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CLINICAL TRIAL

Circulating selenoprotein P levels predict glucose-lowering and insulinotropic effects of metformin, but not alogliptin: A *post-hoc* analysis

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ABSTRACT

Aims/Introduction: Selenoprotein P (SeP; encoded by *SEPP1* in humans) is a hepatokine that causes impaired insulin secretion and insulin resistance. Metformin downregulates *SELENOP* promoter activity through an adenosine monophosphate-activated kinase–forkhead box protein O3a pathway in hepatocytes. This study aimed to test our hypothesis that circulating SeP levels are associated with the glucose-lowering effect of metformin in humans.

Materials and Methods: A total of 84 participants with poorly controlled type 2 diabetes were randomly assigned to receive metformin (1,000 mg, twice daily) or a dipeptidyl peptidase-4 inhibitor, alogliptin (25 mg, once daily) for 12 weeks. We tested metformin and alogliptin on SeP levels and factors associated therewith as a *post-hoc* analysis.

Results: Both metformin and aloglipitin did not change the SeP levels. Although metformin significantly increased the insulin secretory index secretory units of islets in transplantation only in participants with higher baseline SeP (>3.87), both agents similarly reduced fasting plasma glucose and glycated hemoglobin. SeP levels at baseline were correlated negatively with changes in SeP (r = –0.484, P = 0.004) and fasting plasma glucose (r = –0.433, P = 0.011), and positively with changes in C-peptide immunoreactivity (r = 0.420, P = 0.017) and secretory units of islets in transplantation (r = 0.388, P = 0.028) in the metformin, but not alogliptin, group.

Conclusions: Higher baseline levels of SeP significantly predicted metformin-mediated, but not alogliptin-mediated, glucose-lowering and insulinotropic effects. Serum SeP levels might be a novel biomarker for predicting the outcomes of metformin therapy, which might be helpful in tailoring diabetes medication.

INTRODUCTION

Large-scale clinical trials found that intensive antidiabetic therapies causing hyperinsulinemia, hypoglycemia and weight gain were associated with poor cardiovascular outcomes in participants with type 2 diabetes[1]. Treatment with incretin-based agents and metformin might prevent unnecessary hyperinsulinemia. Several clinical trials have shown that metformin and dipeptidyl peptidate-4 inhibitors are well tolerated, and produce sustained glycemic control. We had previously compared the pleiotropic effects of metformin and alogliptin for 12 weeks on various parameters, including body composition, insulin secretion, cardiovascular parameters, serum fatty acid levels and treatment satisfaction, in participants with poorly controlled type 2 diabetes[2]. Accordingly, both

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metformin and alogliptin significantly reduced glycated hemoglobin (HbA1c) levels and fasting plasma glucose (FPG), and significantly elevated 1,5-anhydroglucitol and insulin secretory index[2].

Metformin has proven beneficial in treating non-alcoholic fatty liver disease, polycystic ovary syndrome, cardiovascular disease and cancer, in addition to type 2 diabetes[3,4]. However, clinical trials have recognized that certain patients do not respond to metformin[5]. Although interindividual drug response and disposition could be influenced by various factors, such as nature of the disease and organ function, estimates indicate that genetics can account for 20–95% of the variability in responses to the same medication[6]. Metformin is transported to the liver through organic cation transporter 1 (*OCT1*)[7]. Several non-synonymous polymorphisms of *OCT1* have been found to reduce reduced metformin uptake and activity[8]. However, *OCT1* gene polymorphisms are unique to white people, and have not been identified in Japanese people[9].

