A limonene-derivative from Sudachi peel improves lipid and glucose metabolism with SIRT1 upregulation in high fat diet-fed mice.

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Sudachi (Citrus Sudachi) is a small sour citrus, and it is a typical seasoning for roast as well as raw fish in Japanese cuisine. Interestingly, it grows exclusively in Tokushima region of Japan, and Sudachi is a specialty of Tokushima prefecture. Recently our collaborators have reported that administration of freeze-dried peel of Sudachi decreases serum triglyceride (TG) levels in obese human subjects. Thus we aimed to determine active components from Sudachi peel in the current study. Here we report identification of an active ingredient to ameliorate lipid and glucose metabolism which are dependent on sirt1.

The effects of crude Sudachi peel were evaluated in Zucker diabetic fatty (ZDF) rats. Hexane-extract of Sudachi peel was fractionated by silica gel column and subjected to a cell-based screening using C2C12 myotubes by an index of intracellular TG content. The positive fractions were then purified using octadecylsilyl column. Activities of the compound were evaluated in high fat diet-fed male ddY mice as well as C2C12 cells.

A daily administration of Sudachi peel improved serum TG levels and extended lifespan of ZDF rats. It also reduced TG levels in cultured C2C12 myotubes. We found a limonene-derivative from Sudachi peel as an active compound by the screening. The TG-lowering effects of the compound was sensitive to nicotinamide, a sirt1 inhibitor, and the molecule increased sirt1 expression levels in a dose-responsive manner. In high fat diet-fed mice, repetitive administration of the compound for 10 days improved glucose tolerance, fatty liver, serum TG and cholesterol to the same levels as those of healthy mice with increase in sirt1 activities in the gastrocnemius muscle and liver.

Therefore, it will be one of the active components of Sudachi peel regulating metabolism, which should be mediated by sirt1 activation. The sirt1 activation is supposed principally due to upregulation of the expressions.

In conclusion, we identified limonene-derivative which ameliorates metabolism in cultured cells and in vivo with increase in the activities and expression levels of sirt1 which should be involved in the metabolic action. It will be a novel lead compound regulating metabolic homeostasis by modulating sirt1 expression levels.
GLP-1 action attenuates breast cancer growth and progression

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Incretin therapy has emerged as one of the most popular treatment for type 2 diabetes. GLP-1R agonist, Exendin-4(Ex-4), has received much attention, because of its tissue protective effects beyond glycemic control, such as weight reduction and vascular protection. We have previously reported vascular protective effects (Diabetes 2010, BBRC 2011) and anti-prostate cancer effect (Diabetes 2014, PLOS ONE 2015) of Ex-4. On the other hand, breast cancer is one of the most popular cancers in female with patients with type 2 diabetes and obesity. Then, we next examined whether GLP-1 action could attenuate breast cancer in the present study.

First, we observed abundant GLP-1R expression in early stage breast cancer (Stage I) tissue extracted from patients without diabetes, but not advanced breast cancer (Stage IIb). In human breast cancer cell lines, MCF-7, MDA-MB-231 and KPL-1 cell, GLP-1R was expressed abundantly. 0.1~10nM Ex-4 treatment significantly decreased cell number of breast cancer cells in growth curve, in dose-dependent manner. Although Ex-4 did not induce apoptosis in breast cancer cells, BrdU assay revealed that Ex-4 attenuates cell proliferation of breast cancer cells in dose dependent manner. If we transplanted MCF-7 cells into non-diabetic nude mice subcutaneously and treated them with 300pM/kg/day Ex-4 for 6 weeks, we observed decreased tumor size of MCF-7 in Ex-4-treated mice, in both male and female mice with no change in body weight and blood glucose level. Immunohistochemistry with Ki67, a marker of cell proliferation, revealed that breast cancer cell proliferation was significantly decreased in tumor extracted from mice treated with Ex-4. Further, 60% high fat diet significantly increased breast cancer size and weight in nude mice. Further, Ex-4 treatments decreased tumor size and weight in high fat fed mice similar to that of control diet level.

These data suggest that GLP-1 action could attenuate breast cancer via inhibition of breast cancer cell proliferation.
Activation of heat shock response improves glucose metabolism and inflammation in obese subjects with type 2 diabetes

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Decreased heat shock response (HSR) is reported to be associated with insulin resistance in type 2 diabetes. Activation of HSR improves accumulated visceral adiposity and metabolic abnormalities in type 2 diabetes. This study was designed to identify the optimal intervention strategy of the activation of HSR provided by mild electrical stimulation with heat shock (MES+HS) in obese type 2 diabetes patients. We have previously reported that MES+HS improved HbA1c by -0.43% in male subjects with obese type 2 diabetes.

