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THE PHARMACOLOGY OF ALDOSE REDUCTASE INHIBITORS[1]

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INTRODUCTION

Despite the advent of life-prolonging insulin for the treatment of diabetes, the appearance and progression of many of the disabling complications associated with this disease cannot be prevented through the administration of insulin. Clinically, the onset and rate of progression of diabetic complications, including cataract, corneal epitheliopathy, microangiopathy, nephropathy, neuropathy, and retinopathy, appear to be dependent upon both the duration and the severity of the diabetes. Moreover, many of the cells in tissues displaying diabetic complications are capable of insulin-independent glucose transport so that the intracellular glucose levels in these cells can mirror blood glucose concentrations, with increased blood glucose levels leading to increased levels of intracellular glucose (1).

The intracellular glucose in turn is utilized primarily for energy production, although it can also be used for special reactions such as the synthesis of polysaccharides, collagen, mucin, or for the glycosylation of proteins. Utilization for glycolysis, however, requires that glucose first be phosphorylated to glucose-6-phosphate by the enzyme hexokinase. Alternatively, glucose can be converted to fructose, which can then undergo glycolysis following phosphorylation by fructokinase. This conversion of glucose to fructose proceeds in two steps through the intermediate sorbitol in what is commonly called the sorbitol or polyol pathway. In the first step of this pathway, aldose reductase (alditol: $NADP^+$ oxidoreductase, EC 1.1.1.21) utilizes NADPH to reduce the aldehyde form of glucose to its corresponding sugar alcohol, sorbitol. In the second step, sorbitol dehydrogenase (1-iditol dehydrogenase, ED 1.1.1.14) utilizes NAD^+ to oxidize sorbitol to fructose.

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$$\text{glucose} + \text{NADPH} \xrightarrow{\text{aldose reductase}} \text{sorbitol} + \text{NADP}^+$$

$$\text{sorbitol} + \text{NAD}^+ \xrightarrow{\text{sorbitol dehydrogenase}} \text{f uctose} + \text{NADH}$$

The sorbitol pathway was first observed in 1956 in the seminal vesicles, where it appears to have a physiological function in the production of fructose for sperm (2). Since then, its presence has been observed in a variety of tissues, including those that display diabetes-associated pathology. In these tissues, aldose reductase can compete directly with hexokinase for the utilization of glucose. The affinity of aldose reductase for glucose, however, is much less than that of hexokinase, so that under normal physiological conditions available glucose is rapidly phosphorylated by hexokinase rather than being reduced to sorbitol by aldose reductase. It is only under nonphysiological conditions, such as in diabetes where the elevated levels of glucose can saturate hexokinase, that a physiologically significant level of sorbitol is produced. Moreover, under these conditions sorbitol can be produced more rapidly than it is converted to fructose, resulting in an accumulation of sorbitol. This accumulation is enhanced by the polar nature of the sugar alcohol, since its polarity prevents facile membrane penetration and subsequent removal through diffusion. The intracellular accumulation of a polar sugar alcohol can thus produce an hyperosmotic effect, which results in an infusion of fluid to counteract the osmotic gradient produced. This fluid influx has been observed to lead to membrane permeability changes and the onset of cellular pathology (3).

In addition to reducing glucose, aldose reductase possesses broad substrate specificity, with the ability to reduce a variety of aromatic and aliphatic aldehydes, including the aldoses galactose, xylose, and arabinose (4–8). Moreover, the enzyme displays a greater affinity for galactose than for glucose, so that the increased levels of galactose are more readily reduced to dulcitol (galactitol) than glucose to sorbitol. The dulcitol is not further oxidized by sorbitol dehydrogenase, so that the intracellular levels of this polar sugar alcohol remain elevated.

$$\text{galactose} + \text{NADPH} \xrightarrow{\text{aldose reductase}} \text{dulcitol} + \text{NADP}^+$$

$$\text{dulcitol} + \text{NAD}^+ \xrightarrow{\text{sorbitol dehydrogenase}} \text{NR}$$

The increased intracellular levels of galactose can result in a more rapid and greater hyperosmotic effect than that from glucose. Tissues accumulating dulcitol develop cellular pathology similar to that observed in diabetic tissues. Therefore, through the use of galactosemic and diabetic animal studies, evidence linking the aldose reductase–initiated accumulation of sugar alcohols with the pathogenesis of diabetic complications is rapidly increasing. These observations suggest that the inhibition of aldose reductase represents a novel,

potentially direct pharmacological approach toward the treatment of certain diabetic complications—an approach distinct from the improved control of blood sugar levels. This novel approach has evolved from studies on the mechanisms of diabetic cataract formation.

ALDOSE REDUCTASE IN DIABETIC CATARACTS

Diabetic cataracts have been extensively studied since the 1930s, when it was demonstrated that cataracts could easily be produced in rats either by the removal of the pancreas or through the destruction of pancreatic beta cells by the injection of alloxan or streptozotocin. Despite intensive research, however, it has taken over 50 years to elucidate the mechanism of cataract formation in diabetic animals. The solution to the question of diabetic cataract formation had its beginning in 1959, when van Heyningen found the presence of sorbitol in the lenses of diabetic rats, indicating that the enzyme aldose reductase was functioning in the lens (9). Prior to van Heyningen's finding, it was thought that the sorbitol pathway primarily functioned in certain reproductive tissues; however, this finding spurred others to uncover aldose reductase in other tissues, especially those tissues affected by diabetes.

The lenticular accumulation of sorbitol, which was known to poorly penetrate biological membranes, immediately became suspect as the cause of osmotic changes observed in histopathological studies of diabetic lenses (10). These studies had revealed that the earliest visible change was the appearance of swollen lens fibers caused by an increase in lens hydration. The swollen lens fibers eventually ruptured, with liquifaction of the fibers resulting in vacuole formation. Thus, sorbitol formation within the lens cells created a hypertonicity that was corrected by an influx of water to maintain isotonicity with the environment. This, as events were to prove later, is the initiating step in the development of the diabetic cataract.

