論文の内容の要旨

論文題目　Elucidation of the mechanisms by which Angiotensin II receptor blocker Candesartan improves insulin resistance
（アンジオテンシン II 受容体拮抗薬カンデサルタンのインスリン抵抗性改善メカニズムの解明）

氏名　　山口　麻美子

ABSTRACT

Angiotensin II receptor blocker (ARB) reduces the incidence of type-2 diabetes mellitus. In this study, I examined the effects of candesartan on metabolic syndrome by using diet-induced obesity (DIO)-C57BL/6J mice and diabetic *db/db* mice. One of the ARBs, candesartan ameliorated insulin resistance, increased both the plasma adiponectin and the plasma levels of high molecular weight (HMW) adiponectin and decreased the expression levels of inflammatory genes in white adipose tissue (WAT). In addition, candesartan decreased the adipocyte sizes of WAT in these mice. Moreover, candesartan increased the mRNA expression levels of adiponectin, adiponectin receptors, AdipoR1 and AdipoR2, and peroxisome proliferator-activated receptor (PPAR)γ in WAT of *db/db* mice. To assess the contribution of adiponectin signaling to the effects of candesartan on amelioration of insulin resistance, I next examined by using *Adipor1/r2* double-knockout mice. Candeartan ameliorated insulin resistance in wild-type mice, but not in *Adipor1/r2* double-knockout mice. Candesartan increased the plasma adiponectin levels and the plasma levels of HMW adiponectin in both wild-type mice and *Adipor1/r2* double-knockout mice. Candesartan increased the mRNA expression levels of PPARγ, decreased those of inflammatory genes, and decreased oxidative stress in WAT of wild-type mice, but not in those of *Adipor1/r2* double-knockout mice. Furthermore, candesartan decreased the adipocyte sizes of WAT in wild-type mice, but not in those of *Adipor1/r2* double-knockout mice. In conclusion, the effects of candesartan on amelioration of insulin resistance might be partly through increase of HMW adiponectin and activation of adiponectin receptors (AdipoRs).

INTRODUCTION

Obesity is a major risk factor for the development of hypertension and is also the principal risk factor for insulin resistance and the development of type 2 diabetes. Hypertension in obese individuals is further complicated by the concomitant presence of insulin resistance.

Angiotensin converting enzyme (ACE) inhibitors or ARBs, which are blockers of the rennin-angiotensin system, have been shown to increase insulin sensitivity and are now widely used as therapy for hypertension in diabetic patients. Angiotensin II (Ang II) is considered the major final mediator of the renin-angiotensin system. The actions of Ang II have been implicated in many cardiovascular conditions, such as hypertension, atherosclerosis, coronary heart disease, restenosis and heart failure. Ang II can act through two different receptors: Ang II type 1 (AT1) receptor and Ang II type 2 (AT2) receptor which are members of the G-protein-coupled receptor family. Ang II regulates the production of adipokines. Ang II increases the expression and the release of pro-inflammatory cytokines, and reduces plasma levels and gene expression of adiponectin, an insulin-sensitizing and anti-inflammatory adipokine.

Adiponectin is a hormone secreted by adipocytes that acts as a major anti-diabetic and anti-atherogenic adipokine. Adiponectin acts through two main receptors, AdipoR1 and AdipoR2. AdipoR1 and AdipoR2 are key players in the physiological and pathophysiological significance of adiponectin, and are involved in the regulation of glucose and lipid metabolism, inflammation and oxidative stress.

PPARγ is one of the key regulators of glucose homeostasis. Activation of PPARγ by agonists such as thiazolidinediones stimulates lipid storage in adipocytes, thereby reducing lipotoxicity in liver and skeletal muscle. In addition, PPARγ activation increases small adipocytes, thereby increasing the insulin-sensitizing hormone adiponectin and reducing tumor necrosis factor (TNF)-α, which induces insulin sensitivity. It has previously been reported that a PPAR γ agonist, pioglitazone, increased secretion of total and HMW adiponectin. It has been previously reported that decreased HMW adiponectin plays a crucial and causal role in obesity-linked insulin resistance and metabolic syndrome. Importantly, under pathophysiological conditions, such as obesity and diabetes, only HMW adiponectin was decreased; therefore, strategies to increase only HMW adiponectin may be a logical approach to provide a novel treatment modality for obesity-linked diseases, such as insulin resistance and diabetes.

Candesartan, one of the ARBs, has been reported to improve insulin sensitivity. Moreover, Koh et al reported that candesartan therapy increased serum adiponectin levels and insulin sensitivity in hypertensive patients. In this study, I examined the effects of candesartan in DIO-C57BL/6J mice, diabetic *db/db* mice and differentiated 3T3-L1 adipocytes, and I investigated the mechanisms by which they improve insulin resistance, especially in WAT. Moreover, I used *Adipor1/r2* double-knockout mice to investigate whether candesartan might be capable of ameliorating insulin resistance in the absence of adiponectin signaling.

