CHAPTER IV
RESULTS

4.1 Effect of rice bran peptide hydrolysates and curcumin on hemodynamic status, oxidative stress, endothelial dysfunction and vascular remodeling in 2K-1C hypertensive rats

4.1.1 RBP and CUR attenuates hypertension and hemodynamic disturbances in 2K-1C hypertensive rats

At the beginning of the experiments, baseline systolic blood pressure (SBP) and body weight were not different in all experimental groups (data not shown). SBP was significantly increased in 2K-1C rats after renal artery clipping for five days and continued to increase throughout the study period ($P < 0.05$, Figure 4.1A and B). When compared to untreated controls, RBP or CUR at 50 and 100 mg/kg/day attenuated the ride of the SBP in 2K-1C rats ($P < 0.05$, Figure 4.1A and B). With regard to the hemodynamic data, increased arterial blood pressure (systolic, diastolic and mean arterial blood pressure), decreased HBF and increased HVR were found in 2K-1C hypertensive rats, and this hemodynamic disturbance was significantly alleviated after RBP or CUR treatment ($P < 0.05$, Table 1). The hemodynamic status of the sham groups with or without RBP or CUR treatments were not different (Table 1), suggesting that RBP or CUR had no hypotensive effect on normotensive animals. Moreover, heart rates and body weight were not different among all groups throughout the duration of the experiment (Table 1).

4.1.2 RBP and CUR reduces oxidative stress and plasma ACE activity in 2K-1C hypertensive rats

Increased superoxide level was found in the arteries of 2K-1C rats when compared with sham groups ($P < 0.05$, Table 2). In parallel with these results, we found increased $p47^{phox}$ NADPH oxidase subunit in the aortas from the clipped animals ($P < 0.05$, Figure 4.2A and B), indicating increased ROS production was associated the increased expression of NOX enzyme in renovascular hypertension. Further confirming increased oxidative stress associated with hypertension, we found that 2K-1C rats had higher levels of plasma MDA and protein carbonyl than respective sham controls ($P < 0.05$, Table 2). Treatment with RBP or CUR

significantly attenuated superoxide production, plasma MDA and protein carbonyl levels in 2K-1C rats ($P < 0.05$, Table 2), and these deleterious effects were associated with a downregulation of the $p47^{phox}$ NADPH oxidase subunit (Figure 4.2A and B).

Plasma ACE levels were increased in 2K-1C rats compared to sham-operated animals (Table 2). Treatment with RBP 50 and 100 mg/kg or CUR 100 mg/kg significantly reduced the plasma ACE level in 2K-1C hypertensive rats ($P < 0.05$, Table 2). RBP or CUR did not affect plasma ACE activity in sham-operated controls.

4.1.3 RBP and CUR improve hypertension-induced endothelial dysfunction in 2K-1C hypertensive rats

To examine the effects of RBP or CUR on 2K-1C hypertension-induced endothelial dysfunction, in large (conducing) artery, the aortic rings were isolated and their vascular reactivity was assessed in organ bath experiments. Figure 4.3 and Figure 4.4 show endothelially-dependent and -independent vasorelaxation induced by ACh and SNP (dose range: 10^{-9}–10^{-5} M) in the thoracic aortic rings. A significant impairment of the vascular response to ACh was found in the aortic rings of 2K-1C rats when compared with those isolated from the sham group ($P < 0.05$, Figure 4.3A and 4.4A). Moreover, the study in small (resistant) artery, the mesenteric artery beds were also isolated to evaluate vascular reactivity in organ bath experiments. Figure 4.5 and Figure 4.6 shows vasorelaxation induced by ACh (dose range: 10^{-9}–10^{-4} M) in mesenteric arteries. A significant reduction of the vascular response to ACh was also found in mesenteric arteries of 2K-1C rats when compared with those isolated from the sham group ($P < 0.05$, Figure 4.5A and 4.6A). These results reflect endothelial dysfunction in 2K-1C hypertension. Results in both aortas and mesenteric vascular beds showed that, treatment with RBP or CUR significantly enhanced endothelial-dependent vasorelaxation induced by ACh ($P < 0.05$, Figure 4.3A - 4.6A), but had no effect on the endothelial-independent response induced by SNP (Figure 4.3B - 4.6B). Effect of CUR was reduced to normalize the response better than RBP. In contrast, the vasorelaxant responses to SNP were not different among the groups (Figure 4.3B and 4.4B) and mesenteric arteries (Figure 4.5B and 4.6B).

