Background: Dipeptidyl peptidase 4 (DPP-4, also known as CD26) binds with adenosine deaminase (ADA) to activate T lymphocytes. Here, we investigated whether ADA activity is specifically affected by treatment with DPP-4 inhibitor (DPP4I) compared with other anti-diabetic agents.

Methods: Fasting ADA activity, in addition to various metabolic and biochemical parameters, were measured in 262 type 2 diabetes mellitus (T2DM) patients taking various anti-diabetic agents and in 46 non-diabetic control subjects.

Results: ADA activity was increased in T2DM patients compared with that in non-diabetic control subjects (mean±standard error, 23.1±0.6 U/L vs. 18.6±0.8 U/L; \( P < 0.05 \)). ADA activity was correlated with fasting plasma glucose (\( r = 0.258, P < 0.05 \)), HbA1c (\( r = 0.208, P < 0.05 \)), aspartate aminotransferase (\( r = 0.325, P < 0.05 \)), and alanine aminotransferase (\( r = 0.248, P < 0.05 \)). Compared with the well-controlled T2DM patients (HbA1c<7%), the poorly controlled group (HbA1c>9%) showed significantly increased ADA activity (21.1±0.8 U/L vs. 25.4±1.6 U/L; \( P < 0.05 \)). The effect of DPP4I on ADA activity in T2DM patients did not differ from those of other oral anti-diabetic agents or insulin. T2DM patients on metformin monotherapy showed a lower ADA activity (20.9±1.0 U/L vs. 28.1±2.8 U/L; \( P < 0.05 \)) compared with that of those on sulfonylurea monotherapy.

Conclusion: Our results show that ADA activity is increased in T2DM patients compared to that in non-diabetic patients, is positively correlated with blood glucose level, and that DPP4I has no additional specific effect on ADA activity, except for a glycemic control- or HbA1c-dependent effect.

Keywords: Adenosine deaminase; Diabetes mellitus, type 2; Dipeptidyl peptidase

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) [1,2], an incretin, promotes insulin secretion in a glucose concentration-dependent manner in pancreatic beta cells [3], inhibits glucagon secretion in alpha cells [4], decreases the gastric discharge rate [5], and mediates appetite suppression [6]. However, because GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), oral DPP-4 inhibitor (DPP4I) drugs and GLP-1 analogues have been developed to overcome the GLP-1 degradation effect in the treatment of diabetes [7].

DPP-4, also known as CD26 or adenosine deaminase (ADA) binding protein, is a cellular membrane ectopeptidase in the prolyl oligopeptidase family [8,9]. Mammalian endothelial and epithelial cells generally express DPP-4; it is particularly abundantly expressed in the intestines, bone marrow, kidney, and liver. The enzymatic function of this molecule is not limited to direct action upon various substrates; it is also involved...
in the regulation of cellular functions through interactions with various extracellular substrates [8, 10]. DPP-4 is also expressed in the cells of the immune system, especially in T cells, in which it interacts with other signal transduction pathways (CD3) and acts as a co-stimulator of T cell (particularly CD4+ T cell); the promotion of T-cell responses to foreign antigens, initial signal transduction, increased cytokine secretion, promotion of cell proliferation, increased expression of active T-cell markers (CD25, CD71, and CD69), promotion of effector cell differentiation, increased cellular mobility, and many other actions [8-10]. After new anti-diabetic drugs that selectively inhibit DPP-4 were introduced and administered to diabetic patients, there were several reports that DPP4I might increase the incidences of some infectious diseases (e.g., nasopharyngitis and urinary tract infection), so further experimental and clinical studies are needed to determine the effects of DPP-4 on immune cell function [11-13]. One alleged side effect of DPP-4 inhibition is the nonspecific inhibition of DPP-8 and DPP-9. However, according to a recent study, high doses of vildagliptin, producing nearly complete inhibition of DPP-8 and DPP-9 in vivo, yielded no toxicities in rodents [14]. Therefore, further studies are required regarding the side effects of DPP4Is.

