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専　　攻　　名	先進医療科学専攻
学位論文題名	Ipragliflozin-induced adipose expansion inhibits cuff-induced vascular remodeling in mice （マウスにおいてイプラグリフロジン誘導性の脂肪組織の拡大はカフ誘導性血管障害を抑制する）
論文審査委員	委員長　教　授　久木山　清貴 委　員　教　授　川原　敦雄 委　員　講　師　榊原　賢士

学位論文内容の要旨

Background Perivascular adipose tissue (PVAT) surrounds vasculature and has been suggested to play an important role in the pathogenesis of cardiovascular disease. PVAT not only stores triglycerides and functions as structural support for vessels, but also secretes a wide variety of biologically active molecules to control vascular function. In obesity or type 2 diabetes (T2DM), PVAT dominantly secrete pro-inflammatory and pro-atherogenic cytokines which contribute to the progression of systemic and local vascular diseases. It therefore suggests that PVAT has been considered as a potential therapeutic target for treatment of atherosclerosis associated with obesity and/or diabetes. Sodium glucose cotransporter 2 (SGLT2) inhibitors are a class of oral hypoglycemic agents that work by decreasing glucose reabsorption in the renal proximal tubules to promote urinary glucose excretion. Accumulating evidence suggests that SGLT2 inhibitors provide multiple benefits to reduce major cardiovascular adverse events in patients with T2DM. Recent clinical and experimental evidence have further confirmed that SGLT2 inhibitors have benefits on atherosclerotic cardiovascular events. However, the mechanisms underlying the protective effects of SGLT2 inhibitors on cardiovascular complications among T2DM still remain to be explored. Others and we recently reported that the SGLT2 inhibitors promotes fat accumulation in epididymal adipose tissue (Epi) of diet-induced obese mice without deteriorating adipose inflammation and/or fibrosis, which may be referred to as “healthy adipose expansion”. In this article, we investigated whether SGLT2 inhibitors also alter characters of PVAT to the “healthy adipose expansion”, and if so, whether the SGLT2 inhibitors-induced

changes of adipose tissue, especially the alternation of adipose tissue-derived secretory factors, affect vascular pathophysiology.

Methods Ipragliflozin was dissolved in dimethyl sulfoxide (DMSO) at 0.04% (v/v) and added into the drinking water. In a Western type diet (WD) feeding experiments, 8-week-old WT mice were fed a WD for 8 weeks, and thereafter a WD with the vehicle or Ipra for 10 weeks. WEHI 274.1 and primary vascular smooth muscle cells were incubated with conditioned media (CM) of epididymal adipose tissue (Epi) or abdominal PVAT of Ipra- or vehicle-treated mice fed a WD. WEHI 274.1 cells were plated into the upper chamber, while CM was filled into the lower one with or without anti-MCP-1 blocking antibody. After 12 h, the number of migrated cells was counted. Vascular smooth muscle cells were isolated and cultured from 4-week-old WT male. Scratch was created by a 100 μl pipette tip in the monolayer. The cells were then stimulated with rat recombinant platelet-derived growth factor (PDGF)-BB in CM with or without LY294002. After 24 h, images of the scratch wounds were taken. The femoral artery was isolated from surrounding tissues under anesthesia, and then a polyethylene tube was loosely placed around the artery. Fifty mg of Epi was taken from WD-fed or WD/Ipra-fed mouse, followed by placed onto the artery after cuff placement.

