BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 28(1), 98-103 (2020)

Transforming Growth Factor β Receptor Type I Inhibitor, Galunisertib, Has No Beneficial Effects on Aneurysmal Pathological Changes in Marfan Mice

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Abstract

Marfan syndrome (MFS), a connective tissue disorder caused by mutations in the fibrillin-1 (*Fbn1*) gene, has vascular manifestations including aortic aneurysm, dissection, and rupture. Its vascular pathogenesis is assumed to be attributed to increased transforming growth factor β (TGFβ) signaling and blockade of excessive TGFβ signaling has been thought to prevent dissection and aneurysm formation. Here, we investigated whether galunisertib, a potent small-molecule inhibitor of TGFβ receptor I (TβRI), attenuates aneurysmal disease in a murine model of MFS (*Fbn1*$^{C1039G/+}$) and compared the impact of galuninsertib on the MFS-related vascular pathogenesis with that of losartan, a prophylactic agent routinely used for patients with MFS. *Fbn1*$^{C1039G/+}$ mice were administered galunisertib or losartan for 8 weeks, and their ascending aortas were assessed for histopathological changes and phosphorylation of Smad2 and extracellular signal-regulated kinase 1/2 (Erk1/2). Mice treated with galunisertib or losartan barely exhibited phosphorylated Smad2, suggesting that both drugs effectively blocked overactivated canonical TGFβ signaling in *Fbn1*$^{C1039G/+}$ mice. However, galunisertib treatment did not attenuate disrupted medial wall architecture and only partially decreased Erk1/2 phosphorylation, whereas losartan significantly inhibited MFS-associated aortopathy and markedly decreased Erk1/2 phosphorylation in *Fbn1*$^{C1039G/+}$ mice. These data unexpectedly revealed that galunisertib, a TβRI inhibitor, showed no benefits in aneurysmal disease in MFS mice although it completely blocked Smad2 phosphorylation. The significant losartan-induced inhibition of both aortic vascular pathogenesis and Smad2 phosphorylation implied that canonical TGFβ signaling might not prominently drive aneurysmal diseases in MFS mice.

Key Words: Galunisertib, Marfan syndrome, Thoracic aortic aneurysm, Transforming growth factor-β receptor I inhibitor

INTRODUCTION

Marfan syndrome (MFS) is an autosomal dominant disorder caused by mutations in the gene encoding fibrillin-1 (*Fbn1*) that is a major element of the extracellular matrix, microfibril (Dietz *et al*., 1991; Kim *et al*., 2014). Patients with MFS exhibit several clinical manifestations including bone overgrowth, dislocation of the lens of the eye, and dural ectasia, among which aortic aneurysm, dissection, and rupture are the primary causes of death (Judge and Dietz, 2005). Numerous previous studies of *Fbn1* mutant mice and human patients with MFS have demonstrated significantly enhanced level of transforming growth factor (TGF)-β and increased Smad2 activation in vascular smooth muscle cells, which has been thought to contribute to the cardiovascular manifestation of MFS (Neptune *et al*., 2003; Habashi *et al*., 2006; Matt *et al*., 2009; Kim *et al*., 2013). Suppression of excessive TGFβ signaling would prevent dissection and aneurysm formation. Accordingly, losartan, an angiotensin II type 1 receptor (AT1R) antagonist, was assumed to have a beneficial effect on MFS based on the findings that losartan attenuated the TGFβ signaling in other diseases including chronic renal failure (Lavoie *et al*., 2005). In various murine models of MFS, treatment with losartan significantly prevented the development of thoracic aortic aneurysm. This therapeutic effect of losartan on MFS-associated aortopathy was independent of the blood pressure lowering effect; atenolol, an antihypertensive β blocker, exerted much less pronounced effect on MFS than losartan did. These findings suggest that anti-TGFβ therapy would become an attractive approach to ameliorate the vascular manifestation of MFS

Open Access **https://doi.org/10.4062/biomolther.2019.042**

Received Mar 7, 2019 **Revised** Apr 19, 2019 **Accepted** Jun 4, 2019
Published Online Jul 9, 2019

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(Habashi *et al*., 2006; Xiong *et al*., 2012). However, no TGFβ inhibitors other than losartan has been studied as therapeutic approaches to ameliorate the vascular manifestation of MFS. To my knowledge, the present study might be the first to investigate the preventive effect of small-molecule inhibitor of TGFβ receptor in a murine model of MFS.

