Abstract

Background

The malnutrition-inflammation-atherosclerosis syndrome is an important prognostic factor for hemodialysis (HD) patients. Linagliptin is a bile-excreted dipeptidyl peptidase (DPP)-4 inhibitor, and therefore, dose reduction is not necessary for HD patients. The anti-inflammatory effects of linagliptin were reported in HD patients, and linagliptin may also prevent systemic atherosclerosis. Advanced glycation end products (AGEs)-receptor for AGEs (RAGE) signals as well as inflammation are important and well-known atherosclerotic factors. In this study, we examined the influence of linagliptin on multiple RAGE ligands to further investigate the anti-atherosclerotic mechanism of linagliptin in HD patients.

Methods

Twenty HD patients (16 men) with diabetes with inadequate glycemic control (glycated albumin [GA] levels > 20%) following diet and exercise therapy were eligible to participate in the present study. The mean patient age was 65.5 ± 2.8 years. The patients received once-daily administrations of linagliptin (5 mg). The following parameters of efficacy were examined before treatment, and 1, 3 and 6 months after treatment: S100A12/EN-RAGE, CML, Glycer-AGE and MG levels were measured using corresponding enzyme-linked immunosorbent assay kits.

Results

After 6 months of linagliptin treatment, S100A12 levels significantly decreased from 220.3±38.5 ng/mL at baseline to 94.2±11.5 ng/mL, CML levels significantly increased from 0.05 ± 0.01 μg/mL to 0.21 ± 0.03 μg/mL, and Glycer-AGE levels significantly increased from 0.49 ± 0.06 μg/mL to 1.15 ± 0.12 μg/mL. There were no changes in MG levels after starting linagliptin. No patients exhibited hypoglycemia or any other significant adverse effects during this study.

Conclusion

Linagliptin had a variety of effects on multiple RAGE ligands that are known atherosclerotic factors. It is important that future studies continue to further investigate the anti-atherosclerotic mechanism of linagliptin.

Keywords: Linagliptin; Hemodialysis; S100A12/EN-RAGE; CML; Glycer-AGE; MG.

Introduction

The malnutrition-inflammation-atherosclerosis syndrome (MIA syndrome) is an important prognosis-related factor for hemodialysis (HD) patients [1]. Diabetes mellitus induces end-stage renal disease and also promotes the atherosclerosis and inflammation of the MIA syndrome. Although insulin injections are central to therapy in HD patients, failure of eyesight from diabetic retinopathy and aging-associated dementia can make multiple daily insulin injections impossible [2]. Moreover, in HD patients, many oral antidiabetic drugs induce critical side effects such as hypoglycemia and lactic acidosis. Therefore, there is a need for new oral antidiabetic drugs with fewer side effects for HD patients. Dipeptidyl peptidase-4 (DPP-4) inhibitors are well tolerated and have a lower incidence of hypoglycemia and a good safety profile in HD patients [3,4].

Seven types of DPP-4 inhibitors have been released. There are almost no differences in the anti-diabetic effects of each DPP-4 inhibitors, and the adaptations of each DPP-4 inhibitors are unclear. However, it is important to use each seven DPP-4 inhibitors properly depending on the condition of the patients. For this reason, we must fully understand the pharmacological actions of DPP-4 inhibitors respectively, and further analysis is essential.

The anti-diabetic drug linagliptin is the only bile-excreted DPP-4...
inhibitor; therefore, a reduced dose is not necessary for HD patients with diabetes. Linagliptin therapy reduces the risk of cardiovascular and cerebrovascular diseases, which are related to systemic atherosclerosis [5,6]. Therefore, linagliptin therapy is also expected to prevent systemic atherosclerosis. Systemic atherosclerosis in HD patients is more severe than in patients with non-chronic kidney disease; therefore, understanding the anti-atherosclerotic mechanism is especially more important for HD patients with diabetes.

Inflammation, dyslipidemia, endothelial dysfunction, and AGEs-RAGE signals are important famous and well-known atherosclerotic factors. We previously reported that linagliptin has the anti-inflammatory effects of linagliptin in HD patients with diabetes [7]. However, glycation is the most notable factor involved in atherosclerosis in the study of diabetes. We did not examine the impacts of glycation with linagliptin, to our knowledge, no study has examined the impact of linagliptin monotherapy on AGEs-RAGE signals in HD patients with diabetes. Furthermore, there was no research which investigated the impacts of the multiple RAGE ligands with different effects by linagliptin monotherapy treatment at the same time in HD patients with diabetes.

This study is an extension and a more thorough follow-up of the previous study. We examined the influence of linagliptin on multiple RAGE ligands to further investigate the anti-atherosclerotic mechanism of linagliptin in HD patients with diabetes.