The argument for polyol-initiated osmotic changes was strengthened by the identification of dulcitol in the lenses of galactosemic rats and by studies that indicated that excess galactose feeding of rats could induce cataracts histologically similar to diabetic cataracts. Moreover, from the more favorable substrate affinity of galactose and the lack of metabolism of dulcitol by sorbitol dehydrogenase, one could predict that the lens polyol level should be higher and the onset of cataract earlier than in the diabetic rat. This was indeed the case with polyol levels of 72 mmol per Kg of lens found in the 50% galactose–fed rats, at least twice the level found in diabetic lenses (11). The cataracts also appear earlier in the galactosemic rats, with the dense nuclear cataract occurring after three weeks of the galactosemic state compared to two months for the diabetic rat (11). Thus, the galactose model is much more convenient to study the details of aldose reductase–initiated cataract formation. Moreover, if aldose reductase

is involved in the development of other diabetic complications, then they should be reproducible in the galactosemic state as well.

Osmotic Hypothesis

The sequence of events leading to the formation of diabetic cataract is summarized in Figure 1 (3). Although the K_m for glucose is high, so that at ambient levels of glucose very little sorbitol is formed, the availability of high glucose levels in diabetes in a sense activates aldose reductase to produce substantial amounts of sorbitol, some of which is converted to fructose. 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 the surrounding intraocular fluids. Thus, 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 changes 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 cataract.

Protein synthesis also ceases, even in the early stages of these sugar cataracts (12). Normally, there is continual growth of the lens, due to new fibers being laid down from the elongation of epithelial cells at the lens equator. Concomitant with this growth is the synthesis of new lens proteins. However, in these cataracts growth of the lens is retarded as protein synthesis is decreased. Apparently the potassium-sodium ratio governs the degrees of protein synthesis. In the lens the normally high K^+/Na^+ environment is conducive to protein synthesis. However, in these sugar cataracts, where the intracellular concentration of K^+ is lowered and Na^+ is increased, protein synthesis is depressed. If the cation concentrations are returned to near normal, as in the reversal of galactose cataract, protein synthesis also returns to normal (12). The control of lens protein synthesis by electrolytes appears to be a general phenomenon, since other osmotic cataracts have shown defects in protein synthesis similar to those found in sugar cataracts (13, 14).

The fact that all the secondary changes shown in Figure 1 are related to the osmotic event has been verified by in vitro lens culture studies. By this technique, lenses can be maintained in a control medium with normal glucose concentrations while their contralateral lenses are placed in a medium containing high levels of glucose, simulating the hyperglycemic state (15–17). The lenses in the control medium remain clear and transparent while the lenses exposed to high sugar gain water, become cloudy and develop vacuoles at the

