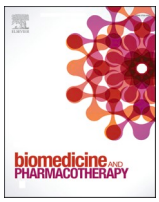
Biomedicine & Pharmacotherapy 167 (2023) 115504

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha



Angiogenesis inhibitor-specific hypertension increases the risk of developing aortic dissection

Kaito Tsujinaka [a,b], Yuki Izawa-Ishizawa [a,c,*], Koji Miyata [a], Toshihiko Yoshioka [a,b], Kohei Oomine [a], Honoka Nishi [a], Masateru Kondo [a,b], Syuto Itokazu [a], Tatsumi Miyata [a], Takahiro Niimura [a,d], Maki Sato [a], Fuka Aizawa [a,b], Kenta Yagi [a,d], Masayuki Chuma [e], Yoshito Zamami [f], Mitsuhiro Goda [a,b], Keisuke Ishizawa [a,b,d]

[a] Department of Clinical Pharmacology and Therapeutics, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
[b] Department of Pharmacy, Tokushima University Hospital, Tokushima, Japan
[c] Department of General Medicine, Taoka Hospital, Tokushima, Japan
[d] Clinical Research Center for Developmental Therapeutics, Tokushima University Hospital, Tokushima, Japan
[e] Department of Hospital Pharmacy & Pharmacology, Asahikawa Medical University & University Hospital, Asahikawa, Japan
[f] Department of Pharmacy, Okayama University Hospital, Okayama, Japan

ARTICLE INFO

Keywords:
Angiogenesis inhibitors
Aortic dissection
Real-world database
Hypertension
FDA Adverse Event Reporting System

ABSTRACT

Aortic dissection is an adverse event of angiogenesis inhibitors; however, the association between the drugs and aortic dissection is unclear. Therefore, we investigated if and how angiogenesis inhibitors increase the onset of aortic dissection using pharmacologically-induced aortic dissection-prone model (LAB) mice, cultured endothelial cells, and real-world databases, which is a novel integrated research approach. Disproportionality analysis was performed and calculated using the reporting odds ratio as a risk signal using a worldwide database of spontaneous adverse events to estimate the risk of adverse events. Angiogenesis inhibitors, but not other hypertension-inducing drugs, showed significant risk signals for aortic aneurysms and dissection. A retrospective cohort analysis using JMDC, a medical receipt database in Japan, showed that the history of atherosclerosis and dyslipidemia, but not hypertension, were significantly associated with the onset of aortic dissection during angiogenesis inhibitor medication administration. For in vivo studies, sunitinib (100 mg/kg/day) was administered to LAB mice. Sunitinib increased systolic blood pressure (182 mmHg vs. 288 mmHg with sunitinib; $p < 0.01$) and the incidence of aortic dissection (40% vs. 59% with sunitinib; $p = 0.34$) in mice. In vivo and in vitro studies revealed that sunitinib increased endothelin-1 expression and induced endothelial cell damage evaluated by intracellular- and vascular cell adhesion molecule-1 expressions. The increased risk of developing aortic dissection with angiogenesis inhibitors is associated with the development of drug-specific hypertension via endothelial cell damage and endothelin-1 expression. Our findings are invaluable in establishing safer anticancer therapies and strategies to prevent the development of vascular toxicity in high-risk patients.

1. Introduction

Aortic dissection is a dynamic condition in which the aortic wall is detached in two layers at the tunica media level, forming a false lumen with blood flow or hematomas within the aortic wall. Aortic dissection is a lethal disease, with sudden onset and death [1]. Aortic dissection is caused by hemodynamic loading against a background of tunica media weakening over time [2]. The etiology of tunica media lesions may be

Abbreviations: Ang II, angiotensin II; BAPN, β-aminopropionitrile; eNOS, endothelial nitric oxide synthesis; ET-1, endothelin-1; FAERS, FDA Adverse Event Reporting System; G-CSF, granulocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; KC, keratinocyte-derived chemokines; L-NAME, *N*ω-nitro-l-arginine methyl ester; MCP-1, monocyte chemoattractant protein 1; ROR, reporting odds ratio; SNT, sunitinib; VEGF, vascular endothelial growth factor; VCAM-1, vascular cell adhesion molecule 1.

* Correspondence to: Department of Clinical Pharmacology and Therapeutics, Tokushima University Graduate School of Biomedical Sciences, 3-18-15 Kuramoto, Tokushima 770-8503, Japan.
E-mail address: yuki.ishizawa@gmail.com (Y. Izawa-Ishizawa).

https://doi.org/10.1016/j.biopha.2023.115504
Received 15 July 2023; Received in revised form 30 August 2023; Accepted 12 September 2023
Available online 16 September 2023

due to decreased elastic lamina and cross-linking fibers between elastic fibers because of hypertension or inherited connective tissue diseases such as Marfan's syndrome [3,4]. Endothelial cell dysfunction may play an essential role in the onset of aortic dissection, adding to the medial fragility and hemodynamic loading [5–7].

Angiogenesis inhibitors are anticancer drugs that target vascular endothelial growth factor (VEGF), including anti-VEGF antibodies, anti-VEGF receptor antibodies, and VEGF tyrosine kinase inhibitors. These anticancer drugs are widely used for various carcinomas, including colorectal, lung, and breast cancer. Major side effects of angiogenesis inhibitors are hypertension, renal failure, neurotoxicity, and appetite loss. A meta-analysis showed a significant increase in blood pressure in 8% of bevacizumab-treated patients [8]. The cardiovascular toxicity of angiogenesis inhibitors is caused by their effects on cardiomyocytes, endothelial cells, and pericytes [9,10]. Depleting the VEGF pathway by inhibiting the VEGF receptor induces vascular endothelial cell apoptosis and may lead to acute arterial thrombotic events [11,12]. VEGF signaling inhibition also decreases nitric oxide production, a vasodilator, leading to increased vascular resistance and hypertension [13]. Endothelin-1 (ET-1) is associated with angiogenesis inhibitor-induced hypertension and renal failure [14]. Thus, various mechanisms have been demonstrated for cardiovascular toxicities associated with angiogenesis inhibitors.

Aortic dissection in patients using angiogenesis inhibitors is a rare but severe side effect [15]. The U.S. Food and Drug Administration (FDA) and other agencies issued a warning in 2018 regarding the risk of aortic dissection. However, how angiogenesis inhibitors cause aortic dissection has not been clarified, nor have preventive methods been established. In the present study, we demonstrate the risk and causes of aortic dissection induced by angiogenesis inhibitors.

We approached this research problem using a novel research methodology that we have recently established, which entails the integration of a large-scale medical information database analysis and in vivo and in vitro experiments. A large-scale medical information database, that is, "a real world database", is useful for researching emergency diseases, rare diseases, and the side effects that occur infrequently like aortic dissection. Many cases of aortic dissection happen suddenly and develop rapidly. Therefore, it is difficult to design a clinical study. This is one of the reasons why aortic dissection has not been studied well to date. The real-world database reflects the clinical situation and allows for comprehensive analysis of a vast number of aortic dissection cases. On the other hand, database analysis only proposes hypotheses and does not prove interrelationships. Therefore, we established a new research approach in which hypotheses obtained from databases are elucidated by basic pharmacological studies. In this study, we utilized the pharmacologically-induced aortic dissection prone model mice, which we established, to clarify the pathogenesis of angiogenesis inhibitor-induced aortic diseases.

