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DisBa-01 inhibits angiogenesis, inflammation and fibrogenesis of sponge-induced-fibrovascular tissue in mice

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ABSTRACT

Integrins are involved in a number of physio-pathological processes including wound healing, chronic inflammation and neoplasias. Blocking its activity is potentially of therapeutic value in these conditions. We investigated whether DisBa-01, a recombinant His-tag RGD-disintegrin from *Bothrops alternatus* snake venom, could modulate key events (inflammatory cell recruitment/activation, neovascularization and extracellular matrix deposition) of the proliferative fibrovascular tissue induced by polyether polyurethane sponge implants in mice. The hemoglobin content (μg/mg wet tissue), blood flow measurements (laser Doppler perfusion imaging) and number of vessels in the implants, used as indices of vascularization, showed that the disintegrin dose-dependently reduced angiogenesis in the implants relative to the Saline-treated group. DisBa-01 inhibited neutrophil and macrophage content as determined by the myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities, respectively. Similarly, down regulation of the fibrogenic component studied (collagen deposition) was observed in DisBa-01-treated implants. VEGF, bFGF, TNF-α, CXCL1 and CCL2 levels were also decreased by the disintegrin. The inhibitory effect of this $\alpha_v\beta_3$-blocking disintegrin on the angiogenic, inflammatory, and fibrogenic components of the fibrovascular tissue induced by the synthetic matrix extends the range of DisBa-01 actions and may indicate its therapeutic potential in controlling angiogenesis in fibroproliferative diseases.

1. Introduction

In a number of pathological conditions such as rheumatoid arthritis, psoriasis, atherosclerosis, and tumor growth, inflammation and angiogenesis are key events that

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act simultaneously and synergistically contributing to disease progression (Folkman, 1995; Carmeliet and Jain, 2011). Analyses of both processes have revealed the involvement of integrins on adhesion and proliferation of the cells involved in these biological events (Silva et al., 2008). Particularly, the αvβ3 integrin plays a major role in angiogenesis and tumorigenesis participating in adhesion signaling, activation of matrix metalloproteinases (MMPs), proliferation, migration, invasion and protection against apoptosis (Eliceiri and Cheresh, 2001; Somanath et al., 2009). Although, poorly expressed in most adult tissues, this integrin is highly expressed and active on metastatic tumor cells and on vascular endothelial cells undergoing angiogenesis (Contois et al., 2009; Robinson and Hodivala-Dilke, 2011). Thus, it has been proposed that inhibition of integrins involved in the inflammatory and angiogenic cascades may be of potential therapeutic value in pathological conditions where these processes co-exist. As a result, they have been widely investigated for their therapeutic potential in a number of conditions in which controlled angiogenesis is sought (wound healing, tumor propagation, diabetic retinopathy) (Rapraeger, 2013; Goodman and Picard, 2012). There is also increasing evidence that integrin-inactivating proteins are crucial for appropriate integrin function *in vitro* and *in vivo* and that the regulation of integrin–ligand interactions is a fine-tuned balancing act between inactivation and activation (Bouvard et al., 2013). A number of molecules have been shown to inactivate the interactions between the integrins and target cells (Cox et al., 2010). Among them, disintegrins, a family of nonenzymatic, low molecular weight, cysteine-rich peptides from snake venom, have been shown to specifically and potently inhibit integrin actions, including tumor development, metastasis, and angiogenesis (Selistre-de-Araujo et al., 2010; Calvete, 2013). In fact, blocking of αvβ3 with DisBa-01 (a αvβ3 binding disintegrin) decreased bFGF-induced angiogenesis in a matrigel plug assay in athymic nude mice and inhibited melanoma metastasis (Ramos et al., 2008). These reports on the effects of this peptide on key events of the angiogenic cascade prompted us to hypothesize that it might also modulate inflammatory angiogenesis in the *in vivo* mouse sponge model. In this chronic inflammation model, the acellular and avascular synthetic matrix, implanted subcutaneously in the animals' dorsa, induces the migration, proliferation, and activation of various cell types responsible for the development of a fibrovascular tissue that underlies chronic pathological conditions (Andrade et al., 1987; Pereira et al., 2012). Thus, it has been proven to be instrumental in evaluating a number of potential compounds and molecules bearing anti-angiogenic, inflammatory and/or fibrogenic activities (Barcelos et al., 2009; Araujo et al., 2010). We report here that DisBa-01 was able to dose-dependently attenuate inflammation, neovascularization

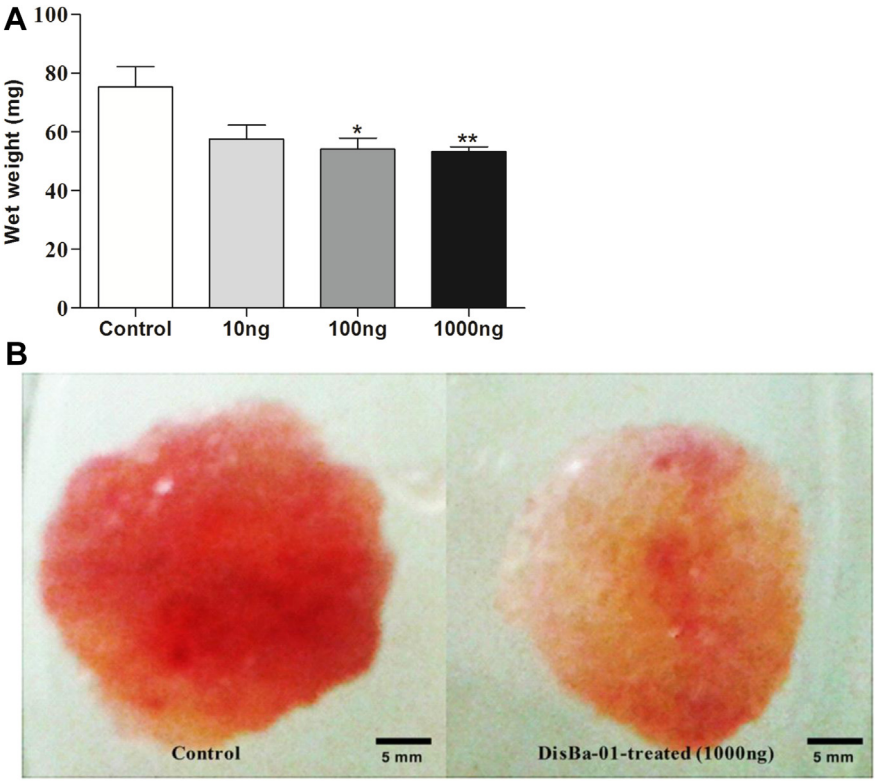

Fig. 1. The sponge tissues were excised, photographed, and weighed. (A) Note that the implant treated with DisBa-01 doses (1000 ng) exhibited reduce weight and intense of staining when compared to the control group. (B) Representative image showing a polyester-polyurethane sponge 9 days post-implantation. Values shown are the means (±SEM) from groups of 8 animals for each group. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. Control group (ANOVA). Bar = 5 mm.