DPP-4 can also bind with ADA. Since ADA degrades adenosine, which inhibits the proliferation of T cells, this interaction of DPP-4 with ADA and the rearrangement of ADA on cell membrane can lead to the increase in T-cell proliferation and cytokine synthesis due to ADA activity on the cell membrane [15-17].

ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction [18]. Previous studies have reported that the highest ADA activity was observed in the lymphoid and fatty tissues, liver, skeletal muscle, and heart, although the activity was widely distributed in most organs [19,20]. An increase in ADA activity in type 2 diabetic (T2DM) patients has been reported [21-23]. While the mechanism that increases serum and tissue ADA activity is not well known, with higher ADA activity in insulin-sensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced [24]. Thus, if ADA activity is suppressed, insulin sensitivity may be improved, and cellular proliferation, inflammation, and T-cell activity, all of which are associated with the pathophysiology of insulin resistance, can also be affected. Therefore, insulin resistance may have an important relationship with ADA activity. However, it is difficult to conclude whether changes in ADA activity are the cause or result of actual insulin resistance [25,26]. In addition to its association with diabetes, serum ADA activity is also increased in patients with liver cirrhosis as well as in patient with infectious diseases such as hepatitis, tuberculosis, brucellosis, and typhoid fever [27-28].

Studies of the many functions of DPP-4, particularly those related to T-cell function, were performed prior to the development of the DPP-4 selective inhibitor. As such, some of those studies used non-selective DPP inhibitors with low specificity and could have non-specific study results due to inhibition of other isoforms of DPPs in addition to DPP-4 [14,29].

Since ADA activity is associated with T-cell activity and insulin resistance and can bind with DPP-4, in the present study, we measured serum ADA activity in T2DM patients to evaluate the relationship between serum ADA activity and various clinical and metabolic parameters including inflammatory markers and to check if selective DPP4I affect ADA activity in T2DM patients.

METHODS

Subjects
The measurement of ADA activity was performed in patients with type 2 diabetes (T2DM, n=262) who were outpatients or who were hospitalized to control their blood glucose levels. All of the patients were on their oral anti-diabetic medications or insulin injection for more than one month. Standard biochemical, metabolic, and anthropometric measurements were also performed in the subjects. Non-diabetic healthy subjects who visited our hospital for health screenings or standard medical examinations were selected as the control group (Non-DM, n=46). Patients with type 1 diabetes, systemic infectious diseases, systemic inflammatory diseases, viral or alcoholic liver disease, advanced cardiovascular disease or other acute diseases, pregnant women, and patients with a glomerular filtration rate less than 60 mL/min/1.73 m² were excluded from this study.

After receiving a summary of the purpose of this study, the participants provided consent, and this study was conducted after approval from the Jeju National University Hospital Institutional Review Board (IRB No. #2009-11).

Methods
Examinations
Blood and urine tests were performed after at least eight hours
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of fasting, and microalbumin tests were calibrated to be measured with urinary creatinine concentration (urine albumin to creatinine ratio [UACR], µg/g of creatinine). For blood cell calculation tests, total lymphocytes were calculated using complete blood cell count. High sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rates (ESR) were measured as inflammation markers. Serum ADA activity was measured using UV spectrophotometry (Asan Pharmaceutical Co., Seoul, Korea). C-peptides were measured using a radioimmunoassay. Glycated hemoglobin (hemoglobin A1c, HbA1c) was measured using ion exchange HPLC (Tosoh Co., Tokyo, Japan).