The impairment of endothelial vasorelaxation was associated with a reduction of plasma nitrate/nitrite concentration ($P < 0.05$, Table 2) and a down-regulation of eNOS protein expression in the aortas of 2K-1C hypertensive rats ($P < 0.05$, Figure 4.5A and B). The improvement of endothelial dysfunction in 2K-1C rats treated with RBP or CUR was associated with increased nitrate/nitrite levels ($P < 0.05$, Table 2) and also upregulated eNOS expressions ($P < 0.05$, Figure 4.7A and B).

(A) **(B)**

Figure 4.1 Effects of RBP (A) and CUR (B) on systolic blood pressure before and after renal artery clipping measured by tail-cuff plethysmography in 2K-1C hypertensive rats. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. In this and all subsequent plots, data are means ± SEM. (n=10/group). *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group and ‡$P < 0.05$ versus 2K-1C+RBP50 or 2K-1C+CUR50 group.

Table 1 Effects of RBP and CUR on hemodynamic status in 2K-1C hypertensive rats.

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; body weight, BW; systolic blood pressure, SBP; diastolic blood pressure, DBP; mean arterial pressure, MAP; heart rate, HR; hindlimb blood flow, HBF; hindlimb vascular resistance, HVR. All hemodynamic variables refer to measurements made on anaesthetized animals 6 weeks after the start of the treatment period. Data are means ± SEM. (n=10/group), *$P < 0.05$ versus sham-operated group, $^{\dagger}P < 0.05$ versus 2K-1C group, ‡$P < 0.05$ versus 2K-1C+RBP50 or 2K-1C+CUR50 group.

Table 2 Effects of RBP and CUR on the levels of oxidative stress markers, nitrate/nitrite and ACE activity in 2K-1C hypertensive rats.

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Malondialdehyde, MDA; Angiotensin converting enzyme, ACE.

Data are means ± SEM. (n=10/group), *$P < 0.05$ versus sham-operated group, $^{\dagger}P < 0.05$ versus 2K-1C group, ‡$P < 0.05$ versus 2K-1C+RBP50 or 2K-1C+CUR50 group.

(A) **(B)**

Figure 4.2 Effects of RBP (A) and CUR (B) on p47^{phox} NADPH oxidase subunit expression in the 2K-1C rat aortas. The bars representative expression of p47^{phox} normalized with β-actin and data are means ± SEM. (n=4/group), *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group.

Figure 4.3 Effect of RBP on endothelial-dependent vasorelaxation in aortic rings. Vasorelaxation was induced by acetylcholine (A) and endothelial-independent relaxation induced by sodium nitroprusside (B) in aortic rings pre-contracted with phenylephrine (1 μM) of 2K-1C hypertensive rats. Data are means ± SEM. (n=6/group), *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group.

Figure 4.4 Effect of CUR on endothelial-dependent vasorelaxation in aortic rings. Vasorelaxation was induced by acetylcholine (A) and endothelial-independent relaxation induced by sodium nitroprusside (B) in aortic rings pre-contracted with phenylephrine (1 μM) of 2K-1C hypertensive rats. Data are means ± SEM. (n=6/group), *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group and ‡$P < 0.05$ versus 2K-1C+CUR50 group.

Figure 4.5 Effect of RBP on endothelial-dependent vasorelaxation in mesenteric artery beds. Vasorelaxation was induced by acetylcholine (A) and endothelial-independent relaxation induced by sodium nitroprusside (B) in mesenteric artery beds pre-contracted with methoxamine (5μM) of 2K-1C hypertensive rats. Data are means ± SEM. (n=6/group), **P* < 0.05 versus sham-operated group, †*P* < 0.05 versus 2K-1Cgroup.

