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Vasomotor dysfunction in the thoracic aorta of Marfan syndrome is associated with accumulation of oxidative stress

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ABSTRACT

We have described that the progression of thoracic aortic aneurysm in Marfan syndrome is accompanied with aortic vascular dysfunction. In the present study, we hypothesized that the impaired contractile function and endothelial-dependent relaxation could be resulted from oxidative stress in the thoracic aorta. Adrenergic contraction and cholinergic relaxation of thoracic aortae from mice ($n=40$; age = 3, 6, 9 months) heterozygous for FBN1 allele ($Fbn1^{C1039G/+}$), a well-defined model of Marfan syndrome, were compared with those from control ($n=40$). The aortic 8-isoprostane level, an oxidative stress marker, was 32–50% greater in the Marfan group than in the control. Pre-incubation with superoxide dismutase (SOD) improved the phenylephrine-induced contraction and the sensitivity to acetylcholine in Marfan aortae, but not in controls. The phenylephrine-contraction in Marfan aortae was potentiated by 1400 W, an inducible nitric oxide synthase (iNOS) inhibitor, and allopurinol, a xanthine oxidase inhibitor. Acetylcholine-induced relaxation was restored by apocynin, an inhibitor of NAD(P)H oxidase. Protein expression of SOD-1 and SOD-2 was decreased in Marfan aortae, whereas that of xanthine oxidase, iNOS, and the enzymatic subunits of NAD(P)H oxidase was increased. The vasomotor dysfunction in Marfan thoracic aortae could be associated with accumulation of oxidative stress due to unbalanced protein expression of superoxide-producing and superoxide-eliminating enzymes.

1. Introduction

Thoracic aortic aneurysm leading to dissection and rupture is the most life-threatening complication of Marfan syndrome (Judge and Dietz, 2005). We have demonstrated clearly that the progression of thoracic aortic aneurysm is associated with a pronounced impairment of aortic contractile function and reduction of nitric oxide (NO)-mediated endothelial-dependent relaxation (Chung et al., 2007a,b,c, 2008a,b). Such vasomotor dysfunction might determine the susceptibility of aneurysm formation (Chew et al., 2004).

Vasomotor function is tightly regulated by reactive oxygen species (ROS) (Lounsbury et al., 2000; Gutterman et al., 2005; Lee and Griendling, 2008). At low concentration, ROS regulates vascular tone, proliferation, and cell signaling (Lee and Griendling, 2008; Faraci and Didion, 2004). However, excessive amount of ROS, termed oxidative stress, is associated with the pathogenesis of cardiovascular diseases including hypertension, atherosclerosis, diabetes, and chronic kidney disease (Lee and Griendling, 2008; Faraci and Didion, 2004; Cai and Harrison, 2000). The elevation of ROS is caused by an imbalance between the production and neutralization of ROS (Cai and Harrison, 2000). All cell types in the vasculature contain enzymes that generate ROS (Schulz et al., 2004). Among the many potential pro-oxidant enzymes, NAD(P)H oxidase, xanthine oxidase, and nitric oxide synthase (NOS) are the most well-studied and are also believed to play a dominant role in vascular diseases (Cai and Harrison, 2000). Superoxide dismutase (SOD) is believed to be the main endogenous antioxidant responsible for superoxide removal (Faraci and Didion, 2004; Didion et al., 2002).

It has been well-recognized that oxidative stress is associated with endothelial dysfunction in cardiac and vascular diseases (Johnstone et al., 1993; Panza et al., 1995). Endothelial dysfunction is commonly described as the impairment of endothelium-dependent vasorelaxation caused by a loss of NO bioavailability in the vasculature (Cai and Harrison, 2000; Schulz et al., 2004). We have shown that in the aorta of a mouse model of Marfan syndrome, NO mediated endothelium-dependent relaxation was impaired (Chung et al., 2007a). However, it is unclear whether the impairment of relaxation could result from oxidative stress.

ROS has been reported to impede calcium signaling, which consequently leads to a reduction in vascular contractility (Lounsbury et al., 2000; Sener et al., 2004). It was reported that preservation of contraction affords protection against aneurysm formation in the abdominal aorta (Chew et al., 2004). The compromised contractile function in the Marfan thoracic aorta (Chung et al., 2007a,b, 2008a),

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which likely increases their susceptibility to aneurysm formation, might be the result of the excessive oxidative stress.

In the present study, we hypothesized that the impaired contractile function and endothelial-dependent relaxation observed in the thoracic aorta of Marfan syndrome would be resulted from the elevation of oxidative stress during the disease progression. We demonstrated that the increase in oxidative stress could be associated with the unbalanced protein expression of superoxide-producing and superoxide-eliminating enzymes.

2. Methods

2.1. Experimental animals and tissue preparation

Heterozygous (*Fbn1*$^{C1039G/+}$) mice were mated to C57BL/6 mice to produce equal numbers of *Fbn1*$^{C1039G/+}$ 'Marfan' subjects ($n=40$) and wild-type 'control' ($n=40$) (Chung et al., 2007a,b,c, 2008a,b). Both strains were housed in the institutional animal facility (University of British Columbia, Child and Family Research Institute) under standard animal room conditions, and all animal procedures were approved by the institutional Animal Ethics Board. Mice at age 3 ($n=30$), 6 ($n=30$) and 9 ($n=30$) months were anesthetized with a mixture of ketamine hydrochloride (80 mg kg^{-1}) and xylazine hydrochloride (12 mg kg^{-1}) intraperitoneally.

