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Case Report

A Case of A Non-Insulin Dependent Diabetic Patient with Regular Spontaneous Hypoglycemic Attacks, Which were Due to Insulin-Binding Antibodies Induced by Human Insulin Therapy

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SUZUKI, K., HIRAYAMA, S. and ITO, S. *A Case of A Non-Insulin Dependent Diabetic Patient with Regular Spontaneous Hypoglycemic Attacks, Which were Due to Insulin-Binding Antibodies Induced by Human Insulin Therapy.* Tohoku T. Exp. Med., 1997, **182** (2), 163–173 —— A 74-year-old diabetic patient had been treated with oral hypoglycemic agents from the age of 40 years until the age of 72, when his treatment regimen was changed to human insulin. He began experiencing hypoglycemic attacks 14 months after the initiation of insulin therapy. He continued to experience hyperglycemia every morning and hypoglycemia every day at 5 : 30 p.m. even after insulin therapy was withdrawn. His plasma levels of C-peptide immunoreactivity, total and free immunoreactive insulins were 4.2 ng/ml, 740 μU/ml and 141.8 μU/ml, respectively. His ^{125}I-insulin binding rate was 94.4%. These findings suggest that his hypoglycemic attacks may have been due to insulin antibodies. Analysis revealed that insulin binding antibodies belonged to IgG with κ light chains. The patient's genotype was HLA-DR4. He had not received animal insulin or any medications containing a sulfhydryl group. Although the IgG antibody was produced against injected human insulin, his HLA type and the characteristics of his antibodies resembled those of a patient with insulin autoimmune syndrome (IAS). We hypothesize that this patient represented a rare instance of a patient constitutionally similar to a patient with IAS, but whose hypoglycemic attacks resulted from the antibodies induced by the administration of human insulin. This case seems to be the first one with hypoglycemic attacks due to anti-human insulin antibody produced by human insulin therapy. —— anti-human insulin antibody; spontaneous hypoglycemia; NIDDM

Hypoglycemia occurring in diabetics treated with either insulin or antidiabetic drugs is numerically, by far the commonest cause of spontaneous hypoglycemia. The hypoglycemia is usually due to overtreatment of insulin or anti-

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diabetic drugs. Therefore, the clinical manifestation of hypoglycemia is not observed in these patients after treatment with either insulin or anti-diabetic drugs is withdrawn. We encountered a patient with non-insulin dependent diabetes mellitus who was treated with exogenous human insulin injections for 14 months and experienced hypoglycemic attacks even after insulin treatment was withdrawn. Various diseases which are known to cause hypoglycemia were not detected. Only insulin-binding antibodies were found in the serum of this patient as a causal factor.

Insulin antibodies are frequently present in the serum of patients who receive exogenous insulin injections, but hypoglycemia is rarely caused by these insulin antibodies (Yasuda et al. 1989). In contrast, the insulin autoimmune syndrome (IAS) is characterized by the presence of insulin-binding antibodies in the serum of patients not receiving insulin injections and spontaneous hypoglycemia (Hirata et al. 1970; Følling and Norman 1972).

We analyzed clinical and immunological characteristics in this patient from the point of view of the relation between IAS and insulin-binding antibodies in this patient.

Case Report

A 74-year-old man was admitted to Akita University Hospital on October 18, 1995, with hypoglycemia. The patient had been in good health until 1961, when non-insulin dependent diabetes mellitus was diagnosed. He was treated with glibenclamide from 1961 until 1993, when insulin therapy was initiated. He began experiencing hypoglycemic attacks for 14 months after the initiation of insulin therapy and he was introduced to our hospital.

Physical findings on admission were unremarkable. The patient was pleasant and well-oriented. His thyroid was not enlarged and lymphadenopathy, hepatomegaly, and splenomegaly were not present. Skin eruptions and lipoatrophy were not observed at insulin injection sites on the arms, abdomen and legs. Skin and mucous membrane showed no hyperpigmentation. He weighed 64.5 kg and his height was 155 cm. His blood pressure was 140/70 mmHg. No abnormalities of the knee or Achilles tendon reflexes or impaired vibration was found. No abnormalities in autonomic function were detected. Examination of the bilateral fundus showed no diabetic retinopathy.

