INDEX COPERNICUS INTERNATIONAL

Cell therapy in surgical treatment of fistulas – preliminary report

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

Marcin Piejko[1,2ABDEF], Michał Romaniszyn[1ABD], Julia Borowczyk-Michałowska[2B], Justyna Drukała[2 GEF], Piotr Wałęga[1 BDEFG]

[1]3rd Department of General Surgery Medical College Jagiellonian University, Krakow, Poland: Head: prof. dr hab. med. Wojciech Nowak
[2] Cell Bank, Department of Cell Biology, Faculty of Biochemistry
[3] Department of Analytical Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland: Head: prof. dr hab. Zbigniew Madeja

Article history: Received: 19.03.2017 Accepted: 22.03.2017 Published: 30.06.2017

ABSTRACT:

Background: Risk of recurrence after surgical treatment of a recurrent fistula is up to 50%. It has be known that more aggressive surgical treatment is associated with a high risk of anal sphincter damage and leads to incontinence. Several studies have been designed to elaborate minimally invasive treatment of rectovaginal and anal fistulas. The properties of Adipose-derived Stem Cells (ASC) significantly enhance a natural healing potency. Here, we present our experience with combined surgical and cell therapy in the treatment of fistulas.

Materials and Methods: Four patients were enrolled in our study after unsuccessful treatments in the past – patients 1-3 with rectovaginal fistulas including two women after graciloplasty, and patient 4 - a male with complex perianal fistula. Adipose tissue was obtained from subcutaneous tissue. ASCs were isolated, cultured up to 10+/-2 mln cells and injected into the walls of fistulas. Follow-up physical examination and anoscopy were performed at 1, 4, 8, and 12 weeks, 6 and 12 months after implantation.

Results: Up to 8 weeks after ASC implantation, symptoms of fistulas' tracts disappeared. At 8 weeks, in patients 1-3, communication between vaginal and rectal openings was closed and at 12-16 w. intestinal continuity was restored in patient 3 and 4. After a 6-month follow-up, the fistula tract of patient 4 was closed. Up to 12 m. after ASC implantation no recurrences or adverse events were observed.

Conclusion: ASCs combined with surgical pre-treated fistula tracts were used in four patients. All of them were healed. This encouraging result needs further trials to evaluate the clinical efficiency and the cost-effectiveness ratio.

KEYWORDS: stem cell-based therapy; recurrent fistula; regenerative medicine; rectovaginal fistula, anal fistula;

INTRODUCTION

Anal fistulas, defined as abnormal channels between the rectum and the surrounding structures, are still a big problem in everyday surgical practice. Aggressive surgical treatment results in the highest healing rate, however, are associated with the risk of sphincter damage and incontinence. On the other hand, less aggressive methods are associated with the risk of recurrence1. The increasing popularity of minimally-invasive treatment and lowering the risk of incontinence requires new techniques promoting healing of fistulas, especially considering Crohn's disease, recurrent or branching fistulas. Thus far, minimally-invasive methods, such as fibrin sealants 2,3 and pig-collagen plugs 4,5 proved to be unsuccessful.

Significant advancement in treating fistulas, especially those most troublesome found in Crohn's disease, has been achieved by Garcia-Olmo team using the adipose-derived stem cells (ASC)6. This type of mesenchymal stem cells derived from the adipose tissue shows a number of features including immunomodulation7,8, releasing growth and proangiogenic factors9, promoting fibroblast migration to the wound and production of collagen10 as well as differentiation of mesenchymal cells11. The properties of ASCs are perfect for healing of large tissue defects. Therefore, a reasonable use of stem cells along with proper surgical treatment allowed to achieve 50% to 90% healing rate of fistulas in Crohn's disease in the study by Garcia-Olmo team12,13, as well as other authors14-17. In those studies, millions of cells were injected into the lumen and walls of the fistula immediately after surgical intervention as part of a single procedure.

The aim of this article is to present the preliminary results of combined surgical and cell therapy of recurrent fistulas. Stem cells were administered to patient, for whom successive surgical interventions would have been associated with an increased risk of incontinence.

