

Mesenchymal Stem Cells and PRP combined therapy promotes gastric leak closure following sleeve gastrectomy

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Received date: May 02, 2022
Accepted date: May 17, 2022

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Citation: Lecorgne E, Amor IB. Mesenchymal Stem Cells and PRP combined therapy promotes gastric leak closure following sleeve gastrectomy. Arch Stem Cell Ther. 2022;3(1):18-22.

Abstract

Sleeve gastrectomy is the most common bariatric surgery worldwide. However, such a surgery carries risk of complications associated with morbidity and mortality. Gastric leak can occur and represents one of the most severe complications following sleeve gastrectomy. Since the two last decades, regenerative medicine has emerged, offering new strategies to face to sleeve gastrectomy complications. Among these, autologous transplantation of material appears to be promising. Indeed, platelet rich plasma-based therapy has gained interest in several fields since it contains many bioactive materials involved in the hemostatic process. Moreover, mesenchymal stem cells-based approaches appear as the gold standard strategy as it takes advantage of stem cells plasticity, renewal and repair of damaged tissue abilities. In this article, we combined plasma rich platelet with mesenchymal stem cells therapy on obese rat models that went under gastric leak following sleeve gastrectomy. The data suggest that this combined therapy appears to be promising since it favors the healing process. Altogether, it suggests that stem cell therapy combined with plasma rich platelet may become a new tool in the treatment of gastric leak following sleeve gastrectomy.

Keywords: Sleeve gastrectomy, Gastric leak, Bariatric surgery, Regenerative medicine, Mesenchymal stem cells, Platelet rich plasma

Introduction

Obesity, defined as an excessive fat accumulation, is growing dramatically over the world, increasing the risk of morbidity and mortality. According to the World Health Organization, this disorder affected 13% of the world's adult population in 2016 [1]. Different treatments exist for obesity, including non-surgical (physical activities, diets) and surgical approaches such as bariatric surgeries, e.g., Roux-en-Y gastric bypass, laparoscopic adjustable gastric band or sleeve gastrectomy (SG). SG has become the most common bariatric surgery worldwide and performed in more than 50% of the bariatric surgeries [2,3]. SG consists in a duodenal switch enabling sustained weight loss. However, SG carries a risk of complications with an overall morbidity rate of 6% and a mortality rate under 0.3% [2,4,5]. Among these, gastric leak represents one of the most severe complications following SG surgery. It occurs between 0.7% and 5% of the cases and is associated to decrease in the quality of life, but also morbidity [6,7].

Early diagnosis and gastric leak treatment are necessary to avoid lethal complication of SG. Gastric leak following SG requires therapeutic management such as drainage, surgical exploration, endoscopic techniques, total enteral/parenteral nutrition or application of plugs. Although new treatments of gastric leak are emerging, chronic leaks or fistula, characterized by a long healing process, are still reported.

Among the new strategies to face to gastric fistula, regenerative medicine has emerged in the last two decades. Regenerative medicine consists in repairing organs or damaged tissue. It encompasses two main strategies to promote regeneration: cell therapy approach, defined by cell injection into the circulation of the injured tissue or tissue engineering, combining cells and biocompatible matrix injection [8]. These strategies rely on autologous transplantation taking advantage of the patient's material to favor healing of damaged tissues.

Among regenerative medicine, Platelet Rich Plasma (PRP)-based therapy has emerged. PRP is defined as a preparation of platelet concentrates contained in plasma, obtained from autologous blood. PRP-based therapy has gained interest in regenerative medicine among several fields such as sport medicine, dental or maxillofacial surgery or even wound healing since it contains many bioactive factors [9]. Platelets, stored into granules, are known to be involved in healing processes through the release of proteins involved in clotting, enabling hemostatic ability.

Moreover, among autologous transplantation strategies, Mesenchymal Stem Cells (MSCs) appear as the gold standard strategy as stem cells exhibit plasticity, renewal and repair damaged tissue capacities [10]. According to the minimum criteria to define MSCs of the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, MSCs have the ability to adhere to plastic, express some specific surface antigen (i.e. CD105, CD73 and CD90) and have multipotent differentiation potential into several mesenchymal lineages including cartilage, bone, tendon, muscle and fat, giving rise to osteoblasts, adipocytes or chondrocytes [11,12]. Although MSCs can be easily isolated from different sources, the primary source is the bone marrow [13,14], facilitating their isolation and making them attractive for regenerative medicine. Since MSCs are multipotent and self-renewal progenitor cells, *in vivo* experiments showed that they can enhance wound-healing process after skin injury through the secretion of cytokines stimulating angiogenesis upon inflammation [15,16]. Since 2003, the use of autologous MSCs as a therapy for the treatment of human complex and chronic anal fistula has shown encouraging results with complete closure [17,18].

Finally, PRP-MSCs combined therapy has been already tested in human for the treatment of complex recurrent cryptoglandular fistula-in-ano allowing long-term healing where no post-operatively adverse effect has been reported [19]. However, the effectiveness of a combinational treatment with PRP and MSCs in gastric leak closure following SG still needs to be investigated.

