EXPERIMENTAL STUDY

Inhibitory Effects of Tofogliflozin on Cardiac Hypertrophy in Dahl Salt-Sensitive and Salt-Resistant Rats Fed a High-Fat Diet

Tomonari Kimura,[1] MD, Kazufumi Nakamura,[1] MD, Toru Miyoshi,[1] MD, Masashi Yoshida,[2] MD, Kaoru Akazawa,[1] MSc, Yukihiro Saito,[1] MD, Satoshi Akagi,[1] MD, Yuko Ohno,[1] MSc, Megumi Kondo,[1] MSc, Daiji Miura,[1,3] PhD, Jun Wada,[4] MD and Hiroshi Ito,[1] MD

Summary

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are drugs for diabetes and might prevent heart failure. In this study, we investigated the effects of tofogliflozin, an SGLT2 inhibitor, on cardiac hypertrophy and metabolism in hypertensive rats fed a high-fat diet. Dahl salt-sensitive (DS) rats, hypertensive model rats, and Dahl salt-resistant (DR) rats, non-hypertensive model rats, were fed a high-salt and high-fat diet containing tofogliflozin (0.005%) for 9 weeks to examine the effects of this drug on cardiac hypertrophy and metabolism. Tofogliflozin tended to suppress a rise of the systolic blood pressure, relative to the control, throughout the treatment period in both DR and DS rats, and significantly suppress a rise of the systolic blood pressure, relative to the control, at the 9th week in DS rats. Tofogliflozin reduced cardiac hypertrophy (heart weight/body weight) not only in DS rats but also in DR rats. Histological analysis showed that tofogliflozin significantly decreased cardiomyocyte hypertrophy and perivascular fibrosis in both DS and DR rats. Tofogliflozin significantly decreased the expression levels of genes related to cardiac hypertrophy (encoding for natriuretic peptides A and B and interleukin-6), and to cardiac fibrosis (encoding for transforming growth factor-β1 and collagen type IV), in DS rats. Recent studies have shown that hypertrophied and failing hearts shift to oxidizing ketone bodies as a significant fuel source. We also performed metabolome analysis for ventricular myocardial tissue. Tofogliflozin reduced 3-hydroxybutyrate, a ketone body, and significantly decreased the expression levels of β-hydroxybutyrate dehydrogenase 1 and 3-oxoacid CoA-transferase, which are related to ketone oxidization. In conclusion, tofogliflozin ameliorated cardiac hypertrophy and fibrosis along with reduction of ketone usage in myocardial tissue.

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Key words: SGLT2 inhibitor, Fibrosis, Hypertension, Ketone

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are antihyperglycemic agents that inhibit the activity of SGLT2, which is involved in glucose reabsorption in proximal tubules.[1)] Recently, several studies have shown that SGLT2 inhibitors reduced cardiovascular events and decreased mortality in patients with diabetes mellitus.[2,3)] Empagliflozin significantly reduced cardiovascular death and heart failure hospitalization in type 2 diabetic patients with a high risk for cardiovascular disease in the EMPA-REG OUTCOME trial.[2)] Canagliflozin significantly decreased cardiovascular events and heart failure hospitalization in patients with a moderate risk for cardiovascular disease in the Canagliflozin Cardiovascular Assessment Study (CANVAS) trial.[3)] The SGLT2 inhibitor tofogliflozin has also been shown to have beneficial effects on impaired glucose tolerance, hypertension, hyperuricemia, and dyslipidemia.[4)] However, the precise mechanisms underlying the beneficial effects of SGLT2 inhibitors for heart failure remain unclear.

In the present study, we used clinically relevant rodent models of hypertension fed a high-salt and high-fat diet. Dahl salt-sensitive (DS) rats develop hypertension and heart failure when they are fed a high-salt and high-fat diet that induces insulin resistance.[5)] Fat loading accelerates hypertension in DS rats.[5-7)]

We hypothesized that an SGLT2 inhibitor could prevent cardiac hypertrophy and change the cardiac metabolism. We investigated the effect of tofogliflozin on cardiac hypertrophy using hypertensive model rats.

From the [1]Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, [2]Department of Chronic Kidney Disease and Cardiovascular Disease, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, [3]Department of Basic Medicine, Nagano College of Nursing, Komagane, Japan and [4]Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

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Address for correspondence: Kazufumi Nakamura, MD, Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan. E-mail: ichibun@cc.okayama-u.ac.jp