Figure 4.6 Effect of CUR on endothelial-dependent vasorelaxation in mesenteric artery beds. Vasorelaxation was induced by acetylcholine (A) and endothelial-independent relaxation induced by sodium nitroprusside (B) in mesenteric artery beds pre-contracted with methoxamine (5 μM) of 2K-1C hypertensive rats. Data are means ± SEM. (n=6/group), **P* < 0.05 versus sham-operated group, †*P* < 0.05 versus 2K-1Cgroup.

(A) **(B)**

Figure 4.7 Effects of (A) RBP and CUR (B) on eNOS protein expression in the aortas of 2K-1C hypertensive rats. The bars representative expression of eNOS normalized with β-actin and data are means ± SEM. (n=6/group), *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group, ‡ $P < 0.05$ versus 2K-1C + RBP 50 group.

4.1.4 RBP and CUR prevented hypertension-induced vascular structural changes and remodeling in thoracic aortas of 2K-1C hypertensive rats

2K-1C renovascular hypertension induced with arterial wall hypertrophy, with significant increase in the thoracic aortic wall thickness, medial cross sectional area (CSA) and media/ lumen (M/L) ratio after 6 weeks of hypertension compared to sham operated animals ($P < 0.001$, Table 3).However, there was no significant difference in the lumen areas (data not shown) between the 2K-1C and the sham groups (Table 3). Cell counting showed an increased number of VSMCs per medial CSA, suggesting medial hyperplasia in the 2K-1C group compared with the sham group ($P < 0.05$, Table 3). In addition, a significant increase in the number of VSMCs and the relative amounts of SMA, collagen and elastin in the aortic wall of 2K-1C rats was also seen ($P < 0.05$,Table 3). Treatment with RBP or CUR prevented the morphological changes of the aortic wall seen in 2K-1C hypertensive rats ($P < 0.05$, Table 3) although only at the high dose did it significantly prevent the increase in SMA, elastin and collagen associated with the 2K-1C treatment. It was found that the contents of SMA, collagen and elastin in the aortic media of sham controls were unaltered by vehicle, RBP, or CUR treatment.

MMP-2 and MMP-9 enzymes are involved in the vascular remodeling in hypertension. The prevention effect of RBP and CUR were attenuated by immunohistochemistry attaining of MMP-2 and MMP-9 in aortas (Table 4 and Figure 4.8A and B).

Representative immunohistochemistry photomicrographs showing MMP-2 and MMP-9 staining in the aortas are seen in Table 4 and Figure 4.8A and B. We found higher MMP-2 ($P < 0.05$, Table 4 and Figure 4.8A) and MMP-9 ($P < 0.05$, Table 4 and Figure 4.8B) levels in the aortas of 2K-1C hypertensive rats compared with sham controls. RBP or CUR, especially at high dose, significantly attenuated the 2K-1C hypertension-induced increase in MMP-2 and MMP-9 levels in the aortic walls, whereas no changes in the MMP levels were observed in the sham + RBP group or sham + CUR group (Table 4 and Figure 4.8A and B).

4.1.5 RBP and CUR improve hypertension-induced vascular structural changes and remodeling in mesenteric arteries of 2K-1C hypertensive rats

A significant increase in CSA and M/L ratio was observed, in the mesenteric arteries of 2K-1C hypertensive rats, confirming the eutrophic nature of small artery remodeling in a rat model of 2K-1C renovascular hypertension (Arribas *et al.*, 2006). The media wall of mesenteric arteries of the 2K-1C hypertensive rats was thickening with a significant increase in the number of VSMCs/CSA, SMA, collagen and elastin contents compared with those from sham control ($P < 0.05$, Table 5 and 6). Supplementation of RBP or CUR prevented the morphological changes of small artery in the 2K-1C rats ($P < 0.05$, Table 5). It was apparent that the contents of SMA, collagen and elastin in the mesenteric media of sham controls were unchanged by vehicle, RBP, or CUR treatment.

MMP-2 and MMP-9 were highly expressed in the mesenteric media of 2K-1C rats as compared with rats in the sham group ($P< 0.05$, Table 6 and Figure 4.9A and B). Interestingly, treatment with RBP or CUR significantly reduced the 2K-1C hypertension-induced increase in MMP-2 and MMP-9 expressions in the mesenteric arteries. There were no changes in the MMP levels in sham control, sham + RBP and sham + CUR treated groups (Table 6 and Figure 4.9A and B).

