

NEW CONCEPTS AND INSIGHTS ON PATHOGENESIS AND TREATMENT OF DIABETIC COMPLICATIONS: POLYOL PATHWAY AND ITS INHIBITION

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ABSTRACT

The polyol pathway is one of the possible biochemical mechanisms by which hyperglycemia could impair the function and structure of the cells affected by diabetic complications. As possible hypothesis for the pathogenesis of diabetic complications, the polyol osmotic theory, alterations in myo-inositol and sodium metabolism, intermediary metabolites, abnormal changes of the redox state (NADH/NAD⁺ ratio) and an abnormality of kinase C dependent protein phosphorylation have been proposed. Recently, increasing evidence suggests that glycation and oxidative stress may have a cross-link with polyol pathway, contributing to the development of diabetic complications.

If hyperglycemia-induced polyol pathway hyperactivity has an important role in the etiology of late-onset diabetic complications, the inhibition of aldose reductase (AR), a rate-limiting enzyme of the pathway, could become a key element in the prevention and reversal of diabetic complications. Recent evidence from both animal experiments and clinical studies has emerged to support this theory, resulting in the development of drugs available for the clinical treatment of diabetic neuropathy. From the results obtained mainly in animal models of diabetic complications, it is well recognized at present that AR inhibitors have a positive inhibitory effect on neuropathy, retinopathy, nephropathy, keratopathy, cataract-formation, possibly infection and atherosclerosis.

It is now clear that AR inhibitors may offer various benefits to patients with diabetic complications. However, more extensive efforts are needed for the evaluation of their effects.

Key Words: Polyol pathway, Diabetic complications, Neuropathy, Retinopathy, Atherosclerosis

INTRODUCTION

The etiology of late-onset diabetic complications is not yet clear but is probably multifactorial, and related to the quality of glycemic control¹⁾ and to genetic factors. Several general theories have emerged, each based on the premise that either hyperglycemia or some related metabolic abnormalities are the primary events which trigger tissue damage and result in the development of diabetic complications (Fig. 1). The polyol pathway is one of the possible biochemical mechanisms by which hyperglycemia can impair the function and structure of cells

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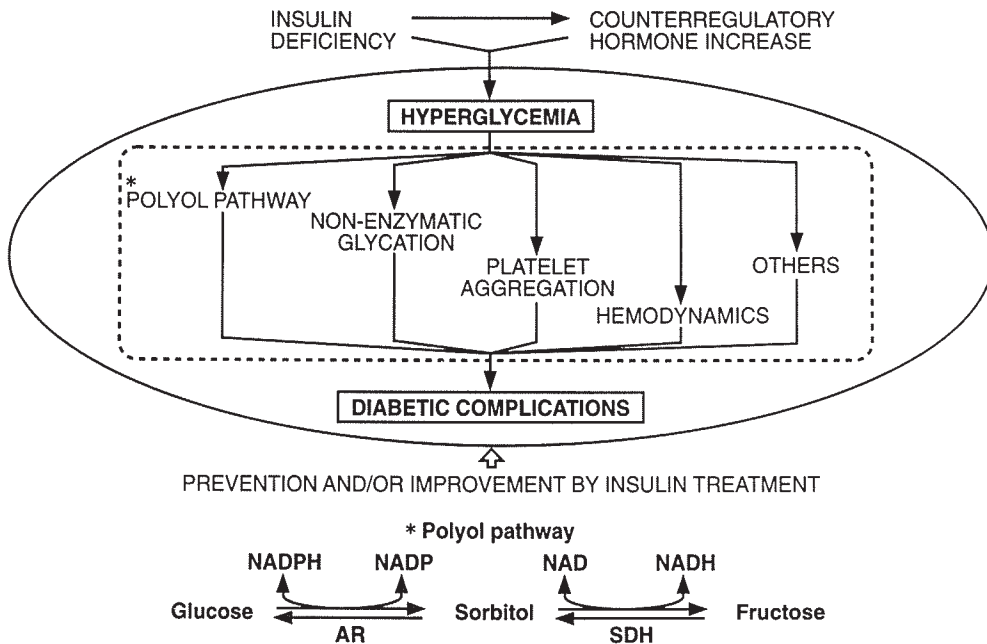


Figure 1: Main hypotheses of pathogenesis on diabetic complications and prevention and/or improvement by insulin treatment.

affected by diabetic complications.²⁾⁻¹²⁾ Other possible contributory factors include alteration in protein structure and metabolism by accelerated glycosylation, microvascular changes leading to reduced availability of oxygen, and abnormalities in platelet functions as well as in growth factors.⁹⁾⁻¹²⁾

If hyperglycemia-induced polyol pathway hyperactivity has an important role in the etiology of late-onset diabetic complications, the inhibition of aldose reductase (AR), a rate-limiting enzyme of the pathway, could become a key element in the prevention and reversal of diabetic complications.^{2)-10),12)} Recent evidence from both animal experiments and clinical studies has emerged to support this theory, resulting in the development of drugs available for the clinical treatment of diabetic neuropathy.

General aspects of polyol pathway and aldose reductase, and their relationship to diabetic complications

The polyol pathway

The polyol pathway consists of two steps: nonphosphorylated glucose is first reduced to sorbitol by the enzyme, AR (alditol: NADP⁺ oxidoreductase [EC 1. 1. 1. 21]) and the resulting sorbitol is then changed to fructose by sorbitol dehydrogenase (L-itol: NAD⁺2-oxidoreductase [EC 1. 1. 1. 14]) (Fig. 1). AR is one of a family of aldehyde reducing enzymes with a broad substrate specificity.⁵⁾⁻¹⁰⁾ Under normal physiological conditions, glucose is converted to glucose-6-phosphate by hexokinase. Saturation of hexokinase in the presence of excess glucose as occurs in the diabetic state results in the conversion of glucose to sorbitol by AR and then to fructose by sorbitol dehydrogenase (SDH). Under euglycemic conditions, the higher affinity of hexokinase for the glucose substrate ensures that very little sorbitol is formed. However, under

