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## DPP-4 inhibition with alogliptin on top of angiotensin II type 1 receptor blockade ameliorates albuminuria *via* up-regulation of SDF-1 $\alpha$ in type 2 diabetic patients with incipient nephropathy

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**Abstract.** Dipeptidyl peptidase-4 (DPP-4) inhibitor is a new class of anti-diabetic drug which exerts its glucose-lowering action by suppressing the degradation of a gut incretin hormone glucagon-like peptide-1 (GLP-1). To elucidate whether treatment with stronger DPP-4 inhibitor on top of angiotensin II type 1 receptor blocker (ARB) provides greater renal protective effects, we performed a crossover study with two DPP-4 inhibitors, sitagliptin and alogliptin, in twelve type 2 diabetic patients with incipient nephropathy taking ARBs. This study consisted of three treatment periods: sitagliptin 50 mg/day for 4 weeks (first period), alogliptin 25 mg/day for 4 weeks (second period), and sitagliptin 50 mg/day for 4 weeks (third period). Significant changes in body mass index, blood pressure, serum lipids, serum creatinine, estimated glomerular filtration rate, and HbA1c were not observed among the three treatment periods. Reduced urinary levels of albumin and an oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG), increased urinary cAMP levels, and elevated plasma levels of stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) which is a physiological substrate of DPP-4 were observed after the switch from sitagliptin to a stronger DPP-4 inhibitor alogliptin. Given a large body of evidence indicating anti-oxidative action of cAMP and up-regulation of cellular cAMP production by SDF-1 $\alpha$ , the present results suggest that more powerful DPP-4 inhibition on top of angiotensin II type 1 receptor blockade would offer additional protection against early-stage diabetic nephropathy beyond that attributed to glycemic control, *via* reduction of renal oxidative stress by SDF-1 $\alpha$ -cAMP pathway activation.

**Key words:** Diabetic nephropathy, DPP-4 inhibition, Glucagon-like peptide-1, Oxidative stress, SDF-1 $\alpha$

**DIPEPTIDYL PEPTIDASE-4 (DPP-4)** inhibitor is a new class of anti-diabetic drug which enhances circulating levels of a gut incretin hormone, glucagon-like peptide-1 (GLP-1), by inhibiting the degradation of GLP-1 [1]. GLP-1 stimulates insulin secretion from pancreatic  $\beta$ -cells in a blood glucose-dependent manner and suppresses glucagon release from pancreatic

$\alpha$ -cells, leading to amelioration of hyperglycemia [1, 2]. Based on such action mode, DPP-4 inhibitors have a low risk of hypoglycemia and are widely used for glycemic control in type 2 diabetic patients.

Recently, several experimental studies have suggested that DPP-4 inhibitors may exert the protective effects on diabetic kidney. For example, a DPP-4 inhibitor, vildagliptin, has been shown to ameliorate diabetic renal injury through reducing the production of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) in the kidneys of streptozotocin (STZ)-induced diabetic rats [3]. Similarly, other DPP-4 inhibitor, linagliptin, has been reported to improve renal oxida-

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tive stress and reduce albuminuria in STZ-induced diabetic mice [4]. In these experimental studies, it is noteworthy that the two DPP-4 inhibitors ameliorated diabetic nephropathy (DN) independent of their glucose-lowering effects. Actually, receptors for GLP-1 are expressed in kidney as well as pancreatic  $\beta$ -cells [5], although the physiological role of GLP-1 receptor activation in kidney has not been fully elucidated. Given these lines of evidence, it seems to be plausible that the elevation of circulating active GLP-1 levels by DPP-4 inhibition contributes to renal protection under hyperglycemic condition.

Stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) is a chemokine initially identified in bone marrow-derived stromal cells, and currently this chemokine is well-known to be expressed in various tissues [6-8]. It has been shown that SDF-1 $\alpha$  contributes to tissue repair by mediating migration of circulating stem cells or bone marrow-derived progenitor cells to tissue injury sites [9, 10]. In this regard, several experimental studies have demonstrated the anti-apoptotic effects of SDF-1 $\alpha$  in pancreatic  $\beta$ -cells of streptozotocin-induced diabetic mice [11] and ischemic injured kidneys of mice [12]. Thus, SDF-1 $\alpha$  plays a crucial role in protection of multiple organs including kidney. Importantly, SDF-1 $\alpha$  (active form SDF-1 $\alpha$  (1-68)) is a physiological substrate of DPP-4, and it is rapidly degraded to inactive form SDF-1 $\alpha$  (3-68) by the DPP-4 enzyme [13]. Actually, a recent clinical study has shown a significant elevation of plasma SDF-1 $\alpha$  levels by treatment with a DPP-4 inhibitor sitagliptin in type 2 diabetic patients [14]. Given these lines of evidence, it is conceivable that treatment with more powerful DPP-4 inhibitors may highly contribute to renal protection including albuminuria reduction in type 2 diabetic patients.

Following the introduction of sitagliptin in 2006, five DPP-4 inhibitors have been launched [15]. Allogliptin is a newly-developed DPP-4 inhibitor which has higher selectivity and stronger inhibitory activity for DPP-4 as compared with sitagliptin [15, 16]. Considering these properties of allogliptin for the DPP-4 enzyme, we hypothesized that stronger DPP-4 inhibition with this drug on top of angiotensin II type 1 receptor blockade may provide more beneficial effects on incipient DN independent of its glucose-lowering effects. To test this hypothesis, we performed a crossover study between sitagliptin and alogliptin in type 2 diabetic patients with incipient DN taking angiotensin

II type 1 receptor blockers (ARBs), and investigated the alterations of albuminuria, oxidative stress, and circulating SDF-1 $\alpha$ .

## Subjects and Methods

### *Subjects and study protocol*

Twelve microalbuminuric type 2 diabetic patients who had been treated with sitagliptin 50 mg/day for more than 4 weeks were recruited. Persistent microalbuminuria (incipient DN) was defined as a urinary albumin-to-creatinine ratio between 30 and 300 mg/g creatinine in two morning spot urine collections performed over 3 months. The patients were under treatment with sulfonylurea,  $\alpha$ -glucosidase inhibitor, metformin, and thiazolidine in addition to sitagliptin, and all of them had been given angiotensin II type 1 receptor blockers (ARBs), telmisartan or valsartan. These anti-diabetic and anti-hypertensive drugs except DPP-4 inhibitors were unchanged during the study. This study consisted of three treatment periods: sitagliptin 50mg/day for 4 weeks (first period; baseline), alogliptin 25 mg/day for 4 weeks (second period), and sitagliptin 50mg/day for 4 weeks (third period). After being recruited, the patients continued taking sitagliptin 50mg/day for 4 weeks. Thereafter, the patients were crossed over to the treatment with alogliptin 25 mg/day for 4 weeks, and then to the treatment with sitagliptin 50mg/day for 4 weeks, without a wash-out period. The study protocol of crossover treatment between sitagliptin and alogliptin is summarized in Fig. 1. The measurements of biochemical parameters and oxidative stress marker were performed at the end of each treatment period. The study protocols were approved by the Ethics Committees of Akita University and Honjo Daiichi Hospital. Written informed consent was obtained from all participants.

### *Measurements*

Blood and urine samples were collected in the morning after an overnight fast. HbA1c values were converted to National Glycohemoglobin Standardization Program (NGSP) equivalent values as calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society [JDS]) + 0.4%. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and cAMP levels were measured using 8-OHdG Check ELISA kit (Nikken Seil, Fukuroi, Shizuoka, Japan) and direct cAMP enzyme immunoassay kit (Arbor Assays, Ann Arbor, MI, USA),

