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Current Perspective

Exploring Mechanisms of Diabetes-Related Macrovascular Complications: Role of Methylglyoxal, a Metabolite of Glucose on Regulation of Vascular Contractility

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Abstract. Methylglyoxal (MGO) is a metabolite of glucose. MGO binds to and modifies arginine, lysine, and cysteine residues in proteins, which leads to formation of a variety of advanced glycation end-products (AGEs) such as argpyrimidine and N^{ε}-(carboxyethyl)lysine. The concentration of MGO significantly increases in plasma from diabetic patients. Increased plasma MGO level seems to be associated with diabetic microvascular complications. In addition, MGO accumulates in large vascular tissues from spontaneous hypertensive rats, which is associated with increased blood pressure. Although it is logical to hypothesize that MGO could directly affect vascular reactivity, available reports are very limited. Our group has examined effects of MGO on vascular reactivity (contraction and relaxation) and explored underlying mechanisms. In this review article, we summarized our recent findings on 1) short-term effects of MGO, 2) long-term effects of MGO, and 3) effects of MGO accumulation in arterial walls on vascular reactivity. These findings may provide further mechanistic insights into the pathogenesis of diabetes-related macrovascular complications including hypertension.

Keywords: glucose metabolite, diabetes, hypertension, vascular reactivity, reactive oxygen species

1. Introduction

It is estimated in Japan that 22 million people are potentially diabetic patients (Ministry of Health, Labour and Welfare, Japan; 2007), 90% of whom are type 2 diabetic patients. It is known that plasma concentrations of glucose, advanced glycation end-products (AGEs), and metabolites of glucose including glyoxal and methylglyoxal (MGO) significantly increase in diabetic complicated patients (Fig. 1).

MGO is an α-dicarbonyl compound that is produced in various biochemical processes. In the case of the non-enzymatic pathway, MGO is spontaneously formed in a process of glycolysis from dihydroxyacetone phosphate as a by-product during the formation of glyceraldehyde 3-phosphate in most mammalian cells including vascular endothelial cells (1) and smooth muscle cells (2). In addition, MGO is formed in some enzymatic processes by the enzymes including MGO synthase, cytochrome P450 2E1, and semicarbazide-sensitive amine oxidase (SSAO) (3). MGO is generated from aminoacetone in the process of protein catabolism by P450 2E1. MGO is also produced from ketone bodies during fatty acid oxidation by SSAO. Because MGO has two carbonyl carbons, it can react with various molecules including RNA, DNA, and protein in various cells (4). Specifically, MGO binds to and modifies arginine, cysteine, and lysine residues in proteins, which causes a non-enzymatic formation of a variety of AGEs (5) such as argpyrimidine (6), N^{ε}-(carboxyethyl) lysine (7), hydroimidazolones N^{ε}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (8), and methyl-glyoxal-lysine dimer (9). MGO is catalyzed to propanediol by aldose reductase (10). In addition, MGO is degraded to D-lactic acid in the presence of glutathione by a glyoxalase system in the cytosol of all mammalian cells (4). The site of action for exogenously applied

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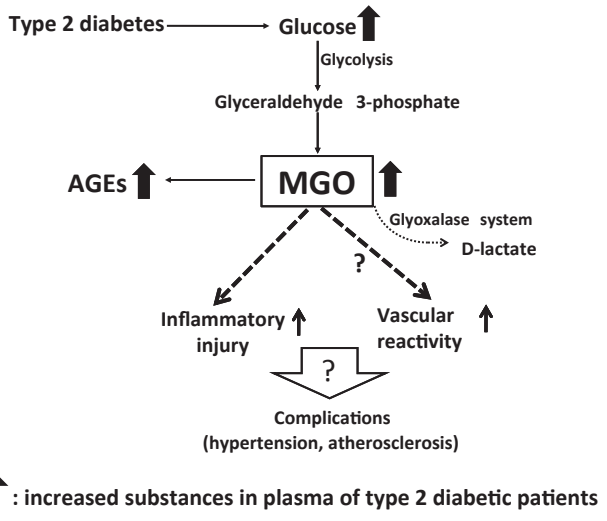

Fig. 1. Hypothesis: MGO is responsible for diabetic macrovascular complications. In type 2 diabetic patients, plasma concentrations of glucose, advanced glycation end-products (AGEs), and metabolites of glucose such as glyoxal, 3-deoxy glucosone, and methylglyoxal (MGO) increase. We previously demonstrated that MGO is a more powerful inducer for vascular endothelial inflammatory injury than AGEs and glucose itself. We hypothesized that MGO could affect vascular reactivity in addition to inflammatory responses, which may lead to diabetic macrovascular complications including hypertension.