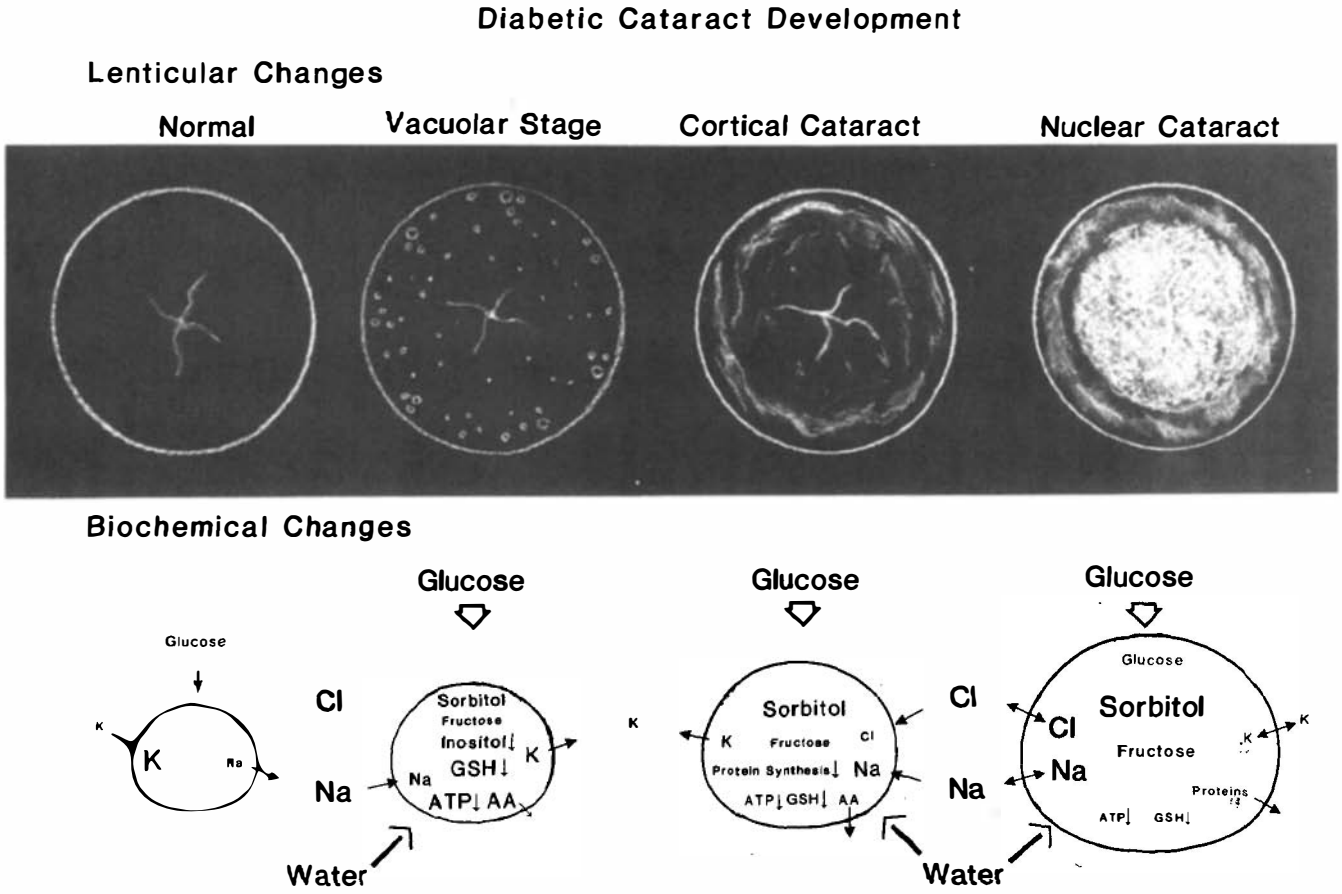


Figure 1 Summary of changes occurring in the rat lens during sugar cataract formation. K indicates potassium; Na, sodium; Cl, chloride; GSH, reduced glutathione; AA, amino acids; ATP, adenosine-5'-triphosphate.

equator region, just as do the lenses in diabetic animals. By this approach, a quantitative relationship showing that sorbitol accumulation is paralleled by an increase in lens hydration was clearly established, and biochemical changes related to the osmotic effect were observed. These biochemical changes can be prevented by preventing the lens swelling from occurring despite the accumulation of polyol by adding an amount of sorbitol to the medium equivalent to that formed in the lens. Under these conditions, normal lens volume was maintained despite the accumulation of sorbitol, and the biochemical parameters remained normal so that no changes in the levels of electrolytes, amino acids, glutathione, or inositol could be observed.

Aldose Reductase Inhibitors

Although biochemical and in vitro culture studies indicated that the aldose reductase–initiated accumulation of polyols induces lens swelling, which in turn results in other changes leading to cataract formation, the most convincing evidence for aldose reductase's role in diabetic cataracts has emerged from studies with aldose reductase inhibitors (3). These studies, which indicate that inhibition of aldose reductase can successfully prevent the onset of cataract, have led to the development of a variety of structurally diverse inhibitors. Through their use, evidence for the involvement of aldose reductase in other serious diabetic complications has evolved (18).

Initial studies in the late 1960s indicated that long-chain fatty acids could inhibit aldose reductase in lens homogenates (7). This led to the development of tetramethylene glutaric acid (TMG), the first inhibitor to reveal that the cataractous process could be altered by modifying the activity of aldose reductase (3, 19). Lens culture studies in which the lens was incubated in a high-galactose medium revealed that TMG is effective in blocking dulcitol synthesis and accumulation, that it minimizes increases in lens hydration, and that it prevents the appearance of vacuoles. Its inability to penetrate membranes, however, made the compound ineffective in vivo. The first in vivo active inhibitor was N-(3-nitro-2-pyridyl)-3-trifluoromethyl-aniline (AY-20,263) (3). Significant solubility problems were encountered with this compound, which was administered by intraocular injection as a dimethylsulfoxide solution. This was shortly followed by the development of the water-soluble inhibitor alrestatin (3-dioxo-1-*H*-benz[de]isoquinoline-2(3*H*)-acetic acid, AY-22,284) (20). This compound, when administered orally to galactosemic rats, delays the appearance of the nuclear opacity. Since alrestatin, the development of a number of more potent organic acids as aldose reductase inhibitors has been pursued. From these studies several compounds, including 3-(4-bromo-2-fluorobenzyl-4-oxo-3*H*-phthalazine-1-ylacetic acid (ICI 128,436), N-[(5-(trifluoromethyl)-6-methoxy-1-naphthalenyl)thioxomethyl]-N-methylglycine (tolrestat, AY 22,773) and (E)-5-[(E)-2-methyl-3-phenylpropenylidene] rhodanine-3-acetic

acid (ONO 2235) have evolved as clinical trial candidates (21–23). By the mid-1970s a number of flavonoids were observed to have aldose reductase–inhibitory activity (24). These included quercetin [2-(3',4'-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4*H*-chromen] and its 3-rhamnoside quercitrin. Solubility problems were also encountered in the evaluation of certain flavonoids; however, replacement of the aromatic 2-phenyl substituents with a nonaromatic carboxyl group increased the water solubility of these inhibitors (25). Moreover, this indicated that the 4-oxo-4*H*-chromen ring system of flavonoids appeared to be necessary for inhibitory activity. Because of the structural similarities of the 2-chromone carboxylic acid with the antiallergy agonist disodium cromoglycate, examination of a variety of other classes of antiallergy agonists have been pursued. These led to the observation that many antiallergy compounds that contained the chromone ring system or analogs of this system, including quinolones, coumarins, chalcones, fluorenones, xanthones, 11-oxo-11*H*-pyrido[2,1*b*]quinazoline-8-carboxylic acids, 1,6-dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic acids, and oxazolidines, could also inhibit aldose reductase (26). The chroman ring has also been combined with a hydantoin ring to form the potent inhibitor sorbinil (S-6-fluoro-spirochroman-4-4'-imidazolidine-2',5'-dione,CP45,634) (27). This compound was the first in vivo effective oral inhibitor that could prevent the entire cataractogenic process when administered to either galactosemic or diabetic rats (28). Moreover, through its use relationships between aldose reductase and a variety of other diabetic complications has been uncovered, so that today sorbinil may be considered a benchmark by which the potency and effectiveness of other aldose reductase inhibitors are measured. Sorbinil in turn has led to the development of several other spirohydantoins and related thiazolidine-2,4-dione and oxazolidine-2,4-dione analogs (29–32).