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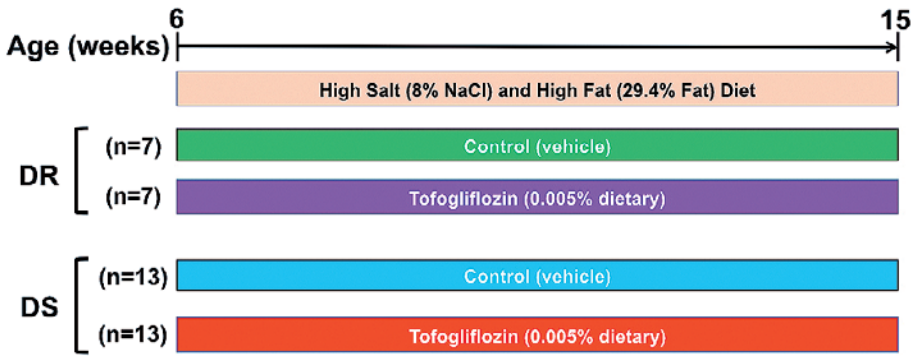

Figure 1. Scheme of the experimental protocol. We used male Dahl salt-resistant (DR) rats and Dahl salt-sensitive (DS) rats at the age of 6 weeks. The rats were divided into four different groups as shown above and treated with a vehicle or tofogliflozin (0.005% dietary) for 9 weeks. All rats were fed a high-salt (8% NaCl) and high-fat (29.4% fat) diet during the same period.

Methods

Protocols for animal experiments: Six-week-old male Dahl salt-resistant (DR) rats (n = 14) and DS rats (n = 26; Japan SLC, Shizuoka, Japan) were fed a high-salt (HS; 8% NaCl) and high-fat (HF; 29.4% fat) diet and were treated with a vehicle or 0.005% tofogliflozin dietary (Kowa Co., Ltd.) for a period of 9 weeks (Figure 1). Chow was purchased from CLEA Japan, Inc. Tokyo, Japan. The experiments were carried out in the following on four groups; (1) DR-Control (n = 7), (2) DR-tofogliflozin (TOFO) (n = 7), (3) DS-Control (n = 13), and (4) DS-TOFO (n = 13). All experimental protocols were approved by and conducted in accordance with the recommendations of the Okayama University Animal Care and Use Committee (permit number OKU-2015660).

Blood pressure measurement: Systolic blood pressure was measured at 9 weeks by tail-cuff plethysmography (MK-2000; Muromachi, Japan; or BP-2000; Visitech Systems, Inc.). The average of three measurements was used.

Plasma and urine collection: At 15 weeks, rats were placed individually in metabolic cages to collect the urine over a period of 24 hours under the condition of feeding and water consumption. The rats were also anesthetized with isoflurane. Whole blood was collected from the abdominal aorta into a chilled tube. After centrifugation at 3000 rpm for 10 minutes at 4℃, serum and plasma were collected and stored at −80°C. Plasma glucose, insulin, serum total cholesterol, triglycerides, free fatty acids, urinary glucose, and electrolytes were measured at SRL, Inc (Tokyo, Japan).

Histological evaluation: The heart was fixed with 4% paraformaldehyde in phosphate buffered saline, embedded in paraffin, and cut into 5-μm-thick sections. Sections were stained with hematoxylin-eosin for morphological analysis and with Masson-trichrome for evaluation of fibrosis. The widths of 30 individual cardiomyocytes in each group were measured as previously described.[8-10] Perivascular fibrosis was measured and the percent of fibrosis was calculated using WinROOF Version 5.7 (MITANI Corporation, Fukui, Japan).

Quantitative real-time polymerase chain reaction analysis: For reverse transcription (RT)-polymerase chain reaction (PCR) analysis, RNA was extracted from cardiac tissue with RNeasy Mini Kit (Qiagen). The total RNA (2 μg) from each tissue sample was used to generate complementary DNA (cDNA) with ReverTra Ace (TOYOBO, Osaka, Japan). The cDNA was subjected to PCR with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA, USA) and predesigned gene-specific primer and probe sets (TaqMan Gene Expresso in Assays; Applied Biosystems). Quantitative real-time PCR was performed using the Applied Biosystems 7300 real-time PCR System (Applied Biosystems) as reported.[11] The PCR primers used were the following: natriuretic peptide A (*Nppa*), Rn00664637; natriuretic peptide B (*Nppb*), Rn 00646450; interleukin-6 (*Il6*), Rn01410330; transforming growth factor beta-1 (*Tgfb1*), Rn00572010; α-myosin heavy chain 6 (*Myh6*), Rn00691721; collagen type IV alpha-1 chain (*Col4a1*), Rn01482927; 3-hydroxybutyrate dehydrogenase 1 (*Bdh1*), Rn00588855; 3-oxoacid CoA-transferase 1 (*Oxct1*), Rn01402438; Acetyl-Coenzyme A acetyltransferase 1 (*Acat1*), Rn00567139 (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) was used as the internal control.

