Nonosmotic Diabetic Cataracts

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ABSTRACT. It has been suggested that sugar cataracts associated with diabetes mellitus result from the accumulation of excess sorbitol within lens fibrils. Swelling of lens fibrils occurs when water moves in to maintain osmotic balance; the excess water causes disruption of fibrils and cataract formation. Other studies have indicated that more than sorbitol-induced osmotic stress is involved. Our study used lenses collected from rats after 21 or 44 d of streptozotocin diabetes. Cataracts formed in untreated 44-d streptozotocin diabetic rats, but were not apparent in the 21-d untreated diabetic animals. Lens sorbitol increased in the diabetic animals both before and after cataract formation. Lens taurine varied inversely with the sorbitol content in a fashion that resulted in no net change in total lens osmoles. Lens water did not increase in the diabetic animals with or without cataracts. The aldose reductase inhibitor Sorbinil prevented the increase in lens sorbitol in both the 21- and 44-d streptozotocin diabetic rats; cataract formation was prevented in the 44-d diabetic animals. The lens water in untreated diabetic animals with cataracts did not differ from lens water in the Sorbinil-treated diabetic animals that did not develop cataracts. Sorbinil treatment of diabetic animals was associated with normalization of both lens sorbitol and taurine levels. Taurine has been shown to serve both as an osmoregulator and as an antioxidant. The apparent increase in lens osmolality attributed to sorbitol was counterbalanced by an equimolar reduction in taurine concentration. The reciprocal relationship between taurine and sorbitol reduces the likelihood of an osmotic mechanism for sugar cataractogenesis; the reduced lens taurine, however, may increase the risk of lens protein oxidation and subsequent cataract formation. Thus *in vivo* sugar cataract formation may be an oxidative process rather than an osmotic phenomenon. (*Pediatr Res* 27: 293–296, 1990)

Abbreviations

STZ, streptozotocin

Histopathologic studies have shown that sugar cataracts follow the swelling and rupture of lens fibers (1). Van Heyningen was the first to report that polyols (sugar alcohols) are present in excess concentrations in the lenses of animals with sugar cataracts (2). The intracellular polyol levels are the direct result of elevated sugar (glucose or galactose) concentrations that increase the activity of the polyol pathway. Inasmuch as cell membranes are relatively impermeable to sugar alcohols, the polyols accumulate intracellularly. It was subsequently reported that intracellular polyol accumulation was accompanied by increased lens water

(3, 4) that caused swelling (termed the vacuolar stage) and subsequent disruption of the lens fibrils. The loss of lens fiber integrity is noted grossly as an opacity or cataract (5).

Animals made hyperglycemic with STZ have been shown to develop lens fiber swelling in 3 wk and nuclear cataracts by 6 wk (6). STZ-diabetic rats untreated with insulin commonly maintain blood glucose values greater than 22.5 mM, have massive polyuria, and do not gain weight in a normal fashion (7). These animals have the required hyperglycemia to develop cataracts; they also appear to be severely dehydrated despite special care to provide abundant water at all times.

Taurine, the most abundant free amino acid in mammalian tissues, has been noted to maintain cell volume by compensating for changes in osmotically active intracellular substances (8). It has been reported that hypernatremic dehydration causes shifts of intra- and extracellular taurine in a fashion that maintains the osmotic balance in animal tissues (9, 10). In addition to osmoregulation, taurine has been shown to have other cellular functions. One of these is as an antioxidant, where taurine inhibits light-induced lipid peroxidation in the frog retina (11) and retinol-induced damage in human lymphoblastoid cells (12).

Chylack and coworkers (13, 14) have reported that oxidative stress plays a major role in the pathogenesis of senile cataracts. It has also been noted that lens taurine decreases with increasing age and that taurine levels in senile cataracts are lower than found in noncataractous lenses of the same age (15, 16).

