Research letter

Diabetology

Inverse correlation between levels of glycated haemoglobin and expression levels of SERCA protein in Mexican patients with type 2 diabetes mellitus

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Abstract

We examined the association between sarco/endoplasmic reticulum calcium ATPase (SERCA) expression and glycated hemoglobin (HbA_{1c}) levels since alterations in this protein expression are associated with the genesis of insulin resistance. HbA1c levels and SERCA protein expression from platelets of Mexican patients diagnosed with type 2 diabetes mellitus (T2DM) were analyzed showing lower values of SERCA expression against the normal values we find in healthy people. Interestingly, as diabetes condition got worse; SERCA protein expression decreased gradually until it was undetectable. The results showed an inverse correlation between HbA_{1c} and SERCA protein expression in T2DM patients. .

Key words: type 2 diabetes mellitus, glycated hemoglobin, SERCA.

Insulin resistance is a systemic condition in which the cell response to this hormone decreases, thus increasing blood glucose levels and inducing the onset of type 2 diabetes mellitus (T2DM), which represents a serious public health issue in Mexico [1]. At the molecular level, there are solid data demonstrating that endoplasmic reticulum stress (ERS) is one of the main causes of insulin resistance genesis [2, 3]. Recent evidence proves that the reduction in SERCA expression induces ERS and the consequent state of insulin resistance [4]. In congruence, overexpression of SERCA protein has been shown to decrease the insulin resistance state in cell cultures and in rodents [4, 5]. All the features previously mentioned could place SERCA as a possible therapeutic target because rosiglitazone reduces insulin resistance while increasing SERCA expression levels [5, 6].

The study protocol received previous approval from the Hospital General 5 de Diciembre of ISSSTE Mexicali, Mexico (Circular letter number 0985/2017), and was carried out in accordance with the principles of the Declaration of Helsinki, as revised in 2000. T2DM patients and apparently healthy people were recruited after a check-up with their Familiar Physician, obtaining informed written consent to carry out a pilot study.

The study included 27 T2DM diagnosed patients by ADA 2017 Standards of medical care in DM fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l), glycated hemoglobin (HbA_{1c}) ≥ 6.5%, or patients with ran-

dom plasma glucose ≥ 200 mg/dl (11.1 mmol/l) with classic symptoms of hyperglycaemia. The control group included eight apparently healthy individuals (FPG < 100 mg/dl, HbA_{1c} < 5.7%, body mass index (BMI) < 25%, no comorbidities or pathologic signs or symptoms) of both genders, aged 19–60 years.

Congestive heart failure, acute or chronic renal failure, type 1 diabetes mellitus, acute or chronic pancreatitis, acute or chronic liver disease, and pregnancy were exclusion criteria.

Glucose and HbA_{1c} levels were determined by standard methods (HITACHI Cobas 600 and Cobas b 101 from Roche, respectively).

Ten millilitres of peripheral blood were drawn into polypropylene tubes containing sodium citrate (Vacutainer System, BD Biosciences). Fractions enriched in platelets were obtained as described previously, with some modifications [7]. Plasma was carefully aspirated from the pellet and centrifuged at 800 g for 15 min to obtain plasma fractions enriched in platelets. Finally, fractions enriched in platelets were analysed by western blot.

The protein level of each sample was determined by the micro Bradford protein assay [8]. Proteins (35 µg of protein/sample) were separated by SDS-PAGE using 10% separating gels followed by transfer to polyvinylidene fluoride (PVDF) membranes. Blots were incubated overnight at 4°C with primary antibodies (SERCA2 and β-actin, Santa Cruz Biotechnology) and washed three times with TBS-T buffer before incubation with horseradish peroxidase-conjugated secondary antibodies for 1 h at 22°C. Blots were then visualised with Millipore Immobilon western HRP substrate peroxide solution. Quantification of immunoblot films was carried out with ImageJ software.

Average intensities from Western blot films were analysed by two-tailed unpaired Student's *t*-test between two groups, and by One-way ANOVA with Dunnett's post-test for multiple comparison using PRISM, version 7.0 (GraphPad Software, San Diego, CA, USA). A *p*-value < 0.05 was considered to be statistically significant. Values were reported as mean ± SEM, and the figures show representative blots.

Initially, in order to corroborate the effectiveness of our antibody to recognise the SERCA protein of human platelets, we processed a rat heart homogenate as a positive control (this antibody recognises the SERCA2 isoform in humans and rats), since