The liver plays a central role in glucose homeostasis, and produces various bioactive secretory proteins, termed hepatokines[10]. Selenoprotein P (SeP; encoded by *SELENOP* in humans) is a hepatokine, the expression of which is correlated with post-challenge glucose levels[11] and peripheral insulin resistance[12]. SeP eliminates reactive oxygen species required for signal transduction[13], and thereby causes pathology similar to type 2 diabetes, such as insulin resistance[12], insulin secretory failure[14], exercise resistance[16] and angiogenesis resistance[15]. Serum SeP levels increase during aging[17], as well as in individuals with type 2 diabetes[12] and non-alcoholic fatty liver disease[18]. Elevated serum SeP levels have been shown to predict the onset of insulin secretory failure and hyperglycemia in a human health checkup cohort[17]. Therefore, SeP might be a potential diagnostic and therapeutic target against type 2 diabetes and its complications. Given that metformin downregulates *SEPP1* promoter activity through an adenosine monophosphate-activated kinase–forkhead box protein O3a pathway in rat hepatocytes[19], circulating SeP levels could perhaps predict the glucose-lowering and insulinotropic effects of metformin in humans. The present study, therefore, tested this hypothesis by investigating the significance of circulating SeP levels in the pleiotropic effects of metformin and a dipeptidyl peptidase-4 inhibitor in participants with type 2 diabetes as the *post-hoc* analysis.

MATERIALS AND METHODS

Study overview

Among the 84 participants of the UMIN000010385 trial as the *post-hoc* analysis, only those who had a complete set of data regarding SeP (n = 71) were considered in the present subanalysis. Individuals (n = 13) without SeP values and glucose levels were excluded from this analysis (Figure S1). Details regarding the study have been described previously[2]. In brief, the main eligibility criteria were as follows: age >20 years; diagnosed with type 2 diabetes mellitus; HbA1c >6.5% within 12 weeks of screening; and undergoing any combination of diet therapy, oral hypoglycemic therapy and insulin therapy for ≥12 weeks. The full eligibility criteria have been discussed in the previously published paper[2].

Participants were randomly assigned to receive metformin or alogliptin treatment at a 1:1 ratio using a computer-generated randomization sequence. Dynamic randomization was used to adjust for demographic differences (HbA1c and age) between treatment groups. In this active-comparator, parallel-group trial, eligible participants received metformin or alogliptin in conjunction with their current treatment for 12 weeks. Metformin (Sumitomo Pharma Co. Ltd., Osaka, Japan) was started at 1,000 mg (500 mg tablets, twice daily) and adjusted at the discretion of the attending physicians. Alogliptin (Takeda Pharmaceutical Co. Ltd., Tokyo, Japan) was started and maintained at 25 mg once daily.

Outcomes and measurements

Body composition (using an InBody 720 analyzer; Biospace Co. Ltd., Seoul, Korea), clinical (weight, body mass index) and laboratory data (FPG, HbA1c, insulin secretory index) were measured at baseline and after 12 weeks of intervention. Secretory units of islets in transplantation (SUIT) was defined as 1,485 × fasting CPR (ng/mL) / (FPG [mg/dL] − 61.8)[20,21]. As we previously reported, plasma SeP levels were measured using a sol particle homogeneous immunoassay[22]. We assessed plasma levels of full-length SeP selectively by using two types of SeP monoclonal antibodies, one recognizing the N-terminal domain of SeP and another recognizing the C-terminal domain[22,23].

Statistical analysis

The Mann–Whitney *U*-test was used for intragroup comparisons, and the Wilcoxon signed-rank test was used for intergroup comparisons. Associations between variables were assessed using Spearman's rank correlation coefficient. The results were expressed as the median (interquartile range). Baseline concomitant medication was analyzed using the χ^2-test (or Fisher's exact test) in the intergroup comparison. IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) was used to carry out the statistical tests, with P-values <0.05 showing statistical significance.

RESULTS

Clinical outcomes

Baseline demographics and disease characteristics including concomitant medication did not significantly differ between the groups (Table S1). Although both metformin and alogliptin did not alter SeP levels, both agents significantly and similarly reduced HbA1c. Metformin, but not alogliptin, significantly elevated SUIT. The median baseline SeP was 3.87. Participants were then stratified into two groups (Q1, >3.87%; Q2, ≤3.87%) according to baseline SeP levels to determine their influence on glucose metabolism (Table 1). Baseline clinical characteristics

Table 1 | Treatment effects of metformin or alogliptin on glucose metabolism according to baseline selenoprotein P