Table 3 Effects of RBP and CUR on the structural modifications in the thoracic aortas of 2K-1C hypertensive rats.

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Cross sectional area, CSA; Media to lumen ratio, M/L ratio; Smooth muscle actin, SMA. Data are means ± SEM. (n=5-6/group), **P* < 0.05 versus sham-operated group, †*P* < 0.05 versus 2K-1C group, and ‡*P* <0.05 versus or 2K-1C+RBP50 groups 2K-1C+CUR50.

Table 4 Effects of RBP and CUR on MMP-2 and MMP-9 localization in the thoracic aortas of 2K-1C rats.

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; matrix metalloproteinase-2, MMP-2; matrix metalloproteinase-9, MMP-9. Data are means ± SEM. (n=5-6/group), ***P* < 0.05 versus sham-operated group, †*P* < 0.05 versus 2K-1C group, and ‡*P* <0.05 versus 2K-1C+RBP50or 2K-1C+CUR50 groups.

(A) MMP-2

(B) MMP-9

Figure 4.8 Representative immunohistochemistry photomicrographs (200X) showing the effects of RBP and CUR on MMP-2 (panel A) and MMP-9 (panel B) localization in the thoracic aortas of all experimental groups.

Table 5 Effects of RBP and CUR on the structural modifications in the mesenteric arteries of 2K-1C hypertensive rats.

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Cross-sectional area, CSA; Media to lumen ratio, M/L ratio; Smooth muscle actin, SMA. Data are means ± SEM. (n=5-6/group), *$P < 0.05$ versus sham-operated group, †$P < 0.05$ versus 2K-1C group, and ‡$P <0.05$ versus 2K-1C+RBP50 group or 2K-1C+CUR50 group.

Table 6 Effects of RBP and CUR on MMP-2 and MMP-9 localization in the mesenteric arteries of 2K-1C hypertensive rats**.**

Rats were induced hypertension by 2K-1C model. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; matrix metalloproteinase-2, MMP-2; matrix metalloproteinase-9, MMP-9. Data are means ± SEM. (n=5-6/group), **P* < 0.05 versus sham-operated group, †*P* < 0.05 versus 2K-1C group, and ‡*P* <0.05 versus 2K-1C + RBP50 group or 2K-1C + CUR50 group.

(A) MMP-2

(B) MMP-9

Figure 4.9 Representative immunohistochemistry photomicrographs (400X) showing the effects of RBP and CUR on MMP-2 (panel A) and MMP-9 (panel B) localization in the mesenteric arteries of 2K-1C hypertensive rats**.**

4.2 Effects of rice bran peptide hydrolysates and curcumin on hemodynamic status, oxidative stress, endothelial dysfunction and vascular remodeling in L-NAME hypertensive rats

4.2.1 RBP and CUR attenuate hypertension and hemodynamic disturbances in L-NAME hypertensive rats

At the beginning of the experiments there were no differences in SBP among experimental groups (Figure 4.10). SBP progressively increased in L-NAME hypertensive rats throughout the study period of 3 weeks. A daily supplementation of RBP or CUR (50 or 100 mg/kg) showed a significant reduction in SBP (P<0.05, Figure 4.10A and B), however, the blood pressure remained above the normal control levels until the end of experiment. There was no change in SBP in normal control and normal control treated with RBP or CUR groups.

At the end of experimental period, arterial blood pressure measured directly by arterial catheterization was assessed. A marked increased in SBP, DBP and MAP were observed in L-NAME rats (p<0.05, Table 7). The increase in arterial blood pressure was accompanied by decrease of HBF and increase of HVR in these animals. RBP or CUR treatment significantly attenuated arterial blood pressure and prevented hemodynamic disturbances in L-NAME hypertensive rats (p<0.05, Table 7). The effect of RBP is found in a dose-dependent manner (Table 7). There were no significant differences in arterial blood pressure and hemodynamic parameters in normal control treated with RBP or CUR, suggesting that RBP or CUR does not possess hypotensive effect in normotensive rats (Table 7).