**Statistical analysis**
All technical data were expressed as means±standard errors, and statistical analysis was performed using the SPSS statistical program version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Clinical features in the control group and in T2DM patients were analyzed using independent sample t-tests, and Pearson's bivariate correlation analysis was used to correlate each variable with ADA activity. We performed multivariate regression analysis using log-substituted ADA activity as the dependent variable. Additionally, T2DM patients were classified into smaller groups based on their glycemic control level, diabetes treatment medications, and liver function, and the differences in ADA activity among these smaller groups were also analyzed. Fasting plasma glucose (FPG) and HbA1c data was used to classify patients into three groups. The difference in ADA activities among the three groups was analyzed by using a one-way ANOVA test. The comparison of ADA activities according to diabetes treatment medication was performed using a one-way ANOVA test. The diabetic patients were divided into three groups as follows: the DPP4i group who were taking DPP4i and metformin combination therapy, the other oral hypoglycemic agent group who were taking metformin and other oral anti-diabetic agent(s) except for DPP4i, and the insulin treatment group (insulin combination therapy or insulin monotherapy groups). To clearly evaluate the efficacy of the DPP4i, the differences in ADA activity and levels of inflammatory markers between the metformin only group and the metformin and DPP4i combination therapy group (DPP4i merged group) were also analyzed using independent sample t-tests. Additionally, in T2DM patients who were taking DPP4i, ADA activity and inflammatory markers were measured prior to administration of DPP4i and 8 to 12 weeks after and were compared using a paired t-test. The comparison of ADA activities according to statin therapy was also performed and independent sample t-tests were used to compare the groups; any statin therapy group vs. no statin therapy group.

When analyzing the effects of drug treatment, ADA activity may be affected secondary to the degree of glycemic control. Therefore, when needed, ADA activity was adjusted using HbA1c value and the ANOVA test was performed.

In patients with T2DM, even mild changes in liver function are considered to have any effect on ADA activity. Prati et al. [30] proposed upper normal limit values in men and women according to alanine aminotransferase (ALT) value. Here, among the T2DM patients, women with ALT values less than 19 IU/L and men with ALT values less than 30 IU/L were placed in the normal liver function group (Normal ALT), those who were not in that group were classified into the dysfunctional liver group (High ALT). The differences in ADA activity between two groups were analyzed using independent sample t-tests. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Clinical characteristics of study subjects**

The clinical characteristics of the study subjects are shown in Table 1. The FPG values of the T2DM group (n=262) and the control group (n=46) were 154.8±3.4 mg/dL and 104.5±2.6 mg/dL (P<0.05), and the HbA1c of the T2DM group was significantly higher than that of the control group (7.7±0.1% vs. 6.2±0.1%, P<0.05). However, because some patients with impaired fasting glucose were included in the control group, FPG and HbA1c levels were slightly increased when compared to the values that would be expected for a group with completely normal glucose metabolism. Compared to the control group, ADA activity in T2DM patients was significantly higher (23.1±0.6 U/L vs. 18.6±0.8 U/L, P<0.05); the ADA activity range in the control group was 5.4 to 30.8 U/L compared to an ADA activity range of 5.8 to 64.6 U/L in the T2DM group. The ADA activity distributions in normal healthy subjects and T2DM patients showed considerable overlap (Table 1, Fig. 1).

**ADA activity and the correlations among various variables**

Regarding the correlations between ADA activity and other variables in all included experimental subjects, age, FPG,
HbA1c, aspartate aminotransferase (AST), ALT, ESR, and serum creatinine levels of both the control group and the T2DM patient group were highly correlated with ADA activity (Table 2). In a multivariate regression analysis that included age, FPG, HbA1c, AST, ALT, ESR, and serum creatinine as input variables and log-substituted ADA activity as a dependent variable, age (\( \beta = 0.164, P = 0.028 \)), FPG (\( \beta = 0.259, P = 0.004 \)), HbA1c (\( \beta = 0.168, P = 0.012 \)), and AST (\( \beta = 0.264, P = 0.014 \)) remained significant.