Methods

Patients

Twenty HD patients (16 men) with diabetes with inadequate glycemic control (glycated albumin [GA] levels > 20%) following diet and exercise therapy were eligible to participate in the present study. The mean patient age was 65.5 ± 2.8 years. Of these 20 patients, 4 had a prior history of insulin therapy, 8 were taking other oral antidiabetic drugs, and 8 had been treated with both insulin and other oral antidiabetic drugs. However, their therapy had been discontinued before or after initiation of maintenance dialysis therapy. Thus, at the start of the study, no patient was using any prescribed oral antidiabetic drug or receiving insulin injections. As a result, each patient’s glycemic control had gradually become inadequate. During the treatment period, the patients received once-daily administration of linagliptin (5mg). In addition, no patients were taking non-steroidal anti-inflammatory drugs or allopurinol. The ethics committee of Saiyu Soka Hospital study approved this study, and informed consent was obtained from each patient.

Efficacy

The following parameters of efficacy were examined before and after 1, 3, and 6 months of treatment: S100A12/extracellular newly identified RAGE binding protein (EN-RAGE)], a RAGE ligand; Ne-(carboxymethyl) lysine (CML); glyceraldehydes-derived AGEs (Glycer-AGE); and methylglyoxal (MG).

Safety Assessments

The safety assessment involved monitoring all adverse events. Hypoglycemia events were assessed by blood glucose measurements. As a countermeasure for possible hypoglycemia, a 24-hour treatment support system was made available to the patients; this system enabled them to receive immediate treatment if they experienced symptoms of potential hypoglycemia. Moreover, all HD patients with diabetes underwent capillary glucose monitoring before commencing HD treatment. Hematological and blood biochemical assessments were performed regularly, and vital signs and physical condition were assessed regularly.

Blood samples

Blood samples were taken from the arterial side of the arteriovenous fistula before the start of HD treatment. This study was conducted using the same blood samples of the patients examined in our previous report [5]. Blood collection was performed 1–2 hours before eating. Caution was exercised to prevent hemolysis of the blood samples; plasma was obtained by centrifugation and was stored at −70°C.

Measurement of GA Levels and Multiple Ligands Levels of RAGE

The serum concentration of S100A12 was measured using a commercially available quantitative sandwich ELISA kit (CircuLex S100A12/EN-RAGE ELISA, CycLex Co., Ltd., Nagano, Japan), according to the manufacturer’s instructions. This kit is employs the quantitative sandwich enzyme immunoassay technique. The monoclonal antibody of S100A12 restrains the S100A12 protein which exists in a sample. Furthermore, the antibody which combined specific HRP is added. The remaining conjugation is allowed to react by substrate H2O2-tetramethlbenzidine. The absorbance which arises as a result of a reaction is measured. CML levels were measured with an OxiSelect™ Nε-(carboxymethyl) lysine(CML) ELISA Kit (Cell Biolabs, Inc., San Diego, CA, USA). The quantity of CML adduct in protein samples is determined by comparing its absorbance with that of a known CML-BSA standard curve.

Glycer-AGE levels were measured with a Glyceraldehyde origin AGE ELISA (Selista Inc. Tokyo, Japan). This kit used the enzyme immunoassay method. The measurement principle of Glyceraldehyde origin AGE concentration used the immunity reaction which arises by scrambling for Glyceraldehyde origin AGE and an enzyme labeled antibody. MG levels were measured with an OxiSelect™ Methylglyoxal (MG) ELISA Kit (Cell Biolabs, Inc., San Diego, CA, USA). This kit is an enzyme immunoassay developed for detection and quantitation of MG-H1 (methyl-glyoxal-hydro-imidazolone) protein adducts. The quantity of MG adduct in protein sample is determined by comparing its absorbance with that of a known MG-BSA standard curve.

Statistical Analysis

JMP (version 10) statistical software (SAS Institute, Cary, NC, USA) was used to conduct all statistical analyses. All results are presented as means ± standard error values. Significance was tested using the paired t-test; P values of < 0.05 were considered statistically significant.
statistically significant.

**Results**

Baseline characteristics of the 20 patients are shown in Table 1. Each patient had received treatment for type 2 diabetes prior to the start of maintenance dialysis therapy; the mean treatment period was 10.4 ± 2.1 years. The mean duration of maintenance dialysis was 6.0 ± 1.9 years. Six patients were smokers, 9 were on antiplatelet drugs, 2 were on vasodilator drugs, 6 were on HMG-CoA reductase inhibitors, and 3 patients were on angiotensin receptor blockers. Dialysis treatment was conducted 3 times per week in 4-hour sessions. During HD treatment, the blood flow was 201.0 ± 4.9 mL/min (range, 160–250 mL/min), quantity of dialysate was 515.0 ± 10.9 mL/min (range, 500–700 mL/min), dialysis time was 4.1 ± 0.1 hours (range, 4–4.5 hours), membrane area was 1.8 ± 0.1 m² (range, 1.5–2.1 m²), and total glucose concentration in the dialysates was 100 mg/dL.

