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FULL PAPER

Pharmacology

Eukaryotic elongation factor 2 kinase inhibitor, A484954 potentiates β-adrenergic receptor agonist-induced acute decrease in diastolic blood pressure in rats

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ABSTRACT. Eukaryotic elongation factor 2 (eEF2) kinase (eEF2K) acts to inhibit protein translation through phosphorylating a specific substrate, eEF2. We previously found that the increased eEF2K expression in mesenteric artery mediates hypertension development in spontaneously hypertensive rats. More recently, we have revealed that a selective eEF2K inhibitor, A484954 induced vasorelaxation via opening inward rectifier K^+ channel and activating β_2-adrenergic receptor in smooth muscle of rat isolated mesenteric artery, which contributes to prevent noradrenaline-induced acute increase in blood pressure (BP). In this study, we further explored acute effects of A484954 on BP in rats, especially focusing the action on β-adrenergic receptor. We also examined whether A484954 affects contraction and heart rate (HR) of isolated heart. BP and HR were measured by a carotid cannulation method in rats. Isometric contraction and HR in rat isolated atria were also measured pharmacologically. A484954 potentiated adrenaline-induced decrease in diastolic BP (DBP) but not increase in systolic BP (SBP). A484954 potentiated isoproterenol-induced decrease in DBP but not SBP. Contrastingly, A484954 prevented a non-β-adrenergic receptor agonist, angiotensin II-induced increase in both SBP and DBP. In isolated left atria, A484954 caused contraction, which was prevented by a β-adrenergic receptor antagonist, propranolol. In isolated right atria, A484954 increased HR. In conclusion, we for the first time demonstrated that A484954 potentiates β-adrenergic receptor agonist-induced decrease in DBP possibly through vasorelaxation mediated via activating β_2-adrenergic receptor. It was also demonstrated that A484954 causes contraction of rat isolated heart via activating β_1-adrenergic receptor.

KEY WORDS: β-adrenergic receptor, blood pressure, cardiac contraction, eukaryotic elongation factor 2 kinase, vasorelaxation

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Eukaryotic elongation factor 2 (eEF2) kinase (eEF2K) is known as calmodulin (CaM)-dependent protein kinase III (CaMKIII). eEF2K phosphorylates its specific substrate, eEF2 and prevents protein translation [8, 10, 11]. We previously reported that eEF2K expression increases in superior mesenteric artery from spontaneously hypertensive rats (SHR) [14] and that eEF2K mediates development of systemic hypertension in SHR through promoting inflammation, proliferation, and migration of vascular cells [12, 13]. In addition to systemic hypertension, we reported that eEF2K mediates development of pulmonary arterial hypertension through promoting structural remodeling of arterial wall via NADPH oxidase-1/reactive oxygen species/matrix metalloproteinase-2 pathway [3]. In addition to vasculature, we further revealed that eEF2K expression increases in hypertrophied left ventricles form SHR, pressure overload-induced cardiac hypertrophy model mice, and isoproterenol-induced cardiac hypertrophy model rats [4]. More recently, we have revealed that eEF2K/eEF2 axis inhibits apoptotic cell death via inducing autophagy in H9c2 cardiomyoblasts during glucose-deprivation state [5]. Other than the pathologies related to hypertension and cardiac hypertrophy, it was demonstrated that eEF2K may participate in the progression of atherosclerosis [1, 16]. Further, it was recently demonstrated that eEF2K may participate in angiogenesis and tumor progression in hepatocellular carcinoma [17].

A484954 (7-amino-1-cyclopropyl-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-6-carboxamide) is a selective small molecule inhibitor for eEF2K, which was identified from a chemical library using a high-throughput screening [2]. It seems to inhibit eEF2K activity in an ATP-competitive but not CaM-dependent manner. We have recently revealed that A484954 causes relaxation partly through activating smooth muscle inward rectifier K^+ (K_{ir}) channel and β_2-adrenergic receptor in rat isolated

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superior mesenteric artery, which contributes to prevent noradrenaline-induced acute blood pressure (BP) rise in normal Wistar rats [6, 7]. In this study, we further explored the acute effects of A484954 on BP, especially focusing the effects on β-adrenergic receptor. Systemic BP is regulated not only by peripheral arterial resistance but also by cardiac output. Cardiac contractile force and heart rate (HR) are two important determinants for cardiac output. Then, in the present study, we also examined whether A484954 affects contraction and HR of isolated heart. Here, we for the first time revealed that A484954 mediates a decrease in BP and increase in cardiac function via β-adrenergic action.