In the present study, we hypothesized that galunisertib (LY2157299 monohydrate), a potent small-molecule inhibitor of the TGFβ receptor I (TβRI) kinase, might prevent the MFS-associated aortopathy by blocking overactivated TGFβ signaling. Galunisertib is an oral chemical inhibitor of TβRI kinase that specifically abrogates the Smad2-dependent canonical pathways of TGFβ (Herbertz *et al*., 2015). It has antitumor efficacy with an acceptable margin of safety and is currently being studied in Phase II clinical trials for the treatment of various cancers (Herbertz *et al*., 2015). In this study, galunisertib or losartan was orally administered to *Fbn1*$^{C1039G/+}$ mice, which are MFS mice with heterozygous missense mutation in *Fbn1*. The histopathological changes (i.e., cystic medial degeneration, elastin loss and fragmentation, and apoptosis) and levels of phosphorylated Smad2 and extracellular signal-regulated kinase 1/2 (Erk1/2) proteins in aortic media were evaluated after an 8-week treatment with galunisertib or losartan and compared, to determine the effect of galunisertib on the aortopathy in MFS mice.

MATERIALS AND METHODS

Animals

All procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH, Bethesda, MD, USA). Protocols for all animal experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University in Seoul, Korea (approval number: 2016-00089). *Fbn1*$^{C1039G/+}$ mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and wild type (WT) mice were littermates of the *Fbn1*$^{C1039G/+}$ mice. Both male and female mice were included in this study. Mice were anesthetized with an intraperitoneal injection of ketamine (79.5 mg/kg) and xylazine (9.1 mg/kg) and underwent immediate laparotomy, descending abdominal aortic transection, and perfusion with saline containing heparin sodium (JW Pharma, Seoul, Korea) through the right and left ventricles. The mouse aortic root and ascending aortas (aortic root to origin of right brachiocephalic trunk) were then harvested, fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm sections, which were deparaffinized and rehydrated in ethanol for histological and morphometric analysis.

Drug treatment

Drug treatment commenced at 8 weeks of age and continued for 8 weeks. *Fbn1*$^{C1039G/+}$ mice received 0.6 g/L losartan *ad libitum* in drinking water and *Fbn1*$^{C1039G/+}$ mice treated with drinking water were included as a control. Galunisertib (Selleck Chemicals, Houston, TX, USA) was reconstituted in 20% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich, St. Louis, MO, USA) and orally administered to *Fbn1*$^{C1039G/+}$ mice once daily (30 mg·kg^{-1}·day^{-1}) with a 5 day on and 2 day off schedule. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice were included as a control. Sixteen-week-old WT littermates were included as a normal control.

Immunohistochemistry

After quenching the endogenous peroxidase activity with 3% hydrogen peroxide and blocking with 10% normal goat serum, sections were treated with 1% Triton X-100 (Sigma-Aldrich) and incubated overnight with anti-phospho-Smad2 (p-Smad2) IgG (Invitrogen, Carlsbad, CA, USA; Cat. Number SF255280, at 1:30) or anti-phospho-Erk1/2 (p-Erk1/2) IgG (Cell Signaling, Danvers, MA, USA; Cat. Number 4370, at 1:200). The sections were then washed and incubated with biotinylated secondary IgG (Vector Laboratories, Burlingame, CA, USA; Cat. Number BA-1000, at 1:200). After washing, positive immunoreactivity was visualized using the ABC-peroxidase kit (Vector Laboratories) and 3,3'-diaminobenzidine tetrachloride (Vector Laboratories). Stained sections were then counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted. The magnitude of Smad2 or Erk1/2 phosphorylation was scored from 1 to 4 by two independent observers based on the percentage of cells that were positive for p-Smad2 or p-Erk1/2 by two independent observers (grade 1, no positive cell; grade 2, <30% positive cells; grade 3, 30-50% positive cells; grade 4, >50% positive cells).