Metabolomic analysis: To determine the amount of 3-hydroxybutyric acid (3HBA), which is one of the ketone bodies, part of the left ventricle (LV) was frozen at −20℃. Frozen LV tissue (n = 3 in the DS-Control and DS-TOFO groups each) was plunged into 750 μL of 50% acetonitrile/Milli-Q water containing internal standards (Solution ID: 304-1002; Human Metabolome Technologies, Inc., Tsuruoka, Japan) at 0℃ in order to inactivate enzymes. The tissue was homogenized thrice at 1,500 rpm for 120 seconds using a tissue homogenizer (Micro Smash MS100R; Tomy Digital Biology Co., Ltd., Tokyo, Japan) and the homogenate was then centrifuged at 2,300 × g at 4℃ for 5 minutes. Subsequently, 800 μL of upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter at 9,100 × g and 4℃ for 120 minutes to remove proteins. The filtrate was centrifugally concentrated and re-suspended in 50 μL of Milli-Q water for CE-MS analysis. Metabolome measurements were carried out through a facility service at Human Metabolome Technologies Inc.

Statistical analysis: Statistical analysis was performed us-

Table I. Effects of TOFO on Physiological Parameters After Treatment for 9 Weeks

	High salt and high-fat diet				Normal diet	
	DR-Control	DR-TOFO	DS-Control	DS-TOFO	DR	DS
Number of rats	7	7	13	13	10	10
HW (g)	1.45 ± 0.02	1.32 ± 0.03**	1.65 ± 0.03	1.55 ± 0.02$^{\#}$	1.35 ± 0.02*	1.20 ± 0.02$^{\#\#}$
BW (g)	422 ± 6	405 ± 7	375 ± 6	383 ± 5	447 ± 8*	347 ± 5$^{\#}$
HW/BW (mg/g)	3.45 ± 0.07	3.25 ± 0.05*	4.43 ± 0.11	4.06 ± 0.06$^{\#\#}$	3.02 ± 0.04**	3.47 ± 0.05$^{\#\#}$
SBP (mmHg)	142 ± 1	140 ± 1	184 ± 1	180 ± 1$^{\#\#}$		
Heart rate (beats/minute)	377 ± 6	385 ± 8	386 ± 5	366 ± 9$^{\#}$		
Food intake (g/24 hours)	16 ± 1	15 ± 2	13 ± 1	13 ± 1		
Water intake (g/24 hours)	63 ± 3	80 ± 8	57 ± 8	58 ± 3		
Urine volume (mL/24 hours)	52 ± 3	71 ± 6*	45 ± 7	47 ± 2		

TOFO indicates tofogliflozin; DR, Dahl salt-resistant; DS, Dahl salt-sensitive; HW, heart weight; BW, body weight; and SBP, systolic blood pressure. Values are mean ± SE. *$P < 0.05$ versus DR-Control. **$P < 0.01$ versus DR-Control. $^{\#}P < 0.05$ versus DS-Control. $^{\#\#}P < 0.01$ versus DS-Control.

Table II. Effects of Tofogliflozin on Systolic Blood Pressure over 9 Weeks

	0 week	3rd week	6th week	9th week
DR-Control	125 ± 2	141 ± 2	142 ± 2	142 ± 1
DR-TOFO	126 ± 2	138 ± 1	140 ± 1	140 ± 1
DS-Control	131 ± 1	149 ± 1	164 ± 1	184 ± 1
DS-TOFO	130 ± 1	146 ± 2	162 ± 1	181 ± 1*

TOFO indicates tofogliflozin; DR, Dahl salt-resistant; and DS, Dahl salt-sensitive. Values are mean ± SE. *$P < 0.05$ versus DS-Control.

ing SPSS version 24 (IBM). All results are expressed as mean ± SE. The two-tailed Student's *t*-test for two groups and one-way analysis of variance followed by Tukey post-hoc test for more than two groups were used for the statistical analysis. *P*-values < 0.05 were considered significant.

Results

Effects of tofogliflozin on body weight, food and water intake, systolic blood pressure, heart rate, and heart weight in Dahl rats: At baseline, the mean body weights of the DR and DS rats were 187 ± 5 g ($n = 14$) and 191 ± 4 g ($n = 26$), respectively, and there was no significant difference between the groups. The body weight, heart weight, and heart-to-body weight ratio after 9 weeks of treatment are shown in Table I. There was no significant difference in body weight between the DR-Control and DR-TOFO groups, or between the DS-Control and DS-TOFO groups at 9 weeks after the start of the treatment with tofogliflozin, respectively. Systolic blood pressure was not different between the DS-Control and the DS-TOFO groups (131 ± 1 versus 130 ± 1 mmHg) at baseline. After treatment for 9 weeks, the systolic blood pressure was significantly lower in the DS-TOFO group compared with that in the DS-Control group (180 ± 1 versus 184 ± 1 mmHg, $P < 0.01$; Tables I, II). Tofogliflozin tended to suppress a rise of the systolic blood pressure, relative to the control, throughout the treatment period in both DR and DS rats, and significantly suppress a rise of the systolic blood pressure, relative to the control, at the 9 th week in DS rats (Table II). The heart rate was also significantly lower in the DS-TOFO group than in the DS-Control group (366 ± 9 versus 386 ± 5 beats/minute, $P < 0.05$; Table I). Also, the change in heart rate was not significantly different between the DR-Control and DR-TOFO groups. Heart weight and heart-body weight ratio were significantly lower in the DR-TOFO and DS-TOFO groups than in the DR-Control ($P < 0.05$) and DS-Control groups ($P < 0.01$). HW/BW were significantly higher in the DR-Control and DS-Control rats fed a high-salt and high-fat diet than in the DR and DS rats fed a normal diet. There was no difference in the food and water intake between the groups. The urine volume was significantly increased in the DR-TOFO group compared to that in the DR-Control group.

