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Low molecular weight fucan prevents transplant coronaropathy in rat cardiac allograft model

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Abstract

Introduction: Transplant arteriosclerosis is the main cause of long-term failure after cardiac transplantation. Vascular rejection is thought to be due to intimal proliferation occurring in response to arterial wall immune-mediated injury. A low molecular weight fucan (LMWF) compound, a sulfated polysaccharide, has been demonstrated to increase plasma levels of stromal cell-derived factor 1 (SDF-1) and consequently to mobilize bone marrow-derived vascular progenitor cells (BMVPC). The aim of this study was to evaluate the capacity of LMWF to prevent coronary intimal proliferation in a rat cardiac allograft model.

Methods: Heterotopic abdominal cardiac graftings were performed in Brown Norway (BN) and Lewis (LEW) rats. Animals were divided into 4 groups of 10 rats. Two groups were treated intramuscularly with LMWF (5 mg/kg/day) (one BN to BN isograft group, and one BN to LEW allograft group); and two control groups were LMWF-untreated (one BN to BN isograft group and one BN to LEW allograft group). All animals were treated by cyclosporin (15 mg/kg/day) sub-cutaneously and sacrificed at day 30. The cardiac grafts were assessed by morphometry of structural parameters and by histological and immunohistochemical analyses.

Results: All cardiac isografts were devoid of any coronary and parenchymal lesions. In contrast, the majority of untreated allografts developed coronary intimal proliferation in close association with intimal and adventitial inflammatory $CD68^+$ cell infiltration. Further, the parenchyma exhibited large areas of $actin^+$ cells (myofibroblasts) of recipient origin colocalized with the $CD68^+$ infiltrating cells. Interestingly, all LMWF-treated allografts were well protected against coronary and parenchymal lesions and the coronary arteries exhibited an intimal monolayer of flat cells, which however were CD34 negative.

Conclusion: treatment with LMWF appeared very effective in this rat cardiac allograft model to prevent arterial and parenchymal lesions occurring in response to alloimmune injury. However this protective effect does not appear to depend on mobilization of bone marrow-derived cells.

Keywords: Transplantation; Arteries; Heart; Cell therapy; Stem cells

1. Introduction

In cardiac transplantation, as in other solid organ transplantations, chronic rejection remains the major cause of organ failure. The most prominent aspect of chronic rejection is the development of transplant arteriosclerosis (TA). Histologically, TA is characterized by intra-luminal obstructive cellular proliferation essentially composed of infiltrating inflammatory cells, smooth muscle cells (SMC), and extracellular matrix deposition [1]. The transplant organ tissue damage and fibrosis

Abbreviations: BN, Brown Norway Rat; Lew, Lewis Rat; LMWF, Low Molecular Weight Fucan; SDF-1, Stromal Cell-derived Factor-1; TA, Transplant Arteriosclerosis.

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appear as an ischaemic consequence of TA [2]. To our knowledge no effective therapy has yet been established to prevent transplant coronaropathy [3,4]. Endothelial cells are thought to be the key target in the development of TA, due to allo-immune reactions [5]. Endothelial cells express molecules that activate the immune system, leading to inflammatory cell recruitment and graft rejection [6]. Endothelial monolayer cell destruction induces a cascade of events resulting in intimal hyperplasia as a response to arterial wall injury. In contrast, the restoration of endothelial integrity promotes favourable remodelling in an experimental arterial model [7].

In our study we hypothesized that early intimal healing by syngeneic cells, after initial allograft endothelial injury, could prevent TA by inhibiting SMCs proliferation. Additionally, intimal cell seeding may result from bone marrow-derived vascular progenitor cells (BMVPC). In fact, bone marrow-derived vascular progenitor cell mobilization has recently been reported to inhibit intimal proliferation in a balloon-induced arterial wall de-endothelialization injury model [8].