MATERIALS AND METHODS

Patients

Patients, in whom the fistula had remained for at least 12 months despite at least 2 surgical interventions were qualified for combined therapy. The group of 4 patients consisted of three women with a rectovaginal fistula and one male with a transsphincteric fistula of cryptoglandular origin (according to Parks18) aged 30 to 50. Their detailed characteristics are shown in Table 1. In two females, the rectovaginal fistula was formed by peripartum perineal tear. In the third female, the fistula was formed as a result of a mechanical injury, probably sexual one. In all three women, fecal incontinence and release of gas through the vagina was seen despite earlier reconstructive interventions. On physical examination as well as on transrectal ultrasound, an extensive defect of rectal and vaginal walls was visible above the sphincter. In the male, a transsphincteric branching fistula was diagnosed with visible internal and external openings. Subjective symptoms, gas release and purulent discharge persisted for more than 12 months despite numerous surgical interventions.

The experimental therapy (or so-called 'compassionate use') included a surgical intervention followed by implantation of stem

DOI: 10.5604/01.3001.0010.1019

Tab. I.

ID	SEX, AGE	TYPE OF FISTULA	ETIOLOGY, DURATION	SURGICAL INTERVENTIONS	RESOLUTION OF SYMPTOMS	NO COMMUNICATION	OTHER
1	Female aged 30	Rectovaginal	Postpartum, 12 months		4-8 weeks	8 weeks	
2	Female, aged 33	Rectovaginal	Postpartum, 6 years	Fistula excision, Gracilloplasty, colostomy	4-8 weeks	8 weeks	Restoration of GI continuity on month 5
3	Female, aged 50	Rectovaginal	Mechanical (sexual practices), 5 years	Fistula excision with wall plasty, Gracillopasty, colostomy	4-8 weeks	8 weeks	Restoration of GI continuity on month 4
4	Male, aged 32	Rectal, transsphincteric, recurrent	cryptoglandular, 12 months	LIFT*	12 weeks	6 months	

* **LIFT** – Ligation of InterSphincteric Fistula Tract

cells conducted in the years 2013 to 2014, which was approved by local bioethics committee and was in accordance with Polish legal regulations as well as the Declaration of Helsinki. Each patient was informed about the risk associated with experimental therapy and gave informed consent to participate. As part of a diagnostic process and preparation for the procedure, all patients underwent endoscopy and transrectal ultrasound, while the degree of defecation control was assessed based on standard questionnaires (FISI).

2.2 Treatment

In order to increase the survival rate of stem cells at the application site, ASCs were applied 2-4 weeks after surgery. In 2 patients, it followed the transposition of the gracilis muscle to the space between vagina and rectum in order to restore the rectovaginal septum. In the third patient, they were applied to the slit-like canal after previous plasty of the vagina and rectum. In patient 4, the first stage was the ligation of the intersphincteric fistula tract (LIFT)19. At the end of the surgical procedure, adipose tissue was taken from the abdomen, which was later placed in a sterile container filled with saline and transported to the cell bank. Cultures were conducted according to the Good Manufacturing Practice (GMP). After intensive rinsing with saline and 1% penicillin / streptomycin, the tissue was cut into small pieces and weighted. Further conduct was according to the protocol described11. For each cultures, three passages were performed, until obtaining 8-12 mln ASCs (Patient 1 – 10 mln, Patient 2 – 10 mln, Patient 3 – 12 mln, Patient 4 – 8.5 mln). After that, the cells were transferred into one falcon tube, centrifuged twice changing the supernatant for Ringer's solution. Finally, the cells were inoculated in 2 mL of Ringer's solution and provided in sterile syringes. The cell survival rate was at least 95%. Phenotyping and differentiation into adipocytes, osteocytes and myocytes was assessed after the first passage and was in accordance with mesenchymal stem cell characteristics.

Approximately ¾ of suspended cells were administered to the fistula walls under visual guidance. The rest of the mixture was administered in a similar manner, however, from the vaginal opening site (patients 1-3) or external opening on the skin (patient 4). After 6h since the application of cells, the patients were discharged home.