**Table 1: Baseline patient characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>65.5 ± 2.8</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>16</td>
</tr>
<tr>
<td>Diabetes treatment period (years)</td>
<td>10.4 ± 2.1</td>
</tr>
<tr>
<td>Dialysis treatment period (years)</td>
<td>6.0 ± 1.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 0.8</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>6</td>
</tr>
<tr>
<td>Antiplatelet drugs (n)</td>
<td>9</td>
</tr>
<tr>
<td>Vasodilator drugs (n)</td>
<td>2</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors (n)</td>
<td>6</td>
</tr>
<tr>
<td>Angiotensin receptor blockers (n)</td>
<td>3</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dL)</td>
<td>55.8 ± 3.6</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein (mg/dL)</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

HMG-CoA = hydroxymethylglutaryl coenzyme A

**Efficacy**

$S100A12(EN-RAGE)$ levels significantly decreased from the 3 through 6 month, decreasing from 220.3 ± 38.5 ng/mL at baseline to 94.2 ± 11.5 ng/mL after 6 months of linagliptin treatment (Figure 1). CML levels significantly increased from 0.05 ± 0.01 μg/mL at baseline to 0.21 ± 0.03 μg/mL 6 months after starting linagliptin (Figure 2). Glycer-AGE levels significantly increased from the 3 through 6 month, increasing from 0.49 ± 0.06 μg/mL at baseline to 1.15 ± 0.12 μg/mL after 6 months linagliptin treatment (Figure 3). There was no change in MG levels after starting linagliptin treatment (Figure 4).

**Figure 1:** $S100A12(EN-RAGE)$ levels before and after starting linagliptin. $S100A12$ levels significantly decreased from the 3 through 6 month after starting linagliptin treatment. Values are expressed as mean ± standard error of the mean (SEM). *: significant difference (P < 0.05).

**Figure 2:** CML (Nε-carboxymethyl lysine) levels before and after starting linagliptin. CML levels significantly increased 6 months after starting linagliptin treatment. Values are expressed as mean ± SEM. *: significant difference (P < 0.05).

**No Adverse Effects of Linagliptin Treatment**

Hypoglycemia is a potential side effect of diabetes therapy, although a meta-analysis of clinical trial data revealed that only a small number of hypoglycemic events were associated with vildagliptin and sitagliptin treatment (other DPP-4 inhibitors) [8]. However, over the 6-month course of the present study, no patients exhibited hypoglycemia or any other significant adverse effects.

**Discussion**

Some studies have examined whether treatment with incretin-related drugs, including DPP-4 inhibitors, prevents atherosclerosis and cardiovascular events [5,6]. However, few studies have examined the impact of linagliptin monotherapy treatment on
atherosclerotic factors in HD patients with diabetes. Inflammation and AGEs-RAGE signals are important factors which promote atherosclerosis. We previously reported linagliptin has the anti-inflammatory effects of linagliptin in HD patients with diabetes. However, we did not also examine the impacts of glycated albumin, which is an intermediate glycation product, significantly decreased with increased levels of active glucagon-like peptide-1 levels in HD patients following linagliptin [7]. In this study, CML and Glycer-AGE increased with linagliptin therapy. However, although CML is one of the AGEs, it does not bind to RAGE [15]. Therefore, CML itself may not promote atherosclerosis. If the levels of other AGEs increase, CML may play a role in avoiding the promotion of atherosclerosis through an averting pathway [16]. On the other hand, Glycer-AGE promotes atherosclerosis. Glycer-AGE originates in the side pathway of glycolysis within the cell, and is released into the blood [17]. These results, which indicate that CML and Glycer-AGE increased despite the decreased S100A12 levels with linagliptin, may be due to the differences in synthetic locations and metabolic pathways for each RAGE ligand.

