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Clinical application of the different cross-reactivities of anti-insulin antibodies to insulin lispro to evaluate endogenous insulin secretion

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ABSTRACT

Background: Insulin analogs are often used to treat patients with diabetes. We evaluated the cross-reactivities of anti-insulin antibodies in two insulin immunoassay kits (Architect and ECLusys) against recombinant human insulin and insulin analogs, and measured insulin concentrations in the serum of the diabetic patients treated with only insulin lispro.

Methods: Ten-fold dilutions of recombinant human insulins and insulin analogs were measured using Architect and ECLusys kits. The serum samples of 4 type 2 diabetic patients at fasting, and several time points after breakfast (25 kcal/kg) following subcutaneous injection of insulin lispro were measured by Architect, ECLusys and LISPro RIA kit.

Results: The ECLusys kit could detect human insulin but not insulin analogs. The Architect kit detected human insulin and insulin analogs with similar recovery ratios. The difference in serum insulin concentrations measured by Architect and ECLusys assays reflected the concentration measured by LISPro insulin kit in the patients. The differences in the AUC between Architect and ECLusys assays were significantly correlated with the AUC for LISPro assay ($p<0.01$).

Conclusions: By exploiting the different cross-reactivities of anti-insulin antibodies to insulin analogs, it may be possible to measure the endogenous and exogenous insulin concentrations in diabetic patients treated with insulin analogs.

1. Introduction

In order to evaluate the plasticity of pancreatic β cells following insulin treatment, it is important to determine the pharmacokinetic properties of injected insulin together with endogenous insulin. Serum insulin concentrations are generally measured using immunoassays with specific antibodies against insulin. These insulin antibodies may express different levels of cross-reactivity to human insulin, proinsulin, and insulin analogs. Consequently, the measured insulin concentrations may differ among these kits. However, these properties of different antibodies may be useful, allowing us to measure the concentrations of insulin and insulin analogs separately.

To generate insulin analogs, the primary sequence of insulin is genetically altered by the substitution of specific amino acids, yielding insulin with ultra-short or long-acting properties [1]. Insulin analogs are now used to achieve good glycemic control by mimicking physiologic insulin secretion, and to prevent the progression of microvascular and macrovascular complications [2–4].

In this study, we evaluated the cross-reactivities of the antibodies provided in two insulin assay kits (Architect and ECLusys) against human insulin and three insulin analogs (insulin lispro [5], insulin aspart [6] and insulin glargine [7]). We also used these kits to measure serum insulin concentrations in patients with type 2 diabetes, who were treated with insulin lispro. The differences in insulin concentrations measured by the two kits, which use anti-insulin antibodies raised against human insulin with or without cross-reactivity to insulin lispro, were calculated to determine endogenous insulin concentrations in patients being treated with insulin lispro. These analyses may be useful to determine the optimal mode of treatment, including the use of insulin, insulin analogs, and oral hypoglycemic agents, taking into account the patient's endogenous insulin secretion.

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2. Materials and methods

2.1. In vitro evaluation of the insulin assays

We used the Architect insulin® (Architect; Abbott Japan, Tokyo, Japan) [8,9] and ECLusys Insulin® (ECLusys; Roche Diagnostics, Basel, Switzerland) [10] assays to measure insulin concentrations. The within-run coefficient of variations (CVs) and the total CVs of both kits, assessed according to the protocols of the Japanese Committee of Clinical Laboratory Standards, did not exceed 5% (data not shown). The specificity of the anti-insulin antibodies contained in these kits was determined with linear dilutions of recombinant human regular insulin (human insulin; Novo Nordisk, Bagsværd, Denmark), neutral protamine Hagedorn insulin (NPH insulin; Novo Nordisk), and three insulin analogs; insulin lispro (Humalog®; Eli Lilly, Indianapolis, IN, USA) [5], insulin aspart (NovoRapid®; Novo Nordisk) [6] and insulin glargine (Lantus®; Sanofi-Aventis, Paris, France) [7]. Each insulin formulation was serially diluted in Tris buffer (50 mmol/l Tris pH 7.2, 1% bovine serum albumin, and 1% lipoprotein) to provide theoretical concentrations ranging from 10 to 10,000 mU/l. Each sample was measured twice.

2.2. Regression analysis between the insulin concentrations measured by Architect and ECLusys assays

One hundred serum samples were obtained from randomly selected individuals who attended our hospital. Informed consent was obtained from all of the individuals who provided serum samples. Insulin concentrations were measured using the Architect and ECLusys assays. Regression analysis was applied to compare the resulting insulin concentrations in the serum samples of diabetic patients between the Architect and ECLusys assays.

