**NOTE**

**Relationship between Anti-Insulin Antibodies and Albuminuria or Proteinuria in Human Insulin-Treated Type 2 Diabetes Mellitus**

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**Abstract.** In order to examine the relationship between anti-insulin antibodies (AIA) caused by extrinsic human insulin and albuminuria or proteinuria, 53 human insulin-treated type 2 diabetics were divided into two groups: (AIA(+) group) 27 patients with a titer of AIA greater than 7.6% and (AIA(-) group) 26 patients with a titer of AIA less than 7.5%. Although no significant difference was found between the two groups for age, gender, body mass index, duration of diabetes, duration of insulin treatment, blood pressure, serum creatinine, glycosylated hemoglobin (HbA1c), daily dose of insulin, daily insulin injection times, or treatment of hypertension, the AIA(+) group had a significantly higher urinary albumin to creatinine ratio and urinary protein to creatinine ratio than the AIA(-) group (p<0.05). It is suggested that AIA in type 1 diabetics might be insulin autoantibodies, which is not the case with type 2 diabetics. To our knowledge this is the first study demonstrating the relationship between AIA induced not by porcine or bovine insulin, but by human insulin and albuminuria or proteinuria in type 2 diabetics.

**Key words:** Anti-insulin antibodies, Diabetic nephropathy, Albuminuria, Proteinuria, Type 2 diabetes mellitus

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**Materials and Methods**

**Patients**

We examined 53 type 2 diabetic patients (18 males aged 25–80 years, 35 females aged 30–76 years) treat-
of human insulin without elevated serum creatinine (<1.5 mg/dl). All patients were treated with conventional insulin preparations (daily insulin dose ranged between 10–46 unit/day) and were on 1 or 2 injections of intermediate-acting human insulin or biphasic-acting human insulin. Diagnosis of type 2 diabetes was arrived at by clinical course and negative anti-glutamic acid decarboxylase (GAD) antibody, with AIA titer measured twice within three months for all patients. Subjects were divided into two groups according to mean titer of AIA: (AIA(+) group) 27 patients with AIA titer greater than 7.6% and (AIA(−) group) 26 patients with AIA titer less than 7.5%.

Urine sampling

Urine samples from all patients were collected 3 times within 6 months for measurement of urinary albumin, urinary protein, and urinary creatinine. In estimating the urinary albumin to creatinine ratio (UACR) and the urinary protein to creatinine ratio (UPCR), we used the average of 3 samples collected within 6 months.

Measurements

AIA titer was assayed by polyethylene glycol assay (SRL, Tokyo, Japan), and expressed as a percentage of 125Iodine-labelled insulin precipitated with γ-globulin. Anti-GAD antibody was measured by GAD antibody radioimmunoassay (RIA) kit (RIP Anti-GAD Hoechst, Tokyo, Japan). Urinary albumin was measured by latex agglutination immunoassay (Eiken Chemical, Tokyo, Japan). Urinary protein was measured by pyrogallol red assay (Wako Pure Chemical, Osaka, Japan). Serum and urinary creatinine were measured by enzyme assay (Creatinase F-DAOS method) (Wako Pure Chemical, Osaka, Japan). Glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (Tosoh, Tokyo, Japan).

Statistics

All data except gender, treatment of hypertension, and daily insulin injection times were expressed as mean±SD. Simple linear regression analysis was performed to calculate correlations. The goodness-of-fit test for chi-square was used to compare the differences of gender and treatment of hypertension. Mann-Whitney’s U test was used to compare the difference of daily insulin injection times. Other data were compared by Student’s t-test for unpaired data. The StatView statistics package for Macintosh, version 4.1, was employed for the present analysis. P values below 0.05 were considered significant.

Results

Control sera

A total of 99 control sera were assayed. The data were skewed within a range of 4.6 to 7.0%. Subjects with an IA titer greater than mean plus 3 standard deviation (X + 3 SD) of normal controls were considered positive (X = 5.9, SD = 0.54, X + 3 SD = 7.52 %).

UACR or UPCR and AIA titer

As shown in Table 1, no significant difference was found between the two groups for age, gender, body mass index, duration of diabetes, duration of insulin treatment, blood pressure, serum creatinine, HbA1c, daily dose of insulin, daily insulin injection times, or treatment of hypertension. However, patients in the AIA(+) group had a significantly higher UACR and UPCR than those in the AIA(−) group (p<0.05, respectively). On the other hand, UACR was significantly correlated with UPCR (r=0.975, p<0.0001).

Discussion

Brun et al. [10] reported a correlation not between proteinuria but rather microalbuminuria only after exercise and AIA in type 1 diabetics. However, since we cannot distinguish insulin autoantibodies (IAA), which are IA detected in type 1 diabetics before insulin treatment, from those produced after insulin treatment [9], it is possible that the IA detected in insulin-treated type 1 diabetics might be IAA. It has been reported that microalbuminuria development may not be associated with IAA in type 1 diabetics [11]. IA in type 2 diabetics may thus
be said to have a different meaning from those in type 1 diabetics. Therefore in this paper where we investigated only type 2 diabetics, we tried to examine the relationship between AIA which were caused by extrinsic human insulin and albuminuria or proteinuria in a narrow sense.

It is reported that when insulin is administered subcutaneously during multiple insulin injections, AIA production increases in contrast with conventional treatment [12]. All diabetics included in this study were treated with conventional treatment, and the number of daily insulin injections made no difference in AIA production.