Results Histological analysis revealed that Ipra treatment increased adipocyte size in abdominal PVAT of WD-fed mice. In abdominal PVAT, inflammation (*Ccl2*, *Ccr2*, and *Emr1*)- and fibrosis (*Col1a1*, *Col1a2*, and *Fn1*)-related genes were upregulated in WD-fed mice compared to SD-fed mice, which were significantly or tended to be inhibited by Ipra treatment. Accordingly, immunostaining for a macrophage marker F4/80 revealed that Ipra treatment effectively suppressed macrophage infiltration and crown-like structure (CLS) formation in abdominal PVAT of WD-fed mice. Ipra significantly decreased the number of TUNEL-positive cells in WD-fed mice as compared to vehicle-treated mice. Ipra tended to reduce protein expression of HMGB1 in abdominal PVAT, and suppressed HMGB1 release from isolated Epi of WD-fed mice into CM. An in vitro chemotaxis assay revealed that CM of Epi from vehicle-treated mice fed a WD significantly enhanced monocyte migration as compared to that from SD-fed mice, whose effect was attenuated in CM of Epi from Ipra-treated mice. Pretreatment with a neutralizing anti-MCP-1 antibody also inhibited the increase of monocyte migration by CM of Epi from vehicle-treated mice, and it also diminished the difference of monocyte migration stimulated by CM of Epi from Ipra- and vehicle-treated mice. Whereas the CM of abdominal PVAT from vehicle-treated mice enhanced platelet-derived growth factor (PDGF)-BB-induced VSMCs migration in vitro, its effect was significantly attenuated in CM of abdominal PVAT from Ipra-treated mice. Neointimal hyperplasia assessed by intima area and intima to media ratio were significantly attenuated in ApoE-knockout mice implanted with Epi from Ipra-treated mice compared to vehicle-treated mice.

Disucussion The present study demonstrated that Ipra increased adipocyte size in abdominal PVAT in WD-induced obese and diabetic mice, which is consistent with previous observation in Epi of diet-induced obese mice treated with SGLT2 inhibitors. The adipocyte hypertrophy in abdominal PVAT accompanied a decrease of inflammation and fibrosis, which corresponded to "healthy adipose expansion". In addition to multiple metabolic benefits by SGLT2 inhibitors, the present study proposed novel mechanisms by which SGLT2 inhibitors prevented vascular complications in T2DM via modulating PVAT characters.

Conclusion The Ipra-induced changes of abdominal PVAT will lead to a better understanding of unveiled mechanisms by which SGLT2 inhibitors prevent cardiovascular complications in T2DM, and the development of new therapeutic strategies targeting PVAT.

論文審査結果の要旨

Sodium glucose cotransporter 2 (SGLT2) inhibitors は糖尿病患者に対して血糖を低下させるとともに心血管病の発生を抑制することが知られている。今回の研究の目的は、SGLT2 inhibitor である Ipragliflozin (Ipra)の心血管病に対する抑制機序を動物実験モデルにて明らかにすることである。Western Diet を 10 週間与えたマウスにおいて Ipra 治療群はプラシーボ投与群に比べて腹腔内血管周囲脂肪組織の脂肪細胞のサイズを大きくし、炎症、線維化、アポトーシスを抑制させた。アポ E ノックアウトマウスの大腿動脈に Ipra 治療群マウスからの腹腔内血管周囲脂肪組織を移植した場合、プラシーボ投与群マウスからの脂肪組織に比べて大腿動脈の内膜肥厚を抑制した。これらはマクロファージおよび血管平滑筋細胞の遊走能を Ipra が抑制しているために生じていることが培養細胞を用いた in vitro 研究結果により示唆された。これらの研究結果から SGLT2 inhibitors による腹腔内血管周囲脂肪細胞のフェノタイプ変化が、SGLT2 inhibitors による心血管病の発生抑制の機序に関わっていることが示唆された。

本研究内容は、まだ不明である SGLT2 inhibitors の心血管病発生抑制機序を血管周囲脂肪細胞の観点から解明したものであり独自性が高いと思われる。研究方法も既に確立された方法を駆使しており得られた結果も信頼性が高いと考えられる。発表内容は、既に 2019 年に peer review の Cardiovascular Diabetology に掲載されており完成度は高いと考えられる。審査委員一致して医学博士の学位論文にふさわしい内容であると認めた。