The Antifibrotic Effect of α2AP Neutralization in Systemic Sclerosis Dermal Fibroblasts and Mouse Models of Systemic Sclerosis

Yosuke Kanno[1], En Shu[2], Hiroyuki Kanoh[2] and Mariko Seishima[2]

Systemic sclerosis (SSc) is a connective tissue disease of autoimmune origin characterized by the fibrosis of skin and visceral organs, and peripheral circulatory disturbance. We recently demonstrated that α2-antiplasmin (α2AP), which is the physiological inhibitor of plasmin, is associated with the development of fibrosis. The aim of this study was to clarify the role of α2AP in the pathogenesis of SSc. The administration of α2AP in mice induced profibrotic changes, such as increased dermal thickness, collagen production, and myofibroblast differentiation. Conversely, the α2AP neutralization prevented not only profibrotic changes, but also the production of autoantibodies in bleomycin-induced mouse models of SSc. The expression of α2AP was elevated in dermal fibroblasts obtained from patients with SSc. Furthermore, α2AP treatment promoted profibrotic changes in human normal dermal fibroblasts, and α2AP neutralization reversed a profibrotic phenotype of SSc dermal fibroblasts, in the absence of plasmin. Our findings demonstrated that α2AP has a profibrotic effect probably not by the action as a plasmin inhibitor, and that the blocking of α2AP exerts an antifibrotic effect in humans and mice with SSc.

Journal of Investigative Dermatology (2016) **136,** 762–769; doi:10.1016/j.jid.2015.12.028

INTRODUCTION

Systemic sclerosis (SSc) is autoimmune rheumatic disease of unknown etiology that is characterized by the fibrosis of skin and visceral organs, and peripheral circulatory disturbance (Gilbane et al., 2013). This process usually occurs over many months and years, and can lead to organ dysfunction or death. It has been known that the progression of SSc is associated with immunologic mechanisms, vascular damage, and the activation of fibroblasts, and transforming growth factor (TGF)-β plays a central role in the pathogenesis of SSc, and that aberrant TGF-β expression is implicated in the process (Varga and Pasche, 2009). However, the precise mechanism of SSc pathogenesis remains unclear, and there are no therapies to halt progression of the disease.

Alpha2-antiplasmin (α2AP), which has a molecular weight of 65 to 70 kDa, is the principal inhibitor of plasmin and inhibits the fibrinolysis (Collen, 1976). α2AP rapidly inactivates plasmin, resulting in the formation of a stable inactive complex, plasmin-α2AP (Lijnen et al., 1994). It has been reported that the levels of plasmin-α2AP complex in plasma are elevated in patients with rheumatic disease including SSc (Jinnin et al., 2003; Kawakami et al., 1989), and that α2AP regulates angiogenesis, tissue repair, and vascular remodeling (Hou et al., 2008; Kanno et al., 2006). Furthermore, α2AP is associated with the production of IgG, IgM, and IgE (Okada et al., 2013; Zhabin and Gorin, 1997), the recruitment of lymphocytes and neutrophils (Eddy et al., 2015; Kager et al., 2013; Okada et al., 2013), and contributes inflammatory response and immune modulation. Recently, we demonstrated that α2AP induces the production of TGF-β through adipose triglyceride lipase (ATGL), which is known to be a receptor of pigment epithelium-derived factor, in fibroblasts (Kanno et al., 2013), and that α2AP is associated with the development of fibrosis in mice (Kanno et al., 2007, 2010, 2013, 2014). These findings suggest that α2AP may be associated with the pathogenesis and progression of SSc. We herein showed that α2AP has a profibrotic effect in humans and mice, and that the neutralization of α2AP exerts an antifibrotic effect in human and mice with SSc.

RESULTS

The profibrotic effect of α2AP in mice

We examined the levels of α2AP in the serum from bleomycin-induced mouse models of SSc. The levels of α2AP in the serum from bleomycin-administrated mice were significantly higher than those in saline-administrated mice (Figure 1a). Next, we examined the profibrotic effect

[1]*Department of Clinical Pathological Biochemistry, Faculty of Pharmaceutical Science, Doshisha Women's Collage of Liberal Arts, Kodo Kyo-tanabe, Kyoto, Japan; and* [2]*Department of Dermatology, Gifu University Graduate School of Medicine, Yanagido Gifu, Japan*

Correspondence: Yosuke Kanno, Department of Clinical Pathological Biochemistry, Faculty of Pharmaceutical Science, Doshisha Women's Collage of Liberal Arts, 97-1 Kodo, Kyo-tanabe, 610-0395 Kyoto, Japan. E-mail: ykanno@dwc.doshisha.ac.jp

Abbreviations: α2AP, α2-antiplasmin; ATGL, adipose triglyceride lipase; α-SMA, α-smooth muscle actin; SSc, systemic sclerosis; TGF, transforming growth factor

Received 26 August 2015; revised 16 November 2015; accepted 14 December 2015; accepted manuscript published online 29 December 2015; corrected proof published online 10 February 2016