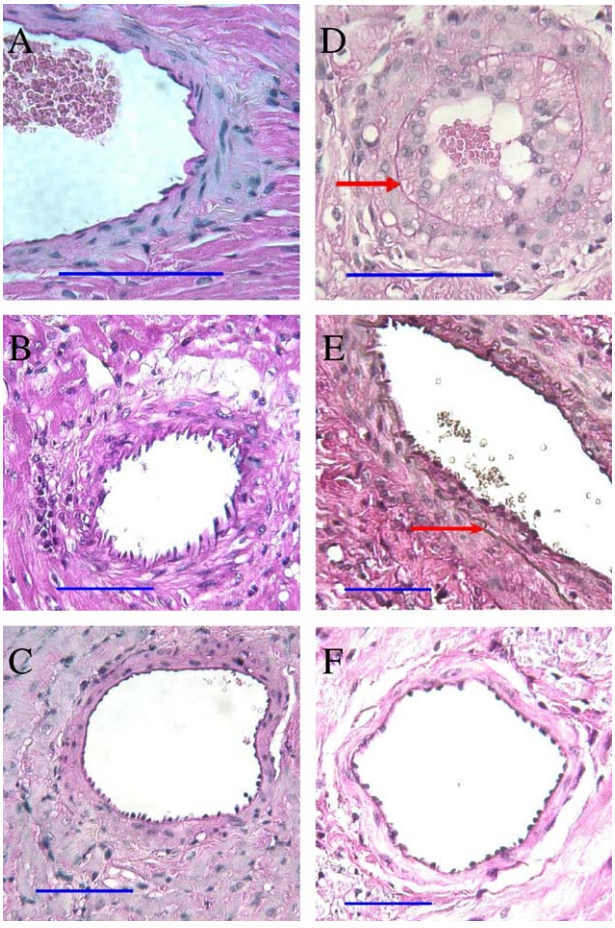

Fig. 1. Histological characteristics of heterotopic cardiac grafts in BN and LEW strains at 30 days post-transplantation. (Scale bar = 50 μm in all pictures. Arrows indicate coronary internal elastic laminae). (Hematein). Aspect of a non-grafted non-treated normal BN heart (A). No coronary lesion was evident in LMWF-untreated isografts (B) nor in LMWF-treated isografts (C). Coronary lesions developed in the LMWF-untreated control allografts (D, E): intimal proliferation is prominent and tends to obliterate coronary lumens. LMWF-treatment well protected coronary wall against intimal proliferation; intimal lining was evidenced by a single layer of flat endothelial-like cells (F).

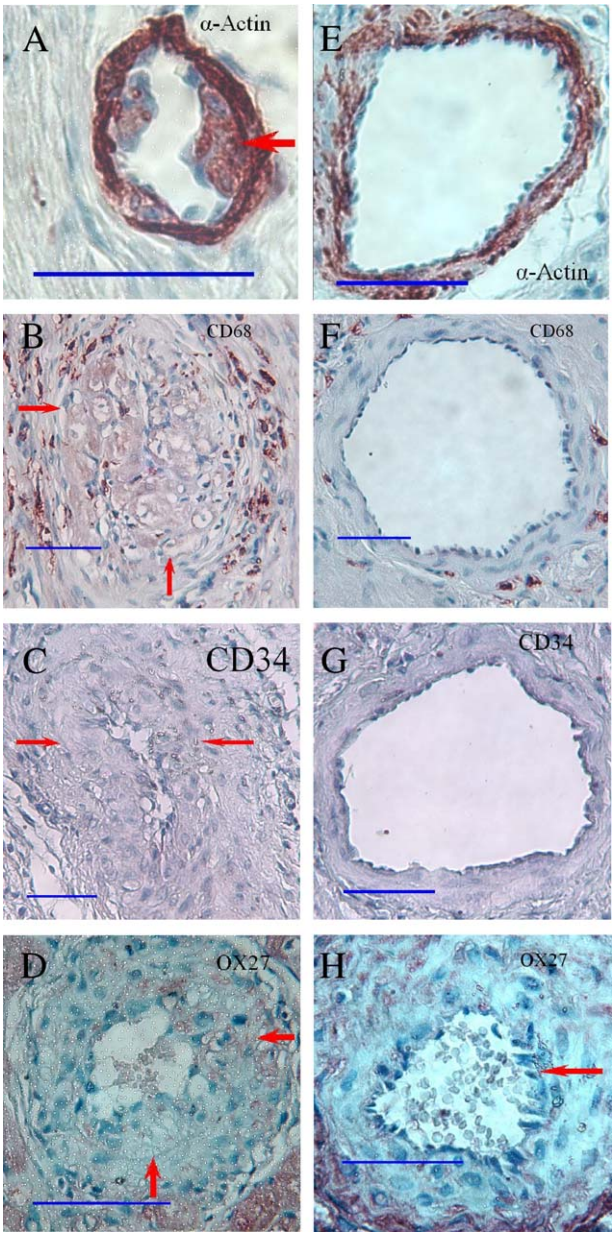

Fig. 2. Transplant coronary artery histological characteristics in control cardiac allografts and LMWF-treated cardiac allografts at 30 days post-transplantation. (Scale bar = 50 μm in all pictures. Arrows indicate coronary internal elastic laminae.) Intimal proliferation is prominent in LMWF-untreated control allografts (A–D), mainly composed of α-actin$^+$ cells (A) and CD68$^+$ inflammatory cells (B). Only few cells (D) are of donor origin (positively stained by OX27). Intimal covering is CD34$^-$ (C). The media is highly actin$^+$ (A), devoid of inflammatory cells (B). Media is positively stained by OX27 indicating its donor origin (D). The adventitia is highly infiltrated by CD68$^+$ inflammatory cells (B). In contrast, LMWF-treatment prevented intimal lesion (E–H). Intima is reduced to a monolayer of flat endothelial-like cells which remains CD34 negative (G). Actin positive cells are found in the medial layer whereas no actin positive cells nests the intima (E). Few CD68$^+$ inflammatory cells infiltrate the adventitia (F). All of coronary wall cells are positively stained by OX27 indicating their donor origin, except the endothelial cells (H).

In this regard, treatment with carbohydrate ligands such as fucans, sulfated polysaccharides derived from brown seaweed [9], have previously been shown to mobilize progenitor cells in mice [10]. In this study, we use a low molecular weight fucan