Hematoxylin and Eosin (H&E) staining

Sections were treated with Mayer's hematoxylin (Sigma-Aldrich), washed with water, and then counterstained with Eosin Y solution alcoholic (Sigma-Aldrich). After washing, the sections were dehydrated, cleared in xylene, and mounted using Canada balsam (Sigma-Aldrich). Stained sections were photographed using an upright microscope (Olympus, Tokyo, Japan). Six to ten sections were examined per mouse. The magnitude of cystic medial degeneration was scored from 1 to 4 by two independent observers based on the percentage of cystic medial degeneration area in sections (grade 1, no focal; grade 2, <5% focal; grade 3, 5-10% focal; grade 4, >10% diffuse).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assays were performed on sections using an *in situ* cell death detection kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. In brief, sections were treated with 0.5% Triton X-100 for permeabilization and incubated with terminal deoxynucleotidyl transferase at 37°C. Sections were then mounted with antifade medium containing 4,6-diamidino-2-phenylindole (Vector Laboratories).

Lawson staining

Sections were stained in Lawson solution (Klinipath, Duiven, Netherlands) to selectively stain elastin and differentiated in 100% ethanol. After washing in water, the sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and images were captured. Two independent observers assessed the aortic wall architecture by grading the degree of gaps between adjacent elastin laminae, elastin loss and fragmentation in sections on a scale of 1 to 4 (grade 1, intact elastic fiber morphology; grade 2, moderate elastin fragmentation; grade 3, increased gaps between adjacent elastin laminae, moderate elastin loss and fragmentation; grade 4, large gaps

between adjacent elastic laminae, diffuse elastin loss and fragmentation).

Statistical analysis

All data are expressed as the means ± standard error of the mean (SEM). Statistical significance was evaluated using an unpaired Student *t*-test (GraphPad Prism, San Diego, CA, USA).

RESULTS

Galunisertib significantly suppressed Smad2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice

In *Fbn1*$^{C1039G/+}$ mice, aortic lesions characterized by cystic medial degeneration, apoptosis, and disrupted aortic wall architecture become more severe as mice get older (Habashi *et al*., 2006; Yang *et al*., 2009). In accordance with these previous findings, we also observed that 8-week-old *Fbn1*$^{C1039G/+}$ mice did not display MFS-associated histopathological feature and Smad2 phosphorylation in their ascending aortas. However, 16-week-old *Fbn1*$^{C1039G/+}$ mice exhibited enhanced phosphorylation of Smad2, cystic medial degeneration, elastin loss and fragmentation, and apoptosis (data not shown). Because the aim of the present study was to determine the preventive effect of the TβRI inhibitor, galunisertib, on MFS-associated aortic vascular pathogenesis, drug treatment was started before the onset of MFS and continued until MFS-associated aortic histopathology became evident. Either galunisertib or losartan was administered to 8-week-old *Fbn1*$^{C1039G/+}$ mice for 8 weeks when they became 16 weeks old (Wisler *et al*., 2015; Hibender *et al*., 2016).