This study was a prospective, frequency-escalating, randomized, open-label, triple-arm trial. A total of 60 obese type 2 diabetes patients were randomized into three groups of two, four, or seven times treatment per week for 12 weeks.

No adverse events were identified. In comparison to the baseline, MES+HS treatment over time significantly improved visceral adiposity (-11.69 cm². p<0.001), glycemic control (HbA1c: -0.36%: from 7.64% to 7.28%. p<0.001), insulin resistance (HOMA-IR:-1.09. p<0.001), systemic inflammation (TNF-α: -0.40 pg/mL. p<0.001. CRP: -663.6 ng/mL. p=0.008), renal function (eGFR: + 2.96 mL/min/1.73m². p<0.001), hepatic steatosis (AST/ALT: + 0.06. p=0.007) and lipid profiles (triglyceride: -30.02 mg/dL. p=0.015). The clinical target of HbA1c less than 7.0% was achieved by 38.3% (n=23) of participants after MES+HS treatment. The reduction in HbA1c was significantly greater in 4 per week (-0.36%. p=0.036) or 7 per week (-0.65%. p=0.001) than that in 2 per week (-0.10%) of treatment. The decrease in the visceral fat area showed similar trend of changes (-5.37, -14.24, -16.45 cm² by two, four, seven per week, respectively), indicating that the beneficial effects depend on its frequency. More pronounced effects were observed in males (HbA1c: -0.44%. from 7.70% to 7.25%. p<0.001) than those in females (HbA1c: -0.17%. from 7.50% to 7.33%. p=0.140).

This research provides additional lines of evidence to support the positive impacts of MES+HS in improving metabolic outcomes in obese type 2 diabetes patients. Those who do not reach the glycemic control goal of HbA1c less than 7.0% could be offered additional personalized medical care including MES+HS treatment.
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Inhibition of semicarbazide-sensitive amine oxidase reduces atherosclerosis in apolipoprotein E-deficient mice

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Background: Inflammation, oxidative stress, and formation of advanced glycated end-products (AGEs) are important mechanisms of atherosclerosis. Vascular adhesion protein-1 or semicarbazide-sensitive amine oxidase (VAP-1/SSAO) participates in inflammation and catalyzes oxidative deamination reaction to produce hydrogen peroxide and aldehydes, leading to subsequent generation of AGEs. We investigated the effect of VAP-1/SSAO inhibition on atherosclerosis.

Methods and Results: We included 127 human subjects with coronary arterial disease (CAD) and 53 subjects without CAD, diagnosed by coronary angiography. Serum VAP-1 concentration was higher in subjects with CAD (579 vs. 544 ng/ml, adjusted p=0.003) and was associated with the number of coronary arteries and coronary arterial segments with significant stenosis (both adjusted p=0.003). In arterial walls, VAP-1 expression was stronger in atherosclerotic plaques in apolipoprotein E-deficient mice and human. VAP-1 was expressed on endothelial cells, smooth muscle cells (SMC), and macrophages in apolipoprotein E-deficient mice. Inhibition of VAP-1/SSAO by a selective inhibitor, PXS-4728A, decreased hydrogen peroxide generation and reduced atherosclerotic plaque area. Expression of adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, and adhesion of U937 monocytes to aorta were reduced by VAP-1/SSAO inhibition. It also decreased the expression of pro-inflammatory cytokines including cyclooxygenase-2, monocyte chemoattractant protein-1, inducible nitric oxide synthase, and tumor necrosis factor-α (TNF-α). Macrophage recruitment and activation were reduced by VAP-1/SSAO inhibition, measured by the expression of ionized calcium-binding adapter molecule 1, CD36, receptor of AGE, lectin-like oxidized low-density lipoprotein receptor-1, and Toll-like receptor-4. It also decreased SMC migration and proliferation, analyzed by the expression of matrix metalloproteinase-9 and proliferative cell nuclear antigen.

In cell model, human umbilical vein endothelial cells over-expressing VAP-1/SSAO (HUVEC/VAP-1) had higher oxidative stress and index of monocyte transmigration across HUVEC monolayer than HUVEC controls, which was further stimulated by TNF-α treatment. Inhibition of VAP-1/SSAO by PXS-4728A reduced TNF-α-stimulated oxidative stress and monocyte transmigration in HUVEC/VAP-1. In A7r5 SMC cell line over-expressing VAP-1/SSAO (A7r5/VAP-1), the oxidative stress was higher than that in A7r5 controls. Inhibition of VAP-1/SSAO by PXS-4728A reduced oxidative stress in A7r5/VAP-1 cells, with or without the stimulation of lipopolysaccharide. Besides, the proliferation and the migration rate of A7r5/VAP-1 cells were decreased by inhibition of VAP-1/SSAO, with or without the stimulation of platelet-derived growth factor.