hyperglycemic conditions, there is a considerable increase in intracellular sorbitol levels. Namely, AR has a high capacity and a low affinity for glucose, but sorbitol dehydrogenase (SDH) has a high affinity and a low capacity for sorbitol. Hence, glucose flux mediated by AR is very low in this pathway except during hyperglycemia, and sorbitol oxidation is relatively independent of the sorbitol concentration within the physiological range.^{13),14)} The activity of this pathway in relation to glucose metabolism is only about 3% under normal conditions. However, this activity increases 2–4 times in a concentration-dependent manner as the ambient glucose concentration rises above physiological levels. Thus, in response to hyperglycemia, sorbitol accumulates in complication-prone tissues that have a high capacity for polyol pathway enzymes and in which glucose entry is not rate-limiting for glycolysis or mediated by insulin. Once sorbitol has been produced, it does not easily diffuse across cell membranes;^{3),15)} this intracellular accumulation of sorbitol may be a factor in the etiology of diabetic complications.

A possible physiological role of AR

AR has been found in a number of tissues, including those affected in diabetes such as nerve, retina, kidney, aorta, lens and cornea.⁵⁾ However, the physiological role of this pathway has remained unclear since its discovery was first made by Hers¹⁶⁾ in sheep seminal vesicles. Recent findings suggest that this pathway may have an important role in the regulatory system of osmolar changes between the intracellular and extracellular milieu.^{17),18)} A progressive rise in AR activity and synthesis of sorbitol in amounts related to the magnitude of the change in extracellular osmolarity was induced by exposing cells derived from rabbit renal medulla to raised extracellular sodium and chloride ions.¹⁸⁾ Also, increasing amounts of AR mRNA in response to increasing osmolarity of the culture medium, were expressed in cells derived from rabbit inner renal medulla.¹⁹⁾ A similar phenomenon was observed in *in vivo* studies using the kidney²⁰⁾ and *in vitro* investigations using other tissues.²¹⁾ Thus, it seems that an increased osmolarity of the extracellular milieu can trigger an expression of the aldose reductase gene and activation of this enzyme in some cells including renal medullary cells.

Current concepts of the mechanisms involved in the development of polyol pathway-associated diabetic complications

The polyol osmotic theory, alterations in myo-inositol and sodium metabolism, intermediary metabolites, abnormal changes of the redox state (NADH/NAD⁺ ratio) and an abnormality of kinase C dependent protein phosphorylation have been proposed as possible hypothesis for the pathogenesis of diabetic complications.^{2)–9)} Recently, increasing evidence suggests that glycation^{22),23)} and oxidative stress^{10),24)} may have a cross-link with polyol pathway, contributing to the development of diabetic complications.

Williamson et al.⁹⁾ have proposed the role of hypoxia and hyperglycemia-induced pseudohypoxia, whereby an increase in NADH/NAD⁺ and associated metabolic imbalances contribute to vascular changes. The proposal is attractive because the hyperglycemia-induced redox imbalance is largely the result of increased oxidation of sorbitol to fructose being coupled to the reduction of NAD⁺ to NADH in the second step of the polyol pathway. It is suggested that hypoxia and hyperglycemia-induced pseudohypoxia may play an important role in the pathogenesis of diabetic complications. Recent findings in experimental models suggest that hyperglycemia-induced polyol pathway hyperactivity may play a role in the neurovascular dysfunction that is accompanied by impaired nerve conduction velocity.^{10),25),26)} In addition to metabolic factors, it is likely that vascular disorders caused by polyol pathway hyperactivity may contribute to the development of some complications of diabetes that involve nerve and the retina. More recently, Ishii et al.²⁷⁾ reported that PKC β inhibitor ameliorated the glomerular filtration rate, albumin

excretion rate, and retinal circulation in diabetic rats in a dose-dependent manner, in parallel with its inhibition of PKC activity. The theory that hyperglycemia-induced PKC hyperactivity may contribute to the development of diabetic complications is attractive, but the relationship between polyol pathway hyperactivity and abnormal PKC activity remains partially unclear.

Effect of AR inhibitors on diabetic neuropathy

AR inhibitors

Although the exact mechanism is unknown, AR appears to be the possible link between increased polyol pathway activity and the development of some diabetic complications. Therefore, in recent years, preventive or therapeutic approaches for diabetic complications based on the polyol pathway theory have focused on the development of potent AR inhibitors. The first report concerning AR inhibitors, published in 1965 by Hayman and Kinoshita,²⁸⁾ discussed the inhibitory effect of these agents on metabolizable fatty acids. However, the first successful prevention of diabetes-associated complications was achieved with tetramethylene glutaric acid (TMG) (AY-20,037), which inhibited cataractogenesis in whole lens incubates after being given to rabbits by intraocular injection.^{5),29)} Of the large number of AR inhibitors developed, alrestatin (AY-22,284) was the first to reach the clinical trial stage and was also the first orally effective inhibitor to be developed.

Since then, a large number of AR inhibitors have been generated and studied in experimental animal models, but only limited number of the drugs have reached the clinical trial stage.⁷⁾ All of the compounds listed in Table 1 are now either available for clinical use, such as epalrestat for neuropathy, or in the clinical trial stage. However, AD-5467 and tolrestat were dropped due to minimal efficacy for neuropathy in diabetic patients. In addition to the AR inhibitors listed in Table 1, zopolrestat is now at the clinical trial stage in USA.³⁰⁾ The developed AR inhibitors can be roughly classified into four groups: carboxylic acids, flavonoids, hydantoins, and other compounds.⁷⁾ Among the AR inhibitors in Table 1, epalrestat, FK-366, AD-5467, tolrestat, TAT and NZ-314 belong to the carboxylic acid group, as does zopolrestat. On the other hand, SNK-860 is classified into the hydantoin group. However, none of the flavonoid compounds has reached the clinical trial stage.