Although aldose reductase inhibitors appear to be diverse, certain similarities can be seen among them. Both kinetic and competition studies with purified enzyme reveal that the inhibitors interact with aldose reductase at a common site independent of either the substrate or the nucleotide cofactor fold. This site appears to be stereospecific and contains a nucleophilic residue that can reversibly interact with the inhibitors (33, 34). The steric requirements of this site may vary with enzymes from different tissues and species. Through the use of computer modeling and molecular orbital calculations, steric and electronic similarities among the inhibitors have also become apparent (33). By superimposing specific aromatic residues common to most inhibitors, a common molecular conglomerate can be formed that consists of a generally planar structure with two aromatic (hydrophobic) regions and a common carbonyl region susceptible to reversible nucleophilic attack. From this conglomerate, a schematic inhibitor site model has been postulated. Inhibitor attachment to this site results from a combination of hydrophobic bonding and a reversible

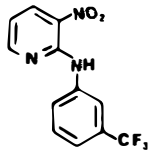
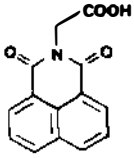
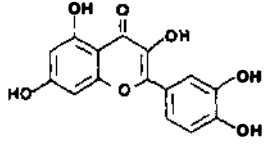
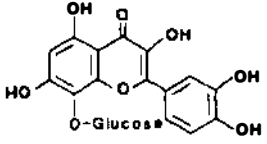
charge-transfer reaction between the nucleophilic residue and the reactive carbonyl moiety. Thus, the inhibitory potency of a compound would be expected to increase either by the addition of selective lipophilic substituents that could through enhanced hydrophobic bonding increase its affinity for the inhibitor site or by the addition of groups that could more readily undergo nucleophilic attack (e.g. thiocarbonyl versus carbonyl). In addition, hydrogen bonding sites located near the two lipophilic regions can also help to orient the inhibitor onto this site. For this orientation several selective hydroxyl groups appear to be required that correspond to regions encompassed by the 7-position on the 4-oxo-4*H*-chromen ring and the 2-(4'-hydroxyphenyl) position. From this model the pharmacophor requirements for an aldose reductase inhibitor have been defined.

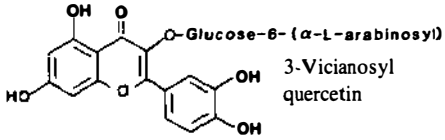
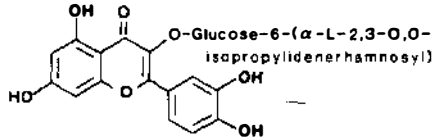
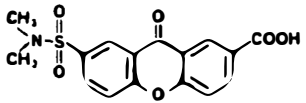
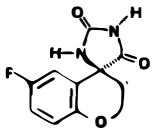
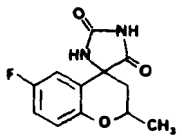
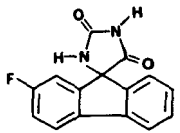
For the in vitro analyses of these inhibitors, aldose reductase from a variety of different sources and species have been employed, including bovine, rabbit, rat, dog, and human lens, human placenta, and the Engelbreth-Holm-Swarm (EHS) tumor cell line (35–40). Although kinetic studies indicate that aldose reductases from different species and tissues display similar substrate affinities, differences in the susceptibility to inhibition of aldose reductases from various sources have been observed through the use of these structurally diverse inhibitors. These studies reveal no specific trends; human placental aldose reductase is generally less susceptible to inhibition, and increasing certain steric bulk on the inhibitors makes the human placental enzyme less susceptible to inhibition than enzyme from other sources. The inhibitory susceptibility of isolated aldose reductase can also change with enzyme purification; highly purified enzyme often is less susceptible to inhibition (41). These studies suggest that no universally potent inhibitor currently exists.

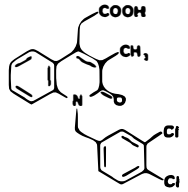
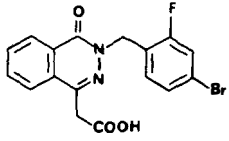
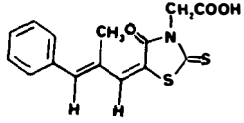
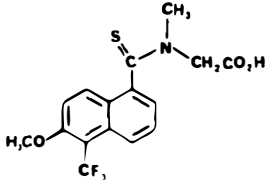
A number of in vivo studies on the effect of aldose reductase inhibitors on the progression of diabetic or galactosemic rats have also been reported (Table 1). These studies, which employed various routes of administration, conclude that the ability of the inhibitor to delay or prevent the onset of cataract formation is proportional to the inhibitory potency of the drug. Their potency can also be gauged by their effectiveness in offsetting the effects produced by diets containing increasing amounts of galactose, with effectiveness against a 50% galactose diet considered the acid test for inhibitory potency.

From the studies illustrated in Table 1, it is very evident that the cataractous process can be prevented if an aldose reductase inhibitor potent enough to completely block polyol formation is administered at the onset of hyperglycemia or hypergalactosemia. However, can an aldose reductase inhibitor reverse the cataractous process once the process gets underway? This question was addressed in galactosemic rat studies, where it was shown that, when rats are fed a diet of 50% galactose, the cataractous process can be reversed only when galactose is withdrawn from the diet six days after its inception (42).

Table 1 In vivo effects of aldose reductase inhibitors on rat sugar cataracts

Inhibitor		Cataract type	Dose	Effect	Reference
Structure	Name				
	AY 20,263	Galactose (50% diet)	Intravitreal injection 0.8 mg in 10 μl DMSO	Eighteen-day delay of nuclear opacity	3
	AY 22,284 Alrestatin	Galactose (30% diet) Galactose (50% diet)	0.96 g/kg/day oral 10% topical 2×/day	80% delay of cataract after 29 days Seven-day delay of nuclear opacity	20 98
	Quercetin	Galactose (50% diet)	Oral, 2.5% of diet	After 12 days lens fiber integrity and growth preserved	99
	Gossypin	Galactose (30% diet)	15 mg/kg oral	60% delay of cataract after 28 days	100