2. Methods

2.1. Data analysis using the FDA adverse event reporting system

The FDA Adverse Event Reporting System (FAERS) is a voluntary adverse event reporting system operated by the U.S. FDA. Of the 14,524,065 spontaneous adverse events report data from the first quarter of 2004 to second quarter of 2020, duplicate reports were excluded according to FDA recommendations, and the remaining 12,190,284 reports were used for analysis (Fig. 1). The Navicat for SQLite database 3.33.0 was used to process the data. The types of adverse events were obtained from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) International Glossary of Pharmaceutical Terms, Medical Dictionary. The term "aortic dissection" was defined and extracted using

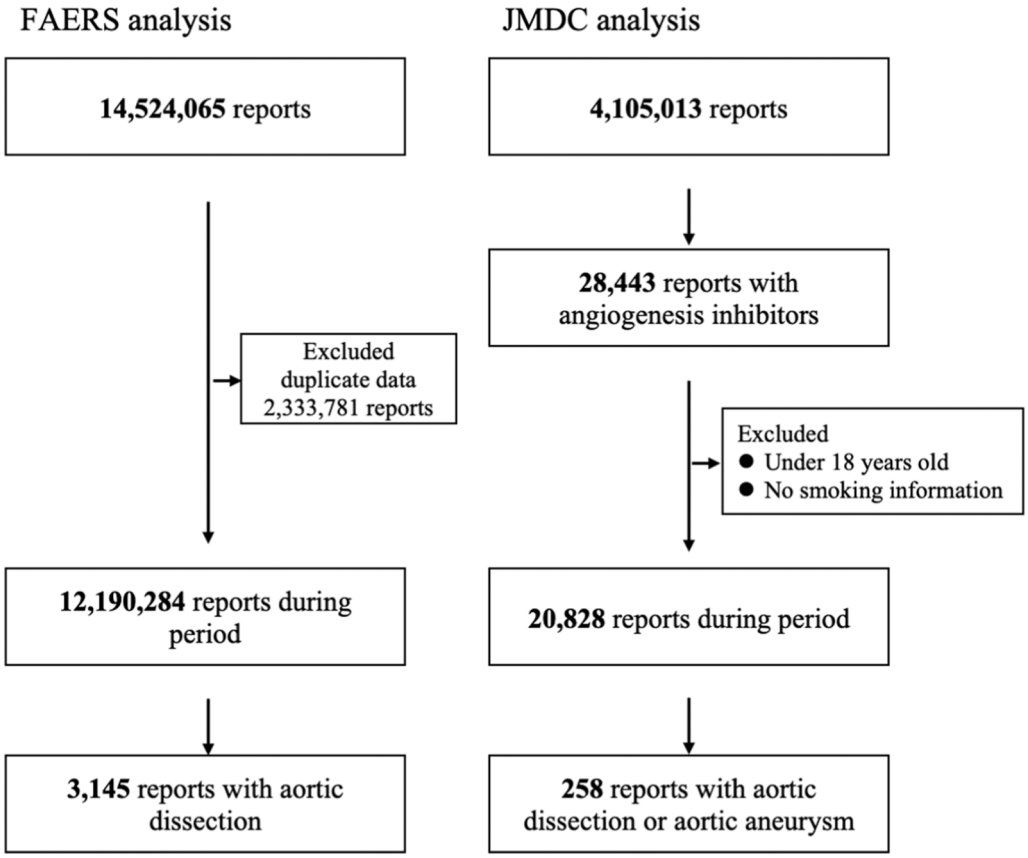


Fig. 1. Flow diagram of the study design. FAERS: FDA Adverse Event Reporting System.

"10002895/aortic dissection/Aortic dissection," based on the ICH Medical Dictionary for Regulatory Activities/J ver. 23.1. The risk of an adverse event was assessed by calculating the reporting odds ratio (ROR) and 95% confidence interval (CI) as previously reported [16]. Briefly, patients were classified into four groups: (A) patients who received a drug and reported aortic dissection as a side effect, (B) patients who received a drug and did not report aortic dissection as an adverse reaction, (C) patients who did not receive a drug and reported aortic dissection as an adverse reaction, and (D) patients who did not receive a drug and did not report aortic dissection as an adverse reaction. The number of reports in each group was calculated based on the above classification, and the ROR and 95% CI were calculated according to the following formula:

$$\mathrm{ROR} = \frac{A/B}{C/D},\ 95\%\ \mathrm{CI} = \exp\left(\ln(\mathrm{ROR}) \pm 1.96\sqrt{\frac{1}{A}+\frac{1}{B}+\frac{1}{C}+\frac{1}{D}}\right)$$

A, B, C, and D represent the number of reports in each group, and ln is the natural logarithm.

Drugs for which A, B, C, and D exceeded 10 and the ROR was > 1 were defined as those with an increased risk of developing aortic dissection, and a marker for dissection development was detected. Statistical analysis was performed using R version 4.2.1 for Windows.

2.2. Data analysis using JMDC

This retrospective cohort analysis used the Diagnosis Procedure Combination (DPC) database, a nationwide Japanese inpatient database provided by JMDC (JMDC Inc. Tokyo, Japan) as previously reported [17]. This database comprises medical fee schedules (receipt data) and DPC data for patients who visited medical institutions contracted by JMDC. As of August 2022, JMDC had contracts with 620 DPC and non-DPC hospitals. The database includes a tabulation of major diagnoses, comorbidities at admission, complications during hospitalization, and treatment information such as medications, procedures, and rehabilitation. Cases in the receipt database from April 2014 to October 2020, including angiogenesis inhibitors (bevacizumab, aflibercept, ramucirumab, sorafenib, regorafenib, lenvatinib, sunitinib, pazopanib, axitinib, cabozantinib and vandetanib by component name), were analyzed, excluding patients younger than 18 years and those without smoking information (Fig. 1). The incidence of aortic aneurysm or dissection ("aortic aneurysm and dissection" International Statistical Classification of Diseases and Related Health Problems (ICD) 10 code: I71) within 1 year of receiving an angiogenesis inhibitor was used as an objective factor. The following were used as objective factors: age 60 years or older, sex, smoking status, history of anthracyclines (doxorubicin, amrubicin, daunorubicin, epirubicin, pirarubicin, idarubicin, and achlorubicin by component name), history of hypertension (ICD-10: I10, I15, I 674, P29), atherosclerosis (ICD-10: I70), dyslipidemia (ICD-10: L94, M30, M31, M32, M33, M34, M35, M36), and connective tissue disorders (ICD-10: E78). The Mann–Whitney U test was used for continuous variables, and Fisher's exact test for categorical variables. Multivariate analysis was performed using multiple logistic analyses. Statistical analysis was performed using R version 4.2.1 for Windows.

2.3. Ethical considerations

Due to the anonymity of the data, the obligation to obtain informed permission was waived to perform database analysis. All animal procedures were performed per the guidelines of the Animal Research Committee of the University of Tokushima Graduate School which complied with ARRIVE 2.0.

2.4. Experimental animals and treatment administration

The experimental animals were 8-week-old male C57BL/6 J mice (CLEA Japan Inc. Tokyo, Japan). Mice were acclimated to obtain similar mean body weight and blood pressure in each group, and allowed to eat and drink freely through the experimental period.