Effect of AR inhibitors on diabetic complications

From the results obtained mainly in animal models of diabetic complications, it is well recognized at present that AR inhibitors have a positive inhibitory effect on neuropathy,^{5)-7),25),26),31)-33)} retinopathy,^{5)-7),34)-37)} nephropathy,^{5)-7),38),39)} keratopathy,⁵⁾⁻⁷⁾ cataract formation,^{5)-7),40),41)} and possibly infection,^{42),43)} and atherosclerosis.⁴⁴⁾⁻⁴⁶⁾

Although, clinically, there has been a massive concentration of efforts on diabetic neuropathy,⁵⁾⁻⁷⁾ not all of the assessed studies support the polyol pathway theory.⁵⁾⁻⁷⁾ A double-blind study in patients with diabetic neuropathy by Sima et al.⁴⁷⁾ gave exciting evidence of the efficacy of sorbinil, an aldose reductase inhibitor, against morphological signs of degeneration in sural nerve biopsies, accompanied by a decrease in the nerve sorbitol level and an increase in the nerve conduction velocity. A similar observation was reported by Greene et al.⁴⁸⁾ using another aldose reductase inhibitor, FK-366 (zenarestat). Although there have been a few positive results in other areas, the treatment of diabetic retinopathy with epalrestat over three years was particularly impressive, with drug-treated patients showing improvements in retinal structure^{49),50)} and electroretinogram.⁴⁹⁾ Cunha-Vaz et al.⁵¹⁾ found that alterations of the blood-retinal barrier increased significantly less in the sorbinil-treated group compared with the placebo group during the 6-mo study period, and that there was also a lower incidence of capillary microaneurysms of the retina in the sorbinil group. The Sorbinil Retinopathy Trial Research Group⁵²⁾ reported that

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Table 1. Chemical structure and activity of aldose reductase inhibitors which have reached the clinical evaluation for "triopathy" complications (neuropathy, retinopathy and nephropathy) in Japan.

Name	Structure	Company	Inhibitory activity of ARI ($IC_{50} \times 10^{-6}$ M)		Pharmacokinetics of ARI ^a	
			HPAR	RLAR	T _{max} (h)	T 1/2 (h)
Epalrestat (ONO-2235)		Ono	0.026	0.01	2	0.76
FK-366		Fujisawa	—	0.044	0.9 ± 0.2	7.7 ± 0.5
AD-5467		Takeda	0.051	0.129	1.3 ± 1.2	0.5 - 3.0
SNK-860		Sanwa		0.022	1.3	1.71
Tolrestat (AY-27773)		Ayerst		0.04	1.3 ± 0.1	9.2
TAT		Wakamoto	0.028	0.021	1.0	1.49
NZ-314		Nihonzoki		0.062	1.4 ± 0.6	1.8 ± 0.4

Note : HPAR = human placenta aldose reductase; RLAR = rat lens aldose reductase.

^aSingle administration in healthy subjects: ONO-2235, AD-5467 → 200 mg p.o., FK-366 → 150 mg p.o., NZ-314 → 50 mg p.o., TAT → 20 mg p.o., SNK-860 → 15 mg p.o.

the treatment of 497 IDDM patients with sorbinil at a dose of 250 mg daily or placebo for 30–50 months resulted in no significant inter-group difference in the severity level but that the number of capillary microaneurysms increased at a slightly lower rate in the sorbinil group than in the placebo group. Although past clinical studies of AR inhibitors on diabetic retinopathy are limited in number, these findings suggest that AR inhibitors play an important role in preventing the development and progression of diabetic retinopathy, indicating the need for further clinical study.

Effect of AR inhibitors on diabetic neuropathy and its possible mechanisms

AR, a principal enzyme of the polyol pathway in the peripheral nerve, is present in the Schwann cell and in pericytes and endothelial cells of endoneurial capillaries.^{53),54)} More recently, intense immunostaining for AR has been found in the paranodal region and the Schmidt-Lanterman clefts as well as in the terminal expansions of paranodal myelin lamellae and nodal microvilli.⁵⁵⁾

Under hyperglycemic conditions, there is a considerable increase in intracellular sorbitol levels in the peripheral nerve, exacerbated by the relative inability of this metabolite to cross the cellular membrane. This results in osmotic damage, and metabolic, structural and functional abnormalities.^{56),57)} It is also possible that damage of the peripheral nerve may result from depletion of intracellular cofactors, such as nicotinamide-adenosine dinucleotide phosphate (NADPH), due to increased flux through the pathway.⁹⁾ Moreover, accumulations caused by hyperglycemia-induced polyol pathway hyperactivity have also been associated with the deple-

tion of myo-inositol. This may in turn decrease sodium-potassium adenosine triphosphatase (Na^+/K^+ -ATPase) activity, resulting in changes in cellular metabolism and the membrane structure of the peripheral nerve.^{3),9),10)} All of these conditions were improved by treatment with AR inhibitors. However, a recent investigation of the reciprocity of sorbitol accumulation and myo-inositol uptake as part of cellular 'osmotic' indicates that changes in nerve myo-inositol might be a parallel and functionally irrelevant event.⁸⁾