Bacteria and much leukocytic infiltration were present in the urine (Table 1). After antibiotic treatment, the urinary excretion rate of albumin was 21.7 μg/min. No abnormalities in biological parameters were detected. The insulin receptor antibody level was 85.5%. HLA typing showed that the patient was DR4 positive.

Because an insulin overdose was suspected as the cause of hypoglycemia, the dose of insulin was gradually tapered off and was discontinued. However, his daily glucose profile continued to indicate hyperglycemia in the morning and

TABLE 1. *Laboratory data on admission*

Urinalysis: Prot. (−) Glu. (4+) Uro. (±)		BUN	21 mg/100 ml	Serological test	
Sed.: RBC 2-3/1F WBC many/1F		Creat.	1.1 mg/100 ml	CRP	0.1 mg/100 ml
Bacteria	(3+)	T-P	6.7 mg/100 ml	STS	(−)
Hyaline cast	(−)	alb	65.5%	anti-DNA ab.	(−)
CBC		α1-globulin	2.9%	IgA	195 mg/100 ml
WBC	4,900 /mm^3	α2-globulin	8.8%	Insulin receptor ab.	85.5%
RBC	456×10^4/mm^3	β2-globulin	9.4%	(normal control	>93%)
Hb	15.2g/100 ml	γ-globulin	13.8%	HLA A locus A2	
Ht	45.1%	T.Chol	185 mg/100 ml	B locus B46, B51(5)	
Plt.	11.1×10^4/mm^3	TG	130 mg/100 ml	C locus Cw1	
Blood chemistry		T. Bil	0.5 mg/100 ml	DR locus DR4, DR6, DR52, DR53	
Na	145 mEq/liter	GOT	29 U/liter	DQ locus DQ4, DQ6(1)	
K	4.4 mEq/liter	GPT	34 U/liter	Serum C-peptide	1.1 ng/ml (0.6-2.8)
Cl	109 mEq/liter	ALP	88 U/liter	Urinary C-peptide	36 μg/day (16-120)
Ca	9.1 mg/100 ml	LDH	170 U/liter	17-OHCS	7.4 mg/day (3.4-12.0)
P	3.4 mg/100 ml	Amy.	311 U/liter	TSH	0.72 μIU/ml (0.29-5.11)
		CPK	106 U/liter	F-T_3	3.5 pg/ml (2.0-3.4)
				F-T_4	1.4 ng/100 ml (0.9-1.7)

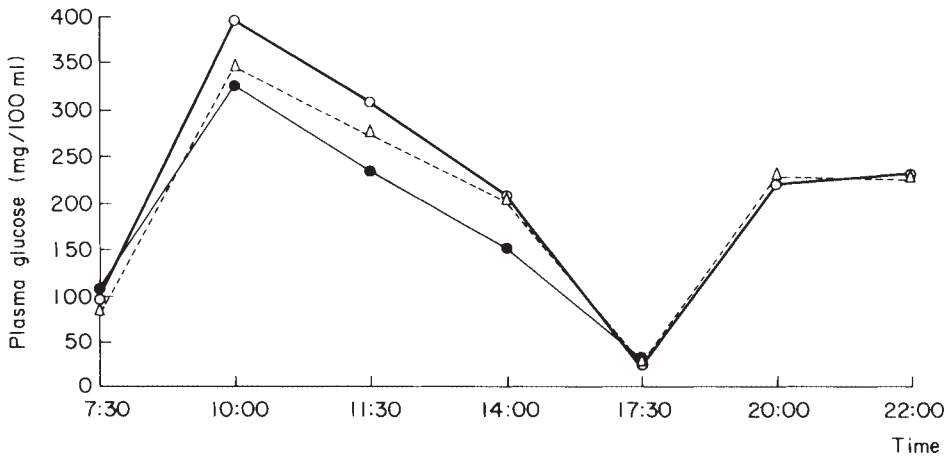

Fig. 1. Daily plasma glucose levels.
——○——, During treatment with Humulin-N (15-0-10 unit);
——●——, During treatment with Humulin-R (6-0-4 unit);
------△------, After discontinuation of insulin.

