are currently not available. Data from the liver suggests that once established, the possibility to spontaneously reverse fibrotic complications seems to be dependent on the

stage of fibrosis.⁸⁹⁻⁹¹ While early stages may be reversed by the removal of the etiological factor, advanced stages may not regress on their own. As shown for liver fibrosis, identification of morphological indicators or biochemical ECM analyses of advanced fibrosis could define mechanisms for a point of no return.⁸⁹ In CD the impact of anti-inflammatory treatment in patients with established fibrostenotic strictures needs to be further elucidated in clinical trials.⁹² Despite the required complex study designs including long follow up periods and large patient cohorts, there are currently first studies ongoing investigating the potential of anti-inflammatory drugs to modify the course of disease and to prevent fibrostenotic complications in CD patients (Clinical Trials.gov Identifier: NCT01698307).

We presented multiple mechanisms that could play a role in the reversal of intestinal fibrosis, a phenomenon that can be observed after strictureplasty surgery, such as suppression of inflammation, the two forms of cellular transformation – epi- and endothelial to mesenchymal transition, macrophage subtypes, fibroblast deactivation, and fibroblast apoptosis. Multiple compounds, whose mechanisms were discussed in this article, are already in clinical development or clinical practice in other organs than the intestine that could be employed in the therapy of Crohn's disease.^{1, 93} We propose strictureplasty as a novel and understudied human model for reversal of fibrosis and potential anti-fibrotic drug approaches should make use of this knowledge to explore novel therapies.

Acknowledgments

Source of funding: This work was supported by a research fellowship from the Faculty of Medicine, Westfälische Wilhelms-Universität Münster to D.B. and grants from the National Institutes of Health (T32DK083251 and P30DK097948) and the European Crohn's and Colitis Foundation to F.R.

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Abbreviations

BMP	bone morphogenic protein
CD	Crohn's disease
CCl4	carbon tetrachloride
ECM	extracellular matrix
EMT	epi to mesenchymal transition
EndoMT	endothelial to mesenchymal transition
FGF	fibroblast growth factor
ERK	extracellular regulated kinase
HCC	hepatocellular carcinoma
HSC	hepatic stellate cell
IL	interleukin
MMP	matrix metalloproteinase
NASH	non-alcoholic steatohepatitis
TIMP	tissue inhibitor metalloproteinases
MET	mesenchymal-to-epithelial transition
NK cells	natural killer cells
TNF- α	tumor necrosis factor alpha
IFN- γ	interferon gamma
SMA	smooth muscle actin
ROCK	rho-associated protein kinase

STAT	signal transducer and activator of transcription
TRF	tocotrienol rich fraction
TGF	transforming growth factor
TLR	toll-like receptor
TRAIL	TNF-related apoptosis-induced ligand receptor

Figure 1. Mechanisms contributing to reversibility of intestinal fibrosis

Depicted are the mechanisms and mediators that drive (green color) or inhibit (red color) reversibility of strictures in Crohn's disease. Abbreviations: Bcl-2: B-cell lymphoma-2; BMP-7: Bone morphogenetic protein; IL, Interleukin; IFN, Interferon; MAPK, MAP kinase; MMP, Matrix metalloproteinase; mTOR, mammalian target of rapamycin; ROCK, Rho-associated protein kinase; TGF, transforming growth factor; TNF, Tumor necrosis factor; TRAIL-R2: TNF-related apoptosis-induced ligand receptor 2