Concerning the pathogenesis of diabetic neuropathy, both metabolic and vascular defects have been implicated but the interrelationships between them are poorly understood. Endoneurial blood flow is diminished shortly after the induction of diabetes in rats. Vasodilator treatment or pharmacologic adrenergic sympathectomy increases endoneurial blood flow and nerve conduction velocity, implicating neural ischemia as well in the early and reversible slowing of nerve conduction velocity.¹⁰⁾ AR inhibitors corrected the delayed nerve conduction velocity and decreased endoneurial blood flow,^{10),25),26)} also improved the impaired endothelium-dependent aortic relaxation (thought to be nitric oxide-[NO]-mediated) in diabetic rats.⁵⁸⁾ A more recent study suggests that the AR inhibitor-sensitive component of conduction slowing and the reduced Na^+/K^+ -ATPase activity in diabetic rats may in part reflect impaired nitric oxide activity, thus comprising a dual metabolic-ischemic pathogenesis.¹²⁾ As for other vascular factors in the development of diabetic neuropathy, 2,3-diphosphoglycerate (DPG) in red blood cells (RBCs), platelet aggregation and RBC deformability are of interest. 2,3-DPG has a high affinity for hemoglobin and plays an important role in regulating the binding of oxygen to hemoglobin. It is well-known that a low concentration of 2,3-DPG reduces the ability of RBCs to release oxygen, resulting in tissue hypoxia, and that the 2,3-DPG concentration in RBCs is decreased in patients with diabetic ketoacidosis.^{59),60)} The AR inhibitor, SNK-860, which restored nerve sorbitol levels, nerve Na^+/K^+ -ATPase activity, and nerve conduction velocity, also increased 2,3-DPG levels in RBCs and endoneurial blood flow in diabetic rats.⁶¹⁾ This increase in RBC 2,3-DPG levels by SNK-860 may cause an increment of oxygen supply to the peripheral nerve in diabetic rats, contributing in part to the improvement of delayed nerve conduction velocity. Takiguchi et al.⁶²⁾ observed that the treatment of diabetic rats with ONO-2235 (epalrestat), an AR inhibitor, for 8 weeks, prevented the anti-aggregating activity of plasma, as did insulin treatment. Moreover, Robey et al.⁶³⁾ reported that sorbinil could partially prevent the decreased RBC deformability in diabetic rats. All of these findings and other observations⁶⁴⁾⁻⁶⁶⁾ strongly suggest that AR inhibitors can cause an increment of endoneurial microcirculation of the peripheral nerve in diabetic rats, contributing to the improvement of diabetic neuropathy.

It is well established that the effect of AR inhibitors on diabetic neuropathy is mainly via the metabolic changes due to the inhibition of the polyol pathway activity. However, considering the above findings, it is strongly suggested that vascular factors may also play an important role in the effects of AR inhibitors on diabetic neuropathy. The possible mechanisms of AR inhibitors in diabetic neuropathy are summarized in Fig. 2.

AR inhibitors' action on diabetic complications in other areas

Polyol pathway and atherosclerosis

It has been hypothesized that a reduction in Na^+/K^+ -ATPase activity is the major pathogenic step in the development of hypertension.⁶⁷⁾ This results in the accumulation of intracellular Na^+ and ultimately leads to significant increases in cytosolic-free Ca^{2+} concentrations [Ca^{2+}]_i in vascular smooth muscle cells. In diabetes, low Na^+/K^+ -ATPase activity in tissues is observed, which may be linked to the polyol pathway.

To examine this possibility, sorbitol, Na^+/K^+ -ATPase activity and [Ca^{2+}]_i were measured using cultured rabbit aortic smooth muscle cells. Epalrestat (100 μM), an AR inhibitor, blocked

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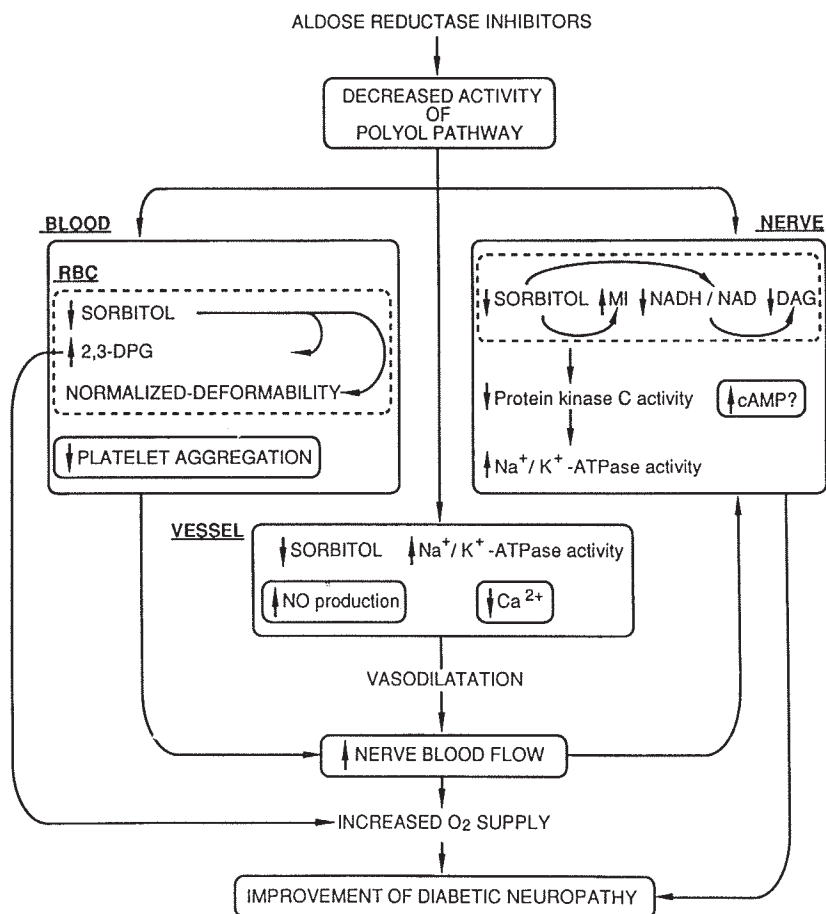


Figure 2: Possible mechanisms of aldose reductase inhibitors in improvement of diabetic neuropathy.

glucose-induced changes in sorbitol and myo-inositol metabolism.⁴⁵⁾ The increment of cytosolic-free Ca^{2+} , induced by 30 mM glucose was completely reduced by epalrestat to control levels.⁶⁸⁾ Although in the presence of 30 mM glucose, the ouabain binding capacity was significantly reduced and ^{32}Rb uptake was also markedly decreased, epalrestat restored the ^{32}Rb uptake but failed to restore the ouabain binding capacity.

These findings suggest that increased sorbitol levels and decreased Na^+/K^+ -ATPase activity in culture rabbit smooth muscle cells may be important pathogenic factors in the development of hypertension and atherosclerosis in diabetes, and that AR inhibitors may be useful for their prevention. From these data and observations by others,^{22),23),44),46),62),63),66)} a hypothesis for the development of atherosclerosis in diabetes based on polyol pathway cascade is summarized in Fig. 3.