MGO in the cells remains to be fully clarified. There is a study demonstrating that MGO can enter the cells and interact with intracellular molecules (11). In addition, it is likely that MGO can directly interact with some of extracellular components such as receptors (12) since it can modify various proteins as described above.

It is known that blood MGO concentration significantly increases in plasma from diabetic patients (13 – 16) and spontaneous hypertensive rats (SHR) (17, 18). It was demonstrated that the blood MGO concentration in human diabetic patients is around 2 μM (14, 15). While there is a report demonstrating that it was much higher (around 400 μM) (16), it was shown that blood MGO level is approximately 33.6 μM in adult SHR compared with 14.2 μM in age-matched Wistar Kyoto rats (WKY) (17). Increased level of blood MGO-derived AGEs seems to be associated with diabetic microvascular complications such as diabetic nephropathy (19), retinopathy (20), and neurological disorders (21). MGO is also known as a reactive oxygen species (ROS) inducer and impairs tissues of diabetic rats such as kidney (22), lenses (23), neuron (21), and heart (24). In addition, we have recently demonstrated that glyoxal and MGO are more powerful inducers for large vascular endothelial inflammatory injury than AGEs and glucose itself (25, 26), suggesting that it may be involved in the pathogenesis of large vascular complications including atherosclerosis and hypertension. In fact, it was reported that MGO increased the risk of cardiovascular diseases in diabetic patients via increasing arterial atherogenicity (27). In addition, it was reported that MGO accumulated in aorta from SHR with aging and that the increased MGO accumulation in aorta correlated with increased blood pressure (18). Furthermore, there are reports demonstrating that treatment with MGO by drinking water not only increased blood pressure (28) but also caused salt-sensitive hypertension and insulin resistance in rats (29). Although accumulating evidence indicates that MGO could directly affect vascular reactivity in addition to inflammation (Fig. 1), there are only few reports available. To overcome this, our group has examined the effects of MGO on vascular reactivity (contraction and relaxation) and explored underlying mechanisms. In this review article, we summarized our recent findings. It should be noted that some of the preliminary unreviewed results are included in this manuscript.

2. Short-term effects of MGO on vascular reactivity of isolated blood vessels (in vitro study)

First, we examined effects of short-term MGO treatment (30 min) on contractility of isolated blood vessels. Treatment of endothelium-intact [E (+)] aorta with MGO did not change the concentration–response curve for noradrenaline (NA). On the other hand, treatment of endothelium-denuded [E (−)] aorta with MGO shifted the concentration–response curve for NA to the right (Fig. 2: A, B). The inhibitory effect was MGO concentration–dependent. Indomethacin, a cyclooxygenase inhibitor, or cimetidine, a histamine H_2-receptor blocker was not able to prevent the inhibitory effect of MGO. However, a non-selective K^+-channel inhibitor, tetramethylammonium, prevented the MGO-mediated inhibition of NA-induced contraction. Glibenclamide, an ATP-sensitive K^+-channel inhibitor or apamin, a small conductance Ca^{2+}-activated K^+-channel inhibitor was ineffective. On the other hand, iberiotoxin (IbTx), a large conductance Ca^{2+}-activated K^+ (BK_{Ca})-channel inhibitor significantly prevented the inhibitory effect of MGO (Fig. 2C). MGO also inhibited the NA-induced contraction in E (–) isolated mesenteric artery. The results indicate that short-term MGO treatment has an inhibitory effect on contractility of isolated blood vessels, which is mediated via opening smooth muscle BK_{Ca} channel (30).

