Journal of Crohn's and Colitis, 2021, 1–5
https://doi.org/10.1093/ecco-jcc/jjab154
Advance Access publication September 2, 2021
Short Report

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Mesenchymal Stem Cell Injection in Crohn's Disease Strictures: A Phase I–II Clinical Study

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Abstract

Background and Aim: Mesenchymal stem cells [MSCs] have anti-inflammatory and anti-fibrotic properties and could be a potential therapy for Crohn's disease [CD] strictures. In this phase I–II pilot trial, we assessed safety and efficacy of local MSC injection to treat CD strictures.

Methods: CD patients with a short [less than 5 cm in length] non-passable stricture accessible by ileocolonoscopy were included. Allogenic bone-marrow derived MSCs were injected in the four quadrants of the stricture. Adverse events and clinical scores were evaluated at each follow-up visit and endoscopy and magnetic resonance enterography were performed at baseline, Week [W]12 and W48. The main judgement criterion for efficacy was the complete [defined by the ability to pass the ileocolonoscope] or partial [defined by a diameter increase] resolution of the stricture at W12. Second efficacy criteria included assessment of the stricture at W48 and evolution of clinical scores at W12 and W48.

Results: We performed 11 MSC injections in 10 CD patients [three primary and seven anastomotic strictures; one stricture injected twice]. MSC injections were well tolerated but four hospitalisations for occlusion were reported. At W12, five patients presented a complete or partial resolution of the stricture [two complete and three partial]. Seven patients were re-evaluated at W48 [one dilated, one operated, and one lost to follow-up] and four patients had a complete resolution. The evolution of clinical scores between W0, W12, and W48 was not statistically significant.

Conclusions: MSCs injection in CD stricture was well tolerated and may offer a benefit.

Key Words: Crohn's disease; stricture; mesenchymal stem cells

1. Introduction

Intestinal fibrosis is a common complication of inflammatory bowel disease [IBD],[1] especially Crohn's disease [CD],[2] leading to strictures formation and bowel obstructions.[3] Beside surgical resection or stricturoplasty, endoscopic balloon dilatation [EBD] is commonly used to treat strictures but, albeit initially successful, such balloon dilatations have often to be repeated or followed by surgical resection.[4] The injection of intralesional steroids [betamethasone[5] or triamcinolone[6–10]] or antitumour necrosis factor α [infliximab[11]] following EBD has not dramatically changed the dilatation and surgery-free survival.[4,12] Whereas fibrosis in CD stricture is often the end result of chronic inflammation, there is an increased recognition that anti-inflammatory agents by themselves are insufficient in controlling or reversing advanced fibrosis in CD, which could explain

the absence of clear beneficial effects of intralesional steroid or biologic injection.[13] Mesenchymal stem cells [MSCs], known for their anti-inflammatory and anti-fibrotic properties, could potentially improve both inflammatory and fibrotic components of these CD strictures, but this has never been tested as local injection. In this phase I–II open-label pilot trial, we aimed to assess safety and efficacy of MSC injection in CD strictures.

2. Material and Methods

2.1. Patients

We included patients diagnosed with CD at least 6 months previously, aged 18 years or older, and having a symptomatic non-passable CD stricture, less than 5 cm in length (according to magnetic resonance enterography [MRE] measurement), endoscopically accessible, and refractory to conventional or biologic therapies [azathioprine, 6-mercaptopurine, methotrexate, anti-tumour necrosis factor, vedolizumab, ustekinumab]. Exclusion criteria were intestinal obstruction or indication for immediate surgery, intra-abdominal fistula or abscess, pregnancy or planning pregnancy within 1 year, terminal organ failure, human immunodeficiency virus positivity, uncontrolled infection, and a history of malignancy within the past 5 years. The study was approved by the ethics committee of Liège University and written informed consent was obtained from all patients.