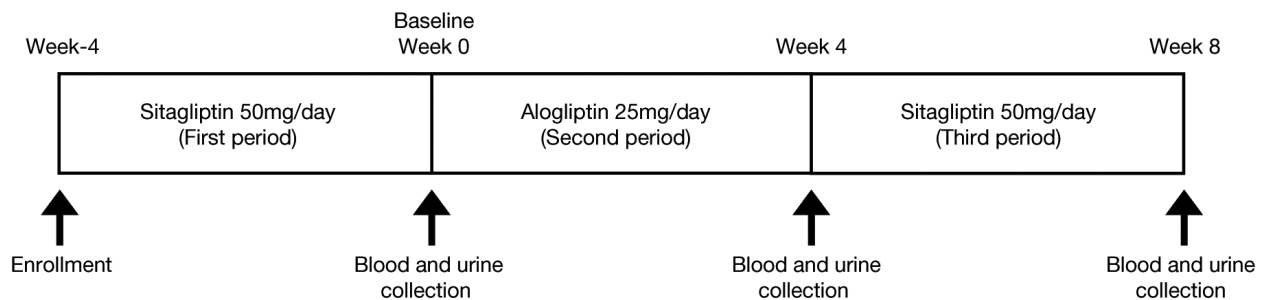
respectively. Plasma SDF-1 $\alpha$  levels were determined by ELISA using human CXCL12/SDF-1 $\alpha$  immunoassay kit (R&D Systems, Minneapolis, MN, USA).

### Statistical analysis

All data were presented as means  $\pm$  SD. Statistical analysis was performed using GraphPad Prism software (GraphPad, San Diego, CA, USA). Data were analyzed by nonparametric Friedman's test followed by Dunn's multiple comparison test.  $P < 0.05$  was considered statistically significant.

## Results

Table 1 shows biochemical and physiological data at enrollment and the end of each treatment period. Significant changes in body mass index, blood pressure, serum lipids, serum creatinine, and estimated glomerular filtration rate (GFR) were not observed after the crossover treatment from sitagliptin to alogliptin and from alogliptin to sitagliptin. It is noteworthy that HbA1c values were not significantly altered by the crossover treatment with sitagliptin and alogliptin. This finding indicates that these two DPP-4 inhibitors exert similar effects on blood glucose reduction.



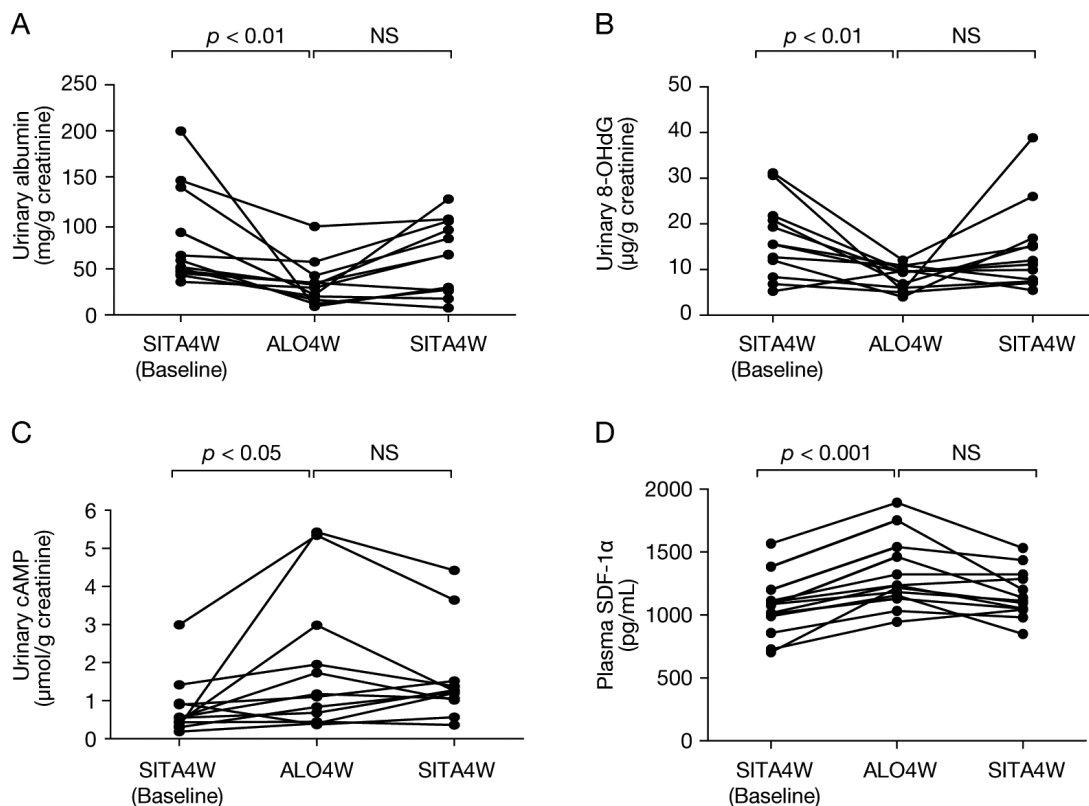
**Fig. 1** Study protocol of crossover treatment between sitagliptin and alogliptin