Effects of tofogliflozin treatment on serum and urine biochemical parameters in Dahl rats: As shown in Table III, after 9 weeks of treatment with tofogliflozin, the urinary glucose concentrations were significantly increased in the DR and DS rats ($P < 0.01$ respectively). Tofogliflozin significantly decreased plasma glucose ($P < 0.05$) in DS rats and also decreased urine sodium concentration and plasma insulin ($P < 0.01$) in DR rats. Tofogliflozin did not decrease urine albumin concentration, serum creatinine, triglycerides, free fatty acids, and total cholesterol in both DR and DS rats compared to the respective control groups.

Effects of tofogliflozin treatment on cardiac hypertrophy and fibrosis: As stated above (Table I), the heart weights were significantly decreased in the DR-TOFO and DS-TOFO groups compared to those in the DR-Control and DS-Control groups. Therefore, we performed histological analysis of the LV tissue in both DR and DS rats. As shown in Figure 2, hematoxylin and eosin staining after 9 weeks of treatment revealed that the cardiomyocyte widths were significantly reduced in the DR-TOFO and DS-TOFO groups compared to those in the DR-Control ($P < 0.05$) and DS-Control ($P < 0.01$) groups, respectively. Masson's trichrome staining showed that the areas of perivascular fibrosis were also significantly decreased in the DR-TOFO and DS-TOFO groups compared to those in the DR-Control ($P < 0.05$) and DS-Control groups ($P < 0.01$; Figure 3).

Effect of tofogliflozin treatment on cardiac gene expression in Dahl rats: Figure 4 shows the mRNA expression levels in the hearts of DR and DS rats. Tofogliflozin

Table III. Effects of TOFO on Blood and Urine Measurements After Treatment for 9 Weeks

	DR-Control	DR-TOFO	DS-Control	DS-TOFO
Urine glucose (mg/dL)	8 ± 2	2337 ± 252*	5 ± 4	889 ± 126##
Urine sodium (mEq/dL)	406 ± 9	272 ± 18*	353 ± 18	312 ± 16
Urine albumin (mg/dL)	64 ± 10	127 ± 36	164 ± 13	198 ± 17
Plasma glucose (mg/dL)	243 ± 11	217 ± 3	270 ± 6	244 ± 9#
Plasma insulin (mg/dL)	3.6 ± 0.3	2.6 ± 0.2*	3.0 ± 0.3	3.0 ± 0.4
Serum creatinine (mg/dL)	0.29 ± 0.01	0.28 ± 0.01	0.31 ± 0.02	0.30 ± 0.01
Total cholesterol (mg/dL)	72 ± 0	78 ± 2	87 ± 3	88 ± 2
Triglyceride (mg/dL)	241 ± 30	164 ± 22	247 ± 40	298 ± 60
Free fatty acid (mg/dL)	706 ± 46	544 ± 31	551 ± 22	514 ± 31

TOFO indicates tofogliflozin; DR, Dahl salt-resistant; and DS, Dahl salt-sensitive. Values are mean ± SE. *$P < 0.01$ versus DR-Control. #$P < 0.05$ versus DS-Control. ##$P < 0.01$ versus DS-Control.