	3-Vicianosyl quercetin	Galactose (20% diet)	100 mg/kg/day ip 4 days after start of diet	28% decrease at nine days of ophthalmoscopic ring opacities	101
	—	Galactose (20% diet)	100 mg/kg/day ip 4 days after start of diet	38% decrease at nine days of ophthalmoscopic ring opacities	101
	RS 7535	Galactose (35% diet)	2% topical 2×/day	30% delay of bilateral cataract after 29 days	102
	CP 45,634 Sorbinil	Diabetic	60 mg/kg/day oral	No lens change after six months	103
		Galactose (50% diet)	60 mg/kg/day oral	No lens change after eight months	28
	M 79175	Galactose (30% diet)	1 mg/kg po/day	No opacity after 33 days	29
	AI 1567	Galactose (30% diet)	4 mg/kg/day oral	No opacity after 32 days	30

	ICI 105,552	Diabetic	50 mg/kg/day intubation	170 day delay for 50% of lenses with greater than punctate opacities	104
	ICI 128,436	Diabetic	25 mg/kg/day oral	No lens change after 74 days	21
	ONO 2235	Diabetic	50 mg/kg/day oral	Significant decrease in lens sorbitol after five months	77
	AY 27,773 Tolrestat	Galactose (50% diet)	56 mg/kg/day oral	No opacities after nine months	See footnote a below

[a]D. Dvornik, personal communication

After six days on the galactose diet, reversal of the cataractous process is not possible, with the vacuolar stage continuing to the dense nuclear cataract even though the rats are on a normal diet. A similar point of no return has been observed with sorbinil, which can reverse the appearance of cataract in the galactose-fed rats as long as it is administered prior to the sixth day of galactose feeding. The reversal of the cataract with sorbinil has been accomplished despite the continuation of the galactose diet. However, when sorbinil is applied after the sixth day the cataractous process continues, leading to opacification of the lens.

Human Diabetic Cataracts

The appearance of true diabetic cataracts seems to occur only rarely in young diabetics. In older individuals, however, diabetes hastens cataracts. Evidence for this clinical impression was provided by an epidemiological study that clearly demonstrated that diabetics between 55 and 64 years of age have a three-fold greater risk of developing cataracts than nondiabetics (43). Diabetes, therefore, appears to be one of many factors that contribute to cataract formation.

In the human lens, aldose reductase activity is not as substantial as in the rat lens. As a result, some have claimed that aldose reductase may not be active enough to accumulate sorbitol to levels sufficient for an osmotic effect (44). Analysis of cataracts extracted from diabetic patients, however, reveals that the amount of sorbitol and fructose recovered parallels the level of HbA1C, with the levels of polyol and fructose as high as 19 mmol per Kg per lens (45, 46). If these products of the sorbitol pathway are confined to the regions of the lens where aldose reductase is found, polyol may have an osmotic effect.

OTHER OCULAR DIABETIC COMPLICATIONS

Cornea

Diabetic corneal effects were unknown prior to the advent of vitrectomy, a surgical procedure used to remove blood or tissues obscuring the path of light onto the retina from the vitreous. Following insertion of the vitrectomy instrument into the eye, the corneal epithelium of diabetics often becomes cloudy, obscuring the vision of the surgeon who must view the surgical procedures through the patient's cornea. Consequently, the epithelium must be removed to permit surgery. Under normal circumstances, stripping the corneal epithelium does not present a problem because reepithelialization occurs rapidly. In diabetic patients, however, it was found that a delay in epithelial regeneration and persistent epithelial defects were serious post-surgical complications.

A similar delay in the reepithelialization of denuded corneas has been

reproduced in diabetic and galactosemic rats (11, 47, 48). Moreover, the reepithelialized corneas of both diabetic and galactosemic rats appeared hazy and edematous, while the corneas stripped of their epithelium were characterized by a swelling of the stroma and marked invasion of polymorphonuclear leucocytes. Treatment with a number of aldose reductase inhibitors completely abolished the delay in the rate of reepithelialization and resulted in resurfaced corneas that were clear and transparent (11, 48). Histologically, the epithelia of the inhibitor–treated corneas also appeared healthier, multilayered, and thicker than those of untreated diabetic or galactosemic corneas, with leucocytes appearing less prominently in the treated ones.

Clinically, the aldose reductase inhibitor sorbinil has been employed in an interesting case of corneal keratopathy involving a 24-year-old female diabetic whose diabetes was poorly controlled (49). Following laser treatment for retinopathy, spontaneous bilateral corneal erosion developed. Since conventional treatment was ineffective, a single case protocol calling for the use of sorbinil eye drops was drafted and a single masked study was arranged in which one eye was treated with sorbinil while the other was given placebo eye drops. The sorbinil-treated eye slowly responded and the inflammatory signs and epithelial defects disappeared, while the placebo-treated eye worsened and eventually perforated, requiring an emergency corneal transplant. The keratoplast appeared successful until a few days after the operation, when the cornea developed the characteristic diabetic defects. At this point this eye was also treated with sorbinil. It responded favorably, and after a year both eyes appear stable.

The presence of aldose reductase in the corneal and conjunctival epithelium has been demonstrated by the immunoperoxidase method, which shows aldose reductase to be particularly rich in the basal layer of cells (50). Moreover, the fact that the delay of reepithelialization can be reproduced in the galactosemic as well as the diabetic rats strongly suggests the involvement of aldose reductase in diabetic corneal epitheliopathy. These observations, combined with the fact that aldose reductase inhibitors produce beneficial effects, indicate that aldose reductase is involved in diabetic abnormalities of the epithelium when the cornea is stressed in vitrectomy or when it is compelled to regenerate the epithelial layer.

Retina

Diabetic retinopathy, one of the leading causes of blindness, increases in prevalence with the duration of diabetes. Among the characteristics of early nonproliferative retinopathy are vascular changes of the retinal capillary bed, with the formation of microaneurysms, exudates, and small intraretinal hemorrhages. The hallmark of early retinopathy is the selective loss of retinal

capillary pericytes (mural cells) versus endothelial cells; however, electroretinogram (ERG) pattern changes, color vision shifts, and capillary basement membrane thickening also have been observed (51).