**Table 1** Changes in clinical parameters by crossover treatment with sitagliptin and alogliptin in type 2 diabetic patients with incipient nephropathy

Parameter	Enrollment	Sitagliptin 4W	Alogliptin 4W	Sitagliptin 4W
n	12			
Age (years)	65 $\pm$ 9			
Gender (male/female)	9/3			
Body mass index (kg/m <sup>2</sup> )	24.6 $\pm$ 4.1	24.5 $\pm$ 4.1	24.6 $\pm$ 4.2	24.6 $\pm$ 4.1
Systolic blood pressure (mmHg)	123 $\pm$ 12	121 $\pm$ 11	120 $\pm$ 9	120 $\pm$ 6
Diastolic blood pressure (mmHg)	73 $\pm$ 6	72 $\pm$ 6	71 $\pm$ 8	69 $\pm$ 9
HbA1c (%)	7.0 $\pm$ 0.4	7.0 $\pm$ 0.4	6.9 $\pm$ 0.4	7.0 $\pm$ 0.5
LDL-cholesterol (mg/dL)	94.6 $\pm$ 24.0	94.9 $\pm$ 27.3	92.9 $\pm$ 25.9	92.3 $\pm$ 23.9
HDL-cholesterol (mg/dL)	50.3 $\pm$ 13.2	48.8 $\pm$ 11.1	50.5 $\pm$ 12.6	50.1 $\pm$ 12.4
Triglyceride (mg/dL)	107 $\pm$ 51	112 $\pm$ 41	123 $\pm$ 81	112 $\pm$ 62
Serum creatinine (mg/dL)	0.87 $\pm$ 0.20	0.87 $\pm$ 0.21	0.87 $\pm$ 0.21	0.84 $\pm$ 0.19
Estimated GFR (mL/min/1.73 m <sup>2</sup> )	66.2 $\pm$ 9.3	66.1 $\pm$ 10.2	64.7 $\pm$ 8.4	65.8 $\pm$ 8.0
ARBs (telmisartan/valsartan)	9/3			
OHAs (SU/ $\alpha$ GI/Met/TZD)	12/4/4/2			

Data are presented as means  $\pm$  SD or number.

LDL, low-density lipoprotein; HDL, High-density lipoprotein; GFR, glomerular filtration rate; ARBs, angiotensin II type 1 receptor blockers; OHAs, oral hypoglycemic agents; SU, sulfonylurea;  $\alpha$ GI, alpha glucosidase inhibitor; Met, metformin; TZD, thiazolidine.



**Fig. 2** Changes in levels of urinary albumin (A), 8-OHdG (B), and cAMP (C) and plasma SDF-1 $\alpha$  (D) by crossover treatment between sitagliptin and alogliptin. NS, not significant; SITA, sitagliptin; ALO, alogliptin

The changes in albuminuria were assessed at the end of each treatment period in this crossover study. Urinary levels of albumin were significantly reduced after the switch from sitagliptin to alogliptin ( $81.0 \pm 52.4$  vs.  $33.9 \pm 23.9$  mg/g creatinine; Fig. 2A). It is noteworthy that these changes in albuminuria were observed despite comparable levels of HbA1c, blood pressure, and serum lipids between the treatment periods.

To elucidate the mechanism underlying albuminuria reduction by alogliptin treatment, we next examined the effects of alogliptin on systemic oxidative stress. In addition, we investigated the changes in renal production of cAMP which is an important second messenger on the GLP-1 receptor signaling pathway and also the alterations in plasma levels of SDF-1 $\alpha$  which is an important physiological substrate of DPP-4 enzyme after the switch from sitagliptin to alogliptin. The degree of systemic oxidative stress was evaluated by determining urinary 8-OHdG levels. Interestingly, urinary 8-OHdG levels were significantly decreased after the crossover from sitagliptin to alogliptin, indicating that alogliptin provides stronger anti-oxidative

effects as compared with sitagliptin (Fig. 2B). Renal cAMP production was assessed by measuring urinary cAMP levels. Notably, urinary cAMP levels were significantly increased after the change from sitagliptin to alogliptin (Fig. 2C). Furthermore, a significant elevation of plasma SDF-1 $\alpha$  levels was observed after the switch from sitagliptin to alogliptin (Fig. 2D). Collectively, these results suggest that more powerful and selective inhibition for DPP-4 by alogliptin treatment may contribute to the amelioration of systemic and renal oxidative stress induced by chronic hyperglycemia and offer renal protective effects, possibly *via* enhancing circulating SDF-1 $\alpha$  levels and increasing renal cAMP production.

## Discussion

The aim of the present study was to investigate whether greater DPP-4 inhibition provides renal protective effects in type 2 diabetic patients with incipient DN. To eliminate the effects of DPP-4 inhibitors on kidney *via* their glucose-lowering action, we per-

formed a crossover study using two DPP-4 inhibitors, sitagliptin and alogliptin, which have a different degree of selectivity and inhibition activity for DPP-4 in type 2 diabetic patients with incipient DN.

In the present study, sitagliptin and alogliptin showed a comparable degree of glucose-lowering effects, as evidenced by similar HbA1c levels at the end of each treatment period. Moreover, the differences in other biochemical and physiological parameters such as serum lipids, body weight, and blood pressure were not observed between the sitagliptin and alogliptin treatment periods. Importantly, the reduction of albuminuria was observed by the switch from sitagliptin to alogliptin. This finding may be explained by the differences in selectivity and inhibition activity for DPP-4 between sitagliptin and alogliptin. Actually, alogliptin is reported to have higher selectivity and stronger inhibitory activity for DPP-4 as compared with sitagliptin [15, 16]. The powerful DPP-4 inhibition by alogliptin is expected to provide two effects in body. One is an elevation of circulating active GLP-1, and the other effect is an increase in circulating SDF-1 $\alpha$ . In the present study, we could not measure plasma active GLP-1 levels. In this regard, unexpectedly, a recent crossover study between sitagliptin and alogliptin reported that higher plasma active GLP-1 levels were observed in the treatment period with sitagliptin than alogliptin despite comparable levels of HbA1c [17]. Although the reason is unknown, this finding does not support the concept that further elevation of circulating GLP-1 by the switch from sitagliptin to alogliptin contributes to albuminuria reduction. On the other hand, we clearly demonstrated an increase in circulating SDF-1 $\alpha$  by the crossover from sitagliptin to alogliptin in the present study. Collectively, the increase in circulating levels of SDF-1 $\alpha$  rather than GLP-1 may be involved in the reduction of albuminuria observed after the switch from sitagliptin to alogliptin.

