CHAPTER V
DISCUSSION

The present study investigated the preventive effects of RBP and CUR on alterations of hemodynamic status, endothelial function, oxidative stress and vascular remodeling in models of 2K-1C and L-NAME-induced hypertensions in rats.

The main findings of this study are that treatment with RBP or CUR blunts the increase in blood pressure and improves endothelial dysfunction and vascular remodeling in 2K-1C and L-NAME hypertensive rats. These beneficial effects of RBP or CUR may contribute to the improvement of hemodynamic status, attenuation of vascular structural alterations, reduction in ACE and MMP levels, alleviation of oxidative stress, and increase in NO bioavailability. In agreement with previous observations (Costa *et al.*, 2009; Martinez *et al.*, 2008; Priviero *et al.*, 2007), we found increased peripheral vascular resistance and reduced endothelium dependent vasorelaxation-induced by ACh in 2K-1C and L-NAME hypertensive rats. These effects were evidently associated with a reduction in blood flow and suppression of eNOS expression, confirming the presence of endothelial dysfunction and reduced NO bioavailability in these hypertensive models.

It is evident that the contribution of the endothelium to the vascular tone regulation is distinct among different vascular beds. In the large artery, like aorta, ACh-induced relaxation is predominantly mediated by NO, whereas in the mesenteric artery, beside NO, EDHF plays a crucial role to ACh-induced relaxation response (Bolz *et al.*, 1999; Nagao *et al.*, 1992). Our data has shown that, clipping renal artery or L-NAME administration to rats leads to a decrease in ACh-induced relaxation in both aortas and mesenteric artery beds, whereas the relaxation responses to NO donor SNP were not alter in all experimental groups, indicating the involvement of endothelium-dependent vasorelaxation in the aorta and mesenteric artery (Chang *et al.*, 2002; Marin *et al.*, 2000). Interestingly, RBP and CUR treatment significantly prevented the impairment of ACh-induced vasorelaxation in both preparations isolated from 2K-1C and L-NAME hypertensive rats. It is therefore conceivable that the enhanced vasorelaxation effects of RBP and CUR in these two models of

hypertension are endothelium-dependent and act via the NO-mediated relaxation system. However, we have not conducted a study to confirm the pathways of RBP and CUR treatment interferes with hyperpolarization of the mesenteric artery smooth muscle cells, and additional studies would be required.

Endothelial dysfunction is often associated with pronounced oxidative stress that is due to, at least in part, increased $O_2^{\bullet-}$from NADPH oxidase and increased degradation of NO by reaction with $O_2^{\bullet-}$, thereby, reducing its bioavailability (Paravicini and Touyz, 2008; Sarr *et al.*, 2006). Thus, eNOS becomes uncoupled, causing $O_2^{\bullet-}$ generation rather than NO production. NADPH oxidase is a multi-subunit enzymatic complex which has been shown to be one of the main sources of $O_2^{\bullet-}$in the vascular wall (Ulker *et al.*, 2003). Upregulation of this oxidase, in particular p47phox contributes to the pathogenesis of oxidative stress in several animal models of hypertension (Litterio *et al.*, 2012; Sanchez *et al.*, 2006; Sarr *et al.*, 2006). In this study, increased $O_2^{\bullet-}$production was associated with increased p47phox expression in the vascular wall of 2K-1C and L-NAME rats. Moreover, increased oxidative stress as indicated by enhanced MDA and protein carbonyl levels was also found in both 2K-1C and L-NAME hypertension. Therefore, reduced NO bioavailability and enhanced oxidative stress in these hypertensive models lead to endothelial dysfunction thereby contributing to less vasodilation, and increased vascular resistance in hypertension.

Several studies have been shown the beneficial properties of rice bran extracts in cardiovascular risk factors for example dyslipidemia, metabolic syndrome and hypertension (Ardiansyah *et al.*, 2007; Justo *et al.*, 2013; Kaup *et al.*, 2013). In addition, It has been reported that polyphenols present in fruits and vegetables are able to modulate the production of NO in vascular endothelium, contributing to the prevention of endothelial dysfunction (Curin and Andriantsitohaina, 2005). In this study we found that, RBP and CUR restored hemodynamic status, enhanced ACh-induced vasodilation, upregulated eNOS expression and increased nitrate/nitrite concentration in 2K-1C and L-NAME hypertensive rats. Therefore, our findings suggest the beneficial effects of RBP and CUR on the improvement of vascular dysfunction and alleviation of oxidative stress in 2K-1C and L-NAME hypertensive rats. Further support for this suggestion comes from the observation that RBP and

CUR treatments inhibit the increases in MDA and protein carbonyl levels, enhanced $O_2^{\bullet-}$ production and p47phox expression in the arteries of 2K-1C and L-NAME hypertensive rats. Recent studies of our group have evidenced the beneficial effect of CUR in animal models of hypertension (Boonla *et al.*, 2014; Nakmareong *et al.*, 2011). Besides, it has been reported that long-term treatment with rice bran enzymatic extract restored endothelial function in obese rats by reducing vascular NADPH oxidase expression and increasing aortic eNOS expression (Justo *et al.*, 2013).

