Distinguished Lecture 2: Dithi Chungcharoen Memorial Lecture

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Impact of functional foods on neural regulation and neurovascular protection

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Abstract

Insulin resistance, which is a physiological condition where insulin becomes less effective at lowering blood glucose, is associated with overweight and obesity in metabolic syndrome. The present study was designed to investigate whether natural products including honey-bee products (Royal Jelly; RJ and Propolis) and Eucommia ulmoides Oliv. leaves extract (ELE) prevent insulin resistance (hyperinsulinemia) and altered neural vascular regulation in fructose-drinking rat (FDR; experimental insulin resistance model) and Otsuka Long-Evans Tokushima Fatty rat (OLETF-R) (spontaneous insulin resistance model). FDR was produced by receiving 15% fructose solution in drinking water for 8 weeks using male Wistar rats (6 week-old). FDR had significant increases in plasma levels of insulin and triglyceride, an index of insulin resistance (HOMA-IR), and systolic blood pressure (SBP), but not blood glucose levels. OLETF-R at 14 week-old also had hyperinsulinemia with normal blood glucose level, increased HOMA-IR and hypertension. FDR and OLETF-R showed increase in adrenergic nerve-mediated vasoconstriction and adrenergic innervation and decrease in calcitonin gene-related peptide (CGRP) nerve-mediated vasodilation and CGRP nerve innervation. Oral treatment with RJ (100 and 300 mg/kg/day) or Propolis (Brazilian Propolis extract; 100 and 300 mg/kg/day) for 8 weeks significantly decreased plasma levels of insulin and triglyceride, HOMA-IR and tended to lower SBP. In OLETF-R, RJ or Propolis treatment for 4 weeks tended to decrease SBP and significantly reduced plasma insulin levels and HOMA-IR. In FDR, Oral treatment with ELE (500 and 1000 mg/kg/day for 4 weeks) significantly decreased plasma insulin levels and HOMA-IR and significantly lowered SBP. Furthermore, ELE treatment in FDR resulted in significant increase in CGRP-LI never density and significant decrease in TH-LI never density in mesenteric arteries of FDR. These results suggest that honeybee products and plant extract ELE could be an effective functional food to prevent insulin resistance associated with the development of hypertension.

Introduction

Insulin resistance, which is a physiological condition where insulin becomes less effective at lowering blood glucose, is associated with overweight and obesity in metabolic syndrome. When insulin resistance exists, more insulin is secreted by the pancreas to compensate insulin action, resulting in hyperinsulinemia. If this compensatory increase does not occur, blood glucose concentrations increase and Type 2 diabetes mellitus occurs. Thus, insulin resistance often progresses to full Type 2 diabetes mellitus. This is often seen when hyperglycemia develops after taking appropriate dieting such as high fat and high calorie diets, when pancreatic β-cells are unable to produce sufficient insulin to maintain normal blood sugar levels (euglycemia) in the face of insulin resistance. The inability of the β-cells to produce sufficient insulin in a condition of hyperglycemia transits from insulin resistance to Type 2 diabetes mellitus. Therefore, it is very important to prevent development of insulin resistance to reduce a risk for developing Type 2 diabetes mellitus.

Patients with Type 2 diabetes mellitus have high risk for complications of vascular diseases including atherosclerosis, angina pectoris and hypertension, and it has been suggested that there is a relationship between insulin levels and blood pressure. Many clinical studies have shown that patients with Type 2 diabetes mellitus have frequently insulin resistance associated with hypertension, suggesting that insulin resistance and hyperinsulinemia contribute to the pathogenesis of hypertension. In fact, insulin increases sympathetic activity and renal sodium reuptake and promotes proliferation of vascular smooth muscle cells, which could increase the blood pressure. Moreover, hyperinsulinemia has been shown to contribute to increase sympathetic activity, and a close association between insulin resistance and hyperinsulinemia has been suggested in essential hypertension. This relationship implies that insulin resistance and hyperinsulinemia contribute to the pathogenesis of hypertension, and furthermore, that insulin might play an important role in malfunction of the cardiovascular system. On the other hand, some investigations have shown that insulin acts as an endogenous vasodilator. Therefore, it is hypothesized that insulin resistance might induce hypertension due to decreased insulin-induced vasodilation and the imbalance between its pressor and depressor effects. However, the association of insulin and blood pressure has remained controversial. Previously, we reported that in pithed rats without a central vasoreflex acute insulin infusion augments adrenergic nerve-mediated vasoconstriction,
which is partially associated with inhibition of calcitonin gene-related peptide (CGRP)-containing vasodilator nerve (CGRPergic nerve) function as well as endothelium function. This finding implies that hyperinsulinemia may elevate blood pressure by augmenting the sympathetic adrenergic activity.

