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Role of Protein Tyrosine Phosphatase in Type-2 Diabetes Mellitus Patients

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Summary Background

Blood glucose level is regulated through insulin.Insulin resistance is mostly caused by impairment in the insulin receptor signal transduction pathway which is the most important characteristic of type-2 diabetes mellitus.The evidence for the physiological role of PTP1B in the regulation of metabolic pathways came from whole body PTP1B knock out mice.The increased incidence of T2DM has intensified search for new therapeutic treatment options.

Methods

Immune Complex Assays

1.Protein Tyrosine Phosphatases

The number of cells depends on the abundance and activity of the specific phosphatase that is assayed.

2.Insulin in serum

The Merocodia Insulin ELISA- two site enzyme immunoassay.

3. Hexokinase method-blood glucose

Results

The present study specified correlation in PTP1B activity,glucose and insulin as compared with control group and T2 DM group based on statistical analysis.In control group PTP1B is 1.04µmol/L(0.18) & T2DM group is 1.00µmol/L(0.15),glucose is 5.6mmol/L(0.3) in control group & 8.7mmol/L(0.6) in T2DM group,insulin is 6.1mU/L(1.5) in control group & 11.9mU/L(1.6) in T2DM group ($p<0.001$).

Conclusion

The study to supports the hypothesis that insulin resistance develops from a coordinated response to various cellular processes that develop upon the exposure of insulin-responsive cells to excess sugar.

Abstract

Blood glucose level is regulated through insulin.Insulin resistance is mostly caused by impairment in the insulin receptor (IR) signal transduction pathway which is the most important characteristic of type-2 diabetes mellitus(T2DM).The evidence for the physiological role of PTP1B in the regulation of metabolic pathways came from whole body PTP1B knock out mice.The increased incidence of T2DM has intensified search for new therapeutic treatment options.PTP's constitute a large family of enzymes which are crucial modulators of cellular phosphorylation events.Recent studies have demonstrated that loss of PTP1B activity resulted in insulin resistance.PTP1B is able to interact with IR & IRS-I to hydrolyse tyrosine phosphorylation induced by insulin action,causing an impairment of glucose uptake.The present study was conducted in CSSH,Meerut in the year 2008.100 controls & 100,T2DM patients of age 40-60 years were included for the study.Decrease in PTP1B can help in the assessment and treatment of T2DM patients

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Key words

IR-insulin receptor,IRS-1-insulin receptor substrate,PTP1B-protein tyrosine phosphatase,T2DM-type 2 diabetes mellitus,RTK-receptor tyrosine kinase,MAPK-mitogen activated protein kinase,

PI3K-phosphatidylinositol 3 kinase.

Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent metabolic disorder [1,2] and global prevalence of this disease is expected to rise to 7079 persons per 100,000 by 2030[3]. T2DM is correlated with a wide range of lifethreatening complications [3]. Main characteristics of

T2DM are insulin resistance, chronic hyperglycemia, and relative β-cell dysfunction [4]. Insulin resistance is defined as a subnormal biological response to normal insulin level [1, 5], which could be mostly caused by impairment in the insulin receptor (IR) signaling pathway [1, 6]. The

IR is a type of receptor tyrosine kinase (RTK), in which the binding of insulin triggers

autophosphorylation of the tyrosine residues. Then, this process causes phosphorylation

of several downstream targets. Insulin activates two major protein–protein interaction signaling pathways: (1) the phosphatidylinositol 3-kinase (PI3K) pathway which mediates the metabolic function of insulin and (2) the mitogen-activated protein kinase (MAPK) pathway which

mediates mitogenic function of insulin [5, 7]. Thus, protein phosphorylation in the

insulin signaling pathway is a key step that mediates the effects of the insulin in target cells.

This phosphorylation is reversed by phosphatases. Various phosphatases, such as protein tyrosine phosphatases (PTPs) and inositol phosphatases, regulate the phosphorylation

of RTK and its downstream substrates [8–10]. Due to importance of PTPs in the regulation of the insulin signaling, it is not surprising that PTPs play a key role in the pathogenesis of insulin resistance and T2DM [11]. Protein tyrosine phosphatase (PTP1B) as an ubiquitously expressed

PTP is broadly expressed in insulin sensitive tissues and considered as the key negative regulator of IR signaling [12]. In addition to insulin-sensitive tissues, PTP1B is also

noticeably expressed in tissues that are affected by diabetes complications [13].Given the important role of PTP1B in controlling of the insulin signaling pathway and alteration

of its expression level in various tissues by diabetes, we have here reviewed the functions of this protein in insulin resistance, diabetes and its complications.