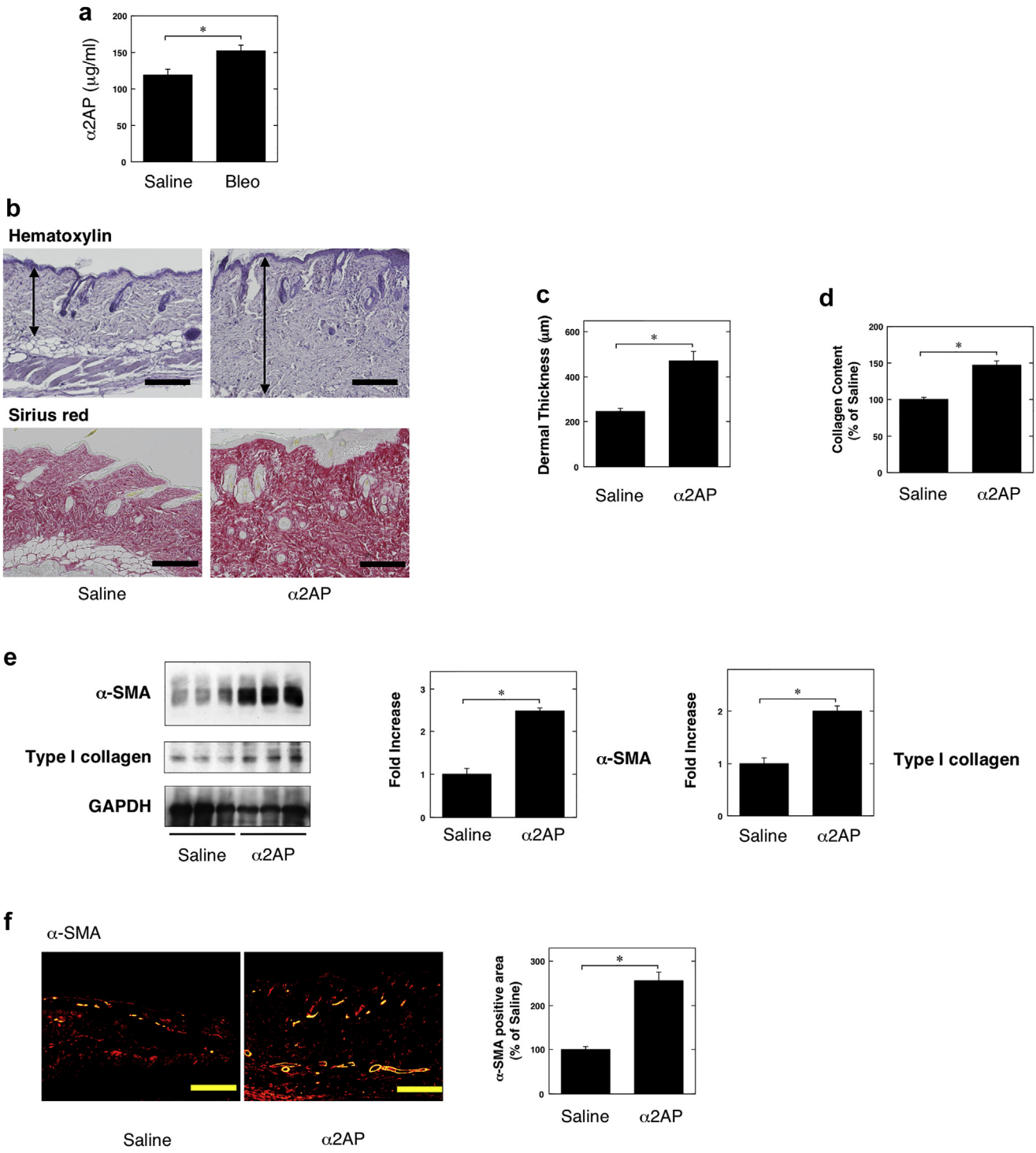

Figure 1. The profibrotic effect of α2AP in mice. (**a**) The level of α2AP in the serum from saline or bleomycin-administered mice was determined by ELISA (n = 4). (**b**) Representative skin sections from saline or α2AP-administered mice (hematoxylin and sirius red stain). Double head arrows indicate the dermal thickness. (**c**) The dermal thickness in the skin sections from mice (n = 6). (**d**) The collagen content in the skin from mice (n = 6). (**e**) The expression of each protein in the skin of mice was examined by a western blot analysis. The histogram shows quantitative representations of each protein (n = 3). (**f**) Paraffin sections were stained with antibodies to α-SMA. The histogram shows quantitative representations of α-SMA (n = 6). The data represent the mean ± SEM. *$P < 0.01$. Scale bar = 200 μm. α2AP, α2-antiplasmin; α-SMA, α-smooth muscle actin; SEM, standard error of the mean.

of α2AP in mice after the administration of α2AP. The administration of α2AP induced profibrotic changes, such as increased dermal thickness (Figure 1b and c), collagen production (Figure 1b, d and e), and the expression of α-smooth muscle actin (α-SMA) (a hallmark of the myofibroblast phenotype) in mice (Figure 1e). In addition, we showed the expression of α-SMA in the skin by immunohistochemical staining, and the administration of α2AP induced the increase of myofibroblasts within the dermis (Figure 1f).