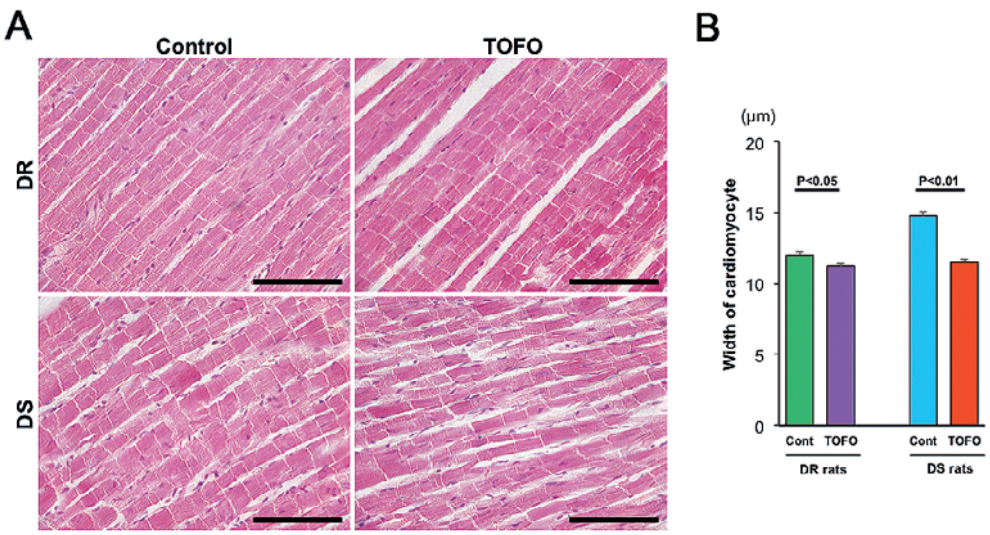

Figure 2. Effect of tofogliflozin on the cardiomyocyte size in Dahl rats fed a high-salt and high-fat diet. **A:** Hematoxylin and eosin staining of hearts in the vehicle and tofogliflozin treatment groups of DR and DS rats. The widths of 30 individual cardiomyocytes from each rat were measured across a line bisecting the nucleus. Scale bar = 100 μm. **B:** The bar graph shows the cardiomyocyte width. Values are mean ± SE. n = 7-13 each.

significantly decreased the mRNA levels of *Nppa* encoding natriuretic hormones in DS rats (Figure 4A (a) and (b)). Tofogliflozin significantly decreased the mRNA levels of *Nppb* encoding natriuretic hormones in both DR and DS rats (Figure 4B (a) and (b)). The expression levels of the inflammatory marker *Il6* were significantly decreased by tofogliflozin in the DS-TOFO group (Figure 4 C). The expression levels of genes that encode fibrotic markers, including *Tgfb1* and *Col4a1*, were significantly decreased in the DS-TOFO group (Figure 4D and E). The mRNA expression level of the gene that encodes Myh6, which is a myosin heavy chain isoform expressed in rodent hearts, was significantly increased in the DS-TOFO group (Figure 4F).

Metabolomic effect of tofogliflozin treatment on ketone oxidation in Dahl rats: Metabolomic analysis showed trends of metabolite changes in glycolysis (Figure 5A). Tofogliflozin decreased 3HBA, which is a ketone body, and increased adenosine triphosphate. Figure 5B shows the expression levels of *Bdh1*, *Oxct1*, and *Acat1*, which are related to ketone oxidation in the myocardium in DS and DR rats. The expression levels of *Bdh1* and *Oxct1* were significantly reduced in the DS-TOFO and DR-TOFO groups. The expression levels of *Oxct1* were also significantly reduced in DS-TOFO group.

Discussion

In this study, we demonstrated that treatment with an SGLT2 inhibitor, tofogliflozin, prevented LV hypertrophy caused by feeding an HS/HF diet in different types of rats. We also showed that tofogliflozin decreased the expression levels of genes related with cardiac hypertrophy, inflammation, fibrosis and ketone oxidation, indicating that tofogliflozin possibly changed the cardiac metabolism in rats fed a HS/HF diet.

Recently, several clinical trials have shown that SGLT2 inhibition significantly reduced cardiovascular death and heart failure hospitalization in type 2 diabetic patients at high risk for cardiovascular disease.[2,3,12] However, the underlying mechanism has remained to be elucidated. Empagliflozin significantly reduced cardiovascular

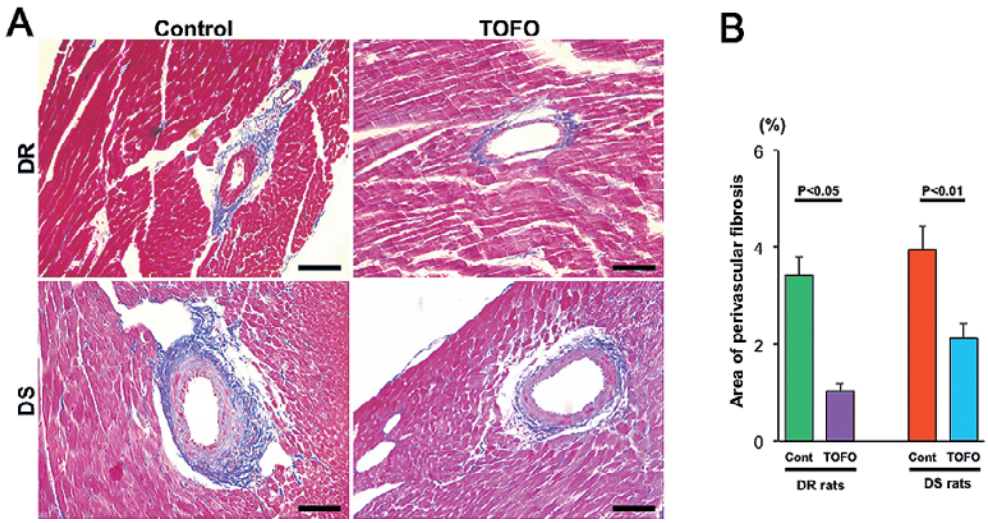

Figure 3. Effect of tofogliflozin on perivascular fibrosis in Dahl rats fed a high-salt and high-fat diet. **A:** Masson's trichrome staining of hearts in the control and tofogliflozin (TOFO) treatment groups of DR and DS rats. Scale bar = 100 µm. **B:** The bar graph shows the area of fibrosis (%) in each case. Values are mean ± SE. *n* = 7-13 each.