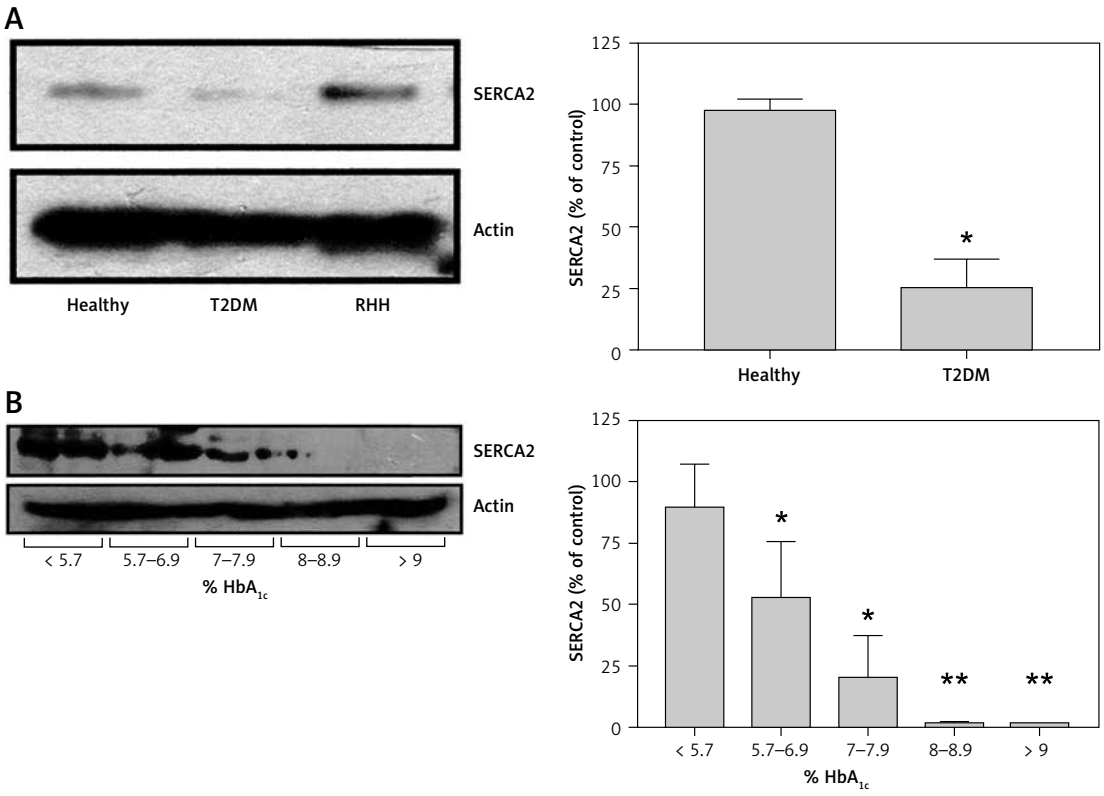

Figure 1. An increase in percentage of glycosylated HbA_{1c} is negatively correlated with SERCA2 protein expression levels in platelets from T2DM patients. **A** – Fractions enriched in platelets from blood samples of healthy individuals (n = 3) and T2DM patients (n = 3) compared to rat heart homogenates (RHH). **B** – Fractions enriched in platelets from blood samples of healthy individuals (< 5.7, n = 8), and T2DM patients with increasing percentages of HbA_{1c} (5.7–6.9%, n = 8; 7–7.9%, n = 8; 8–8.9%, n = 7; and > 9, n = 4). Bar graphs indicate mean ± S.E of SERCA. Western blots were also probed for actin as a loading control

**P < 0.05, **p < 0.01 vs. control group (healthy individuals).*

SERCA protein levels are high in this tissue [6]. Then, we evaluated SERCA protein expression levels in platelets of apparently healthy people and T2DM patients. Our data show that in platelets of people with uncontrolled T2DM, SERCA expression was significantly diminished (mean ± SEM: 97.11 ±2.8 vs. 25.45 ±6.46, respectively; $p < 0.005$) (Figure 1 A).

HbA_{1c} levels are an indicator of different degrees of uncontrolled T2DM [9]. The T2DM Mexican patients were divided according to HbA_{1c} haemoglobin percentage, in order to determine if SERCA expression levels in platelets were disturbed in a manner that correlated with HbA_{1c} [4]. The results confirmed that there is a decrease in SERCA expression levels that depends on HbA_{1c} increase (mean ± SEM: 89.28 ±6.78, 52.65 ±8.15, 19.67 ±6.29, to < 5.7, 5.7–6.9, and 7–7.9, respectively; $p < 0.005$) and that, from levels of HbA_{1c} > 8%, SERCA expression levels are undetectable (Figure 1 B).

Different scientific reports have established the relevance of SERCA expression disturbances in the genesis of ERS and insulin resistance [4, 5]. In the same context, we carried out this work because few reports in the literature address the alterations in SERCA expression in T2DM patients [10]. To begin our study, we decided to compare platelets of apparently healthy people and T2DM patients, who had HbA_{1c} between 7% and 7.9%. The result of this experiment shows that the levels of SERCA expression are diminished in T2DM patients as well as during the state of insulin resistance [5]. On the other hand, we also wanted to assess if at different degrees of uncontrolled T2DM (defined by HbA_{1c} > 7%) SERCA expressed disturbances. Finding that there is a progressive decrease in SERCA levels until it disappears, inversely correlated with haemoglobin levels. We believe that SERCA levels can become so low that they are undetectable by the antibody.

In conclusion, as was demonstrated in previous reports, the SERCA expression decrease is not only involved in the genesis of insulin resistance [4, 5]; in fact, once the T2DM is established, SERCA levels decrease even more, depending on the degree of disease control. Moreover, there are solid data proving that overexpression of SERCA in cell culture decreases insulin resistance [4]; likewise, another study showed in uncontrolled T2DM patients that rosiglitazone administration increased SERCA protein expression levels and decreased the insulin resistance [10]. The foregoing centralises the increase in the expression of SERCA as a probable therapeutic strategy.

Conflict of interest

The authors declare no conflict of interest.

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