Currently, only secondary evidence links aldose reductase with diabetic retinopathy. Histochemically, this enzyme has been localized in all human retinal regions displaying diabetic pathology. These include the pericytes of retinal capillaries, the Mueller cells, ganglion cells, and selective cone cells (50, 52, 53).

The first evidence suggesting the pathological involvement of aldose reductase in diabetic retinopathy, especially pericyte loss, evolved from studies with isolated monkey retinal capillary cells (54). Endothelial cells cultured in high glucose medium remained viable, while pericytes cultured in high glucose showed a threefold increase in sorbitol and cellular degeneration. However, rapid progress in this area has been hampered by the lack of a convenient, short-term animal model capable of displaying human-like retinal pathology, especially the selective loss of pericytes.

In streptozotocin diabetic rats, increased sorbitol levels have been detected in the retina and these increased levels have been reduced by the administration of the aldose reductase inhibitor 1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid (ICI 105552) (55). Retinal capillary basement membrane thickening, which has been observed to occur in both diabetic and galactosemic rats (see below), can also be controlled by the administration of aldose reductase inhibitors (56, 57). Through histochemistry, aldose reductase has been localized in the Mueller cells, ganglion cells, and pericytes of rat retinas (58, 59). The formation of pericyte ghosts as observed in human retinopathy, however, has not been equivocably demonstrated in either diabetic or galactosemic rats (57).

Long-term studies with alloxan diabetic dogs indicate that these animals are unique in developing retinopathy with a selective loss of pericytes demonstrable after 60 months of diabetes (60). Recently, an identical retinopathy, which includes the presence of retinal capillary aneurysms, hemorrhages, exudates, pericyte ghosts, and capillary basement membrane thickening, has been demonstrated to occur in dogs after 32 months of 30% galactose feeding (61). Increased polyol levels have also been detected in isolated canine retinal vessels cultured with high levels of either glucose or galactose (62). This formation of polyols was reduced by the addition of the aldose reductase inhibitor sorbinil to the culture medium. Upon histochemical examination of isolated canine retinal capillaries with antibodies prepared against purified dog lens aldose reductase, the presence of aldose reductase could only be detected in the pericytes (37).

These canine studies clearly suggest the involvement of aldose reductase in

the pathogenesis of diabetic retinopathy. Confirmation, however, must wait until current long-term galactose-feeding studies with aldose reductase inhibitors are complete.

DIABETIC NEUROPATHY

A majority of all diabetics are afflicted to some degree with neuropathy. Diabetic neuropathy may express itself in many ways, ranging from subtle changes in nerve conduction velocity and axoplasmic flow to complete loss of various neurons and a myriad of clinical manifestations, which can include some of the most disabling complications of long-term diabetes mellitus (63). The role of aldose reductase in the pathogenesis of this diabetic complication has been established through basic laboratory and animal studies as well as through clinical trials. Aldose reductase has been localized to the Schwann cells of the myelin sheath, and the accumulation of sorbitol in these cells may result in osmotic swelling similar to that observed in the lens (64, 65). In vitro incubation of rat sciatic nerves in high glucose medium has been demonstrated to result in the accumulation of sorbitol, and this accumulation can be prevented by the administration of a variety of aldose reductase inhibitors, including alrestatin, sulindac, and sorbinil (65, 66). In streptozotocin diabetic rats, nerve sorbitol levels have been observed to increase within three days following induction of diabetes (67). This increase in the nerve sorbitol concentration was accompanied by significant decreases in the motor nerve conduction velocities of the sciatic nerves, whether the duration of diabetes was two, five, or nine months (68). The accumulation of sorbitol was therefore associated with impairments of nerve conduction velocities, which are among the earliest and most easily quantifiable signs of diabetic neuropathy. Treatment for four weeks with the potent aldose reductase inhibitor sorbinil reduced the sorbitol concentration in the sciatic nerve and increased motor nerve conduction to normal levels, even though high blood glucose persisted.

Similar neurological changes have been observed in galactosemic animals, with nerve swelling in a galactosemic model similar to that reported in streptozotocin-induced diabetic rats (69). The swollen sciatic nerves of galactosemic rats have been shown to contain increased polyol (dulcitol) concentrations and increased water content, concomitant with decreased nerve conduction velocity. These changes can be reversed either by removal of the rats from the galactose diet or by the administration of aldose reductase inhibitors, suggesting that the water content and nerve conduction velocity are related to the polyol pathway. In rats fed a 50% galactose diet for forty-four weeks, the sciatic nerves are decidedly swollen compared to those of rats on a normal,

control diet (35% increase in diameter) (70). Nerve swelling has not been found in rats receiving the galactose diet containing the inhibitor sorbinil.

In addition to nerve conduction velocity, the axonal transport of choline acetyltranferase has been demonstrated to decrease in either diabetic or galactosemic rats (71). This decreased axonal transport, another early sign of diabetic neuropathy, can be reversed through the use of aldose reductase inhibitors. Impaired orthograde axonal transport of choline acetyltransferase has been demonstrated by ligature of the sciatic nerve in diabetic rats and has been reversed by treatment with ICI 105,552.

Several clinical trials with aldose reductase inhibitors have been reported. Early trials with alrestatin on long-term diabetics suggested qualitatively that treatment could improve peripheral nerve function and the relief of pain (72, 73). Recent reports suggest that within a few days of treatment with the more potent inhibitor sorbinil, several patients with severely painful diabetic neuropathy experienced decreased pain, improved sensory perception, increased muscle strength, and normalization of nerve conduction velocities (74, 75).