For the sunitinib (SNT) alone study, mice were divided into four groups; the control, 4 mg/kg/day SNT (SNT4), 40 mg/kg/day SNT (SNT40), and 100 mg/kg/day SNT (SNT100), and received 1% carboxymethyl cellulose (CMC, Wako, Osaka, Japan) or SNT (Selleck Chemicals, Houston, TX, USA) suspended in 1% CMC at concentrations of 4, 40, or 100 mg/kg/day, orally via a feeding needle. Body weight was measured daily, and blood pressure was measured weekly using a tail-cuff plethysmography (BP-98AL, Softron, Tokyo, Japan). Vascular toxicity was assessed 4 weeks after the start of treatment as explained under the following section.

In the SNT study in the aortic dissection-prone mouse model (LAB model), LAB and SNT+LAB groups received *N*ω-nitro-L-arginine methyl ester (L-NAME; Sigma-Aldrich, St. Louis, MO, USA) (10 mg/kg/day) orally in drinking water for 4 weeks starting at 8 weeks of age [18]. Angiotensin II (Ang II; 1000 ng/kg/min) and β-aminopropionitrile (BAPN; 150 mg/kg/day) (both from Sigma-Aldrich, St. Louis, MO, USA) were administered continuously for 1 week using an osmotic pump (MINI-OSMOTIC PUMP MODEL 2002; Alzet, Muromachi Kikai Co.) from 3 weeks later starting with L-NAME administration. The SNT+LAB group was administered SNT (100 mg/kg day) dissolved in 1% CMC continuously for 4 weeks starting at 8 weeks of age. The onset of aortic dissection and vascular pathology were assessed 1 week after osmotic pump implantation.

2.5. Blood and tissue collection

After the measurement of body weight, mice were anesthetized by intraperitoneal administration with a three-drug mixture of 1 mg/mL medetomidine hydrochloride (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 5 mg/mL midazolam (Sandoz K.K. Tokyo, Japan), and 5 mg/mL butorphanol tartrate (Meiji Seika Pharma Co., Ltd. Tokyo, Japan), mixed with saline. Blood was collected from the orbital venous plexus of the mice and centrifuged at 1000 $\times g$ for 10 min in a tube to separate serum. The systemic circulation was perfused with 25–50 mL of saline, and tissues including aorta, heart, and kidney were harvested. Tissues were weighed and frozen at − 80 °C or fixed using 4% paraformaldehyde (Wako) and embedded in paraffin. The aortic diameter was measured using ImageJ v.1.37 (National Institutes of Health, Bethesda, Maryland, USA).

2.6. Histology

Section (5 μm thick) were subjected to Elastica van Gieson (EVG) staining (Merck, Darmstadt, Germany) according to the manufacturer's instructions. The development of aortic dissection was determined by false lumen formation under EVG staining. Elastin degradation was graded as follows: grade 1, intact and well-organized elastic lamina; grade 2, elastic lamina with some interruption and breakage; grade 3, severe elastin fragmentation or loss; grade 4, aortic rupture [19].

2.7. Real-time PCR

RNA was extracted from aortic homogenates collected following SNT monotherapy and the LAB model using the RNeasy® Fibrous Tissue Mini Kit (QIAGEN, Hilden, Germany) and from cultured cells using the RNeasy® Plus Mini Kit (QIAGEN). The extracted RNA was dissolved in RNase-free water, and the concentration was measured using a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was then performed using a PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan). Quantitative analysis of gene expression

levels was performed by the relative quantification method using the MyiQ2 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and THUNDERBIRD® SYBR qPCR Mix (TOYOBO LIFE SCIENCE, Osaka, Japan) was used to prepare the PCR reaction solution. The primer sequences used are listed in Supplementary Table 1. All primers were purchased from Eurofins Genomics (Tokyo, Japan).

2.8. Multiplex assay

Serum concentrations of granulocyte colony-stimulating factor (G-CSF), CCL11/eotaxin, monocyte chemoattractant protein 1 (MCP-1), interleukin-6 (IL-6), CXCL10/IP-10 (IP-10), keratinocyte-derived chemokines (KC), and vascular endothelial growth factor A (VEGF-A) were analyzed using the MILLIPLEX® kit (Merck) using Luminex®200TM (Merck) according to the manufacture's instruction.

2.9. Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza K.K. (Tokyo, Japan). The culture medium was EGM-2 Bullet Kit (Lonza K.K.) in 4% fetal bovine serum at 37 °C and 5% CO_2 until the passages of 3–8. Cells were stimulated with SNT (0.1, 1, 3, 5, 10 μM) for 24 h when they became confluent.

2.10. Statistical analysis

Data are expressed as the mean ± standard error (S.E.) for each value. Statistical analysis was performed using R version 4.2.1 for Windows. One-way analysis of variance was performed for comparisons of three or more groups. Tukey's test was performed as a post hoc analysis. The Kruskal–Wallis test was also used for ordinal scale data. Two-tailed $p < 0.05$ values were considered statistically significant.

3. Results

3.1. Disproportionality analysis using the FAERS database

Of the 12,190,284 adverse event cases, 3145 cases of aortic dissection were reported (Fig. 1). The reported aortic dissection rates were significantly higher in the patients receiving angiogenesis inhibitors, including bevacizumab (ROR, 5.12; 95% CI, 4.19–6.27), SNT (ROR, 5.50; 95% CI, 4.15–7.29), sorafenib (ROR, 5.41; 95% CI 3.67–7.96), and lenvatinib (ROR, 6.57 95% CI 3.96–10.92) compared to the patients not receiving these drugs (Table 1). All cases were statistically significant; therefore, angiogenesis inhibitors were strongly suggested to be associated with aortic dissection onset.

3.2. Drugs that are risk factors for drug-induced hypertension do not necessarily cause aortic dissection

Among the representative drugs which may induce hypertension as an adverse drug event, significant ROR signals for aortic dissection were observed in prednisolone (ROR, 2.71; 95% CI, 2.19–3.36), but not in epinephrine (ROR, 0.82; 95% CI, 0.39–1.72), norepinephrine (ROR, 1.87; 95% CI, 0.47–7.49), cyclosporine (ROR, 1.24; 95% CI, 0.84–1.83), methylprednisolone (ROR, 1.19; 95% CI, 0.76–1.87), and celecoxib (ROR, 0.75; 95% CI, 0.48–1.17). In the case of dexamethasone, significant protective signals were observed (ROR, 0.39; 95% CI, 0.25–0.62) (Table 1). Therefore, blood pressure elevation alone cannot cause drug-associated aortic dissection.

3.3. Retrospective cohort analysis using the JMDC receipt database

Of the 4,105,013 cases accumulated from April 2014 to November 2020, 20,828 cases with angiogenesis inhibitors were subjected to analysis (Fig. 1). Bevacizumab users were the most (37.6%). Patients using angiogenesis inhibitors tended to be men over 60 years of age (Table 2, Supplementary Fig. 1 A). Of these, 258 (1.24%) developed an aortic aneurysm or dissection within 1 year of the first angiogenesis inhibitor administration (Supplementary Fig. 1B). Higher percentages of the patients were 60 years or older, male, and had a history of hypertension, atherosclerosis, or dyslipidemia in the group of the patients with the aortic aneurysm or dissection compared to the group without these aortic diseases (Table 3). Only atherosclerosis and dyslipidemia

Table 1
Risk signal for aortic dissection with angiogenesis inhibitors and other drugs which cause hypertension in FAERS.