2.2. Mesenchymal stem cell preparation and injection

MSC donors were healthy adult volunteers, unrelated to the recipient, and eligible for allogeneic haematopoietic cell donation. No human leukocyte antigen [HLA] matching was required. Bone marrow collection and MSC cultures were carried out at the Laboratory of Cell and Gene Therapy [LTCG] at the CHU of Liège, as previously described.[14,15] Freshly P3-harvested cells were washed and resuspended at 3.75 x 10^6 cells/mL in a 75% NaCl 0.9%/25% HAS 20% solution and conditioned in 2-mL syringes at room temperature. The MSC solution was locally injected into the stricture during ileocolonoscopy performed by a gastroenterologist with an expertise in IBD and therapeutic endoscopy. A total of 3 x 10^7 MSCs[16] were equally injected into the four quadrants of the submucosa of the strictured bowel wall.

2.3. Study design and endpoints

Adverse events, clinical scores [Crohn's Disease Activity Index,[17] Short Health Scale,[18] and Crohn's Disease Obstructive Score,[19]] as well as biomarkers [C-reactive protein and faecal calprotectin] were evaluated at each follow-up visit [at Weeks 4, 12, 24, 36, and 48] and endoscopy and MRE were performed at baseline, Week 12 and Week 48. The Crohn's Disease Obstructive Score is a 0–6 scale assessing the intensity of obstructive symptoms, taking into account duration and intensity of obstructive pain, associated signs such as nausea and vomiting, dietary restriction, and hospitalisation.[19] The main judgement criterion for efficacy was the complete or partial resolution of the stricture, assessed during ileocolonoscopy, at 12 weeks. Complete resolution was defined by the ability to pass an adult ileocolonoscope through the stricture, and partial resolution was defined by an increase of the diameter of the stricture as measured in comparison with the size of an open biopsy forceps [7 mm]. Secondary efficacy criteria included complete or partial resolution of the stricture at 48 weeks as well as the evolution of clinical scores and MRE images [stricture length, bowel wall thickness, pre-stricture dilatation, intramural T2 hyperintensity, three-layered target sign, homogeneous mural enhancement, late enhancement, and presence of a comb sign] between Weeks 0, 12, and 48. These images were reviewed by a single radiologist blinded for the clinical and endoscopic outcomes [PM]. All concomitant medications including conventional therapies, immunomodulators, or biologics were allowed during the study but had to remain stable till evaluation for primary endpoint at Week 12.

2.4. Statistical analysis

Demographic and disease-specific data are given descriptively or tabulated. Results are presented as medians and quartiles for continuous parameters or as frequency tables for qualitative parameters. Statistical tests were performed by using GraphPad Prism, version 8.1.2 for Windows [GraphPad Software, San Diego, CA].

3. Results

3.1. Patient and stricture characteristics

A total of 11 MSC injections were performed in the strictures of 10 patients [six females; median age 45 years, range 38–65 years; 70% were smokers] between May 2018 and July 2020; one patient was injected twice in the same anastomotic stricture at a 26-month interval. The stricture [three de novo and seven anastomoses] locations were as followed: seven at an ileo-colonic or ileo-rectal anastomosis, one colonic, and two of the terminal ileum. The median stricture length was 23 mm [range, 6.3–150 mm; three patients were protocol violations after central reading by PM, with a length of 70 mm for two and 150 mm for one], and five patients [50%] presented abnormal mucosa [inflammation/ulceration] on endoscopy. A previous EBD was performed in six patients. Patient and stricture characteristics are detailed in Table 1 [see also Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online, for details by patient].

3.2. Safety

MSC injection was safe, and no immediate side effect was identified. No patient developed abscess or fistula in the follow-up. One patient had actually a small intestinal fistula at baseline [not diagnosed by the routine imaging report but disclosed by the central reading by PM]. A total of five hospitalisations were reported: one for a disease flare-up and four for occlusion [one secondary to a different stricture, at Week 37] leading to a surgical resection for one patient [at Week 22] and to an EBD, twice, for the patient who underwent two procedures [at Week 39 for the first and Week 2 for the second one]. One patient developed a basocellular carcinoma [Week 24] which was completely resected.