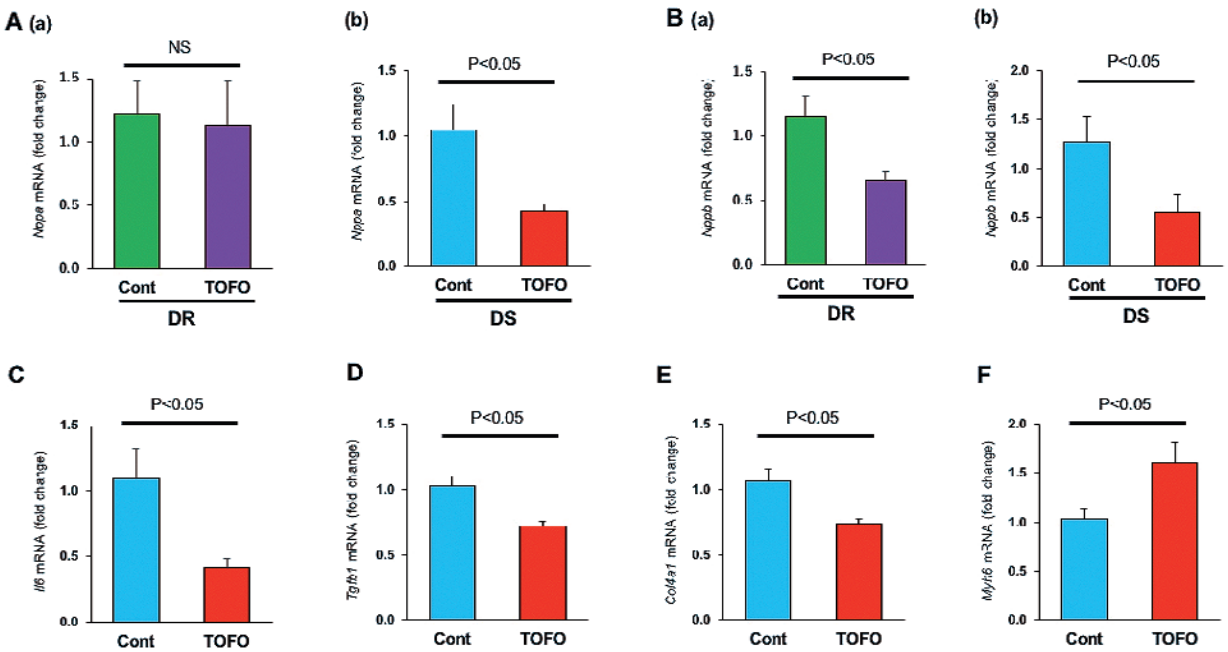

Figure 4. Cardiac gene expression in Dahl rats fed a high-salt and high-fat diet. Changes in the mRNA expression levels of natriuretic peptide **A** (*Nppa*) in DR rats (**A (a)**), in DS rats (**A (b)**), natriuretic peptide **B** (*Nppb*) in DR rats (**B (a)**), in DS rats (**B (b)**), interleukin 6 (*Il6*) (**C**), tissue growth factor beta 1 (*Tgfb1*) (**D**), collagen type IV alpha 1 chain (*Col4a1*) (**E**), myosin heavy chain 6 (*Myh6*) (**F**). The mRNAs were analyzed using quantitative real-time PCR. Cont indicates control. Values are mean ± SE; *n* = 5-9.

death and heart failure hospitalization in the EMPA-REG OUTCOME trial.[2] Canagliflozin was also shown to have similar cardioprotective effects in the CANVAS trial.[3] Thus, a cardioprotective effect was considered to be a class effect of SGLT2 inhibitors. A study showed that empagliflozin ameliorated cardiac hypertrophy and fibrosis in SHr/NDmcr-cp (+/+) rats, prediabetic model animals.[13] Another study showed that dapagliflozin ameliorated cardiac fibrosis in an infarct rat model.[14] Tofogliflozin is an SGLT2 inhibitor with high selectivity to SGLT2, and was shown to have effects similar to those of other SGLT2 inhibitors in rodent model.[4] Therefore, tofogliflozin showed a cardioprotective effect.