Protein tyrosine phosphatase 1B (PTP1B)

PTP1B is a ubiquitously expressed non-receptor classical protein tyrosine phosphatases (PTPs) from class 1 PTPs. The *PTPN1* gene encodes PTP1B, which is located in 20q13 [14, 15]. PTP1B, as a 435 amino acids (50 kDa) monomeric enzyme, is mostly targeted to the

endoplasmic reticulum (ER) by cleavable hydrophobic and proline-rich C-terminal segment [16, 17]. The C-terminal non-catalytic domain of PTP1B has a regulatory role [18].PTP1B controls several signaling events including cell growth, apoptosis, differen

tiation, and cell movement through dephosphorylation of the target molecules [19].

In the following section, PTP1B regulation at the mentioned levels will be reviewed.

Material and methods

Immune Complex Assays for Protein Tyrosine Phosphatases

Principle

The number of cells that need to be lysed per data point depends on the abundance and activity of the specific phosphatase that is assayed. While 5×106 – 5×107 cells should provide a reasonable range to achieve measurable phosphatase activity for most of commonly tested PTPs of the immune system, it is recommended that the optimal number of cells be experimentally determined for each phosphatase and cell type. As a general rule, the amount of assayed phosphatase and the assay time should allow measurement of the enzymatic activity in the linear range, i.e. where there is a direct correlation between the amount of protein and the enzymatic activity.

Materials

Cells of desired type Phosphate-Buffered Saline (PBS, Appendix 2A), ice cold 5× NP 40 lysis ,1× NP 40 lysis buffer (freshly supplemented with protease inhibitors), ice-cold Antibodies against protein of interest (polyclonal or monoclonal) Protein A- or Protein G-Sepharose (GE Healthcare) ,1× Phosphatase buffer Phosphatase substrate; p-Nitrophenyl Phosphate (pNPP) ,NaOH [1 N]

Insulin in Serum

Principle:-

Insulin is the primary hormone responsible for controlling glucose metabolism, and its secretion is governed by plasma glucose concentration. The insulin molecule is synthesized in the pancreas as pro-insulin and is later cleaved to form C-peptide and insulin. The principal function of insulin is to control the uptake and utilization of glucose in the peripheral tissues. Insulin concentrations are severely reduced in insulindependent diabetes mellitus (IDDM) and some other conditions, while concentrations are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, and some endocrine dysfunctions.The Merocodia Insulin ELISA is a two-site enzyme immunoassay utilizing the direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants of the insulin molecule. Specimen, control, or standard is pipetted into the sample well, then followed by the addition of peroxidase-conjugated anti-insulin antibodies. Insulin present in the sample will bind to anti-insulin antibodies bound to the sample well, while the peroxidase-conjugated anti-insulin antibodies will also bind to the insulin at the same time. After washing to remove unbound enzyme-labelled antibodies, TMB-labelled substrate is added and binds to the conjugated antibodies. Acid is added to the sample well to stop the reaction and the colorimetric endpoint is read on a microplate spectrophotometer set to the appropriate light wavelength.

Quantitative analysis of blood glucose- Hexokinase method

Principle:-

The enzyme hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). In the presence of nicotinamide adenine dinucleotide (NAD), G-6-P is oxidized by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

Results

Table I:-

Demographic data

Mean age		Cases	Control
53 ± 8	Males	51	52
48 ± 6	Females	49	48
Hypertensive	Yes	70 %	30 %
	No	30 %	70 %
Smokers	Yes	80 %	20 %
	No	20 %	80 %

Table II:-

Subject	No.of patients	PTP1B (µmol/L)	Glucose (mmol/L)	Insulin (mU/L)	P value
Control	100	>>1.04(0.18)<<	>>5.6 (0.3)<<	>>6.1 (1.5)<<	p<0.001
T2DM	100	>>1.00(0.15)<<	>>8.7 (0.6)<<	>>11.9(1.6)<<	p<0.001

In the present study,control group and study group biochemical parameters included .The baseline measure of the studied groups are represented in table II. The mean values sharing the significantly at 0.001 level. Both

groups include total number of 200 patients. Mean values of PTP1B, were significantly decreased as compared to control group ($p<0.001$) ,blood glucose increased as compared to control group ($p<0.001$) and insulin increased significantly in T2DM group ($p<0.001$) as compared to control group.