3.3. Efficacy

At 12 weeks, five patients presented a complete [two patients] or partial [three patients] resolution of the stricture. The stricture diameter could not be measured in one patient due to poor preparation. Out of the 11 MSC injections, seven strictures were re-evaluated at Week 48 [one operated, one dilated twice, and one lost to follow-up] and four patients had a complete resolution [Figure 1]. Figure 2 shows the evolution of stricture diameter, Crohn's Disease Obstructive Score, and pre-stricture dilatation between Weeks 0, 12, and 48. There was no statistically significant change of these parameters nor of other clinical scores, biomarkers, and the stricture characteristics analysed

by MRE [Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online] except the disappearance of T2 submucosal oedema in two endoscopic responders.

Table 1. Patient characteristics

Parameter	*n* = 10 [%]
Female gender	6 [60.0]
Age at inclusion (years, median [IQR])	45 [42.5–49]
Disease duration (years,median [IQR])	21 [14.5–25.5]
Smoking habits	
Never	1 [10.0]
Past smoker	3 [30.0]
Active smoker	6 [60.0]
Medication during follow-up[a]	
None	2 [20.0]
Budesonide	3 [30.0]
Methylprednisolone	1 [10.0]
Immunomodulators	4 [40.0]
Anti-TNF therapy	4 [40.0]
Ustekinumab	4 [40.0]
Previous luminal surgery	
Yes	7 [70.0]
No	3 [30.0]
CDOS(median [IQR])	3 [2-4]
Disease activity at inclusion	
CDAI (median [IQR])[b]	204 [127–271.5]
CRP (mg/L, median [IQR])[b]	4.75 [1.8–7.7]
Faecal calprotectin (µg/g, median [IQR])[b]	67 [32–195]
CDEIS (median [IQR])[b]	3 [3–6.5]

IQR, interquartile range; CDAI, Crohn's Disease Activity Index; CDOS, Crohn's Disease Obstructive Score; CRP, C-reactive protein; TNF, tumour necrosis factor α; CDEIS,Crohn's Disease Endoscopic Index of Severity.

[a]Some patients are under combination therapy.

[b]Data not available for all subjects.

4. Discussion

Whereas clinical studies have demonstrated the safety and variable efficacy of intravenous [IV] MSC administration to treat luminal CD[15] and of local MSC administration to treat perianal CD fistulas,[16,20,21] the present study is the first, to our knowledge, to evaluate MSC injection in CD strictures. Similarly to intralesional injection of steroids[4,6,22] or infliximab,[11,23] local MSC injection was well tolerated in the short term and no complication such as intestinal perforation, abscess, or new intra-abdominal fistula was reported. Several occlusions were reported in the follow-up, probably related to the insufficient efficacy of the MSC injection in those cases.

The efficacy of MSC injection in the strictures remains difficult to interpret in this study in absence of a control group. We cannot exclude that the diameter increase observed in some patients was related to the medical CD concomitant treatment[24] rather than the MSCs. However, if we evaluate the need for re-intervention [EBD or surgery] within 24 to 48 weeks, generally considered as an appropriate endpoint according to expert consensus,[13] only two patients [20%] needed further endoscopic or surgical intervention. This result is in fact quite close to what has been observed with EBD,[25,26] and seems encouraging for a local treatment of symptomatic non-passable CD stricture. As EBD implies elongation and rupture of fibrous tissue rich in collagen and could result in further tissue damage by inflammation, oedema, additional fibrosis, and scarring, a combined treatment with MSCs might be beneficial and could prevent re-scarring.[5]

