DOI 10.1002/mnfr.201600233 *Mol. Nutr. Food Res.* 2016, *60*, 2304–2311

FOOD & FUNCTION

Morus alba extract modulates blood pressure homeostasis through eNOS signaling

Albino Carrizzo[1], Mariateresa Ambrosio[1], Antonio Damato[1], Michele Madonna[1], Marianna Storto[1], Luca Capocci[1], Pietro Campiglia[2,8], Eduardo Sommella[3,8], Valentina Trimarco[4,5], Francesco Rozza[4,6], Raffaele Izzo[4,7], Annibale A. Puca[9,10] and Carmine Vecchione[1,10]

[1] IRCCS Neuromed, Pozzilli (IS), Italy
[2] European Biomedical Research Institute of Salerno, Salerno, Italy
[3] Department of Agriculture, Laboratory of Food Chemistry, University of Reggio Calabria, Reggio Calabria, Italy
[4] Hypertension Research Center, Federico II University Hospital, Naples, Italy
[5] Department of Neurosciences, Federico II University, Naples, Italy
[6] Department of Advanced Biomedical Sciences, University Federico II of Naples, Naples, Italy
[7] Department of Translational Medical Sciences, Federico II University, Naples, Italy
[8] Department of Pharmacy, School of Pharmacy, University of Salerno, Fisciano (SA), Italy
[9] IRCCS Multimedica, Milan, Italy
[10] Department of Medicine and Surgery, University of Salerno, Baronissi (SA), Italy

Scope: *Morus alba* is a promising phytomedicine cultivated in oriental countries that is extensively used to prevent and treat various cardiovascular problems. To date, despite its beneficial effects, the molecular mechanisms involved remain unclear. Thus, we investigate the vascular and haemodynamic effects of *Morus alba* extract in an experimental model focusing our attention on the molecular mechanisms involved.

Methods and results: Through vascular reactivity studies, we demonstrate that *Morus alba* extract evokes endothelial vasorelaxation through a nitric oxide-dependent pathway. Our molecular analysis highlights an increase in endothelial nitric oxide synthase (eNOS) phosphorylation. In vivo administration of *Morus alba* extract reduces blood pressure levels exclusively in wild-type mice, whereas it fails to evoke any haemodynamic effects in eNOS-deficient mice. Molecular analyses revealed that its beneficial action on vasculature is mediated by the activation of two important proteins that act as stress sensors and chaperones: PERK and heat shock protein 90. Finally, *Morus alba* extract exerts antihypertensive action in an experimental model of arterial hypertension.

Conclusion: Through its action on eNOS signaling, *Morus alba* extract could act as a food supplement for the regulation of cardiovascular system, mainly in clinical conditions characterized by eNOS dysfunction, such as arterial hypertension.

Received: March 14, 2016
Revised: May 12, 2016
Accepted: May 17, 2016

Keywords:
Endothelium / Hypertension / *Morus alba* / Nitric oxide / Nutraceuticals

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

The extracts of *Morus alba* leaves, marketed as dietary supplements, modulate cholesterol metabolism, prevent atherosclerosis, and modulate blood pressure levels [1–3].

Correspondence: Professor Carmine Vecchione
E-mail: cvecchione@unisa.it

Abbreviations: **DAF-FM**, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate; **eNOS**, endothelial nitric oxide; **HSP90**, heat shock protein 90; **L-NAME**, N^G-nitro-L-arginine methyl ester; **MAE**, *Morus alba* extract; **NO**, nitric oxide; **PERK**, PKR-like endoplasmic reticulum kinase; **SHR**, spontaneously hypertensive rats

A thorough understanding of the molecular mechanisms responsible for the beneficial effects of *Morus alba* leave extract (*MAE*) could contribute to the development of therapies aimed at reducing cardiovascular risk factors, improving vascular biology and vascular health, reducing cardiovascular target organ damage and reducing healthcare expenditures.

Here, we demonstrate for the first time that *MAE* evokes endothelial-dependent vasorelaxation of resistance vessels through the endothelial nitric oxide (eNOS) pathway in mice. This effect is mediated by an interaction with PKR-like endoplasmic reticulum kinase (PERK) and heat shock protein 90 (HSP90) signaling. *MAE* reduces blood pressure levels in vivo, and such hemodynamic actions are consistent with the direct endothelial-mediated vasorelaxation on resistant arteries through a nitric oxide (NO)-mediated mechanism. Finally, *MAE* exerts anti-hypertensive action in an experimental model with a genetic background of arterial hypertension.

2 Materials and methods

2.1 Reagents and animals

Extract from leaves of *Morus alba* was obtained from Akademy Pharma (Italy). All experiments involving animals conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and were approved by the IRCCS INM Neuromed review board (1070/2015 PR). C57BL/6 and spontaneously hypertensive rats (SHR) were generated in our animal facility. eNOS knockout mice were obtained from the Jackson Laboratory. All efforts were made to minimize the number of animals used and their suffering.