Results

In this study, we compared the efficacy of a combination of PRP and MSCs in the treatment of a gastric fistula after SG in adult male obese Zucker rat model [20]. Two groups of rats went under SG with an artificial leak. For the experimental group, a mixture containing PRP and MSCs from a donor rat was injected on the gastric leak edges first, then a PRP-MSCs solution also containing calcium chloride was applied in order to form a clotted gel. Indeed, adding calcium chloride into PRP solution, has been reported to induce platelet activation facilitating clot formation and stability, without affecting MSC survival [21,22].

PRP and MSC extraction were achieved from the blood and bone marrow of Zucker donor rat, respectively. Bone marrow-derived MSCs were resuspended with the PRP solution, then calcium chloride solution was added or not, in order to obtain the mixtures for cellular therapy. The efficacy on leak closure was evaluated by comparing stomach histological samples after hematoxylin-eosin-saffron (HES) staining from the two groups following one, two, three or four weeks after the SG.

Traditionally, the healing process occurs by steps, all regulated and drove by different cells, growth factors or cytokines. First, after an

injury, platelets are activated to form a fibrin clot. Then, an increase of permeability promotes the migration of inflammatory cells (e.g., neutrophils) to the wounded site. This results in a promoting hemostasis process and epithelial regeneration. Then, fibroblast proliferation, which produces collagen, leads to the establishment of granulation tissue. Finally, fiber reorganization and remodeling, thickening of collagen fibers and wound contractions appear [23-25]. Altogether, these different steps contribute to the regeneration of the tissue.

In our study, we defined three histological characterizations of the samples: inflammation, fibrosis and mucosae regeneration phases. Inflammation phase was subdivided into acute, sub-acute or chronic signs of inflammation. Acute signs were characterized by the presence of a high density of polymorphonuclear neutrophils. Sub-acute signs were defined by a lower density of polymorphonuclear neutrophils and higher density of lymphocytes and plasmacytes. Fibrosis was evaluated and subdivided into no fibrosis, early signs of fibrosis with cell granulomas, advanced fibrosis or major fibrosis groups. Finally, mucosae regeneration was analyzed and sorted into no regeneration, early signs of mucosae renewal, advanced or complete mucosae regeneration phases.

We demonstrated that using regenerative medicine combining PRP and MSCs significantly promotes tissue regeneration and therefore fistula healing process providing a closure of the leak site occurring after SG (Figure 1). Indeed, we showed that cellular therapy has induced early signs of fibrosis and mucosae regeneration from the first week after SG, whereas none of these signs have been observed where no therapy has been applied. Moreover, these effects were more obvious three weeks after the SG where mild signs of fibrosis and complete mucosae renewal were observed when rats have received PRP-MSCs combination while only early mucosae regeneration signs were described in a non-treated strategy. Lastly, the healing process positive effect was significant four weeks after the SG with major fibrosis and complete mucosae renewal in all rats receiving the cellular therapy whereas none of the control ones presented complete mucosae regeneration. Samples anatomopathological examination from all time points following SG shows that combination of PRP and MSCs promotes faster recovery. Altogether, these data suggest that PRP-MSCs cellular therapy following artificial leak after SG significantly favors the healing process.

Discussion

Since the two last decades, regenerative medicine is a promising alternative option in the treatment of a chronic wound. One of the first application of MSCs in the gastro-enterology field consisted in treating a rectovaginal fistula in perianal Crohn's disease where autologous MSC transplantation has been successfully achieved, resulting in fistula closure [26]. Indeed, MSCs can exert their regenerative properties through their ability to migrate toward the injury site where C-X-C motif Chemokine 12 (CXCL12), growth factors such as basic Fibroblast Growth Factor (bFGF), Insulin-like Growth Factor-1 (IGF-1), Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF) or Transforming Growth Factor- β 1 (TGF- β 1) appear to be important as homing factors [27-32].

PRP therapy has several clinical applications such as in dental and maxillofacial surgery but more recently in a context of traumatic injury for its regenerative effect on several tissues (e.g. bone, tendon,

