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Flavin associated Sulfhydryl oxidase and Ero1β in Insulin activity in Type 2 Diabetes Mellitus

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ABSTRACT

DM is proving to be a global public health burden as this number is expected to rise to another 200 million by 2040. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both. Type 1 diabetes mellitus accounts for 5% to 10% of DM and is characterized by autoimmune destruction of insulin-producing beta cells in the islets of the pancreas.

Type 2 diabetes mellitus accounts for around 90% of all cases of diabetes. In Type 2 diabetes mellitus the response to insulin is diminished, and this is defined as insulin resistance. During this state, insulin is ineffective and we now hypothesize that this ineffectiveness of Insulin is due to its improper folding affecting its activity.

Insulin consists of two chains: A chain with 21 amino acids and B chain consists of 30 amino acids with intra and interchain disulphide bonds. These disulphide linkages help to stabilize the structure of insulin thus resulting in its proper biological activity.

The flavin-dependent Endoplasmic Reticulum oxidoreductase 1 beta family of sulfhydryl oxidase enzymes Quiescin-sulfhydryl oxidase rapidly inserts disulfide bonds into unfolded proteins thereby stabilising their tertiary and quaternary structures. As this enzyme is Flavin dependent, a dietary deficiency of Riboflavin leads to improper activity of Sulfhydryl oxidase resulting in abnormal folding of proteins affecting their biological activity. Riboflavin is one of the most common vitamin deficiencies seen in Indian population. The overall prevalence of deficiency of vitamin B2 (Riboflavin) was strikingly high as documented in various studies.

Looking at the rising trend of Type 2 Diabetes Mellitus cases in worldwide and in India, it is always the need of time to look into the pathophysiology of Insulin inactivity in these cases. Sulfhydryl oxidase can be the target molecule and improving its actions with Riboflavin supplementation can prove to be an easy and cost-effective way for the target population to overall improve the Insulin activity. For this the biochemical link has to be established between Sulfhydryl oxidase, Riboflavin and Insulin.

Hence, this study aims to establish the mechanistic link between serum Sulfhydryl oxidase and Ero1β with serum Riboflavin and Insulin in Type 2 Diabetes Mellitus patients.

Introduction

Diabetes Mellitus is proving to be a global public health burden as this number is expected to rise to another 200 million by 2040. The hall mark of Diabetes Mellitus is Insulin deficiency which can be quantitative as seen in Type 1 Diabetes Mellitus (T1DM) or qualitative as seen in Type 2 Diabetes Mellitus (T2DM). In type 2 Diabetes Mellitus there is improper action of Insulin which is seen as Insulin resistance. Type 2 Diabetes Mellitus is the most common type of Diabetes accounting for about 90% of all Diabetes cases[1].

Pathophysiology of Insulin Resistance in Type 2 DM

With the elucidation of the amino-acid sequence of insulin by Sanger in the mid 1950's it became known that insulin was a two-chain heterodimer consisting of a 21-residue A-chain linked to a 30-residue B chain by two disulfide bonds derived from cysteine residues (A7-B7 and A20-B19). An intrachain disulfide bond also exists within the A-chain (A6-A11)[2]. The three native disulfide bonds have been conserved in the insulin structure for more than half a billion years and are of major importance for the stability of the molecule. The structure of Insulin has been well characterized by X-ray crystallography and NMR spectroscopy. Structural and biological studies revealed that all three disulfide bonds are essential for the receptor binding activity of insulin, whereas the different disulfide bonds make different contributions to the overall structure of insulin. Deletion of the A20-B19 disulfide bond had the most substantial influence on the structure as indicated by loss of ordered secondary structure, increased susceptibility to proteolysis, and markedly reduced compactness[3]. A study showed that the two inter-chain disulfide bonds are important for efficient in vivo folding/secretion of PIP (porcine insulin precursor) from yeast, especially the A20-B19 disulfide bond, and that the A7-B7 disulfide bond is crucial for maintaining the native conformation and biological activity of insulin[4]. Another study revealed that the removal of disulfide A7-B7 will result in serious loss of biological activity and the native conformation of insulin[5]. It was documented by Vinther et al[6] that several active four disulfide bonded insulin analogues markedly improved stability and gained insights into the instability of analogues with seven cysteine residues, importance of dimerization for stability, insulin fibril formation process, and the conformation of insulin binding to its receptor.

Hence, the importance of the disulphide linkages was well established with respect to stability and receptor binding activity of Insulin resulting in its proper biological function.

Mechanistic link between flavin-dependent Endoplasmic Reticulum oxidoreductase 1 beta family of sulfhydryl oxidase and Insulin Resistance

Sulfhydryl oxidase 1 (QSOX1 or quiescin Q6) oxidizes sulfhydryl groups in peptide and protein thiols to disulfides with the reduction of oxygen to hydrogen peroxide, permitting disulfide bond formation. QSOX1 is predominantly found in the Golgi and Endoplasmic reticulum[7,8]. Sulfhydryl oxidases are flavin-dependent enzymes[9].

Members of the Quiescin-sulfhydryl oxidase (QSOX) family catalyse the direct introduction of disulfide bonds into unfolded reduced proteins with the reduction of molecular oxygen to generate hydrogen peroxide[10].The oxidase is shown to exhibit a high catalytic activity toward a range of reduced peptides and proteins including insulin A and B chains, lysozyme, ovalbumin, riboflavin-binding protein, and RNase[11].Klyosova et al showed that polymorphism of the GFER gene encoding FAD-dependent sulfhydryl oxidase was associated with a low risk of Diabetes Mellitus in non-obese patients[12].

Endoplasmic reticulum oxidoreductase 1 beta (Ero1β) family of flavin-dependent sulfhydryl oxidase enzymes are believed to play key roles in

disulfide generation in yeast and higher eukaryotes. Ero1β is particularly abundant in the pancreas where it is an important disulfide oxidase in insulin-producing β cells[13]. Mice homozygous for the Ero1β mutation developed a diabetic phenotype, as would be expected if this oxidase were a significant contributor to disulfide bond generation in proinsulin[14]. The defect in insulin is triggered by Ero1β deficiency which thus put emphasis on the special role of Ero1β in disulfide bond formation and protein folding homeostasis in the lumen of the ER of insulin-producing cells. Retarded oxidative folding of proinsulin in islets lacking ERO1-β are consistent[15].

Riboflavin deficiency is highly prevalent in developing countries. In a study from India, it was shown that amongst all vitamin deficiencies, Vitamin B2(Riboflavin) accounted for about 50% of the cases[16]. Sulfhydryl oxidase is a Flavin dependent enzyme. Dietary Riboflavin deficiency leads to improper action of the enzyme resulting in abnormal disulphide linkage associated protein folding. This attributes to ineffective action of the protein molecule[17,18]. Proinsulin misfolding is a phenotype that is very much linked to deficient insulin production and diabetes[19]. Alam et al suggested supplementation with Riboflavin may help in reduction of Diabetic complications[20].

Conclusion

Hence, the mechanistic link between Sulfhydryl oxidase, Ero1 β and Riboflavin needs to be established in relation to ineffective Insulin action and resistance. The reduced Insulin activity may result due ineffective disulphide linkages integral to Insulin activity thus affecting its structural integrity. This may get aggravated in Riboflavin deficiency affecting the activity of Flavin dependent Sulfhydryl oxidase and Ero1β. Riboflavin supplementation in this scenario may prove to be beneficial in control of type 2 Diabetes Mellitus.

Conflict of Interest:

None

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