Drug A	AD without Drug A /total, n (%)	AD with Drug A /total, n (%)	ROR (95% CI)	*P* value *
Angiogenesis inhibitors				
Bevacizumab	3048/ 12,114,970 (0.025)	97/ 75,314 (0.13)	5.12 (4.19–6.27)	< 0.01
Lenvatinib	3130/ 12,181,388 (0.026)	15/8896 (0.17)	6.57 (3.96–10.92)	< 0.01
Sorafenib	3119/ 12,171,499 (0.026)	26/ 18,785 (0.14)	5.41 (3.67–7.96)	< 0.01
Sunitinib	3096/ 12,155,257 (0.025)	49/ 35,027 (0.14)	5.50 (4.15–7.29)	< 0.01
Other representative drugs which cause hypertension				
Epinephrine	3138/ 12,157,254 (0.026)	7/33,030 (0.021)	0.82 (0.39–1.72)	0.732
Norepinephrine	3143/ 12,186,139 (0.026)	2/4145 (0.048)	1.87 (0.47–7.49)	0.29
Celecoxib	3126/ 12,091,998 (0.026)	19/ 98,286 (0.019)	0.75 (0.48–1.17)	0.231
Cyclosporine	3119/ 12,108,911 (0.026)	26/ 81,373 (0.032)	1.24 (0.84–1.83)	0.272
Dexamethasone	3126/ 12,004,712 (0.026)	19/ 185,572 (0.010)	0.39 (0.25–0.62)	< 0.01
Methylpredonisolone	3126/ 12,128,410 (0.026)	19/ 61,874 (0.031)	1.19 (0.76–1.87)	0.449
Predonisolone	3059/ 12,065,172 (0.025)	86/ 125,112 (0.069)	2.71 (2.19–3.36)	< 0.01

Footnotes: AD; aortic dissection, ROR; reporting odds ratio, CI; confidential intervals,
* Fisher's exact test

Table 2
Number of patients using each angiogenesis inhibitor by JMDC.

Drug	Number of patients (%)
Bevacizumab	10,702 (51.4)
Aflibercept	4325 (20.8)
Ramucirumab	3437 (16.5)
Sorafenib	1174 (5.6)
Regorafenib	1160 (5.6)
Lenvatinib	1086 (5.2)
Sunitinib	537 (2.6)
Pazopanib	495 (2.4)
Axitinib	510 (2.4)
Cabozantinib	17 (0.1)
Vandetanib	0 (0.0)

Footnotes: Total number of patients using angiogenesis inhibitors were 20,828. Some patients received more than one angiogenesis inhibitor.

Table 3
Patient background of patients using angiogenesis inhibitors with different onset of aortic dissection in JMDC.

Variable	Angiogenesis inhibitors user		Univariate analysis	Multivariate analysis	
	non-AD/AA (n = 20,570)	AD/AA (n = 258)	*P* value	OR (95%CI)	*P* value
Age (min,max)	70 (19,90)	73 (32,90)	< 0.001[a]		
Over 60 years old	16,870 (82%)	223 (86%)	0.072[b]	1.39 (0.92–2.1)	0.12
Male	12,224 (59%)	174 (67%)	0.009[b]	1.31 (0.94–1.82)	0.11
Smoking	11,439 (56%)	154 (60%)	0.207[b]	1.13 (0.84–1.54)	0.42
History of Anthracycline use	959 (4.7%)	15 (5.8%)	0.371[b]	1.62 (0.90–2.93)	0.11
Hypertension	9464 (46%)	138 (54%)	0.017[b]	1.19 (0.89–1.58)	0.24
Atherosclerosis	673 (3.3%)	17 (6.6%)	0.007[b]	1.81 (1.04–3.14)	0.036
Dyslipidemia	4603 (22%)	77 (30%)	0.005[b]	1.46 (1.07–1.99)	0.017
Connective tissue disorders	265 (1.3%)	2 (0.78%)	0.777[b]	0.31 (0.0044–2.25)	0.25

Footnotes: AD; aortic dissection, AA; aortic aneurysm, OR; odds ratio, CI; confidential intervals,
[a] Mann–Whitney U test,
[b] Fisher's exact test

were significantly associated with the onset of aortic aneurysm or dissection in patients prescribed angiogenesis inhibitors. In contrast, a history of hypertension was not significantly associated with aortic aneurysm onset or dissection. These findings suggest that hypertension before angiogenesis inhibitor administration may not influence aortic aneurysm or dissection development (Table 3).

3.4. SNT alone does not cause vascular injury in mice

To confirm the relationship between angiogenesis inhibitor and aortic dissection, in vivo studies were performed using sunitinib (SNT). First, the single SNT administration was examined in mice. Systolic blood pressure (SBP), body, heart, and kidney weights were unchanged after SNT administration (4, 40, and 100 mg/kg/day) after 4 weeks (Supplementary Figure 2 A, Supplementary Table 2). No elastin degradation was observed at any dose following EVG staining of aortic sections (Supplementary Figure 2B). Thus, SNT alone, up to 100 mg/kg/day, did not worsen the general condition, increase SBP, or form vascular injuries, including aortic dissection in mice.

3.5. Sunitinib aggravates vascular injury in LAB mice

As SNT had no toxicity on control mice up to 100 mg/kg/day, the effects of SNT on the aortic dissection onset were examined at 100 mg/kg/day. Consequently, neither LAB nor SNT affected body and kidney weight (Fig. 2A and B). SNT did not aggravate cardiac hypertrophy, as observed in LAB mice (Fig. 2C). However, SBP showed a significant increase in the SNT group compared to in the LAB group at week 4 ($p < 0.01$), 1 week after adding Ang II (Fig. 2D). The incidence of aortic dissection increased in the SNT-treated mice (59%) compared to the LAB group (40%). In the SNT group, 16% of mice died from aortic rupture, but no mice died in the LAB group (Table 4, Fig. 2E). Although aortic diameter enlargement was not observed in the LAB group, the diameter was significantly increased in the SNT+LAB group compared to in the control group (Fig. 2F). The elastin degradation score evaluated using EVG staining was also significantly higher in the SNT+LAB group than in the LAB group (Fig. 2G).

In LAB mice, the serum concentrations of inflammatory cytokines, G-CSF, IL-6, KC, eotaxin, and IP-10 were significantly increased (Fig. 3A). These increases in serum concentration were significantly decreased (IP-10 and MCP-1) or unchanged (G-CSF, IL-6, KC, and eotaxin) after SNT administration (Fig. 3A). Thus, systemic inflammation was suppressed by SNT administration in LAB mice.

The gene expressions for endothelial cell specific molecules, such as ICAM-1, VCAM-1, ET-1, eNOS, and VEGFR2, in the aortae were examined. No statistically significant changes were observed in all parameters, but SNT increased these endothelial cell-derived gene expressions compared to those in the LAB group (Fig. 3B).