2.3 Observation

After application of the adipose-derived stem cells, follow-up visits were continued for at least 12 months at the following intervals: 1, 4, 8, 12 weeks and 6, 12 months (Figure 1A). In addition to subjective evaluation, physical examination and transrectal ultrasound were performed. As full healing, we assumed a lack of clinical symptoms, a lack of communication between the primary openings on physical examination, and as a lack of recurrence – no channel present on the 12th month after cell therapy.

RESULTS

The interventions and observations were conducted according to the scheme shown in Figure 1A. In all patients with rectovaginal fistula, no fistula was noticed on physical examination 8 weeks afterwards. However, between 4 and 8 weeks, the subjective symptoms resolved (stool and gas release through the vagina) (Table I). In the fourth patient, no discharge from the external opening was observed after 8 weeks. In patients with a stoma, the continuity of the alimentary tract was restored 4 months after application of cells. Six months after application of ASCs, full healing of fistula openings was observed (Figure 1B). On one-year observation, no recurrence or adverse reactions were noted. Due to the limited size of the patient group and the therapeutic nature of the intervention, no statistical analysis was performed.

4. DISCUSSION

Our preliminary experience suggests that combination of surgical treatment with cell therapy is an interesting alternative for patients with difficult forms of anal fistulas, especially rectovaginal fistulas. In the group of patients receiving such treatment, full healing of a fistula was achieved up to 8 weeks in the case of small tissue defects and within 3-6 months in the case of a very large soft tissue defect, while using a relatively low number of ASCs (only 10 mln vs. tens or hundreds of millions14,15,17) on single application (vs sequential application13-15). On observation for over a year, no recurrence was noted. A natural limitation of our observational study is the small size of the patient group, however, in spite of that, our results are similar to those presented by the Garcia-Olmo team20. It should be mentioned that only patients with fistulas that were difficult to treat were enrolled in the compassionate-use study, where further surgical treatment would be associated with the risk of fecal incontinence (patients 1 and 4) or sustaining stoma (patients 2 and 3) and hence it would lower the quality of life.

Despite encouraging results of numerous clinical studies12-17,21,22, cell therapy in fistula management is still considered an investiga-