Given that severe aneurysm is found mainly in the ascending thoracic aorta and the aortic arch, both parts were dissected and examined in this study (Chung et al., 2007a,b, 2008a,b, Habashi et al., 2006).

2.2. Measurement of isoprostanes (8-isoprostane)

Whole blood was collected via cardiac puncture and plasma was separated by centrifugation. 8-Isoprostane (8-epi-$PGF_{2\alpha}$) level was determined in plasma and aortic homogenate (ascending aorta = 1.2 mm; arch = 5 mm in length) using an enzyme immunoassay kit according to the manufacturer's procedures.

2.3. Measurement of isometric force

Aortic arch segment (1.8 mm in length) was mounted isometrically in a small vessel myograph (A/S Danish Myotechnology, Aarhus N, Denmark) for force generation measurement (Chung et al., 2007a,b,c). Aortic segment was stretched to the resting tension (6.0 mN) for 20 min and challenged twice with 60 mM KCl before experiments were continued. To assess whether removal of superoxide affected contractile function, aortic segments were pre-incubated with SOD (150 U mL^{-1}) or SOD plus catalase (1000 U mL^{-1}) for 30 min before the addition of phenylephrine (1 nM–3 μM). After sustained pre-contraction was obtained, cumulative concentrations of acetylcholine (ACh; 1 nM–100 μM) were added. Aortic segments were also pre-incubated with three inhibitors that block the potential superoxide-generating enzymes: the xanthine oxidase inhibitor allopurinol (300 μM), the NAD(P)H oxidase inhibitor apocynin (100 μM), and the inducible NOS (iNOS) inhibitor 1400 W (1 μM). They have all been shown to be selective towards the targeted proteins. Specifically, apocynin blocks the translocation of the regulatory unit of NADPH oxidase to the catalytic component; allopurinol is an allosteric inhibitor of xanthine oxidase; 1400 W is a tightly bound competitive inhibitor.

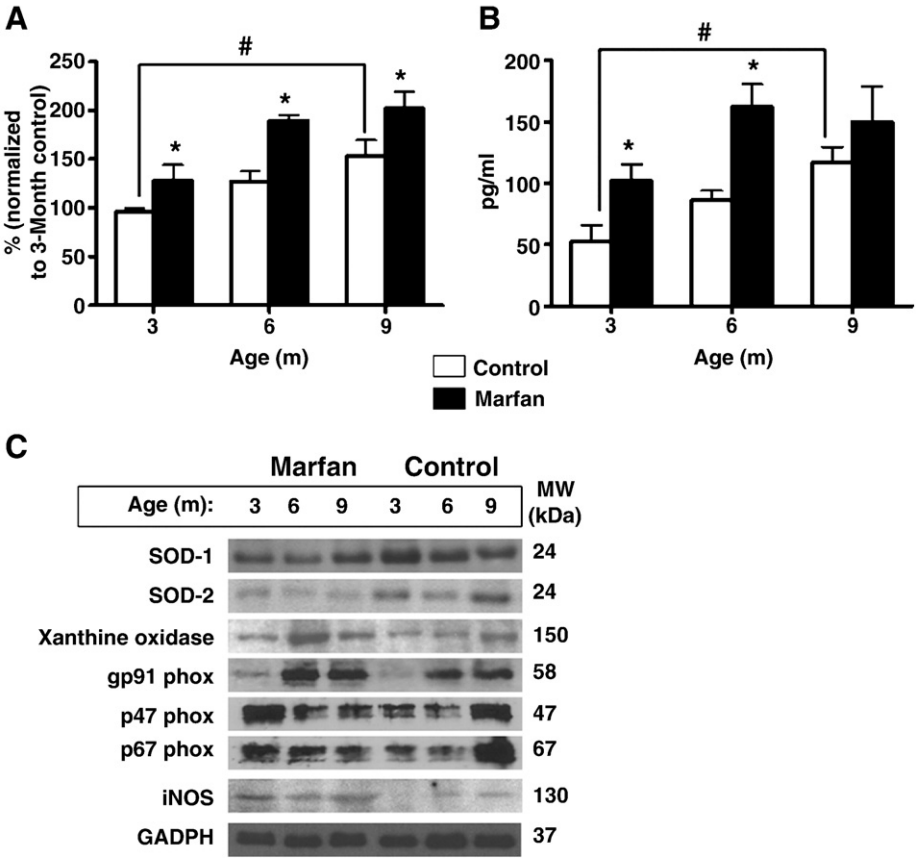

Fig. 1. Bar graph presenting the levels of isoprostane 8-epi-$PGF_{2\alpha}$ in the (A) aortic homogenate and (B) plasma from control and Marfan group. ($n=4$, $^{*}p<0.05$, compared with the age-matched control. $\#p<0.05$, compared between 3 and 9 months control). (C) Western immunoblots showing the protein expression of SOD-1, SOD-2, xanthine oxidase, gp91phox, p47phox, and p67phox subunits of NAD(P)H oxidase, iNOS, and GADPH in the Marfan and control aorta during aging (3, 6, and 9 months old). The densitometric analysis is shown in Table 1.