4.2.2 RBP and CUR reduce oxidative stress and plasma ACE activity in L-NAME hypertensive rats

Superoxide production in the vascular tissue was significantly elevated in L-NAME rats compared with normal controls (P < 0.05, Table 8). It was associated with an increase of $p47^{phox}$ NADPH oxidase subunit in the aortas of the L-NAME treated animals (P < 0.05, Figure 4.11A and B), suggesting the source of ROS production in L-NAME hypertension. Further confirming increased oxidative stress associated with hypertension, we found that L-NAME had higher levels of plasma MDA and protein carbonyl than respective normal controls (P < 0.05, Table 8). Treatment with RBP or CUR significantly attenuated superoxide production, plasma

MDA and protein carbonyl levels in L-NAME treated rats ($P < 0.05$, Table 2), and these deleterious effects were associated with a downregulation of the $p47^{phox}$ NADPH oxidase subunit (Figure 4.11A and B).

The ACE activity was measured in plasma of normal control and L-NAME-treated animals. ACE activity in plasma of L-NAME rats was higher than that of normal control rats ($P < 0.05$, Table 8). Treatment with RBP or CUR significantly reduced the plasma ACE level in L-NAME hypertensive rats ($P < 0.05$, Table 8), whereas there was no change in plasma ACE activity in normal controls.

4.2.3 RBP and CUR improve hypertension-induced endothelial dysfunction in L-NAME hypertensive rats

To examine the effect of RBP or CUR on L-NAME hypertension-induced endothelial dysfunction, aortic rings and mesenteric vascular bed were isolated and their vascular reactivity was assessed in organ bath experiments. A significant attenuation of endothelium-dependent vasorelaxation in response to ACh was found in the aortic ring and mesenteric vascular beds of L-NAME hypertensive rats when compared with those isolated from normal control rats ($P < 0.05$, Figure 4.12-4.15). Treatment with RBP or CUR improved ACh-induced vasorelaxation, whereas endothelial-independent vasorelaxation to SNP was comparable in both aortic rings and mesenteric vascular beds obtained from L-NAME hypertensive and normotensive rats **(**Figure 4.12-4.15).

The impairment of endothelial vasorelaxation in L-NAME-treated rats is conforming by a decrease in plasma nitrite/ nitrate level ($P < 0.05$, Table 8) and a suppression of eNOS protein expression in the aortas ($P< 0.05$, Figure 4.16 A and B). RBP or CUR dose-dependently improve endothelial function of L-NAME hypertension rats as shown by an increase in plasma nitrate/nitrite levels ($P < 0.05$, Table 8) and upregulation of eNOS expressions ($P < 0.05$, Figure 4.16A and B).

(A) **(B)**

Figure 4.10 Effects of RBP (A) and CUR (B) on systolic blood pressure measured by tail-cuff plethysmography method throughout the experimental period in L-NAME hypertensive rats. In this and all subsequent plots, data are means ± SEM. (n=8/group). *$P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group.

Table 7 Effects of RBP and CUR on hemodynamic status in L-NAME hypertensive rats.

Rats were induced hypertension by L-NAME. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Body weight, BW; Systolic blood pressure, SBP; Diastolic blood pressure, DBP; Mean arterial pressure, MAP; Heart rate, HR; Hindlimb blood flow, HBF; Hindlimb vascular resistance, HVR. All hemodynamic variables refer to measurements made on anaesthetized animals 6 weeks after the start of the treatment period. Data are means ± SEM. (n=8-10/group), ***P* < 0.05 versus normal control group, †*P* < 0.05 versus L-NAME group and ‡*P* < 0.05 versus L-NAME +RBP50 group or L-NAME +CUR50 group.

Table 8 Effects of RBP and CUR on the levels of oxidative stress markers, nitrate/nitrite and ACE activity in L-NAME hypertensive rats.

Rats were induced hypertension by L-NAME. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Malondialdehyde, MDA; Angiotensin converting enzyme, ACE. Data are means ± SEM. (n=6-8/group), *$P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group and ‡$P < 0.05$ versus L-NAME +RBP50 group or L-NAME +CUR50 group.