2.2 Extraction solvent (crude extract)

Briefly, 0.5 g of pulverized extract from leaves of *MAE* were solubilized in 25 mL of EtOH (60% v/v) and stirred for 3 h. Then, the solution was centrifuged at 6000 *g* for 10 min. The supernatant was recovered, and the process was repeated twice. Supernatants were pooled and concentrated under reduced pressure as previously described [4] (Supporting Information online-only).

2.3 Vascular reactivity studies

Second-order branches of the mesenteric arterial tree were removed from mice to perform vascular studies. Vessels were placed in a wire or pressure myograph system filled with Krebs solution maintained at pH 7.4 at 37°C as previously described [5]. Some mesenteric arteries mounted on a pressure myograph were pretreated with phosphatidylinositol-4,5-bisphosphate 3-kinase inhibitor (Wormannin, 10 μM, 1 h) PERK inhibitor (GSK2606414, 1 μM, 1 h), HSP90 inhibitor (SNX2112, 400 nM, 1 h) or the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM, 30 min) before data for *MAE* dose-response curves were obtained (Supporting Information online-only).

2.4 Evaluation of NO production by DAF

Production of NO was assessed as previously described [6]. *MAE* (8 mg/mL) or acetylcholine (10^{-6} M) was administered to the mesenteric artery in the last 30 min of 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM) incubation, alone and after 20 min exposure to L-NAME (300 μmol/L, 30 min). Mesenteric segments were cut in 5-μm thick sections, observed under a fluorescence microscope, subsequently counterstained with haematoxylin and eosin and observed under a light microscope.

2.5 Immunoprecipitation

Mesenteric artery homogenates were prepared in 5 mM Tris-HCl buffer, pH 7.5, containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $NaHCO_3$. Samples were processed as previously described to perform immunoblotting analyses [7] (Supporting Information online-only).

2.6 Blood pressure measurement

Blood pressure was evaluated in WT (C57BL/6), eNOS KO mice, and SHR under basal conditions and after single orally administration of 200 μL of *MAE* (100, 200, or 400 mg/kg). Animals used as controls were treated in a similar manner, but *MAE* was replaced with the vehicle alone (saline solution). The tail cuff method was performed as previously described [8]. At the end of blood pressure measurements, some vessels were excised to perform vascular reactivity or molecular analyses (Supporting Information online-only).

2.7 Assessment of in vivo oral toxicity of *MAE* in mice

Toxicity was evaluated at the haematological and biochemical level and in organs after the administration of *MAE* (Supporting Information online-only).

2.8 Statistical analysis

For vascular reactivity studies, statistical analysis was performed by 2-way ANOVA followed by Bonferroni post hoc test. Immunoblot experiments were presented as the mean values and standard deviation. The chemiluminescence quantification was performed using ImageJ software. Group comparisons were made by paired *t*-test. Differences were considered to be statistically significant at $p < 0.05$.

3 Results

3.1 HPLC analysis of compounds contained in *MAE*

HPLC analyses of polyphenolic compound levels in the *MAE* are reported in Supporting Information Table 1. Rutin, also called rutoside (quercetin-3-*O*-rutinoside), was the most concentrated phenolic compound (1071.70 ± 0.13 μg/g) present in the extract followed by another phenolic compound, chlorogenic acid (199.90 ± 0.02 μg/g). Moreover, we also detected high concentrations of the flavonoid astragalin (kaempferol-3-O-glucoside) (187.42 ± 0.01 μg/g). All other polyphenolic compounds detected at lower concentrations are reported in Supporting Information Table 2 (Supporting Information online-only).

3.2 *MAE* evokes dose-dependent endothelial nitric oxide vasorelaxation in mesenteric mice vessels

Our first aim was to explore whether *MAE* modulates vascular function. Our data demonstrate that the administration of increasing doses of *MAE* evoked dose-dependent vasorelaxation in mice vessels, which was abolished by endothelium removal (Fig. 1A). In agreement, in presence of the eNOS inhibitor L-NAME, *MAE*-induced vasorelaxation was significantly reduced, demonstrating the involvement of NO-signaling in its vascular action (Fig. 1B). Molecular analyses revealed that *MAE* induced the phosphorylation of eNOS on serine^{1177}, an activation site of the enzyme, and a reduction of the phosphorylation of the inhibitor site threonine^{495} [9], whereas no changes were recorded at serine^{633} of eNOS, an alternative activation site of the enzyme [10]. Overall, these data further confirm that the main activation site of eNOS involved in the vascular effect evoked by *MAE* is represented by serine^{1177} (Fig. 1C). Interestingly, the degree of eNOS phosphorylation is positively related to the increasing doses of *MAE*. Finally, DAF-FM fluorescence induced by *MAE* was comparable to that obtained with a classical agonist that evokes NO release, such as acetylcholine (Fig. 1D) (Supporting Information online-only).

3.3 PERK-HSP90 signaling is essential for eNOS phosphorylation evoked by *MAE*

Together with the modifications on eNOS phosphorylation described above, we also demonstrate that treatment with *MAE* enhances phosphorylation of PERK at threonine^{980} and HSP90 at threonine$^{5/7}$, which represents the activation sites of these proteins (Fig. 2A).