In an effort to quantitate the effects of the aldose reductase inhibitor sorbinil on neuropathy, a multicenter, randomized, doubly masked crossover trial was undertaken (76). Following a six-week baseline study, patients chosen for good blood glucose control were randomized as to treatment received. Half received 250 mg sorbinil per day for nine weeks while the other half received placebo for nine weeks; then the treatments were reversed for a second nine-week period. Finally, all patients received placebo treatment for another three weeks to allow a masked assessment of drug washout for each patient. In order to evaluate the effect of the drug, nerve conduction velocities of peroneal motor nerve, median motor nerve, and median sensory nerve were measured at three-week intervals throughout the twenty-seven-week study. As expected, during their placebo treatment, whether this was received first or last, all patients exhibited decreased nerve conduction velocities in all three nerves. During treatment with sorbinil, however, the nerve conduction velocities of all three nerves rose significantly. Conduction decreased again during the three-week sorbinil washout.

The apparent initially positive clinical results of sorbinil further strengthen the link between the polyol pathway and the neuropathy of diabetes mellitus. Successful utilization of several unrelated inhibitors of aldose reductase has demonstrated the improbability of nonspecific inhibition of the polyol pathway. Moreover, the possibility that the hydantoin entity common to many of the aldose reductase inhibitors might affect mainly the central nervous system, having only indirect effects on the polyol pathway, has been eliminated by work with several hydantoin-free compounds such as the 1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid (ICI

105552) and (E)-5-[(E)-2-methyl-3-phenylpropenylidene]rhodanine-3-acetic acid (ONO 2235) (71, 77).

DIABETIC ANGIOPATHY

Capillary Basement Membrane Thickening

A striking morphological change occurring consistently in all tissues of diabetics and animal models of diabetes is the frank thickening of capillary basement membranes (78). These so-called membranes are thin, extracellular matrices consisting of a unique type IV collagen bound to varying degrees with several proteoglycans, glycoproteins, and an amyloid (79). They form enveloping sheaths that surround the capillary and separate the pericytes and endothelial cells of the capillary wall from adjacent tissues. Pathophysiologically, capillary basement membrane thickening has been considered the fundamental structural lesion of the small blood vessels in diabetic patients and the ultrastructural hallmark of diabetic microangiopathy (78, 80). It is believed to be involved in several diabetic complications, including both diabetic retinopathy and nephropathy, since the basement membranes of both retinal capillaries and kidney glomerular capillaries thicken progressively in diabetics (80, 81). Failure of glomerular filtration is accompanied by massive accumulations of basement membrane material surrounding the endothelial cells and pericytes (masangial cells) of the capillaries, leaving little surface area for filtration to occur. Basement membrane thickness in capillaries of muscle biopsies has been utilized as a sign of microangiopathy in asymptomatic diabetic patients (82).

An understanding of the pathogenesis of capillary basement membrane thickening in diabetics has been complicated because factors other than abnormal carbohydrate metabolism can contribute to basement membrane thickening (78). These include hypertension and aging. Moreover, many of the results obtained have been controversial due to the measurement methodologies employed (78, 83). However, a possible breakthrough in the study of basement membrane thickening has come from studies using precise, reproducible computer planimetry techniques that indicate that galactosemic animals can form thickened capillary basement membranes that appear to be ultrastructurally similar to those of diabetics (56). Basement membranes from galactosemic and diabetic rats and dogs contain fibrous collagen with banding patterns, clear vacuoles, and areas of irregular thicknesses and multilaminar composition, all of which are seen seldom in controls (56, 57, 61).

In rats fed from weaning a diet containing 50% galactose, a 57% thickening of capillary basement membranes in the outer plexiform layer of the retina was observed after 28 weeks, and a twofold thickening after 44 weeks, compared to controls fed a normal diet (56). This thickening was prevented in another

galactose-fed group by the concomitant daily administration of .04% sorbinil mixed into the diet (56). Similar results were obtained with the structurally unrelated aldose reductase inhibitor tolrestat at both .03% and .04% levels in the diet. Rats fed a 30% galactose diet also developed significant thickening of retinal capillary basement membranes after 15 to 21 months that was more pronounced in hypertensive than in normotensive rats (57). This thickening was also prevented by sorbinil. Significant retinal capillary basement membrane thickening has also been reported in rats two months after the induction of diabetes with streptozotocin (84). This thickening was prevented by treatment with the inhibitor dl-spiro-(2-fluorofluoren-9'4'-imidazolidine)-2'5'-dione (AL 1567).

It is known that basement membrane thickening can result from high serum hexose levels, and the prevention of thickening by these diverse aldose reductase inhibitors suggests that aldose reductase may play a role in the biochemical mechanism leading to the excessive formation of basement membranes. This possibility is currently being investigated in the Engelbreth-Holm-Swarm (EHS) tumor, a tissue that produces relatively large quantities of basement membrane (85). When grown in galactosemic mice, these tumor cells can accumulate dulcitol (40). Moreover, enzyme studies indicate the presence of an apparent aldose reductase in this tumor tissue that can be inhibited by a variety of aldose reductase inhibitors.

The observation that diabetes-like basement membrane thickening occurs in galactosemic animals, combined with the fact that aldose reductase inhibitors can prevent this thickening in either galactosemic or diabetic animals, strongly suggests that aldose reductase may regulate basement membrane thickening, although the mechanism remains unknown. While the regulation of blood sugar levels has been known to mediate basement membrane thickness, the prevention of thickening by inhibition of aldose reductase represents a novel approach toward the regulation of basement membrane thickness, an approach independent of blood sugar control.