hypoglycemia at 5 : 30 p.m. every day even after discontinuation of insulin (Fig. 1). Examination of the abdomen by ultrasound, superior mesenteric angiography, computed tomography and magnetic resonance imaging revealed no evidence of a pancreatic tumor. Urinary 17-hydroxycorticosteroids were within the normal range, and thyroid function was normal. We thus excluded possible diagnoses of insulinoma, adrenocortical insufficiency, and thyroid disease.

Determination of insulin

Total and free insulins were measured by radioimmunoassay according to the method of Nakagawa et al. (1973). ^{125}I-insulin binding rate was determined as follows: 0.1 ml of serum was incubated with ^{125}I-insulin and 0.1 ml of phosphate buffer for 2 days at 4°C. Then, 2.5% dextran-coated charcoal was added, and the mixture was centrifuged at 3000 rpm for 30 minutes at 4°C. Radioactivity of the supernatant (bound) was measured. Binding rate was expressed as per cent of bound ^{125}I-insulin to total radioactivity. The kinetics of insulin antibody was analyzed according to the Scatchard's analysis (Goldman et al. 1978). Briefly, insulin-free serum was prepared from patient serum by adding acidified, dextran-coated charcoal according to the method of Jayarao et al. (1973). The supernatant after centrifugation was diluted with 0.04 M phosphate buffer pH 7.4, so as to obtain a suitable range of B/F ratio to avoid the paradoxic binding phenomenon which is found when the antibody of high titer is used. To 0.1 ml of insulin-free serum was added 0.1 ml of ^{125}I-insulin and 0.1 ml of buffer or various concentrations of unlabeled insulin. The mixtures were incubated for 2 days at 4°C. To separate free insulin from bound insulin, 0.3 ml of 25% polyethylenglycal was added to the incubation mixture and the mixtures were centrifuged for 30 minutes at 4°C. Bound insulin was measured in each mixture. Human insulin was used in this experiment as unlabeled insulin. ^{125}I-insulin was purchased from Dinabot Co., Ltd., Tokyo.

Clinical course

Serum levels of C-peptide immunoreactivity (CPR) and total and free immunoreactive insulins (IRI) were elevated, when the afternoon episodes of hypoglycemia occurred (Table 2). The ^{125}I-insulin binding rate was 94.4%. These findings suggest that the patient's hypoglycemic attacks may have been caused by insulin antibodies. Antibodies against CPR should not have been present in this patient because CPR assays performed with and without acid-polyethyleneglycol extraction of serum showed approximately the same in the CPR levels. We did not measure proinsulin directly, but we estimated the proinsulin level by subtracting the free-CPR level (determined after the procedure with neutral-polyethyleneglycol extraction of serum) from the total-CPR level (determined

TABLE 2. *Laboratory values after discontinuation of insulin therapy*

	Time						
	7 : 30	10 : 10	11 : 30	14 : 00	17 : 30	20 : 00	22 : 00
PG (mg/100 ml)	96	334	353	191	27	222	216
Total-IRI (μU/ml)	361	730	948	1359	740	547	586
Free-IRI (μU/ml)	36.8	56.8	78.6	88.2	141.8	44	38.6
C-peptide (ng/ml)	2.1	9.1	9.1	11	4.2	3	4.1
Total-C-peptide (ng/ml)	2.8	8.4	6.4	10.4	3.2	4.4	4.4
Free-C-peptide (ng/ml)	1.8	5.6	5.4	7.8	2.2	3.4	3.2

Daily profiles of plasma glucose (PG), total and free-IRI, C-peptide, total and free-H-peptide levels 2 weeks after stopping insulin injection in this patient. Diurnal variation of total-IRI was almost the same as that of free IRI. Values of C-peptide were approximately the same as those of total-C peptide. Differences between levels of total and free-C-peptide were considered to be proinsulin.