Recent reports indicate that oxidative damage is major contributor to sugar-induced cataractogenesis (17, 18) that can be prevented by antioxidants such as vitamin E (19, 20) glutathione (21), and butylated hydroxytoluene (18). Current theory about oxidative stress in the pathogenesis of sugar-induced cataractogenesis focuses on the increased turnover of NADPH caused by the increased activity of the polyol pathway. Reduced NADPH availability impairs production of glutathione, an important antioxidant.

Thus sugar-induced cataractogenesis appears to be a multifactorial process combining both osmotic and oxidative factors. Inasmuch as taurine is a substance that functions both as an osmoregulator and an antioxidant we decided to evaluate the relationship between lenticular sorbitol and taurine during the evolution of cataracts in the STZ-diabetic rat.

MATERIALS AND METHODS

Experimental diabetes was induced in fasted 60-d-old male Wistar rats (Charles River, Wilmington, NC) by the intraperitoneal injection of STZ (65 mg/kg) dissolved in cold 0.1 mM sodium citrate buffer (pH 4.5). The STZ (Sigma Chemical Company, St. Louis, MO) was dissolved in the buffer less than 20 s before injection. Citrate buffer was administered i.p. to control animals. Blood was collected on two or more occasions after STZ administration for the measurement of glucose concentration by a glucose oxidase method.

Seven d after STZ injection, one-half of the hyperglycemic group were given the aldose reductase inhibitor Sorbinil (Pfizer Inc., Groton, CT) (10 mg/kg) intraperitoneal daily for 37 d (first

study) and 14 d (second study) while the other hyperglycemic rats were given vehicle alone. The Sorbinil was dissolved in a buffered isotonic sodium chloride solution (pH 9.5) and the concentration adjusted to 4 mg/mL and a final pH 7.4. The animals were provided abundant food and water and were weighed every 3rd d. The animals were examined with a flashlight every 3rd d in a darkened room for lenticular opacities. The animal was said to have a cataract when a central opacity corresponding to stage III [nuclear cataract as described by Sipple (22)] was observed (Fig. 1).

Borosilicate test tubes (12 × 75 mm) were preweighed for the collection of the rat lenses. Sodium pentobarbital (85 mg/kg) was given intraperitoneally as a general anesthetic; while the animals were alive, the lenses were removed, blotted dry, placed in the preweighed test tubes, weighed, flash frozen with liquid nitrogen, and stored at −80°C. The tubes containing the frozen

Fig. 1. *A*, transparent rat lens. *B*, nuclear opacity (44 d). *C*, total opacification (60 d).

lenses were lyophilized for 48 h and reweighed. Drying for 72 and 96 h did not change the dry weights. Lens water is expressed as a hydration index (mm H_2O/mg dry wt × 10) (23).

The dried lenses were homogenized in 3.5 mL of deionized water with a motor driven ground glass homogenizer. Somogyi-Nelson protein free filtrates were prepared (24) and lyophilized before derivatization with acetic anhydride (25). Acetate derivatives of lens polyols were identified and quantitated on a Perkin-Elmer (Norwalk, CT) model 900 gas-liquid chromatograph equipped with a DB-225 Megabore column (J & W Scientific Company, Folsom, CA) with α-methylmannoside used as the internal standard.

Taurine concentrations were determined in protein free filtrates by HPLC utilizing a 6 × 250 mm column containing 17 μm Aminex A-6 (Bio-Rad Laboratories, Richmond, CA) eluting with a citrate buffer (pH 3.0) and using *o*-phthaldialdehyde (Sigma) as the indicator reagent. A fluorometer with an excitation wave length of 360 nm and emission wave length of 560 nm was used to detect amino acid peaks that were analyzed by a Perkin-Elmer LC-100 integrator. Serum osmolality was determined with a Precision System, Inc. (Sudbury, MA) Micro Osmette model 5004 freezing point depression osmometer.

Statistical analyses were performed on an IBM 3033 computer using the VM/CMS operating system and SAS (SAS Institute, Cary, NC) statistical programs. Analysis of variance coupled with Duncan's new multiple range test were used to detect mean differences among three or more treatment groups. Differences between two group means were detected by using the Student's *t* test. Prudent precautions were used to avoid type I errors (26). Results are expressed as mean ± SD.