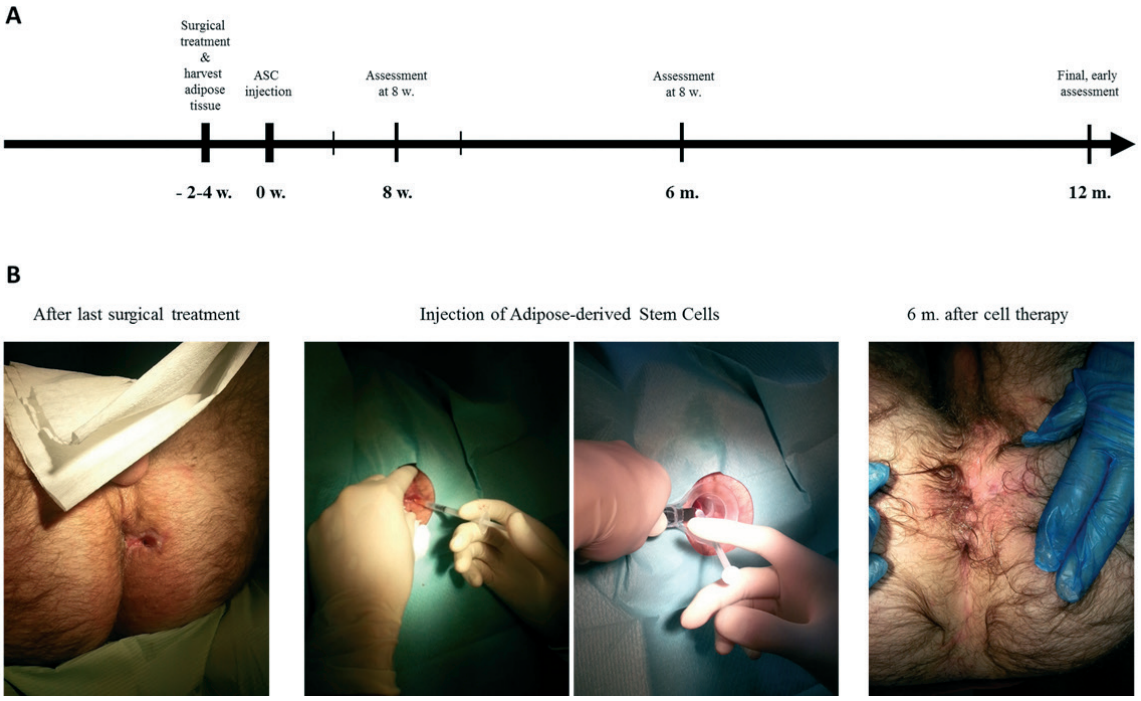

Fig. 1. A. Conduct scheme during therapy. B. Patient 4: on the left – photography of fistula following LIFT, in the middle – photography of cell administration, on the right – photography taken 6 months after administration of cells (full healing was noted with epithelialization of the external opening).

tional method. It is hard to unequivocally determine the efficacy of ASC therapy due to the fact that each of the previously mentioned studies had a slightly different study design20. Most studies regarded fistulas formed in the course of Crohn's disease12,14-16, while two studies covered recurrent fistulas in the course of Crohn's disease as well as other diseases13,20. In those studies, mesenchymal stem cells derived from the adipose tissue6,12-15,22,23 or bone marrow16,17 were used, suspending those cells in fibrin sealant13,14 or not13,17,20,21,23. In all studies conducted so far, surgical intervention was combined with cell application as a single-step procedure, reaching the healing rate of 50-85% 12,14-17,21,22 and 50-90%13,20 for fistulas in the course of Crohn's disease and other diseases respectively.

Somatic cell therapy is classified in the European Union as an advanced therapy medicinal product (ATMP). It imposes an obligation to produce cells according to the Good Manufacturing Practice (GMP), which raises production costs (according to our estimation, around ten times). GMP rules also imply strict control of cooperation between clinicians and the cell bank while transferring the source tissue and the final cell product. However, implementation of those rules significantly increases patient safety during cell therapy. Total cost of producing 10-40 mln ASCs in Poland is similar to that reported by Garcia-Olmo20. Production-related cost in a classic cell bank can only be lowered by increasing the production scale (maximal decrease by 30%) or shortening the incubation time (ca. 1-5% per day).

SUMMARY

In conclusion, healing of the fistulas was achieved in all patients regardless of the type and etiology of the fistula, the size of soft tissue defect and previous interventions.

During our observation, no side effects relating to cell therapy and surgical treatment were noted. However, further research is needed on a larger group of patients, which would allow to determine the clinical efficacy and precise indications for ASC application. Despite a relative high cost of cell products, ASC therapy brings a chance of complete cure for patients with recurrent fistulas, and hence it can drastically lower the costs of chronic treatment and successive surgical interventions, as well as of work absence, hygienic products and psychological counseling.

REFERENCES

1. Blumetti J., Abcarian A., Quinteros F., et al.: Evolution of treatment of fistula in ano. World J. Surg. 2012; 36: 1162–1167.
2. Cintron J.R., Park J.J., Orsay C.P. et al.: Repair of fistulas-in-ano using fibrin adhesive: long-term follow-up. Dis. Colon Rectum. 2000; 43: 944–949.
3. de Parades V., Far H.S., Etienney I., et al.: Seton drainage and fibrin glue injection for complex anal fistulas. Colorectal Dis. 2010; 12: 459–463.
4. O'Connor L., Champagne B.J., Ferguson M.A., et al.: Efficacy of anal fistula plug in closure of Crohn's anorectal fistulas. Dis. Colon Rectum. 2006; 49: 1569–1573.
5. Garg P., Song J., Bhatia A., et al.: The efficacy of anal fistula plug in fistula-in--ano: a systematic review. Colorectal Dis. 2010; 12: 965–970.
6. Garcia-Olmo D., Garcia-Arranz M., Garcia L.G., et al.: Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. Int. J. Colorectal Dis. 2003; 18: 451–454.
7. Keyser K.A., Beagles K.E., Kiem H.P.: Comparison of mesenchymal stem cells from different tissues to suppress T-cell activation. Cell Transpl. 2007; 16: 555–562.
8. Gonzalez M.A., Gonzalez-Rey E., Rico L., et al.: Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology. 2009; 136: 978–989.
9. Lee E.Y., Xia Y., Kim W.S., et al.: Hypoxia-enhanced wound-healing function of adipose-derived stem cells: Increase in stem cell proliferation and up-regu-

lation of VEGF and bFGF. Wound Repair Regen. 2009; 17: 540–547.

