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POLYOL PATHWAY: A REVIEW ON A POTENTIAL TARGET FOR THE PREVENTION OF DIABETIC COMPLICATIONS

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ABSTRACT: Diabetes mellitus is a complex metabolic disorder arising from lack of insulin production or insulin resistance. It is a leading cause of morbidity and mortality in the developed world, particularly from diabetic complications. Activation of polyol pathway under chronic hyperglycemic conditions by accumulation of sorbitol is the major pathway responsible for the incidence of diabetic complications and further development of oxidative stress. Several mechanisms have been identified by which hyperglycemia induces increased generation of free radicals resulting development of oxidative stress. One of the important mechanisms by which hyperglycemia induces oxidative stress is polyol pathway. The polyol pathway may be implicated in diabetic complications that result in microvascular damage to nervous tissue, retina and kidney. Under normoglycemia, most of the cellular glucose is phosphorylated into glucose-6-phosphate hy hexokinase. A minor part of non-phosphoylated glucose enter the poyol pathway, the alternate route of glucose metabolism. However, under hyperglycemia, because of saturation of hexokinase with ambient glucose aldose is activated leading to excessive production of sorbitol. Intracellular accumalation of sorbitol is through to results in irreversible damage. The review also analyzes the potential mechanism underlying Aldose reductase involvement in pathogenesis of diabetic complication and discusses interations between aldose reductase and other pathogenic factors such as formation of advanced glycation end-products, oxidative- stress, protein kinase C, inflammation and growth factors imbalances. Therefore, aldose reductase enzyme inhibition is becoming one the of the therapeutic strategies that have been proposed to prevent or ameliorate long term diabetic complications.

Keywords: Diabetes, risk factors, oxidative stress, polyol pathway, diabetic complications.

INTRODUCTION:

Diabetes mellitus is a group of metabolic diseases charcterized by hyperglycemia resulting from defects of insulin action, insulin secretion or both. Diabetes has taken place as one of the most important disease worldwide, reaching epidemic proportions. Global estimates predict that the proportion of adult population with diabetes will increase 69% for the year 2030 [1]. Hyperglycemia in the course of diabetes usually leads to the development of microvascular complications, and diabetic partients are more prone to accelerated atherosclerotic macrovascular disease. These complications acount for permature mortality and most of the social and economical burden in the long term of diabetes. Increasing evidence suggests that oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications. Hyperglycemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, insulin action and insulin secreation. Most diabetic patients suffer from long-term complications such as neuropathy, nephropathy, retinopathy, cataracts and even stroke arise from chronic hyperglycemia, and all forms of diabetes increase the risk of long-term complications. These typically develop after many years (10-20), but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels and peripheral nerves. Aldose reductase inhibition represents an attractive strategy for prevention of diabetic complications. The beneficial effect of aldose reductase inhibition in preveting or substantially delaying the onset of diabetic complications in experimental models provides strong support to this hypothesis [2]. The aim of this review is to revise the current knowledge of the role of oxidatve stress in the pathogenesis of diabetes mellitus and its complications. In this review, recent advances in the understanding of the pathophysiological significance of aldose reductase are presented that would be relevent to the efficacy of the enzyme inhibitors in clinical intervention trials of diabetic complications.

METABOLIC AND SIGNALING PATHWAYS INVOLVED IN OXIDATIVE STRESS IN DIABETES:

There are several molecular pathways involved in ROS formation and ROS induced damage. Here we will review the ones that have been related to oxidative stress in diabetes. Not surprisingly, most of them are related to glucose and/or lipid metabolism.

Glucose oxidation and GAPDH:

In order to generate energy, glucose needs to be first oxidized inside the cells by glycolysis. In this process, once glucose enters the cells, it is phosphorylated to form glucose-6-phosphate, a reaction mediated by hexocinases. Glucose-6-P is then converted to Fructose-6-P by phosphoglucoisomerase, which can undergo two fates: The pentose phosphate pathway, where reduction of $NADPH^+$ to NADPH occurs, or to continue glycolysis to yield

Gliceraldehyde- 3-P. Glyceraldehyde-3-P dehydrogenase (GAPDH) phosphorylates this product and glycolysis is further completed until its end product pyruvate, which enters the Krebs cycle and mitochondrial metabolism. It has been proposed that hyperglycemia-induced mitochondrial superoxide production activates damaging pathways by inhibiting glyderaldehyde-3-phosphate dehydrogenase (GAPDH), [3-4] an enzyme that normally translocates in and out of the nucleus. ROS inhibit glyderaldehyde-3-phosphate dehydrogenase through a mechanism involving the activation of enzyme poly-ADP-ribose-1 (PARP-1). This enzyme is involved in DNA repair and apoptotic pathways. ROS cause strand breaks in nuclear DNA which activates PARP-1. PARP-1 activation result in inhibition of glyderaldehyde-3-phosphate dehydrogenase by poly-ADP-ribosylation [5]. This results in increased levels of the glycolytic intermediates upstream of GADPH. Accumulation of glyceraldehydes 3-phosphate activates two major pathways involved in hyperglycemia-complications: a) It activates the AGE deriving glyceraldehydes phosphate and dihydroxyacetone phosphate to the non-enzymatic synthesis of methylglyoxal. b) Increased glyceraldehydes 3-phosphate favours diacylglycerol production which activates PKC pathway. Further upstream, levels of the glycolytic metabolite fructose 6-phosphate increase, which then increases flux through the hexosamine pathway where fructose 6-phosphate is converted by the enzyme glutamine-fructose-6-phosphate amidortransferase (GFAT) to UDP-N-Acetylglucosamine. Finally, inhibition of GAPDH favors the accumulation of the first glycolytic metabolite, glucose. This increases its flux through the polyol pathway, consuming NADPH in the process.

