



Modern Treatments and Stem Cell Therapies for Perianal Crohn's Fistulas

Al-Maawali AKS¹, Nguyen P² and Phang PT^{1*}

¹Department of Surgery, St Paul's Hospital, Canada

²Department of Surgery, University of British Columbia, Canada

Abstract

Crohn's disease (CD) is a complex disorder with important incidence in North America. Perianal fistulas occur in about 20% of patients with CD and are almost always classified as complex fistulas. Conventional treatment options have shown different success rates, yet there are data indicating that these approaches cannot achieve total cure and may not improve quality of life of these patients. Fibrin glue, fistula plug, topical tacrolimus, local injection of infliximab and use of hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are newly suggested therapies with variable success rates. Here, we aim to review these novel therapies for the treatment of complex fistulizing CD. Although initial results are promising, randomized studies are needed to prove efficacy of these approaches in curing fistulizing perianal CD.

Keywords: Crohn's disease; Perianal fistula; Fibrin glue; Fistula plug; Infliximab; Stem cell therapy

Introduction

Crohn's disease (CD) is a complex disorder of uncertain etiology characterized by chronic recurrent inflammation of the bowel. The disease incidence in North America ranged from 3.1 to 20.2 cases per 100,000 persons per year in published epidemiological studies [1,2]. Perianal fistulas occur in about 20% of patients with CD and are almost always classified as complex fistulas [3]. The ideal outcome from treatment of these fistulas is complete closure with prevention of infection and abscess formation. However, intensive medical and surgical therapy has only success rates ranging from 30 to 80%. In view of incomplete fistula closure, treatment strategies have shifted from cure to reduction of fistula drainage and quality life improvement until more effective therapies become available.

Conventional Medical Treatments

Antibiotics, immunosuppressive drugs such as thiopurines, oral tacrolimus and anti-TNF alpha's role in the management of fistulizing CD have been reported with variable success rates when used as single agents or in combination. Antibiotics use in uncontrolled studies of fistulizing CD report symptom reduction but fail to result in fistula closure [4,5]. There was no significant difference between antibiotics and placebo in achieving complete fistula closure or/and improvement of fistula in a small sampled, randomized, double blinded, placebo control trial [6]. Effectiveness of thiopurines, including 6-metacaptopirine and azathioprine, studied by Pearson et al., [7] has been investigated in a meta-analysis of 5 controlled trials reporting complete fistula closure or reduction in fistula drainage in 54% of patients. Multiple randomized controlled trials showed that anti-TNF alpha treatments including infliximab, adalimumab and certolizumab are superior to placebo in induction treatment and maintenance therapy for perianal fistulas in CD [8-13]. However, development of antibodies against these agents has been reported and can result in loss of clinical response [14]. In addition, anti-TNF agents have been associated with opportunistic infections, serum sickness like reaction, autoimmune disorders and sepsis [15]. In a randomized control trial, although oral tacrolimus was effective in closure of 50% of CD fistulas, there was no difference in complete closure of all fistulas when compared to placebo [16].

Surgical Options

Fistulotomy with sphincterotomy is the preferred management for simple fistulas that results in high cure rates without fecal incontinence in non-CD fistulas. In CD fistulas with any degree of diarrhea, seton placement, advancement flaps and ligation of the intersphincteric fistula tract

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*Correspondence:

Phang PT, Department of Surgery, St Paul's Hospital, University of British Columbia, 1081 Burrard St. Vancouver BC, V6Z 1Y6, Canada, Tel: 604-806-8711; Fax: 606-806-9604; E-mail: tphang@providencehealth.bc.ca

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(LIFT) are surgical options that have higher recurrence rates in an attempt to avoid fistulotomy with sphincterotomy that could result in incontinence. Seton placement for chronic drainage does not cure fistulas but limits recurring perianal sepsis and is the standard surgical option for CD fistulas that is meant to improve quality of life in patients living with chronic disease [17-21]. Advancement flaps have healing rates from 60 to 70% but have increased complications over seton drains [17,22,23]. Data regarding effectiveness of the LIFT procedure in CD patients are lacking [24-26]. Best practice guidelines recommend that surgical interventions apart from seton placement to allow continuous drainage should be avoided [27,28].

Current combined medical and surgical management are reported to have better outcomes in the treatment of perianal fistulas in CD [29-31]. Yet, these approaches do not achieve cure and fail to sufficiently improve quality of life of these patients such that there is need for new and improved treatments. Fibrin glue, fistula plug, topical tacrolimus, local injection of infliximab and the use of hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are newly suggested therapies for these fistulas.