Cultured HUVECs were stimulated with 0, 0.1, 1, 3, 5, and 10 μM SNT for 24 h, and gene expressions were examined to clarify the impact of SNT on endothelial cells. The gene expressions for ICAM-1, VCAM-1, ET-1, and VEGFR2 significantly increased concentration-dependently. Moreover, eNOS expression was decreased by SNT stimulation in a concentration-dependent manner (Fig. 3C). Therefore, ICAM-1, VCAM-1, and eNOS expressions indicated that SNT induced endothelial damage.

In summary, SNT increases the likelihood of developing aortic dissection via ET-1-related hypertension and endothelial dysfunction, but does not exacerbate systemic inflammation.

4. Discussion

We elucidated the background factors and mechanisms underlying aortic dissection development induced by angiogenesis inhibitors using a novel approach that combines real-world databases, in vivo, and in vitro studies. The pathogenesis mechanism may involve impaired vasoconstrictive capacity resulting from elevated ET-1, decreased eNOS, and vascular endothelial cell damage with increased ICAM-1 and VCAM-1 expressions. Elevated blood pressure after angiogenesis inhibitor use, but no history of hypertension, before treatment initiation had a greater impact on the risk of developing aortic dissection. Furthermore, drugs that increase blood pressure do not necessarily increase the risk of developing aortic dissection or aneurysms, which are adverse events specific to angiogenesis inhibitors.

In our study, SNT administration aggravated vascular injury in LAB mice. The expressions of inflammatory markers were elevated in patients who developed aortic dissection [20]. Similar to what is observed in human patients, an inflammatory response may be involved in the aortic dissection development in the LAB model [5,18]. Therefore, we first hypothesized that enhancing inflammatory responses might be one of the mechanisms of SNT-induced vascular injury. However, G-CSF, IL-6, KC, and eotaxin serum concentrations were unchanged, and IP-10 and MCP-1 were somewhat decreased by SNT administration. The effect of SNT on these inflammation-related molecules is controversial, with reports of exacerbation[21] and suppression [22]. The effect of SNT may depend on the base inflammation level, but in our LAB model, SNT showed a suppressive trend. SNT-associated aortic dissection was not dependent on inflammatory response in mice.

Aortic dissection is known to be associated with endothelial dysfunction [6,7], and the LAB model is unique because it allows for a high rate of aortic dissection based on endothelial damage caused by L-NAME [5]. Although the effects of SNT on the expressions of endothelial cell-specific genes were not apparent in mice aorta (Fig. 3), significant increases in ICAM-1 and VCAM-1, and a decrease in eNOS by SNT, were observed in cultured HUVECs, consistent with previous reports [23,24]. These results indicate that SNT-induced vascular endothelial damage in the aorta may be involved in developing aortic dissection. Moreover, ET-1 expression increased in the aorta of SNT-treated LAB mice and significantly increased in HUVECs (Fig. 3B and C). Although systemic ET-1 elevation does not correlate with blood

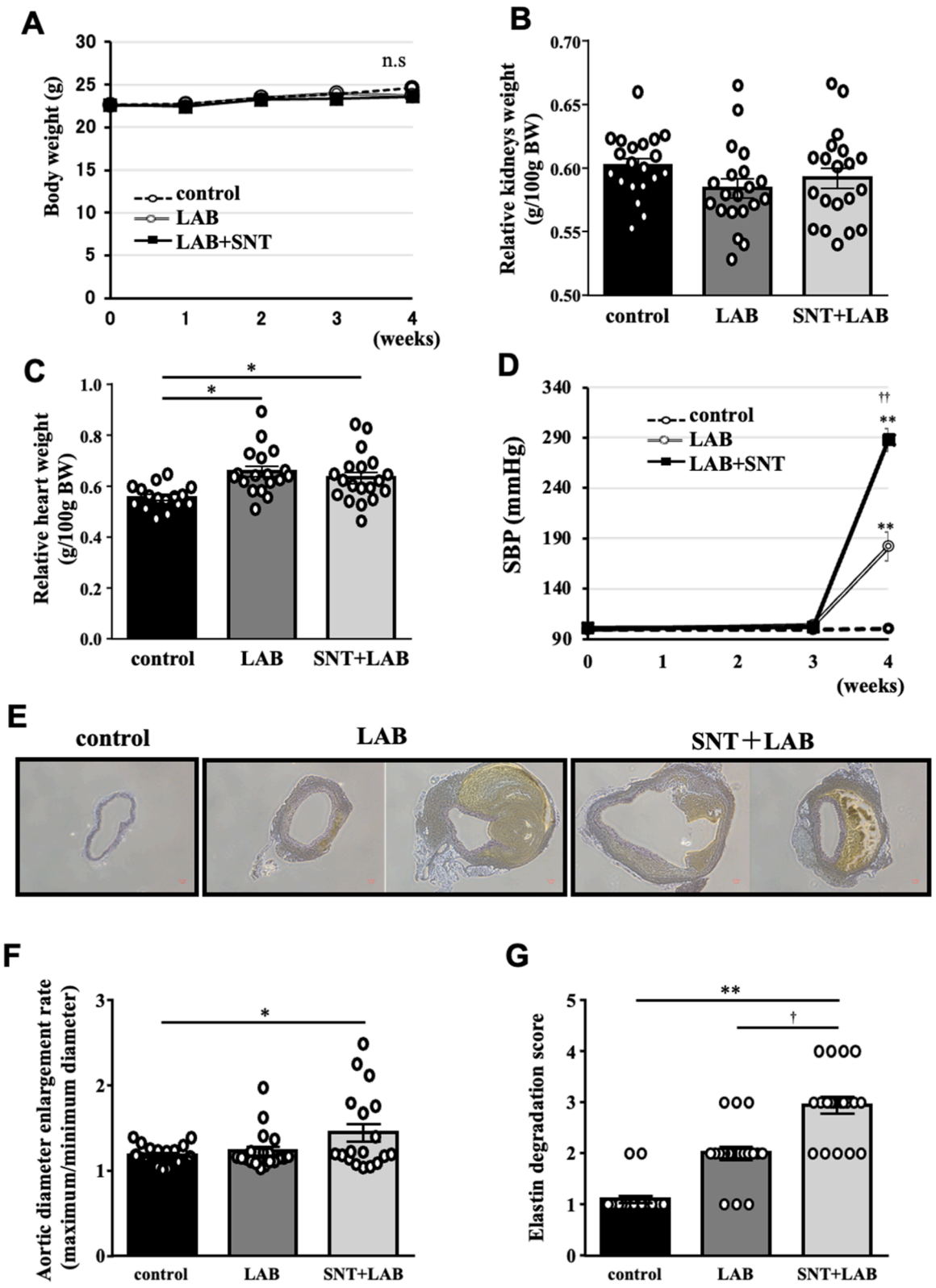


Fig. 2. Effects of oral administration of sunitinib on the aortic dissection mouse model (LAB). (A) Body weight was monitored weekly until 4 weeks after starting sunitinib administration. (open circles: control; double circles: LAB group; black squares: LAB + SNT group). (B) Relative kidney weight is expressed as a ratio of kidney weight to 100 g body weight. (C) Relative heart weight was expressed as a ratio of heart weight to 100 g body weight. (D) Systolic blood pressure (SBP) was monitored from week 0 which indicates starting time of sunitinib (SNT) administration until week 4. (open circles: control group; double circle: LAB group; black square: LAB+SNT group). (E) Representative images of Elastica van Gieson's staining (bar: 10 μm). (F) The enlargement rate of aortic diameter was calculated by the ratio of maximum to minimum diameter. (G) Average values of elastin degradation score estimated under EVG staining. Values are expressed as mean ± SEM from n = 19–20, and statistically analyzed using Tukey's test (a-d and f) or Kruskal–Wallis test followed by Dunn's test (g). *$p < 0.05$, * *$p < 0.01$ vs control. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs LAB. n.s: not significant.