The polyol pathway:

The family of aldo-keto reductase enzymes catalyzes the reduction of a wide variety of carbonyl compounds to their respective alcohols. These reactions utilize nicotinic acid adenine dinucleotide phosphate (NADPH). Aldo-keto reductase has a low affinity (high Km) for glucose, and at the normal glucose concentrations, metabolism of glucose b y this pathway is a very small percentage of total glucose metabolism. However, in a hyperglycemic environment, increased intracellular glucose results in its increased enzymatic conversion to the polyalcohol sorbitol, with concomitant decreases in NADPH. Since NADPH is a cofactor required to regenerate reduced glutathione, an antioxidant mechanism, and this compound is an important scavenger of reactive oxygen species (ROS), this could induce or exacerbate intracellular oxidative stress [3, 4]. Moreover, sorbitol is oxidated to fructose by sorbitol dehydrogenase, which can lead to PKC activation via the increased $NADH/NAD^+$ ratio. Although this mechanism does not produce ROS in a direct way, it takes part in the redox imbalance causing oxidative stress.

Hexosamine Pathway:

When glucose levels are within normal range, a relatively low amount of fructose-6-P is derived away from glycolysis. If intracellular glucose rises, excess fructose-6-phosphate is diverted from gylcolysis to provide substrate for the rate-limiting enzyme of this pathway, GFAT. This enzyme converts fructose 6-phosphate to glucosoamine 6-phosphate, which is then converted to UDP-NAcetylglucosamine, which is essential for making the glycosyl chains of proteins and lipids. Specific O-Glucosamine-N-Acetyl transferases use this metabolite for post-translational modification of specific serine and threonine residues on cytoplasmic and nuclear proteins [4, 6].

Diacylglycerol formation and PKC activation:

The protein Kinase C (PKC) family comprises at least eleven isoforms of serine / threonine kinases, which participate in signaling pathways activated by phosphatidyl serine, Calcium and Diacylglycerol (DAG). DAG levels are elevated chronically in the hyperglycemic or diabetic environment due to increase in the glycolytic intermediate dihydroxyacetone phosphate. This intermediate is reduced to glycerol-3-phosphate, which conjugated with fatty acids, increases de novo synthesis of DAG. Evidence suggests that the enhanced activity of PKC isoforms could arise from inhibition of the glycolytic enzyme glyceraldehide-3-phosphate dehydrogenase by increased ROS intracellular [3-4]. Other studies suggest that enhanced activity of PKC isoforms could also result from the interaction between AGEs and their extracellular receptors. PKC isoforms constitute a wide range of cellular signals, including activation of NADPH oxidase and NF-κB, resulting in excessive ROS production. They also increase vascular permeability, stabilize vascular endothelial growth factor (VEGF) mRNA expression and increase leukocyte-endothelium interaction.

Glyceraldehyde autoxidation:

Accumution of glyceralehyde 3-phosphate, besides activating the AGE, formation and the PKC pathway, it can oxidate itself. This autoxidation generates H_2O_2, which further contributes to oxidative stress [7].

Advanced glycation end-products (AGEs):

Intracellular hyperglycaemia is the primary initiating event in the formation of both intracellular and extracellular AGEs. [8] AGEs can arise from intracellular auto oxidation of glucose to glyoxal, decomposition of the Amadori product (glucose-derived 1-amino-1-deoxyfructose lysine adducts) to 3-deoxyglucosone (perhaps accelerated by an amadoriase), and nonenzymatic phosphate elimination from glyceraldehydes phosphate and dihydroxyacetone phosphate form methylglyoxal. These reactive intracellular dicarbonyl glyoaxl, methylglyoxal and 3-deoxyglucosone react with amino groups of intracellular and extracellular proteins to form AGEs.[3] Intracellular production of AGE precursors can damage cells by three general mechanisms: 1) Intracellular proteins modified by AGEs have altered function, 2) Extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with matrix receptors (integrins) that are expressed on the surface of cells, and 3) plasma proteins modified by AGE precursors bind to AGE receptors (such as RAGE and AGE-R1, 2 and 3) on cells such as macrophages, vascular endothelial cells and vascular smooth muscle cells. AGE receptor binding induces the production of ROS, which in turn activates PKC. It also activate NF-κB and NADPH oxidase, and disturbs MAPK signaling [7].

Stress–sensitive signaling pathways:

In addition to direct damage of bio-molecules in the cells, oxidative stress is also involved in activation of several stress-sensitive signaling pathways, which can result in inflammation, cytokine release, and even apoptosis. Among these pathways we find the transcription factor N-κB, which together with PARP acts as transcriptional co-activator of inflammation molecules such as iNOS, intracellular adhesion molecule-1 (ICAM-I), and histo compatibility complex class II. p38 MAPK pathway and c-Jun Nterminal kinase (JNK) (also known as stress-activated protein kinase (SAPK) participate in cellular responses to stress due to osmotic shock, cytokines and UV light, playing a role in cellular proliferation, apoptosis, and inflammatory responses. Jak/STAT is another important signaling pathway, which initiates and mediate s cellular responses to cytokines such as interferons and interleukins [9].

MECHANISM OF HYPERGLYCAEMIA-INDUCED DAMAGE:

How do these diverse microvascular and macrovascular pathologies all result from hyperglycaemia? Four main hypotheses about how hyperglycaemia causes diabetic complications have generated a large amount of data, as well as several clinical trials based on specific inhibitors of these mechanisms. The four hypotheses are: increased polyol pathway flux; increased advanced glycation end-product (AGE) formation; activation of protein kinase C (PKC) isoforms; and increased hexosamine pathway flux are explained above. Until recently there was no unifying hypothesis linking these four mechanisms.