10. Kim W.-S., Park B.-S., Sung J.-H., et al.: Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J. Dermatol. Sci. 2007; 48: 15–24.
11. Zuk P.A., Zhu M., Mizuno H., et al.: Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001; 7: 211–228.
12. Garcia-Olmo D., Garcia-Arranz M., Herreros D., et al.: A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis. Colon Rectum. 2005; 48: 1416–1423.
13. Herreros M.D., Garcia-Arranz M., Guadalajara H., et al.: Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: fistula Advanced Therapy Trial 1) and long-term evaluation. Dis. Colon Rectum. 2012; 55: 762–772.
14. Lee W.Y., Park K.J., Cho Y.B., et al.: Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. Stem Cells. 2013; 31: 2575–2581.
15. Cho Y.B., Lee W.Y., Park K.J., et al.: Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. Cell Transplant. 2013; 22: 279–285.
16. Ciccocioppo R., Gallia A., Sgarella A., et al.: Long-Term Follow-Up of Crohn Disease Fistulas After Local Injections of Bone Marrow-Derived Mesenchymal Stem Cells. Mayo Clin. Proc. 2015; 90: 747–755.
17. Molendijk I., Bonsing B.A., Roelofs H., et al.: Allogeneic Bone Marrow–Derived Mesenchymal Stromal Cells Promote Healing of Refractory Perianal Fistulas in Patients With Crohn's Disease. Gastroenterology. 2015; 149: 918–927.
18. Parks A.G., Gordon P.H., Hardcastle J.D.: A classification of fistula-in-ano. Br. J. Surg. 1976; 63: 1–12.
19. Rojanasakul A.: LIFT procedure: a simplified technique for fistula-in-ano. Tech. Coloproctol. 2009; 13: 237–240.
20. Garcia-Olmo D., Guadalajara H., Rubio-Perez I., et al.: Recurrent anal fistulae: limited surgery supported by stem cells. World J. Gastroenterol. 2015; 21: 3330–3336.
21. Garcia-Olmo D., Herreros D., Pascual M., et al.: Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. Int. J. Colorectal Dis. 2009; 24: 27–30.
22. de la Portilla F., Alba F., Garcia-Olmo D., et al.: Expanded allogeneic adipose--derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. Int. J. Colorectal Dis. 2013; 28: 313–323.
23. Wałęga P: Mesenchymal Stem Cells in Treatment of Perianal and Rectovaginal Fistulas. Gastroenterol Hepatol Open Access 2016; 5
24. 24. Guadalajara H., Herreros D., De-La-Quintana P., et al.: Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. Int. J. Colorectal Dis. 2012; 27: 595–600.

Word count: 1900 Page count: 4 Tables: 1 Figures: 1 References: 24

DOI: 10.5604/01.3001.0010.1019

Table of content: http://ppch.pl/resources/html/articlesList?issueId=9993

Competing interests: The authors declare that they have no competing interests.

Corresponding author: Piotr Wałęga, III Katedra i Klinika Chirurgii Ogólnej, Uniwersytet Jagielloński Collegium Medicum, Kraków, Polska; ul. Prądnicka 35-37, 31-202 Kraków; tel. +48 12 633 19 95, fax: +48 12 633 31 05; e-mail: pwalega@mp.pl

Cite this article as: Piejko M., Romaniszyn M., Borowczyk-Michałowska J., Drukała J., Wałęga P.; Cell therapy in surgical treatment of fistulas. Preliminary report; Pol Przegl Chir 2017: 89 (3): 48-51