**Degree of glycemic control in patients with T2DM according to the ADA activity comparison**

Patients with T2DM were classified into three groups, those

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**Table 1. Baseline characteristics of the subjects**

| Characteristic   | Non-DM (n=46) | T2DM (n=262) |
|-----------------|--------------|--------------|
| Age, yr         | 57.8±2.0     | 60.1±0.7     |
| BMI, kg/m²      | 24.9±0.8     | 25.9±0.3     |
| FPG, mg/dL      | 104.5±2.6    | 154.8±3.4    |
| HbA1c, %        | 6.2±0.1      | 7.7±0.1*     |
| C-peptide, ng/mL| 2.4±0.3      | 2.1±0.1      |
| hsCRP, mg/L     | 1.2±0.2      | 2.7±0.5      |
| ADA, U/L        | 18.6±0.8     | 23.1±0.6*    |
| Uric acid, mg/dL| 5.0±0.3      | 5.2±0.2      |
| AST, IU/L       | 25.3±1.6     | 24.7±0.8     |
| ALT, IU/L       | 27.4±2.1     | 29.3±1.2     |
| BUN, mg/dL      | 14.9±0.8     | 16.2±0.3     |
| Creatinine, mg/dL| 0.9±0.0 | 1.0±0.0*    |
| Total cholesterol, mg/dL | 185.9±6.6 | 176.8±2.4 |
| Triglyceride, mg/dL | 145.6±13.1 | 138.6±5.4 |
| HDL-C, mg/dL    | 48.7±2.1     | 47.2±0.8     |
| LDL-C, mg/dL    | 119.3±5.7    | 109.7±2.2    |
| γ-GTP, U/L      | 19.7±2.3     | 36.1±3.2     |
| WBC, ×10³/μL   | 6.5±0.2      | 6.7±0.1      |
| ESR, mm/hr      | 10.2±1.3     | 12.4±1.0     |
| Lymphocyte, %   | 34.8±1.3     | 34.9±0.5     |
| Lymphocyte, n   | 2,040.6±137.8| 2,242.7±52.8|
| UACR, mg/g      | 75.0±29.0    | 108.2±14.4   |

Data are presented as means±standard error. T2DM, type 2 diabetes mellitus; BMI, body mass index; FPG, fasting plasma glucose; ADA, adenosine deaminase; hsCRP, high sensitivity C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; γ-GTP, gamma GTP; WBC, white blood cell; ESR, erythrocyte sedimentation rate; UACR, urine albumin to creatinine ratio. *\(P<0.05\) vs. Non-DM group.

HbA1c, aspartate aminotransferase (AST), ALT, ESR, and serum creatinine levels of both the control group and the T2DM patient group were highly correlated with ADA activity (Table 2). In a multivariate regression analysis that included age, FPG, HbA1c, AST, ALT, ESR, and serum creatinine as input variables and log-substituted ADA activity as a dependent variable, age (\( \beta = 0.164, P = 0.028 \)), FPG (\( \beta = 0.259, P = 0.004 \)), HbA1c (\( \beta = 0.168, P = 0.012 \)), and AST (\( \beta = 0.264, P = 0.014 \)) remained significant.

**Degree of glycemic control in patients with T2DM according to the ADA activity comparison**

Patients with T2DM were classified into three groups, those

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**Table 2. The correlations between serum ADA activity and other parameters in all subjects**