Second, we examined effects of short-term MGO treatment on both endothelium-dependent and -independent relaxations in rat isolated thoracic aorta. Treatment of E (+) aorta with MGO did not change acetylcholine (ACh)-induced endothelium-dependent relaxation. In

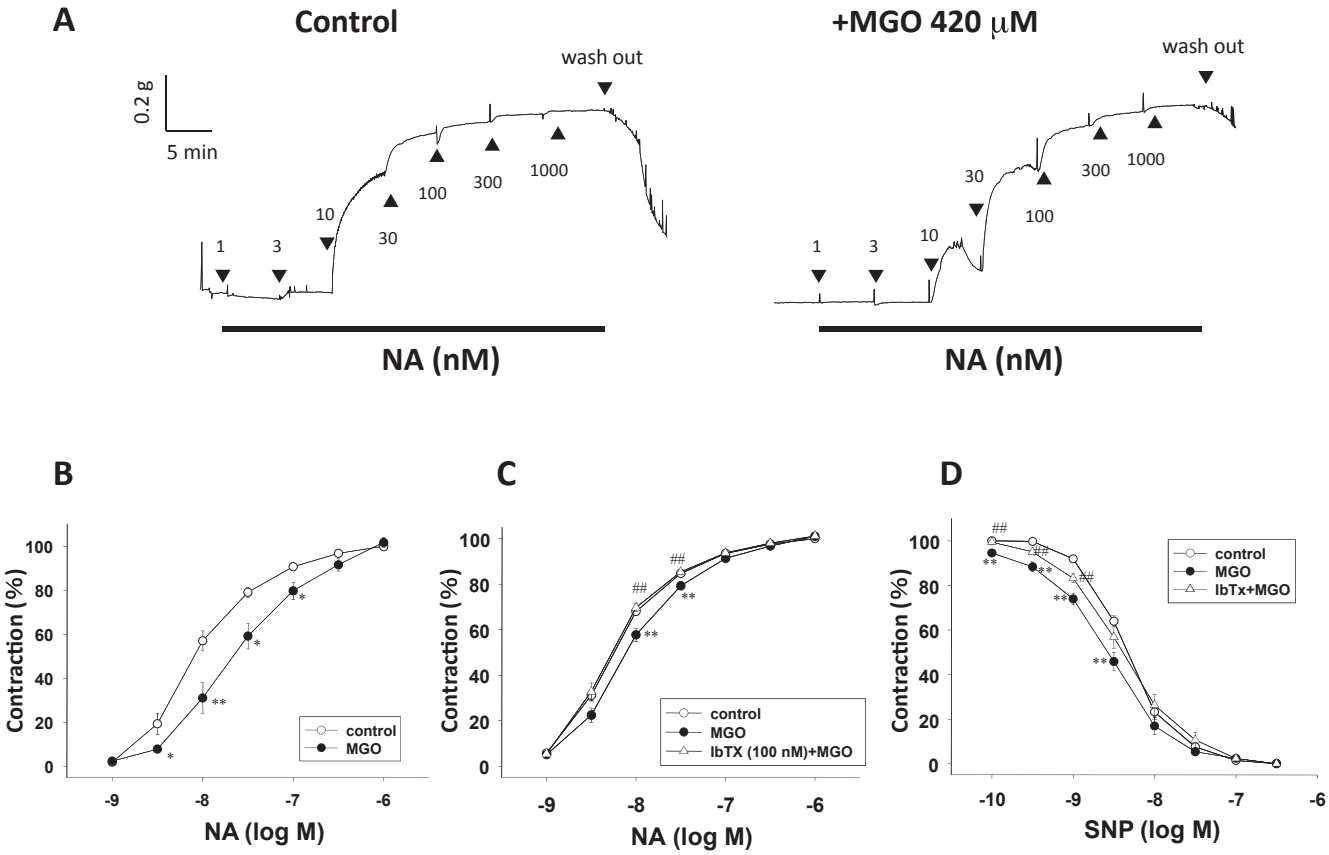

Fig. 2. Short-term effects of MGO on vascular reactivity of rat isolated aorta. A, B: Effects of pretreatment with MGO on noradrenaline (NA)-induced concentration-dependent contraction. A: NA (1 – 1000 nM) was cumulatively applied to endothelium-denuded rat aorta in the absence (control) or presence of MGO (420 μM, 30 min). B: Concentration–contraction relationships for NA in endothelium-denuded artery in the absence (control: open circle) or presence of MGO (MGO: closed circle). C: Concentration–contraction relationships for NA in endothelium-denuded rat aorta in the absence (control, open circle) or presence of MGO (420 μM, 30 min) pretreated without (MGO, closed circle) or with iberiotoxin (IbTX; 100 nM, 15 min, open triangle). 100% represents NA (1000 nM)-induced maximal contraction in control artery. D: Concentration–contraction relationships for sodium nitroprusside (SNP) in endothelium-denuded rat aorta in the absence (control: open circle) or presence of MGO pretreated without (MGO: closed circle) or with IbTx (100 nM, 15 min, open triangle). 100% represents NA (100 nM)-induced pre-contraction. Results were expressed as the mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, control vs. MGO; $^{\#\#}P < 0.01$, MGO vs. IbTx + MGO. The figures were rearranged from original publications (Ref. 30, 31) with permission.