Hypertension is strongly implicated in the progression of functional and structural alterations in the vascular system and vascular remodeling is an adaptive response to elevation of BP. In hypertension, large artery remodeling is characterized by an increase in M/L ratio and CSA, the change is identified as hypertrophic remodeling, while resistance artery (diameter < 300 μm) remodeling is eutropic and/or hypertrophic remodeling. Inward eutrophic remodeling associated with a reduced lumen and increased M/L ratio but without CSA increase (Mulvany *et al.*, 1996). As we have found in this study, the process of vascular remodeling leads to increased arterial wall thickness which appears to be due to changes in VSMCs and ECM components (Arribas *et al.*, 2006; Castro *et al.*, 2008). Consistent with this idea, we observed arterial wall hypertrophy, increased collagen and elastin deposition, VSMC hyperplasia and hypertrophy (as suggested by the increased area staining-intensity product) in the aortae and mesenteric arteries of 2K-1C and L-NAME hypertensive rats, and that these alterations are reversed by dietary supplementation with RBP or CUR. Since the lumen area of the aortas of 2K-1C and L-NAME rats did not alter, this suggests that the aortic remodeling is eccentric, thus leaving the resistance of the aorta unchanged.

The main function of MMPs, a family of structurally related, zinc containing enzymes, has been reported to be the degradation and removal of ECM components from the tissue, encouraging VSMC migration and proliferation and the production of adhesion molecules (Jacob, 2003; Newby, 2006). The gelatinases MMP-2 and MMP-9, which cleave the basement membrane, type IV collagen, laminin and elastin, are the most studied MMPs in the vasculature (Dollery *et al.*, 1995). Increased expression and activity of MMPs, especially MMP-2 and MMP-9 have been reported to induce vascular changes in animal models of hypertension (Bouvet *et al.*, 2005; Castro *et al.*,

2008; Castro *et al.*, 2009), suggesting that MMP activation contributes to vascular remodeling associated with hypertension. The apparently paradoxical result that the scleroprotein content is raised in the hypertensive animals is associated with raised levels of the proteolytic enzymes MMP-2 and MMP-9 may be explained by the concept that hypertension-induced vascular remodeling is associated with increased MMP activity (Bouvet *et al.*, 2005; Castro *et al.*, 2008; Castro *et al.*, 2009; Rodriguez *et al.*, 2008). Furthermore, there is evidence that MMPs are actually directly involved in the blood pressure increase (Odenbach *et al.*, 2011; Schmid-Schonbein, 2011). Consistent with our results, increased MMP-2 expression and excessive collagen deposition have been found in the arterial wall of 2K-1C and L-NAME hypertensive rats, and the antioxidant therapy inhibited hypertension-induced upregulation of MMP-2 expression (Castro *et al.*, 2008). Moreover, it has been suggested that increased ROS levels in 2K-1C and L-NAME rats may produce vascular changes that are mediated by MMPs (Luchtefeld *et al.*, 2005). Our results support the interplay between MMP-2, MMP-9 and the extracellular matrix proteins during the remodeling process. However, more work is needed to identify their exact role.

The expression of MMPs can be activated by Ang II (Walter *et al.*, 2008) and ROS involved in preservation of MMP latency by reaction with thiol groups (Van Wart and Birkedal-Hansen, 1990). In fact, it has been shown that, increased Ang II, excessive vascular $O_2^{\bullet -}$production and NO depletion are implicated in vascular cell growth, inflammation, increased ECM deposition, vascular remodeling, promotion of MMP-2 expression and activity, and also reduced tissue inhibitor of MMP levels in hypertensive rat models (Bouvet *et al.*, 2005; Castro *et al.*, 2012; Castro *et al.*, 2009). This study showed an increase in MMP-2 and MMP-9 expressions in the arterial wall of 2K-1C and L-NAME rats and these alterations are associated with increased ACE activity, thus indicating an important activation of the RAAS in the 2K-1C (Sharifi *et al.*, 2003) and L-NAME hypertensive models (Koyanagi *et al.*, 2000; Yang *et al.*, 2008). Interestingly, we found that RBP and CUR significantly attenuated MMP-2 and MMP-9 expressions and reduced ACE activity in these hypertensive rats. Therefore, one of the possible mechanisms to explain the antihypertensive effect of RBP and CUR may be related to ACE-inhibitory activity since many ACE inhibitors inhibit MMPs. In addition, antioxidants can down-regulate MMPs as previously

shown in the 2K-1C hypertensive model (Castro *et al.*, 2009), suggesting a major role of ROS in MMP activity.