RESULTS

To examine the anti-diabetic effects of candesartan, I treated DIO-C57BL/6J mice with candesartan. I examined the effects of candesartan on the improvement of glucose intolerance and insulin resistance, using oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). Plasma glucose levels during both test and plasma insulin levels during OGTT in candesartan-treated mice were significantly lower than those in vehicle-treated mice, suggesting that candesartan treatment ameliorated insulin resistance in DIO-C57BL/6J mice. Moreover, candesartan significantly increased the plasma adiponectin levels, in particular of HMW adiponectin.

Candesartan significantly increased the mRNA expression levels of Cu,Zn-superoxide dismutase (SOD)1 in WAT, whereas it decreased the mRNA expression levels of TNF-α and monocyte chemoattractant protein (MCP)-1 in WAT and plasma MCP-1 levels. In addition, candesartan decreased the adipocyte sizes of WAT in DIO-C57BL/6J mice.

Next, to examine the anti-diabetic effects of candesartan on severe diabetic model, I treated *db/db* mice with candesartan. Plasma glucose levels during OGTT and ITT in candesartan-treated mice were significantly lower than those in vehicle-treated mice, suggesting that candesartan treatment ameliorated insulin resistance in *db/db* mice. Moreover, candesartan increased both the plasma adiponectin and the plasma levels of HMW adiponectin in *db/db* mice.

Candesartan increased the mRNA expression levels of adiponectin, AdipoR1 and AdipoR2 in WAT. Moreover, candesartan significantly increased the mRNA expression levels of PPARγ and tended to decrease those of MCP-1 in WAT. In addition, candesartan decreased the adipocyte sizes of WAT in *db/db* mice.

As described above, candesartan increased the plasma adiponectin levels, in particular of HMW adiponectin, in DIO-C57BL/6J mice and *db/db* mice. To further investigate the contribution of adiponectin signaling to the effects of candesartan on amelioration of insulin resistance, I examined whether or not candesartan would ameliorate insulin resistance in *Adipor1/r2* double-knockout mice. Candeartan ameliorated insulin resistance in wild-type mice, but not in *Adipor1/r2* double-knockout mice. Candesartan increased the plasma adiponectin levels and the plasma levels of HMW adiponectin in both wild-type mice and *Adipor1/r2* double-knockout mice. On the other hand, candesartan increased the mRNA expression levels of PPARγ and SOD1, and decreased those of TNF-α and MCP-1 in WAT of wild-type mice, but not in those of *Adipor1/r2* double-knockout mice. Moreover, candesartan decreased the adipocyte sizes of WAT in wild-type mice, but not in those of *Adipor1/r2* double-knockout mice.

Finally, to investigate the direct effect of candesartan on adiponectin expression, I treated differentiated 3T3-L1 adipocytes with candesartan. Candesartan significantly increased the mRNA expression levels of adiponectin, AdipoR2 and glucose transporter 4. In addition, candesartan tended to increase the mRNA expression levels of PPARγ.

DISCUSSION

I examined the effects of candesartan on insulin resistance and metabolic syndrome *in vivo* and *in vitro*. Candesartan ameliorated insulin resistance in DIO-C57BL/6J mice and *db/db* mice. In the current study, I showed for the first time that candesartan significantly increased both the plasma adiponectin levels and the plasma levels of HMW adiponectin in DIO-C57BL/6J mice and *db/db* mice. Candesartan also significantly increased the mRNA expression levels of adiponectin, AdipoR1 and AdipoR2 in WAT of *db/db* mice.

To assess the contribution of adiponectin signaling to the effects of candesartan on amelioration of insulin resistance, I next examined by using *Adipor1/r2* double-knockout mice. Candeartan ameliorated insulin resistance in wild-type mice, but not in *Adipor1/r2* double-knockout mice. These results indicate that adiponectin signaling is casually involved in the candesartan-mediated amelioration of insulin resistance. Interestingly, candesartan increased the plasma adiponectin levels and HMW adiponectin in both wild-type and *Adipor1/r2* double-knockout mice. Moreover, candesartan reduces expression levels of proinflammatory adipokines, increased expression levels of SOD1 involved in reduction of oxidative stress and indeed reduced oxidative stress in WAT dependently on AdipoRs actions. These data suggest that candesartan increases adiponectin independently of AdipoRs, and also that candesartan ameliorate insulin resistance dependently on AdipoRs actions.

To investigate the direct effect of candesartan on adiponectin expression, I next examined the effects of candesartan in differentiated 3T3-L1 adipocytes. Candesartan significantly increased the mRNA expression levels of adiponectin and AdipoR2 in differentiated 3T3-L1 adipocytes. These results imply that increasing of HMW adiponectin and AdipoR2 by treatment of candesartan might alter insulin sensitivity and metabolism of adipocytes in a paracrine manner, in part.

In conclusion, the effects of candesartan on amelioration of insulin resistance might be partly through increase of HMW adiponectin and activation of adiponectin receptors (AdipoRs).