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Impact of platelet rich plasma and adipose stem cells on lymphangiogenesis in a murine tail lymphedema model

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ABSTRACT

Background: Lymphedema is an underdiagnosed pathology which in industrialized countries mainly affects cancer patients that underwent lymph node dissection and/or radiation. Currently no effective therapy is available so that patients' life quality is compromised by swellings of the concerned body region. This unfortunate condition is associated with body imbalance and subsequent osteochondral deformations and impaired function as well as with an increased risk of potentially life threatening soft tissue infections.
Methods: The effects of PRP and ASC on angiogenesis (anti-CD31 staining), microcirculation (Laser Doppler Imaging), lymphangiogenesis (anti-LYVE1 staining), microvascular architecture (corrosion casting) and wound healing (digital planimetry) are studied in a murine tail lymphedema model.
Results: Wounds treated by PRP and ASC healed faster and showed a significantly increased epithelialization mainly from the proximal wound margin. The application of PRP induced a significantly increased lymphangiogenesis while the application of ASC did not induce any significant change in this regard.
Conclusions: PRP and ASC affect lymphangiogenesis and lymphedema development and might represent a promising approach to improve regeneration of lymphatic vessels, restore disrupted lymphatic circulation and treat or prevent lymphedema alone or in combination with currently available lymphedema therapies.

Introduction

Acute lymphedema, the clinical manifestation of a lymph transport dysfunction, concerns a high percentage of patients undergoing oncologic surgery with lymphadenectomy and/or radiation therapy. In these cases, if treatment is delayed, lymphedema will progress towards the chronic accumulation of protein-rich fluid, local inflammation, adipose tissue hypertrophy and fibrosis. Chronically affected patients present with a significant swelling of the concerned body region which may cause reduced mobility and function.

Upper and lower extremity lymphedema with limited mobility of the concerned extremity and body imbalance due to unilateral swelling is a common condition following the treatment of breast cancer, melanoma, gynecological and genitourinary cancer. Incidence of lymphedema may vary between 1 and 66% depending on type of cancer, extent of the disease, treatment protocol, study population and used lymphedema measurement methods (Petrek and Heelan, 1998; Armer, 2005; Ryan et al., 2003; Cormier et al., 2010). Neck lymphedema with limited jaw and neck mobility and/or dysphagia has been described in up to 30% of patients undergoing head and neck cancer treatment (Cormier et al., 2010; Murphy and Gilbert, 2009; Murphy et al., 2007).

Fibrosis plays a crucial role in the pathophysiology of lymphedema and has been shown to impair lymphatic regeneration, lymphatic endothelial cell proliferation and migration, to interfere with tubule formation, and to impair lymphatic function (Clavin et al., 2008; Zhang et al., 2005; Shin et al., 2008). One of the mechanisms through which fibrosis acts is by impeding the interaction of vascular endothelial growth factor-C (VEGF-C) with its receptors VEGFR-2 and VEGFR-3 (Zhang et al., 2005; Maruyama et al., 2007; Goldman et al., 2007). Through the activation of VEGFR-3, VEGF-C improves secondary lymphedema by promoting lymphangiogenesis, stimulating the recanalization of injured lymphatic vessels, restoring the lymph flow, and ameliorating the pump activity of the collecting lymphatics (Saaristo et al., 2004; Yoon et al., 2003; Breslin et al., 2007). Through the activation of VEGFR-2, VEGF-C improves lymphangiogenesis and promotes the

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organization of lymphatic endothelial cells into functional lymphatic vessels (Goldman et al., 2007).

Platelet-rich plasma (PRP) is a part of blood plasma which is obtained through repeated centrifugation procedures and is characterized by an increased platelet concentration. Secondary to the presence of numerous pro-angiogenic growth factors and cytokines in cytoplasmatic granulas, platelets have been shown to positively affect tissue regeneration (Eppley et al., 2004). Among the most important factors are platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), insulin-like growth factor 1 and 2 (IGF-1, IGF-2), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin 8 (IL-8), keratinocyte growth factor (KGF) and connective tissue growth factor. The relevance of platelets for lymphangiogenesis and tissue repair has been described recently (Uhrin et al., 2010). It was shown that platelets take the lead in the separation between lymphatic and blood vessels during the embryonic development (Carramolino et al., 2010).

Adipose tissue is another autologous and easily accessible source of pro-angiogenic and tissue regenerating factors. This is mainly due to the presence of multi-potent mesenchymal stem cells in adult adipose tissue referred to as adipose-derived mesenchymal stem cells (ASCs) (Gimble et al., 2012). The phenotypic and gene expression profiles of ASCs are similar to mesenchymal stem cells (MSCs) obtained from bone marrow (De Ugarte et al., 2003) and these cells may be expanded in culture for extended periods. Additionally to their transdifferentiation potential in different adult tissues, ASCs present a superior pro-angiogenic potential and produce collagen, fibronectin and growth factors able to promote tissue repair (Kim et al., 2011). In previous in vitro and in vivo studies ASCs proved among others to be able to acquire a lymphatic phenotype (Conrad et al., 2009; Hwang et al., 2011), and therefore to potentially be used to regenerate injured lymphatic vessels (Hwang et al., 2011; Cherubino et al., 2011; Ko et al., 2011).