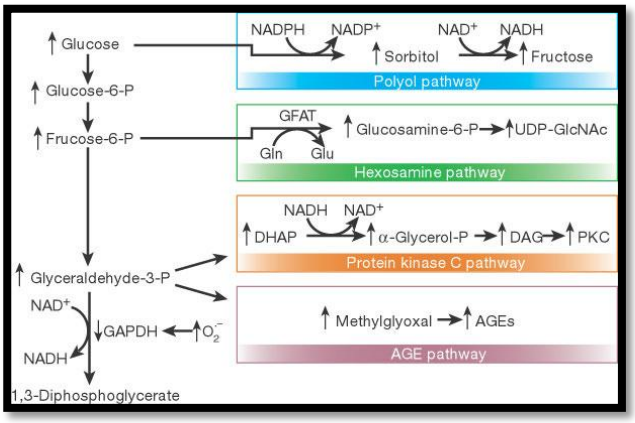

Fig.1. Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycaemic damage

Excess superoxide partially inhibits the glycolytic enzyme GAPDH, thereby diverting upstream metabolites from glycolysis into pathways of glucose overutilization. This results in increased flux of dihydroxyacetone phosphate (DHAP) to DAG, an activator of PKC, and of triose phosphates to methylglyoxal, the main intracellular AGE precursor. Increased flux of fructose-6-phosphate to UDP-*N*-acetylglucosamine increases modification of proteins by *O*-linked *N*-acetylglucosamine (GlcNAc) and increased glucose flux through the polyol pathway consumes NADPH and depletes GSH (Fig.1) [10].

Current concept of mechanisms involved in the development of polyol pathway associated diabetic complications:

Hyperglycemia induced increased generation of free radicals and consequent development of oxidative stress has been recognized as one of the crucial pathway for the development of diabetic complications. Several mechanisms have been identified by which hyperglycemia induces increased

generation of free radicals resulting development of oxidative stress. One of the important mechanisms by which hyperglycemia induces oxidative stress is polyol pathway. Although, under euglycemic condition only trace amounts (~3%) of glucose enter polyol pathway, increased flux (>30%) of glucose through polyol pathway has been noticed under hyperglycemic condition[11, 12] . The rate limiting step of polyol pathway is reduction of glucose to sorbitol catalyzed by enzyme aldose reductase (ALR) at the expense of reduced nicotinamide adenosine dinucleotide phosphate (NADPH) [12]. Sorbitol is, in turn converted to fructose by sorbitol dehydrogenase (SDH) with the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as a co-factor. Depletion of NADPH by ALR hampers regeneration of reduced glutathione (GSH), an important intracellular antioxidant (Fig.2) leading to ineffective scavenging of reactive oxygen species (ROS) and development of oxidative stress. Furthermore, during conversion of sorbitol into fructose by SDH, the co-factor NAD^+ is converted into NADH. NADH is substrate for NADH oxidase responsible for generation of superoxide anions (Fig.2) [13]. Taken together, reduction in antioxidant enzyme GSH and increased generation of free radicals (ROS) through polyol pathway contributes to the development of oxidative stress (Fig. 2). Oxidative stress and free radicals induced damage to bio-molecules results imbalance in their normal physiological functions and consequent development of diabetic complications. These advances in understanding pathophysiology of diabetic complications have increased interest in determining beneficial effects of antioxidant therapy that can complement to intensive glucose control. Even though, the efficacy of classical antioxidants in preventing diabetic complications is still uncertain, it is being advocated that development of mechanism-based antioxidant therapies may become more promising therapeutic strategy[14].

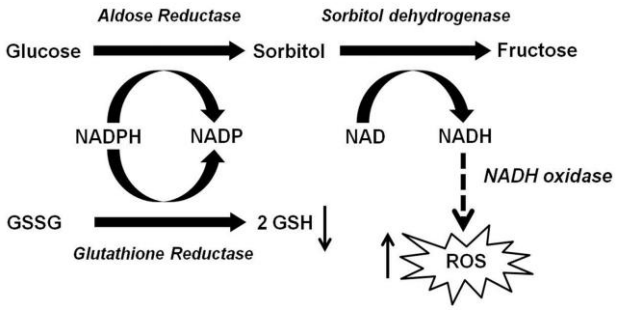

Fig.2.Role of aldose reductase (AR) in hyperglycemia-induced oxidative stress

ALDOSE REDUCTASE:

1. Polyol Pathway First Identified in the Seminal Vesicle:

Aldose reductase is a cytosolic enzyme present in most of the mammalian cells, although the distribution of the enzyme is not uniform among tissues. The polyol pathway was first identified in the seminal vesicle by Hers [15]. Who demonstrated the conversion of blood glucose into fructose, an energy source of sperm cells. Later Van Heyningen reported the presence of sorbitol in diabetic rat lens[16]. This work provided the basis for new research concerning the pathological role of aldose reductase and the polyol pathway in the development of diabetic complications. Several studies have suggested that increased reduction of glucose by aldose reductase contribute to the development of secondary diabetic complications[17]. During hyperglycemia event, the elevated glucose level enhanced the activity of Aldose reducatse by increasing glucose flux through this pathway. In fact, aldose reductase messenger ribonucleic acid (mRNA) in rat was highly expressed in the lens, the retina, and the sciatic nerve, the major "target" organs of diabetic complications[18]. The increased activity of aldose reductase results in decrease $NADPH/NADP^+$ ratio which impact other NADPH-dependent enzymes, such as Nitric Oxide (NO) synthase and gluthathione reductase[19].The retarded activity of the antioxidant enzyme, glutathione reductase causes oxidative stress under diabetic conditions. Increased sorbitol flux through the polyol pathway cause increases in $NADH/NAD^+$ ratio, which blocks the glycolytic pathway at the triose phosphate levels and Consequently, developed above mentioned pathway which cause pathological changes by disrupting protein function and interfering with cellular receptors and have been implicated in the etiology of diabetic complications[20].