(A) (B)

Figure 4.11 Effects of RBP (A) and CUR (B) on $p47^{phox}$ protein expression in the aortas of L-NAME hypertensive rats. The bars representative of $p47^{phox}$ normalized with β-actin and data are means ± SEM. (n=6/group), $*P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group, ‡ $P < 0.05$ versus L-NAME + RBP 50 group or L-NAME + CUR50 group.

Figure 4.12 Effect of RBP on endothelial-dependent vasorelaxation in aortic rings. Vasorelaxation was induced by acetylcholine (**A**) and endothelial-independent relaxation induced by sodium nitroprusside (B) in aortic rings pre-contracted with phenylephrine (1 μM) of L-NAME hypertensive rats. Rice bran peptides, RBP. *$P < 0.05$ versus control group, †$P < 0.05$ versus L-NAME group and ‡$P < 0.05$ versus L-NAME+RBP50 group.

Figure 4.13 Effect of CUR on endothelial-dependent vasorelaxation in aortic rings. Vasorelaxation was induced by acetylcholine (**A**) and endothelial-independent relaxation induced by sodium nitroprusside (B) in aortic rings pre-contracted with phenylephrine (1 μM) of L-NAME hypertensive rats. Curcumin, CUR. *$P < 0.05$ versus control group, †$P < 0.05$ versus L-NAME group.

Figure 4.14 Effect of RBP on endothelial-dependent vasorelaxation in mesenteric artery beds. Vasorelaxation was induced by acetylcholine (**A**) and endothelial-independent relaxation induced by sodium nitroprusside (B) in mesenteric artery beds pre-contracted with methoxamine (5μM) of L-NAME hypertensive rats. Rice bran peptides, RBP. *$P < 0.05$ versus control group, †$P < 0.05$ versus L-NAME group.

Figure 4.15 Effect of CUR on endothelial-dependent vasorelaxation in mesenteric artery beds. Vasorelaxation was induced by acetylcholine (**A**) and endothelial-independent relaxation induced by sodium nitroprusside (B) in mesenteric artery beds pre-contracted with methoxamine (5μM) of L-NAME hypertensive rats. Curcumin, CUR. *$P < 0.05$ versus control group, †$P < 0.05$ versus L-NAME group.

(A) **(B)**

Figure 4.16 Effect of RBP (A) and CUR (B) on eNOS protein expression in the aortas of L-NAME hypertensive rats. The bars representative expression of eNOS normalized with β-actin and data are means ± SEM. (n=6/group),
*$P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group, ‡ $P < 0.05$ versus L-NAME + RBP50 group or L-NAME + CUR50 group.

4.2.4 RBP and CUR improve hypertension-induced vascular structural changes and remodeling in thoracic aortas of L-NAME hypertensive rats

Three weeks of L-NAME-induced hypertension caused a significant increase in the thoracic aortic wall thickness, CSA and M/L ratio compared to normotensive animals ($P < 0.05$, Table 9). A significant increase in the number of VSMCs and the relative amounts of SMA and collagen in the aortic wall of L-NAME rats was found ($P < 0.05$, Table 9). However, the relative content of elastin was significantly decreased in L-NAME hypertensive animals ($P < 0.05$). RBP or CUR reduced the aortic wall hypertrophy and attenuated the increase in VSMCs, SMA and collagen in L-NAME hypertensive rats. An increase in elastic content in the aortic wall of L-NAME rats was also found after RBP or CUR treatment ($P < 0.05$, Table 9). Administration of vehicle, RBP, or CUR to the normotensive rats did not cause any significant changes in the contents of SMA, collagen and elastin in the aortic wall.

Representative immunohistochemistry photomicrographs showing MMP-2 and MMP-9 staining in the aortas are seen in Table 11 and Figure 4.17A and B. We found higher MMP-2 ($P < 0.05$, Table 10 and Figure 4.17A) and MMP-9 ($P < 0.05$, Table 10 and Figure 4.17B) levels in the aortas of L-NAME-induced hypertensive rats compared with normal controls. Treatment with RBP or CUR was significantly decreased in MMP-2 and MMP-9 levels in the aortic walls, whereas no changes in the MMP levels in the normal control treated with vehicle, RBP, or CUR (Table 10 and Figure 4.17A and B).

