Colchicine Inhibits Smooth Muscle Cell Phenotypic Switch and Aortic Dissection in Mice

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Abstract

Aortic dissection is a serious cardiovascular disease characterized by intimal tearing and vascular delamination. Smooth muscle cell phenotypic transformation, extracellular matrix degradation and vascular inflammation are the main pathogenic mechanisms of aortic dissection. Colchicine is an alkaloid derived from the plant *Colchicum autumnale* L. and was the first FDA-approved anti-inflammatory drug in the cardiovascular field. In this study, we investigated the protective effects of colchicine in aortic dissection in mice and found that colchicine resisted aortic dissection by inhibiting vascular inflammation and smooth muscle cell phenotypic transformation, and that colchicine reversed smooth muscle cell phenotypic transformation by targeting the transcriptional activator myocardin. This study provides new directions for the development of drug therapies for aortic dissection.

Key words: Aortic dissection, Colchicine, Inflammation, Smooth muscle cell phenotypic transformation

Aortic dissection is a cardiovascular condition where a tear in the intimal layer allows blood to separate the vessel wall layers, causing delamination. Open and endovascular repair surgery is the primary treatment option[1]. The pathogenesis involves vascular smooth muscle cells (VSMCs) transitioning from a contractile to a synthetic phenotype, along with inflammatory cell infiltration that releases matrix metalloproteinases and cytokines, promoting extracellular matrix degradation and dissection progression[2]. Colchicine is an alkaloid compound found in the *Colchicum* plant, recognized for its potent anti-inflammatory effects and considered for treating various cardiovascular diseases, such as pericarditis, atrial fibrillation, and coronary syndromes[3]. The FDA has approved colchicine as the first anti-inflammatory drug for the treatment of atherosclerotic cardiovascular disease. However, it remains unknown whether colchicine can inhibit the development of aortic dissection.

To investigate the protective effect of colchicine in aortic dissection, we used a β-Aminopropionitrile (BAPN)-induced aortic dissection model in mice. Three-week-old male C57BL/6J mice were fed a standard chow diet, and BAPN (A3134, Sigma-Aldrich, USA) was administered via drinking water at a concentration of 1 g/kg/day for 3 weeks. Simultaneously, mice were gavaged with either colchicine (200 μg/kg/day) (C3915, Sigma-Aldrich, USA) or an equivalent dose of saline (Fig. A). All mouse experiments were approved by Institutional Animal Care and Use Committee. Mice were monitored daily for survival, and any mice died were immediately dissected to examine the aorta. Mice in the colchicine group showed a higher survival rate (Fig. B). Gross evaluation of the aorta revealed that the control group exhibited a severe disease phenotype with an 80% incidence of dissection, whereas the colchicine-treated group showed a significantly reduced incidence of dissection at 36% (Fig. C). Images of the aorta are shown in Fig. D. Pathological analysis revealed that the vehicle group developed significant dissections characterized by intimal tearing and the formation of a false lumen. H&E staining showed erythrocytes passing through the lumen, filling the detached vascular layers, while EVG staining indicated elastic lamina fragmentation. In contrast, the aortic vessels in the colchicine group displayed better structural integrity (Fig. E).

Transcriptome sequencing analysis of the thoracic aorta from control and colchicine-treated mice (data are available at GEO: GSE279545), using differentially expressed genes (DEGs) defined by $\log2(FC) > 1$ and $p < 0.05$, identified 502 significantly upregulated genes and 1,471 significantly downregulated genes (Fig. F). Reactome pathway enrichment analysis of these DEGs revealed significant enrichment in pathways related to the immune system and signal transduction. Similarly, KEGG enrichment analysis showed that cytokine-cytokine receptor interactions and chemokine signaling pathways were notably affected (Fig. G). Analysis of molecular genes related to smooth muscle cell transformation and vascular inflammation showed that contractile phenotype-related genes (*Myocd*, *Myh11*, *Cnn1*, *Acta2*) were significantly upregulated in the colchicine-treated group,

while synthetic phenotype-related genes (*Fn1*, *Spp1*), inflammation-related genes (*IL1b*, *IL6*, *Ccl2*), and extracellular matrix degradation genes (*Mmp9*) were markedly reduced compared to