First, we performed immunohistochemical analysis to investigate whether treatment with galunisertib would effectively inhibit the canonical TGFβ signaling pathway that is highly activated in murine models and patients with MFS (Fig. 1A). *Fbn1*$^{C1039G/+}$ mice treated with gastric juice showed substantial increase in Smad2 phosphorylation in their ascending aortas relative to age-matched normal WT mice. Smad2 phosphorylation in *Fbn1*$^{C1039G/+}$ mice treated with galunisertib was significantly lower than that of *Fbn1*$^{C1039G/+}$ mice with gastric juice and indistinguishable from that observed in normal WT mice (Fig. 1B). In accordance with other previous findings, mice

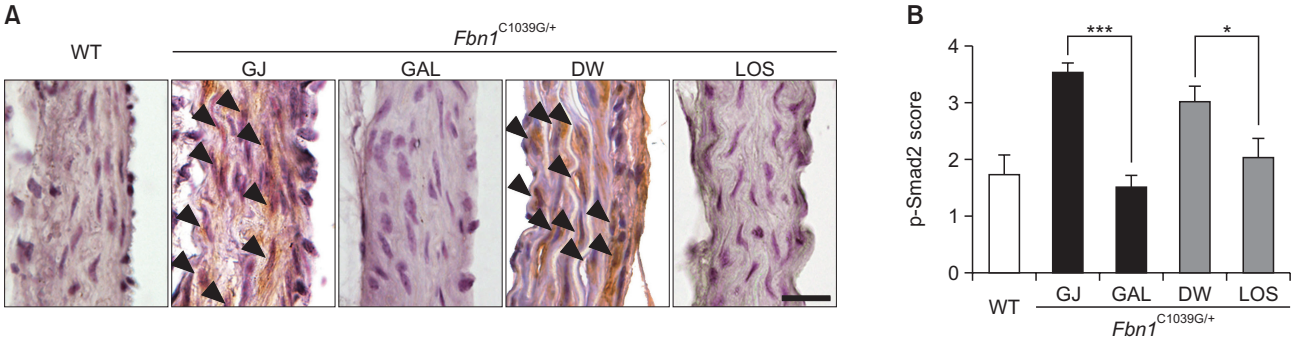

Fig. 1. Treatment with either galunisertib or losartan significantly suppressed Smad2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. (A) Representative images of immunohistochemical staining of phospho-Smad2 (p-Smad2) on ascending aortas harvested from 16-week-old WT and *Fbn1*$^{C1039G/+}$ mice. *Fbn1*$^{C1039G/+}$ mice were treated with galunisertib (GAL; n=6) or losartan (LOS; n=6) for 8 weeks. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice (GJ; n=10) or drinking water (DW; n=9) were used as controls. WT littermates of same age (n=7) were included as normal control. Arrowheads indicate p-Smad2-positive nuclei. Scale bar: 20 μm. (B) Quantitative analysis of p-Smad2 staining intensity in immunohistochemical images. Data are expressed as the mean ± SEM (**p*<0.05; ****p*<0.001).