contrast, treatment of E (−) aorta with MGO shifted the concentration–response curve for sodium nitroprusside (SNP) to the left. MGO increased ROS production in smooth muscle on analysis of protein carbonylation. However, tempol, a superoxide scavenger; catalase which metabolites hydrogen peroxide to water; or N^{G}-nitro-L-arginine methylester, a nitric oxide (NO) synthase inhibitor, had no effect on the MGO-induced enhancement of SNP-induced relaxation. On the other hand, IbTx prevented the effect of MGO on SNP-induced relaxation (Fig. 2D). Glibenclamide, apamin, or 4-aminopyridine, a voltage-gated potassium channel inhibitor was ineffective. The results indicate that short-term MGO treatment enhances SNP-induced relaxation in part by opening smooth muscle BK_{Ca} channel (31). We suppose that short-term MGO treatment may inhibit the ACh-induced endothelial NO production or alternatively enhance the endothelium-derived contracting factor such as superoxide. This may explain the discrepancy why ACh-induced relaxation was not enhanced even though NO sensitivity of smooth muscle was augmented by MGO.

It remains to be clarified whether MGO could affect vascular reactivity in isolated blood vessels other than aorta and mesenteric artery. Furthermore, effects of MGO on contractions induced by contractile agonists other than NA are not determined yet. Therefore, we examined effects of MGO on NA- and angiotensin (Ang) II-induced contraction as well as ACh-induced relaxation in rat isolated carotid artery. Short-term (30 min) treatment of E (−) carotid artery with MGO inhibited NA-

induced contraction similar to the results in aorta and mesenteric artery. Treatment of E (+) carotid artery with MGO did not change ACh-induced relaxation, which is also consistent with the results in aorta. In contrast, treatment of E (+) carotid artery with MGO augmented Ang II-induced concentration-dependent contraction (Fig. 3A). The effect was abolished by the removal of endothelium (Fig. 3B). BQ-123, an endothelin A-receptor blocker had no effect on the MGO-induced enhancement of Ang II-induced contraction. AL8810, a prostaglandin $F_{2\alpha}$-receptor blocker, or SQ29548, a thromboxane A_2-receptor blocker, was also ineffective. However, tempol or catalase significantly prevented the effect of MGO. Combined MGO and Ang II treatment increased ROS production as revealed by a fluorescence staining using a ROS-sensitive dye, 2′,7′-dichlorohydrofluroscin