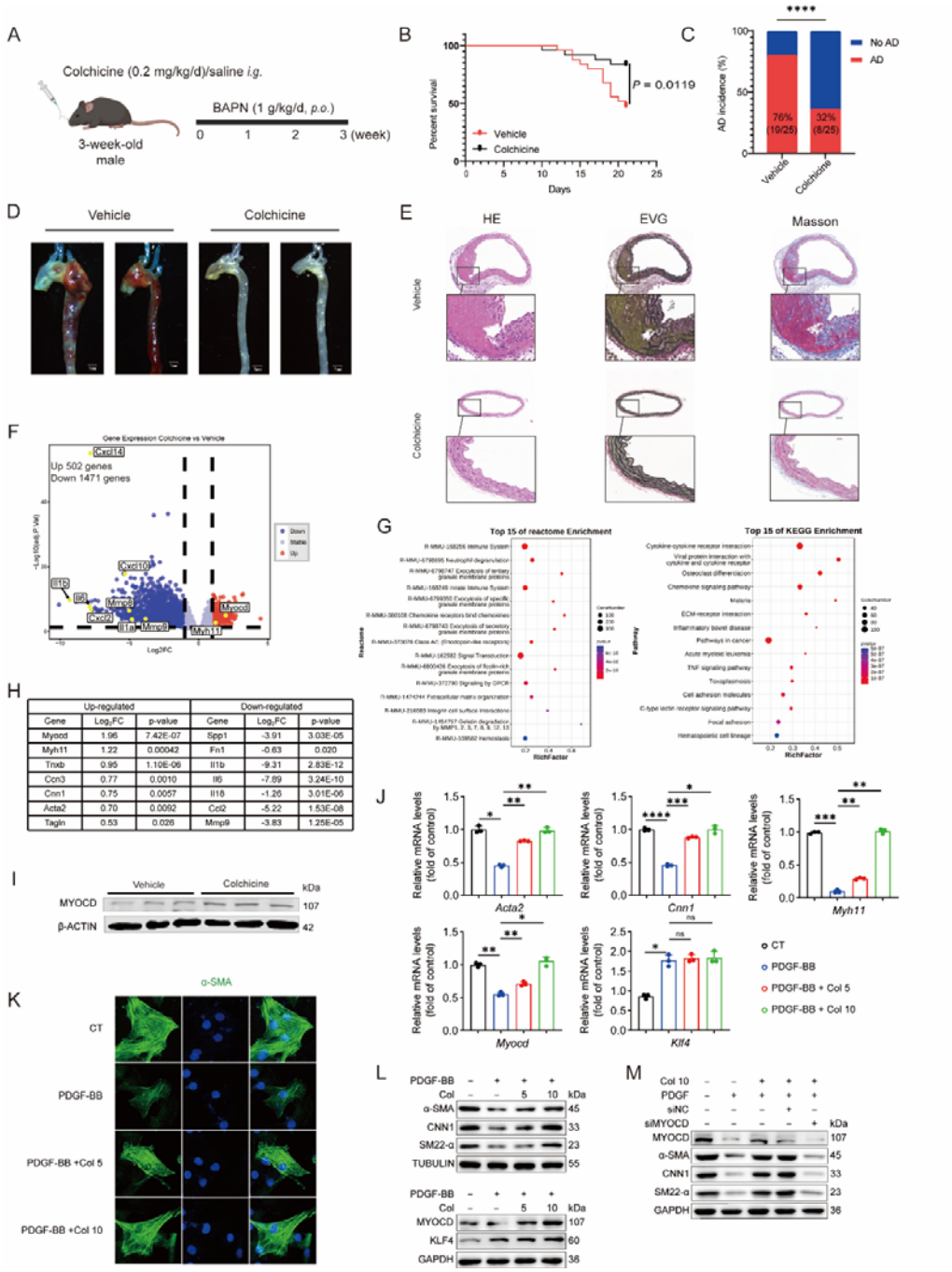

Figure. Colchicine resists aortic dissection in mice.

(A) Schematic representation of aortic dissection induction in three-week-old male mice by administering β-aminopropionitrile monofumarate (BAPN) in drinking water, along with colchicine or saline gavage treatment. (B) Survival curves of colchicine-treated and control mice. Survival rates were analyzed using the Log-rank (Mantel-Cox) test (n = 25 per group). (C) Incidence of aortic dissection in