We have previously described intracellular signaling that involves HSP90 and PERK for the activation of eNOS [11]. Thus, to better characterize the signaling recruited by *MAE* at the vascular level, we performed experiments with the PERK inhibitor GSK2606414 and the HSP90 inhibitor SNX2112. The treatment of vessels with GSK2606414 completely abolished the phosphorylation of HSP90, resulting in a significant reduction of eNOS phosphorylation (Fig. 2A). At the functional level, PERK inhibition significantly reduced endothelial vasorelaxation evoked by *MAE* (Fig. 2B). Furthermore, we evaluated the action on *MAE* in the presence of the HSP90 inhibitor SNX2112. In this experimental condition, we observed a reduction in HSP90 and eNOS phosphorylation associated with an impaired *MAE* vascular effect (Fig. 2C). Of note, during HSP90 inhibition, PERK is still phosphorylated. Overall, these data suggest that PERK is a target of *MAE* and upstream modulator of HSP90, thus leading to eNOS activation (Supporting Information online-only).

3.4 *MAE* treatment induces PERK/HSP90/eNOS complex formation

The interplay of the phosphorylation state of the enzyme and the physical interaction of eNOS with various intracellular proteins plays a crucial role in the regulation of eNOS activity [12]. Based on the results described above, we performed immunoprecipitation studies in vessels treated with *MAE* to interrogate possible interactions among PERK, HSP90, and eNOS. Indeed, we demonstrate that *MAE* treatment leads to the formation of a molecular complex between eNOS and PERK/HSP90, as shown by the immunoblot performed on vessel extracts immunoprecipitated for eNOS (Fig. 2D). We only detected the presence of both PERK and HSP90 in the protein extract from *Morus alba*-treated vessels. Interestingly, using GSK2606414, we are not able to detect the presence of both HSP90 and PERK, suggesting that the recruitment of PERK by *MAE* is the key step in the induction of the complex formation with eNOS enzyme. Of note, in presence of SNX2112, eNOS was unable to immunoprecipitate both PERK and HSP90 (Fig. 2D). These data are consistent with the results described above, in which we demonstrated that the recruitment of both proteins, PERK and HSP90, is needed to evoke the enhancement of eNOS phosphorylation, leading to dose-dependent endothelial vasorelaxation.

3.5 *MAE* reduces blood pressure and enhances endothelial function in mice

We assessed the effect on blood pressure homeostasis evoked by the oral administration of different single doses of *MAE* (100, 200, and 400 mg/kg). In particular, we observed that the administration of 100 *MAE* mg/kg did not modify blood pressure and vascular function (Fig. 3A–C). Interestingly, the administration of 200 mg/kg *MAE*, although did not modify blood pressure levels, it was able to enhance endothelial vasorelaxation and PERK/HSP90/eNOS phosphorylation (Fig. 3C). The highest dose of MAE (400 mg/kg) significantly reduces blood pressure levels (Fig. 3A), and the action on both endothelial vasorelaxation and PERK/HSP90/eNOS