2. Aldose Reductase as a Member of the Aldo-Keto Reductase Superfamily:

The Aldo-Keto Reductase (AKR) comprises a large number of structurally related enzymes that catalyze the pyridine the nucleotide-dependent reduction of carbonyl groups. Examples include aldehyde reductase (AKR1A), aldose reductase (AKR1B), which are responsible for NADPH-linked formation of alcohol products from aldehydic functional groups of aromatic and aliphatic hydrocarbons and aldo- and keto-sugar, [21,22] hydroxysteroid dehyrogenase (AKR1C), which catalyze steroid conversion and detoxification of polycyclic aromatic

hydrocarbons [23], ketosteroid reductase (AKR1D) and prostaglandin synthase [24]. Aldose reductase may be considered as a typical enzyme of the AKR superfamily. The enzyme is a small monomeric protein composed of 315 amino acid residues. The primary structure was first determined on rat lens aldose reductase[25,26]. They demonstrated high similarity to another NADPH-dependent oxido-reductase, human liver aldehyde reductase (EC 1.1.1.2) [27] and to p-crystallin, a major structural component of the lens of frog Rana pipiens[28]. The degree of similarity clearly suggests that these proteins belong to the same family, namely aldoketo reductase superfamily, with related structures and evolutionary origins. Graham *et al*[29]. reported that aldose reductase gene has located on chromosome $7q^{35}$. Complementary Deoxyribonucleic Acid (cDNA) clones of human placenta, retina and muscle aldose reductase were isolated[30,31, 32]. The identification of amino acids of aldose reductase from different species revealed a relatively low sequence identity (82-85%) conserved among human, rat, and other animal species. This could account for the species differences in the sensitivity of aldose reductase to some of the inhibitors. Molecular cloning techniques have identified many amino acid sequences for cellular proteins (39 proteins) as members of the aldo-keto reductase superfamily [33]. A wide variety of proteins from various species constitute this family, including aldehyde and xylose reductases from plants, yeast, and bacteria[34]. Nonetheless, the majority of this family is represented by mammalian aldehyde reductases, aldose reductases and hydroxysteroid dehydrogenases. These enzyme are now categorized into functionally and evolutionarily related group bases on their genetic origins. [35,36] Thus, the superfamily of AKRs is organized into a series of families and subfamilies. While the overall structural features of the AKR family member are well conserved, subtle differences near the C-terminal domain are thought to be responsible for differences in substrate specificity among closely related enzymes.

3. Tertiary structure of Aldose Reductase:

X-ray Crystallographic and site directed mutagenesis studies have provided important insight into the structures of aldose reductase. Wilson *et al.* [37] Showed the structure of human placenta aldose reductase. The enzyme molecule contains α/β barrels which are still the most prevalent motif with a core of eight parallel β strands connected by peripheral α helices with a large hydrophobic active site (Fig3). The structure of active site of aldose reductase differs significantly from that of aldehyde reductase[38]. However, it has been shown that the co-enzyme binding site is the same for aldose reductase aldehyde reductase [2] . The $NADP^+$ co-enzyme molecule was bound to the carboxyl terminus of the β barrel in an extended conformation. The nicotinamide ring was centered in the active site cavity. This highly hydrophobic active site pocket is formed by aromatic residues (Trp 20, Tyr 48 Trp, 79, Trp 111, Phe 121, Phe 122 and Trp 219) apolar residues (Val 47, Pro 218, Leu 300 and Leu 301) and polar residues (Glu 49, Cys 298 and His 110). The pyrophosphate bridge of NADPH is tied down by residues 214-230 amino acids which hold NADPH tightly in place[39].The interactions that favor the binding of NADPH are due to Lys 263 and Arg 269 salt linked to the 2'-phosphate of adenosine moiety of NADPH2.

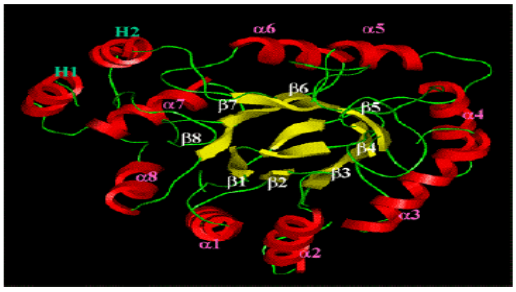

Fig.3.Tertiary Barrel structure of aldose reductase. There is eight α helices surrounding a core of eight β strands all in parallel orientation [2]

PHYSIOLOGICAL SIGNIFICANCE OF ALDOSE REDUCTASE:

Current evidence suggests that aldose reductase contributes to metabolic imbalance associated with diabetes and its complications in the eye and peripheral nerves system[40] . While it is generally accepted that aldose reductase-mediated pathogenesis is dependent on chronically elevated ambient hexose levels as in diabetes mellitus and galactosemia. However, the beneficial role(s) of aldose reductase in the cells when hexose levels are normal still under investigations[41]. Aldose reductase gene expression is widespread, as evidenced by the presence of gene transcripts in a large number of human tissues. It is suggested that the enzyme might function physiologically as a general housekeeping enzyme under

normal conditions. In addition, new evidence points to a potential role for aldose reductase in cytokin-mediating signaling processes.

1. Osmoregulatory Role in the Kidney:

The kidney is one of the richest tissue sources of aldose reducatase, with most of the enzyme localized in the medullary portion [42] from which quantities of the enzyme have been isolated for biochemical studies[43]. Sorbital is one of the organic osmolytes that balance the osmotic pressure of extracellular NaCl, fluctuating in accord with urine osmolality[44]. These findings, therefore, suggest the osmoregularoty role of aldose reductase in the renal homeostasis. The functional consequence of aldose reductase expression in kidney medulla was made clear when it was discovered that exposure of a line of renal papillary epithelial cells to increased extracellular osmolarity stimulated an increase in intracellular sorbitol[45]. In addition, ablation of aldose reductase gene in mice results in defect in kidney function. The mice become unable to concentrate their urine to normal level[42]. However, it is not clear why the absence of aldose reducatse leads to a defect in water resorption, as sorbitol constitute only 2% of the total osmolality of mouse kidney [46]. No such phenotype has been reported when animal models from other species (rat) are treated with aldose reductase inhibitors. This suggests either that aldose reductase inhibitors are not sufficiently effective to reduce sorbitol levels enough to cause a defect in urinary concentration or that other mechanisms, not present in the mouse kidney are able to functionally compensate for the loss of aldose reductase activity. This increased expression of aldose reductase under hyperosmotic stress was subsequently reported in a variety of cells of non-renal origin, such as Chinese hamster ovary cells[47]. Cultured human retinal pigment epithelial cells [48] and human embryonic epithelial cells [49].Transient transfection studies with luciferase or chloramphenicol acetyltransferase reporter constructs, containing various 59-flanking regions of aldose reductase gene, identified the osmotic response element mediating this hypersomotic stress-induced increase in transcription of aldose reductase gene[50 51, 52]. Studies on the factors that interact with these response elements and augment transcription of aldose reductase gene are now in progress and may provide insight into the regulatory mechanisms of the gene expression.