Several food proteins have been reported to be an important source of bioactive peptides with antihypertensive, antioxidative, immunomodulationg and hypocholesterolemic effects (Moller *et al.*, 2008). It has been demonstrated that ACE inhibitory peptides-derived from enzymatic hydrolysis of different food proteins shown *in vivo* antihypertensive effect in SHR and humans (Hernandez-Ledesma *et al.*, 2011; Yang *et al.*, 2004). Similar results have been observed that rice protein and rice dreg protein hydrolysates exerted *in vitro* ACE inhibitory activity and exhibited antihypertensive effect in hypertensive and obese-induced rats (Chen *et al.*, 2007; Justo *et al.*, 2013; Li *et al.*, 2007). It is well known that di- and tripeptides are easily absorbed in the intestine (Adibi, 1971; Hara *et al.*, 1984; Vermeirssen *et al.*, 2004). Although, most of food proteins are degraded during their transit through the small intestine, they may be derived bioactive peptides throughout the whole intestine and they can display bioactivity in the small and large bowel. Moreover, previous results indicated the rice protein hydrolysates slightly decreased ACE inhibitory activity after treatment with digestive enzymes (Li *et al.*, 2007), this suggested hydrolysate peptide resisted to gastrointestinal enzyme. Increasing evidence is indicated that the mechanism other than ACE inhibition can be involved in the blood pressure lowering effect exerted by many food-derived peptides (Fuglsang *et al.*, 2003; Vermeirssen *et al.*, 2004), such as vasorelaxant that exert their effects through the stimulation of opioid receptors (Sipola *et al.*, 2002) or indirect action on vascular smooth muscles (Kouno *et al.*, 2005; Maes *et al.*, 2004). Furthermore, experimental evidence has provided oxidative stress is mediator in cardiovascular pathologies, and thus antioxidant activity can also be responsible for blood pressure lowering effects. Indeed, it should be noted that RBP possess not only ACE inhibitory effect but also induce vasorelaxation (Tuangpolkrung, 2012), scavenge free radicals(Adebiyi *et al.*, 2009; Sereewatthanawut *et al.*, 2008), and reduce inflammation via suppression of cytokine signaling pathways (Boonloh *et al.*, 2014). Therefore, these may be explained the mechanisms implicated in the antihypertensive activity of RBP in 2K-1C and L-NAME hypertensive rats.

Previous studies have demonstrated that CUR possesses antioxidant activity by directly scavenging free radicals and hydrogen donors and that it also exhibits anti-inflammatory properties (Castro *et al.*, 2009; Kim *et al.*, 2008; Sreepriya and Bali, 2006). Our previous results on L-NAME hypertensive rats demonstrated that CUR effectively restored NO production in association with a reduction of the over production of $O_2^{\bullet-}$ in vascular tissue (Nakmareong *et al.*, 2011). In addition, treatment with the antioxidant tempol and the polyphenol-rich plant E. oleracea Mart. was associated with a downregulation of vascular MMP-2 expression and activity in 2K-1C hypertensive rat model (Castro *et al.*, 2009; da Costa *et al.*, 2012). The antioxidant effect of CUR is probably mediated by phenolic compounds in turmeric since recent evidence has demonstrated that polyphenols inhibit expression and activation of MMP-2 in VSMCs (Lee and Griendling, 2008). Increased MMP-2 expression and activity lead to less vasodilation or increased vasoconstriction, contributing to endothelial dysfunction and increased vascular resistance in hypertension (Martinez *et al.*, 2008; Rodriguez *et al.*, 2008; Tran *et al.*, 2010).

Structurally, CUR have identical-diketone structures and phenolic group, which is the most active site reacting with the free radicals. On the contrary, the sequence and structure of RBP have not been clarified. Further study, the sequence and structure of the purified peptide of RBP are needed to be clarified for providing much more information. However, previous studies have reported the compounds of rice bran are rich in the phenolic compounds oryzonal, ferulic acid, tocopherols, tocotrienols and unsaturated fatty acids (Kennedy and Burlingame, 2003). In addition, protein from rice bran are highly nutritional and have functional properties such as, hypoallergenic and hypocholesterolemic effect (Fabian and Ju, 2011; Jariwalla, 2001). Therefore, apart from the nutritional values, RBP and CUR are the promising dietary antioxidants.

In conclusion, the results of this study suggest that RBP and CUR moderate development of hypertension, endothelial dysfunction, vascular remodeling and oxidative stress in 2K-1C and L-NAME hypertensive rat models. The mechanisms of these effects might involve an increase in antioxidant activity and NO bioavailability, together with a decrease in ACE, MMP-2 and MMP-9 levels after RBP and CUR

treatment. Our findings suggest that supplementation of RBP and CUR in the daily diet may be useful for the prevention of hypertension. Epidemiological and clinical studies are needed to support this contention.