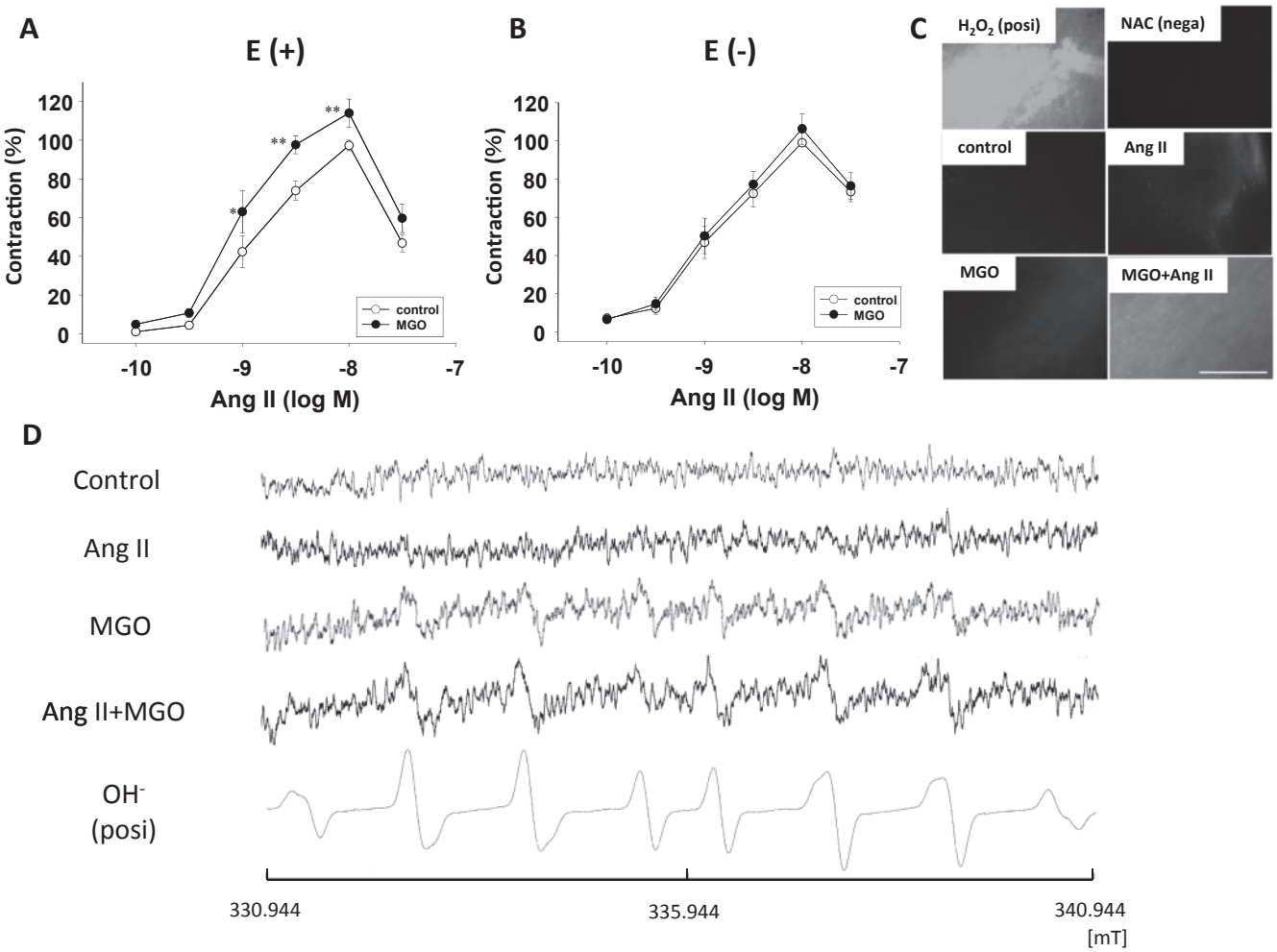

Fig. 3. Short-term effects of MGO on angiotensin (Ang) II-induced contraction of rat isolated carotid artery. A, B: Concentration–contraction relationships for Ang II in endothelium-intact [E (+), A] or -denuded [E (−), B] artery in the absence (control: open circle) or presence of MGO (MGO: closed circle). Results were expressed as the mean ± S.E.M. 100% represents Ang II (1 – 10 nM)-induced maximal contraction in control artery. *$P < 0.05$, **$P < 0.01$, control vs. MGO. C, D: Effects of MGO on Ang II-induced reactive oxygen species (ROS) production. C: ROS production in E (+) rat carotid artery was determined by a fluorescence staining using 2′,7′-dichlorohydrofluroscin diacetate (H_2DCFDA). After treatment with Ang II (3 nM, 5 min) in the absence or presence of MGO (420 μM, 30 min pretreatment) or with MGO alone, carotid arteries were loaded with H_2DCFDA (10 μM, 20 min). H_2O_2 (300 μM, 15 min) and *N*-acetyl-L-cysteine (NAC, 10 mM, 30-min pretreatment before Ang II) were used as a positive and negative control, respectively. Images of the endothelial surface were obtained with a fluorescence microscope. Scale bar: 50 μm. D: Electron spin resonance spectra of 5-(2,2-dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline *N*-oxide (CYPMPO)-OH adduct obtained from the 10 mM CYPMPO–containing normal physiological salt solution collected after treatment of E (+) rat carotid artery with Ang II (10 nM, 5 min) in the absence or presence of MGO (420 μM, 30 min) or with MGO alone. The positive control (CYPMPO-OH adduct) was obtained by reacting H_2O_2 (600 μM) and $FeSO_4$ (600 μM). The figures were rearranged from the original publication (Ref. 33) with permission.

diacetate (H_2DCFDA) (Fig. 3C). The increased ROS production was further confirmed by a electron spin resonance technique using a 5-(2,2-dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline *N*-oxide (CYPMPO) (32) (Fig. 3D). Gp91ds-tat, an inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), or losartan, an Ang II type 1–receptor (AT1R) blocker prevented the effect of MGO. These results indicate that short-term MGO treatment augments Ang II-induced contraction by increasing AT1R-mediated NOX-derived ROS production in endothelium of rat carotid artery (33).