MATERIALS AND METHODS

Materials

Reagent sources were as follows: A484954 (Merck, Darmstadt, Germany), noradrenaline (NA), adrenaline, isoproterenol, angiotensin (Ang) II, and (±)-propranolol hydrochloride (Sigma-Aldrich, St. Louis, MO, U.S.A.).

Measurement of BP

BP and HR were measured in male Wistar rats (183–369 g; 6–11-week-old) under urethane (1.5 g/kg, i.p.) anesthesia as described previously [7]. A catheter filled with a heparin-saline solution was inserted into carotid artery, which was connected to a BP transducer (ADInstruments, Dunedin, New Zealand). Systolic BP (SBP), mean BP (MBP), and diastolic BP (DBP) were measured and recorded using a BP Amp (ADInstruments) and PowerLab system (ADInstruments). HR was calculated by a cyclic measurement of BP recording as described previously [7]. Adrenaline (0.02–40 μg/kg), isoproterenol (0.01–1 μg/kg), or Ang II (0.02–40 μg/kg) was injected for 5 min through a catheter inserted into femoral vein 10 min after pretreatment with A484954 (122 μg/kg) or dimethyl sulfoxide (DMSO; 0.1%), a vehicle. The changes of BP and HR before and after the injection of drug were calculated. Animal care and treatment were conducted in conformity with the institutional guidelines of The Kitasato University. Animal experiments were approved by the Institutional Animal Care and Use Committee of The Kitasato University.

Measurement of isometric contraction and HR in isolated heart

Isometric contraction and HR were measured as described previously [9, 15]. Male Wistar rats (127–313 g; 5–9-week-old) were deeply anesthetized with urethane (1.5 g/kg, i.p.) and sacrificed. The left and right atria were isolated for the measurement of isometric contraction and HR, respectively. Left and right atria were placed in a 15-m*l* organ bath filled with Krebs-Henseleit solution, which contained the following compositions (mM): 119 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 24.9 $NaHCO_3$, and 10 glucose. The solution was saturated with a 95% O_2–5% CO_2 mixture at 37°C. Left atrium, which was electrically stimulated via a pair of platinum electrodes (field stimulation, 1 Hz, 5 msec, and 1.5 times threshold voltage) connected to an electronic stimulator (SEN-3301; Nihon Kohden, Tokyo, Japan), was equilibrated at least for 70 min. Right atrium, which was not electrically stimulated, was also equilibrated at least for 70 min. The resting tension was adjusted to 0.5 g. Isometric contraction and HR were measured and recorded with a force-displacement transducer (TB-651T; Nihon Kohden) and PowerLab system.

Statistics

Data were shown as means ± standard error of the mean (SEM). Statistical evaluations were performed with 2-way ANOVA followed by Bonferroni's *post-hoc* test. Results were considered significant when P value was <0.05.

RESULTS

Effects of A484954 on adrenaline-induced acute changes of BP in rats

We first examined the effects of pretreatment with A484954 on adrenaline-induced change of BP in Wistar rats. A484954 (122 μg/kg, 10 min-pretreatment) significantly inhibited adrenaline (40 μg/kg)-induced increase in MBP (Fig. 1b, n=5, P<0.01) and also potentiated adrenaline (14, 40 μg/kg)-induced decrease in DBP (Fig. 1c, n=5, P<0.01). On the other hand, A484954 had no effect on adrenaline-induced increase in SBP (Fig. 1a, n=5) as well as decrease in HR (Fig. 1d, n=5).

Effects of A484954 on isoproterenol-induced acute changes of BP in rats

We next examined the effects of pretreatment with A484954 on isoproterenol-induced change of BP in Wistar rats. A484954 (122 μg/kg, 10 min-pretreatment) significantly potentiated isoproterenol (0.3 μg/kg)-induced decrease in MBP [Fig. 2b, n=6 (Vehicle), n=8 (A484954), P<0.05] and DBP [Fig. 2c, n=6 (Vehicle), n=8 (A484954), P<0.01] but not SBP [Fig. 2a, n=6 (Vehicle), n=8 (A484954)]. A484954 had no effect on HR [Fig. 2d, n=6 (Vehicle), n=8 (A484954)].

