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Use of cross-reactivity immunoassay to orient insulin replacement in diabetic patients with high levels of insulin antibodies

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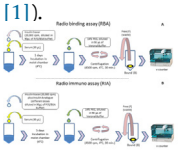
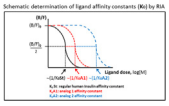
GRAPHICAL ABSTRACT

Buffer P/G/BSA: 0,1 M Phosphate, 0,25% of non specific gamma globulin and 0,5% of bovin serum albumin, pH 7,4. Veronal Buffer: 0,05 M sodium barbital and 0,01% tween 20, pH 8,6. PEG: Polyethylene glycol 6000. A) RBA: IA binding rate measured as tracer binding percent (B%) over a cutoff of nonspecific binding. B) RIA: B and F results are transformed in plots of B/F = f (ligand dose, M) to calculate the respective K_0 values. The molar concentration of the tracer in the test must be lower than the inverse of K_0 value. This condition precludes the preparation of the respective labeled competitors to perform specific single RIAs for each homologous ligand (Berzofsky-Schechter [1]).





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A B S T R A C T

The prevalence and high levels of anti-insulin antibodies (IA) have frequently been associated with brittle diabetes, lipodystrophy in the areas where the insulin is injected and/or poor metabolic control. When this happens the usual criterion adopted is the empirical change of insulin type and/or formulation intending to diminish the IA level and then to decrease the undesirable side-effects. Here, we present a rational two step radiometric method consisting in: A) a first-line radioligand binding assay (RBA) to assess IA in sera of these patients and detecting those with high levels. B) applying a displacement assay (RIA) to determine the in vitro cross-reactivity parameters (affinity constants and selectivity ratios) that quantify the relative degree of interaction between antibodies and alternative insulin analogs. From these results we conclude that conventional criteria for selection of insulin analogs, in terms of pharmacokinetic and pharmacodinamic parameters, should be complemented with an appropriate test to assess affinity parameters when high IA title is demonstrated.

- This manuscript introduces a rational method to determine the appropriated insulin replacement when high insulin antibodies levels are present.
- This protocol provides instructions and details in mathematical tools and laboratory processes for the analysis of serum samples.
- This method proved to be successful in a single case and requires confirmation using a large group of patients.

A R T I C L E I N F O

Method name: Radio binding assay (RBA), Radio immuno assay (RIA)

Keywords: Insulins analogues, Diabetes type 1, Insulin antibodies, Brittle diabetes

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Introduction

The prevalence and levels of anti-insulin antibodies (IA) elicited nowadays during insulin therapy have decreased remarkably in comparison to the original treatments as a consequence of the high purity of recombinant human insulin preparations and hypoimmunogenicity of insulin analogs. However, some patients still present relatively high IA titer levels, frequently associated with brittle diabetes, lipodystrophy in injection sites and/or poor metabolic control despite the high insulin doses administered. When this happens the usual criterion adopted is the empirical change of insulin type and/or formulation intending to diminish the IA level and then to decrease the undesirable side-effects. However, the consequent problem is that such an empirical procedure requires relatively long periods of time and clinical surveillance during each interval of change, whilst immediate and long-term complications may persist or aggravate.

Here, we present a rational method supporting the best choice of alternative insulin variants according to the immune cross-reactivity parameters exhibited by a panel of candidate formulations assayed in vitro with serum from patients who present with a high positive IA titer. We report the preliminary results obtained after applying the approach mentioned above to a unique model sample consisting of a high level IA positive serum from a diabetic patient exhibiting persistent poor metabolic control despite the high dose of regular and NPH insulin administered.

Methods details

Patient

The patient selected for this study was a 69 year old woman who presented type 2 diabetes, diagnosed 10 years ago and treated with insulin only for the last two years. The study followed the recommendations of WMA Declaration of Helsinki [2]. She was admitted to hospital (Hospital J. R. Vidal, Corrientes City, R. Argentina) presenting with hyperglycaemia [U1] and inadequate metabolic control despite treatment with more than 3 IU/kg/day of NPH human insulin (Humulin – Eli Lilly) complemented with crystalline human insulin during the last two years. Moreover, frequently the glycaemia reached values as high as 500 mg/dL (27.7 mmol/L) and HbA_{1C} values of 11% (IFCC 97 mmol/