Indeed, besides their known anti-inflammatory properties, MSCs have been shown to have an anti-fibrotic effect in several organs[27] by acting through different pathways including release of anti-fibrotic molecules [such as hepatocyte growth factor[28] and tumour necrosis factor TNF]-stimulated gene 6,[29]] and by inhibiting extracellular matrix [ECM] remodelling[30] and TGF-β activation,[27,31] known to play a key role in intestinal fibrosis. In animal models, it has been shown that MSC culture supernatant can prevent luminal stricture

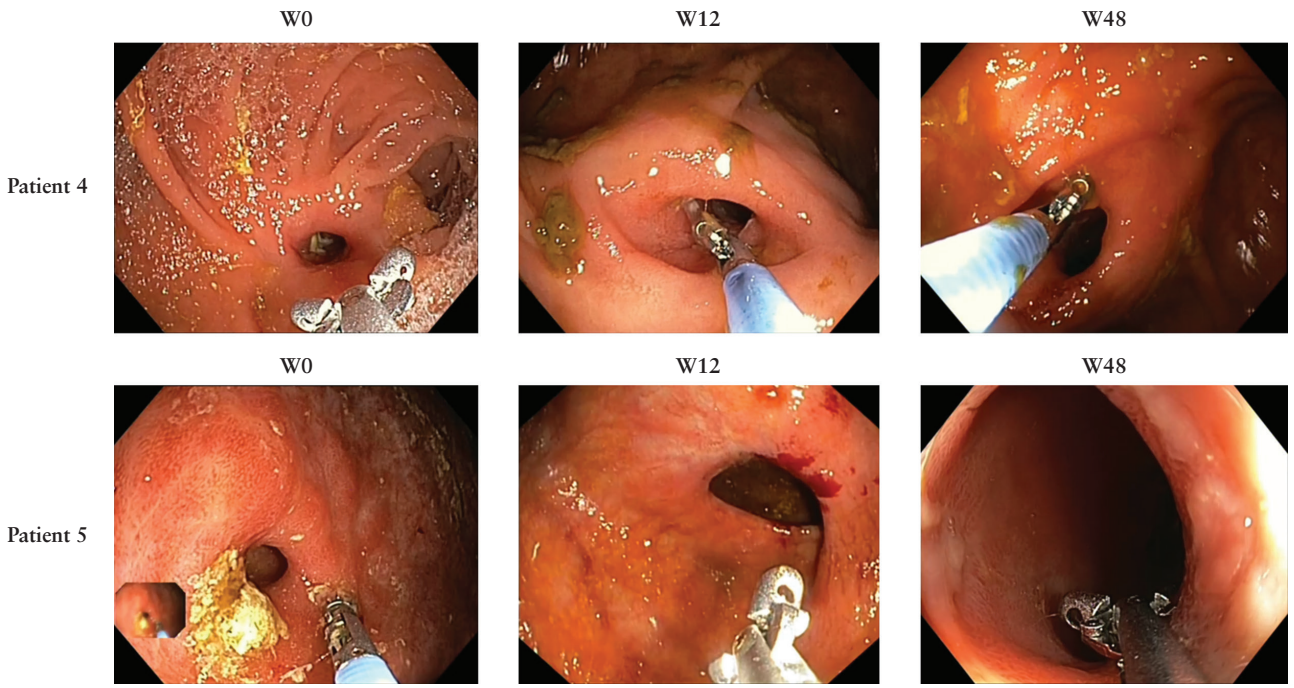

Figure 1. Evolution of the stricture diameter in patients 4 and 5 at Weeks 0, 12, and 48.