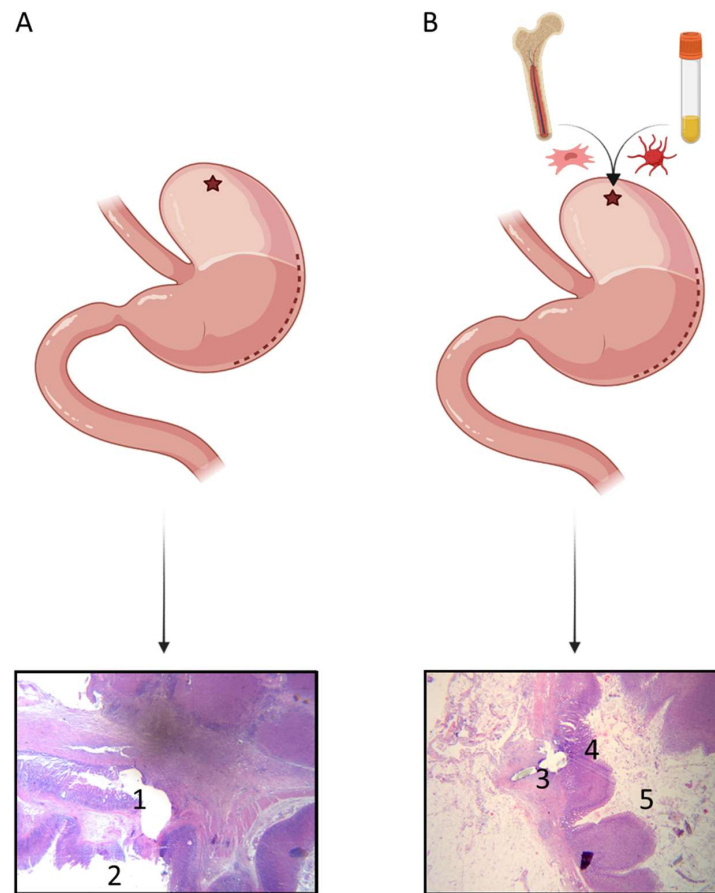


Figure 1: Summary chart recapitulating gastric leak healing process after SG with (B) or without (A) PRP-MSCs cellular therapy. The dot line and the star represent the SG and the fistula, respectively. 1: neo mucosae with mucosae renewal signs. 2: intestinal lumen. 3: fibrosis with leak orifice closure. 4: completely regenerated mucosae. 5: intestinal lumen.

muscle, cartilage) [33,34]. Importantly, it has been shown that platelet degranulation provides growth factors and cytokines such as TGF- β , PDGF, IGF, bFGF and VEGF [35,36]. Furthermore, platelet containing PRP is used in tissue regeneration strategies where all these factors can activate MSCs promoting angiogenesis, proliferation, differentiation or stem cell homing, which can in return enhance the healing process [10,37].

Numerous studies have demonstrated the feasibility of using PRP in order to activate MSCs in many fields [38,39]. As a matter of fact, it has been shown that PRP isolated from rats enhances proliferation rates and self-renewal of MSCs from rat endometrium *in vitro*, suggesting that such a combination may be beneficial in a context of infertility treatment [38]. Moreover, other teams have demonstrated the *in vitro* synergistic effect of PRP and MSCs where such a combination promotes tissue regeneration of rats irradiated submandibular salivary glands [40] or enhances mice myofibroblast proliferation and differentiation as a therapeutic strategy for skeletal muscle damages [41]. In this context, autologous formulation derived from patients and combining PRP and MSCs may have a synergistic effect on tissue regeneration.

However, despite PRP is known to be source of essential

growth factors participating in the healing process, growth factor concentration has to be taken into consideration as it can affect biological affects in a dose-dependent manner. Indeed, Cho et al. have demonstrated that PRP induced MSC proliferation in a dose-dependent manner [42]. For that reason, characterizing growth factors composition and concentration within the PRP could bring new insights considering PRP activity. In addition, PRP component variability, PRP concentration, and PRP harvesting methods will need to be standardized in order to treat patients in the clinic.

Moreover, PRP-MSCs takes advantage of the patient autologous material, where PRP can be extracted from the peripheral blood sample and MSCs from a bone marrow puncture, avoiding transplant resection issues.

Then, this strategy appears to be promising despite raising questions concerning the characterization of the different actors involved into the wound healing process and molecular pathways mediating beneficial effects of such a therapy. Indeed, in depth characterization of the cellular and molecular mechanisms driving PRP-MSCs-induced wound healing response will help to optimize this innovative technique for the treatment of fistula. In addition, PRP-MSCs-based therapy could also be used in other

fields of regenerative therapy including sport medicine, dental or maxillofacial surgery. While fibrosis is a non-physiological scarring process, it can affect tissue architecture and reduce tissue function. Considering that, more research will need to be conducted in order to characterize the fibrotic tissue after PRP-MSCs treatment and to define whether or not this fibrosis response became uncontrolled and pathologic. Understanding the mechanisms driving gastric leak closure after SG may facilitate to extent this therapy to another range of disease.

Finally, after this study on rats, Debs et al., have reported complete fistula resolution after SG for two patients that went under PRP-MSCs cellular therapy injection around the fistula tract [43]. More importantly, complete fistula resolution have occurred faster with the cellular therapy and without any adverse effect compared to the recovery time needed for a more common strategy [43,44].

Conclusion

Managing acute fistulas following SG remains a challenge since actual treatments require emergency surgical exploration and long-term care treatment regarding leak closure. Moreover, acute fistula can evolve into chronic leak, requiring surgical approaches. Unfortunately, this treatment remains problematic since there is still a high percentage of leakage with very low healing process [45]. Here we show that PRP and MSCs promote tissue regeneration enabling the fistula closure occurring after SG. In conclusion, it is now getting clear that using PRP-MSCs-based regeneration medicine is promising for the treatment of gastric leak after SG. However, more research will need to be conducted to study the impact of PRP-MSCs on the leak closure and also to characterize long term and potential adverse effects on stomach and surrounding tissues.

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