| Parameter                  | Correlation coefficient (r) | \(P\) value |
|----------------------------|----------------------------|-------------|
| Sex                        | 0.061                      | 0.287       |
| Age, yr                    | 0.172                      | 0.003*      |
| BMI, kg/m²                 | 0.146                      | 0.060       |
| FPG, mg/dL                 | 0.258                      | 0.000*      |
| HbA1c, %                   | 0.208                      | 0.001*      |
| C-peptide, ng/mL           | 0.005                      | 0.948       |
| hsCRP, mg/L                | 0.019                      | 0.741       |
| Uric acid, mg/dL           | -0.072                     | 0.374       |
| AST, IU/L                  | 0.325                      | 0.000*      |
| ALT, IU/L                  | 0.248                      | 0.000*      |
| BUN, mg/dL                 | 0.020                      | 0.727       |
| Creatinine, mg/dL          | 0.116                      | 0.046       |
| Total cholesterol, mg/dL   | -0.062                     | 0.292       |
| Triglyceride, mg/dL        | -0.045                     | 0.435       |
| HDL-C, mg/dL               | -0.028                     | 0.627       |
| LDL-C, mg/dL               | -0.081                     | 0.165       |
| γ-GTP, U/L                 | 0.098                      | 0.248       |
| WBC, ×10³/μL               | 0.002                      | 0.971       |
| ESR, mm/hr                 | 0.220                      | 0.003*      |
| Lymphocyte, %              | 0.009                      | 0.882       |
| Lymphocyte, n              | 0.015                      | 0.795       |
| UACR, mg/g                 | 0.009                      | 0.889       |

ADA, adenosine deaminase; BMI, body mass index; FPG, fasting plasma glucose; hsCRP, high sensitivity C-reactive protein; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; γ-GTP, gamma GTP; WBC, white blood cell; ESR, erythrocyte sedimentation rate; UACR, urine albumin to creatinine ratio. *\(P<0.05\).

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**Fig. 1.** (A) The comparison of serum adenosine deaminase (ADA) activity levels (means±standard error) between non-diabetic control subjects (Non-DM) and patients with type 2 diabetes mellitus (T2DM). (B) Serum ADA activities show wide variations within each group. *\(P<0.05\) vs. Non-DM subjects.
with HbA1c levels less than 7% \((n=108)\), those with levels ranging from 7 to 9% \((n=100)\), and those with levels greater than 9% \((n=52)\). When the ADA activity levels of these groups were compared, the group with HbA1c level less than 7% had a significantly lower ADA activity compared to that of the groups with HbA1c levels between 7-9% and greater than 9% \((21.1\pm0.8 \text{ U/L}, 24.5\pm1.0 \text{ U/L}, \text{and} 25.4\pm1.6 \text{ U/L} \text{ for the less than 7%, 7-9%, and greater than 9%} \text{ groups, respectively}; P<0.05)\). However, the ADA activities in the 7-9% and greater than 9% HbA1c groups were not significantly different \((P=0.849, \text{Fig. 2A})\).

T2DM patients were classified into three groups according to FPG level: less than 155 mg/dL \((n=157)\), 155-212 mg/dL \((n=66)\), and greater than 212 mg/dL \((n=37)\). These groups were then analyzed for their ADA activity. The respective ADA activities were 21.5\pm0.7 \text{ U/L}, 24.6\pm1.2 \text{ U/L}, \text{and} 28.2\pm1.8 \text{ U/L} \text{ for the less than 155 mg/dL, 155-212 mg/dL, and greater than 212 mg/dL FPG} \text{ groups, respectively; there were no significant differences between the less than 155 mg/dL FPG group and the 155-212 mg/dL FPG group (} P=0.075) \text{ or between the 155-212 mg/dL FPG group and the greater than 212 mg/dL FPG group (} P=0.171, \text{Fig. 2B})\).

Comparative ADA activity levels between the DPP4I treatment group and other treatment groups of T2DM patients

T2DM patients were classified into three separate groups according to treatment type. Thirty-four patients were placed in the DPP4I treatment group, 195 patients were in the other oral anti-diabetic agent group, and 33 patients were in the insulin combination therapy group. The ADA activity values for the three groups were 23.6\pm1.3 \text{ U/L}, 22.5\pm0.7 \text{ U/L}, \text{and} 27.3\pm1.8 \text{ U/L} \text{ for the DPP4I, other oral hypoglycemic agent, and insulin combination therapy groups, respectively}. There was a significant difference between the other oral hypoglycemic agent group and the insulin combination therapy group \((P<0.05)\). There were no significant differences in ADA activity level between the DPP4I treatment group and the other oral hypoglycemic agent group \((P=0.834)\).
or between the DPP4I treatment group and the insulin combination therapy group \((P=0.278, \text{Fig. 3A})\). When HbA1c level of these groups were compared, the insulin combination therapy group had a significantly higher HbA1c level compared to those of two other groups (6.8±0.1%, 7.6±0.1%, and 8.9±0.3% for DPP4I treatment group, the other oral hypoglycemic agent group, and the insulin combination therapy group, respectively; \(P<0.05\)). However, there was no significant difference in the ADA activity values that were adjusted for HbA1c among the three groups (Fig. 3B).