	Metformin				Alogliptin				P**	P***
	Week 0	Week 12	P*	Changes from baseline	Week 0	Week 12	P*	Changes from baseline		
ALL (Met *n* = 34, Alo *n* = 37)										
Selenoprotein P	4.02 (3.53–4.48)	4.06 (3.64–4.41)	0.443	0.07 (–0.21 to 0.25)	3.77 (3.30–4.25)	3.80 (3.40–4.27)	0.213	0.05 (–0.18 to 0.23)	0.179	0.356
FPG (mg/dL)	139.5 (119.8–174.5)	130.0 (106.5–154.5)	0.004	–15.0 (–35.3 to 0.0)	142.5 (124.5–176.5)	131.0 (118.0–166.8)	0.088	–7.5 (–28.5 to 6.8)	0.275	0.433
HbA1c (%)	6.95 (6.53–7.85)	6.55 (6.23–7.28)	0.003	–0.40 (–0.93 to 0.10)	7.30 (6.90–7.93)	6.80 (6.50–7.40)	<0.001	–0.35 (–0.70 to 0.20)	0.755	0.351
CPR (ng/mL)	1.60 (1.23–2.70)	1.75 (1.13–3.00)	0.514	0.10 (–0.48 to 0.58)	1.95 (1.10–2.75)	2.00 (1.30–2.78)	0.126	0.00 (–0.17 to 0.60)	0.632	0.564
SUIT	28.9 (16.0–58.4)	43.4 (21.7–79.0)	0.019	7.0 (–0.4 to 16.3)	35.4 (18.5–61.0)	38.8 (22.0–68.4)	0.183	3.1 (–1.5 to 8.8)	0.310	0.321
High baseline SeP (SeP >3.87) (Met *n* = 19, Alo *n* = 16)										
Selenoprotein P	4.34 (4.11–4.82)	4.29 (4.08–4.94)	0.256	–0.00 (–0.34 to 0.26)	4.33 (3.98–4.72)	4.27 (4.00–4.73)	0.641	–0.01 (–0.23 to 0.14)	0.427	0.758
FPG (mg/dL)	142.0 (126.3–199.8)	124.5 (109.5–162.5)	0.007	–26.0 (–79.0 to 5.0)	151.5 (132.5–174.3)	127.0 (112.8–166.8)	0.013	–14.0 (–30.8 to 1.5)	0.367	0.981
HbA1c (%)	7.00 (6.60–7.93)	6.65 (6.30–7.35)	0.031	–0.30 (–1.05 to 0.08)	7.25 (6.93–8.15)	6.75 (6.60–7.55)	0.006	–0.30 (–0.58 to 0.20)	0.341	0.355
CPR (ng/mL)	1.45 (1.28–2.23)	1.70 (1.10–2.85)	0.092	0.40 (–0.43 to 0.83)	2.15 (1.53–3.30)	2.50 (1.70–3.57)	0.126	0.10 (–0.25 to 0.68)	0.613	0.237
SUIT	22.7 (10.8–54.0)	42.1 (20.1–72.7)	0.031	9.9 (0.8 to 21.3)	39.2 (19.2–61.0)	59.7 (25.4–70.1)	0.291	3.7 (–0.8 to 22.7)	0.297	0.847
Low baseline SeP (SeP ≤3.87) (Met *n* = 15, Alo *n* = 21)										
Selenoprotein P	3.50 (3.24–3.69)	3.62 (3.32–3.77)	0.624	0.11 (–0.15 to 0.26)	3.37 (3.16–3.64)	3.41 (3.24–3.78)	0.064	0.09 (–0.13 to 0.33)	0.411	0.998
FPG (mg/dL)	135.0 (117.5–152.3)	128.5 (104.0–132.8)	0.291	–14.0 (–19.3 to 2.8)	134.5 (121.5–176.5)	133.0 (118.3–171.8)	0.677	–5.5 (–22.3 to 13.5)	0.751	0.319
HbA1c (%)	6.95 (6.50–7.63)	6.30 (6.10–7.08)	0.037	–0.50 (–0.80 to 0.18)	7.30 (6.75–7.93)	6.80 (6.23–7.30)	<0.001	–0.40 (–1.08 to 0.13)	0.458	0.577
CPR (ng/mL)	2.00 (1.15–4.00)	1.85 (1.18–3.23)	0.047	–0.10 (–0.53 to 0.10)	1.45 (0.93–2.45)	1.80 (1.00–2.18)	0.461	0.00 (–0.18 to 0.58)	0.067	0.831
SUIT	37.0 (22.8–111.3)	49.9 (23.8–85.4)	0.374	4.9 (–1.1 to 8.5)	28.1 (18.5–59.8)	27.0 (21.7–44.6)	0.434	3.1 (–3.3 to 7.7)	0.982	0.262