3. Long-term effects of MGO on vascular reactivity of isolated blood vessels as revealed by an organ culture technique

Because hypertension and diabetes-related vascular dysfunction are chronically developed diseases, we examined long-term effects of MGO using an organ culture technique of isolated rat mesenteric artery. Organ culture of blood vessels is a useful technique for investigating the long-term direct effects of drugs or physiological substances. This model has several advantages beyond the conventional in vivo or in vitro model including preservation of a differentiated cell function, easy handling of experimental conditions, and dissociation from complicated factors existing in vivo (34). In recent studies, we have successfully established an organ-culture technique for isolated rat mesenteric artery (35, 36). We demonstrated that 3-day organ-cultured rat mesenteric arteries in a serum-free condition preserved sufficient contractility and endothelium-dependent relaxing function to enable the analysis of long-term effects. Long-term (3 days) MGO treatment inhibited NA or KCl-induced contraction (Fig. 4A). The inhibitory effect was higher in endothelium-denuded than endothelium-intact artery. *N*-acetyl-L-cysteine (NAC) or gp91ds-tat prevented the inhibitory effect of MGO. MGO increased NOX-derived superoxide production as detected by a lucigenin assay. In the medial layer of arteries cultured with MGO, apoptotic morphological change was observed and NAC or gp91ds-tat prevented it (Fig. 4B). MGO significantly increased protein expression of a