RESULTS

The initial study lasted for 44 d after the induction of hyperglycemia by STZ. During that interval the control animals gained a mean of 153 g; the hyperglycemic animals gained a mean of 35 g (Table 1). The wt gain of Sorbinil-treated animals was not statistically different from the wt gain of diabetic animals not treated with the aldose reductase inhibitor (Table 1). The untreated diabetic animals began to develop cataracts at 40 d of
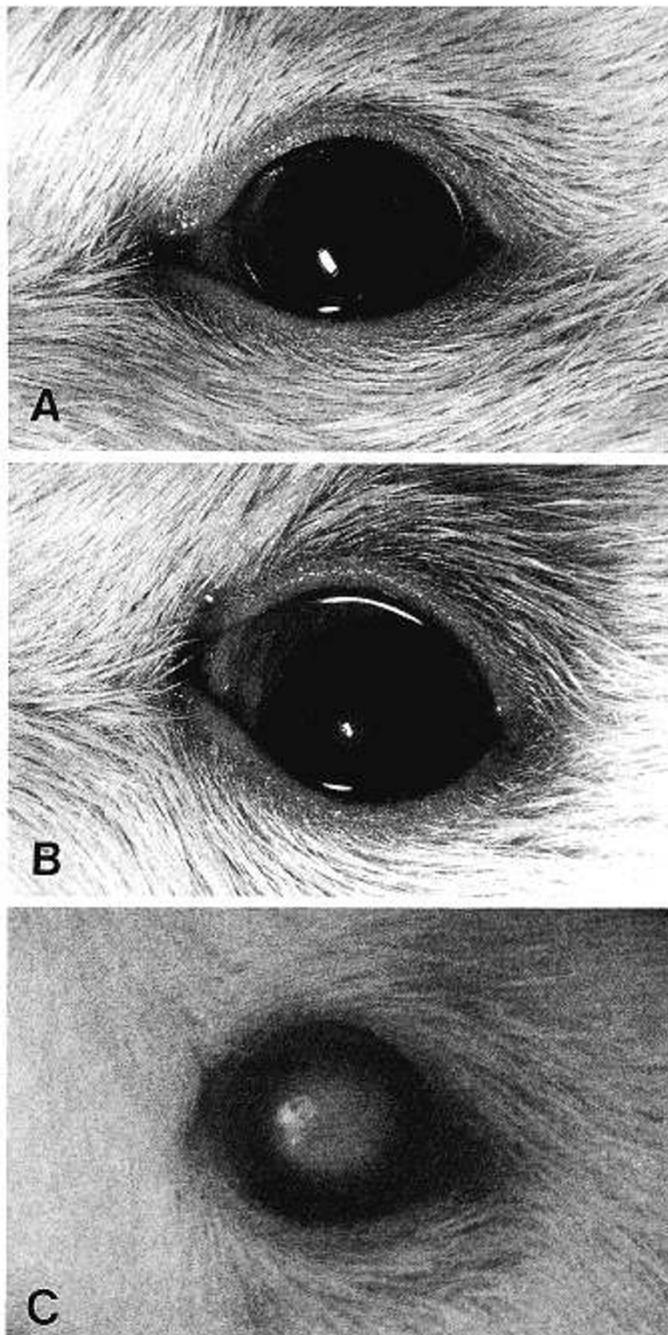
Table 1. *Rat physical features*