Data at baseline, week 12 and changes from baseline are presented as median (interquartile range). **P*-values for intragroup comparison (baseline vs 12 weeks); ***P*-values for intergroup comparison (changes from baseline between groups). ****P*-values for intragroup comparison (12 weeks). Alo, alogliptin; CPR, C-peptide immunoreactivity; Met, metformin; SUIT; secretory units of islets in transplantation.

Table 2 | Factors associated with baseline selenoprotein P and changes in selenoprotein P

	Baseline selenoprotein P						Changes in selenoprotein P					
	Metformin		Alogliptin		ALL		Metformin		Alogliptin		ALL	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
SU use	−0.051	0.774	−0.125	0.461	−0.091	0.449	0.298	0.087	−0.094	0.581	0.172	0.152
Insulin use	−0.335	0.053	0.037	0.830	−0.149	0.215	0.210	0.233	−0.224	0.182	0.003	0.981
ΔSeP	−0.484	0.004	−0.198	0.241	−0.387	0.001						
ΔFPG	−0.433	0.011	−0.213	0.206	−0.306	0.009	0.207	0.241	0.196	0.245	0.191	0.110
ΔHbA1c	−0.102	0.568	0.138	0.416	0.013	0.912	0.272	0.120	−0.031	0.854	0.126	0.293
ΔCPR	0.420	0.017	0.003	0.988	0.189	0.123	−0.098	0.595	−0.054	0.755	−0.067	0.588
ΔSUIT	0.388	0.028	0.162	0.345	0.284	0.019	−0.168	0.357	−0.231	0.175	−0.173	0.159
ΔBodyweight	0.115	0.524	0.207	0.233	0.096	0.437	−0.158	0.380	−0.017	0.922	−0.095	0.440
ΔBMI	0.139	0.441	0.114	0.513	0.054	0.664	−0.175	0.330	−0.064	0.717	−0.127	0.301
ΔFat mass	−0.102	0.570	0.277	0.107	0.013	0.915	0.106	0.557	0.078	0.655	0.117	0.341
ΔAST	−0.011	0.997	−0.081	0.634	−0.023	0.848	0.108	0.544	0.047	0.784	0.050	0.677
ΔALT	0.017	0.925	0.105	0.534	0.074	0.541	−0.036	0.839	0.083	0.625	0.004	0.971
ΔsdLDL	−0.042	0.827	−0.017	0.921	−0.009	0.944	0.016	0.931	0.093	0.590	0.083	0.508

BMI, body mass index; CPI, C-peptide immunoreactivity index; CPR, C-peptide immunoreactivity; FPG, fasting plasma glucose; sdLDL, small dense low-density lipoprotein; SeP, selenoprotein P; SU, sulfonylurea.

including baseline SeP were similar in both groups (Table S1). Among participants with higher baseline SeP levels (Q1), metformin significantly elevated the insulin secretory index SUIT, whereas both agents similarly reduced FPG and HbA1c. Among participants with lower baseline SeP (Q2), both metformin and alogliptin did not alter FPG and SUIT, but significantly and equally reduced HbA1c levels.

Factors associated with baseline SeP levels and changes in SeP levels

Univariate analysis was carried out to determine the relationships between baseline SeP levels (SeP0) and various clinical factors (Table 2). The concomitantly used drugs, body composition, liver enzymes and lipid levels were not associated with SeP0 in either group. SeP0 levels were positively correlated with changes in CPR in the metformin group, but not in the alogliptin group and all (Table 2). SeP0 levels were significantly negatively correlated with changes in SeP (ΔSeP; ΔSeP = 1.17–0.3 × SeP0, $r = -0.484$, $P = 0.004$) and ΔFPG (ΔFPG = 96.6–29.1 × Sep0, $r = -0.433$, $P = 0.011$; Table 2, Figure S2) and positively correlated with changes in CPR (ΔCPR; ΔCPR = −1.88 + 0.48 × SeP0, $r = 0.420$, $P = 0.017$) and SUIT (ΔSUIT; ΔSUIT = −81.8 + 23.9 × SeP0, $r = 0.388$, $P = 0.028$) in the metformin, but not alogliptin, group (Table 2, Figure S2). No association was observed between change in SeP and changes in FPG, CPR, CPI and SUIT in both groups (Table 2).