2. Diverse Substrates for Aldose Reductase:

While aldose reductase is thought to play a major role in the synthesis of sorbitol as an osmolyte in the kidney medulla, its distribution among tissues unaffected by extacelluar osmatic stress suggests an alternate metabolic role. In addition, the marked hydrophobic nature of the active site is unusual for an enzyme thought to be involved with metabolism of aldo-sugars. It has been demonstrated that hydrophobic substrate is the catalytic preference of aldose reductase. Srivastava *et al* [53] indicated that aldose reductase reduced lipid perioxidation-derived aldehydes as well as their glutathione conjugates. The role of aldose reductase in metabolizing products of lipid perioxidation or their metabolites is further supported by the observations that the expression of aldose reductase is enhanced under conditions of oxidative stress both in cell culture system and *in vivo*[54] and that inhibition of aldose reductase increases the accumulation of lipid perioxidation products during inflammation and ischemia[55]. In a series of aldehyde substrates for human aldose reductase investigated, isocorticosteroids [56] and isocaproaldehyde [57], both with KM values of approximately 1mM or less, are the best physiological substrates known to date. The next preferred substrates for aldose reductase may be aldehydes derived from biogenic amines [58,59] and methylglyoxaql, a toxic aldehyde produced nonenzymatically from triosephospate and enzymatically from acetone/acetol metabolism[60]. 17 a-hydroxyprogesterone [61] and 4-hydroxynonenal [62], a reactive aldehyde produce by oxidative damage to unsaturated fatty acids, are also excellent substrates for the enzyme with KM values of 20-30 mM. Another line of study demonstrated that 3-deoxyglucosone, one of the cross-linking agents formed as intermediates in nonenzymatic glycation, is a good substrate for aldose reductase[63]. Aldose reducatse also catalyzes the reduction of acroline, a highly reactive and mutagenic molecule generated during lipid peroxidation and as a metabolic by-product of cyclophosphamide[64]. Both 3-deoxyglucoson and acroline exhibited a similar range of KM values (40-80mM) in the kinetic analysis[65]. Whereas glucose is one of the endogenous substrates for aldose reductase, comparison with other endogenous aldehydes unequivocally indicates that glucose is a

rather poor substrate with a KM valve of 70 mM[66]. The interpretation of these findings is that aldose reductase in the adrenal gland and reproductive organs may normally participate in the synthesis and catabolism of steroid hormones, whereas it is involved in the metabolism of biogenic amines is the central nervous system. The enzyme may also act as extrahepatic detoxification enzyme in various tissues. Thus the significance of aldose reductase in the polyol pathway may be quite limited under non-diabetic conditions: it provides an osmolyte sorbitol in the renal medulla and supplies fructose as an energy source of sperm in the seminal vesicle. Recent studies hence illustrate the diversity in biological significance of aldose reductase in different tissues and in different animal species. The interactions of the inhibitors with aldose reductase in various organs along with other structurally related proteins in aldo-keto reductase family, may become potential source of their ineffectiveness and/or side effects when drugs are administered to diabetic patients for a prolonged period of time. Experimental data on the efficacy and side effects of inhibitors obtained from animal models should be cautiously interpreted, as significant species-specific differences in the localization and in physiological functions of aldose reductase were noted. Nevertheless, it should be appended that aldose reductase is not the only enzyme participating in most of the above–mentioned pathways of endogenous aldehyde metabolism. The suppression of aldose reductase activity with enzyme inhibitors may thus have moderate effects on such aldehyde metabolism aside from polyol pathway.

3. Unique Tissue Distribution Pattern of Aldose Reductase:

Recent investigations disclosed the unexpected distribution pattern of aldose reductase not only in different species but in tissues other than "target" organs of diabetic complications. In mouse, aldose reductase mRNA was most abundantly expressed in the testis, whereas a very low level of the transcript was detected in the sciatic nerve and lens[67]. These results suggest that mouse aldose reductase may possess a significant role in the testicular metabolism. On the other hand, the low expression of the enzyme in the nerve and lens was in marked contrast with the findings in rat, which indicated the localization of the enzyme transcript in these "target" organs of diabetic complications[18]. Consistent with these findings is the absence of cataract formation during the course of hyperglycemia in mouse, [68] in contrast with finding in rat, the first experimental model of sugar cataract formation. [1] Immunoblot and immunhistochemical analyses in rat tissues further showed high levels of aldose reductase protein in the adrenal gland and various reproductive organs, including the granulose cells of rat ovary[69]. Of particular interest is the fact that cyclic changes in the expression and localization of aldose reductase were observed in rat ovary during the estrous cycle[70]. These changes in the enzyme expression were indicated to be under hormonal control, and the study suggests another functional role of aldose reductase in the female reproductive organ, which can be deranged under diabetic conditions.

ALDOSE REDUCTASE IN GLUCOSE TOXICITY:

Aldose reductase is the first and rate-limiting enzyme of the polyol pathway[17].Under euglycemic conditions, aldose reductase plays a minor role in glucose metabolism, however , during dibetes its contribution is significantly enhance. Increased aldose reductase activity by hyperglycemia has been proposed to greatly influence the development and progression of secondary diabetic complications (retinopathy, neuropathy and nephropathy) [71]. Retinal capillary pericytes contain the enzyme aldose reductase and the accumulation of excess sugar a alcohol catalyzed by aldose reductase in pericytes, has been linked to their degradation[72].