Effects of A484954 on Ang II-induced acute changes of BP in rats

We further examined the effects of pretreatment with A484954 on Ang II-induced change of BP in Wistar rats. A484954 (122 μg/kg, 10 min-pretreatment) significantly inhibited Ang II-induced increase in SBP (Fig. 3a, n=5, P<0.01 at 40 μg/kg), MBP (Fig. 3b, n=5, P<0.01 at 14 and 40 μg/kg), and DBP (Fig. 3c, n=5, P<0.01 at 14 and 40 μg/kg). On the other hand, A484954 had no effect on Ang II-induced decrease in HR (Fig. 3d, n=5).

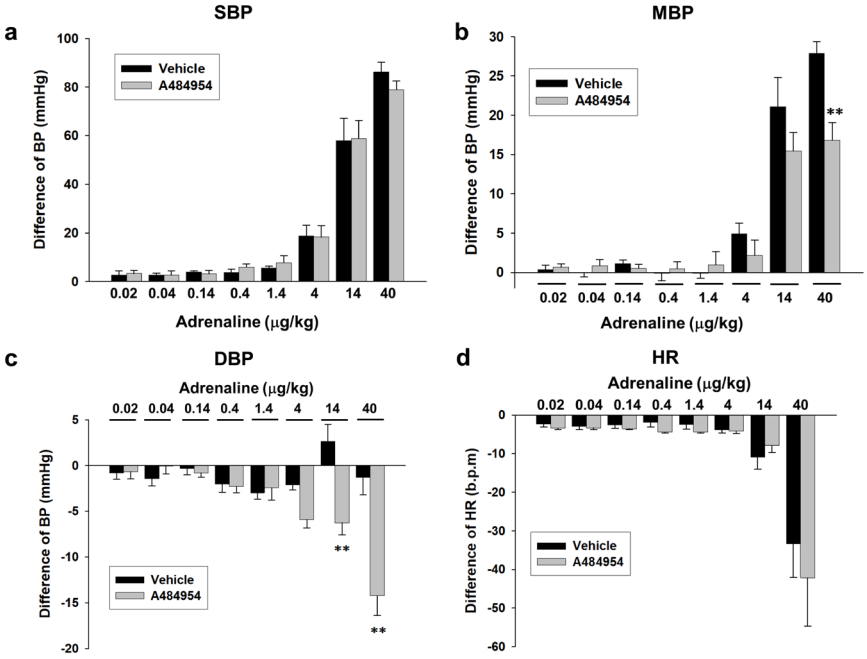

Fig. 1. Effects of A484954 on adrenaline-induced changes of blood pressure (BP) in Wistar rats. BP and heart rate (HR) were measured by a carotid cannulation method. Adrenaline (0.02–40 μg/kg, 5 min-interval) was cumulatively injected through femoral vein 10 min after pretreatment with A484954 (122 μg/kg) or (vehicle: dimethyl sulfoxide, DMSO; 0.1%). We chose and measured the highest point for systolic BP (SBP) and the lowest point for diastolic BP (DBP) after injection with adrenaline. Mean BP (MBP) was calculated between the 2 points and shown as the average value. Bar graphs indicated the changes of BP [(a) SBP, (b) MBP, and (c) DBP] and (d) HR after injection with adrenaline. The results were shown as means ± standard error of the mean (SEM) (n=5). $^{**}P<0.01$ vs. Vehicle.