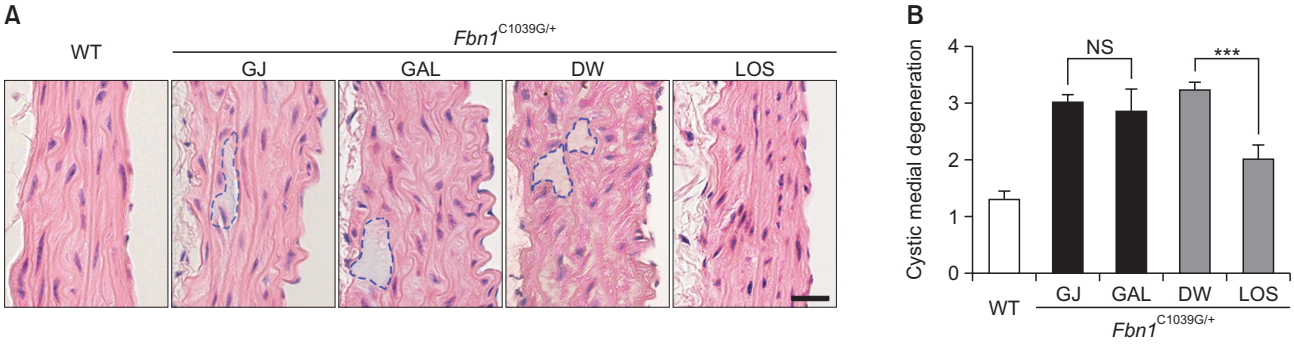

Fig. 2. Treatment with galunisertib did not prevent cystic medial degeneration in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. (A) Representative H & E stained images of ascending aortas harvested from 16-week-old WT and *Fbn1*$^{C1039G/+}$ mice. *Fbn1*$^{C1039G/+}$ mice were treated with galunisertib (GAL; n=6) or losartan (LOS; n=6) for 8 weeks. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice (GJ; n=10) or drinking water (DW; n=9) were used as controls. WT littermates of same age (n=7) were included as normal control. Blue dotted lines indicate the lesion with cystic medial degeneration. Scale bar: 20 μm. (B) Quantitative analysis of cystic medial degeneration in stained images. Data are expressed as the mean ± SEM (****p*<0.001; NS=not significant).

treated with losartan also exhibited much less phosphorylation of Smad2 in their ascending aortas than mice treated with drinking water (Habashi *et al*., 2006). This result indicates that 8-week treatment with either galunisertib or losartan almost completely suppressed the canonical TGFβ signaling in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice.

Galunisertib did not inhibit aortic vascular pathogenesis in *Fbn1*$^{C1039G/+}$ mice

Next, we carried out histopathological analysis to investigate whether treatment with galunisertib would prevent aortic vascular pathogenesis in *Fbn1*$^{C1039G/+}$ mice. We first examined H & E stained sections for the presence and degree of cystic medial degeneration (Fig. 2A). The ascending aortas of galunisertib-treated *Fbn1*$^{C1039G/+}$ mice displayed substantial cystic medial degeneration, which was as severe as those of gastric juice-treated control mice (Fig. 2B). However, losartan-treated *Fbn1*$^{C1039G/+}$ mice exhibited a significant decrease in cystic medial degeneration relative to drinking water-treated controls. In addition, TUNEL analysis was performed to assess the cellular apoptosis in aortic media. As shown in Fig. 3, substantial medial apoptosis occurred in the ascending aortas of *Fbn1*$^{C1039G/+}$ control mice. However, administration of losartan significantly reduced the number of TUNEL-positive cells, whereas galunisertib treatment did not attenuate the medial apoptosis in *Fbn1*$^{C1039G/+}$ mice. Additionally, we carried out Lawson staining analysis to evaluate the integrity of aortic wall architecture in *Fbn1*$^{C1039G/+}$ mice (Fig. 4A). While ascending aortas of losartan-treated *Fbn1*$^{C1039G/+}$ mice displayed almost intact aortic wall architecture with little loss and fragmentation of elastin, those of galunisertib-treated *Fbn1*$^{C1039G/+}$ mice exhibited severely disrupted aortic wall architecture with substantial loss and fragmentation of elastin, which was not statistically different from those of control mice (Fig. 4B). These findings indicate that treatment with galunisertib was not effective in preventing cystic medial degeneration, elastin loss and fragmentation, and apoptosis in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice.

Losartan, but not galunisertib, significantly suppressed Erk1/2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice

We then investigated how galunisertib and losartan exerted differential effects on the aortic vascular pathogenesis in MFS

