The Effect of an Aldose Reductase Inhibitor (Sorbinil) on the Level of Metabolites in Lenses of Diabetic Rats

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SUMMARY

This study examined the effect of an aldose reductase inhibitor (Sorbinil, CP 45634, Pfizer, Sandwich, Kent, United Kingdom) on the metabolite profile of the lens during the first week after induction of diabetes with alloxan. The lens content of sorbitol, fructose, glycerol 3-phosphate, and glucose 6-phosphate was, respectively, 0.33 ± 0.03, 0.55 ± 0.05, 0.10 ± 0.01, and 0.074 ± 0.006 μmol/g (means ± SEM) in the control group rising to 12.2 ± 0.52, 3.20 ± 0.10, 0.76 ± 0.10, and 0.200 ± 0.009 in lenses from alloxan-diabetic rats. Sorbinil treatment (40 mg/kg) decreased the lens content of sorbitol to 0.60 ± 0.06, fructose to 0.85 ± 0.08, and glycerol 3-phosphate to 0.36 ± 0.03 μmol/g; glucose 6-phosphate remained unchanged. Significantly, the lens content of glutathione was decreased to 60% of the normal value in the diabetic group, but was sustained at normal levels with Sorbinil treatment. The ATP content of the lens was not altered by diabetes or Sorbinil treatment at this time interval. Sorbinil has no significant effect on the above metabolites in the normal rat lens. The effect of Sorbinil in restoring normal levels of glutathione and glycerol 3-phosphate may be a potentially important facet of the action of this drug. The interlocking of metabolic pathways by the redox state of NAD^+/NADH and $NADP^+$/NADPH, their derangement in diabetes, and the wider effects of Sorbinil on the network of reactions in the lens are discussed. DIABETES 32:482–485, May 1983.

There is now strong evidence to suggest that the excessive conversion of glucose to sorbitol via the polyol pathway and the accumulation of sorbitol in the lens is an important factor in cataract formation in diabetes.[1–4] Several lines of evidence point to this conclusion. First, the formation of a number of sugar alcohols via aldose reductase, including dulcitol from galactose and xylitol from xylose,[4] is associated with cataract formation. Second, studies from diabetic mutant animals have shown that strains with a low aldose reductase activity do not develop cataracts despite persistent hyperglycemia.[5] Furthermore, induction of diabetes in normal laboratory mice, a species that has an exceptionally low aldose reductase in the lens,[5] also fails to lead to lens opacification. Finally, the administration of aldose reductase inhibitors has been shown to lower the sorbitol content of the lens and to retard cataract formation in experimental diabetes.[1,5,6]

While the flux of glucose to sorbitol in diabetes is undoubtedly of prime importance in cataractogenesis, it must be borne in mind that there are a number of independent and interlocking routes that are subject to change in diabetes.

One consequence of the exposure of the lens to high ambient glucose concentrations is the glycosylation of ε amino groups of lysine residues of lens crystallins, which impart an increased susceptibility to sulphydryl oxidation.[7] The resultant disulphide crosslinks lead to the formation of high-molecular-weight aggregates of lens proteins.[7] A further significant change is the increased hexokinase activity with an accompanying increase in the lens content of glucose 6-phosphate found in the alloxan-diabetic rat lens.[8–10] Glucose 6-phosphate is a key intermediate for the glycolytic route, pentose phosphate pathway, and glycogen metabolism as well as a potential factor in the nonenzymatic glycosylation of lens crystallin.[7]

Thus, three distinct strands, all arising from increased ambient glucose concentration, combine and may contribute to the process of cataract formation. These are (1) sorbitol formation, (2) nonenzymatic glycosylation of lens proteins, and (3) altered metabolism resulting from changes in glucose 6-phosphate content and the associated variation in the redox state of the nicotinamide nucleotides.

The effects of diabetes on the sorbitol route on the one hand and the pathways arising from glucose 6-phosphate on the other hand cannot easily be dissociated in the lens since these multiple routes are linked via the redox state of

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the $NADP^+/NADPH$ and $NAD^+/NADH$ couples which are of key regulatory significance in the pentose phosphate pathway, the glycolytic route, and the glycerophosphate route. The advent of the aldose reductase inhibitors provides a valuable probe in dissecting the complex interactions of these processes in diabetes.

The present experiments, in which the aldose reductase inhibitor Sorbinil is administered immediately after induction of diabetes with alloxan, seek to throw light on the following problems: (1) to examine the changes in lens metabolism in relation to the increased capacity for formation of glucose 6-phosphate in diabetes, (2) to examine the relative contribution of the glycerol 3-phosphate dehydrogenase and aldose reductase routes of glycerol 3-phosphate formation in the diabetic rat lens, and (3) to determine the effects of aldose reductase inhibitors on the sharp fall in lens glutathione, which is known to occur as an early event in cataractogenesis.[10–13]

MATERIALS AND METHODS

Materials. Substrates, coenzymes, and enzymes used in the assay procedures were purchased from Boehringer Corporation Ltd. (London, England) or Sigma London (Poole, England). Aldose reductase inhibitor, Sorbinil CP 45634, was a gift from Pfizer, Central Research Ltd.