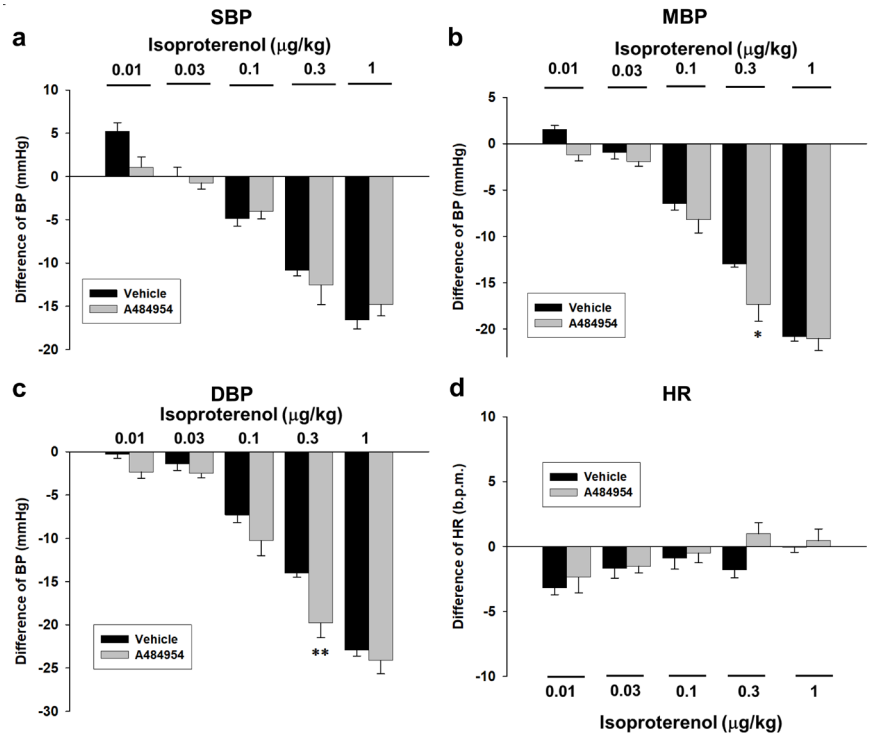

Fig. 2. Effects of A484954 on isoproterenol-induced changes of blood pressure (BP) in Wistar rats. Isoproterenol (0.01–1 μg/kg) was cumulatively injected through femoral vein 10 min after pretreatment with A484954 (122 μg/kg) or vehicle. The lowest point was chosen for measurement of both diastolic BP (DBP) and systolic BP (SBP) after injection with isoproterenol. Mean BP (MBP) (for 10 seconds) was calculated and shown as the average value. Bar graphs indicated the changes of BP [(a) SBP, (b) MBP, and (c) DBP] and (d) heart rate (HR) after injection with isoproterenol. The results were shown as mean ± SEM [n=6 (Vehicle), n=8 (A484954)]. $^{*}P<0.05$, $^{**}P<0.01$ vs. Vehicle.

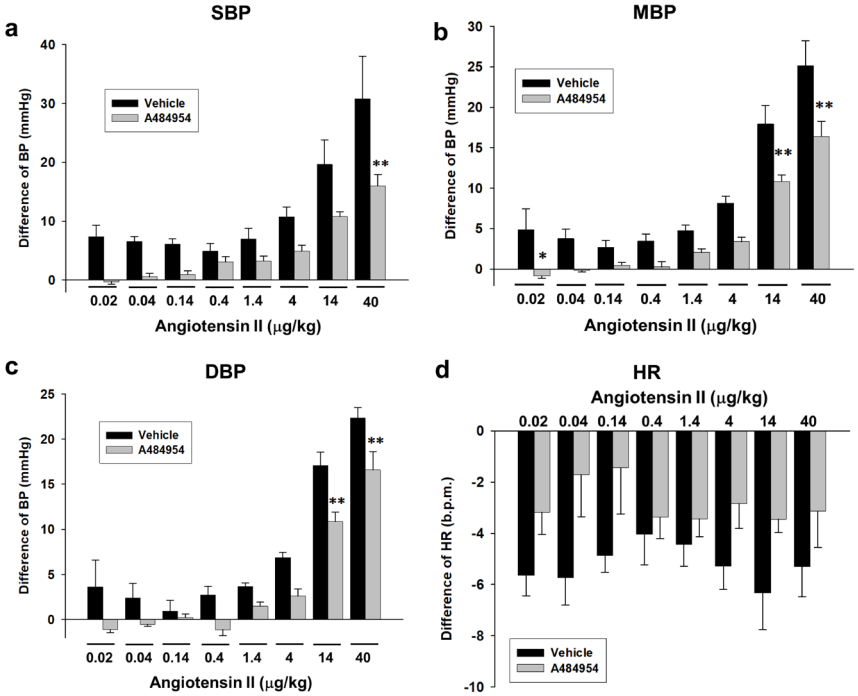

Fig. 3. Effects of A484954 on angiotensin II (Ang II)-induced changes of blood pressure (BP) in Wistar rats. Ang II (0.02–40 μg/kg) was cumulatively injected through femoral vein 10 min after pretreatment with A484954 (122 μg/kg) or vehicle. The highest point was chosen for measurement of both systolic BP (SBP) and diastolic BP (DBP) after injection with Ang II. Mean BP (MBP) (for 10 seconds) was calculated and shown as the average value. Bar graphs indicated the changes of BP [(a) SBP, (b) MBP, and (c) DBP] and (d) heart rate (HR) after injection with Ang II. The results were shown as mean ± SEM (n=5). ***P*<0.01 vs. Vehicle.