The ADA activity results between the metformin monotherapy group \((n=51)\) and the DPP4I combination therapy group \((n=34)\) did not differ significantly (20.9±1.0 \text{U/L} vs. 23.6±1.3 \text{U/L}, \(P=0.09\), Fig. 3C). In addition, there was no significant difference in HbA1c level between the two groups (7.1±0.2% vs. 6.8±0.1%, \(P=0.285\)), and there was very little difference in the ADA activity values that were adjusted for HbA1c (Fig. 3D).

In order to better understand the effects of metformin monotherapy on ADA activity, we made independent comparisons of the metformin monotherapy group \((n=50)\) and the sulfonylurea monotherapy group \((n=9)\). These results showed that, despite the small difference in HbA1c value between them (7.1±0.2% vs. 6.9±0.4%, \(P=0.624\)), the ADA activity in the metformin monotherapy group was significantly lower than that of the sulfonylurea monotherapy group (20.9±1.0 \text{U/L} vs. 28.1±2.8 \text{U/L}, \(P<0.05\), Fig. 3E). This suggests the possibility that metformin can directly influence ADA activity. Additionally, the ADA activity levels adjusted for HbA1c maintained a significant statistical difference (Fig. 3F).

**ADA activity comparison before and after DPP4I treatment in T2DM patients**

We measured HbA1c and ADA activity levels before DPP4I treatment and 8 to 12 weeks after treatment in a small subgroup of the DPP4I treatment group \((n=24)\). Despite the reductions in the comparatively analyzed results of HbA1c (7.4±0.2% vs. 6.6±0.1%, \(P<0.05\)), ADA activity (24.7±1.7 \text{U/L} vs. 23.0±1.6 \text{U/L}, \(P=0.154\)) showed no change (Fig. 4A). Additionally, ADA activity levels before and after DPP4I administration were adjusted for HbA1c, but the comparatively analyzed results did not show a significant difference (3.4±0.2 \text{U/L}/% vs. 3.5±0.2 \text{U/L}/%, \(P=0.347\), Fig. 4B).

These results suggest that ADA activity is unaffected by DPP4I administration regardless of glycemic control. Also, leukocytes, lymphocyte count, lymphocyte percentage, ESR, and hsCRP before and after the administration of DPP4I were not significantly statistically different.

**Comparison of ADA activity according to statin therapy in T2DM patients**

T2DM patients were classified into two groups according to statin treatment or not. Between the statin-treated \((n=114)\) and non-treated \((n=146)\) groups, the comparatively analyzed results showed little difference with regard to HbA1c level (7.7±0.1% vs. 7.7±0.1%, \(P=0.851\)) or FPG level (154.0±5.7 mg/dL vs. 155.6±4.2 mg/dL, \(P=0.833\)). However, ADA activity was significantly lower in the statin-treated group than in the group not treated with statins (22.0±0.8 \text{U/L} vs. 24.2±0.9 \text{U/L}, \(P<0.05\), Fig. 5A). Even after ADA activity was adjusted for HbA1c, there was a strong tendency toward a lower ADA activity in the statin-treated group compared to the non-treated group \((P=0.086\), Fig. 5B). Additionally, the comparable levels of AST, ALT, and hsCRP between the two groups sug-

![Fig. 4.](https://example.com/fig4) The comparisons of (A) adenosine deaminase (ADA) activity and (B) ADA activity adjusted for HbA1c from before and after the addition of DPP4 inhibitor (DPP4I) therapy to an existing regimen of metformin monotherapy \((2 g/day)\) in patients with type 2 diabetes mellitus. Vildagliptin 50 mg bid or sitagliptin 50-100 mg daily. ADA activity was measured before and after treatment with vildagliptin 50 mg bid or sitagliptin 50-100 mg daily for at least 8 to 12 weeks.