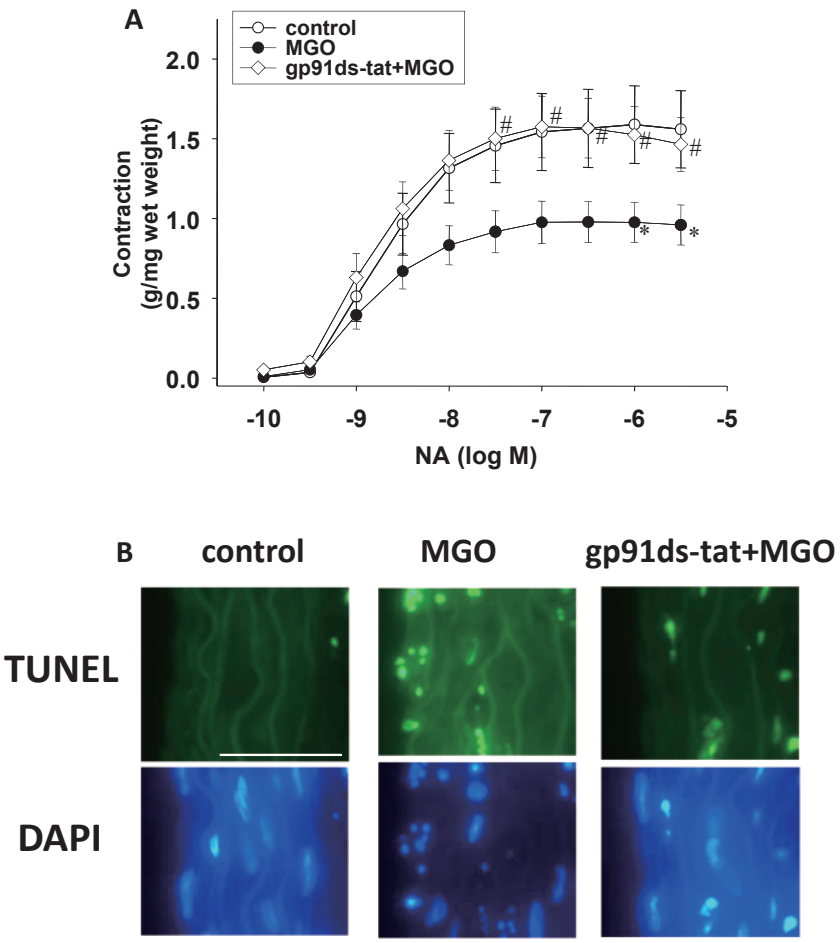

Fig. 4. Long-term effects of MGO on contractility of rat mesenteric artery as revealed by an organ culture technique. A: Concentration–contraction relationships for NA in E (−) rat mesenteric artery cultured for 3 days in the absence (control: open circle) or presence of MGO (42 μM) treated without (MGO: closed circle) or with gp91ds-tat (0.1 μM, open diamond). NA (0.1 nM – 3 μM) was cumulatively applied. Results were each expressed as the mean ± S.E.M. Contraction was expressed as an absolute value (g/mg wet weight). $^{*}P < 0.05$, control vs. MGO; $^{\#}P < 0.05$, MGO vs. gp91ds-tat + MGO. B: Representative photomicrographs of TdT-mediated dUTP nick end labeling (TUNEL) and 4′,6-diamidino-2-phenylindole (DAPI)-stained sections of E (−) rat mesenteric artery cultured in the absence (control) or presence of MGO (42 μM, 3 days) treated without or with gp91ds-tat (0.1 μM). Scale bar = 50 μm. The figures were rearranged from the original publication (Ref. 37).

homolog of gp91^{phox}, NOX1, but not NOX2. An NF-κB inhibitor, pyrrolidine dithiocarbamate, prevented the MGO-induced increased NOX1 expression. On the other hand, MGO had no effect on protein expression of the components of NOX including p22^{phox}, p67^{phox}, and p47^{phox} as well as superoxide dismutase (SOD)-1, SOD-2, and SOD-3. The results indicate that long-term MGO treatment has an inhibitory effect on contractility of isolated blood vessel, which is likely mediated via increased NOX1-derived superoxide production and subsequent apoptosis in smooth muscle (37).

Long-term effects of MGO on endothelial function were next examined using an organ culture technique. Long-term MGO treatment impaired ACh-induced endothelium-dependent relaxation in organ-cultured rat mesenteric artery. In contrast, long-term MGO treatment had no effect on SNP-induced endothelium-independent relaxation. MGO decreased the ACh-induced NO production as measured by a fluorescence NO indicator diaminofluorescein-2. MGO inhibited the ACh-induced phosphorylation of vasodilator stimulated phosphoprotein (an indicator of tissue cyclic GMP production). Long-term MGO treatment caused apoptotic morphological change as well as accumulation of superoxide in endothelium. MGO decreased the endothelial NO synthase (eNOS) protein expression. Gp91ds-tat prevented the impaired ACh-induced relaxation and the decrease in eNOS expression caused by a long-term MGO. The results indicate that long-term MGO treatment impairs endothelium-dependent relaxations through down-regulation of eNOS via NOX-derived increased superoxide-mediated endothelial apoptosis (Mukohda et al., preliminary observation, abstract in Ref. 39).

4. Effects of MGO accumulation in arterial walls on vascular reactivity (in vivo study)

As described above, we have determined the direct effects of MGO on vascular reactivity in vitro. However, it remains to be fully clarified how MGO affects vascular