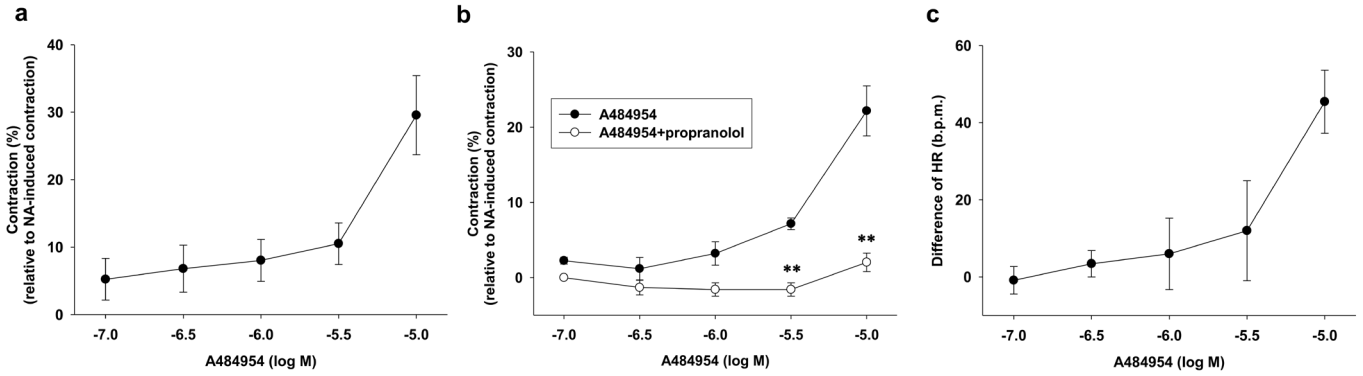

Fig. 4. Effects of A484954 on contractility and heart rate (HR) of rat isolated atria. (a) A484954 (0.1–10 μM) was cumulatively added to isolated left atria. Contraction was normalized to noradrenaline (NA; 1 μM)-induced contraction. (b) A484954 (0.1–10 μM) was cumulatively added to isolated left atria after pretreatment with propranolol (3 μM, 15 min). Contraction was normalized to NA (1 μM)-induced contraction. (c) A484954 (0.1–10 μM) was cumulatively added to isolated right atria. The change (beat per minutes; b.p.m.) in HR after the addition of A484954 was calculated. The results were shown as mean ± SEM (a: n=6, b: n=4, c: n=7). ***P*<0.01 vs A484954.

Effects of A484954 on contractility and HR in rat isolated atria

We finally examined whether A484954 (0.1–10 μM) affects basal contractility and HR in rat isolated atria. A484954 (10 μM) caused contraction in isolated left atria (Fig. 4a, n=6). Vehicle did not affect the basal contractility (n=6, data not shown). A β blocker, propranolol (3 μM) prevented the A484954-induced contraction (Fig. 4b, n=4, P<0.01). A484954 (10 μM) increased HR in isolated right atria (Fig. 4c, 15.54 ± 2.46% increase relative to stable state, n=7). Vehicle did not affect the HR (n=7, data not shown).

DISCUSSION

In the present study, we examined the acute effects of an eEF2K inhibitor, A484954 on BP in rats and explored underlying mechanisms especially focusing on β-adrenergic receptor. The major findings of the present study are as followings; 1) A484954 (122 μg/kg, 10 min-pretreatment) potentiated α- and β-adrenergic receptor agonist, adrenaline (40 μg/kg)-induced decrease in DBP but had no effect on increase in SBP (Fig. 1). 2) A484954 (122 μg/kg) potentiated β-adrenergic receptor agonist, isoproterenol (0.3 μg/kg)-induced decrease in DBP but not SBP (Fig. 2). 3) A484954 (122 μg/kg) inhibited non-β-adrenergic receptor agonist, Ang II (40 μg/kg)-induced increase in both SBP and DBP (Fig. 3). 4) A484954 (0.1–10 μM) caused contraction in rat isolated left aria, which was prevented by a β blocker, propranolol (Fig. 4). In conclusion, it was for the first time demonstrated that A484954 potentiates β-adrenergic receptor agonist-induced decrease in DBP and that A484954 causes contraction in isolated heart via activating β_1-adrenergic receptor.