![Fig. 5.](https://example.com/fig5) The comparisons of (A) adenosine deaminase (ADA) activity and (B) ADA activity adjusted for HbA1c between patients with type 2 diabetes mellitus on statin therapy and those not on statin therapy. \(P<0.05\) vs. no statin.
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Comparison of ADA activity according to liver function in T2DM patients

We applied the upper limit of the normal values of liver function tests in healthy male and female subjects proposed by Prati et al. [30] to patients with T2DM, divided them according to ALT value into normal ALT and high ALT groups, and their ADA activities were compared. In female patients, ADA activity in the normal ALT group (n=75) was significantly lower than that in the high ALT group (n=75) (21.8±1.2 U/L vs. 26.0±1.1 U/L, P<0.05). The same was true in male patients (20.9±1.0 U/L vs. 24.2±1.3 U/L, P<0.05). All T2DM patients were classified as normal ALT (n=125) or high ALT (n=128), and a comparison of ADA activity showed that the normal ALT group had significantly lower ADA activity than did the high ALT group (21.2±0.8 U/L vs. 25.9±0.8 U/L, P<0.05).

T2DM patients with normal liver function were classified into the following groups according to FPG level: less than 155 mg/dL (n=74), 155–212 mg/dL (n=34), and greater than 212 mg/dL (n=17). The ADA activities of the groups were 19.7±1.0 U/L, 22.5±1.4 U/L, and 25.3±2.5 U/L, respectively, and there was a considerable difference in the ADA activity between the group with FPG less than 155 mg/dL and the group with FPG greater than 212 mg/dL.

The comparisons of ADA activity and other factors in T2DM patients

T2DM patients were separated into subgroups based on several variables, including hsCRP level (hsCRP<3 mg/L and ≥3 mg/L), white blood cell count (<1.0×10^10/μL group and ≥1.0×10^10/μL), and lymphocyte percentage (<44% and ≥44%), and comparisons of ADA activity were made for each pair of subgroups. ADA activity did not show a significant difference between the groups with high and low levels of each variable.

DISCUSSION

Because DPP-4 is an ADA-binding partner and ADA is closely related to T lymphocyte function [9] and insulin resistance, in the present study, we measured ADA activity in T2DM patients to evaluate whether there is an effect from selective DPP4I treatment and whether ADA activity is affected by other therapeutic drugs. According to our results, ADA activity in T2DM patients was significantly higher than that in the control group, and had positive correlations with both FPG and HbA1c.

T2DM patients were also classified according to drug treatment type, and the ADA activity analysis results showed that the DPP4I treatment group did not have a significant difference in ADA activity from that of the other oral agent group or from that of the insulin treatment group. Also, the comparison of ADA activity between the metformin monotherapy group and the DPP4I combination therapy group showed no significant difference. Even when ADA activity was compared before and after DPP4I treatment, and regardless of glycemic control status, DPP4I treatments were confirmed not to have any specific effect on ADA activity. Conversely, when HbA1c had a tendency to be lower, ADA activity remained proportional, which suggests that ADA activity can increase as a result of selective DPP4I treatment. At least, in the present study, it was confirmed that DPP4I did not reduce ADA activity. One of the possible reasons that ADA activity was not affected by selective DPP4Is is evident through a recent analysis of the DPP-4 protein structures which revealed the active sites of DPP-4, active binding site of DPP4Is, and ADA binding site. Since the actions of these small molecules with selective DPP-4 inhibitory actions are limited to the DPP-4 catalytic pocket, the selective DPP4Is are unlikely to affect conformational structure of the DPP-4 protein, its dimerization, ant its interactions with other binding partners [31]. However, recent cellular and animal experimental results have shown that the mobility of CD4+ T lymphocytes derived from the spleens of non-obese diabetic (NOD) mice is increased by soluble DPP-4 (sDPP-4) [32]. Also, sitagliptin suppressed sDPP-4-induced CD4+ T cell infiltration in the islets. So, the diabetes reduction by sitagliptin treatment in NOD mice seems to involve a reduction in T-cell mobility, and then the reduction of insulitis [32]. Thus, although selective DPP4Is did not have an effect on ADA activity, more studies of the effects of DPP4Is on T-cell functions are needed.