	Wt (grams)	Mean serum glucose (mM)	Lens hydration index	Serum osmolality (mosmol/ kg)
Streptozotocin diabetic rats (21 d)				
Control				
Initial	231.4 ± 5.1			
Final	345.8 ± 8.7	3.5 ± 0.7	5.63 ± 0.60	296 ± 9.8
Diabetic				
Initial	231.8 ± 9.0			
Final	228.7 ± 19.4	22.2 ± 1.8*	5.20 ± 1.20	318 ± 10.1†
Sorbinil				
Initial	235.7 ± 5.9			
Final	225.2 ± 13.8	21.5 ± 2.4*	5.20 ± 1.50	311 ± 7.5†
Streptozotocin diabetic rats (44 d)				
Control				
Initial	230.7 ± 9.3			
Final	383.8 ± 17.6	7.8 ± 0.8	4.92 ± 1.20	293 ± 2.0
Diabetic				
Initial	234.3 ± 15.3			
Final	255.7 ± 27.2	27.6 ± 4.3*	4.75 ± 0.67	312 ± 2.0*
Sorbinil				
Initial	227.1 ± 13.6			
Final	278.8 ± 66.2	27.4 ± 4.7†	4.00 ± 0.69	306 ± 1.5†

* Different from control $p < 0.001$.
† Different from control $p < 0.01$.

hyperglycemia and by 43 d all six had cataracts, whereas none of the Sorbinil-treated nor any of the control animals showed opacification of the lens (Table 2). Sorbitol values were higher in the lenses of the diabetic animals than the controls but approached control levels in the lenses of diabetic animals treated with Sorbinil. Taurine and sorbitol concentrations varied inversely within a similar range in both the diabetic and the Sorbinil-treated diabetic animals (Table 2). The serum osmolality in the diabetic animals (with or without Sorbinil) was significantly more than control animals (Table 1). Lens water content did not increase either before or after cataract formation associated with hyperglycemia (Table 1); lens water after cataract formation did not differ from the water content of lens removed from STZ hyperglycemic rats treated with Sorbinil to prevent cataract formation. Lens wet wt and dry wt were not different for control, diabetic, or Sorbinil-treated diabetic animals (data not shown).

This study was repeated with a second group of animals after 3 wk of STZ-induced hyperglycemia. The diabetic animals did not regain their pre-STZ wt (Table 1) and appeared to be dehydrated even though excess drinking water was available. At the time of death, the serum osmolality was significantly higher in the diabetic animals (with or without Sorbinil) than in the control animals. Again, lens water in the STZ diabetic rat did not differ from control rats and was not influenced by Sorbinil treatment (Table 1). Sorbitol increased in the lens of the diabetic animals but was normal in the diabetic animals treated with Sorbinil (Table 2); taurine levels in the lens varied inversely with the sorbitol levels. Lens inositol levels were significantly reduced in the diabetic animals, increased in those animals treated with Sorbinil but did not return to control inositol levels after 2 wk of Sorbinil treatment (Table 2).

DISCUSSION

We were able, as have others, to demonstrate sugar cataract formation in the STZ hyperglycemic rat; cataracts did not form in diabetic animals receiving the aldose reductase inhibitor Sorbinil (27–29). Lens sorbitol content was four to six times higher in the STZ diabetic rats both before and after lenticular opacification than in control animals; lens taurine concentration varied inversely to sorbitol with a 3- to 7-fold reduction. Kinoshita et al. (30) also found reductions in total free amino content of lenses after cataract formation; this was thought to reflect the loss of cellular integrity. We observed a reduction of lens taurine before lens fibril disruption and a return to normal after Sorbinil treatment. Inasmuch as taurine is not linked in a metabolic fashion to the activity of the polyol pathway, its inverse relation-

ship to sorbitol suggests a process that maintains the intracellular osmotic balance. It has been reported that changes in intracellular taurine concentration are mediated by the activity of a sodium-dependent taurine pump (31). The inverse relationship between intracellular taurine and sorbitol in hyperglycemic animals of 21 and 44 d indicates that the system controlling intracellular osmolality continues to operate during the evolution of the sugar cataract. Inositol, a cyclic hexatol believed to play a role in hyperglycemic tissue damage (32), normally has an intracellular gradient maintained by a sodium-dependent pump (33). This study as well as others (34) show that intracellular inositol also varies inversely with sorbitol levels in the lens of the STZ diabetic rat. The reduced intracellular inositol found in lens of STZ diabetic rats has been attributed to impaired (Na^+,K^+)-ATPase activity (35). Inositol levels in the lens of the 21-d STZ diabetic rats varied directly with the taurine levels but the inositol concentration was so small that it was not considered to be a significant lens osmol. The quantitative changes of sorbitol, taurine, and inositol content (Table 2) in the lens of the STZ diabetic rat when summed did not differ from the total mosmol contributed by these substances in the normal lens of control animals. The significant reduction in lens inositol found in the STZ diabetic rats may be secondary to normal changes in energy dependent pump activity required to maintain a normal osmotic gradient.