Table 9 Effects of RBP and CUR on the structural modifications in the thoracic aortas of L-NAME hypertensive rats.

Rats were induced hypertension by L-NAME. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Cross-sectional area, CSA; Media to lumen ratio, M/L ratio; Smooth muscle actin, SMA. Data are means ± SEM. (n=5-6/group), *****$P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group, and ‡$P < 0.05$ versus L-NAME+RBP50 group or L-NAME+CUR50 group.

Table 10 Effects of RBP and CUR on MMP-2 and MMP-9 localization in the thoracic aortas of L-NAME hypertensive rats.

Rice bran peptides, RBP; Curcumin, CUR; Matrix metalloproteinase-2, MMP-2; Matrix metalloproteinase-9, MMP-9. Data are means ± SEM. (n=5-6/group), *****$P < 0.05$ versus normal control group, †$P < 0.05$ versus L-NAME group, and ‡$P < 0.05$ versus L-NAME+RBP50 group.

(A) MMP-2 **(B) MMP-9**

Figure 4.17 Representative immunohistochemistry photomicrographs (200X) showing the effects of RBP and CUR on MMP-2 (panel A) and MMP-9 (panel B) localization in the thoracic aortas of L-NAME hypertensive rats**.**

4.2.5 RBP and CUR improve hypertension-induced vascular structural changes and remodeling in mesenteric arteries of L-NAME hypertensive rats

A significant increase in CSA and M/L ratio was observed in the mesenteric arteries of L-NAME hypertensive rats. The medial wall of mesenteric arteries of L-NAME-treated rats was thickening with a significant increase in the number of VSMCs/CSA, SMA and collagen contents as compared to those from normal controls ($P < 0.05$, Table 11). The elastin content was also decreased in L-NAME hypertensive rats. Treatment with RBP or CUR prevented the morphological changes of the mesenteric arteries of L-NAME hypertensive animals ($P < 0.05$, Table 11). It was found that the contents of SMA, collagen and elastin in the mesenteric media of normal controls were unchanged by treatment with vehicle, RBP or CUR treatment.

MMP-2 and MMP-9 were highly expressed in the mesenteric media of L-NAME rats when compared with normotensive rats ($P< 0.05$, Table 12 and Figure 4.18A and B), and treatment of RBP or CUR significantly decreased the MMP-2 and MMP-9 expressions in the mesenteric arteries of hypertensive rats. There were no changes in the MMP levels in normal controls treated with vehicle, RBP or CUR (Table 12 and Figure 4.18A and B).

Table 11 Effects of RBP and CUR on the structural modifications in the mesenteric arteries of L-NAME hypertensive rats.

Rats were induced hypertension by L-NAME. They were treated with RBP50, RBP100 (50 or 100 mg/kg/day) or CUR50 or CUR100 (50 or 100 mg/kg/day), respectively. Rice bran peptides, RBP; Curcumin, CUR; Cross sectional area, CSA; Media to lumen ratio, M/L ratio; Smooth muscle actin, SMA. Data are means ± SEM. (n=5-6/group), **P* < 0.05 versus normal control group, †*P* < 0.05 versus L-NAME group, and ‡*P* <0.05 versus L-NAME+CUR50 group.

Table 12 Effects of RBP and CUR on MMP-2 and MMP-9 localization in the mesenteric arteries of L-NAME hypertensive rats.

Rice bran peptides, RBP; Curcumin, CUR; Matrix metalloproteinase-2, MMP-2; Matrix metalloproteinase-9, MMP-9. Data are means ± SEM. (n=5-6/group), *****P < 0.05 versus normal control group, †P < 0.05 versus L-NAME group, and ‡P <0.05 versus L-NAME+RBP50 group.

(A) MMP-2 **(B) MMP-9**

Figure 4.18 Representative immunohistochemistry photomicrographs (400X) showing the effects of RBP and CUR on MMP-2 (panel A) and MMP-9 (panel B) localization in the mesenteric arteries of L-NAME hypertensive rats**.**