2.3. Patients

Four patients with type 2 diabetes naïve to insulin were enrolled for this phase of the study. Three of the patients were males in their 50s; the other was a 72-year-old female. Their BMI ranged from 24 to 27 kg/m^2. Fasting immunoreactive insulin (IRI) ranged from 2.7 to 11.1 mU/l and urinary C-peptide immunoreactivity (CPR) ranged from 56.6 to 122.1 μg/day. Insulin autoantibodies were negative in all four patients. Table 1 summarizes the characteristics of the patients. All of the patients provided written informed consent. The study was conducted in accordance with the guidelines approved by the local research ethics committee.

All four patients started a short-term intensive insulin therapy (insulin lispro) 4 days after attending our hospital. Two weeks later, blood samples were collected before (fasting) and at 30, 60, 90, and 120 min after breakfast (25 kcal/kg) following a subcutaneous injection of insulin lispro. Each patient administered an appropriate dose of insulin lispro just before breakfast. The resulting blood samples were used to measure insulin concentrations.

2.4. Measurement of serum insulin concentrations

Insulin concentrations in each serum sample were measured using the Architect and ECLusys assays. Insulin lispro concentrations were measured using a LISPro RIA kit (Linco Research, St. Charles, MO, USA). This assay is highly specific for insulin lispro (100%) with negligible cross-reactivity for native human insulin or proinsulin (<0.5%) [11,12].

2.5. Other laboratory tests

CPR was measured in serum and urine samples stored for 24 h using a chemiluminescence enzyme immunoassay with Lumipulse Presto C-peptide (Fujirebio, Tokyo, Japan). Hemoglobin (Hb) A_{1c} was determined using a high-performance liquid chromatographic method (HA-8160; Arkray, Kyoto, Japan). HbA_{1c} (Japan Diabetes Society, JDS) values were transformed to HbA_{1c} (National Glycohemoglobin Standardization Program, NGSP) values using the formula HbA_{1c} (NGSP) = 1.019 × HbA_{1c} (JDS) + 0.3% [13]. The presence of free anti-insulin antibodies, which were not complexed with circulating insulin, was determined using a liquid-phase radio-binding assay. In brief, ^{125}I-labeled insulin was added to 0.1 ml of serum and incubated at 4 °C for 16–20 h. After incubation, a solution containing 25% polyethylene glycol was added and the insulin–insulin antibody complex was precipitated by centrifugation from free ^{125}I-labeled insulin, and radio-counted. Then B (bound)/T (total) % was calculated. Samples exceeding a binding value of 7.0% were defined as insulin antibody positive.

3. Results

3.1. Analyses of human insulin and insulin analogs

Both assay kits were able to measure the concentrations of human insulin, providing values similar to the theoretical values (Table 2). The results of NPH insulin concentrations measured by ECLusys were showed slightly higher than those by Architect. The concentrations of insulin analogs were also successfully measured by the Architect assay with good recovery ratios. By contrast, the ECLusys assay was unable to detect the insulin analogs including insulin lispro.

3.2. Regression analysis between Architect and ECLusys

The regression equation between the ECLusys (x) and Architect (y) assays was $y = 0.841x - 2.01$ mU/l, and $p < 0.0001$.

3.3. Measurement of serum insulin concentrations

The serum insulin concentrations were measured using the Architect and ECLusys assays in all 4 patients at fasting, and at 30, 60, 90, and 120 min after injecting insulin lispro after breakfast. To provide an accurate comparison of the insulin concentrations measured by both kits, the insulin concentrations measured by the ECLusys assay were evaluated according to the regression equation. The samples were also analyzed

Table 1
Subject characteristics.

Patient no.	Age (years)	Sex (M/F)	Type of diabetes	BMI (kg/m^2)	FPG (mg/dl)	HbA_{1c} (%)	F-IRI (mU/l)	F-CPR (ng/ml)	U-CPR (μg/day)	Insulin Ab status
1	54	M	2	25	193	8.0	5.0	1.3	80.0	–
2	72	F	2	24	228	14.4	7.2	1.2	78.5	–
3	59	M	2	27	188	10.3	11.1	1.8	56.6	–
4	58	M	2	24	180	9.7	2.7	2.1	122.1	–

FPG, fasting plasma glucose; F-IRI, fasting immunoreactive insulin; F-CPR, fasting C-peptide immunoreactivity; U-CPR, urinary C-peptide immunoreactivity; Ab, antibody.