Chronic administration of fructose to rats has been reported to cause insulin resistance, which is characterized by increased serum insulin and euglycemia. Especially, a hyperinsulinemic state associated with hypertension was more prominently induced by fructose drinking than by fructose feeding. Our previous studies demonstrated that chronic hyperinsulinemia in fructose-drinking rat (FDR), an experimental model for insulin resistance, increased adrenergic nerve-mediated vasoconstriction and decreased CGRPergic nerve-mediated vasodilation, resulting in abnormal neuronal regulation of vascular tone leading to hypertension. Therefore, it is expected that inhibition of insulin resistance prevents development of cardiovascular diseases such as hypertension.

We previously reported that chronic hyperinsulinemia and insulin resistance induced by fructose-drinking loading elicited hypertension associated with abnormal neuronal regulation of vascular tone in an in vivo study using pithed rats, which have no vasoreflex. Recently, we reported that FDR showed significant increases in plasma levels of insulin, the glucose-insulin index, blood noradrenaline levels and systolic blood pressure (SBP), but not blood glucose levels, when compared with normal water-drinking rats (control rats). In perfused mesenteric vascular beds of FDR, enhanced adrenergic nerve-mediated vasoconstriction and decreased calcitonin gene-related peptide (CGRP)ergic nerve-mediated vasodilation. Furthermore, immunohistochemistry studies showed increased density of neuropeptide Y (NPY) immunopositive adrenergic fibers and reduced density of CGRP-immunopositive fibers in mesenteric arteries of FDR, suggesting that dysfunction of the neuronal vascular control system resulting from abnormal innervation of mesenteric perivascular nerves induced by the hyperinsulinemic state is responsible for the development of hypertension in FDR.

Otsuka Long-Evans Tokushima Fatty rats (OLETF-R) (spontaneous insulin resistance and Type 2 diabetic animal model) in early stages at 8 to 25 weeks of ages increases body weight and develops spontaneously insulin resistance with hyperinsulinemia and euglycemia and elevates SBP. In late stages at more than 25 week-old, they showed decreased blood glucose levels (hypoglycemia) to develop Type 2 diabetes.

Insulin resistance silently develops because of no symptoms. Also, it is very difficult to diagnose as insulin resistance, since complicated clinical tests need. At preset, a very few drugs (thiazolines derivatives) are available to treat insulin resistance. Therefore, if we could find natural foods and their ingredients, which have some preventive effects on development of insulin resistance, and take these functional foods as daily diets, it would be effective to prevent insulin resistance, which is considered to be a cause of various lifestyle-related diseases such as Type 2 diabetes mellitus and hypertension. Thus, the present study was designed to investigate the effects of long-term administration of natural products including Royal Jelly (RJ) and Propolis and plant extract Eucommia ulmoides Oliv. leaves extract (ELE) on insulin resistance (hyperinsulinemia) using FDR and OLETF-R.

**Effects of RJ in FDR**

RJ is a honey bee secretion that is used in the nutrition of larvae, as well as adult queens. RJ is secreted from the glands in the hypopharynx of worker bees, and fed to all larvae in the colony. RJ is known to contain three major nutrients including amino acids, vitamins and minerals. Additionally, RJ has various biological activities such as a hypotensive effect and insulin-like action. Therefore, it is possible that RJ may have some effects on insulin resistance.

In FDR, which received 15% fructose solution in drinking water from 6 week-old to 14 week-old for 8 weeks and showed significant increases in plasma levels of insulin and triglyceride, Homeostasis Model Assessment ratio (HOMA-IR, an index of insulin resistance), and SBP, but not blood glucose levels, when compared with control rats. Oral treatment with RJ (100 and 300 mg/kg/day) for 8 weeks immediately after fructose loading significantly decreased the plasma levels of insulin and triglyceride, HOMA-IR, without affecting blood glucose or total cholesterol levels and tended to lower SBP. In isolated and perfused mesenteric vascular beds of FDR, RJ treatment resulted in a significant reduction in sympathetic nerve-mediated vasoconstrictor response to periarterial nerve stimulation and tended to increase the CGRPergic nerve-mediated vasodilator response to periarterial nerve stimulation, compared with those in untreated FDR. However, RJ treatment did not significantly affect noradrenaline-induced vasoconstriction or CGRP-induced vasodilation. These results suggest that RJ could be an effective functional food to prevent insulin resistance associated with the development of hypertension.