Table 4
Incidence of aortic dissection and death from aortic rupture.

	Control, *n* (%)	LAB, *n* (%)	SNT+LAB, *n* (%)	*P* value*
AD	0/20 (0)	8/20 (40)	11/19 (59)	0.34
Rupture	0/20 (0)	0/20 (0)	3/19 (16)	0.11

Footnotes: Values are shown as n (%). Data are statistically analyzed by chi-squared test. AD, aortic dissection; LAB, L-NAME+AngII+BAPN treated group; SNT+LAB, SNT 100 mg/kg per day+L-NAME+Ang II+BAPN treated group.
* LAB vs SNT+LAB by Fisher's test.

pressure elevation under normal conditions [25], ET-1 elevation is thought to be essential in angiogenesis inhibitor-induced hypertension [26]. Therefore, SNT-exacerbated hypertension in LAB mice (Fig. 2D) was determined to be due to increased ET-1 expressions. A decrease in nitric oxide production capacity and an increase in ET-1 may be involved in the SNT-induced increase in blood pressure [27]. In the LAB model, SNT significantly aggravated SBP at the 4-week point, which was elevated after Ang II loading, consistent with the ET-1 increase. This model required a rapid change in blood pressure after Ang II loading to induce aortic dissection development. The degree of blood pressure variability exacerbation was the most crucial factor for the increased incidence of aortic dissection with SNT. SNT administration alone did not affect SBP in mice up to 100 mg/kg/day for 4 weeks (Supplementary Figure 2) and aggravated SBP only after Ang II loading in the LAB model. Therefore, SNT likely increases blood pressure and may worsen vascular lesions if the patients or mice are initially at risk for blood pressure elevation.

Therefore, we tested the background factors for angiogenesis inhibitor-associated vascular injury in clinical situations. Higher percentages of patients with a history of hypertension in the group with the aortic aneurysm or dissection compared to the group without these aortic diseases were observed. However, no significant differences were found for hypertension. Therefore, the risk factor for developing arterial dissection by angiogenesis inhibitors may involve increased blood pressure after drug administration, regardless of whether the patient had hypertension before drug use. In contrast, a significant relevance between the onset of aortic dissection and atherosclerosis or dyslipidemia was observed. These database analyses and the results of in vivo experiments suggest that worsening hypertension after angiogenesis inhibitor use plays a vital role in developing aortic dissection.

As shown in Table 1, there was no increased risk from other drugs that may induce hypertension as a drug adverse event. This result indicates that hypertension was not a necessary and sufficient condition to induce aortic aneurysms and dissection. Thus, the mechanism of aortic dissection development caused by drug-induced hypertension may be an angiogenesis inhibitor-specific event.

Animal experiments showed a marked increase in blood pressure

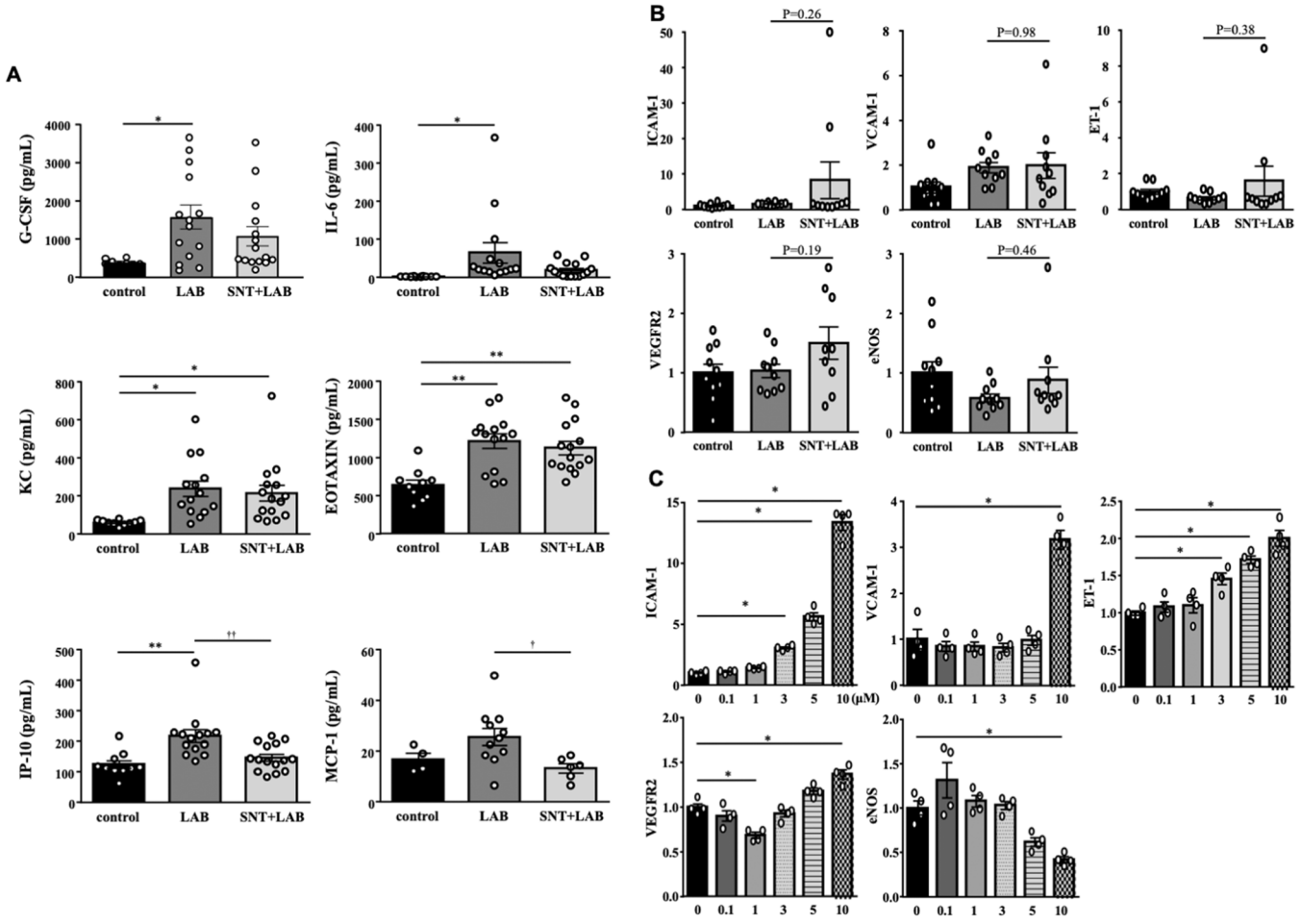


Fig. 3. The effects of sunitinib on the inflammatory response and endothelial cell damage in LAB mice or in cultured human umbilical vein endothelial cells (HUVECs). (A) Serum concentrations of G-CSF, IL-6, KC, EOTAXIN, IP-10, and MCP-1 in LAB mice with our without sunitinib (SNT) were analyzed using the LUMINEX assay. (B) Gene expressions for ICAM-1, VCAM-1, ET-1, eNOS, and VEGFR2 in aortas from LAB mice with or without SNT were determined using real-time reverse transcription-polymerase chain reaction. (C) Gene expressions for VCAM-1, ICAM-1, ET-1, eNOS, and VEGFR2 in HUVECs stimulated by SNT at indicated concentrations for 24 hr. Data represent the mean ± SEM from n = 9–10 (for A and B), or 4 (for C). Values are statistically analyzed by Tukey's test. *$p < 0.05$, * *$p < 0.01$ vs control group, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs LAB.