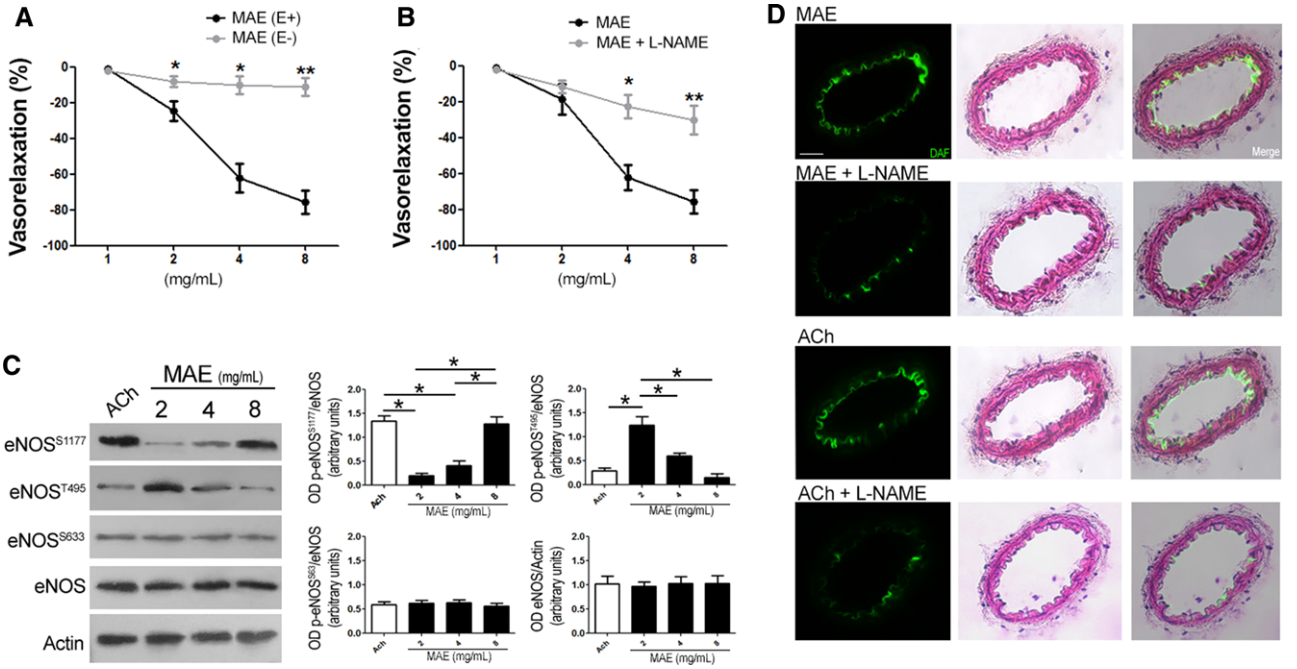

Figure 1. (A) Vascular response of phenylephrine-precontracted mice mesenteric arteries to increasing doses of *MAE* (1 – 8 mg/mL) in the presence (E+) or absence of endothelium (E-) ($n = 7$); *$p < 0.05$ versus *MAE* alone, (B) and after 30 min of pretreatment with L-NAME (300 μmol/L; $n = 7$); (C) *left*, Representative immunoblot of mesenteric arteries treated with acetylcholine (Ach, 10^{-6} M) or with increasing doses of *MAE* (1 – 8 mg/mL); *right*, Columns are the mean ± SD of five independent experiments. *$p < 0.05$; (D) Representative high-power micrographs of 10-μm sections of mice mesenteric arteries loaded for 2 h with 4,5-diaminofluorescein (DAF) reveal nitric oxide production after treatment with acetylcholine (Ach 10^{-6} M) or *MAE* (8 mg/mL) and after 30 min of pretreatment with L-NAME (300 μmol/L; $n = 5$), counterstained with haematoxylin and eosin (HE) and also presented as merged images. Scale bar, 50 μm.

phosphorylation was significantly increased compared with that observed at the lower dose (Fig. 3B–C). Taken together, these results allow us to correlate the potentiation of acetylcholine vasorelaxation observed both at 200 and at 400 mg/Kg of MAE with the enhancement of PERK/HSP90/eNOS phosphorylation.

Smooth muscle vasorelaxation induced by nitro-glycerine was not affected by *MAE* administration (Fig. 3D), thus corroborating an endothelial-mediated mechanism evoked by *MAE*. In our observation period, we detected that the hypotensive effect started 2 days after the oral administration of a single dose of *MAE*. After 4 days, blood pressure levels returned to baseline, and the administration of a second dose evoked the same haemodynamic effect previously observed (Supporting Information online-only).

3.6 Blood pressure reduction and endothelial vasorelaxation evoked by *MAE* are nitric oxide dependent

To definitively confirm the involvement of NO in the haemodynamic effect evoked by *MAE*, we performed experiments in eNOS knock-out (KO) mice. Our data showed that the oral administration of a single dose of *MAE* failed to evoke a reduction of blood pressure levels in eNOS KO mice compared with wild-type mice (Fig. 4A). Interestingly, *MAE* administration did not improve the impaired acetylcholine vasorelaxation in eNOS KO mice treated with vehicle alone in contrast to that observed in wild-type mice (Fig. 4B). These results clearly indicate that eNOS mediates the in vivo effects of *MAE* on the modulation of blood pressure levels and endothelial function.

Finally, to assess the effect of *MAE* in the scenario of hypertension, we tested the effect of the higher dose of *MAE* on a genetic rat model of hypertension, such as SHR. In this experimental setting, *MAE* exhibits a clear anti-hypertensive effect as it normalized blood pressure levels compared with control rats treated only with vehicle (Fig. 4C).

3.7 Single oral dose of *MAE* did not exert toxicity

In our observation period, the administration of the higher dose of MAE did not influence haematological, biochemical, and histopathological evaluation, as shown in Supporting Information Tables 3 and 4 (Supporting Information online-only).

4 Discussion

Our data demonstrate that *MAE* evokes dose-dependent endothelial vasorelaxation given that the mechanical removal of the endothelium completely abolished its vasorelaxant effect.

