Journal of Cardiology (2012) 59, 299–306

Available online at www.sciencedirect.com

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Original article

High-mobility group box 1 protein blockade suppresses development of abdominal aortic aneurysm

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Received 28 July 2011; received in revised form 9 December 2011; accepted 9 January 2012
Available online 24 February 2012

KEYWORDS
Aorta;
Aneurysms;
Cytokines

Summary
Background: Abdominal aortic aneurysm (AAA) expansion is characterized by chronic inflammatory cell infiltration and extracellular matrix degradation. High-mobility group box 1 protein (HMGB1) is one of the damage-associated molecular pattern molecules derived from injured/necrotic and activated inflammatory cells. We investigated the expression of HMGB1 in human AAA and mouse experimental AAA. Then, we evaluated the effect of HMGB1 blockade on AAA formation in the mouse model.
Methods and results: Human AAA samples showed increased HMGB1 expression compared with normal aortic wall. In a mouse $CaCl_2$-induced AAA model, the expression of HMGB1 was increased compared with that in sham, and was positively correlated with matrix metalloproteinase

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0914-5087/$ – see front matter
doi:10.1016/j.jjcc.2012.01.007

(MMP)-2 and MMP-9 activity. We administered neutralizing anti-HMGB1 antibody (AAA/anti-H) or control antibody (AAA/C) to AAA mice subcutaneously every 3 days for 6 weeks. Treatment with neutralizing anti-HMGB1 antibody suppressed AAA formation, and attenuated elastin fragmentation. HMGB1 blockade markedly reduced the number of macrophages and MMP-2 and MMP-9 activity in aneurysmal tissue. The mRNA level of tumor necrosis factor-α and CD68 in the aorta was reduced in AAA/anti-H compared with AAA/C.

Conclusions: Elevation of HMGB1 level in aneurysmal tissue was observed in human AAA and mouse experimental AAA. HMGB1 blockade in a mouse AAA model reduced AAA progression, in association with reduced infiltration of macrophages and MMPs activity. These findings suggest a significant role for HMGB1 in the pathogenesis of AAA.

Introduction

Chronic inflammation of the aortic wall has been implicated in the development, expansion, and rupture of abdominal aortic aneurysm (AAA) [1—6]. Inflammatory cells in the media and adventitia are significant sources of cytokines that stimulate proteolytic enzymes such as matrix metalloproteinases (MMPs) [3,7,8]. MMPs mediate fragmentation of extracellular matrix (ECM) elastin fibers with a reduction of their concentration in ECM, which is the pathologic hallmark of AAA [7—9]. However, the molecular mechanism that maintains the inflammatory response and MMP activation in aneurysmal tissue is still not fully understood.

High-mobility group box 1 protein (HMGB1) was originally identified as a nonhistone DNA-binding nuclear protein produced by nearly all cell types [10]. It stabilizes nucleosomes and enables bending of DNA, which facilitates gene transcription. Recently, it has been clarified that HMGB1 is released passively from injured/necrotic cells, and is actively secreted by inflammatory cells in response to proinflammatory cytokines [10—12]. As extracellular HMGB1 itself can elicit proinflammatory responses by increasing the expression of proinflammatory cytokines [10,13], HMGB1 participates in perpetuation of inflammation and has been reported to play an essential role in the pathogenesis of inflammatory diseases such as sepsis, rheumatoid arthritis, and atherosclerosis [10,12,14—18]. Various stimuli have been linked to the inflammation observed in AAA, including oxidative stress and proinflammatory cytokines [2,3,19]. These stimuli also activate HMGB1 expression in macrophages, monocytes, and smooth muscle cells (SMCs) [10,12,16,17,20]. Increased HMGB1 expression is also reported to be linked to MMP activation. A previous study demonstrated that blockade of HMGB1 suppressed inflammatory cell infiltration and MMP-9 induction during ischemia/reperfusion injury [21]. Additionally, HMGB1 directly stimulated the production of MMPs in vitro [17,22,23]. The importance of HMGB1 in inflammation and MMP activation led us to hypothesize that HMGB1 might play a key role in the progression of AAA through the persistence of inflammation and ECM degradation.

To test this hypothesis, we first examined the expression level and distribution of HMGB1 in aortic tissue in both human and experimental AAA. We then evaluated whether blockade of HMGB1 could attenuate aneurysm formation in a mouse AAA model.

Materials and methods

Clinical study protocol

For determination of serum HMGB1 level, serum was collected from 40 patients with atherosclerosis-associated AAA who were admitted to Keio University Hospital. A diagnosis of AAA was made on the basis of abdominal computed tomography showing dilatation of the abdominal aorta, with a diameter $\geq$5 cm. The study population consisted of patients who had survived a ruptured aorta (n = 6) and patients in whom the diagnosis had been made through computed tomography performed for other reasons (n = 34). There was no patient with recent infection, active inflammatory disorder, malignancy, collagen disease, or chronic renal failure. The shoulders (between the edge and the maximal side) of the AAA were obtained from patients during elective surgical repair of AAA (n = 5). Control aortic samples were obtained from autopsy specimens of patients who had died of unrelated causes (n = 5). This clinical investigation was approved by the institutional medical ethical committee and conducted according to the ethical guidelines outlined in the Declaration of Helsinki.

Mouse model of experimental AAA

We induced AAA in 8-week old mice (C57BL/6J) by periaortic application of 0.5 M calcium chloride ($CaCl_2$) as described previously, with minor modifications [24,25]. The sham group received saline instead of $CaCl_2$. To determine HMGB1 expression, mice were killed 6 weeks after operation for histological (n = 6) and protein analyses (n = 6).

In the HMGB1 blockade study, AAA mice were randomly assigned to two groups: (1) neutralizing polyclonal chicken IgY anti-HMGB1 antibody (10 mg/kg/day, donated by Shino-Test Corporation, Sagamihara, Japan) administered subcutaneously 24 h after operation and subsequently every 3 days for 6 weeks (AAA/anti-H), and (2) control chicken IgY antibody (AAA/C). To prepare neutralizing anti-HMGB1 antibody, IgY class antibody from the egg yolk of HMGB1-immunized hens was isolated and purified [14,18]. The dosage of neutralizing anti-HMGB1 antibody was determined according to a previous study showing improved survival in a rodent sepsis model [14]. Mice were killed 6 weeks after operation for histological (n = 6), mRNA (n = 6), and protein