Aldose Reductase and Other Factors in Glucose Toxicity:

Along with the increased flux of glucose through the polyol pathway, there are other putative mechanisms that may take part in the toxic effects of hyperglycemia (fig.4). Among the well-documented factors are activation of protein kinase C [73,74], enhanced non enzymatic glycation [75], and augmentation of oxidative stress. [76,77] Polyol pathway has been recognised as an important mechanism that contributes to the development of oxidative stress. Under hyperglycaemic conditions, glucose is channelled into aldose reductase dependent polyol pathway which depletes NADPH and consequently reduces the level of enzymatic antioxidant GSH. During SDH mediated conversion of sorbitol into fructose, NAD^+ is converted to NADH which is substrate for NADH oxidase responsible for increased generation of free radicals. [78] Conversion of fructose into Fructose-3-phosphate and 3-deoxyglucosone leads increased formation of non-enzymatic glycation end products. Reduction

in antioxidant enzymes, increased generation of free radicals and consequently oxidative modification of biomolecules increases level of oxidative stress (Fig.2). Some of these are postulated to be correlated with each other. Both increased aldose reductase activity and oxidative stress have been implicated in the pathogenesis of diabetic complications. [79] The important role of two mechanisms in diabetic retinopathy is supported biochemically [80] functionally [81] and biologically [82] abnormalities in the retina. Both aldose reductase inhibitors and antioxidants prevent formation of retinal pericyte ghosts and a cellular capillaries [80] increased vascular permeability [83] and decreased blood flow. Various mechanisms are postulated to account for augmented oxidative stress in diabetes. A generation of oxygen free radicals was enhanced because of auto-oxidation of glucose. The protection against oxidative stress was attenuated because of reduced gluthathion availability and inactivation of superioxde dismutase. Vascular dysfunction and resulting derangement in tissue perfusion under diabetic condition would induce ischemia and reperfusion process, which further generate oxygen free radicals[77]. On the other hand, it has been generally accepted that advanced glycation end product participates in the production of oxygen free radicals[84]. Inactivation of superioxide dismutase in diabetes was demonstrated to results from glycation of the two lysine residues on the enzyme protein[85]. The polyol pathway may act upon this enhanced glycation process, supplying a reactive glycation agent fructose. Reduced glutathione availability under hyperglycemia is attributed to the accelerated polyol pathway flux, depleting the cofactor NADPH for glutathione reductase. In this context, most of the putative mechanisms implicated in the toxic effects of hyperglycemia can be interrelated to each other and linked to enhanced polyol pathway activity. Obrosova et al[86]. Indicated a major contribution of aldose reductase to high glucose-induced oxidative stress in retinal epithelial cells and showed that fidarestate aldose reductase inhibitor can arrest hyperglycemia-induced reactive oxygen. Diabetes associated phenomena as increased retinal aldose reductase activity, oxidative stress and increased vascular permeability are interrelated. Aldose reductase triggers the whole cascade by causing oxidative stress which in turn leads to over expression of Vascular Endothelial Growth Factor (VEGF)[87] responsible for increased vascular permeability [88] . Indeed all three components of this cascade have been found preventable by an a ldose reductase inhibitors treatment. [86] Activation of protein kinase C was reported in vascular smooth muscle and endothelial cells after the exposure to hyperglycemia. The rise in NADPH/NAD^+ ratio via enhanced polyol pathway may also facilitate the Diacylglycerol (DAG) synthesis by increasing the availability of dihydroxyacetone phosphate as well as favoring it reduction to glycerol 3-phosphate, the intermediates of DAG synthesis[89].The increased protein kinase C activity would attenuate contractile responses of aortic vascular smooth muscle cells to such pressor hormones as angiotensin II and arginine vasopressin. The activation protein kinase C increases sodium-proton antiport activity that regulates intracellular pH, cell growth, and differentiation an also augments expression of various matrix proteins such as fibronectin, types IV collagen [74], and laminin[73]. All these biochemical changes could be relevant to diabetes-induced vascular dysfunction. This phenomenon was demonstrated in several tissues including retina, aorta, and renal glomeruli. Recently a specific inhibitor for the β isoform of protein kinase C was shown to ameliorate vascular dysfunctions in diabetic rat[90].

A POTENTIAL TARGET FOR THE PREVENTION OF DIABETIC COMPLICATIONS:

Diabetic retinopathy is the most common microvascular complication of diabetes and the most severe of diabetic ocular complications and risk factors associated with this devastating disease. Special attention is focused on aldose reductase, the first enzyme of the sorbitol pathway of glucose metabolism. The current knowledge on the enzyme localization in the retina and the role for increased aldose reductase activity in retinal capillary cell loss and formation of acellular capillaries, increased vascular permeability and disruption of blood-retinal barrier and increased leukocyte adhesion to endothelial cells associated with early diabetic retinopathy, as well as neovascularization associated with advanced (proliferative) diabetic retinopathy, gained through the experimental studies in animals models of diabetes and galactose feeding. Aldose reductase has been implicated in the etiology of diabetic complications. A variety of compounds have been observed to inhibit aldose reductase. Orally active inhibitors of the enzyme have been investigated for many years. Although, several of

these compounds have progressed to the clinical level, only one such drug is currently on the market. [91] Due to the limited number of a valuable drug for the treatment of diabetic complication, a number of rational approaches for the discovery of aldose reductase inhibitors have been taken since the determination of the 3-dimensional structure of the enzyme. There are a variety of structurally diverse aldose reductase inhibitors.