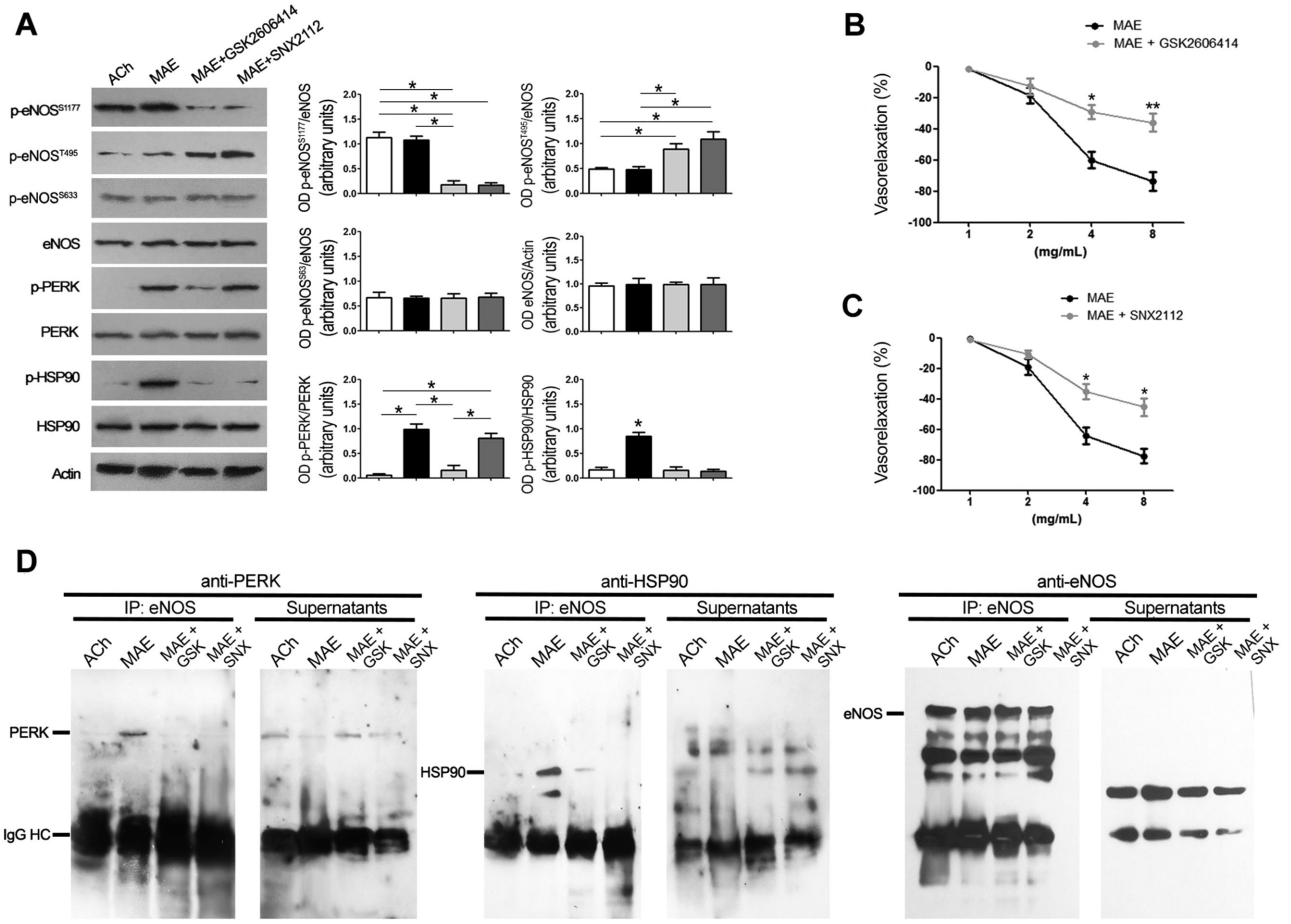

Figure 2. . (A) Representative immunoblot of mice mesenteric arteries treated with acetylcholine (Ach, 10^{-6} M), *MAE* alone (8 mg/mL) or *MAE* (8 mg/mL) plus PERK inhibitor (GSK2606414, 1 μM, 1 h) or HSP90 inhibitor (SNX2112, 400 nM, 1 h). Columns are the mean ± SD of five independent experiments. *$p < 0.05$. (B) Vascular response of phenylephrine-precontracted mice mesenteric arteries to increasing doses of *MAE* (1 – 8 mg/mL) in presence of PERK inhibitor ($n = 7$) (C) or HSP90 inhibitor ($n = 7$); *$p < 0.05$ versus *MAE* alone; **$p<0.05$ versus *MAE* alone. (D) Endogenous PERK and HSP90 immunoprecipitated with eNOS in mesenteric artery lysate from wild-type mice treated with acetylcholine (Ach, 10^{-6} M), *MAE* alone (8 mg/mL), *MAE* plus PERK inhibitor (MA+ GSK) or *MAE* plus HSP90 inhibitor (MA + SNX). Extracts were immunoprecipitated for eNOS and then probed with anti–PERK, anti HSP90 or anti-eNOS antibodies ($n = 3$).

In addition, its action is specifically mediated by NO production, as demonstrated by the inhibition of eNOS by L-NAME, which completely abolished its vascular action.

MAE has been extensively used to prevent and treat various cardiovascular problems acting as an anti-aging plant [13, 14].

Based on this evidence, we investigated a new molecular pathway that modulates eNOS metabolism that we recently associated with an anti-aging protein, which is represented by PERK and HSP90 intracellular signaling [15]. PERK is a regulatory kinase that is known as a transducer of the unfolded protein response, reducing ER protein loading through the inhibition of protein synthesis [16]. Genetic and pharmacological experiments have demonstrated that PERK signaling also confers prosurvival effects given that genetic deletion of PERK activity impaired cell survival [17]. Conversely, transient artificial PERK activation enhanced cell survival in response to ER protein misfolding [18]. Moreover, we recently demonstrated that an alternative pathway converging on NO release is represented by PERK/ HSP90, which are activated by a longevity associated variant (LAV) of BPIFB4 to stimulate NO production [15]. Thus, these data suggest that PERK has an important role in the maintenance of cellular and vascular homeostasis.