TABLE 3. *Response to an oral glucose tolerance test*

	Time (min)											
	0	15	30	45	60	75	90	120	150	180	240	300
PG (mg/100 ml)	110	141	196	248	275	326	259	303	323	300	210	94
Total-IRI (μU/ml)	88		140			340		432	507	404	377	236
Free-IRI (μU/ml)	38.4		36.8			63.6		70.8	89.0	87.4	89.0	75.4
C-peptide (ng/ml)	2.2	3.1	5.5	6.8	5.7	7.5	8.0	10.0	12.0	12.0	7.8	5.1

Plasma glucose, total and free IRI, and C-peptide responses to 75 g oral glucose load. All parameters showed the peak response at 15 minutes. This patient suffered a hypoglycemic attack at 9 : 00 p.m. on that day. (not described)

after the procedure with acid polyethyleneglycol of serum). Levels of proinsulin and insulin appeared to increase after meals (Table 2). An oral glucose tolerance test showed that the plasma levels of glucose demonstrated characteristic diabetic variations. Serum levels of total and free IRI were markedly elevated and parallel with elevated plasma levels of glucose (Table 3). The immunoglobulin class and light-chain type of the insulin binding antibody were examined using the specific precipitation method (Nakagawa et al. 1972; Uchigata et al. 1989a, b; Wasada et al. 1989). The specific precipitation method revealed IgG type insulin-binding antibodies with κ light chains. Scatchard analysis suggested two classes of binding sites: one with a high affinity and a low capacity and the other with a low affinity and a high capacity. The high affinity site had an equilibrium constant, K_1 of 1.7×10^8 liter/mol; the low affinity site had a K_2 of 0.0923×10^8 liter/mol. Binding capacities were 0.6×10^{-8} mol/liter and 3.9×10^{-8} mol/liter, respectively.

Hypoglycemic attacks continued for 2 weeks after discontinuation of insulin injections. Morning hyperglycemia was suspected due to hypersecretion of insulin, which may bind to circulating insulin antibodies. When the insulin becomes disassociated from insulin antibodies, hypoglycemia may result. Therefore, the patient was treated with an α-glucosidase inhibitor to prevent the hyperglycemia-hyperinsulinemia reaction in the morning (Table 4). The peak morning plasma level of glucose decreased by 50 mg/100 ml and the late afternoon plasma level of glucose increased slightly to 61 mg/100 ml on the first day of treatment. However, after a few days of treatment, the glucose profile showed the same pattern as before treatment and the hypoglycemic attacks recurred. Because the titer of insulin-binding antibody remained elevated about 1 month after admission, steroid therapy was initiated to treat hypoglycemia and to suppress production of the autoimmune antibody. Prednisolone was administered in a dose of 30 mg/day for the first 10 days, the dosage was tapered 10 mg/day at 10-day intervals. The antibody titer decreased significantly after steroid therapy (Fig. 2). As insulin or

TABLE 4. *Effect of an α-glucosidase inhibitor*

	Time						
	7 : 30	10 : 00	11 : 30	14 : 00	17 : 30	20 : 00	22 : 00
PG (mg/100 ml)	85	282	211	194	61	207	218
Total-IRI (μU/ml)	193	618	932	619	449	340	400
Free-IRI (μU/ml)	19.2	64.0	100.0	67.8	48.6	36.0	42.6
C-peptide (ng/ml)	1.5	9.5	8.5	5.8	3.3	4.2	3.9

Daily profiles of plasma glucose, total and free IRI, and C-peptide levels after administration of an α-glucosidase inhibitor. Hypoglycemic attack was slightly attenuated.