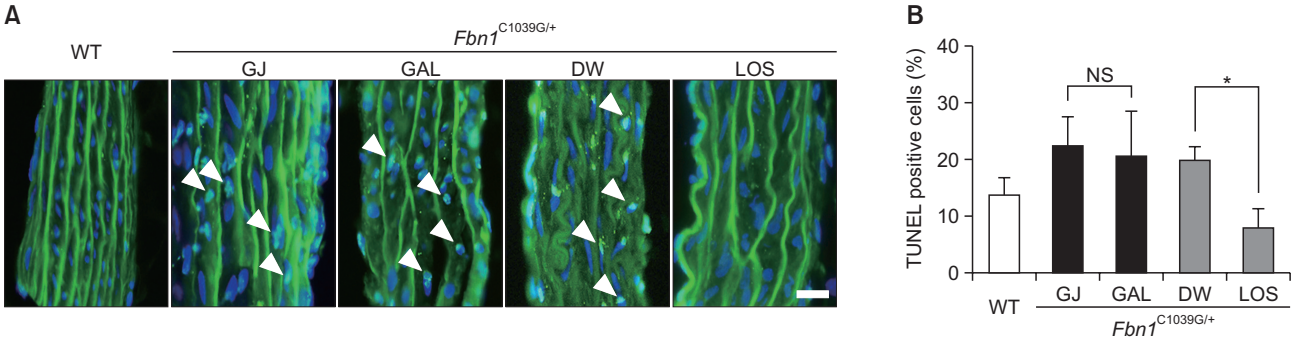

Fig. 3. Treatment with galunisertib did not reduce medial apoptosis in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. (A) Representative TUNEL stained images of ascending aortas harvested from 16-week-old WT and *Fbn1*$^{C1039G/+}$ mice. *Fbn1*$^{C1039G/+}$ mice were treated with galunisertib (GAL; n=6) or losartan (LOS; n=6) for 8 weeks. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice (GJ; n=10) or drinking water (DW; n=9) were used as controls. WT littermates of same age (n=7) were included as normal control. Arrowheads indicate TUNEL-positive apoptotic cells. Scale bar: 20 μm. (B) Quantitative analysis of apoptosis in stained images. Data are expressed as the mean ± SEM (*p<0.05; NS=not significant).

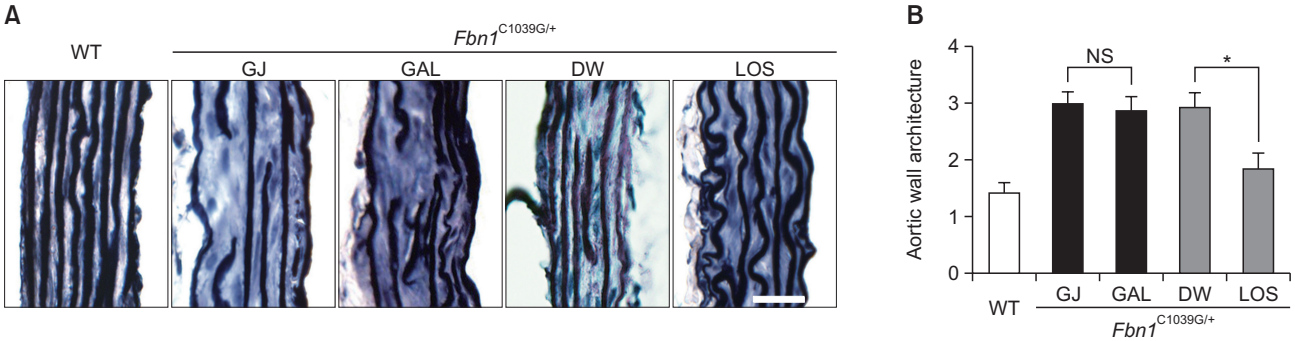

Fig. 4. Treatment with galunisertib did not ameliorate elastin loss and fragmentation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. (A) Representative Lawson-stained images of ascending aortas harvested from 16-week-old WT and *Fbn1*$^{C1039G/+}$ mice. *Fbn1*$^{C1039G/+}$ mice were treated with galunisertib (GAL; n=6) or losartan (LOS; n=6) for 8 weeks. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice (GJ; n=10) or drinking water (DW; n=9) were used as controls. WT littermates of same age (n=7) were included as a normal control. Scale bar: 20 μm. (B) Quantitative analysis of aortic wall architecture in stained images. Aortic wall architecture was assessed by grading the degree of elastin loss and fragmentation. Data are expressed as the mean ± SEM (*p<0.05; NS=not significant).