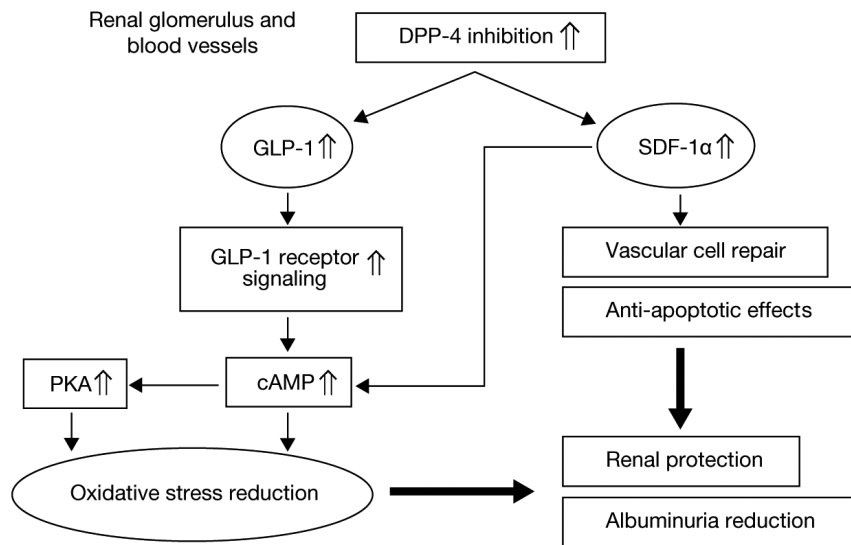
In the present study, urinary cAMP excretion was increased by the switch from sitagliptin to alogliptin. It is well known that cAMP is abundantly produced in kidney [18]. In addition, estimated GFR values did not significantly alter by the switch from sitagliptin to alogliptin. Therefore, it is plausible that increased urinary cAMP excretion in the alogliptin-treated period possibly results from enhanced renal cAMP production, but not from elevated clearance of serum cAMP through the kidney. Various cellular signaling pathways are involved in cAMP production. Interestingly,

the results from recent experimental studies indicate that SDF-1 $\alpha$  induces an increase in cellular cAMP production and activates a cAMP-mediated signaling pathway [19, 20]. Given these lines of evidence, it is conceivable that the increase in urinary cAMP levels after the switch from sitagliptin to alogliptin were triggered mainly by SDF-1 $\alpha$  action.

cAMP is an important second messenger on various signal transduction pathways, and also works as the primary effector of GLP-1-induced insulin secretion from pancreatic  $\beta$ -cells [21-23]. Recent experimental studies have reported that cAMP reduces reactive oxygen species (ROS) including superoxide anion (O<sub>2</sub><sup>-</sup>) and exerts anti-oxidative effects by inhibiting the major source of superoxide anion, NAD(P)H oxidase [24, 25]. Similarly, our study showed a reduction in urinary levels of an oxidative stress marker 8-OHdG in parallel with the increase in urinary cAMP levels after the switch from sitagliptin to alogliptin, suggesting the contribution of increased renal cAMP to the amelioration of renal oxidative stress.

Oxidative stress induced by superoxide anion excess is considered a major cause of DN [26-29]. We have recently reported that renal oxidative stress is highly produced in the incipient DN stage, but not the normoalbuminuric stage, and that the incipient DN stage is more suitable than other stages for antioxidative intervention [30]. Treatment with DPP-4 inhibitors which have higher selectivity and stronger inhibitory activity for DPP-4 is expected to contribute greatly to the amelioration of renal oxidative stress and albuminuria in patients with incipient DN. ARBs are widely used for the amelioration of proteinuria (albuminuria) in patients with DN, and provide more beneficial effects especially in incipient DN stage as shown in the INNOVATION study [31, 32]. A large body of evidence from experimental studies and our clinical study suggests that ARBs exert greater anti-oxidative effects and ameliorate diabetic and oxidative renal injury [30, 33-35]. Moreover, a recent experimental study has reported that DPP-4 inhibition on top of angiotensin II type 1 receptor blockade provides more powerful renoprotective effects through highly reducing oxidative stress in diabetic kidney [4]. Similarly, the present clinical study demonstrates that stronger DPP-4 inhibition adding to treatment with ARBs would be considerably beneficial for renal protection in the incipient DN stage.

Finally, our data support a model for an intra-renal



**Fig. 3** Proposed DPP-4 inhibition signal transduction pathway in kidney

signaling pathway following DPP-4 inhibition as summarized in Fig. 3. The present study verifies a renal protective role of DPP-4 inhibition on top of angiotensin II type 1 receptor blockade in incipient DN stage independent of its glucose-lowering effects. In addition, the present study illustrates that more powerful DPP-4 inhibition contributes to reduction of albuminuria possibly *via* up-regulation of circulating SDF-1 $\alpha$ . However, there are some limitations related to the patient number and treatment term in the present study. Therefore, large-population and long-term studies would be required to clarify whether DPP-4 inhibition offers a new therapeutic approach for patients with incipient DN.

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## Disclosure Summary

The authors declare no conflict of interest.

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