colchicine-treated and control mice. Incidence was compared using Fisher's exact test (n = 25 per group). (D) Representative images of the aortas from colchicine-treated and control mice (n = 25 per group). (E) Representative histological sections of the aorta from colchicine-treated and control mice stained with hematoxylin-eosin (HE), elastic van Gieson (EVG), and Masson's trichrome (Masson). Scale bar, 100 μm for original image; Scale bar, 20 μm for enlarged inset. (F) Volcano plot of differentially expressed genes in the aorta of colchicine-treated versus control mice. Blue dots indicate downregulated genes, while red dots represent upregulated genes. (G) KEGG and Reactome pathway enrichment analyses of differentially expressed genes in the aortas of colchicine-treated and control mice. (H) Levels of smooth muscle cell phenotype-switching and inflammatory genes in colchicine-treated mice compared to control mice. (I) Western blot analysis of MYOCD protein expression in the aorta of colchicine-treated and control mice (n = 3 per group). (J) Relative mRNA levels of contractile markers and transcription factors in rat smooth muscle cells under PDGF stimulation and treated with various concentrations of colchicine (5 μM and 10 μM) (n = 3 per group). Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001; two-tailed unpaired t-test. (K) Immunofluorescence staining of α-SMA in rat smooth muscle cells under PDGF stimulation and treated with different concentrations of colchicine (5 μM and 10 μM) (n = 3 per group). (L) Western blot analysis of contractile markers and transcription factor levels in rat smooth muscle cells stimulated by PDGF and treated with different concentrations of colchicine (5 μM and 10 μM) (n = 3 per group). (M) Western blot analysis of contractile markers in rat smooth muscle cells under PDGF-BB stimulation with 10 μM colchicine treatment and MYOCD knockdown (siMYOCD) (n = 3 per group).

the control group (Fig. H). Furthermore, colchicine treatment resulted in elevated levels of MYOCD protein in the aortic tissues of mice (Fig. I). These findings suggest that colchicine may exert its protective effects by reducing vascular inflammation and inhibiting smooth muscle phenotypic transformation.

To further investigate the mechanism by which colchicine inhibits the development of aortic dissection, we examined its ability to suppress platelet-derived growth factor BB (PDGF-BB)-induced phenotypic transition in primary rat VSMCs. Colchicine effectively reversed the PDGF-BB-induced downregulation of VSMC contractile molecules at both the gene level—including *Acta2*, *Cnn1*, *Myh11*, and *Myocd*—and the protein level, including α-SMA, CNN1, SM22-α, and MYOCD, but did not affect KLF4 at the gene or protein level (Fig. J, K and L). Since MYOCD is a co-activator of serum response factor, a transcription factor that regulates the contractile phenotype of VSMCs[4], we hypothesized that colchicine may influence the expression of downstream VSMC contractile molecules by modulating MYOCD expression. To test this hypothesis, we constructed MYOCD-specific siRNA. Upon

knockdown of MYOCD, the ability of colchicine to reverse the VSMC phenotype was lost, suggesting that MYOCD is a key target of colchicine's action (Fig. M). Additionally, colchicine did not exhibit a significant inhibitory effect on TNF-α-induced inflammation in VSMCs (data not shown).

In conclusion, this study highlights that colchicine, a well-known anti-inflammatory drug, holds significant promise for the treatment of aortic dissection, a critical cardiovascular disease. In a mouse model of aortic dissection, colchicine markedly improved survival rates by inhibiting the phenotypic transformation of VSMCs and reducing vascular inflammation. Since colchicine's known mechanism involves binding to microtubule proteins and inhibiting their polymerization, which suppresses inflammatory cell movement and cytokine release[5], further research is needed to confirm whether colchicine reduces inflammation by affecting immune cells in vascular milieu. More diverse animal models and clinical trials are also necessary to confirm its efficacy in treating aortic dissection.

ARTICLE INFORMATION

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Disclosures

The authors declare no competing interests.