Polyol pathway and neutrophil function

Diabetic patients have increased susceptibility to infection because of an impaired host defense mechanism. Although many factors may contribute to impaired host defense, in particu-

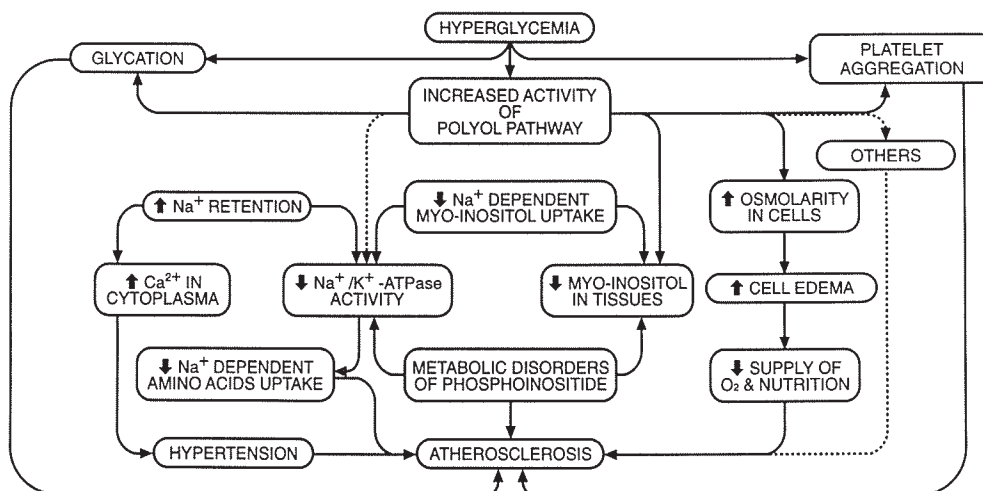


Figure 3: Possible mechanisms by which development of atherosclerosis in diabetes mellitus may be linked to increased polyol pathway activity.

lar, polymorphonuclear leukocyte (hereafter referred to as neutrophil) function plays an important role. Neutrophil function in well-controlled diabetic patients differs little from that in normal subjects,⁶⁹⁾ suggesting that dysfunction is in part related to poor metabolic control induced by high glucose concentrations.

Wilson⁴²⁾ reported that neutrophils have an active polyol pathway, which may be the cause of the decreased killing ability of neutrophils observed in diabetes. However, the mechanisms of altered cell function in diabetes are not fully understood. As bactericidal function is partially mediated by oxidative killing, the biochemical events necessary for causing the oxidative burst are those most susceptible to impaired glucose metabolism. These abnormalities may therefore be associated with the induction of the polyol pathway, which competes for supplies of nicotinamide adenine dinucleotide phosphate (NADPH). Boland et al.⁷⁰⁾ demonstrated that the impaired killing of *Escherichia coli* in diabetic patients was improved by treatment with an AR inhibitor, ponalrestat. More recently, Kawamura et al.⁴³⁾ measured lucigenin-enhanced chemiluminescence stimulated by phorbol myristate acetate as a function of neutrophils in high glucose concentration. The peak value of lucigenin-enhanced luminescence in human neutrophils was decreased by 86% by exposure to 40 mM glucose. This reduction was reversed by 91% with the addition of 10 μ M SNK-860. It has been reported that the lucigenin-enhanced luminescence response, induced by superoxide and only weakly by the hydrogen peroxide and myeloperoxidase- H_2O_2 -halide systems, as an indicator of superoxide anion production and PMA, is decreased in diabetic patients⁷¹⁾ and in high-glucose medium in vitro.⁷²⁾ In the in vivo study, using neutrophils from poorly controlled diabetic patients, epalrestat, an aldose reductase inhibitor significantly improved the impaired *Cypridina* luciferin-analog-dependent chemiluminescence and L-dependent chemiluminescence in patients with diabetes mellitus.⁷³⁾ The results obtained in human neutrophils suggest that, in diabetic patients, the increase of the polyol pathway activity by high glucose concentrations may reduce the depletion of superoxide anion on the membrane of human neutrophils, resulting in the dysfunction of the oxidative killing. Therefore, it appears that AR inhibitors may also be capable of preventing the aggravation of various infections in

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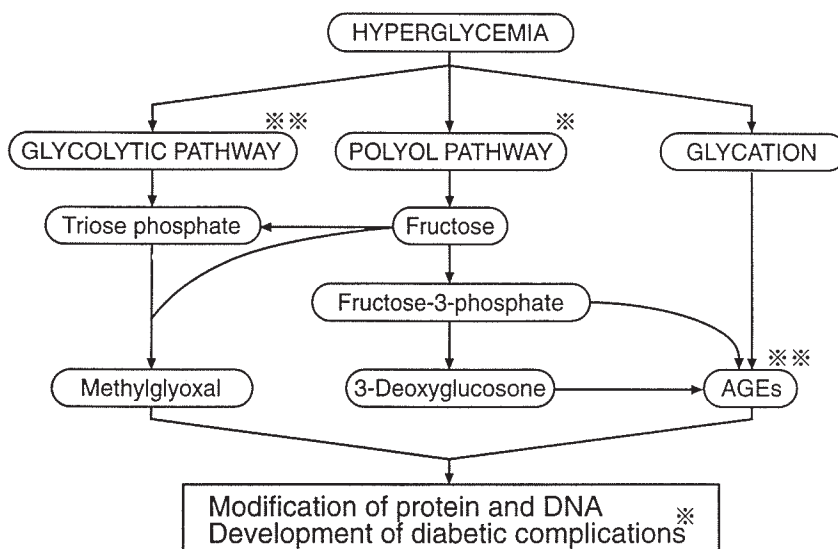


Figure 4: Relationship on the development of diabetic complications between glycolytic pathway, polyol pathway and glycation.

diabetic patients.

CONCLUSION

Recent findings have shown a strong cross-linking between polyol pathway and glycation in the pathogenesis of diabetic complications. In addition to this, it is an interested possibility that increased synthesis of methylglyoxal modifying nucleic acid and protein⁷⁴) may be partially related to hyperglycemia-induced polyol pathway hyperactivity, resulting in diabetic complications (Fig.4). It is now clear that AR inhibitors may offer various benefits to patients with diabetic complications. However, more extensive efforts are needed for the evaluation of their effects. Time will tell whether the hopes for these drugs are to be fulfilled.

REFERENCES

- 1) The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Eng. J. Med.*, 329, 977–986 (1993).
- 2) Gabbay, K.H.: The sorbitol pathway and the complications of diabetes. *N. Eng. J. Med.*, 288, 832–836 (1973).
- 3) Greene, D.A., Lattimer, S.A., Ulbrecht, J.S. and Carroll, P.: Glucose-induced alterations in nerve metabolism: current perspective on the pathogenesis of diabetic neuropathy and future direction for research and therapy. *Diabetes Care*, 8, 290–299 (1985).
- 4) Kador, P.F., Kinoshita, J.H. and Sharpless, N.E.: Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. *J. Med. Chem.*, 28, 841–849 (1985).
- 5) Dvornik, D.: *Aldose Reductase Inhibition: an approach to the prevention of diabetic complications*. McGraw-Hill, New York (1987).

- 6) Sakamoto, N., Kinoshita, J.H., Kador, P.F. and Hotta, N.: *Polyol pathway and its role in diabetic complications*. Excerpta Medica, Amsterdam (1988).
- 7) Hotta, N. and Sakamoto, N.: Aldose Reductase Inhibitors. In *The Diabetes Annual/5* edited by Alberti, K.G.M.M. and Krall, L.P., pp. 330–361 (1990), Elsevier Science Publishers BV, Amsterdam.
- 8) Tomlinson, D.R.: Aldose reductase inhibitors and the complications of diabetes mellitus. *Diabetic Med.*, 10, 214–230 (1993).
- 9) Williamson, J.R., Chang, K., Frangos, M., et al.: Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes*, 42, 801–813 (1993).
- 10) Cameron, N.E. and Cotter, M.A.: The relationship of vascular changes to metabolic factors in diabetes mellitus and their role in the development of peripheral nerve complications. *Diabetes Metab. Rev.*, 10, 189–224 (1994).
- 11) Nathan, D.M.: Long-term complications of diabetes mellitus. *N. Eng. J. Med.*, 328, 1676–1685 (1993).
- 12) Stevens, M.J., Feldman, E.L. and Greene, D.A.: The aetiology of diabetic neuropathy: the combined roles of metabolic and vascular defects. *Diabetic Med.*, 12, 566–579 (1995).
- 13) Gabbay, K.H.: Purification and immunological identification of bovine retinal aldose reductase. *Isr. J. Med. Sci.*, 8, 1626–1629 (1972).
- 14) Travis, S.F., Morrison, A.D., Clements, R.S. Jr., Winegrad, A.I. and Oski, F.A.: The role of the polyol pathway in metahemoglobin reduction in human red cells. *Br. J. Haematol.*, 27, 597–605 (1974).
- 15) Wick, A.N. and Cathcart, E.S.: Insulin and permeability of cells to sorbitol. *Am. J. Physiol.*, 166, 421–423 (1951).
- 16) Hers, H.G.: Le mecanisme de la transformation de glucose en fructose par les vesicules seminales. *Biochim. Biophys. Acta*, 22, 202–203 (1956).
- 17) Bagnasco, S.M., Balaban, R., Fales, H.M., Yang, Y-M. and Burg, M.: Predominant osmotically active solutes in rat and rabbit renal medullas. *J. Biol. Chem.*, 261, 5872–5877 (1986).
- 18) Bagnasco, S.M., Murphy, H.R., Bedford, J.J. and Burg, M.B.: Osmoregulation by slow changes in aldose reductase and rapid changes in sorbitol flux. *Am. J. Physiol.*, 254, C788–C792 (1988).
- 19) Garcia-Perez, A., Martin, B., Murphy, H.R., Uchida, S., Murer, H. and Cowley, B.D. Jr.: Molecular cloning of cDNA coding for kidney aldose reductase. Regulation of specific mRNA accumulation by NaCl-mediated osmotic stress. *J. Biol. Chem.*, 264, 16815–16821 (1989).
- 20) Bondy, C.A. and Lightman, S.L.: Developmental and physiological regulation of aldose reductase mRNA expression in renal medulla. *Mol. Endocrinol.*, 3, 1409–1416 (1989).
- 21) Kaneko, M., Carper, D.A., Nishimura, C., Millen, J., Bock, M. and Hohman, T.C.: Induction of aldose reductase expression in rat kidney mesangial cells and Chinese hamster ovary cells under hypertonic conditions. *Exp. Eye Res.*, 188, 135–140 (1990).
- 22) Hamada, Y., Araki, N., Koh, N., Nakamura, J., Horiuchi, S. and Hotta, N.: Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. *Biochem. Biophys. Res. Commun.*, 228, 539–543 (1996).
- 23) Hamada, Y., Araki, N., Horiuchi, S. and Hotta, N.: Role of polyol pathway in nonenzymatic glycation. *Nephrol. Dial. Transplant.*, 11 (Suppl 5), 95–98 (1996).
- 24) Giugliano, D., Paolisso, G. and Geriello, A.: Oxidative stress and diabetic vascular complications. *Diabetes Care*, 19, 257–267 (1996).
- 25) Hotta, N., Kakuta, H., Fukasawa, H., et al.: Effects of a potent new aldose reductase inhibitor, (5-(3-thienyl)tetrazol-1-yl) acetic acid (TAT), on diabetic neuropathy in rats. *Diabetes Res. Clin. Pract.*, 27, 107–117 (1995).
- 26) Hotta, N., Koh, N., Sakakibara, F., et al.: Effect of propionyl-L-carnitine on motor nerve conduction, autonomic cardiac function, and nerve blood flow in rats with streptozotocin-induced diabetes: Comparison with an aldose reductase inhibitors. *J. Pharmacol. Exp. Ther.*, 276, 49–55 (1996).
- 27) Ishii, H., Jirousek, M.R., Koya, D., et al.: Amelioration of vascular dysfunctions in diabetic rats by an oral PKC β inhibitor. *Science*, 272, 728–731 (1996).
- 28) Hayman, S. and Kinoshita, J.H.: Isolation and properties of lens aldose reductase. *J. Biol. Chem.*, 240, 877–882 (1965).
- 29) Kinoshita, J.H., Dvornik, D., Kraml, M. and Gabbay, K.H.: The effect of an aldose reductase inhibitor on the galactose-exposed rabbit lens. *Biochim. Biophys. Acta.*, 158, 472–475 (1968).
- 30) Inskip, P.B., Ronfeld, R.A., Peterson, M.J. and Gerber, N.: Pharmacokinetics of the aldose reductase inhibitor, zopolrestat, in humans. *J. Clin. Pharmacol.*, 34, 760–766 (1994).
- 31) Yue, D.K., Hanwell, M.A., Satchell, P.M. and Turtle, J.R.: The effect of aldose reductase inhibition on nerve conduction velocity in diabetic rats. *Diabetes*, 31, 789–794 (1982).

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- 32) Tomlinson, D.R., Moriarty, R.J. and Mayer, J.H.: Prevention and reversal of defective axonal transport and motor nerve conduction velocity in rats with experimental diabetes by treatment with the aldose reductase inhibitor sorbinil. *Diabetes*, 33, 470–476 (1984).
- 33) Hotta, N., Kakuta, H., Fukasawa, H., et al.: Effect of a fructose-rich diet and the aldose reductase inhibitor, ONO-2235, on the development of diabetic neuropathy in streptozotocin-treated rats. *Diabetologia*, 28, 176–180 (1985).
- 34) Robison, W.G. Jr., Kador, P.F. and Kinoshita, J.H. Retinal capillaries: Basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. *Science*, 221, 1177–1179 (1983).
- 35) Hotta, N., Kakuta, H., Fukasawa, H., et al.: Prevention of diabetic retinopathy by an aldose reductase inhibitor in fructose-fed streptozotocin-diabetic rats. In *Polyol pathway and its role in diabetic complications*, edited by Sakamoto, N., Kinoshita, J.H., Kador, P.F. and Hotta, N., pp. 311–318 (1988), Excerpta Medica, Amsterdam.
- 36) Akagi, Y., Yajima, Y., Kador, P.F., Kuwabara, T. and Kinoshita, J.H.: Localization of aldose reductase in the human eye. *Diabetes*, 33, 562–566 (1984).
- 37) Kador, P.F., Akagi, Y., Takahashi, Y., Ikebe, H., Wyman, N. and Kinoshita, J.H.: Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Arch. Ophthalmol.*, 108, 1301–1309 (1990).
- 38) Beyer-Mears, A., Murray, F.T., Delval, M., Cruz, E. and Siadini, M.: Reversal of proteinuria by sorbinil, an aldose reductase inhibitor in spontaneously diabetic (BB) rat. *Pharmacology*, 36, 112–120 (1988).
- 39) Mauer, S.M., Steffes, M.W., Azar, S. and Brow, D.M.: Effects of sorbinil on glomerular structure and function in long term diabetic rats. *Diabetes*, 38, 839–846 (1989).
- 40) Datiles, M.B., Fukuoi, H. and Kinoshita, J.H.: Galactose cataract prevention with sorbinil, an aldose reductase inhibitor: a light microscopic study. *Invest. Ophthalmol. Vis. Sci.*, 22, 174–179 (1982).
- 41) Simard-Duquesne, N., Greselin, E., Gonzalez, R. and Dvornik, D.: Prevention of cataract development in severely galactosemic rats by the aldose reductase inhibitor, tolrestat. *Proc. Soc. Exp. Biol. Med.*, 178, 599–605 (1985).
- 42) Wilson, R.M.: Neutrophils function in diabetes. *Diabetic Med.*, 3, 509–512 (1986).
- 43) Kawamura, T., Suzuki, K., Matsumae, H., Sano, T., Sakamoto, N. and Hotta, N.: Effect of glucose and SNK-860, an aldose reductase inhibitor, on the polyol pathway and chemiluminescence response of human neutrophils in vitro. *Diabetic Med.*, 12, 392–396 (1995).
- 44) Morrison, A.D.: Linkage of polyol pathway activity and myo-inositol in aortic smooth muscle. *Diabetes*, 34 (Suppl 1), 12A, (1985).
- 45) Sakakibara, F., Hotta, N., Koh, N. and Sakamoto, N.: Effects of high glucose concentrations and epalrestat on sorbitol and myo-inositol metabolism in cultured rabbit aortic smooth muscle cells. *Diabetes*, 42, 1594–1660 (1993).
- 46) Hotta, N., Kasuya, Y., Koh, N., et al.: An aldose reductase inhibitor prevents galactose-induced macroangiopathy in beagle dogs. *Diabetologia*, 39 (Suppl 1), A287, (1996).
- 47) Sima, A.A.F., Bril, V., Nathaniel, V., et al.: Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. *N. Engl. J. Med.*, 319, 548–555 (1988).
- 48) Greene, D.A., Arrezo, J. and Brown, M., the Zenarestat Study Group: Dose-related effects of the aldose reductase inhibitor Zenarestat on nerve sorbitol levels, nerve conduction velocity and nerve fiber density in human diabetic neuropathy. *Diabetes* 45, (Suppl 2), 5A, (1996).
- 49) Hotta, N., Kakuta, H., Fukasawa, H., et al.: Diabetic retinopathy. In *Current status of prevention and treatment of diabetic complications*, edited by Sakamoto, N., Kinoshita, J.H., Kador, P.F. and Hotta, N., pp. 195–205 (1990), Elsevier Science Publishers BV, Amsterdam.
- 50) Hotta, N., Kakuta, H., Ando, F. and Sakamoto, N.: Current progress in clinical trials of aldose reductase inhibitors in Japan. *Exp. Eye. Res.*, 50, 625–628 (1990).
- 51) Cunha-Vaz, J.C., Mota, C., Leite, E.C., Abreu, J.R.F. and Ruas, M.A.: Effect of sorbinil on blood-retinal barrier in early diabetic retinopathy. *Diabetes*, 35, 574–578 (1986).
- 52) Sorbinil Retinopathy Trial Research Group.: A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. *Arch. Ophthalmol.*, 108, 1234–1244 (1990).
- 53) Ludvigson, M.A. and Sorenson, R.L.: Immunohistochemical localization of aldose reductase. 1. Enzyme purification and antibody preparation: localization in peripheral nerve, artery and testis. *Diabetes*, 29, 438–449 (1980).
- 54) Chakrabarti, S., Sima, A.A.F., Nakajima, T., Yagihashi, S. and Greene, D.A.: Aldose reductase in the BB rat: isolation, immunological identification and localization in the retina and peripheral nerve. *Diabetologia*, 30, 244–251 (1987).

- 55) Powell, H.C., Carrett, R.S., Kador, P.F. and Mizisin, A.P.: Fine-structural localization of aldose reductase and ouabain-sensitive, K(+)-dependent p-nitro-phenylphosphatase in rat peripheral nerve. *Acta Neuropathol.*, 819, 529–539 (1991).
- 56) Powell, H.C., Costello, M.L. and Myers, R.R.: Galactose neuropathy: permeability studies, mechanism of edema, and mast cell abnormalities. *Acta Neuropathol.*, 55, 89–95 (1981).
- 57) Griffey, R.H., Eaton, R.P., Gasparovic, C. and Sibbitt, W.: Galactose neuropathy: structural changes evaluated by nuclear magnetic resonance spectroscopy. *Diabetes*, 36, 776–778 (1987).
- 58) Cameron, N.E. and Cotter, M.A.: Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway. *Diabetologia*, 35, 1011–1019 (1992).
- 59) Alberti, K.G.M.M., Darley, J.H., Emerson, P.M. and Hockaday, T.D.R.: 2,3-Diphosphoglycerate and tissue oxygenation in uncontrolled diabetes mellitus. *Lancet*, ii, 391–395 (1972).
- 60) Ditzel, J. and Stand, I.E.: The oxygen transport system of red blood cells during diabetic ketoacidosis and recovery. *Diabetologia*, 11, 255–260 (1975).
- 61) Nakamura, J., Koh, N., Sakakibara, F., et al.: Polyol pathway, 2,3-diphosphoglycerate in erythrocytes and diabetic neuropathy in rats. *Eur. J. Pharmacol.*, 294, 207–214 (1995).
- 62) Takiguchi, Y., Wada, K. and Nakashima, M.: Effect of aldose reductase inhibitor on the inhibition of platelet aggregation induced by diabetic rat plasma. *Eur. J. Pharmacol.*, 251, 289–291 (1992).
- 63) Robey, C., Dasmaharatra, A., Cohen, M.P. and Suarez, S.: Sorbinil partially prevents decreased erythrocyte deformability in experimental diabetes mellitus. *Diabetes*, 36, 1010–1013 (1987).
- 64) Hotta, N., Kakuta, H., Fukasawa, H., et al.: Effect of nickeritol on streptozotocin-induced diabetic neuropathy in rats. *Diabetes*, 41, 587–591 (1992).
- 65) Nakamura, J., Koh, N., Sakakibara, F., et al.: Diabetic neuropathy in sucrose-fed Otsuka Long-Evans Tokushima Fatty rats: Effect of an aldose reductase inhibitor, TAT. *Life Sci.*, 60, 1847–1857 (1997).
- 66) Hara, T., Nakamura, J., Koh, N., et al.: An aldose reductase inhibitor, TAT, reduces ADP-induced platelet hyperaggregation in streptozotocin-induced diabetic rats with neuropathy. *J. Lab. Clin. Med.*, 126, 641–647 (1995).
- 67) Ohara, T., Sussman, K.E. and Draznin, B.: Effect of diabetes on cytosolic free Ca^{2+} and Na^+/K^+ -ATPase in rat aorta. *Diabetes*, 40, 1560–1563 (1991).
- 68) Sakakibara, F., Hotta, N., Koh, N., et al.: Effect of high glucose concentrations and epalrestat on cytosolic free Ca^{2+} in cultured rabbit smooth muscle cells. In *Pathogenesis and treatment of NIDDM and its related problems*, edited by Sakamoto, N., Alberti, K.G.M.M. and Hotta, N., pp. 437–440 (1994), Excerpta Medica, Amsterdam.
- 69) Bagdade, J.D., Root, R.K. and Bulger, R.J.: Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes*, 23, 9–15 (1974).
- 70) Boland, O.M., Blackwell, C.C., Clarke, B.F. and Ewing, D.J.: Effects of ponalrestat, an aldose reductase inhibitor, on neutrophil killing of *Escherichia coli* and autonomic function in patients with diabetes mellitus. *Diabetes*, 42, 336–340 (1993).
- 71) Altomonta, L., Negrini, A.P., Ghirlande, G., Littarru, G.P. and Greco, A.V.: Chemiluminescence response of polymorphonuclear neutrophils in diabetes mellitus. *IRCS. Med. Sci.*, 8, 318 (1980).
- 72) Nielson, C.P. and Hindson, D.A.: Inhibition of polymorphonuclear leukocyte respiratory burst by elevated glucose concentrations in vitro. *Diabetes*, 38, 1031–1035 (1989).
- 73) Sato, N., Kashima, K., Uehara, Y., Ohtani, K., Shimizu, H. and Mori, M.: Epalrestat, an aldose reductase inhibitor, improves an impaired generation of oxygen-derived free radicals by neutrophils from poorly controlled NIDDM patients. *Diabetes Care*, 20, 995–998 (1997).
- 74) Thornalley, P. J.: Pharmacology of methylglyoxal: Formation, modification of proteins and nucleic acids, and enzymatic detoxification: a role in pathogenesis and antiproliferative chemotherapy. *Gen. Pharmac.*, 27, 565–573 (1996).