Erythrocytes and Platelets

Among the factors contributing to diabetic angiopathy are altered blood flow characteristics, including enhanced erythrocyte aggregation, increased plasma viscosity, increased resistance to blood flow, and increased platelet aggregation (1). Human erythrocyte sorbitol levels in insulin-dependent diabetics have been clearly demonstrated to be above those of nondiabetics after an eight-hour fast (86). Statistically significant correlations have also been observed between the levels of red cell sorbitol and erythrocyte deformability measured as a filtration index. In diabetics this factor is substantially diminished (87). In vitro culture of intact human erythrocytes in high glucose medium also results in the increased intracellular accumulation of sorbitol, and this accumulation of

sorbitol can be inhibited by either tetramethylene glutaric acid or sorbinil (86). Moreover, the sorbitol levels in red blood cells of diabetic rats have been shown to be directly related to nerve sorbitol levels (88). Both red blood cells and nerves appear to be equally susceptible to inhibition by the aldose reductase inhibitor sorbinil. These results clearly demonstrate the presence of aldose reductase in the red blood cell. Although no beneficial effect has been reported to result from the inhibition of red blood cell aldose reductase, measuring its levels has become a convenient method for monitoring the plasma levels of aldose reductase inhibitor in clinical trials (88).

Sorbitol is also present in the platelets of diabetics. The accumulation of sorbitol has been demonstrated in human platelets incubated in high glucose medium, and this accumulation can be inhibited with the aldose reductase inhibitor alrestatin (89). However, no correlation between platelet aggregation and sorbitol accumulation has been reported. Therefore, it has been concluded that the sorbitol accumulation in platelets is not responsible for the abnormal platelet formation or morphology observed in diabetes.

DIABETIC NEPHROPATHY

Loss of renal function associated with diabetic nephropathy leads to death in about half of all insulin-dependent diabetics. Diabetic changes of the kidney generally involve alterations of the glomerular capillaries and associated arterioles, which lead to changes in filtration, proteinuria, and eventually impaired renal failure. Clinically, the earliest feature of diabetic nephropathy is symptomless proteinuria (1, 80).

Currently, little evidence implicating aldose reductase in the pathogenesis of diabetic nephropathy exists. The renal presence of aldose reductase has been demonstrated in the interstitial cells, Henle's loop, and collecting tubules of the dog; in Henle's loop, collecting tubules, and glomerular podocytes of the rat; and in the glomeruli of the human (59, 90, 91). The polyol pathway has also been observed to be present in cultured monkey kidney epithelial cells, which accumulate sorbitol upon culture in a high glucose medium (92). Moreover, the addition of either of the aldose reductase inhibitors tetramethylene glutaric acid, 1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid (ICI 105552), 7-0-(β-hydroxyethyl)quercetin, or 5,7,3',4,'-tetra-0-(β-hydroxyrutin) to the culture medium results in the decreased formation of sorbitol (93).

However, some implications of a potential role for aldose reductase in nephropathy have recently emerged. Consistently higher kidney wet weights have been observed in rats fed a 50% galactose diet for 25 days, than in those fed a normal diet, despite the fact that the body weights of the galactosemic rats were 55% lower (94). Differences in the wet to dry weight ratios suggest that

the apparent hypertrophy involved both increased fluid content and renal mass. Furthermore, this apparent hypertrophy has been prevented by concomitant treatment of the galactose-fed rats with the aldose reductase inhibitor sorbinil. Increased levels of sorbitol have also been observed in isolated glomeruli from streptozotocin diabetic rats, and the level of sorbitol in the glomeruli from similar rats treated with sorbinil has also been reduced (95). Unique protein pattern changes in the urine of these diabetic rats can also be detected with the prolonged onset of diabetes (96). These changes, suggestive of proteinuria, were diminished by sorbinil treatment.

These preliminary observations, suggesting potential beneficial effects of the aldose reductase inhibitor sorbinil for the treatment of nephropathy, should stimulate further work in establishing the relationships between aldose reductase and the pathogenesis of diabetic nephropathy.

CONCLUSION

Work on the role of aldose reductase in diabetic complications has progressed from initial studies limited to cataracts to current studies of virtually all tissues that display diabetic pathology. For these studies, recently developed potent aldose reductase inhibitors used on appropriate diabetic and galactosemic animal models have provided powerful tools for elucidating the relationship between aldose reductase and diabetic complications. Inhibition of aldose reductase has been demonstrated to prevent the onset of cataract, to reverse problems in the reepithelialization of denuded corneas, to reverse decreases in both nerve conduction velocity and axonal transport, and to prevent retinal capillary basement membrane thickening. Evidence for the involvement of aldose reductase in retinopathy and possibly nephropathy is also mounting. Results suggest that in order to be effective, aldose reductase inhibitors must be used at the onset or during very early stages of diabetes. Moreover, while the physiological role of this enzyme remains unknown, no significant adverse effects have been reported from long-term aldose reductase inhibition in rats or dogs. These studies have provided the basis for several clinical trials that will eventually determine the effect of aldose reductase inhibition on diabetic man.

The aldose reductase–initiated intracellular accumulation of polyols has been shown to result in an hyperosmotic effect on either the lens or nerve. However, the possibility that aldose reductase has adverse effects other than osmotic changes must also be considered. Recent nuclear magnetic resonance (NMR) studies have revealed that the flux of glucose through the sorbitol pathway appears to be more substantial than indicated by the levels of polyols observed (97). If this is the case, then the amount of NADPH utilized may be substantial and it may be diverted from reactions in which it is normally used. This deflection of the cofactor to the aldose reductase reaction can result in

adverse metabolic consequences. Another interesting observation in all tissues displaying diabetic complications in which aldose reductase is thought to be involved is the inverse relationship between the levels of sorbitol and myo-inositol. As the sorbitol levels increase in these tissues, the inositol levels decrease. Inositol loss in the lens appears to be due to leakage; however, other possibilities may also exist. Except for experimental diabetic cataracts, the exact mechanism by which aldose reductase is involved in the diabetic complication needs to be clarified.

Until then, we propose that certain guidelines be established in linking aldose reductase to diabetic complications: (*a*) aldose reductase should be present in the tissue in question; (*b*) the complication should occur in the galactosemic as well as in the diabetic state; (*c*) the complication should occur earlier and be more severe in galactosemia than in diabetes; (*d*) aldose reductase inhibitor should prevent or delay the appearance of the complication; and (*e*) more than one aldose reductase inhibitor should be effective.

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