Conclusions: Serum VAP-1 concentrations are associated with CAD in human. Inhibition of VAP-1/SSAO reduces atherosclerosis in apolipoprotein-E deficient mice, through reducing oxidative stress, endothelial cell activation, SMC migration and proliferation, and macrophage recruitment and activation.
Efficacy of heparinoid supplementation on albumin excretion and azotemia in diabetic kidney disease

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Heparinoids, which are heparin-derived glycosaminoglycans (GAGs), have been proposed by studies to be beneficial in diabetic kidney disease (DKD) patients with proteinuria partially resistant to renin-angiotensin-aldosterone system (RAAS) blocking drugs. This meta-analysis aims to evaluate the benefit of heparinoid supplementation in delaying the progression of albuminuria and azotemia in DKD patients.

Trials evaluating the efficacy of heparinoid supplementation on top of standard RAAS therapy among adult DKD patients of any ethnicity or gender, versus either placebo or an alternative drug, were included. Outcomes were changes in urinary albumin excretion rate (UAER), albumin-creatinine ratio (ACR), serum creatinine (SCr), creatinine clearance (CrCl), and the proportion of patients achieving therapeutic success (either an ACR <20 mg/g and at least a 25% drop from baseline or at least a 50% drop from baseline.) A literature search was performed by two independent authors with eligible studies undergoing validity screen, data extraction, and statistical analysis using Review Manager 5.1 software. Results were presented as mean differences, standard deviations, and 95% confidence intervals, and graphically presented as forest plots. Estimates were calculated using the Mantel-Haenszel odds ratio for dichotomous variables and the inverse variance method for continuous variables, and pooled using either a random or fixed effects model depending on heterogeneity as assessed by the I² test.

Thirty-eight studies were initially retrieved for consideration, but only eleven trials were included. Seven involved sulodexide while two each involved low molecular weight heparin and danaparoid, all given for at least three weeks and compared to placebo. We found a statistically significant reduction in UAER in Type 1 DM (95% CI, -1.15 [-2.79, -0.21], p=0.02) but not in Type 2 DM (95% CI, 0.13 [-0.42, 0.68], p=0.65) heparinoid-treated patients. This subgroup analysis was performed due to initial heterogeneity (I²=57%). No statistically significant difference was seen between groups in terms of patient proportion reaching therapeutic success (95% CI, 0.97 [0.71, 1.33], p=0.87), rate of adverse events, SCr (95% CI, 2.55 [-0.54, 5.65], p=0.11) and CrCl (95% CI, -8.55 [-18.28, 1.18], p=0.09), although a trend towards benefit favoring the heparinoid group was seen in the latter.

Heparinoid supplementation in DKD patients for at least three weeks was associated with a statistically significant reduction in UAER in Type 1 DM. No significant reduction was seen in Type 2 DM, as well as in SCr, CrCl, and the proportion of patients reaching therapeutic success for both subgroups.
Dipeptidyl peptidase-4 inhibitor prevents diabetic nephropathy through STRA6 signaling

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We recently found that O-GlcNAcosylation of RBP4 receptor (STRA6) with a decrease of RBP4 binding activity suppressed CRBP-1 and RARα expression and thereafter activate apoptosis and fibrosis in high-glucose cultured renal cells and in the kidneys of diabetic mice. Dipeptidyl peptidase-4 inhibitors (DPP-4i) was reported to capably ameliorate kidney fibrosis in diabetic mice. We hypothesized that DPP-4i can produce its pleiotropic action to prevent kidney damage beyond glycemic control.

STRA6, CRBP1, RARα, caspase 3, collagen 1 and fibronectin was measured by Western blot analysis for protein and PCR for mRNA expression in the kidney in normal fat diet(NFD)-fed, high fat diet(HFD)-fed mice and sitaglitipin-treated HFD-fed mice. We aimed to investigate whether the reciprocal appearance of STRA6 cascade down-regulation and fibrosis increase in the kidney of HFD-fed mice, and whether DPP-4i reverses these alterations beyond glycemic control.

The expression of STRA6, CRBP1 and RARα protein and mRNA expression remarkably decreased, while caspase 3, collagen 1, and fibronectin significantly increased in kidney of HFD-fed mice as compared with NFD-fed mice. All these changes in the aorta of HFD-fed mice were reversed in sitaglitipin-treated HFD-fed mice. The blood glucose values in HFD-fed mice and sitaglitipin-treated HFD-fed mice are not different, but are higher than NFD-fed mice.

We conclude that DDP-4 inhibitor can produce its beneficial action to prevent HFD-induced fibrosis and apoptosis in kidney of HFD-treated mice by reversing the suppression of RBP4 receptor/CRBP-1/RARα signaling beyond its glycemic control.