1. Clinical Trials of Aldose Reductase Inhibitors:

EI-Kabbani et al.[2] showed that the incidence of retinopathy was significantly influenced by mean blood glucose levels and study participants practicing intensive as compared to conventional glycemic control experienced a marked reduction in the incidence and progression of diabetic retinopathy. The protective effect was less noticeable if intensive control was instituted after some progression has occurred. Similar results were found in study of galactosemic dogs, which showed that correction of hyperglycemia through removal of dietary galactose did not stop the progressive appearance of retinal abnormalities associated with diabetic retinopathy. [92] Taken together, these studies emphasize that retinopathy followed a long term pathogenesis and that once initiated, pharmacological efforts to interfere are far less likely to be successful than prophylactic treatment prior to onset of early and perhaps irreversible tissue changes. Therefore, even if the therapeutic basis for aldose reductase inhibition is valid, efficacy for a given drug might be impossible to establish if the clinical study design dose not taken into consideration known risk factors for retinopathy such as duration of diabetes, rigor of glycemic control and existence of early changes in retinal vasculature. It is encouraging that new inhibitors are becoming available[93]. Some with novel structures free from the hydantoin nucleus found in inhibitors such as sorbinil, better tissues penetration and activity than the carboxylic acid inhibitors and higher selectivity against aldose reductase[94]. A similar rationale applies for aldose reductase inhibitors studies to establish efficacy toward diabetic neuropathy. In addition to these potential design flaws in prior clinical trials, failure to demonstrate inhibitor efficacy may be related to poor pharmacokinetic profiles of the investigated compounds. For example, inadequate nerve penetration almost certainly contributed to the failure of ponalrestat in clinical trials for diabetic neuropathy. In addition, unexpected toxicity was a factor leading to the termination of clinical trials of sorbinil and tolrestat[95].However, a promising effect of aldose reductase inhibitor on nerve conduction velocity was reported. When diabetic patients without any symptomatic neuropathy were treated with the aldose reductase inhibitor sorbinil, significant improvement in the conduction velocity was observed in all three nerves tested: the peroneal motor nerve, the median motor nerve, and the median sensory nerve[96]. Subsequently, numerous clinical studies were carried out to evaluate the efficacy of sorbinil. However, the major adverse reaction of sorbinil was a hypersensitivity reaction in the early weeks of therapy, which is similar to that seen with other hydantoins. The efficacy of another class of inhibitor, tolrestat, was modest in diabetic patients already symptomatic of neuropathy [97], although the progress of mild diabetic autonomic and peripheral neuropathy could be halted[98]. The only adverse reaction reported on tolrestat was an increase in serum levels of alanine aminotransferase or aspartate aminotransferase, although some patients in the placebo group also exhibited similar clinical findings during the study[99].

2. Classes Of Aldose Reductase Inhibitors:

A list of the most commonly available inhibiotors contains either a cyclicimide groups such as spirohydantoin group or spirosuccinimide group, an acetic acid moiety. The best known of the spironhydantoin-containing compounds, sorbinil, has been thoroughly analyzed by structural analysis of the aldose reductase and NADPH and several clinical studies. Other examples of these groups include fidarestat and its sterisomers. There are numerous compounds that incorporate the acetic acid moiety and they include tolrestst, ponalrestat (statil) and zopolrestat. It is noted that the cyclicimide or acetic acid moieties bind to an essentially hydrophilic area of the active side of aldose reductase, which contains the Tyr-48, His-110 and Trp-111 residues. Another common features among the various inhibitors is the presence of one or more aromatics groups, which may include phthalazinyl group (ponalrestat), a naphthyl group (tolrestat), a benzothiazole group (zopolrestat), a 2'-thioxo-1, 3-thiazolan-4-one group (epalrestat) and a halogenated benzylgroup (ponlrestat). These aromatic grouped bind in the hydrophobic pocket of alodse reductase, Trp-111, Phe-122 and Leu-300 residues, either through hydrogen

bonding or hydrophobic contact. Inhibitors containing the cyclicimide or carboxylic groups exhibit similar *in vitro* but different *in vivo* activities[100]. The carboxylic acid-containing inhibitors have lower *in vivo* activity, which has been attributed to relatively lower pKa values, thus causing ionization at physiological pH and an inability to traverse cell membranes. Cyclicimides have higher pKa and are only partially ionized at physiological pH, thus able to pass through cell membrane and have better pharmacokinetic properties. Sorbinil possessed all these attributes but its development as a therapeutic agent was halted due to hypersensitive reaction[101].

The flavonoids (2-phenyl-4H-1-benzopyran-4-one) are also good inhibitors of aldoes reductase [102] but do not contain either the carboxylic acid or cyclicimides moieties. This class of inhibitors, both naturally occurring and synthetic, has higher pKa values than carboxylic acid [103] and also has antioxidant properties[104], which prevents 4-hydroxy-2,3-trans-nonenal(HNE)-induced cataract formation. A newer class of aldose reductase inhibitors is the phenylslfonyl-nitromethanes [105] which exhibited potent activity against aldose reductase and some of which also showed irreversible inhibition. The departure of the $NADP^+$ from the enzyme is believed to be the rate-determining step and this is when current aldose reductase inhibitors that bind to hydrophobic pocket were better aldose reductase inhibitors. Such knowledge has been utilized in the design of potent and specific inhibitors of aldose reductase. However, modeling studies have shown that inhibitors specific to aldose reductase should interact with the C-terminal residues by binding to specificity pocket.[106] The hydrogen-bonding interactions between the inhibitors and active site residues (Try-48, His-110, Trp-111) oriented the inhibitor in the active site.

Emblica officinals, commonly known as amla or the Indian gooseberry, is extensively used in the practice of Ayurveda, Indian traditional medicine, as a treatment for diabetes related complications[107]. Previous work has shown that crude aqueous extracts from amla fruit delayed the onset and progression of cataracts and normalized diabetes-induced markers of lipid peroxidation and protein carbonlys[108,109]. Moreover, these studies demonstrated that the active component(s) of the aqueous extract penetrate the lens and subtantially delay the progression of cataracts through (ALR) aldose reductase inhibition. Puppala et al. [110] demonstrated that that β-glucogallin inhibition of a aldose reductase is specific over other aldos-keto reductases (AKRs) and active in an ex vivo transgenic lens organ cultures, preventing the accumulation of sorbitol under hyperglycemic conditions.