ADA activity comparisons of oral hypoglycemic monotherapy groups showed that ADA activity was significantly lower in the metformin monotherapy group than it was in the sulfonylurea monotherapy group. Metformin decreases insulin resistance, so ADA activity is expected to decrease in conjunction with metformin therapy. Reports from one study conducted on red blood cell lysates showed that metformin did not directly inhibit ADA activity [33]. The ADA activity in the statin-treated group was significantly lower when compared to...
the activity in the non-treated group; this difference was not related to glycemic control, but statin itself may potentially affect ADA activity. In previous studies, it was reported that simvastatin-treated groups have significantly reduced ADA activity [34]; however, the exact mechanism still remains unclear. Statin’s lipid-lowering effects and various other effects such as an anti-inflammatory actions, T-cell differentiation inhibition, TNF-α inhibition, and other immune regulatory effects [35-37] may be considered to have an effect on ADA activity.

In line with previous reports done by other researchers, ADA activity in T2DM patients in the present study was significantly higher than that in the control group [21-23]. Moreover, compared to that of T2DM patients with relatively good glycemic control, the ADA activity in T2DM patients with poor glycemic control was significantly lower. These results are consistent with those reported by Hoshino et al. [38].

Although subjects with active liver diseases were excluded from this study, the patients with even mild liver dysfunction which seemed to be related to metabolic syndrome, had significantly higher ADA activity regardless of glycemic control. The differences in ADA activity corresponding to differences in the AST and ALT levels that reflect liver dysfunction are consistent with the reports from other studies [21,30]. A correlation between γ-GTP and ADA activity had also been reported [21], but was not shown to be statistically significant in the present study. When the ADA activity in diabetic patients with normal liver function was analyzed, the results showed that patients with poor glycemic control had significantly higher ADA activity. In the analysis of all patients and several subgroups, ADA activity did not have a significant correlation with hsCRP, white blood cell count, or lymphocyte count.

The limitations of this study were as follows: 1) ADA activity differences based on glycemic control level, treatment medications and liver functions were compared in T2DM patients through a simple cross-sectional study, 2) comparisons before and after medical treatments were not performed, 3) because national health insurance does not cover DPP4I monotherapy, patients undergoing metformin baseline maintenance treatment who were treated with an additional DPP4I were classified into the DPP4I treatment group and analyzed as such. Additionally, the control group was composed of non-diabetic adults, and patients with diabetes were excluded in the group; however, adults with impaired fasting glucose may have been included in the control group.

In conclusion, compared to the control group, ADA activity in T2DM patients was higher. When glycemic control was relatively good, ADA activity was low. However, although neither ADA activity nor inflammatory-response-related variables changed with DPP4I treatment regardless of changes in glycemic control.

Thus, the results of this study suggest that the selective DPP4Is currently used by diabetic patients do not have any effect on the non-enzymatic action of DPP-4. In addition to this study focused on ADA activity, additional studies are needed to evaluate the effects of selective DPP4Is on other physiologic functions related to the non-enzymatic action of DPP-4.

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