REFERENCES

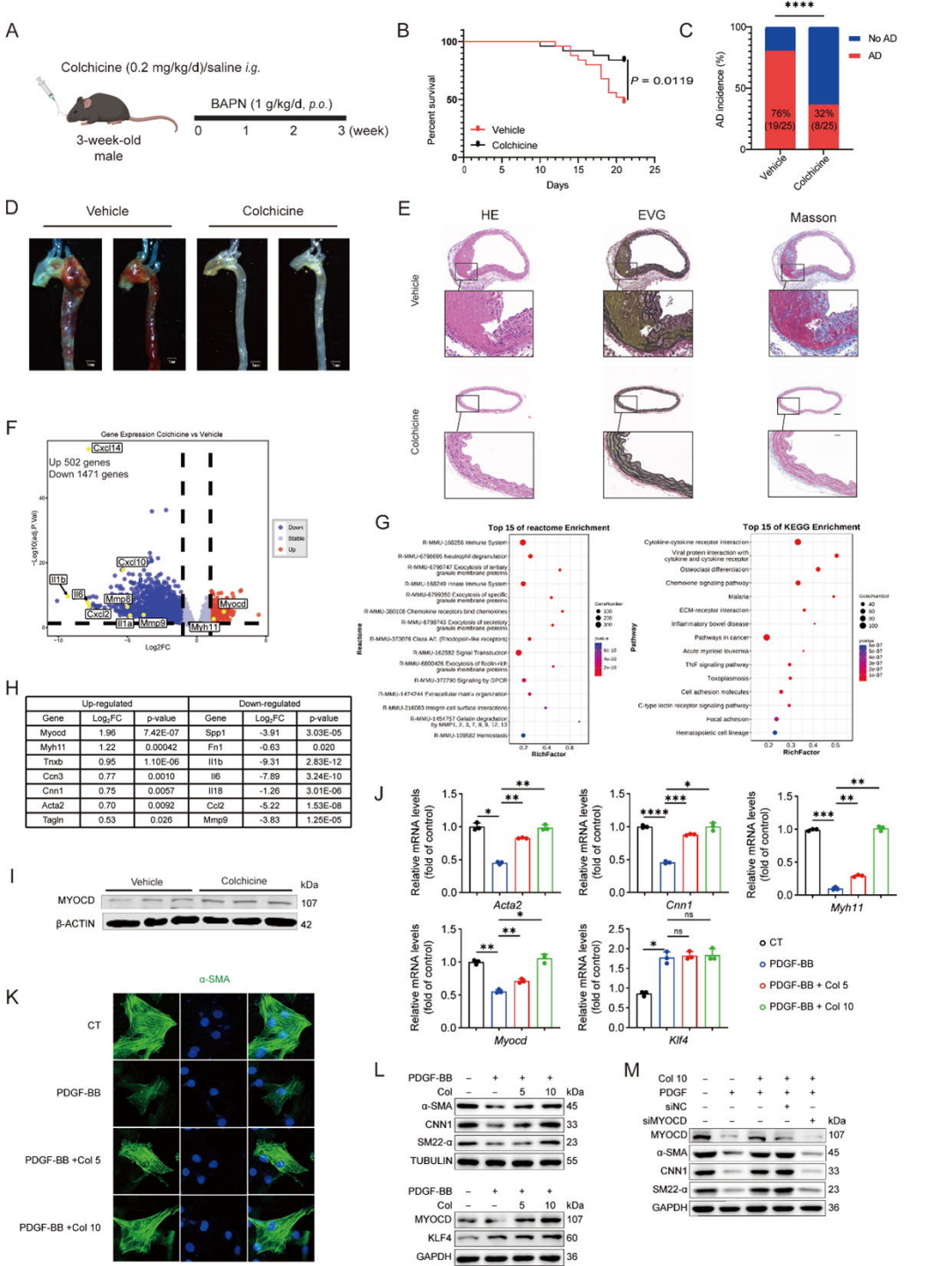
(1) Silaschi, M.; Byrne, J.; Wendler, O. Aortic dissection: medical, interventional and surgical management. *Heart* **2017**, *103* (1), 78-87. DOI: 10.1136/heartjnl-2015-308284 From NLM.

(2) Shen, Y. H.; LeMaire, S. A.; Webb, N. R.; Cassis, L. A.; Daugherty, A.; Lu, H. S. Aortic Aneurysms and Dissections Series. *Arterioscler Thromb Vasc Biol* **2020**, *40* (3), e37-e46. DOI: 10.1161/atvbaha.120.313991 From NLM.

(3) Deftereos, S. G.; Beerkens, F. J.; Shah, B.; Giannopoulos, G.; Vrachatis, D. A.; Giotaki, S. G.; Siasos, G.; Nicolas, J.; Arnott, C.; Patel, S.; et al. Colchicine in Cardiovascular Disease: In-Depth Review. *Circulation* **2022**, *145* (1), 61-78. DOI: 10.1161/circulationaha.121.056171 From NLM.

(4) Fan, C.; Plaxco, K. W.; Heeger, A. J. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc Natl Acad Sci U S A* **2003**, *100* (16), 9134-9137. DOI: 10.1073/pnas.1633515100 From NLM.

(5) Angelidis, C.; Kotsialou, Z.; Kossyvakis, C.; Vrettou, A. R.; Zacharoulis, A.; Kolokathis, F.; Kekeris, V.; Giannopoulos, G. Colchicine Pharmacokinetics and Mechanism of Action. *Curr Pharm Des* **2018**, *24* (6), 659-663. DOI: 10.2174/1381612824666180123110042 From NLM.



Up-regulated			Down-regulated		
Gene	Log_2FC	p-value	Gene	Log_2FC	p-value
Myocd	1.96	7.42E-07	Spp1	-3.91	3.03E-05
Myh11	1.22	0.00042	Fn1	-0.63	0.020
Tnxb	0.95	1.10E-06	Il1b	-9.31	2.83E-12
Ccn3	0.77	0.0010	Il6	-7.89	3.24E-10
Cnn1	0.75	0.0057	Il18	-1.26	3.01E-06
Acta2	0.70	0.0092	Ccl2	-5.22	1.53E-08
Tagln	0.53	0.026	Mmp9	-3.83	1.25E-05