Fibrin Glue

Fibrin glue is a mixture of fibrinogen, calcium ions and thrombin that gets injected using a catheter into the fistulas tract and clot within 60 seconds. Preservation of anal sphincter function is a main advantage of this procedure, but early extravasation of the mixture from the fistulous tract and failure of exact identification of all fistula branches results in high recurrence rate. Data on fibrin glue effectiveness in CD is very limited. In a randomized control trial assessing fibrin glue effectiveness in CD patients, clinical remission was reported in 38% of the study group compared to 16% in the control group [32]. Lindsey et al. [33] reported that only two out of six CD patients treated with fibrin glue (33%) reported healing defined as no drainage. Longer-term follow-up data for fibrin glue in CD fistula has not been reported.

Fistula Plug

Fistula plug is a cone shaped plug synthesized from lyophilized porcine small intestine mucosa that is threaded through the fistulous tract and fixed in place with a suture. The strategy of plugs is similar to fibrin glue in simplicity of use and avoidance of injury to the anal sphincter muscle but is meant to decrease failure from dislodgement of glue. Chung et al. [17] reported healing rates of 75% at 12 weeks in 51 inflammatory bowel disease (IBD) patients of which 40 were CD patients.

However, most failures were reported to be due to early extrusion of the plug within 1 week after placement. In a systematic review published in 2012 by O'Riordan et al. [34] healing rates in non-CD and CD fistulas were reported to be 54.8% and 54.3% respectively.

Topical Tacrolimus

Use of topical tacrolimus in CD perianal fistulas was suggested from its effectiveness in treatment of immune mediated skin diseases [35] but studies about its usage in CD are very limited. In a recently published systematic review of tacrolimus use in CD [36], only 1 randomized controlled trial (RCT) was found addressing topical tacrolimus use in fistulizing perianal CD. Hart et al. [37] tested 12 CD fistulizing patients and concluded that there was no benefit from topical tacrolimus.

Intra-Lesional Infliximab

Multiple local injections of infliximab have been placed into the fistulous tract, internal opening and external opening. Two uncontrolled small sampled studies were published with healing rates around 70% [38,39]. Asteria et al. [39] excluded patients who received previous treatment with infliximab systemically or had rectal or perianal complications like proctitis or abscess. Local injections of infliximab was effective in 72.7% showing reduction in fistula drainage higher or equal to 50% and 36.4% achieving complete cessation of drainage for a minimum of 4 weeks. Alessandrini et al. [40] combined repeated peri-fistular infliximab injection with core-out fistulectomies as a treatment for refractory complex perianal fistulas in CD. Fistula closure at 12 months was observed in 7 out of 8 patients (87.5%) who completed the treatments. These are promising initial data and controlled randomized studies are needed to prove efficacy [40].

Stem Cell Therapy

Use of HSC and MSC in treatment of fistulizing CD was proposed after the serendipity of discovery using stem cells in treatment of CD in 1993. The first report of stem cells' ability to treat IBD patients was on a 41-year-old female who underwent an autologous HSC transplantation for non-Hodgkin's lymphoma [41]. The patient had also suffered from Crohn's disease since 1970, had partial colectomy in 1985 and developed rectovaginal fistula in 1986. After receiving a total of 3.07×10^8 /kg nucleated viable CD34+ HSCs transplanted, for 6 consecutive months being followed up in the outpatient clinic, she was surprisingly asymptomatic and needing no treatment for Crohn's disease. Another case of successful long-term control of Crohn's disease was subsequently reported in a 20-year-old male with non-Hodgkin's lymphoma who also developed a CD perianal fistula. The CD fistula was in stable condition for 7 years following autologous bone marrow transplantation. There was no clinical or laboratory evidence of recurrence of either Crohn's disease or non-Hodgkin's lymphoma in this patient [42].

CD fistulas likely result mainly from a long-term effect of an autoimmune condition. The concept of re-setting the exaggerated immune response with a brand new immune system using HSC transplant is strongly supported by observations of patients after undergoing HSC transplantation [43]. Before new HSCs are administered intravenously into the patient, the HSCs need to be mobilized from the bone marrow into the peripheral blood for harvest. The patient then needs to go through preparative treatments that result in ablation of their current immune system. This procedure will rescue the body from the exaggerated autoimmune condition and permit the new hematopoietic precursors to generate a new tolerant T-cell population [44].

From 1995 to 2007, 22 out of 25 IBD patients who underwent autologous HSC transplant for blood and bone marrow cancer were reported to achieve clinical remission over a median follow-up for 20 months, only two of which received ongoing treatment for Crohn's disease. Even though the original studies were not aimed to investigate the effect of autologous HSC transplant on IBD progression, the notion of long-lasting remission from CD and the potential improvement of related fistulizing disease by resetting new self-tolerant lymphocytes through chemotherapy was supported [45].

Burt et al. [46] published a long-term follow-up report on HSC therapy for IBD, in which 24 patients with severe anti-TNF refractory

Crohn's disease received non-myeloablative hematopoietic stem cell transplantation. Among these, 19% stayed in remission for 5 years, 57% for 3 years and 91% for 1 year after the transplantation. In another trial of HSC transplantation in severe CD patients, there was closure of the fistula tract in three out of four patients [47].