SGLT2 inhibition induced various effects such as lowering the blood pressure, promoting natriuresis, improving glycemic control, body weight loss, renal protective effect, and uricosuric effect.[15,16] SGLT2 inhibitors exhibit reduction in blood pressure, and the natriuretic effect of SGLT2 inhibitors has been considered to be the main mechanism. A previous study showed that urine volumes

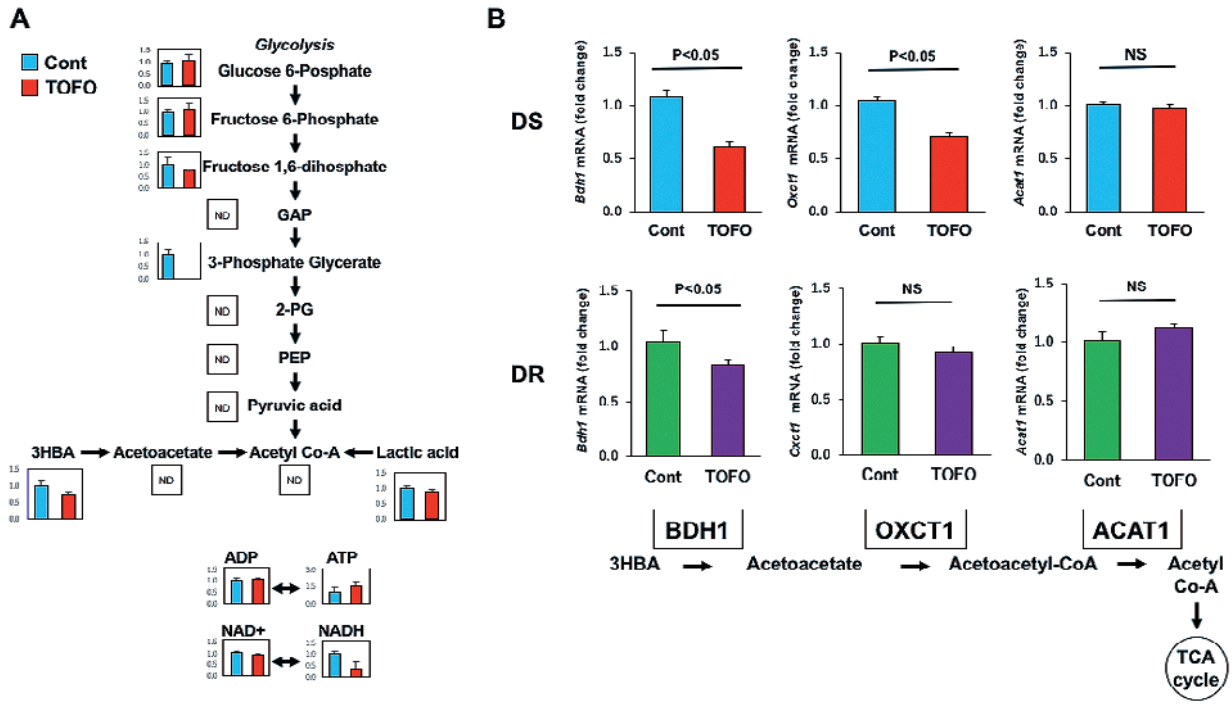

Figure 5. Metabolomic analysis of myocardial tissue in Dahl rats fed a high-salt and high-fat diet. **A:** Metabolomic analysis showing trends of metabolite changes in glycolysis. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; GAP, D-glyceraldehyde 3-phosphate; 3HBA, 3-hydroxybutyric acid; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ND, not detected; PEP, phosphoenolpyruvate; and PG, phosphatidylglycerol. $n = 3$. **B:** Expression of cardiac genes related to ketone oxidation in DS and DR rats fed a high-salt and high-fat diet. The changes in the mRNA expression levels of 3-hydroxybutyrate dehydrogenase 1 (*Bdh1*), 3-oxoacid CoA-transferase 1 (*Oxct1*), and acetyl-CoA acetyltransferase 1 (*Acat1*) were analyzed using quantitative real-time PCR. Values are mean ± SE; $n = 5$-9. NS indicates not significant.

return to baseline a few weeks after the initiation of treatment with SGLT2 inhibitors, though the blood pressure-lowering effects remained,[17] indicating that the diuretic and natriuretic effects are not the only mechanisms underlying the blood pressure-lowering effects. Moreover, there are data showing that the blood pressure-lowering effect of SGLT2 inhibitors persists in patients with reduced glomerular filtration rate, diuresis, and natriuresis, suggesting that there is a mechanism other than natriuresis.[18] In the present study, tofogliflozin did not increase the natriuretic activity, but tofogliflozin tended to decrease a rise of the systolic blood pressure, relative to the control, throughout the treatment period in both DR and DS rats, and significantly decreased a rise of the systolic blood pressure, relative to the control, at the 9th week in DS rats. Other mechanism rather than increase of natriuresis might play an important role in lowering the blood pressure in this study.