This study has several important limitations that should be considered when interpreting our results. First, the present study did not include a control group and only evaluated a small number of participants. However, as none of the patients were receiving diabetic drugs before or after the initiation of maintenance dialysis therapy, any improvement that we observed in the parameters was likely related to the linagliptin treatment. Second, only four AGE receptor ligands were examined. Therefore, we hope to evaluate the effects of linagliptin on other AGE receptor ligands, such as

Interestingly, we observed that S100A12 levels decreased significantly after linagliptin treatment. S100A12 is abundantly expressed and secreted from leucocytes (neutrophils, monocytes, or macrophages) [9], is a ligand for the AGE receptor [10], and acts as a potent chemo attractant. When S100A12 binds to the AGE receptor on the endothelium of mononuclear phagocytes and lymphocytes, it triggers cellular activation by generating proinflammatory cytokines, such as interleukin 6, through NF-kB activation; this process may further promote the underlying inflammation in atherosclerosis [11]. Furthermore, significant associations have been observed between increased plasma S100A12 concentrations and the prevalence of cardiovascular disease and all-cause mortality in HD patients [11-13]. Interestingly, as DPP-4 (CD26) is expressed on the membranes of leucocytes, and S100A12 is abundantly expressed and secreted from leucocytes, the suppression of DPP-4 might be involved in the decreased S100A12 levels that we observed. Other DPP-4 inhibitors, as with linagliptin, might decrease S100A12 levels. However, there is no study which is examined the S100A12 levels with other DPP-4 inhibitors treatment. In a clinical trial, when diabetic patients were given a single daily dose of linagliptin (5mg), the DPP-4 inhibition percentages were >80% for 24 hours after treatment. Compared to other DPP-4 inhibitors, linagliptin shows stronger inhibitory activity and selectivity for DPP-4 [14].

In our previous research, the levels of glycated albumin, which is an intermediate glycation product, significantly decreased with increased levels of active glucagon-like peptide-1 levels in HD patients following linagliptin [7]. In this study, CML and Glycer-AGE increased with linagliptin therapy. However, although CML is one of the AGEs, it does not bind to RAGE [15]. Therefore, CML itself may not promote atherosclerosis. If the levels of other AGEs increase, CML may play a role in avoiding the promotion of atherosclerosis through an averting pathway [16]. On the other hand, Glycer-AGE promotes atherosclerosis. Glycer-AGE originates in the side pathway of glycolysis within the cell, and is released into the blood [17]. These results, which indicate that CML and Glycer-AGE increased despite the decreased S100A12 with linagliptin, may be due to the differences in synthetic locations and metabolic pathways for each RAGE ligand.

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3-deoxy-D-erythro-hexos-2-ulose.

In conclusion, linagliptin had a variety of effects on multiple RAGE ligands that are known atherosclerotic factors. These effects may be related to the anti-atherosclerotic effects of linagliptin. It is important that future studies continue to further investigate the anti-atherosclerotic mechanism of linagliptin.

**Summary**

**Sub-headings:** The variety RAGE ligand effects by linagliptin

The malnutrition-inflammation-atherosclerosis syndrome is an important prognostic factor for hemodialysis (HD) patients. Linagliptin is a bile-excreted dipeptidyl peptidase (DPP)-4 inhibitor, and therefore, dose reduction is not necessary for HD patients. We previously reported that linagliptin has the anti-inflammatory effects of linagliptin in HD patients, and linagliptin may also prevent systemic atherosclerosis. Advanced glycation end products (AGEs)-receptor for AGEs (RAGE) signals as well as inflammation are important and well-known atherosclerotic factors. However, no study has examined the impact of linagliptin monotherapy on AGEs-RAGE signals in HD patients with diabetes. Furthermore, there was no research which investigated the impacts of the multiple RAGE ligands with different effects by linagliptin monotherapy treatment at the same time in HD patients with diabetes. We examined the influence of linagliptin on multiple RAGE ligands to further investigate the anti-atherosclerotic mechanism of linagliptin in HD patients with diabetes. 20 HD patients (16 men) with diabetes with inadequate glycemic control (glycated albumin [GA] levels > 20%) following diet and exercise therapy were eligible to participate in the present study. The patients received once-daily administrations of linagliptin (5mg). The following parameters of efficacy were examined before treatment, and 1, 3 and 6 months after treatment: S100A12 [extracellular newly identified RAGE binding protein (EN-RAGE)]; Ne-(carboxymethyl) lysine (CML); glyceraldehyde-derived AGEs (Glycer-AGE); and methylglyoxal (MG). S100A12, which promotes atherosclerosis as for the ligand for RAGE, levels significantly decreased after 6 months of linagliptin treatment. Meanwhile, CML and Glycer-AGE levels significantly increased after 6 months of linagliptin treatment. These results may be due to the differences in synthetic locations and metabolic pathways for each RAGE ligand. Linagliptin had a variety of effects on multiple RAGE ligands that are known atherosclerotic factors. It is important that future studies continue to further investigate the anti-atherosclerotic mechanism of linagliptin.

**References**