**Effect of RJ in OLETF-R**

OLETF-R at 14 weeks of age showed increase in plasma insulin levels and HOMA-IR, normal blood glucose, and high SBP. OLETF-R at 10 week-old was orally treated for 4 weeks with RJ (10, 30 and 300 mg/kg). RJ treatment tended to decrease SBP and significantly decreased serum insulin levels and HOMA-IR. In isolated and perfused mesenteric vascular beds of OLETF-R, RJ treatment resulted in significant reduction of sympathetic nerve-mediated vasoconstriction and augmentation of CGRPergic nerve-mediated vasodilation, compared with that in non-treated OLETF-R. However, RJ treatment did not significantly affect noradrenaline-induced vasoconstriction and CGRP-induced vasodilation, suggesting that RJ could be an effective and functional food to prevent development of insulin resistance.
Effect of Propolis in FDR

Propolis is a resins mixture that honeybees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is known to contain effective ingredients including flavonoids, vitamins and minerals. Propolis has various biological activities such as an anti-bacterial activity, anti-inflammatory activity, hypotensive effect, insulin-like action, anti-tumor activity and antioxidant activity.

Brazilian Propolis extract at doses of 100 and 300 mg/kg/day was orally administered for 8 weeks immediately after Male Wistar rats (6-week-old) received 15% fructose solution in drinking water for 8 weeks. Treatment with Propolis in FDR significantly decreased plasma level of insulin, HOMA-IR and body weight, plasma triglyceride levels without affecting blood glucose and total cholesterol level and tended to decrease SBP. In isolated and perfused mesenteric vascular beds of FDR, Propolis treatment resulted in significant reduction of sympathetic nerve-mediated vasoconstriction and tended to augment CGRPergic nerve-mediated vasodilation, compared with those in non-treated FDR. However, Propolis treatment did not significantly affect the noradrenaline-induced vasoconstriction and CGRP-induced vasodilation, suggesting that Propolis could be an effective and functional food to prevent development of insulin resistance and hypertension.

Effect of Propolis in OLETF-R

10-week old OLETF-R were orally treated for 4 weeks with Propolis (100 and 300 mg/kg/day) or vehicle (Control). Propolis treatment significantly decreased the plasma levels of insulin and HOMA-IR without affecting blood glucose levels and tended to lower SBP, compared with Control. In isolated and perfused mesenteric vascular beds of OLETF-R, Propolis treatment resulted in significant reduction in sympathetic nerve-mediated vasoconstriction and tended to augment CGRPergic nerve-mediated vasodilation, compared with those in vehicle-treated OLETF-R. However, Propolis treatment did not significantly affect noradrenaline-induced vasoconstriction and CGRP-mediated vasodilation. These results suggest that Propolis could be an effective and functional food to prevent development of insulin resistance in spontaneous insulin resistance model.

Effect of Eucommia ulmoides Oliv. leaves extract (ELE) in FDR

The leaf of Eucommia ulmoides Oliv is commonly used as a traditional Chinese medicine to treat hypertension. Recently, several pharmacological studies have reported that leaf extract of Eucommia ulmoides Oliv (ELE) also exhibits effects such as anti-hypercholesterolemia, anti-fatty liver, anti-oxidative stress and anti-obesity.

Lee et al. reported that powdered Eucommia ulmoides Oliv leaves and water extract improved hyperglycemia in streptozotocin-induced Type 1 diabetic rats. Furthermore, Park et al. reported that water extract of Eucommia ulmoides Oliv leaves ameliorated hyperglycemia and hyperlipidemia in Type 2 diabetes by modulating the glucose and lipid metabolic enzyme activities. Accordingly, we investigate whether ELE treatment prevents the development of insulin resistance in FDR and whether ELE treatment ameliorates the abnormal distribution of perivascular nerves in the isolated mesenteric vascular beds of FDR using immunohistochemical methods.

In FDR, ELE at doses of 500 and 1000 mg/kg/day were orally administered once daily for 4 weeks immediately after 15% fructose-drinking loading. A 4 weeks treatment of FDR with ELE significantly decreased plasma insulin levels and HOMA-IR without affecting blood glucose levels and significantly lowered SBP in FDR. In immunohistochemical study, FDR showed significantly greater density of tyrosine hydroxylase (TH)-like immunoreactivity (LI)-containing nerves and significantly lower density of CGRP-LI-containing nerves in mesenteric arteries of FDR than those in control. ELE treatment in FDR resulted in significant increase in CGRP-LI never fiber density and significant decrease in TH-LI never fiber density in mesenteric arteries of FDR. These results suggest that long-term ELE treatment effectively prevents insulin resistance development and ameliorates abnormal perivascular innervation in FDR.

Conclusion

These results suggest that RJ and PPL of honeybee products and ELE of plant extract could be an effective functional food to prevent insulin resistance, which produces dysfunctional neural regulation of vascular tone associated with the development of hypertension.

References