Discussion

In previous finding ,the case of human studies, PTP1B gene and protein expression were remarkably higher in skeletal muscle, liver and adipose tissues of insulin-resistant, obese, or diabetic humans [20,21]. T-Cell Protein Tyrosine Phosphatase (TC-PTP) is a homologous protein of PTP1B with a high degree of homology in the catalytic structural domain (74% of the amino acid sequence). Meanwhile, TC-PTP is a phosphatase associated with the regulation of T-cell activation and plays an essential role in human hematopoiesis [22].It has found that the activity of PTP1B was 1.02 ± 0.17 µmol/L.study shows normal insulin binding, but inhibited insulin-induced receptor tyrosine kinase activity in human muscle in the two most common forms

of insulin resistance - obesity and Type 2 diabetes. Furthermore, the decrease in enzyme activity was in the same order of magnitude in both conditions (about 40%) and the combination of the two disorders did not produce any additional changes. These observations

were made in a very homogenous group of 60-year-old 439 men. It is therefore not known if women or younger subjects show other or similar changes in insulin action in obesity and Type 2 diabetes. Insulin resistance at the target cellular level may be caused by several events, the earliest being an alteration in the receptor.The insulin dose-response characteristics for the activation of the receptor kinase were similar for control and study groups. Therefore,

the coupling between the insulin binding site of the receptor and the catalytic subunit remained intact in receptors that could respond to insulin by an increase in kinase activity. The lack of an alteration in insulin binding argues against a change in the number of insulin receptors in

the muscle of the insulin-resistant subjects. This interpretation must be tempered somewhat since the small amount of tissue available for the present study required detergent extraction rather than the use of isolated

membranes. Thus, we cannot comment on the number of receptors available to bind inulin in the intact tissue. However, it is unlikely that a large reduc

tion in "active" receptors exists in these subjects unless the distribution between intracellular and cell surface receptors was markedly altered. While the present study reports on the muscle insulin receptor in obesity and diabetes, other studies have examined the insulin receptor in the liver of subjects affected with both disorders. For example, insulin binding by liver membranes of extremely obese subjects with and without diabetes is reduced,where as binding is increased in normal weight Type 2 diabetic patients. Thus, the tissue examined and the degree of obesity evidently influences the level of the insulin receptor. However, it is evident from results that moderate obesity in the presence or absence of Type 2 diabetes does not result in an overall loss of insulin receptors in muscle. Because of the limited amount of muscle tissue that was available it was not possible to investigate the molecular structure of the insulin receptor in the previous study. Although we cannot relate the insulin resistance in muscle to loss of insulin receptors, the alteration found in the tyrosine kinase from these patients suggests an underlying cause, an alteration in the signalling property of the receptor. Insulin stimulation of glucose transport in muscle tissue plays a major role in glucose disposal. If there is a role for the tyrosine kinase activity in the stimulation of glucose transport the 40% reduction in this activity observed in all three groups should alter the insulin response of affected muscle tissue and contribute to the overall insulin resistance in glucose utilisation. The reduction in the kinase activity of muscle receptors in the absence of an insulin binding or coupling alteration suggests that some receptors have lost their phosphotransferase capability[23].In the present study altered levels of PTP1B,blood glucose and insulin have important role in the assessment and treatment of type-2 diabetes mellitus patients.

Conclusion

There is essential study in support of the hypothesis that insulin resistance develops from a coordinated response to various cellular processes and that develop upon the exposure of insulin-responsive cells to excess sugar. This knowledge will help in the design of better strategies for the prevention and management of insulin resistance and increased blood glucose in reference to PTP1B activity.

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