mice, although both drugs completely suppressed the canonical TGFβ signaling. Prior studies of *Fbn1*$^{C1039G/+}$ mice have reported that Erk1/2 contributes to the development and progression of MFS-related aortic diseases (Holm *et al*., 2011). Selective inhibition of Erk1/2 substantially ameliorated aortic root growth, and losartan treatment also attenuated aortic aneurysm in MFS mice through suppressing Erk1/2 phosphorylation (Holm *et al*., 2011). Given the prominent role of Erk1/2 in MFS-associated aortic vascular pathogenesis, we examined Erk1/2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. Erk1/2 phosphorylation in losartan-treated mice was substantially reduced relative to that of drinking water-treated control mice (Fig. 5). However, galunisertib-treated mice exhibited a marginal decrease in Erk1/2 phosphorylation compared with gastric juice-treated control mice, in which the difference between groups was not statistically significant. This result suggested that galunisertib treatment only partially attenuated the phosphorylation of Erk1/2 in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice, while losartan treatment completely inhibited the phosphorylation of Erk1/2.

DISCUSSION

TGFβ is implicated in many pathological conditions, including cancer, fibrosis, inflammation, and cardiovascular disorders (Akhurst and Hata, 2012). It is often chronically overexpressed and/or TGFβ signaling is aberrantly activated in patients, which drives disease progression by modulating cell growth, migration, or phenotype (Akhurst and Hata, 2012). In MFS caused by mutations in *Fbn1* gene, TGFβ expression and Smad2 phosphorylation in aortic tissues are substantially elevated, and administration of losartan, an anti-hypertension drug that inhibits TGFβ signaling, effectively reduced the cardiovascular manifestations in murine models (Dietz *et al*., 1991; Neptune *et al*., 2003; Habashi *et al*., 2006; Matt *et al*., 2009). These findings reveal that dysregulation of TGFβ might contribute to the development and progression of MFS-related aortic aneurysm and dissection, implying the novel therapeutic potential of pharmacological inhibitors of TGFβ signaling, such as the anti-tumor agent galunisertib for the treatment of aneurysmal disease in MFS.

In the present study, we demonstrated that 8-week administration of either galunisertib or losartan effectively blocked the canonical TGFβ signaling, i.e., Smad2 phosphorylation, in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. Galunisertib did not exert noticeable inhibitory effect on histopathological changes in aortic medial layers of *Fbn1*$^{C1039G/+}$ mice, while losartan significantly inhibited cystic medial degeneration, elastin loss and fragmentation, and apoptosis, as reported previously (Habashi *et al*., 2006). This result revealed that blockade of canonical TGFβ signaling with TβRI inhibitors might not be effective in preventing early aortic vascular pathogenesis in *Fbn1*$^{C1039G/+}$ mice. A recent study showed that genetic haploinsufficiency of Smad4, a crucial mediator of canonical TGFβ signaling pathway, exacerbated rather than improved aortic aneurysm and rupture in *Fbn1*$^{C1039G/+}$ mice, suggesting that Smad-dependent TGFβ signaling is not principally responsible for MFS-associated aortic disease (Holm *et al*., 2011). Furthermore, several recent studies have proposed Erk1/2, but not Smad2 as a prominent signaling pathway mediating MFS-associated aortic vascular pathogenesis, because MFS mice exhibited a substantial increase in phosphorylation of Erk1/2 as well as Smad2 in their aortic tissues, and Erk1/2 inhibitors effectively prevented the progression of aortic disease in MFS mice (Holm *et al*., 2011). Thus, we examined Erk1/2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice and found that Erk1/2 phosphorylation was completely inhibited by losartan treatment, but only partially reduced by galunisertib treatment. There might be several possible reasons for the incomplete blockade of Erk1/2 phosphorylation in galunisertib-treated groups. Galunisertib might not be much effective in blocking noncanonical TGFβ signaling pathways such as Erk1/2, when compared with inhibition of canonical signaling pathway. Other studies with hepatocellular carcinoma cells reported that galunisertib exhibited a strong potency in blocking TGFβ-induced Smad2 phosphorylation but exerted only moderate inhibitory effect on noncanonical TGFβ signaling pathways, including Erk1/2 and Akt, in several cell lines (Serova *et al*., 2015). Moreover, signals other than TGFβ might induce Erk1/2 phosphorylation by activating AT1R. Loss of elastic fiber integrity, a typical aortic manifestation of MFS, makes thoracic aortic walls predisposed to dilatation in response to high blood pressure, which highly increases the mechanical stress