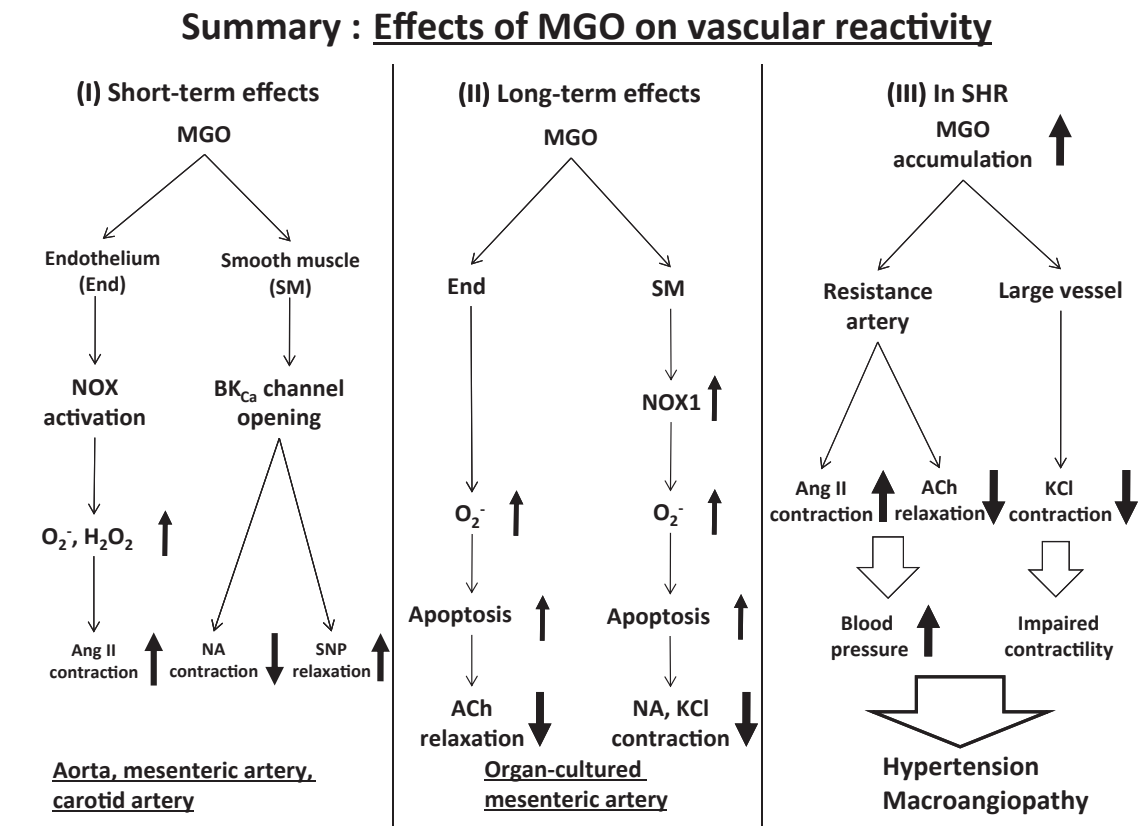

Fig. 5. Summary of the present results. The present results demonstrated that 1) short-term effects of MGO on arterial contractility are different dependent on the types of agonists: MGO inhibits NA-induced contraction, while it enhances Ang II-induced contraction and SNP-induced relaxation; 2) long-term MGO treatment impairs smooth muscle contractility and endothelium-dependent relaxation via inducing apoptosis in smooth muscle and endothelium, respectively, and 3) effects of MGO accumulation in arterial walls of spontaneous hypertensive rats (SHR) on vascular reactivity were similar to the direct effects of MGO on arterial contractility observed in the in vitro study.

reactivity in vivo. To explore it, we finally examined influences of MGO accumulation in arterial walls on vascular reactivity of isolated blood vessels by using SHR (18). Five-week-old SHR were treated with an MGO inhibitor, aminoguanidine (AG), for 5 weeks. AG significantly decreased the increased blood pressure in SHR, which is confirmative of the previous results by Wang et al. (38). In mesenteric artery and aorta from SHR, accumulation of MGO-derived AGEs, N^{ε}-caroxyethyl-lysine and argpyrimidine increased, which was prevented by an AG treatment. In mesenteric artery from SHR, Ang II-induced contraction largely increased, which was prevented by an AG treatment. In addition, ACh-induced endothelium-dependent relaxation was impaired in SHR, which was prevented by AG. Contrastingly, in aorta from SHR, KCl-induced contraction decreased, which was prevented by an AG treatment. ROS production increased in aorta from SHR, which was reversed by AG. NOX1 expression increased in aorta from SHR, which was reversed by AG. The results indicate that MGO accumulation in resistance artery mediates the development of hypertension in SHR likely via increasing Ang II-induced contraction and impairing endothelium-dependent relaxation. On the other hand, MGO accumulation in a large artery induces the impaired contractility likely via NOX1-derived increased ROS production. (Mukohda et al., preliminary observation, abstract in Ref. 40).

5. Conclusions and perspectives

The present results demonstrated that 1) short-term effects of MGO on arterial contractility are different depending on the types of agonists; MGO inhibits NA-induced contraction, while it enhances Ang II-induced contraction and SNP-induced relaxation; 2) long-term MGO treatment impairs smooth muscle contractility and endothelium-dependent relaxation via inducing apoptosis in smooth muscle and endothelium, respectively; 3) effects of MGO accumulation in arterial walls on vascular reactivity are similar to the direct effects of MGO on arterial contractility observed in the in vitro study (Fig. 5). Based on these results, we suggest that MGO would be associated with dysfunctions of vascular reactivity in diabetes-related hypertensive vascular diseases. Further studies on MGO may contribute to elucidate the pathogenesis of diabetes-related hypertensive vascular diseases and to develop pharmaceutical applications.

Acknowledgments

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