DISCUSSION

The present study is the first to address whether circulating SeP levels could predict metformin-mediated hypoglycemic and insulin secretory effects in participants with type 2 diabetes based on our previous *SELENOP* promoter study[19]. We previously reported that metformin and alogliptin significantly reduced HbA1c and FPG[2]. Given that *OCT1* gene polymorphisms that determine metformin transport and activity are unique to white people and have not been identified in Japanese people[9], our Japanese cohort allows for the identification of additional predictors for metformin. However, clinical parameters, such as concomitantly used drugs, body composition, liver function, and dyslipidemia, did not predict the glucose-lowering effects of metformin and alogliptin[2]. The present study found that metformin significantly reduced FPG in individuals with higher baseline circulating SeP levels. Furthermore, baseline SeP levels were associated with a reduction in SeP and FPG in the metformin, but not the alogliptin group. The aforementioned findings suggest the possible involvement of SeP in metformin-mediated glucose-lowering and insulinotropic effects.

Selenoprotein P impairs both insulin secretion[14] and insulin action in the skeletal muscle and liver[12]. In our previous human health checkup cohort, SeP levels at baseline correlated positively with fasting and post-glucose challenge glucose levels, and negatively with initial insulin secretion after glucose challenge[17]. Metformin lowers glucose mainly by reducing hepatic glucose production[24] while also enhancing insulin secretion[2]. Indeed, metformin can be effective in participants with type 2 diabetes who have lower homeostasis model assessment of β-cell function[25]. The present study found that metformin elevated SUIT in participants with higher baseline circulating SeP levels, and that baseline SeP levels were associated with reduction in SeP and elevation in CPR and SUIT in the metformin, but not the alogliptin, group. These findings support the hypothesis that metformin reduces SeP levels and thereby elevates insulin secretion and reduces glucose. However, changes in SeP levels were not significantly associated with alterations

in glucose and insulin levels. Japanese patients with type 2 diabetes have a relatively slender physique compared with Westerners. Thus, the mean metformin dose used in the present study was 1,076 ± 430 mg/day, which seems relatively lower compared with those used in the studies on white people[26,27]. Higher metformin doses might further clarify the association between changes in SeP and metformin effects. The present study at least concludes that higher SeP levels can predict metformin-mediated glucose-lowering and insulinotropic effects. Monitoring circulating SeP levels might provide an opportunity for personalized medicine in participants with type 2 diabetes.

The present study had some limitations. This study was a *post-hoc* analysis of comparing metformin and alogliptin for 12 weeks on various parameters in participants with poorly controlled type 2 diabetes[2]. We found that metformin significantly reduced FPG in participants with higher baseline circulating SeP levels. However, SeP levels did not change under the metformin intervention. Such discrepancies might be attributed to the short study duration and the small number of participants. Because this trial was a pilot exploring study, long-term larger-scale trials are required to confirm the present findings and establish evidence of the metformin–SeP axis mediating glucose-lowering and insulinotropic effects.

In summary, higher baseline levels of SeP can significantly predict metformin-, but not alogliptin-mediated glucose-lowering and insulinotropic effects. The serum levels of SeP might be a novel biomarker to predict the outcomes of metformin therapy, which might be helpful in the individualized diabetes treatment.

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DISCLOSURE

The authors declare no conflict of interest.

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Registry and the registration no. of the study/trial: UMIN000010385.

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Patient flow.

Figure S2 | Relationships between baseline selenoprotein P (SeP) and the changes in SeP, fasting plasma glucose (FPG), C-peptide immunoreactivity (CPR) and secretory units of islets in transplantation (SUIT).

Table S1 | Baseline characteristics of the participants according to baseline selenoprotein P.