Interestingly, our data demonstrate that *MAE* activates the kinase PERK through its phosphorylation in resistance vessels in mice. A number of key regulatory kinases, including PERK, interact with the molecular chaperone for stability and function [19]. Thus, we investigated the activation status of HSP90, an important intracellular chaperone that regulates the activity of many proteins, including eNOS [20]. We found that *MAE* promotes PERK activation and HSP90 phosphorylation on threonine, an activation site of the chaperone [21]. The use of a pharmacological inhibitor of PERK, GSK2606414, reduces the effect of *MAE* on vascular function

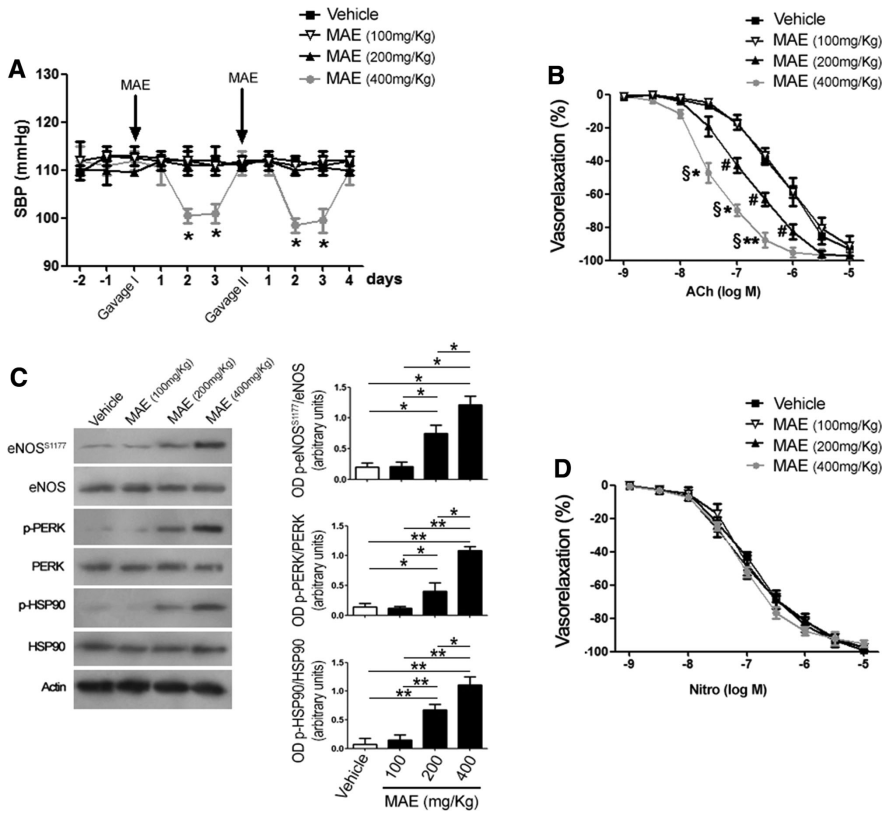

Figure 3. (A) Systolic blood pressure (SBP) in C57BL/6 mice treated with vehicle or with increasing dosage of *MAE* (100, 200, 400 mg/kg) ($n = 8$/group). Arrows indicate a single oral dose of *MAE* (or Vehicle). Data are presented as the mean ± SEM. $^*p < 0.05$ versus all (unpaired *t*-test). (B) Graphs show the dose-response curves of ex-vivo mesenteric arteries, excised after blood pressure measurement from mice after single orally administration with Vehicle or *MAE* at different dosage, to acetylcholine (Ach, from 10^{-9} M to 10^{-5} M). Values are the means ± SEM. $n = 8$ experiments. $^*p < 0.05$ versus vehicle and 100 mg/kg; $^{**}p < 0.01$ vehicle and 100 mg/kg; $\#p < 0.05$ versus vehicle and 100 mg/Kg; $^{\S}p < 0.05$ versus 200 mg/kg. (C) Representative immunoblot in mesenteric arteries obtained from mice treated in vivo with vehicle or with different doses of *MAE* (100, 200, 400 mg/kg) after acetylcholine stimulation; columns are the mean ± SD of three independent experiments. $^*p < 0.05$; $^{**}p < 0.01$. (D) Dose-response curves of ex vivo mesenteric arteries excised after blood pressure measurement from mice after a single oral dose of vehicle or *MAE* at different dose ratios to nitro-glycerine (Nitro, from 10^{-9} M to 10^{-5} M).

and HSP90 and eNOS phosphorylation. In addition, the use of HSP90 inhibitor, SNX2112, blunts *MAE* evoked vasorelaxation and HSP90 and eNOS phosphorylation. Of note, in this experimental setting, PERK is still phosphorylated possibly due to its upstream role in modulating HSP90 and eNOS. Taken together, our data clearly demonstrate for the first time that the vascular complex PERK/HSP90/eNOS is crucial for *MAE* vasorelaxant action.