Although T-cell depletion of the graft will result in self-tolerance, safety with use of HSC transplantation is of significant concern because of the risks of infectious complication due to prolonged lymphopenia [48]. As such, all studies to date only focus on severe refractory Crohn's disease cases in which the risks are out-weighed by potential benefits. Because allogeneic HSC transplantation has high complication and mortality rates, it is not presently recommended treatment for autoimmune diseases [49]. A phase III trial on allogeneic HSC transplantation in Crohn's disease is currently recruiting participants ("Non-myeloablative allogeneic HSC transplantation for patients with refractory Crohn's disease", ClinicalTrials.gov Identifier: NCT01288053). This trial aims to evaluate the effects of allogeneic non-myeloablative HSC transplantation on patients with high-risk Crohn's disease.

Local Mesenchymal Stem Cell (MSC) Therapy in Fistulizing CD

A population of bone-marrow-derived mononuclear cells in culture will adhere to plastic forming colony units of fibroblast morphology [50]. With the ability to differentiate into various lineages such as adipocytes, osteocytes and cartilage cells, this population is identified as mesenchymal stem cells (also called mesenchymal stromal cells) [51,52]. These cells can self-renew and be maintained for prolonged periods in *ex vivo* condition [53]. Adipose tissue derived MSCs (ad-MSCs) can also be isolated from liposuction aspirates and have capability of exerting immunosuppressive functions and are a promising therapy for tissue regeneration in inflammatory related tissue injury at the local level [54]. With 90% similarity in the immunophenotypes, bone marrow derived MSCs (bm-MSCs) and ad-MSCs are alternatively being used for treatment of IBD. However, most current experiments use ad-MSCs due to their higher abundance in human adipose tissue [55,56]. Ad-MSCs' multi lineage capacity have been reported in *in vitro* and animal studies using a colitis mice model have shown ad-MSCs' ability to inhibit inflammatory and autoimmune responses [54].

In 2003, a case report was published as one of the first clinical treatments documented on cell therapy treating IBD using ad-MSCs [55]. A 33 year-old woman with Crohn's colitis for 11 years developed perianal suppurations and rectovaginal fistula. Her symptoms did not improve with infliximab and seton drainage. After receiving 9x106 adipose tissue derived-MSC local injection, her surgery wound closed completely within one week (with minor signs of inflammation) and there was no fecal incontinence or vaginal flatus being observed. The patient remained asymptomatic with no recurrence of a rectovaginal fistula within 3 months. In 2007, Ciccocioppo et al. [57] conducted a clinical trial involving 12 patients with complex perianal fistula and enterocutaneous fistula. Each patient received two to five intra-fistula injections with bm-MSCs every 4 weeks. At 12 months after the final injection, closure of external opening was evident with appearance of regenerative tissue. In trials performed by Garcia-Olmo et al., [58-60] ad-MSC therapy for complex perianal fistula had significant efficacy (more than 70%) versus fibrin glue in both Crohn's and non-Crohn's patients.

The phase III ADMIRE-Crohn's disease study will assess efficacy of ad-MSCs from healthy donors for the treatment of complex perianal fistulas in patients with Crohn's disease over a 24-week period and extended follow-up period up to 104 weeks ("Adipose derived MSCs for induction of remission in perianal fistulizing Crohn's disease", ClinicalTrials.gov Identifier: NCT01541579). Patients with perianal fistulizing Crohn's disease will be treated with 120x106 expanded ad-MSCs administered by intralesional injection. This study is currently enrolling patients with estimated completion in 2017. Another phase I study of autologous mesenchymal stromal cell coated fistula plug in patients with fistulizing Crohn's disease is recently recruiting participants ("Stem cell fistula plug in perianal Crohn's disease"; ClinicalTrials.gov Identifier: NCT01915927). The primary endpoint of this study is to determine the safety and feasibility of using ad-autologous MSC coated Gore Bio-A Fistula Plug as an option for new treatment.

Of note, a number of clinical trials have been terminated because they did not provide a feasible uniform protocol in cell isolation and selection, and cell expansion technique was not developed. It is suggested that for higher clinical efficacy robust priming for best MSCs culture needs to be done in advance together with a careful donor selection process and identification/isolation of its subpopulation with enhanced immunosuppressive properties [48]. Further, we do not yet have a good experiment set up *in vivo* showing the multilineage ability of MSCs and how to prevent MSCs from turning into something undesirable within patients.

Conclusion

Treatment of CD fistulas remains challenging with persisting detrimental effect on quality of life in these patients living with chronic disease. New local treatments including fistula plugs and local injection of infliximab after abscess drainage report promising early results but randomized trials of larger patient numbers and longer term follow-up are required. A handful of clinical studies using stem cell therapy have shown positive results. HSC and MSC therapies are not yet fully developed for technical procedure to be routinely and safely performed. There is a therapeutic gap between conventional treatments and cure of fistulizing CD until clinical trials prove efficacy of these novel treatments.

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