3. Variable Levels of Aldose Reductase in Diabetic patients:

Substantial variations in the levels of aldose reductase expression in various tissues exist among individuals with or without diabetes. Marked variability in aldose reductase activity was reported for enzyme preparations isolated from human placentas[67]. Aldose reductase purified from erythrocytes exhibited a nearly three-fold variation in activity among diabetic patients[111]. Such differences in the activity of aldose reductase may influence the susceptibility of patients to glucose toxicity via acceleratation of polyol pathway when these individuals are maintained under equivalent glycemic control. To test this hypothesis, it is necessary to determine the levels of aldose reductase in numerous diabetic subjects. In the previous studies, investigators examined variations in aldose reductase by isolating the enzyme from placenta or erythrocytes and assaying its activity [67, 111].The isolation of the enzyme was necessary because of the presence of other structurally related members of aldo-keto reductase family, particularly aldehyde reductase, in crude tissue preparations. These enzymes share overlapping substrate specificity with aldose reductase. A newly developed immunoassay method using a specific antibody against aldose reductase could circumvent such difficulties[112]. The amount of the enzyme determined by the immunoassay highly correlates with the activity of aldose reductase isolated from the erythrocytes of the same individuals [113]. By using this assay method, the association between the Aldose reductase level in the erythrocyte and various clinical parameters determined in patients with non-insulin-dependent diabetes mellitus (NIDDM). Several-fold difference in the erythrocyte enzyme level was depicted among diabetic patients, whereas no significant difference in the mean enzyme level was demonstrated between the healthy and diabetic individuals. The enzyme level did not correlate with age, duration of diabetes, fasting blood glucose, or glycosylated hemoglobin (HbA1c) levels, which represent glycemic control of the patient. However, data obtained from two different groups of diabetic subjects suggest that a high level of erythrocyte aldose reductase may affect the susceptibility and prognosis of

diabetic retinopathy[114-115]. In another study group, 95 NIDDM patients were classified according to the results of seven nerve function tests, and the association between the enzyme level and the clinical findings was investigated. [116] The erythrocyte aldose reductase level was significantly higher in those patients showing overt neuropathy compared with those without demonstrable neuropathy. A higher level of aldose reductase is one of the independent risk factors for overt neuropathy. Accordingly, these results support hypothesis that a difference in the level of aldose reductase is responsible for the susceptibility of diabetic patients to toxic effects of glucose. The activity of aldose reductase fractionated from the erythrocytes was reported to be significantly higher in IDDM patients with complication compared with those showing no sign of complication[117]. Increased levels of aldose reductase protein were also demonstrated immunoblot analysis in the mononuclear cells isolated from IDDM patients with apparent diabetic complication[118]. The level of aldose reductase expressed in the erythrocyte seems to be stable, as no apparent alteration in the enzyme level was observed during the follo-up period of 12 months in the studied patients. [116] In this study, enzyme level remained unchanged irrespective of improved or stably high HbA1c levels during the follow-up period. These findings indicate that the expression of the erythrocyte enzyme is unaffected by the glycemic control of the patients. It can therefore, be speculated that different levels of aldose reductase observed in diabetic patients may be genetically determined. To explore this possibility, two regions on the aldose reductase gene relevant to the enzyme expression were examined: the promoter region containing a TATA box [12], and the region containing the recently identified osmotic response sequences[52]. However, in the DNA sampled from 700 NIDDM patients with different enzyme levels in the erythrocyte, there was no change in either of these regions associated with differences in the expression of aldose reductase levels[119-123]. (Nishimura, unpublished observations).Thus, the reason for the variable expression of aldose reductase in human subjects has yet to be elucidated. The understanding of the mechanisms defining the expression levels in the targeted tissues may lead to new avenues of preventive therapy for diabetic complications. A hypothesis as to whether the high enzyme level predisposes the patients to the development of complications has to further tested by the prospective study carried out through the prolonged time course of diabetes. Also to be considered is the relevance of the of the aldose reductase level in the erythrocyte in predicting the enzyme level in the "target" tissues of diabetic complications. Whether a high level of enzyme expression in the erythrocyte reflects the level in the different cell lineage has to be determined. It will take some before all the data become available; nontheless, a high level of aldose reducatase in the erythrocyte was demonstrated to be a risk factor for vascular and neural derangement observed in diabetic patients. Identification of a subset of patients, who have a high level of aldose reductase expression and thereby are more susceptible to toxic effects of glucose, may enable us to target these patients for clinical intervention trial by use of new aldose reductase inhibitors. The data on the enzyme levels may also laid in the optimization of administration of the inhibitors to match the extent of enzyme suppression when exploring their efficacy in diabetic individuals.

CONCLUSION:

Diabetes mellitus is recognized as a leading cause of diabetic complications a new case of retinopathy and is associative with increased risk for painful neuropathy, heart diseases and kidney failure. Many theories have been advanced to explain mechanism leading to diabetes complications including accelerated protein glycation, altered signaling involving PKC, excessive oxidation stress and stimulation of glucose metabolism by the polyol pathway. It has been demonstrated that polyol pathway is the major source of diabetes induced oxidative stress as aldose reducatse activity depletes its co-factors NADPH which is required for glutathione reductase to regenerate GSH. In addition, to the formation of fructose and its metabolites which are potent non-enzymatic glycating agents therefore, aldose reducatse will increase AGE. Therefore designing and screening specific inhibitors for aldose reductase is becoming one of the therapeutic strategies that have been proposed to delay or prevent diabetic complications. The tertiary structure of aldose reductase, including the active site and the interaction with inhibitors of diverse chemical structures has been resolved. However, much still remains to be elucidated regarding the pathphysiological significance of the enzyme and the regulatory mechanisms of aldose reductase

expression in various human tissues. In diabetic animal models, promising effects of aldose reductase inhibitors were demonstrated. However most of the clinical trials carried out so far, produced rather modest or disappointing effects of the inhibitors on the functional and morphological improvements in diabetic complications. There could be several reasons that account for the disparity in the inhibitor effects observe between animal and clinical studies. Possible explanations include the chronic nature of diabetes in human subject its and the ensuing loss of ability to reconstitute in structural derangement once triggered under hyperglycemia. Secondly, the relative abundance of the aldo-keto reductase family, such as aldehyde reductase which are co-localized in human tissues and latter may interfere with the action of inhibitors may suppress aldose reductase. Lastly, the efficacy of aldose reducase inhibitors may depend on the enzyme level expressed in diabetic individuals. The variable levels of the enzyme expressed in the "target'' tissues may affect the extent of involvement of the polyol pathway in the pathogenic mechanisms of diabetic complications. If multiple mechanisms are involved in the pathogenesis of diabetic complications, the extent of the effectiveness of AR inhibitors is likely to be determined by the extent of the polyol pathway involvement in the toxic effects of hyperglycemia. This extent is likely to be variable among individuals having different levels of AR in "target'' tissues.

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