In the present study, we revealed that A484954 potentiated the β-adrenergic receptor agonist, adrenaline or isoproterenol-induced change in DBP but not SBP, while A484954 inhibited the non-β-receptor agonist, Ang II-induced increase in both SBP and DBP. We have previously revealed that A484954 induces relaxation via activating β_2-receptor and/or K_{ir} channel in smooth muscle of rat isolated superior mesenteric artery [6, 7]. Thus, it is suggested that A484954 potentiated the hypotensive effects induced by adrenaline and isoproterenol mainly through the action on β_2-adrenergic receptor in vascular smooth muscle. On the other hand, it is suggested that A484954 inhibited Ang II-induced increase in BP via acting on K_{ir} channel in vascular smooth muscle. In addition, it seems likely that the effect of A484954 on SBP was different dependent on the β-adrenergic action. BP is regulated by cardiac output as well as peripheral arterial resistance. The cardiac contraction mediated by β_1-adrenergic receptor is an important determinant for cardiac output. In the present study, we have revealed that A484954 induces contraction at least in part via activating β_1-adrenergic receptor in rat isolated left atria. Therefore, it is suggested that A484954 had no influence on SBP specifically mediated via β-adrenergic receptor agonist perhaps due to the potentiated cardiac function through activating β_1-adrenergic receptor in heart.

Because we have revealed in this study that A484954 mediates not only hypotensive effect but also cardiotonic action similar to a phosphodiesterase inhibitor, we can expect application of A484954 as a cardioprotective agent against cardiovascular diseases including hypertension and heart failure. On the other hand, A484954 increased HR in rat isolated right atria (HR increasing rate by A484954 was ~15.5%). However, we have recently reported that acute injection with A484954 had no influence on HR in rats [7]. Therefore, it seems likely that the increase in HR by A484954 in isolated right atria has no big influence in the living body.

In conclusion, we have for the first time demonstrated that A484954 potentiates adrenaline- or isoproterenol-induced decrease in DBP possibly though vasorelaxation via the action on smooth muscle β_2-adrenergic receptor. We have also demonstrated for the first time that A484954 induces contraction in rat isolated heart via the action on β_1-adrenergic receptor. Because A484954 mediates both hypotensive and cardiotonic effects, further research might contribute to develop innovative drug for cardiovascular diseases including hypertension and heart failure.