Molecules able to regulate vascular function through NO-modulation influence blood pressure homeostasis [12]. Consistent with previous studies [22, 23], we demonstrate that a single oral dose of *MAE* reduces blood pressure levels in vivo.

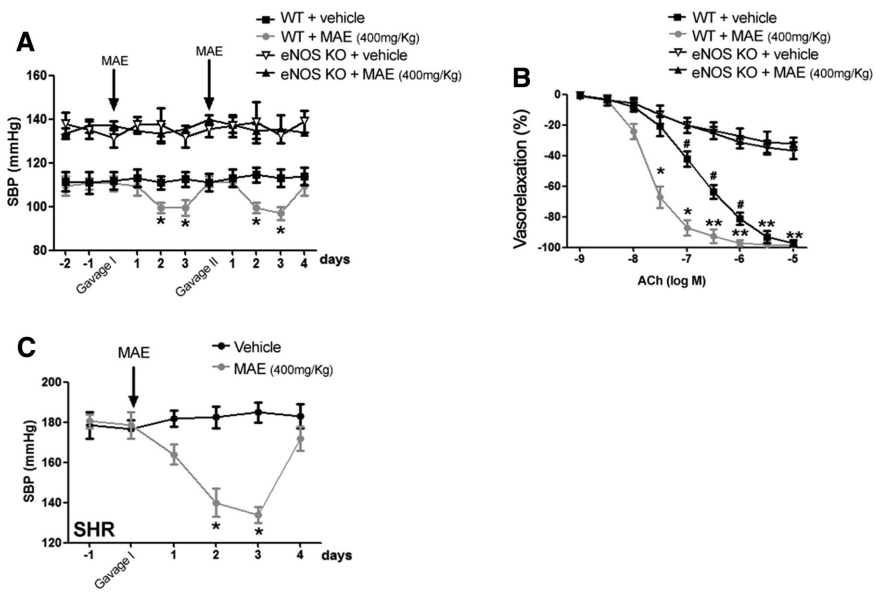

Figure 4. (A) Systolic blood pressure (SBP) in C57BL/6 and eNOS knockout mice (eNOS KO) treated with vehicle or *MAE* (400 mg/kg) ($N = 8$/group). Arrows indicate single oral dose of *MAE* (or vehicle). Data are provided as the mean ± SEM. $^*p < 0.05$ versus WT + vehicle. (B) Vascular response of phenylephrine-precontracted mice mesenteric arteries excised from mice treated with a single oral dose of *MAE* (400 mg/kg) in wild-type (WT) and eNOS knockout mice (eNOS KO). $^*p < 0.05$ versus eNOS KO plus vehicle or eNOS KO plus MAE; $\#p < 0.01$ versus WT plus *MAE*. (C) Systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) treated with vehicle or 400 mg/kg *MAE*. Values are the means ± SEM. ($n = 8$/group). $^*p < 0.05$ versus vehicle.

However, in the present study although we observed a direct vasorelaxant effect of *MAE* in vitro, we were unable to directly correlate this effect with the modulation of blood pressure levels. A pharmacokinetic characterization of *MAE*, which represents the target of future studies, is necessary to address this point. On the other hand, it is important to note that *MAE* still enhances NO mediated vasorelaxation at lower doses without influencing blood pressure homeostasis. This result clearly indicates that the enhancement of endothelial function evoked by *MAE* is independent from its ability to modulate blood pressure levels and could be due to a sensitizing effect on acetylcholine-evoked vasorelaxation mediated by PERK/HSP90 signaling. Taken together, these data prompt us to hypothesize that *MAE* administration at lower doses may help to contain vascular diseases without influencing blood pressure.

To further strengthen the involvement of eNOS signaling in the in vivo vascular effects of *MAE*, we administered the agent to eNOS-KO mice. In conditions of eNOS deficiency, *MAE* failed to exert any hemodynamic effects. Finally, the administration of a higher dose of *MAE* in spontaneously hypertensive rats induced anti-hypertensive effects, thus supporting its possible application in clinical fields.

5 Conclusion

Our data demonstrate for the first time that *MAE* modulates vascular tone and blood pressure through enhancement of endothelial NO release. In addition, *MAE* did not evoke toxicity in our experimental setting. However, more studies are needed to characterize *MAE* effects after a longer period of administration. In conclusion, our results suggest that *MAE* could be used as a dietary supplement for the regulation of the cardiovascular system, mainly in those clinical conditions characterized by endothelial NO dysfunction.

A.C., M.A., and A.D. performed experiments and statistical analysis; M.S., L.C., P.C., and E.M.S. performed haematological and biochemical analysis and the characterization of compounds contained in the extract; C.V. and A.A.P. conceived and designed the study; M.M. was responsible for animal welfare; V.T., F.R., and R.I. made critical revisions to the draft; A.C., C.V., and A.A.P. interpreted data and drafted the paper.

The authors have declared no conflicts of interest.

6 References

[1] Enkhmaa, B., Shiwaku, K., Katsube, T., Kitajima, K. et al., Mulberry (*Morus alba* L.) leaves and their major flavonol quercetin 3-(6-malonylglucoside) attenuate atherosclerotic lesion development in LDL receptor-deficient mice. *J. Nutr.* 2005, *135*, 729–734.