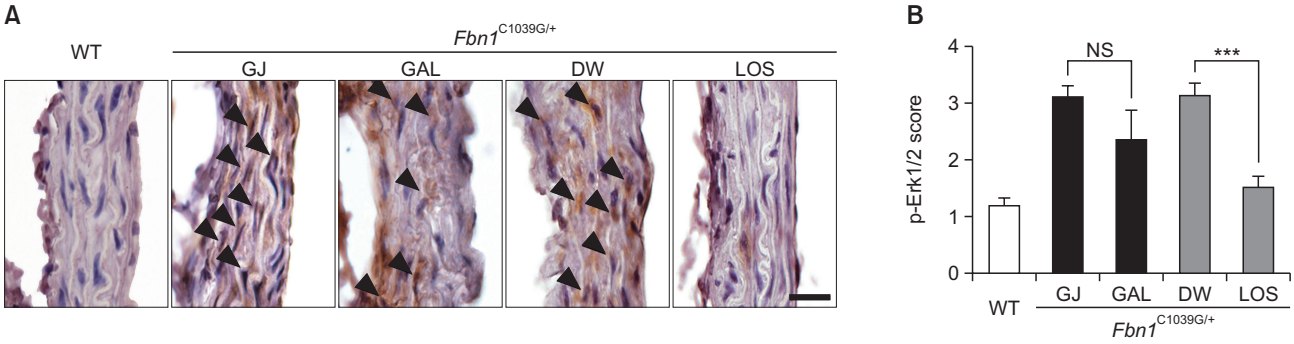

Fig. 5. Treatment with galunisertib did not significantly suppress Erk1/2 phosphorylation in the ascending aortas of *Fbn1*$^{C1039G/+}$ mice. (A) Representative images of immunohistochemical staining of phospho-Erk1/2 (p-Erk1/2) on ascending aortas harvested from 16-week-old WT and *Fbn1*$^{C1039G/+}$ mice. *Fbn1*$^{C1039G/+}$ mice were treated with galunisertib (GAL; n=6) or losartan (LOS; n=6) for 8 weeks. *Fbn1*$^{C1039G/+}$ mice treated with gastric juice (GJ; n=10) or drinking water (DW; n=9) were used as controls. WT littermates of same age (n=7) were included as a normal control. Arrowheads indicate p-Erk1/2-positive nuclei. Scale bar: 20 μm. (B) Quantitative analysis of p-Erk1/2 staining intensity in immunohistochemical images. Data are expressed as the mean ± SEM (***p<0.001; NS=not significant).

on vascular smooth muscle cells. In muscle cells, including cardiomyocytes and vascular smooth muscle cells, elevated mechanical stress activates AT1R and its downstream signaling pathway, Erk1/2, without the involvement of angiotensin II (Zou *et al*., 2004; Schleifenbaum *et al*., 2014). Such angiotensin II-independent mechanical activation of AT1R and Erk1/2 can be inhibited by AT1R inhibitors, but not directly by TβRI inhibitors.

In summary, contrary to our expectation, galunisertib, a TβRI inhibitor, did not exert beneficial effect on aneurysmal disease in MFS mice despite its complete inhibition of Smad2 phosphorylation. However, losartan that inhibited Smad2 phosphorylation as effectively as galunisertib did, substantially blocked aortic vascular pathogenesis in MFS mice. These findings suggest that canonical TGFβ signaling might not be principally responsible for MFS-associated aneurysmal diseases in mice, and blockade of other signaling pathways, such as Erk1/2, might be a therapeutic strategy for MFS.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korea government [2017M3A9C8061192] and the Chung-Ang University Graduate Research Scholarship in 2017.

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