The aim of this study was to evaluate the effect of PRP and ASCs on lymphatic regeneration, wound repair and blood vessel formation and functionality in a murine lymphedema tail model.

Methods

Mouse tail excision model

Acute lymphedema was induced in the tails of 10 week old adult male wild-type C57B16 mice (Charles River, Sulzfeld, Germany). Mice were housed in an approved animal facility with 12-hour light dark cycles. Food and water were provided ad libitum. The care of the animals was consistent with legal guidelines and experiments were conducted following the approval of the local animal welfare authorities.

Under inhalation anesthesia (Isoflurane 2%) and disinfection of the tail base with alcohol patches, a circumferential 5-mm-wide full-thickness excision was performed at a 10 mm distance from the base of the tail according to the previously described procedure (Avraham et al., 2009). The superficial and deep lymphatic network running alongside the major blood vessels were removed conserving the tail blood supply. Depending on the groups either PRP, ASCs or saline were topically applied to the wounds which were then covered by a semi-permeable Tegaderm™ dressing (3 M, St. Paul, MN). Ten mice where included in each group.

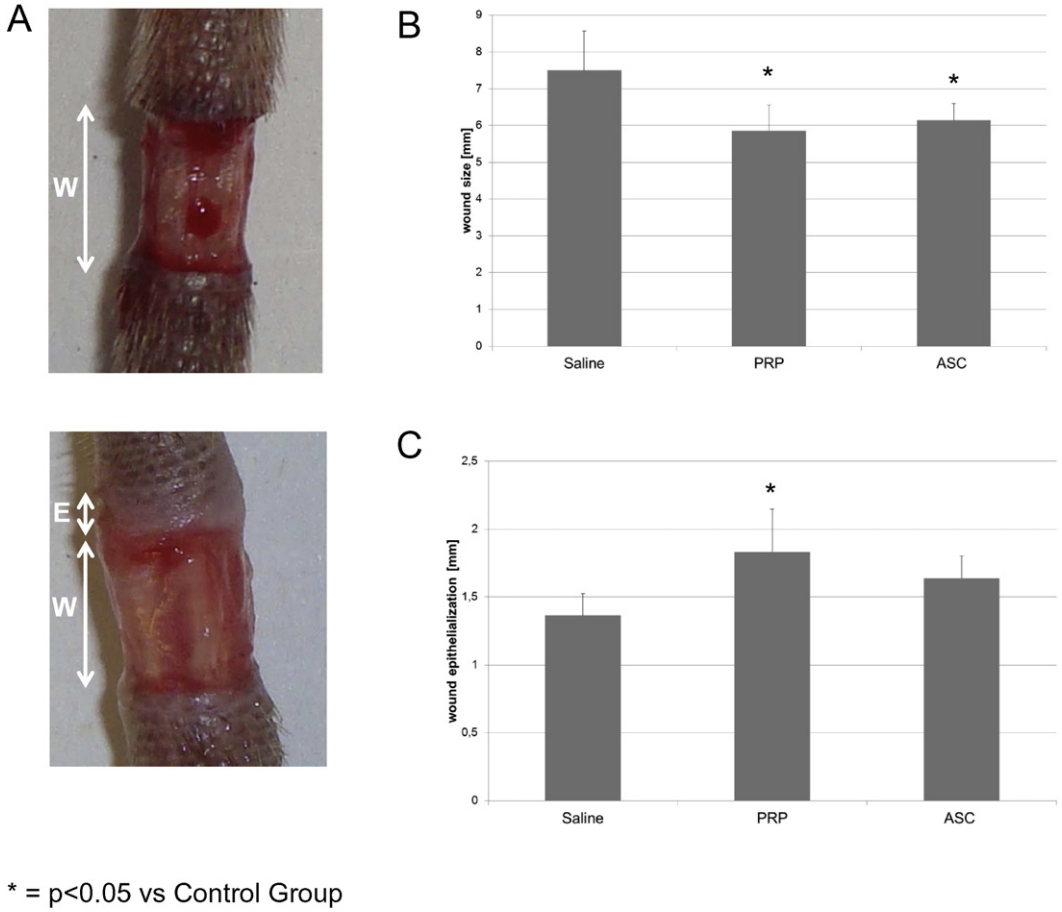

Fig. 1. Assessment of wound healing. A. shows the photographs of a tail wound from day of surgery (upper image) and from day 14 (lower image) for digital planimetric assessment of wound size (W) and wound epithelialization (E) that was carried out 7 and 14 days after wounding. B. shows the significant superiority of PRP- and ASC mice in reducing the wound size, $p < 0.05$. C, in PRP-treated mice a significant higher wound epithelialization was revealed.