Although many investigators reported that there was little or no relationship between AIA and glycemic control [13, 14] or insulin resistance [15], it has been suggested that AIA induced by porcine or bovine insulin might play a role in the progression of diabetic complications [16-18]. Especially, a link between circulating AIA and diabetic glomerulopathy has been suggested by Wehner [7]. However, whether AIA affect albuminuria or proteinuria is controversial. Virella et al. [19] using different screening techniques reported that significant correlations were not always seen between AIA levels and albuminuria or proteinuria, although there were significant correlations between some of these screening tests for immune complexes and some screening tests for diabetic nephropathy. Furuta et al. [20] reported that nephrotic syndrome developed after the beginning of the therapy with porcine insulin and that proteinuria was ameliorated after porcine insulin was replaced by human insulin. Heding et al. [21] also reported that human monoclonal insulin has a lower immunogenicity than porcine insulin. However, in that study [21], AIA were produced in human insulin-treated diabetics (44%) as well as porcine insulin-treated ones (59%), thus it remains unknown whether AIA induced by human insulin affect albuminuria or proteinuria. In the current study, we found that both albuminuria and proteinuria occurred more in the AIA(+) group than in the AIA(-) group, even in human insulin-treated type 2 diabetics. In a previous study of diabetic nephropathy by Furuta et al. [20], it was suggested that the immune complexes mediated by porcine insulin might not cause diabetic glomerulosclerosis, but may cause the membranous nephropathy associated with diabetes mellitus. Moreover, they suggested that endogenous human insulin might be trapped in the immune complexes because antihuman insulin antiserum also detected glomerular insulin deposits in

| Table 1. Statistical comparison between diabetics of the AIA(+) group and the AIA(-) group. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Age (years)                                  | 59.2±12.8                                     | 62.1±11.2                                     | N.S.                                          |
| Gender (male/female)                         | 10/16                                         | 8/19                                          | N.S.                                          |
| BMI (kg/m²)                                  | 23.9±3.5                                      | 23.1±3.7                                      | N.S.                                          |
| Duration of diabetes (years)                 | 14.0±8.8                                      | 12.9±8.0                                      | N.S.                                          |
| Duration of insulin treatment (years)        | 2.6±3.0                                       | 3.8±4.6                                       | N.S.                                          |
| Systolic blood pressure (mmHg)               | 138.5±18.4                                    | 137.8±18.1                                    | N.S.                                          |
| Diastolic blood pressure (mmHg)              | 75.2±10.7                                     | 70.9±13.1                                     | N.S.                                          |
| Serum creatinine (mg/dl)                     | 0.65±0.21                                     | 0.71±0.17                                     | N.S.                                          |
| HbAlc (%)                                    | 7.5±0.9                                       | 7.8±1.1                                       | N.S.                                          |
| Daily dose of insulin (unit/day)             | 21.7±8.7                                      | 22.7±8.3                                      | N.S.                                          |
| Daily insulin injection times (once/twice)   | 10/16                                         | 12/15                                         | N.S.                                          |
| Treatment of hypertension (none/ACEI/others) | 16/4/6                                        | 19/4/4                                        | N.S.                                          |
| AIA titer (%)                                | 6.5±0.6                                       | 29.4±21.9                                     | p<0.0001                                      |
| UACR (mg/g.creatinine)                       | 82.2±110.0                                    | 701.4±1440.7                                  | p<0.05                                        |
| UPCR (mg/g.creatinine)                       | 271.3±169.4                                   | 919.1±1458.0                                  | p<0.05                                        |

Values are the mean±SD. AIA, anti-insulin antibodies; BMI, body mass index; HbAlc, hemoglobin Alc; ACEI, angiotensin converting enzyme inhibitor; UACR, urinary albumin to creatinine ratio; UPCR, urinary protein to creatinine ratio; N.S., not significant.
porcine insulin-treated diabetics, and that these detections were blocked by both porcine and human insulin. However, they also suggested that this hypothesis seems doubtful since insulin deposition was not found using anti-insulin antisera in membranous nephropathy associated in type 2 diabetes mellitus without insulin therapy. Since the immune complexes mediated by human insulin may cause membranous nephropathy associated with diabetes mellitus and since AIA are induced by human insulin with a lower immunogenicity than porcine insulin, we speculated that our results might be due to the same mechanism seen in porcine insulin-treated diabetics.

Although albuminuria as well as proteinuria is generally supposed to reflect serious renal disease specific to diabetes, it has been hypothesized that it might be caused by primary glomerulonephritis such as membranous nephropathy or IgA nephropathy [22-26] and secondary glomerulonephritis other than diabetic nephropathy. In this study, the AIA(+) group had a significantly higher UACR and UPCR than the AIA(-) group, and it is possible that the AIA(+) group had a higher incidence of renal diseases resulting in albuminuria or proteinuria than the AIA(-) group. Meanwhile, it has been reported that no marked prevalence of undiagnosed glomerulonephritis was found in type 2 diabetics [27], and in future pathological examinations it might be useful to determine whether AIA accelerate diabetic nephropathy. To our knowledge this is the first study demonstrating a relationship between AIA induced not by porcine or bovine insulin, but by human insulin and albuminuria or proteinuria. Further investigations should be expected in this field.

References