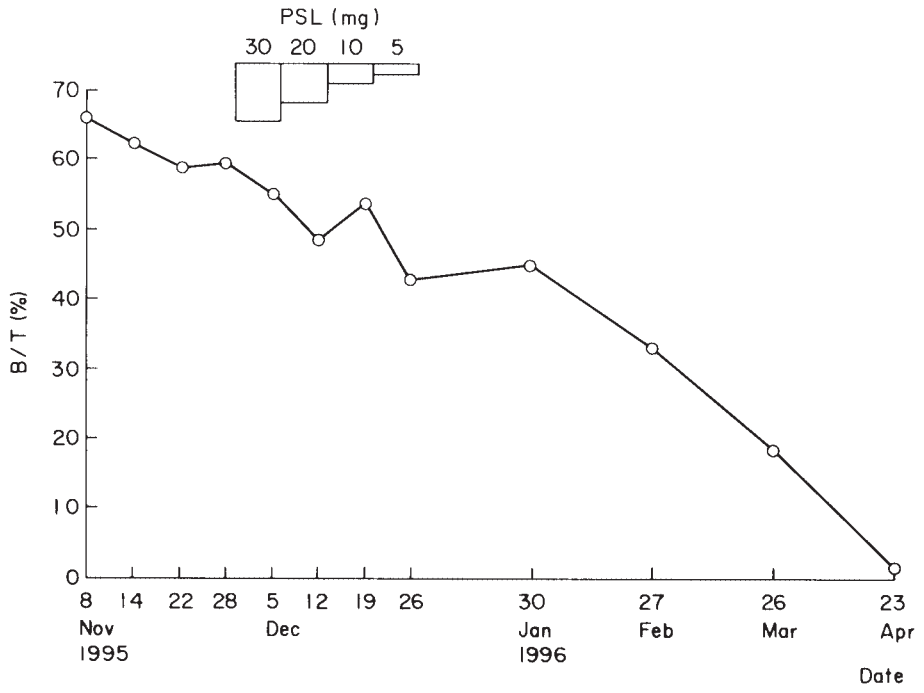

Fig. 2. Clinical course after steroid therapy.
B/T, The antibody-bound insulin/total insulin.
Titer of the insulin antibodies decreased gradually after administration of PSL.

sulfonylureas could not be administrated for the treatment of diabetes, the patient was then treated with diet therapy combined with an α-glucosidase inhibitor. His plasma glucose level remained elevated (250–300 mg/100 ml) for 1 month after steroid therapy, but after 4 months, the average postprandial glucose value was about 150 mg/100 ml, as determined by a self monitoring of blood glucose at home. In April 1996, total and free IRI levels had decreased to 87 μU/ml and 14 μU/ml, respectively, and the ^{125}I-insulin binding rate was 2.2%. He has experienced no episodes of hypoglycemia since completion of steroid therapy.

Discussion

Symptomatic hypoglycemia in patients receiving insulin therapy is often due to an insulin overdose, or surreptitious administration of insulin or inadequate glucose intake. However our patient continued to experience hypoglycemic attacks, despite discontinuation of insulin therapy. Other diseases associated with hypoglycemia, including insulinoma and pituitary-adrenal dysfunction were excluded. Therefore, hypoglycemia in our patient appeared to be caused by the human insulin antibody produced by human insulin injection. The hypoglycemic attacks disappeared in parallel with the decrease in the titer of the human insulin antibody, supporting this hypothesis.

Yasuda et al. (1989) previously described a patient with hypoglycemic attacks thought to have been caused by insulin antibodies against animal insulin. The insulin antibody in that patient had both λ and κ-type light chains whereas

the antibodies in the present case had mainly κ light chains (Uchigata et al. 1989b). Scatchard analysis of the insulin antibody produced by animal insulin injections has shown that it has a high affinity constant and a low binding capacity. The insulin antibody in our patient had a low affinity constant and a high binding capacity. Thus, the insulin antibody in our patient was distinct from that produced by injection of animal insulin, which may explain why the time-lag between the onset of hypoglycemic attacks and initiation of insulin therapy differed between our patient and the patient described by Yasuda et al. (1989). Our patient began experiencing hypoglycemic attacks 14 months after starting insulin therapy whereas the patient treated with animal insulin experienced hypoglycemic attacks 1 month after starting animal insulin therapy.