[2] Liu, L. K., Lee, H. J., Shih, Y. W., Chyau, C. C. et al., Mulberry anthocyanin extracts inhibit LDL oxidation and macrophage-derived foam cell formation induced by oxidative LDL. *J. Food Sci.* 2008, *73*, H113–H121.

[3] Jo, S. P., Kim, J. K., Lim, Y. H., Antihyperlipidemic effects of stilbenoids isolated from *Morus alba* in rats fed a high-cholesterol diet. *Food Chem. Toxicol.* 2014, *65*, 213–218.

[4] Katsube, T., Imawaka, N., Kawano, Y., Yamazaki, Y. et al., Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. *Food Chem.* 2006, *97*, 25–31.

[5] Zacchigna, L., Vecchione, C., Notte, A., Cordenonsi, M. et al., Emilin1 links TGF-beta maturation to blood pressure homeostasis. *Cell* 2006, *124*, 929–942.

[6] Maffei, A., Poulet, R., Vecchione, C., Colella, S. et al., Increased basal nitric oxide release despite enhanced free radical production in hypertension. *J. Hypertens.* 2002, *20*, 1135–1142.

[7] Carrizzo, A., Lenzi, P., Procaccini, C., Damato, A. et al., Pentraxin 3 induces vascular endothelial dysfunction through a P-selectin/matrix metalloproteinase-1 pathway. *Circulation* 2015, *131*, 1495–1505; discussion 1505.

[8] Carnevale, D., Vecchione, C., Mascio, G., Esposito, G. et al., PI3Kgamma inhibition reduces blood pressure by a vasorelaxant Akt/L-type calcium channel mechanism. *Cardiovasc. Res.* 2012, *93*, 200–209.

[9] Carrizzo, A., Puca, A., Damato, A., Marino, M. et al., Resveratrol improves vascular function in patients with hypertension and dyslipidemia by modulating NO metabolism. *Hypertension* 2013, *62*, 359–366.

[10] Chen, Z., Peng, I. C., Sun, W., Su, M. I. et al., AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. *Circ. Res.* 2009, *104*, 496–505.

[11] Puca, A. A., Carrizzo, A., Ferrario, A., Villa, F. et al., Endothelial nitric oxide synthase, vascular integrity and human exceptional longevity. *Immunity Ageing* 2012, *9*, 26.

[12] Forstermann, U., Munzel, T., Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 2006, *113*, 1708–1714.

[13] Sharma, R., Sharma, A., Shono, T., Takasugi, M. et al., Mulberry moracins: scavengers of UV stress-generated free radicals. *Biosci. Biotechnol. Biochem.* 2001, *65*, 1402–1405.

[14] Pan, G., Lou, C., Isolation of an 1-aminocyclopropane-1-carboxylate oxidase gene from mulberry (*Morus alba* L.) and analysis of the function of this gene in plant development and stresses response. *J. Plant Physiol.* 2008, *165*, 1204–1213.

[15] Villa, F., Carrizzo, A., Spinelli, C. C., Ferrario, A. et al., Genetic analysis reveals a longevity-associated protein modulating endothelial function and angiogenesis. *Circ. Res.* 2015, *117*, 333–345.

[16] Lin, J. H., Li, H., Zhang, Y., Ron, D. et al., Divergent effects of PERK and IRE1 signaling on cell viability. *PloS One* 2009, *4*, e4170.

[17] Scheuner, D., Song, B. B., McEwen, E., Liu, C. et al., Translational control is required for the unfolded protein response

and in vivo glucose homeostasis. *Mol. Cell* 2001, *7*, 1165–1176.

[18] Lu, P. D., Jousse, C., Marciniak, S. J., Zhang, Y. H. et al., Cytoprotection by pre-emptive conditional phosphorylation of translation initiation factor 2. *Embo. J.* 2004, *23*, 169–179.

[19] Marcu, M. G., Doyle, M., Bertolotti, A., Ron, D. et al., Heat shock protein 90 modulates the unfolded protein response by stabilizing IRE1alpha. *Mol. Cell. Biol.* 2002, *22*, 8506–8513.

[20] Obermann, W. M., Sondermann, H., Russo, A. A., Pavletich, N. P. et al., In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* 1998, *143*, 901–910.

[21] Kotula, E., Faigle, W., Berthault, N., Dingli, F. et al., DNA-PK target identification reveals novel links between DNA repair signaling and cytoskeletal regulation. *PloS One* 2013, *8*, e80313.

[22] Naowaboot, J., Pannangpetch, P., Kukongviriyapan, V., Kukongviriyapan, U. et al., Mulberry leaf extract restores arterial pressure in streptozotocin-induced chronic diabetic rats. *Nutr. Res.* 2009, *29*, 602–608.

[23] Lee, Y. J., Choi, D. H., Kim, E. J., Kim, H. Y. et al., Hypotensive, hypolipidemic, and vascular protective effects of *Morus alba* L. in rats fed an atherogenic diet. *Am. J. Chin. Med.* 2011, *39*, 39–52.