The in vitro studies of Kinoshita et al. (5) indicated that movement of excess water into lens fibrils was in response to increased intracellular dulcitol; the extra water caused cellular swelling and later disruption recognized as lenticular opacification. However, others have failed to show any net increase in lens water before cataract formation (23, 36); Mizuno et al. (37) reported increased water content in the lens cortex near the equator during the evolution of sugar cataracts. This regionalized swelling was attributed to localization of aldose reductase to specific areas of the lens (38), which caused a localized increase of intracellular sorbitol and therefore increased cell water mediated by an osmotic mechanism. Our observation that lens taurine is reduced proportionately to the increased sorbitol suggests that the changes in taurine concentration are likely to occur in the lens areas of increased sorbitol. The lens fibril swelling that has been reported may not have an osmotic mechanism. The lens water did not increase in these STZ diabetic rats during the evolution or at the time of cataract formation. If one considers that no change occurred in lens osmolality as the result of sorbitol accumulation whereas the extracellular osmolality increased 18 to 22 mosmol in response to the osmotic diuresis of hyperglycemia it seems unlikely that water would move from the extracellular space of increased osmolality into the lens of the same or reduced osmolality.

Although osmotic stress has long been held accountable for sugar-induced cataracts, evidence is accumulating to support an oxidative mechanism for this process (17–21). Reduced taurine levels (15, 16) have been reported to be associated with human senile cataracts which have an "oxidative stress" etiology (13). One of the functions of taurine may be to protect lens protein from oxidative damage. Taurine concentrations in human lenses with senile cataracts are 73% lower than in noncataractus human lenses of similar age (15). Taurine levels in the lens of 21- and 44-d STZ diabetic rats were 68 and 85% lower than found in the lenses of control animals. The reduction of taurine, to preserve intracellular osmolality during polyol accumulation, may be sufficient to allow oxidative damage of lens fibrils. The cellular swelling noted in the lens during the evolution of the sugar-induced cataract (28) may represent the movement of water across cell membranes injured by an oxidative mechanism, rather than a net increase in lens water in response to increased intracellular osmoles.

Thus, sugar cataract and senile cataract formation may have a common, nonosmotic, final pathway that is associated with reduced lens taurine concentrations.

Table 2. *Lens chemical features*

	Lens sorbitol, inositol, and taurine in STZ-diabetic rats (21 d)			
	Sorbitol (μM/g dry wt)	Taurine (μM/g dry wt)	Inositol (μmol/g dry wt)	Cataract occurrence
Control	2.12 ± 3.3	15.56 ± 2.3	0.68 ± 0.1	(0/8)
Diabetic	13.39 ± 3.5*	4.96 ± 2.1*	0.01 ± 0.01*	(0/9)
Sorbinil	3.80 ± 1.7†	10.96 ± 2.6†	0.11 ± 0.1†	(0/10)

	Lens sorbitol and taurine in STZ rats (44 d)		
	Sorbitol (μM/g dry wt)	Taurine (μmol/g dry wt)	Cataract occurrence
Control	3.66 ± 0.38	12.90 ± 1.10	(0/6)
Diabetic	13.36 ± 1.85*	1.83 ± 0.54*	(6/6)
Sorbinil	4.59 ± 3.23†	11.60 ± 1.38†	(0/6)

* Different from control $p < 0.001$.
† Different from diabetic $p < 0.001$.

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