IAS has been well known as a disease which is characterized by spontaneous hypoglycemia and human insulin-binding antibodies in serum without previous insulin injection. IAS was first described by Hirata et al. (1970), and has been documented in a number of patients (Hirata and Arimura 1972; Hirata and Ishizu 1972; Hirata et al. 1972, 1974; Uchigata et al. 1994). Approximately 40% of patients with IAS develop hypoglycemia 2 to 6 weeks after initiation of therapy with a drug that contains a sulfhydryl (S-H) group (e.g. Methimazole). (Hirata et al. 1974; Ichihara et al. 1977; Berson et al. 1984; Masuda et al. 1986; Sklenar et al. 1987) Hypoglycemia usually occurs in the fasting state early in the morning and is often self-limiting. IAS is associated with IgG insulin antibodies with almost exclusively κ light chains (Hirata 1987; Wasada et al. 1989). The antibodies appeared to have two classes of binding sites: one with a high affinity and a low binding capacity and the other with a low affinity and a high binding capacity (Hirata 1987; Wasada et al. 1989). HLA DR4, DR3 and DRB1*0406 genotypes are common in patients with IAS (Hirata 1987; Uchigata et al. 1992a, b, 1993). The findings in the present case suggest that our patient represented a rare case which was constitutionally similar to a patient with IAS, but whose hypoglycemic attacks resulted from human insulin antibodies produced by injection of human insulin. Although Yasuda et al. (1989) reported a case with spontaneous hypoglycemia caused by anti-animal insulin antibody, HLA typing was not analyzed in their patient. Therefore, it remains unclear whether only patients with the specific HLA genotype experience spontaneous hypoglycemia or not.

The assay method used to detect the insulin receptor antibody in the present study may have led to a slight error in measurement. The insulin antibodies in the serum of our patient bound to ^{125}I-insulin, which was added to the assay system to detect the presence of insulin receptor antibody. Thus, the part of ^{125}I-insulin that binds to the placental membrane insulin receptor was decreased. We speculate that this phenomenon may have led to overestimation of the insulin receptor antibody titer.

In our patient, glucose levels after breakfast were extremely higher than those

after the other meals. Although the insulin secretion response to postmeal glucose is thought to be low in non-insulin dependent diabetes mellitus, the pancreatic B-cells response to glucose in our patient may be exaggerated since insulin antibody was present. Therefore, the hypersecreted insulin which responded to remarkable hyperglycemia bound to the antibodies in the morning and became dissociated from them in the evening. Thus, hypoglycemia caused by insulin binding antibodies has been attributed to the dissociation of a large portion of insulin bound to insulin antibodies and is independent of the plasma level of glucose. Antibody-bound insulin is biologically inactive, while a large quantity of free insulin dissociated from antibody-bound insulin is biologically active (Ichihara et al. 1977). In our patient, hypoglycemic attacks occurred at 5 : 30 p.m. even though free IRI peaked at 2 : 00 p.m. The time lag between the peak value of free insulin and hypoglycemic attacks may have been related to glucose intake at lunch.

Based on the hypothesis, we treated the patient with an α-glucosidase inhibitor to inhibit morning hyperglycemia and hypersecretion of insulin. However, although the α-glucosidase inhibitor caused a slight decrease in the plasma level of glucose, there was no improvement in the hypoglycemia. Thus we could not improve the morning hyperglycemia, and hypoglycemia was not prevented.

In contrast to regular hyperglycemia in the morning and hypoglycemia in the afternoon in our patient, patients with IAS experienced hypoglycemia at various times. Although the exact reason for this discrepancy remains to be elucidated, one possibility is that the character of insulin antibody in each patient was different. Another possibility is that our patient was diagnosed as non-insulin dependent diabetes mellitus and patients with IAS are not affected by diabetes mellitus. The total IRI and ^{125}I insulin binding rates were decreased to 25% and 2.2% respectively 5 months after initiation of steroid therapy. The hypoglycemic attacks disappeared as the insulin antibody titer decreased.

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