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REFERENCES

1. Chen, J. H., Riazy, M., Smith, E. M., Proud, C. G., Steinbrecher, U. P. and Duronio, V. 2009. Oxidized LDL-mediated macrophage survival involves elongation factor-2 kinase. *Arterioscler. Thromb. Vasc. Biol.* **29**: 92–98. [Medline] [CrossRef]
2. Chen, Z., Gopalakrishnan, S. M., Bui, M. H., Soni, N. B., Warrior, U., Johnson, E. F., Donnelly, J. B. and Glaser, K. B. 2011. 1-Benzyl-3-cetyl-2-methylimidazolium iodide (NH125) induces phosphorylation of eukaryotic elongation factor-2 (eEF2): a cautionary note on the anticancer mechanism of an eEF2 kinase inhibitor. *J. Biol. Chem.* **286**: 43951–43958. [Medline] [CrossRef]
3. Kameshima, S., Kazama, K., Okada, M. and Yamawaki, H. 2015. Eukaryotic elongation factor 2 kinase mediates monocrotaline-induced pulmonary arterial hypertension via reactive oxygen species-dependent vascular remodeling. *Am. J. Physiol. Heart Circ. Physiol.* **308**: H1298–H1305. [Medline] [CrossRef]
4. Kameshima, S., Okada, M., Ikeda, S., Watanabe, Y. and Yamawaki, H. 2016. Coordination of changes in expression and phosphorylation of eukaryotic elongation factor 2 (eEF2) and eEF2 kinase in hypertrophied cardiomyocytes. *Biochem. Biophys. Rep.* **7**: 218–224. [Medline]
5. Kameshima, S., Okada, M. and Yamawaki, H. 2019. Eukaryotic elongation factor 2 (eEF2) kinase/eEF2 plays protective roles against glucose deprivation-induced cell death in H9c2 cardiomyoblasts. *Apoptosis* **24**: 359–368. [Medline] [CrossRef]
6. Kodama, T., Okada, M. and Yamawaki, H. 2018. Mechanisms underlying the relaxation by A484954, a eukaryotic elongation factor 2 kinase inhibitor, in rat isolated mesenteric artery. *J. Pharmacol. Sci.* **137**: 86–92. [Medline] [CrossRef]
7. Kodama, T., Okada, M. and Yamawaki, H. 2019. Eukaryotic elongation factor 2 kinase inhibitor, A484954 inhibits noradrenaline-induced acute increase of blood pressure in rats. *J. Vet. Med. Sci.* **81**: 35–41. [Medline] [CrossRef]
8. Nairn, A. C. and Palfrey, H. C. 1987. Identification of the major Mr 100,000 substrate for calmodulin-dependent protein kinase III in mammalian cells as elongation factor-2. *J. Biol. Chem.* **262**: 17299–17303. [Medline]
9. Okada, M., Noma, C., Yamawaki, H. and Hara, Y. 2013. Negative inotropic effect of carbachol and interaction between acetylcholine receptor-operated potassium channel (K.ACh channel) and GTP binding protein in mouse isolated atrium—a novel methodological trial. *J. Vet. Med. Sci.* **75**: 377–380. [Medline] [CrossRef]
10. Price, N. T., Redpath, N. T., Severinov, K. V., Campbell, D. G., Russell, J. M. and Proud, C. G. 1991. Identification of the phosphorylation sites in elongation factor-2 from rabbit reticulocytes. *FEBS Lett.* **282**: 253–258. [Medline] [CrossRef]
11. Ryazanov, A. G., Ward, M. D., Mendola, C. E., Pavur, K. S., Dorovkov, M. V., Wiedmann, M., Erdjument-Bromage, H., Tempst, P., Parmer, T. G.,

Prostko, C. R., Germino, F. J. and Hait, W. N. 1997. Identification of a new class of protein kinases represented by eukaryotic elongation factor-2 kinase. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 4884–4889. [Medline]
12. Usui, T., Nijima, R., Sakatsume, T., Otani, K., Kameshima, S., Okada, M. and Yamawaki, H. 2015. Eukaryotic elongation factor 2 kinase controls proliferation and migration of vascular smooth muscle cells. *Acta Physiol. (Oxf.)* **213**: 472–480. [Medline] [CrossRef]
13. Usui, T., Okada, M., Hara, Y. and Yamawaki, H. 2013. Eukaryotic elongation factor 2 kinase regulates the development of hypertension through oxidative stress-dependent vascular inflammation. *Am. J. Physiol. Heart Circ. Physiol.* **305**: H756–H768. [Medline] [CrossRef]
14. Usui, T., Okada, M., Hara, Y. and Yamawaki, H. 2011. Exploring calmodulin-related proteins, which mediate development of hypertension, in vascular tissues of spontaneous hypertensive rats. *Biochem. Biophys. Res. Commun.* **405**: 47–51. [Medline] [CrossRef]
15. Yasuda, J., Takada, L., Kajiwara, Y., Okada, M. and Yamawaki, H. 2015. Endostatin inhibits bradykinin-induced cardiac contraction. *J. Vet. Med. Sci.* **77**: 1391–1395. [Medline] [CrossRef]
16. Zhang, P., Riazy, M., Gold, M., Tsai, S. H., McNagny, K., Proud, C. and Duronio, V. 2014. Impairing eukaryotic elongation factor 2 kinase activity decreases atherosclerotic plaque formation. *Can. J. Cardiol.* **30**: 1684–1688. [Medline] [CrossRef]
17. Zhou, Y., Li, Y., Xu, S., Lu, J., Zhu, Z., Chen, S., Tan, Y., He, P., Xu, J., Proud, C. G., Xie, J. and Shen, K. 2019. Eukaryotic elongation factor 2 kinase promotes angiogenesis in hepatocellular carcinoma via PI3K/Akt and STAT3. *Int. J. Cancer* (In Press). [Medline] [CrossRef]