In this study, tofogliflozin decreased HW, HW/BW, and fibrosis in DS rats, and also in the DR rats. Although tofogliflozin significantly decrease a rise of the systolic blood pressure, relative to the control, only at the 9th week in DS rats, tofogliflozin tended to decrease a rise of the systolic blood pressure, relative to the control, throughout the treatment period even in DR rats, suggesting that lowering the blood pressure could have affect amelioration of cardiac hypertrophy and fibrosis in not only DS rats but also DR rats. Several studies showed the cardioprotective effect of SGLT2 inhibition in humans and rats; however, many of them targeted diabetes or metabolic syndrome.[2,3,13] In the present study, we used hypertensive model rats fed a high-fat diet. Tofogliflozin showed cardioprotective effects, suggesting that inhibition of SGLT2 could be effective also for the condition of hypertension without diabetes.

In this study, we used a diet containing 0.005% tofogliflozin. In several previous experiments, tofogliflozin was administered by feeding a diet containing 0.005% to 0.015% tofogliflozin.[19-21] Although the dose depends on the amount of food intake, the amounts of food intake were not different between the control and tofogliflozin groups for both the DR and DS rats. Thus, the dose of tofogliflozin was not different between groups.

Regarding the mechanisms underlying the protective effect of tofogliflozin in preventing HF/HS diet-induced LV hypertrophy in this study, one possible explanation is that tofogliflozin may suppress the activity of adrenergic nerves. A previous study showed that a high-fat diet induces adrenergic activation, leading to increases in blood pressure and heart rate.[22] In this study, systolic blood pressure and heart rate were significantly lowered after 9 weeks of treatment with tofogliflozin, although the blood insulin levels were not lowered in the DS rats, indicating that tofogliflozin ameliorated adrenergic activation without a glucose-lowering effect. Tofogliflozin decreased cardiac hypertrophy in both DR and DS rats, though tofogliflozin

significantly lowered the blood pressure and heart rate only in DS rats. On the other hand, tofogliflozin significantly decreased metabolic parameters such as plasma insulin in DR rats. The HS/HF diet increased the blood pressure to a much higher level in DS rats than in DR rats. These results indicated that tofogliflozin might have a cardioprotective effect in a different manner depending on its pathophysiological setting.

Several mechanisms underlying the cardioprotective effect of SGLT2 inhibition have been proposed. SGLT2 inhibitors increase the blood ketone body levels in humans and animals.[4, 23-26] Ketone bodies are emerging as potent anti-inflammatory molecules, and inflammation is a recognized risk factor for the development of cardiovascular events.[27] Shimazu, *et al*. recently revealed that beta-hydroxybutyrate could act as an inhibitor of histone deacetylases, leading to suppression of oxidative stress.[28] Interestingly, Aubert, *et al*. demonstrated that increased utilization of ketone occurs in failing hearts and hypertrophic hearts.[29] Moreover, Bedi, *et al*. reported that upregulation of the ketone oxidation pathway occurs in failing hearts.[30] Regarding enzymes involved in ketone oxidation, Uchihashi, *et al*. reported that the cardiac-specific *Bdh1* expression increased ketone body utilization and decreased oxidative stress, leading to an amelioration of the cardiac remodeling in failing heart.[31] In our study, metabolome analysis for myocardial tissue showed that tofogliflozin reduced 3HBA, a main ketone body. Moreover, the mRNA expression levels of *Bdh1* and *Oxct1*, which are related to ketone oxidation, were also significantly decreased. These results suggested that ketone utilization was decreased, leading to reduction of ketone oxidation. Taken together, the results indicate that tofogliflozin ameliorated cardiac hypertrophy, leading to reduction of ketone body oxidation in myocardial tissue.

Several investigators have reported that pro-inflammatory cytokines including tumor necrosis factor alpha and IL-6 induce cardiac hypertrophy.[32,33] Shi, *et al*. reported that higher concentration of 3HBA increased the expression levels of NF-kB-regulated inflammatory cytokines, namely those of the tumor necrosis factor alpha and IL-6, in hepatocytes.[34] In the present study, tofogliflozin also decreased the amount of 3HBA in the myocardium as well as the expression levels of *Il-6*. Decrease of higher levels of 3HBA might affect the expression levels of Il-6 in the myocardium. Further studies are needed to clarify this point.

There are several limitations in this study. First, the urine and serum ketone levels were not been measured. The circulating ketone levels may have affected the metabolism in the myocardium. Second, investigation for detailed renal function is insufficient. Although several items of urinalysis were performed, further study was needed to explain the effects of the changes in renal function on cardioprotective effect. Third, the mechanism underlying the inhibitory effect on cardiac hypertrophy was not fully elucidated.

In conclusion, tofogliflozin ameliorated cardiac hypertrophy and fibrosis and reduced ketone usage in myocardial tissue.

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Disclosure

Conflicts of interest: Nakamura K, Miyoshi T and Ito H received speaker honoraria from Kowa Pharmaceutical Co., Ltd.

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