Development of an Immunoassay for the Detection of Tf-receptors

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ABSTRACT

The purpose of this study was to develop an immunoassay for Tf-receptors that could be used to investigate a possible relationship between iron and type 2 diabetes. Tissue samples were collected from normal mice and the amounts of Tf-receptors present were measured by a Western blot. As the molecular weight of the immunostained protein (25kD) was less than one third of the known size of the Tf-receptor (95kD) it suggests that the Tf-receptor had been degraded by proteolytic enzymes released during sampling. Adding more protease inhibitors during the sampling process could prevent the degradation of the Tf-receptor.

INTRODUCTION

Iron is an essential element for the production of many molecules such as hemoglobin, and for numerous metabolic processes including DNA synthesis and electron transport for cellular respiration.\textsuperscript{1} In the steady state, iron is bound to transferrin and circulates in the blood in a nonreactive form.\textsuperscript{2--3} Iron is taken up from the blood by a high-affinity specific transferrin receptor (Tf-receptor), and the Tf-receptor complex is incorporated by endocytosis.\textsuperscript{3} Following incorporation iron is released into a non-acidic cellular compartment where it can either be used in the synthesis of essential cellular components or stored in complex with a protein called ferritin.\textsuperscript{2--3}

Excessive amounts of iron have been speculated to cause non-insulin-dependent mellitus (type 2 diabetes).\textsuperscript{4--6} Increased iron levels have been correlated with the failure of insulin to inhibit glucose production by the liver as well as with the reduction of insulin metabolism.\textsuperscript{3} These effects cause the liver to become resistant to insulin and results in peripheral hyperinsulinemia.\textsuperscript{3}

Iron is also thought to cause type 2 diabetes through oxidative stress.\textsuperscript{3--5} Iron acts as a catalyst in the formation of hydroxyl radicals that can oxidize other molecules resulting in cellular and tissue damage.\textsuperscript{2--5,7} Since the cells responsible for the production of insulin are
sensitive to oxidative damage, the iron-mediated formation of free radicals contributes to insulin resistance and subsequently to the development of type 2 diabetes.⁴⁻⁶

Despite the findings on the association between iron and type 2 diabetes, the supporting evidence for an iron-diabetes relationship are inconsistent. Studies previously performed have tested the iron-diabetes hypothesis by using serum ferritin concentrations to measure iron status.⁴⁻⁶ However, the use of serum ferritin levels can be misleading because ferritin levels are also elevated by inflammation.⁵⁻⁶ A better marker of iron status is the amount of transferrin receptors in the blood or tissues because the Tf-receptor is less influenced by disease. The purpose of this study was to develop an immunoassay for Tf-receptors that could be used to investigate the possible relationship between iron and type 2 diabetes.

**METHODOLOGY**

Tissue samples from the liver, kidney, and intestine of two mice were collected. The samples were placed into individual test tubes and a sucrose lysis buffer solution was added to each tube to obtain a 1:1 (g:mL) ratio between the sample and the buffer. Each sample was then homogenized with a homogenizer for approximately 20 seconds. Following homogenization, the samples were centrifuged at 12,000 rpm for five minutes. The supernatant of each sample was divided into 1mL aliquots and placed into eppendorf tubes.

The samples were mixed with a 2% sodium dodecyl sulfate (SDS) reducing buffer, and the wells of a 12% SDS polyacrylamide gel were loaded with 10μL of the resulting mixture. A pre-stained molecular weight standard served as a control. Electrophoresis was carried out at 200V for 60 minutes.

Following separation, the proteins were transferred to a nitrocellulose membrane using a BIORAD Transblot apparatus according to the instructions of the manufacturer. The membrane
was blocked with a 2% non-fat dry milk solution and the samples were probed with a primary antibody specific to the Tf-receptor. The samples were incubated with rabbit anti-mouse IgG antibody conjugated with alkaline phosphotase (Sigma-Aldrich Company, St. Louis, MO) and the membrane was stained with BCIP/NBT liquid substrate (Sigma-Aldrich Company, St. Louis, MO) to reveal the immunoreactive proteins. The membrane was photographed and the bands from the samples were compared to the bands of the standard to determine the molecular weight of the Tf-receptor of each sample.

RESULTS

When compared to the pre-stained molecular weight standard, the Tf-receptor of the intestine and kidney samples correlated with a size of approximately 25kD. While a single band was observed in both the intestine and kidney samples, multiple bands were evident in the liver sample (Fig. 1). Accordingly, the weight of the Tf-receptor in this sample was not identified.

![Figure 1. Photograph of the immunostained membrane. ML = mouse liver, MK = mouse kidney, MI = mouse intestine, PS = pre-stained standard. Approximate sizes of the pre-stained standard molecular weight standard proteins are indicated.](image-url)
DISCUSSION & CONCLUSION

The purpose of this study was to develop an immunoassay for Tf-receptors that could be used to verify a relationship between iron and type 2 diabetes. As the molecular weight of the intestine and kidney samples (25kD) were less than one third of the repeated size of the Tf-receptor (95kD) (Fig. 2) it suggests that the transferrin receptor had been degraded. This degradation of the Tf-receptor was likely due to the release of proteolytic enzymes by lysosomes present in the cell during sample preparation.

The bands present in the liver sample indicated that the antibodies had bound non-specifically to numerous proteins (Fig. 2).

![Figure 2. Photograph of the immunostained membrane. ML = mouse liver, MK = mouse kidney, MI = mouse intestine, PS = pre-stained standard. The repeated size of the Tf-receptor (95kD) is also indicated.]

Since the data obtained is inconclusive, the immunoassay must be modified and improved to further support and verify the iron-diabetes hypothesis. This immunoassay can be improved by adding more protease inhibitors during the sampling process to prevent degradation of the Tf-receptor. Also, lowering the concentration of both antibodies used, and lowering the
amount of protein loaded on to the gel could stop nonspecific binding from occurring on the membrane.

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Literature sources:


