M E D I C I N E G R A N D R O U N D S

ALDOSE REDUCTASE INHIBITOR DRUGS: NEW HOPE FOR DIABETIC PATIENTS?

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INTRODUCTION

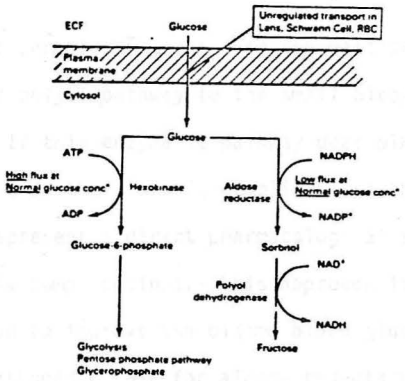
My discussion today is about a new series of compounds, the aldose reductase inhibitors. These agents are still only experimental and in various stages of development, but, I believe they will have an important impact in terms of the management of people with diabetes mellitus. They offer a potential alternative to normoglycemia as a way to prevent or retard the microvascular complications. Since normoglycemia is practically impossible to achieve and wrought with hazards, (1) these drugs offer enormous hope to those with diabetes. For these reasons there is a great activity in terms of developing and testing drugs of this class.

Although the use of aldose reductase inhibitors in patients with diabetes is only a few years old, interest in the polyol pathway is not. It was Mann (2) in 1946 who discovered that the main sugar in seminal fluid was fructose and that the fructose in seminal fluid was derived from blood glucose. Furthermore, there was a relationship between the level of blood glucose and the seminal fluid fructose concentration (3). Hers (4) was the first to demonstrate that seminal fructose fluid was formed via the polyol pathway.

Figure 1 shows the polyol pathway (5). Aldose reductase (alditol:$NADP^+$ oxidoreductase, E.C. 1.1.1.21) is the rate limiting and key enzyme of this pathway. It catalyzes the reduction of hexoses to their respective sugar alcohol (e.g. glucose to sorbitol and galactose to dulcitol); the latter is then oxidized by the second enzyme of this pathway, polyol (sorbitol) dehydroxgenase [L-iditol NAD^+5-oxidoreductase EC 1.1.1.14,(e.g. sorbitol to fructose)]. Sorbitol dehydrogenase has broad substrate specificities for many sugar alco-

hols, additionally converting xylitol to D-xylulose and ribitol to D-ribulose. It has limited ability however to further metabolize duclitol, leading to inhanced accumulation of this sugar alcohol in galactosemia (6). Generally, hexoses including glucose are poor substrates for aldose reductase. However, despite the fact that aldose reductase has a high K_m (≅ 150 mM) for glucose and galactose, when levels of the hexose are elevated as in diabetes with hyperglycemia or in hypergalactosemia significant polyol formation can occur. (6)

Figure 1



(from: Clarke et al, Diabetic Medicine 1:88, 1984)(5)

The measurement of aldose reductase activity in tissues is complicated by the fact that there are at least 3 different enzymes that catalize the converension of hexoses to their sugar alcohols. Table 1 shows some of the biochemical characteristics of this family of enzymes. The similarities between aldose reductase and the aldehyde reductases (I & II) make isolation and measurement of aldose reductase in tissues a difficult task (7,8,9,10,11).

TABLE 1

BIOCHEMICAL CHARACTERISTICS OF THE FAMILY OF "ALDOSE REDUCTASE" ENZYMES

Common Name	Aldose Reductase	Aldehyde Reductase I	Aldehyde Reductase II
Biochemical Name	Alditol:$NADP^+$ 1-Oxidoreductase	Alcohol: NADP Oxidoreductase	Alcohol: NADP Oxidoreductase
Enzyme Number	EC 1.1.1.21	EC 1.1.1.2	EC 1.1.1.2
Molecular Weight	≅ 35,000	≅ 77,000	≅ 36,200
Isozyme	Monomer	Dimer	Monomer
P.I.	5.8	8.25	5.3
Co-Factor	NADPH	NADPH,NADH	NADPH

There has been considerable interest over the past decade (6) in terms of the relationship of the polyol pathway to the small blood vessel complications of diabetes mellitus. If this enzymatic pathway does play some pathogenic mechanism in all or some of the diabetic complications then the inhibition of aldose reductase may represent a direct pharmacological approach in the treatment of certain diabetic complications. This approach is distinct and separate from treatments designed to improve the plasma blood glucose levels (12). In order to establish a pathogenic role for aldose reductase and the polyol pathway in diabetic complications certain criteria should be met. These so called "Koch's postulates" for aldose reductase are listed in Table 2.

Table 2

Criteria Necessary to Establish a Pathogenic Role for Aldose Reductase in Diabetic Complications

1. Aldose reductase must be present in the affected tissue.
2. Similar pathology must occur in the galactosemic as well as the diabetic state.
3. The onset of the complications must be earlier and more severe in the galactosemic state.
4. The onset of the complications should be delayed or prevented with an aldose reductase inhibitor
5. More than one aldose reductase inhibitor must be effective.

Aldose Reductase In Tissues

Aldose reductase has been demonstrated in many tissues in the body. Table 3 lists the tissues in which aldose reductase has been directly demonstated (13-18). As noted above the measurement of aldose reductase activity in human tissues is complicated because of the presence of either aldehyde I and/or II in many of these tissues.

Table 3

Tissue Distribution of Aldose Reductase

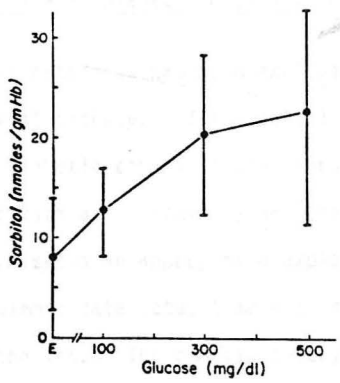
Organ	Tissue	Localization
Eye	Cornea	Epithelium and endothelial
	Conjunctiva	Single layer of blood cells
	Retina	Ganglion, Mueller cells
		Pericytes(mural cells) of retinal capillaries
		Retinal pigment epithelial cells
	Lens	Epithelium
Nerves	Peripheral nerves	Schwann Cell
	Optic nerve	Axons within mylin sheath
Kidney	Medulla	Loop of Henle, conducting tubule
	Glomeruli	Podocytes

In other studies the presence of aldose reductase in various tissues has been implied by the demonstration that sorbitol was present in the tissues and/or sorbitol levels were found to increase in the presence of diabetes or upon incubation of that tissue in a high glucose medium in vitro. Thus, it is thought that aldose reductase is present in spinal cord (19) and in erythrocytes

(20-23). It is now possible to measure the specific activity of aldose reductase separately from the activity of aldehyde reductase II in human erythrocytes (24,25).

The finding that sorbitol levels can be measured in human erythrocytes and that it will change proportionally to plasma glucose levels is an important issue. This makes it possible to relate tissue sorbitol levels to those of plasma glucose and gives us a relatively simple but indirect way to assess the effectiveness of the various aldose reductase inhibitors. The measurement of the specific activity of the isolated aldose reductase enzyme from human erythrocytes would be much more direct but it is unfortunately a very labor intensive task (24,25). If the assay could be streamlined it would be of help in the evaluation of these drugs. Figure 2 shows the effect of increasing the glucose concentration on the production of intact human erythrocites in vitro. Figure 3 shows the effect of the in vivo administration of intravenous glucose and the hyperglycemia that results on erythrocyte sorbitol concentration. Figure 4 shows some data (26) of our own. These data shows the very nice relationship between fasting plasma glucose and erythrocyte sorbitol levels. This relationship has been seen by others as well (20,22). Thus, the use of erythrocyte sorbitol levels are fairly well accepted by most investigators in the field to be representative of sorbitol levels in other tissues. They also are used to measure the relative effectiveness of various aldose reductase inhibitor agents in vivo.

Figure 2



(From: Malone et al Diabetes 29:861-864, 1980)(22)

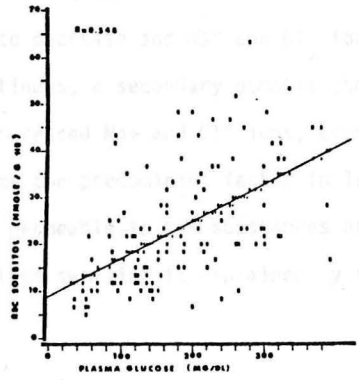
Figure 3

THE EFFECT OF GLUCOSE INFUSION ON ERYTHROCYTE SORBITOL CONCENTRATION

Time (min)	Plasma glucose (mmol/L)	Plasma Sorbitol (μmol/L)	Erythrocyte Sorbitol (μmol/L)
0	3.3	12.6	64.7
45	13.3	12.5	106.0
120	9.8	10.9	75.6
180	3.4	12.0	59.4

(From Hubinont et al Clin Biochem 14(1):19-20, 1981)(21)

Figure 4

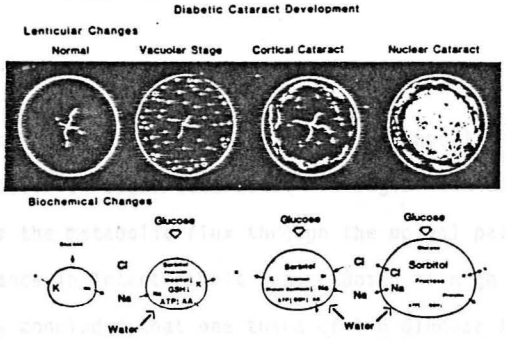


Aldose Reductase in Diabetic Cataract Formation

The formation of diabetic cataracts has been exclusively studied in terms of aldose reductase and the polyol pathway. If the polyol pathway is important in terms of the development of diabetic complications, then how does this occur? The pathogenesis of diabetic cataracts seemingly has been well worked out. Although the "osmotic theory" seems an appropriate explanation for the development of diabetic and galactosemic cataracts, I am not certain that it is operative in tissues other than the lens. The osmotic theory has been promulgated by Kinoshita (28,29) and his coworkers. Stated simply the theory suggests that the intracellular accumulation of polyols initiated by aldose reductase leads to an influx of water that results in permeability changes and eventually leads to a loss of cellular integrety. Figure 5 (98) shows a schematic of biochemical changes that occur during the development of a sugar cataract as well as pictures of actual in vitro cataract formation that correlates with the proposed biochemical changes. The increase in osmolality caused by the accumulation of sorbitol and fructose draws water into the lens fibers, causing them to swell. The swelling has adverse effects as it increases the permeability to substances normally retained in the lens at concentrations higher than surrounding intraocular fluids. Thus, the concentrations of K^+, amino acids, glutathione, inositol and ATP begin to decrease and Na^+ and Cl^- ions slowly begin to build up. As the process continues, a secondary osmotic change results from the electrolyte change of increased Na+ and Cl^- ions; eventually the increases in these electrolytes become the predominant factor in lens swelling. The lens membranes become freely permeable to all substances other than the larger proteins. In this later stage swelling is explained by the Donnan principle. It

is accompanied by the appearance of the dense nuclear cataracts. In the formation of diabetic cataracts the earliest visable change is the appearance of swollen lens fibers caused by an increase in lens hydration. The swollen lens fibers eventually rupture with the liquification of the fibers resulting in vacuole formation. In my judgement the only tissue in which osmotic changes play any role is in the lens relative to cataract formation. In other tissues the amount of water influx is insufficient to cause tissue damage. Other pathogenic mechanisms seem to be more important. These changes will be discussed subsequently.

Figure 5

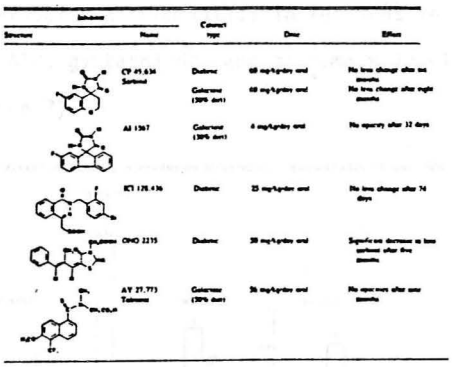


(From: Kador et al Ann. Rev. Pharmacol. Toxicol. 25:691-714, 1985)(29)

The inhibition of <u>in vitro</u> cataract formation is a well established method for evaluating the effectiveness and relative potency of aldose reductase inhibitors. Table 3 (29) shows the structure and potency of the five aldose reductase inhibitors presently being developed.

Table 3

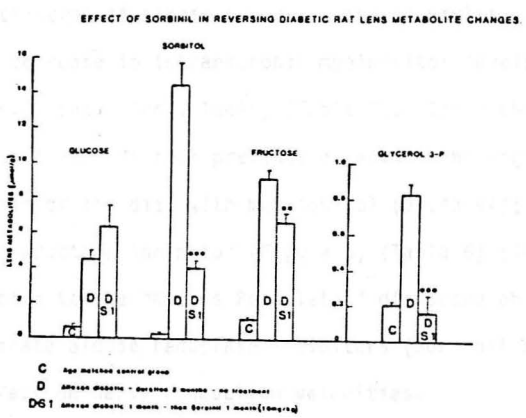
In Vivo Effects of Several Aldose Reductase Inhibition on Sugar Cataracts



There is considerable experimental data to support the effect of aldose reductase inhibitor on sugar cataracts. To begin with Gonzalez et al, (30) were able to measure the metabolic flux through the polyol pathway using nuclear magnetic resonance in intact rabbit lens exposed to high glucose concentrations in vitro. They concluded that one third of the glucose turnover in this model is via the polyol pathway. Hu et al (31) and Datiles et al (32) were able to prevent cataract formation in the galactose fed rat model with the aldose reductase inhibitor sorbinil. Beyer-Mears et al (33) were able to preserve lens growth, cell hydration and protein components (alpha, beta, and gamma crystallins) with the topical administration of sorbinil to neonatal rats during galactose cataractogenesis. Gonzales et al (34), studied the effect of sorbinil on the metabolic profile of the lens following induction of alloxan diabe-

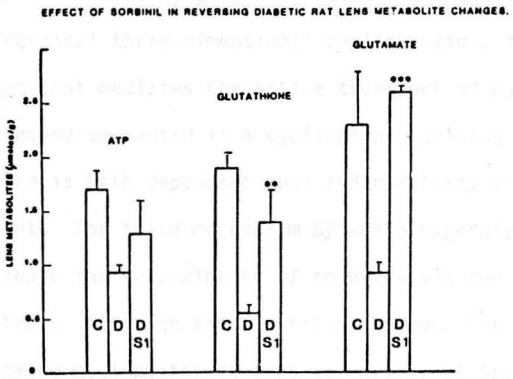
tes in the rat. The lens content of sorbitol, fructose, were markedly increased with induction of diabetes. Administration of the aldose reductase agent, sorbinil, either normalized these abnormalities or partially corrected them. (Figure 6) Other metabolic abnormalities in the lens in alloxan diabetes included diminished ATP, glutathione, and glutamate levels were also corrected with sorbinil. (Figure 7)

Figure 6



Adapted from Gonzales et al Diabetes 32:482-485, 1983 (34)

Figure 7



Adapted from Gonzales et al Diabetes 32:482-485, 1983 (34)

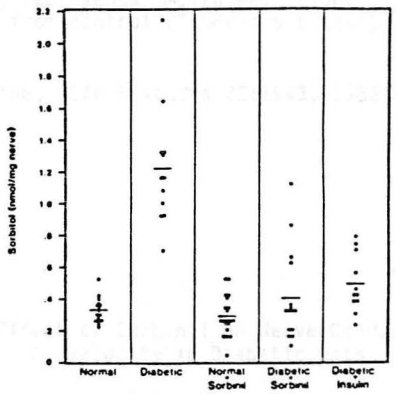
Aldose Reductase in Diabetic Neuropathy

Another mechanism by which aldose reductase and the accumulation of sorbitol may be implicated in the development of diabetic complications is best illustrated in terms of diabetic neuropathy. There is considerable evidence (34) that diabetic neuropathy may be related to changes in nerve myoinositol (35-38) content and the activity of Na-K ATPase (33-35). It is quite clear that in experimental diabetes in the rat that hyperglycemia with resulting increased activation of aldose reductase and accumulation of sorbitol in nerve tissue and a decrease in intraneuronal myoinositol levels (Table 4) results in decreased nerve conduction velocity (Table 5). These changes can be reversed by; 1) insulin treatment that prevents detectable hyperglycemia (36-42); 2) supplementation of the diet with myoinositol (33,43,44); and 3) administration of an aldose reductase inhibitor (Figure 8) (Table 6) (44-48). It is important to note relative to the "Koch's Postulates" discussed above of aldose reductase that two separate aldose reductase inhibitors (Sorbinil and ONO-2235) have the identical effect on nerve conduction velocities.

Hyperglycemia appears to lower intraneuronal myoinositol levels by at least three separate mechanisms. The first is a competitive inhibition of myoinositol uptake by the nerve cell by glucose. Because glucose and myoinositol have nearly the identical three dimensional configuration, the two compete for the carrier system that mediates the active transport of myoinositol into peripheral nerve. The second mechanism is a cyclical one arising from the fact that myo-inositol uptake is both dependent upon and necessary to a normal transmembrane sodium gradient. The third mechanism by which hyperglycemia blocks myoinositol uptake is through the accumulation of sorbitol via the increased activity of aldose reductase. Although the connection between the polyol pathway and the decrease in nerve myoinositol content is unclear at present the use of an aldose

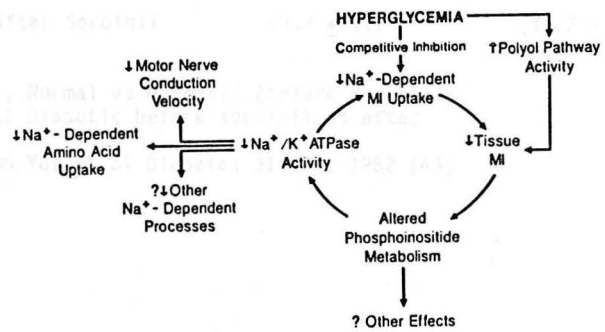
reductase inhibitor will prevent the fall in nerve myoinositol (49). Figure 9 shows the proposed interaction of hyperglycemia on sodium-dependent myoinositol uptake, inositol phospholipid metabolism and sodium-potassium ATPase activity and the polyol pathway in the pathogenesis of diabetic neuropathy (49,50,51). Of interest and importance is that these same metabolic changes, i.e. accumulation of sorbitol with a subsequent reduction in tissue myoinositol and a reduction in Na-K ATPase activity have been demonstrated in both diabetic retina and glomeruli.

Figure 8



Yue et al Diabetes 31:789, 1982 (45)

Figure 9



(From: Greene et al, Diabetes Care 8:290, 1985) (51)

Table 4

Polyol and Sugar Concentrations in Sciatic Nerve after Sorbinil or Inositol

	Fructose	Sorbitol	Glucose	Inositol
Control	1.14±0.09	0.24±0.02	2.56±0.06	3.92±0.07
Diabetic	6.51±0.59[b]	2.90±0.22[b]	8.21±0.41[b]	2.98±0.11[b]
Diabetic/ Sorbinil	1.74±0.14[b]	0.15±0.01[a]	9.55±0.52[b]	3.78±0.10
Diabetic/ Inositol	6.70±0.70[b]	2.20±0.19[b]	8.44±0.53[b]	3.73±0.10

Figures (means ± S.E.M.) represents mol/g wet weight.
Significantly different from control (Student's t test)
a, $p<0.01$; b, $p<0.001$.

From: Gillon and Hawthorne, Life Sciences 22:1943, 1983 (47)

Table 5

The Effect of Sorbinil on Nerve Conduction Velocity in Diabetic Rats

	Motor Nerve Conduction Velocities m/s	
	Normal	Diabetic
Before Sorbinil	50.1 ± 4.5	39.8 ± 5.7*
After Sorbinil	49.7 ± 1.2	47.7 ± 6.7**

* = $p<0.01$, Normal vs diabetic (before sorbinil)
** = $p<0.011$ Diabetic before sorbinil vs after

Adapted from Yue et al Diabetes 31:789, 1982 (45)

Table 6

The Effect of ONO-2235 on Sorbitol Content of the Sciatic Nerve and Motor Nerve Conduction Velocity in Diabetic Rats

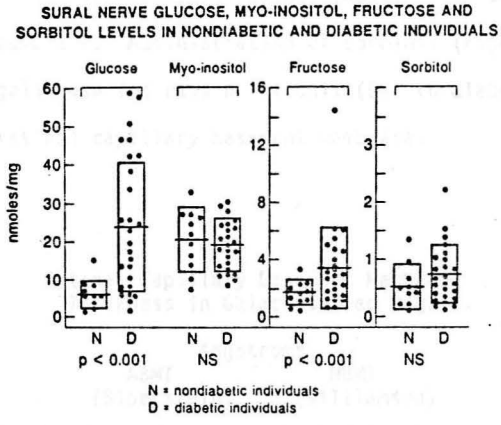
Group	Sorbitol Content nmol/g wet weight	Motor Nerve Conduction Velocity m/s	
		Day 0	Day 7
Normal	0.229 ± 0.015	25.1 ± 0.5	26.4 ± 1.0
Diabetic non-treated	1.309 ± 0.080	----	21.5 ± 0.8
Diabetic ONO-2235 Treated	0.607 ± 0.018	----	25.5 ± 1.5

Adapted from Kikkawa, et al Metabolism 33:212, 1984 (48)

The "myoinositol depletion theory"(51) is widely accepted as being the initiator of the connecting link between hyperglycemia and increased polyol pathway activity and the eventual tissue damage that results in diabetes. Recently there have been studies that put this theory in question, however. In experimental models of diabetes in animals a decrease in tissue myoinositol (41,43,47) is quite uniformly demonstrable. In human diabetes these biochemical changes have been more difficult to show. Dyck et al (52) measured alcohol sugars and myoinositol content in sural nerve biopsies from patients with diabetes and in non-diabetic controls. Interestingly, (Figure 10) although sorbitol levels were increased in erythrocytes from the diabetic patients, myoinositol levels in the peripheral nerves were not decreased. They concluded that myoinositol deficiency might not be a part of the pathogenesis of human diabetic neuropathy. Their work agrees with that of others in nerve tissue obtained from amputated limbs (53). In addition, Suarez et al, have noted an increase in the

fluorescence of collagen from streptozotocin diabetic rats that can be prevented by the administration of the aldose reductase inhibitor drug, sorbinil. They interpret their findings as indicative of the nonenzymatic fructosylation of collagen (54). This is potentially an important finding as it links the polyol pathway with nonenzymatic glycosylation. Previously it has been assumed that either one or the other of these potential mechanisms was pathogenically important in linking hyperglycemia to tissue damage.

Figure 10



(From Dyck et al N Engl J Med 319:542-548, 1988) (52)

Aldose Reductase in Diabetic Retinopathy

The polyol pathway and aldose reductase have been implicated in two histological features of diabetic retinopathy that involve retinal capillaries; 1) the selective loss in numbers of mural cells (pericytes); and 2) a thickening of the retinal capillary basement membrane. Mural cells contain aldose reductase (16) and accumulate sorbitol. Of interest is the fact that dogs (55) and rats

(56,57) made galactosemic by the feeding of a high galactose diet, develop retinopathy that appears to be similar to diabetic retinopathy in humans. The retinopathy in galactosemic dogs (55) is marked by saccular capillary aneurysms, hemorrhages, nonperfused or acellular vessels, tortuous hypertrophic capillaries, loss of capillary pericytes and other lesions typical of diabetic patients. Very recently the presence of the aldose reductase enzyme has been demonstrated in retinal pigment epithelial cells in diabetic human retinas (58).

Furthermore, in both galactose fed dogs (57)(Table 7) and rats (56,57) and in experimental diabetes in rats (59) retinal capillaries developed a thickening of their basement membrane. Administration of sorbinil (Figure 11)(58) or Tolrestat (60) to galactose fed rats or alconil (59) to diabetic rats prevents the thickening of retinal capillary basement membrane.

Table 7

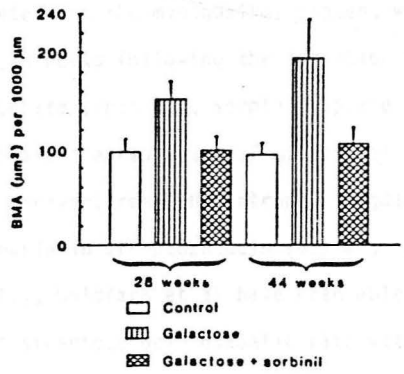
Retinal Capillary Basement Membrane
Thickness in Galactose Fed Dogs

	Angstroms	
	ABMT (Siperstein)	MBMT (Williamson)
Normal Dogs	1689 ± 315	1217 ± 288
Galactosemic Dogs	2497 ± 51*	1773 ± 136*

* = $p<0.05$ Normal vs. Galactosemic

Adapted from Frank et al Invest. Ophthal. Visual Sci. 24:1519, 1983 (57)

Figure 11



(From: Robison et al, Science 221:1177, 1983 (58)

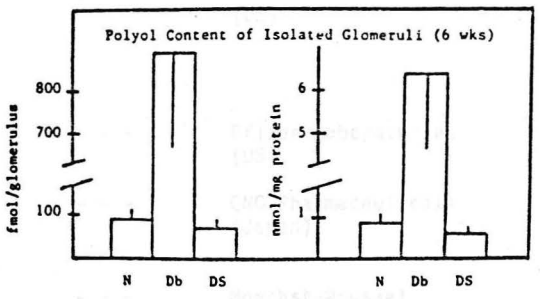
Recently other more functional abnormalities and their relationship to the polyol pathway have been described. Kador and his group (61) have described permeability changes in the blood-retinal barrier in galactosemic rats. These increases in the permeability to small molecules of the blood-retinal barrier are preventable by the aldose reductase inhibitor, sorbinil. Finally, MacGregor and Matschinsky (62) showed that myoinositol feeding or the administration of sorbinil to streptozotocin diabetic rats could prevent deterioration of the electroretinogram that is seen in these animals.

Aldose Reductase in Diabetic Nephropathy

The role of aldose reductase in the pathogenesis of experimental diabetic nephropathy has only recently been investigated. Beyer-Mears (63) and her colleagues recently measured polyols in glomeruli isolated from control and streptozotocin diabetic rats. Compared with control (nondiabetic) animals, the

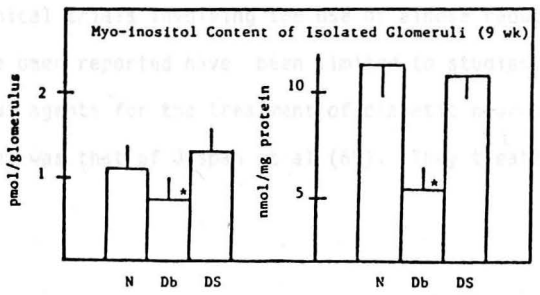
polyol content (Figure 12) was increased ten-fold at 6 weeks following the induction of diabetes and the myoinositol content was reduced in diabetic glomeruli (Figure 13) 9 weeks following the induction of diabetes. Administration of the aldose reductase inhibitor, sorbinil, prevented both the accumulation of polyol (Figure 12) and the reduction of myoinsitol (Figure 13). Sorbinil has also been shown to prevent renal hypertrophy in galactose fed rats (64) and to diminished proteinuria in streptozotocin (65) and spontaneously diabetic rats (BB/W) (66). Finally, Goldfarb et al have been able to reduce glomerular hyperfiltration in streptozotocin diabetic rats with myoinositol feeding and the administration of aldose reductase inhibitors (67). Tolrestat has also been shown to prevent increased urinary albumin excretion in streptozotocin diabetic rats (68).

Figure 12



Adapted from Beyer-Mears et al. Diabetes 33:604, 1984(63)

Figure 13



Adapted from Beyer-Mears et al. Diabetes 33:604, 1984(63)

Aldose Reductase in Clinical Trials

Clinical trials with the various aldose reductase inhibitor agents are now underway. Table 8 lists the various aldose reductase inhibitor drugs that are now in various stages of development.

Table 8

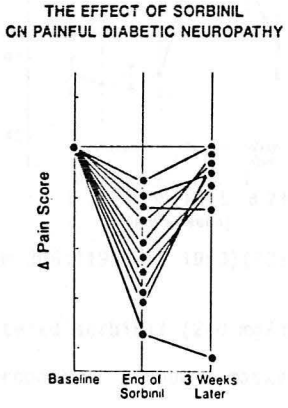
Development of Aldose Reducatase Inhibitor Drugs

Drug	Name	Pharmaceutical Company	Status
ICI-128,436	Statil	Stuart Pharmaceuticals Imperial Chemical Industries (UK)	Phase 2/3 studies (neuropathy) well underway
AY-27,773	Tolrestat	Ayerst-American Home Products (US)	Some Phase 2/3 studies (neuropathy) have been completed. Retinopathy trial ongoing. New studies beginning
CP-45,634	Sorbinil	Pfizer Laboratories (US)	Some Phase 2/3 studies (neuropathy and retinopathy) have been completed. Future unclear (safety)
CP-73,850	- - -	Pfizer Laboratories (US)	Phase 2/3 neuropathy studies beginning
ONO-2235	- - -	ONO Pharmaceuticals (Japan)	Several open clinical trials (neuropathy) completed in Japan
HOE-843	- - -	Hoechst-Roussel	Phase 1/2 studies beginning

The clinical trials involving the use of aldose reductase inhibitors whose results have been reported have been limited to studies involving the use of these various agents for the treatment of diabetic neuropathy. The first of these studies was that of Jaspan et al (69). They treated 11 patients with

severe painful diabetic neuropathy with a single daily dose of 250 mg of sorbinil for a period of 3 to 6 weeks. Eight of the eleven patients also received placebo for an variable period of time ranging from several days to a few weeks. Response was assessed according to a 0-20 graphic scale for pain and by tests for motor and sensory nerve conduction velocities and cardiac autonomic nerve function. Eight patients had moderate to marked relief of their pain, two had equivocal responses and one had no change. Upon discontinuation of the medication, 7 of the 8 responders had a worsening of pain. (Figure 14) During the course of treatment, autonomic nerve function improved significantly in 6 of 7 patients tested. Nerve conduction velocities improved in 4 of the 7 tested.

Figure 14



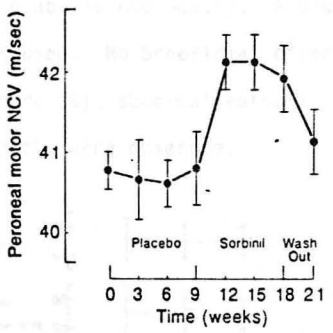
(From: Jaspan et al The Lancet 2:758-762, 1983)(69)

Judzewitsch et al (70) conducted a double masked, placebo crossover trial in 39 diabetic patients without clinical evidence of diabetic neuropathy. Each patient during each 9 week study period received either placebo or a single

daily dose of 250 mg of sorbinil. During the 9 weeks of treatment with sorbinil, nerve conduction velocity was greater than during a 9 week placebo period for all three nerves tested (Figure 15). There were no changes in glycemic control during the study. Nerve conduction velocity for all three nerves declined significantly within 3 weeks after cessation of the drug. As one can tell from Figure 15, the improvement in nerve conduction velocity was small, approximately 1 meter/second. Such a small change hardly warrants an enthusiastic response.

Figure 15

THE EFFECT OF SORBINIL ON
MOTOR NERVE CONDUCTION VELOCITY
IN DIABETIC NEUROPATHY



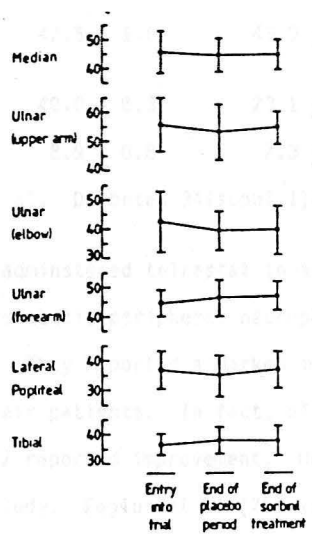
From: Judzewitsch et al NEJM 308:119-125, 1983)(70)

Young et al (71) administered sorbinil (200 mg/day) to 15 patients with chronic painful diabetic neuropathy. A double-masked crossover study design was used. Treatment was evaluated by subjective pain responses, clinical examination, vibration perception threshold, nerve electrophysiology and cardiovascular reflex tests. Among the many measurements only pain, tendon reflex scores and sural sensory potential amplitude improved. Four patients developed an idosyncriatic reaction (rash) that rapidly disappeared upon termination of the drug.

It is important to note that the use of the aldose reductase inhibitor drug, sorbinil, has been associated with an alarmingly high incidence of side effects. These side effects are primarily skin lesions that are most likely related to the hydantoin structure of the drug. Unfortunately, attempts to use a lower dose of the drug have not been effective in reducing the high incidence of side effects. The future of this particular drug seems quite dim because of its high side effects profile. The FDA has not permitted any new human studies with sorbinil for several years.

Lewin et al (72) administered sorbinil 200 mg daily for four weeks in 13 patients with symptomatic diabetic neuropathy. A double-masked placebo crossover study design was used. No beneficial effects of the sorbinil on nerve conduction velocities (Figure 16), abnormal autnomic nervous system function or symptoms of painful neuropathy were observed.

Figure 16



(From: Lewin et al Diabetologia 26:445, 1984)(72)

Two other studies are worth mentioning. The first by Hotta et al (73) using a 300 mg daily dose of the aldose reductase inhibitor drug, ONO-2235, in 13 patients with painful diabetic neuropathy. The drug was given for 12 weeks without any placebo control. Significant improvement in motor nerve conduction velocity in the ulnar nerve was observed over the 12 week treatment. The improvement in electrophysiological studies as well as those of subjective symptoms (pain & numbness) correlated with the reduction of erythrocyte sorbitol levels (Table 9).

TABLE 9

EFFECTS OF ONO-2235 IN DIABETIC PATIENTS

	Baseline	12 Week Drug	4 week Washout
Ulnar Nerve motor conduction velocity (m/s)	43.8 ± 1.8	48.9 ± 2.1	45.9 ± 2.4
Ulnar Nerve sensory conduction velocity (m/s)	47.5 ± 1.6	45.0 ± 1.3	41.3 ± 2.9
RBC Sorbitol (nmoles/gHb)	40.0 ± 6.3	23.1 ± 3.4	30.2 ± 2.9
HbA1c (%)	8.9 ± 0.8	7.3 ± 0.6	8.1 ± 0.5

Adapted From Hotta et al. Diabetes 34(suppl 1):98A, 1985.)(73)

Koglan et al (74) adminstered tolrestat in a dose of 200 mg/day to 19 patients with painful diabetic peripheral neuropathy. There was no placebo control in this study. They reported a marked improvement in symptoms in a large proportion of their patients. In fact, of the 8 patients who had been treated for 24 weeks, 7 reported improvement. Unfortunately, there was no placebo control in this study. Fagius et al (75) studied the effect of 250 mg daily

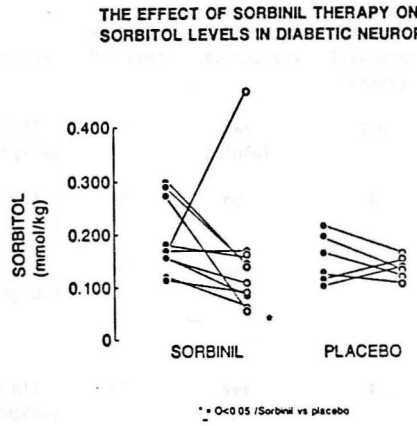
dose of sorbinil in patients with symptomatic diabetic polyneuropathy during a six-month double-masked placebo-controlled trial. There was no improvement in the symptoms of diabetic neuropathy in those patients treated with sorbinil. There were, however, some improvements noted in the results of three of nine neurophysiologic tests and one of five tests of autonomic nerve function.

Rosenstock et al (76) have recently studied the short-term effect of sorbinil in 32 patients with severe painful diabetic neuropathy unresponsive to conventional analgesic and tricyclic therapy. A double-masked randomized study design was used. Patients received either placebo of 250 mg per day of sorbinil. Assessment was made using a 20-point self-administered neuropathy and pain symptom score. Pain scores and overall neuropathy improved after the six-week study in the sorbinil-treated group as compared with those patients who received placebo. They concluded, however, that longer studies will be required to assess the full effect of aldose reductase inhibitors, preferably in patients with less advanced diabetic neuropathy.

The most exciting data regarding the clinical use of aldose reductase drugs has come from Sima et al (79). These workers did sural nerve biopsies at baseline and after one year of treatment with either sorbinil or placebo (randomly assigned) in a group of 16 patients with symptomatic diabetic peripheral neuropathy. In contrast to the patients who received placebo the sorbinil treated pateints had a 42% decrease in nerve sorbitol content (Figure 17) and a 3.8 fold increase in the number of regenerating myelinated nerve fibers (Figure 18), reflected by a 33 percent increase in the number of myelinated fibers seen in a cross-sectional area of nerve. The sorbinil treated patients also had quantitative improvement in terms of the degree of paranodal demylination, segmen-

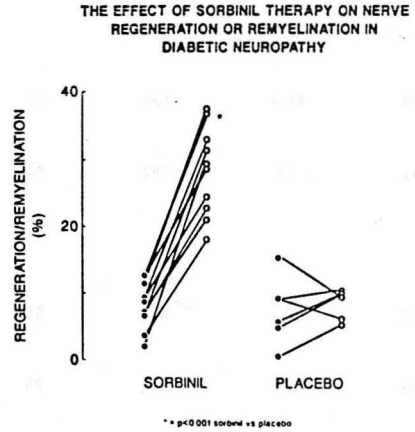
tal demyelination and myelin wrinkling. The increase in the number of normal fibers was accompanied by electrophysiological and clinical evidence of improved nerve function.

Figure 17



(From Sima et al N Eng J Med 319:548-555, 1988)(79)

Figure 18



(From Sima et al, N Eng J Med 319:548-555, 1988)(79)

TABLE 10

Summary of Clinical Trials With Aldose Reductase Inhibitors

Study	Drug/Dose	Number of Patients	Diabetic Neuropathy	Duration of Treatment (weeks)	Placebo Controlled	Results
Jaspan et al(69)	Sorbinil 250 mg/day	11	yes painful	3-6	no	improvement in 8/11
Judzewitsch et al(70)	Sorbinil 250 mg/day	39	no	9	yes	small improvement in MNCV
Young et al(71)	Sorbinil 250 mg/day	15	yes	16	yes	improvement in pain and tendon reflex scores
Lewin et al(72)	Sorbinil 200 mg/day	13	yes	4	yes	no improvement in symptoms or electrophysiological tests
Hotta(73)	ONO-2235 300 mg/day	13	yes	12	no	improvement in symptoms and MNCV
Koglin(74)	Tolrestat 200 mg/day	19	yes	4-24	no	improvement in symptoms
Fagius et al(75)	Sorbinil 250 mg/day	55	yes	24	yes	no improvement in symptoms; some improvement in neurophysiological tests
Rosenstock et al(76)	Sorbinil 250 mg/day	32	yes	6	yes	improvement in pain and neuropathy
Sundkvist et al(77)	Statil 300 mg/day	29	yes	4	yes	no improvement in autonomic or peripheral nerve function
Martyn et al(78)	Sorbinil 125 mg/day	22	no	24	yes	no improvement in autonomic or peripheral nerve function

Conclusion

Aldose reductase inhibitors, from a theoretical perspective, seem to offer promise as possible agents for the treatment or even the prevention of the microvascular complications of diabetes. Although there is considerable theoritical data supporting their effectiveness in experimental diabetes, there have been relatively few studies in humans. Those that have been done often were poorly designed, i.e. lacked an appropriate control group, or were performed on patients with markedly advanced disease. I feel we must await more data before advocating these compounds, except in experimental situations. My bias is, however, (80) that the real utility of these drugs will be as agents used for the prevention of the complications of diabetes in some tissues (nerve and retina seem the most likely candidates) rather than as treatment for advanced disease.

REFERENCES

1. The DCCT Research Group: Diabetes Control and Complications Trial (DCCT): Results of feasibility study. Diabetes Care 10:1-19, 1987.

2. Mann T: Studies on the metabolism of semen. 3. Fructose as a normal constituent of seminal plasma: site of formation and function of fructose in semen. Biochem Journal 40:481-491, 1946.

3. Mann T, Parsons U: Studies on the metabolism of semen. 6. Role of hormones, effect of castration, hypophysectomy and diabetes: relation between blood glucose and seminal fructose. Biochem Journal 46:440-450, 1950.

4. Hers HG: Le mecanisme de la transformation de glucose en fructose par les vesicules seminales. Biochim Biophys Acta 22:202-203, 1956.

5. Clarke BF, Young, RJ, Martyn, CN, Ewing, DJ: Aldose reductase inhibition in diabetes. Diabetes Medicine 1:88-90, 1984.

6. Gabbay KH: The sorbitol pathway and the complications of diabetes. NEJM 288:831-836, 1973.

7. Wermuth B, Von Wartburg J-P: Aldose reductase from human tissues. IN Methods in Enzymology Volume 89. pp 181-186. Academic Press 1982.

8. Ballabh D, Srivastava SK: Purification and properties of aldose reductase and aldehyde reductase II from human erythrocyte. Arch Biochem and Biophys 238: 670-679, 1985.

9. Petrash JM, Srivastava SK: Purification and properties of human liver aldehyde reductases. Biochemic et Biophysica Acta 707:105-114, 1982.

10. Hoffman, PL, Wermuth B, vonWartburg J-P: Human brain aldehyde reductases: relationship to succinic semialdehyde reductase and aldose reductase. J Neurochem 35:354-366, 1980.

11. Chakrabarti S, Sima AAF, Nakajima T, Yagihasi S, Greene DA: Aldose reductase in the BB rat: isolation, immunological identification and localization in the retina and peripheral nerve. Diabetologia 30:244-251, 1987.

12. Raskin, P: Treatment of insulin-dependent diabetes with portable insulin infusion devices. Med. Clin of No Amer 66:1269-1283, 1982

13. Kador, F, Kinoshita, JH, Tung, WH, Chylack, LT: Differences in the susceptibility of various aldose reductases to inhibition. Invest. Ophthalmol. Vis. Sci. 19:980-982,1980

14. Akagi, Y, Yajima, Y, Kador, PF, Kuwabara, T, Kinoshita. JH.: Localization of aldose reductase in the human eye. Diabetes 33:562-566, 1984.

15. Ludvigson, MA, Sorenson, RL: Immunohistochemical localization of aldose reductase. Diabetes 29:450-459, 1980.

16. Akagi, Y, Kador, PF, Kuwabara, T, Kinoshita, JH: Aldose reductase localization in Human Retinal Mural Cells. Invest. Ophthal. Vis. Sci. 24:1516-1519, 1983.

17. Gabbay, Kenneth H., Cathcart, Edgar S.: Purification & immunologic identification of aldose reductases. Diabetes 23:460-468, 1974.

18. Corder, CN, Braughler, JM, Culp, PA,: Quantitative histochemistry of the sorbitol pathway in glomeruli and small arteries of human diabetic kidney. Folia Histochem. Cytochem. (Krakow) 17:137-146, 1979.

19. Gabbey, K, Merola, LO, Field, RA,: Sorbitol Pathway: Presence in nerve and cord with substrate accummulation in diabetes. Science 151:209, 1966.

20. Malone, John I, Knox, Gail, Benford, Stephen, Tedesco, Thomas A: Red cell sorbitol an indicator of diabetic control. Diabetes 29:861-864, 1980.

21. Hubinont, Corinne, Sener, Abdullah, Malaisse, Willy J: Sorbitol content of plasma & erythrocytes during induced short-term hyperglycemia. Clin. Biochem 14:19-20, 1981.

22. Malone, JI, Knox, G, Harvey, C: Sorbitol accumulation is altered in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 27:509-513, 1984.

23. Popp-Snijders, C, Lomecky-Janousek, MZ, Schouten, JA, van der Veen, EA: myo-inositol and sorbitol in erythrocytes from diabetic patients before and after sorbinil treatment. Diabetologia 27:514-516, 1984.

24. Srivastava SK, Ansarai NH, Hair GA, Ballabh D: Aldose and aldehyde reductases in human tissues. Biochemica et Biophysica Acta 800:200-227, 1984.

25. Srivastava SK, Hair GA, Ballabh D: Activated and unactivated forms of human erythrocyte aldose reductase. Proc Natl Acad Sci 82:7222-7226, 1985.

26. Raskin, P., Rosenstock, J., Challis, P., Ryder, S., Mullane, J.F., Gonzalez, R., Hicks, D, Smith, T., Dvornik, D.: Effect of tolrestat on red blood cell sorbitol levels in diabetic subjects. Clinical Pharmacology and Therapeutics 38:625, 1985.

27. Kinoshita, JH: Cataracts in galactosemia. Invest. Ophthalmol. 4:786-799, 1965.

28. Kinoshita, JH, Kador, PF, Robison, WG, Datilis, MB, Cobo, LM, Kupfer C: Aldose reductase and complications of diabetes. Ann. Int. Med. 101:82-91, 1984.

29. Kador, P, Robison, WG, Kinoshita, JH: The pharmacology of aldose reductase inhibitors. Ann. Rev. Pharmacol. Toxicol 25:691-714, 1985.

30. Gonzalez, R, Gilberto, Barnett, Patrick, Aguayo, James, Chen, Hong-Ming, Chylac,Jr, LT: Direct measurement of polyol pathway activity in the ocular lens. Diabetes 33:196-199, 1984.

31. Hu, Tian-Sheng, Datiles, Manuel, Kinoshita, Jin H: Reversal of galactose cataract with sorbinil in rats. Invest.Ophthalmol.& Vis. Sci.24L640-644m 1983

32. Datiles, M, Fukui, H, Kuwabara, T, Kinoshita, JH: Galactose cataract prevention with sorbinil and aldose reductase inhibitor: A light microscopic study. Inves. Ophthalmol. Vis. Sci. 22:174-179, 1982.

33. Beyer-Mears, A, Cruz, E, Nicolas-Alexandre, J, Varagiannis, E: Sorbinil protection of lens protein components and cell hydration during diabetic cataract formation. Pharmacology 24:193-200, 1982.

34. Gonzalez, Ana-Maria, Sochor, Milena, McLean, Patricia.: The effect of an aldose reductase inhibitor (Sorbinil) on the level of metabolites in lenses of diabetic rats. Diabetes 32:482-485, 1983.

35. Winegrad, Albert I, Simmons, David A, Martin, Donald B: Has one diabetic complication been explained?. NEJM 308:152-154, 1983.

36. Greene, DA, De Jesus, PV Jr, Winegrad, AI: Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute strentozotocin diabetes.

37. Greene, DA, Winegrad, AI: Effects of acue experimental diabetes on composite energy metabolism in peripheral nerve axons and Schwann cells. Diabetes 30:967-974, 1981.

38. Simmons, DA, Winegrad, AI, Martin, DB: Significance of tissue myo-inositol concentrations in metabolic regulation in nerve. Science 217:848-851, 1982.

39. Mayhew, JH, Gillon, KRW, Hawthorne, JN: Free and lipid inositol, sorbitol and sugars in sciatic nerve obtained post-mortem from diabetic patients and control subjects. Diabetologia 24:13-15, 1983.

40. Finegold, David, Lattimer, Sarah A, Nolle, Sandra, Bernstein, Marcy, Green, Douglas A: Polyol pathway activity and myo-inositol metabolism - a suggested relationship in the pathogenesis of diabetic neuropathy. Diabetes 32:988-992, 1983.

41. Greene, Douglas A, Lattimer, Sarah A: Impaired rat sciatic nerve sodium-potassium adenosine triphosphatase in acute streptozocin diabetes and its correction by dietary myo-inositol supplementation. J. Clin. Invest. 72:1058-1063, 1983.

42. Pietri, A, Ehle, AL, Raskin, P: Changes in nerve conduction velocity after six weeks of glucoregulation with portable insulin infusion pumps. Diabetes 29:668-671, 1980.

43. Greene, Douglas A, Lewis, Richard A, Lattimer, Sarah A, Brown, Mark J: Selective effects of myo-inositol administration on sciatic and tibial motor nerve conduction parameters in the streptozocin-diabetic rat. Diabetes 31:573-578, 1982.

44. Clements, Rex S, Vourganti, Bhaskar, Kuba, Tadashi, Oh, Shin J, Darnell, Betty: Dietary myo-inositol intake and peripheral nerve function in diabetic neuropathy. Metabolism 28:477-483, 1979.

45. Yue, DK, Hanwell, MA, Satchell, PM, Turtle, JR: The effect of aldose reductase inhibition on motor nerve conduction velocity in diabetic rats. Diabetes 31:789-794, 1982.

46. Kikkawa, R, Hatanaka, J, Yasuda, H, Kobayashi, N, Shigeta, Y, Terashima, H, Morimura T, Tsuboshima, M: Effect of a new aldose reductase inhibitor, (E)-3-carboxymethyl-5-[(2E)-methyl-3-phenylpropenylidene]rhodanine (ONO-2235) on peripheral nerve disorders in streptozotocin-diabetic rats. Diabetologia 24:290-292, 1983.

47. Gillon, KRW, Hawthorne, JN: Sorbitol, inositol and nerve conduction in diabetes. Life Sciences 32:1943-1947, 1983.

48. Kikkawa, Ryuichi, Hatanaka, Ikuo, Yasuda, Hitoshi, Kobayashi, Nobuyuki, Shigeta, Yukio: Prevention of peripheral nerve dysfunction by an aldose reductase inhibitor in streptozotocin-diabetic rats. Metabolism 33:212-214, 1984.

49. Greene, Douglas A, Lattimer, Sarah A: Action of sorbinil in diabetic peripheral nerve-relationship of polyol (Sorbitol) pathway inhibition to a myo-inositol-mediated defect in sodium-potassium ATPase activity. Diabetes 33:712-716, 1984.

50. Greene, DA: Myo-Inositol: The mystery molecule of diabetic complications. Clinical Diabetes 3:80-81, 1985.

51. Greene DA, Lattimer BS, Ulbrecht J, Carroll P: Glucose-induced alterations in nerve metabolism: current perspective on the pathogenesis of diabetic neuropathy and future directions for research therapy. Diabetes Care 8:290-299, 1985.

52. Dyck, PJ, Zimmerman, BR, Vilen TH, et al: Nerve glucose, fructose, sorbitol myo-inositol, and fiber degeneration and regeneration in diabetic neuropathy. New Engl J Med 319:542-548, 1988.

53. Hale, PJ, Nattrass, M, Silverman SH, Sennit, C., Perkin, CM et al: Peripheral nerve concentrations of glucose, fructose, sorbitol and myoinositol in diabetic and non-diabetic patients. Diabetologia 30:464-467, 1987.

54. Suarez, G, Rajaram R., Bhuyan KC, Oronsky, AL, Goidl, JA: Administration of an aldose reductase inhibitor induces a decrease of collagen fluorescence in diabetic rats. J Clin Invest 82:624-627, 1988.

55. Engerman, Ronald L, Kern, Timothy S: Experimental galactosemia produces diabetic-like retinopathy. Diabetes 33:97-100, 1984.

56. Vinores, SA, Campochiaro, PA, Williams, EH, May, EE, et al: Aldose reductase expression in human diabetic retina and retinal pigment epithelium. Diabetes 27:1658-1664, 1988.

57. Frank, Robert N, Keirn, Richard J, Kennedy, Alexander, Frank, Karni W: Galactose-induced retinal capillary basement membrane thickening: Prevention by Sorbinil. Invest. Opthalmol.& Vis. Sci. 24:1519-1524, 1983.

58. Robison, WG, Kador, PF, Kinoshita, JH: Retinal capillaries: Basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. Science 221:1177-1179, 1983.

59. Chandler, ML, Shannon, WA, DeSantis, L: Prevention of retinal capillary basement membrane thickening in diabetic rats by aldose reductase inhibitors. Invest. Ophthalmol. Vis. Sci. 25(Suppl 2):159, 1984.

60. Robison, WG, Kador, PF, Akagi, Y, Kinoshita, JH, et al: Prevention of basement membrane thickening in retinal capillaries by a novel inhibitor of aldose reductase, tolrestat. Diabetes 35:295-299, 1986.

61. Lightman, S., Rechthand, E., Terubayashi, H., Palestine, A., Rapoport, S., Kador, P: Permeability changes in blood-retinal barrier of galactosemic rats are prevented by aldose reductase inhibitors. Diabetes 36:1271-1275, 1987.

62. MacGregor, LC and Matschinsky, FM: Treatment with aldose reductase inhibitor or with myo-inositol arrests deterioration of the electroretinogram of diabetic rats. J Clin Invest 76:887-889, 1985.

63. Beyer-Mears, Annette, Ku, Ling, Cohen, Margo: Glomerular polyol accumulation in diabetes and its prevention by oral Sorbinil. Diabetes 33:604-607, 1984.

64. Beyer-Mears, A, Cruz, E, Dillon, P, Tanis, D, Roche, M: Diabetic renal hypertrophy diminished by aldose reductase inhibition. Fed. Proc. 42:505, 1983.

65. Beyer-Mears, A, Cruz, E., Edelist, T., Varagiannis, E: Dimished proteinuria in diabetes mellitus by sorbinil, an aldose reductase inhibitor. Pharmacology 32:52-61, 1986.

66. Beyer-Mears, A, Murray FT, Del Val, M, Cruz, E, Sciadini, M: Reversal of proteinuria by sorbinil, an aldose reductase inhibitor in spontaneously diabetic (BB) rats. Pharmacology 36:112-120, 1988.

67. Goldfarb, S, Simmons DA, Kern, EFO: Amelioration of glomerular hyperfiltration in acute experimental diabetes mellitus by dietary myo-inosistol supplementation and aldose reductase inhibition. Trans Assoc Amer Phys 99:67-72, 1986.

68. McCaleb, ML, Sredy, J, Millen, J, Ackerman, DM, Dvornik, D: Prevention of urinary albumin excretion in 6 month streptozocin-diabetic rats with the aldose reductase inhibitor Tolrestat. J Diabetic Comp 2:16-18, 1988.

69. Jaspan, J, Maselli, R, Herold, K, Bartkus, C: Treatment of severely painful diabetic neuropathy with an aldose reductase inhibitor: relief of pain and improved somatic and autonomic nerve function. Lancet 2:758-762 1983.

70. Judzewitsch RG, Jaspan JB, Polonsky KS, Weinbert, CR et al: Aldose reductase inhibition improves nerve conduction velocity in diabetic patients New Eng J Med 308:119-125, 1983.

71. Young, RJ., Ewing, DJ., Clarke, BF.: A Controlled trial of sorbinil, an aldose reductase inhibitor, in chronic painful diabetic neuropathy. Diabetes 32:938-942, 1983.

72. Lewin, IG, O'Brian, IAD, Morgan, MH, Corrall, RJM: Clinical and neurophysiological studies with the aldose reductase inhibitor, sorbinil, in symptomatic diabetic neuropathy. Diabetologia 26:445-448, 1984.

73. Hotta, N, Kakuta, H, Kimura, M, Fukasawa, H, Koh,N, Terashima, H, IIda, M, Sakamoto N: Experimental and clinical trial of aldose reductase inhibitor in diabetic neuropathy. Diabetes 34(Suppl 1):98A, 1985.

74. Koglin, L, Clark, C, Ryder, S, Mullane, JF: The results of the long-term open-label administration of ALREDASE in the treatment of diabetic neuropathy. Diabetes 34(Suppl. 1):202A, 1985.

75. Fagius J, Brattberg A, Jameson S, Berne, C: Limited benifits of treatment of diabetic polyneuropathy with an aldose reductase inhibitor: a 24 wseek controlled trial. Diabetologia 28:323-329, 195.

76. Rosenstock J, Challis P, Raskin P: The effect of sorbinil on severe painful diabetic neuropathy (abstr) Diabetes 35(suppl 2):101AA.

77. Sundkvist G, Lilja B, Rosen I, Agardh, C: Autonomic and peripheral nerve function in early diabetic neuropathy. Acta Med Scand 221:445-453, 1987.

78. Martyn CN, Reid W, Young RJ, Ewig DJ, Clarke BF: Six-month treatment with sorbinil in asymptomatic diabetic neuropathy. Failure to improve abnormal nerve function. Diabetes 36:987-990, 1987.

79. Sima AAF, Bril V, Nathaniel V, Thomas AJ, McEwen MS, Brown MB, Lattimer, BS Greene DA: Regenereation and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. New Eng J Med 319:548-555, 1988.

80. Raskin P, Rosenstock J: Aldose reductase inhibitors and diabetic complications. Amer J Med 83:298-306, 1987.