induced by angiogenesis inhibitors when loaded with Ang II and BAPN. In addition, database analysis revealed that a history of atherosclerosis or dyslipidemia was highly associated with the development of aortic dissection, independent of a history of hypertension. Therefore, vascular damage from such diseases may be the underlying pathology that exacerbates the increase in blood pressure induced by angiogenesis inhibitors. In the present study, we demonstrated the importance of angiogenesis inhibitor specifically induced hypertension in the onset of aortic dissection. However, our study also has several limitations. The database analysis of aneurysms and dissections included only the aorta but not peripheral areteries such as carotid, cerebral, and coronary arteries. In addition, ascending and descending dissections have not been distinguished neither in silico or in vivo studies. With regard to in vivo study, the direct association between VEGF inhibition and aortic dissection were not elucidated. Therefore, further basic and clinical investigations are desired.

5. Conclusion

Aortic dissection that develops after administering angiogenesis inhibitors might be related to elevated blood pressure associated with ET-1 and vascular endothelial damage, indicating that this phenomenon might be specific to angiogenesis inhibitors. This concept was proven by both a real-world database and basic pharmacological research with certainty. It is important to keep in mind that regardless of the blood pressure prior to initiation of administration, patients are at risk of developing aortic dissection if their blood pressure increases after initiation of angiogenesis inhibitor administration. Since it has been shown that endothelin-1-induced blood pressure elevation may play an important role in the development of dissection, it is expected that strategies to prevent adverse events targeting endothelin-1 will be established in the future. Thus, our findings are quite meaningful to establish safer anticancer therapies using angiogenesis inhibitors and strategies to prevent the development of vascular toxicity in high-risk patients with atherosclerosis and dyslipidemia, but not hypertension.

Sources of Funding

This work was supported by Taiho Pharmaceutical, Inc. as collaborative research, and by grants from Grants-in-Aid for Scientific Research of Japan Society for the Promotion of Science, KAKENHI, Grant Number, JP22H04283 (K.T.), JP22K06863 (Y.I.-I.), and JP21H02646 (K.I.).

CRediT authorship contribution statement

Kaito Tsujinaka: Conceptualization, Methodology, Formal analysis, Investigation, Writing-original draft, Funding acquisition. **Yuki Izawa-Ishizawa**: Conceptualization, Methodology, Resources, Data Curation, Writing-Review and Editing, Visualization, Supervision, Funding acquisition. **Koji Miyata:** Investigation, Validation. **Toshihiko Yoshioka:** Investigation. **Kohei Omine:** Investigation. **Honoka Nishi:** Investigation. **Masateru Kondo:** Investigation. **Syuto Itokazu:** Investigation. **Tatsumi Miyata:** Investigation. **Takahiro Niimura:** Methodology, Software, Validation. **Maki Sato:** Validation. **Fuka Aizawa:** Methodology, Visualization. **Kenta Yagi:** Methodology, Formal analysis. **Masayuki Chuma:** Methodology, Formal analysis. **Yoshito Zamami:** Software, Resources. **Mitsuhiro Goda:** Methodology, Resources, Supervision. **Keisuke Ishizawa:** Resources, Supervision, Project administration, Funding acquisition. supervised this project. All authors critically reviewed and revised the manuscript draft and approved the final version for submission.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yuki Izawa-Ishizawa reports financial support was provided by Taiho Pharmaceutical, Inc. Keisuke Ishizawa reports financial support was provided by Japan Society for the Promotion of Science. Kaito Tsujinaka reports financial support was provided by Japan Society for the Promotion of Science. Yuki Izawa-Ishizawa reports financial support was provided by Japan Society for the Promotion of Science.

Acknowledgments

We acknowledge the expert assistance by the Support Center for Advanced Medical Sciences, Tokushima University Graduate School of Biomedical Sciences in technical support for basic experiments. English writing support was provided by Editage of Cactus Communications K.K. (Tokyo, Japan).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115504.

References

[1] P.S. Mody, Y. Wang, A. Geirsson, N. Kim, M.M. Desai, A. Gupta, J.A. Dodson, H. M. Krumholz, Trends in aortic dissection hospitalizations, interventions, and outcomes among medicare beneficiaries in the United States, 2000-2011, Circ. Cardiovasc Qual. Outcomes 7 (6) (2014) 920–928.
[2] B.O. Aicher, J. Zhang, S.C. Muratoglu, R. Galisteo, A.L. Arai, V.L. Gray, B.K. Lal, D. K. Strickland, A.A. Ucuzian, Moderate aerobic exercise prevents matrix degradation and death in a mouse model of aortic dissection and aneurysm, Am. J. Physiol. Heart Circ. Physiol. 320 (5) (2021) H1786–H1801.
[3] V. Marque, P. Kieffer, B. Gayraud, I. Lartaud-Idjouadiene, F. Ramirez, J. Atkinson, Aortic wall mechanics and composition in a transgenic mouse model of Marfan syndrome, Arterioscler. Thromb. Vasc. Biol. 21 (7) (2001) 1184–1189.
[4] E. Bossone, K.A. Eagle, Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes, Nat. Rev. Cardiol. 18 (5) (2021) 331–348.
[5] Y. Izawa-Ishizawa, M. Imanishi, Y. Zamami, H. Toya, T. Nagao, M. Morishita, K. Tsuneyama, Y. Horinouchi, Y. Kihira, K. Takechi, Y. Ikeda, K. Tsuchiya, M. Yoshizumi, T. Tamaki, K. Ishizawa, Development of a novel aortic dissection mouse model and evaluation of drug efficacy using in-vivo assays and database analyses, J. Hypertens. 37 (1) (2019) 73–83.
[6] L. Pan, Z. Lin, X. Tang, J. Tian, Q. Zheng, J. Jing, L. Xie, H. Chen, Q. Lu, H. Wang, Q. Li, Y. Han, Y. Ji, S-nitrosylation of plastin-3 exacerbates thoracic aortic dissection formation via endothelial barrier dysfunction, Arterioscler. Thromb. Vasc. Biol. 40 (1) (2020) 175–188.
[7] M. Toral, A. de la Fuente-Alonso, M.R. Campanero, J.M. Redondo, The NO signalling pathway in aortic aneurysm and dissection, Br. J. Pharm. 179 (7) (2022) 1287–1303.
[8] M.M. An, Z. Zou, H. Shen, P. Liu, M.L. Chen, Y.B. Cao, Y.Y. Jiang, Incidence and risk of significantly raised blood pressure in cancer patients treated with bevacizumab: an updated meta-analysis, Eur. J. Clin. Pharm. 66 (8) (2010) 813–821.
[9] J.M. Duran, C.A. Makarewich, D. Trappanese, P. Gross, S. Husain, J. Dunn, H. Lal, T.E. Sharp, T. Starosta, R.J. Vagnozzi, R.M. Berretta, M. Barbe, D. Yu, E. Gao, H. Kubo, T. Force, S.R. Houser, Sorafenib cardiotoxicity increases mortality after myocardial infarction, Circ. Res 114 (11) (2014) 1700–1712.
[10] S. Lee, T.T. Chen, C.L. Barber, M.C. Jordan, J. Murdock, S. Desai, N. Ferrara, A. Nagy, K.P. Roos, M.L. Iruela-Arispe, Autocrine VEGF signaling is required for vascular homeostasis, Cell 130 (4) (2007) 691–703.
[11] J. Herrmann, E.H. Yang, C.A. Iliescu, M. Cilingiroglu, K. Charitakis, A. Hakeem, K. Toutouzas, M.A. Leesar, C.L. Grines, K. Marmagkiolis, Vascular toxicities of cancer therapies: the old and the new–an evolving avenue, Circulation 133 (13) (2016) 1272–1289.
[12] R.M. Touyz, S.M.S. Herrmann, J. Herrmann, Vascular toxicities with VEGF inhibitor therapies-focus on hypertension and arterial thrombotic events, J. Am. Soc. Hypertens. 12 (6) (2018) 409–425.
[13] E.S. Robinson, E.V. Khankin, S.A. Karumanchi, B.D. Humphreys, Hypertension induced by vascular endothelial growth factor signaling pathway inhibition: mechanisms and potential use as a biomarker, Semin Nephrol. 30 (6) (2010) 591–601.
[14] K.M. Mirabito Colafella, K.B. Neves, A.C. Montezano, I.M. Garrelds, R. van Veghel, R. de Vries, E. Uijl, H.J. Baelde, A.H. van den Meiracker, R.M. Touyz, A.H. J. Danser, J. Versmissen, Selective ETA vs. dual ETA/B receptor blockade for the prevention of sunitinib-induced hypertension and albuminuria in WKY rats, Cardiovasc Res 116 (10) (2020) 1779–1790.
[15] N. Niwa, T. Nishiyama, C. Ozu, Y. Yagi, S. Saito, Acute aortic dissection in a patient with metastatic renal cell carcinoma treated with axitinib, Acta Oncol. 54 (4) (2015) 561–562.