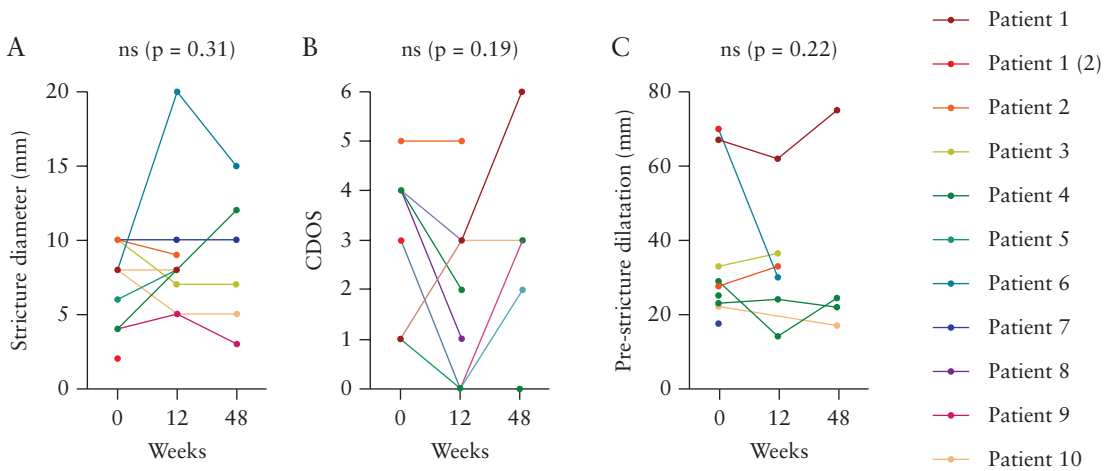

Figure 2. Evolution of the stricture diameter [mm] [A], Crohn's Disease Obstructive Score [CDOS] [B], and pre-stricture dilatation [mm] [C] between Weeks 0, 12, and 48. ns, not significant.

development after endoscopic submucosal dissection [ESD] in the oesophagus[32] and rectum[33] of pigs by inhibiting inflammatory cell infiltration, myofibroblast activation, fibre accumulation, and hypertrophy of the muscularis propria. Recently, the role of MSCs as prophylactic or therapeutic treatment for CD-associated intestinal fibrosis was investigated in a 2,4,6-trinitrobenzene sulphonic acid [TNBS]-induced mouse model.[34] Whereas prophylactic treatment with MSCs allowed inhibition of the expression of fibrotic proteins as well as the accumulation of fibrotic tissue, the use of MSCs as therapeutic treatment reversed the established intestinal fibrosis. It was shown that MSCs down-regulated the secretion of fibrogenic factors [such as IL-1beta, IL-6, and IL-13] and up-regulated anti-fibrogenic factors [such as IL-10], reduced the epithelial-to-mesenchymal transition process [which is a contributing source of fibroblasts in intestinal fibrosis[35]], and inhibited the expression of the TGF-beta/Smad signalling pathway [the most important signalling pathway associated with CD intestinal fibrosis[36]], which makes it a promising adjuvant treatment for CD strictures.

This study has several limitations. First, the small sample size does not allow e conduct of a meaningful statistical analysis to identify predictive factors of response. Second, the lack of control group and the fact that patients received concomitant systemic biologic therapy does not allow evaluation of the beneficial role of MSCs.

In conclusion, local MSC injection in non-passable CD strictures was well tolerated over the short term, although several occlusions occurred in the follow-up, indicating insufficient therapeutic effect in those cases. Combining the effects of MSCs with the proven effect of EBD could possibly improve the outcome of CD stricture.

Funding

The LTCG is supported in part by the Fonds National de la Recherche Scientifique [FNRS] and the 'Federal cancer plan — Belgium'. SV was financially supported by the FNRS [grant numbers 32729160, 40001034]. This project was supported by a research grant [FIRS] from the CHU Liège.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online, contains all the data, which are available at [https://dox.ulg.ac.be/index.php/s/NsjDIkI19W0qlp7], and can be accessed with the following password: 13072021.

Author Contributions

LB, YB, and EL designed the clinical study. JPL performed MSC injection. PM performed the MRE central reading. AB, CL, and EB contributed to the MSC production. SV and EL analysed the data and wrote the paper. All authors revised the manuscript and improved its intellectual content.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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