[16] Y. Zamami, T. Niimura, N. Okada, T. Koyama, K. Fukushima, Y. Izawa-Ishizawa, K. Ishizawa, Factors associated with immune checkpoint inhibitor-related myocarditis, JAMA Oncol. 5 (11) (2019) 1635–1637.
[17] Y. Zamami, T. Niimura, T. Koyama, Y. Shigemi, Y. Izawa-Ishizawa, M. Morita, A. Ohshima, K. Harada, T. Imai, H. Hagiwara, N. Okada, M. Goda, K. Takechi, M. Chuma, Y. Kondo, K. Tsuchiya, S. Hinotsu, M.R. Kano, K. Ishizawa, Search for therapeutic agents for cardiac arrest using a drug discovery tool and large-scale medical information database, Front Pharm. 10 (2019) 1257.
[18] M. Kondo, Y. Izawa-Ishizawa, M. Goda, M. Hosooka, Y. Kagimoto, N. Saito, R. Matsuoka, Y. Zamami, M. Chuma, K. Yagi, K. Takechi, K. Tsuneyama, K. Ishizawa, Preventive effects of quercetin against the onset of atherosclerosis-related acute aortic syndromes in mice, Int J. Mol. Sci. 21 (19) (2020).
[19] M. Imanishi, Y. Izawa-Ishizawa, T. Sakurada, Y. Kohara, Y. Horinouchi, E. Sairyo, Y. Zamami, K. Takechi, M. Chuma, K. Fukushima, Y. Ikeda, H. Fujino, M. Yoshizumi, K. Tsuchiya, T. Tamaki, K. Ishizawa, Nitrosonifedipine, a photodegradation product of nifedipine, suppresses pharmacologically induced aortic aneurysm formation, Pharmacology 102 (5–6) (2018) 287–299.
[20] H. Liu, Z. Luo, L. Liu, X. Yang, Y. Zhuang, G. Tu, G. Ma, Y. Zhang, J. Zheng, D. Zhu, C. Wang, Inflammatory biomarkers to predict adverse outcomes in postoperative patients with acute type A aortic dissection, Scand. Cardiovasc J. 54 (1) (2020) 37–46.
[21] M.E. Asker, A.A. Abdelmeged, M.A. Shaheen, R.G. Eissa, Sunitinib displays pulmonary fibrosis in experimental rats: Role of IL-17A dependent pathway, Int Immunopharmacol. 119 (2023), 110173.
[22] L. Chen, P. Xu, Q. Xiao, L. Chen, S. Li, J.M. Jian, Y.B. Zhong, Sunitinib malate inhibits intestinal tumor development in male Apc(Min/+) mice by down-regulating inflammation-related factors with suppressing beta-cateinin/c-Myc pathway and re-balancing Bcl-6 and Caspase-3, Int Immunopharmacol. 90 (2021), 107128.
[23] P. Nowak-Sliwinska, J.R. van Beijnum, C.J. Griffioen, Z.R. Huinen, N.G. Sopesens, R. Schulz, S.V. Jenkins, R.P.M. Dings, F.H. Groenendijk, E.J.M. Huijbers, V. Thijssen, E. Jonasch, F.A. Vyth-Dreese, E.S. Jordanova, A. Bex, R. Bernards, T. D. de Gruijl, A.W. Griffioen, Proinflammatory activity of VEGF-targeted treatment through reversal of tumor endothelial cell anergy, Angiogenesis 26 (2) (2023) 279–293.
[24] Y. Liu, L.L. Tang, C. Liang, M.M. Wu, Z.R. Zhang, Insulin resistance and pellino-1 mediated decrease in the activities of vasodilator signaling contributes to sunitinib-induced hypertension, Front Pharm. 12 (2021), 617165.
[25] Y.Y. Kisanuki, N. Emoto, T. Ohuchi, B. Widyantoro, K. Yagi, K. Nakayama, R. M. Kedzierski, R.E. Hammer, H. Yanagisawa, S.C. Williams, J.A. Richardson, T. Suzuki, M. Yanagisawa, Low blood pressure in endothelial cell-specific endothelin 1 knockout mice, Hypertension 56 (1) (2010) 121–128.
[26] M.H. Kappers, F.M. Smedts, T. Horn, J.H. van Esch, S. Sleijfer, F. Leijten, S. Wesseling, H. Strevens, A.H. Jan Danser, A.H. van den Meiracker, The vascular endothelial growth factor receptor inhibitor sunitinib causes a preeclampsia-like syndrome with activation of the endothelin system, Hypertension 58 (2) (2011) 295–302.
[27] H.C. Liu, X.T. Zhou, Y.S. Zheng, H. He, X.Q. Liu, PK/PD modeling based on NO-ET homeostasis for improving management of sunitinib-induced hypertension in rats, Acta Pharm. Sin. 41 (5) (2020) 719–728.