Animals. Male albino rats of the Wistar strain were used. The initial weight was 250 g. Diabetes was induced by the subcutaneous injection of alloxan (200 mg/kg body wt) into rats previously starved for 24 h. To study the effect of Sorbinil on the lens metabolism, the drug was administered to the animals orally as a daily dose of 40 mg/kg body wt on the day of induction of diabetes and thereafter for 6 days; a 0.05-mg/rat dose of Sorbinil was also administered daily to each eye in the form of eyedrops. The rats were killed on the seventh day of experiment. The severity of diabetic state was judged by failure of normal growth and blood glucose measurements. Blood glucose was estimated by a spectrophotometric method with hexokinase and glucose 6-phosphate dehydrogenase.[14]

Metabolite estimations. Rat lens tissue, pooled from four rats, was used. The lenses were removed rapidly, in approximately 10 s, and placed in liquid nitrogen. Frozen lenses were weighed and homogenized for 1 min in 5 vol of ice-cold 0.5 N perchloric acid using Ultra Turrex homogenizer, and the protein precipitate removed by centrifugation at 4°C for 10 min. The ice-cold supernatants were neutralized to pH 6.5–7.0 with KOH (glycerol-free) and the insoluble $KClO_4$ removed by centrifugation. The final concentration of extract was adjusted to 1:10 (wt/vol).

Metabolites were assayed according to the standard methods described by Bergmeyer.[15] For all metabolite assays the reduction of NAD^+ and $NADP^+$ or oxidation of NADH or NADPH was measured in a Unicam SP 800 recording spectrophotometer.

Results are expressed as mean ± SEM for the indicated number of observations. Student's *t* test was used for statistical analysis of significance.

RESULTS

The effects of Sorbinil on the metabolite profile of lenses from normal and diabetic rats are presented in Table 1. There was no significant effect of Sorbinil on the body weight or blood glucose values of normal or diabetic rats during this time period and no significant effects of Sorbinil on the metabolite profile of lenses from normal rats treated with doses similar to those given to diabetic rats.

In line with the role of Sorbinil as an aldose reductase inhibitor, there is a very marked decline in the sorbitol content of the lenses from the diabetic group treated with this drug relative to the untreated diabetic rats, 0.60 and 12.2 μmol/

TABLE 1
The effect of Sorbinil on the metabolite profile of normal and alloxan-diabetic rat lens

Lens metabolites	Control (μmol/g)	Control + Sorbinil (μmol/g)	Diabetic (μmol/g)	Diabetic + Sorbinil (μmol/g)
Glucose	0.80 ± 0.08 (12)	1.00 ± 0.10 (4)	4.23 ± 0.28 (12)	4.64 ± 0.47 (7)
(%)	100	125	100	110
Sorbitol	0.33 ± 0.03 (6)	0.30 ± 0.02 (4)	12.2 ± 0.52 (12)	0.60 ± 0.06 (6)
(%)	100	91	100	5***
Fructose	0.55 ± 0.05 (6)	0.50 ± 0.05 (4)	3.20 ± 0.10 (6)	0.85 ± 0.08 (6)
(%)	100	91	100	26***
Glycerol 3-P	0.10 ± 0.01 (6)	0.10 ± 0.01 (4)	0.76 ± 0.10 (6)	0.36 ± 0.03 (6)
(%)	100	100	100	47**
Glucose 6-P	0.074 ± 0.006 (8)	0.090 ± 0.010 (4)	0.200 ± 0.009 (8)	0.210 ± 0.03 (6)
(%)	100	121	100	105
ATP	1.23 ± 0.10 (8)	1.25 ± 0.10 (4)	1.24 ± 0.12 (8)	1.57 ± 0.15 (6)
(%)	100	102	100	127
Glutathione	2.03 ± 0.23 (9)	1.75 ± 0.10 (4)	1.29 ± 0.09 (9)	2.52 ± 0.27 (6)
(%)	100	86	100	195***
Body weight	239 ± 11 (12)	235 ± 9 (8)	219 ± 12 (12)	205 ± 6 (8)
(g)	100	98	100	94
Blood glucose	6.0 ± 0.3 (12)	5.8 ± 0.2 (8)	28.3 ± 1.5 (12)	28.4 ± 1.2 (8)
(mmol/L)	100	97	100	100

The effect of Sorbinil on normal rat lens is shown as a percentage of the normal control group; the effect on the diabetic rats is shown as a percentage of the untreated diabetic group. Sorbinil treatment by mouth and with eyedrops, each given once daily (40 mg/kg body wt), was begun in parallel with the induction of diabetes. The duration of the experiment was 1 wk. Values are given as mean ± SEM. Fisher's P values are shown by asterisks: **$P < 0.01$; ***$P < 0.001$. The number of observations are given in parentheses. Each observation is from lenses pooled from four rats.

g lens, respectively; the equivalent values for fructose are 0.85 and 3.2 μmol/g. It may be noted that there remains a residual elevated sorbitol and fructose in the diabetic group treated with Sorbinil when compared with the normal control rats, a point which is considered further in the DISCUSSION.

The elevated content of glucose and glucose 6-phosphate in the diabetic rat lens is unchanged by Sorbinil treatment. One week after induction of diabetes the ATP content of the lens has not decreased, and this key compound remained unchanged in the Sorbinil-treated group.

The two most interesting findings are, first, that lens glutathione is present at the normal concentration in the diabetic group treated with Sorbinil, in contrast to the untreated diabetic rats in which the lens glutathione was only 60% of the normal value. Second, the markedly elevated glycerol 3-phosphate content of the diabetic rat lens is significantly decreased in the Sorbinil-treated diabetic group. This latter finding is of interest in relation to the possible direct involvement of aldose reductase in the formation of lens glycerol 3-phosphate.

DISCUSSION

Previous studies from this laboratory have pointed out aspects of "glucose overutilization" in the lens in diabetes. The activity of hexokinase has been shown to increase twofold in the diabetic rat lens 6 wk after induction of diabetes,[8] leading to a marked increase in the glucose 6-phosphate content of the lens.[9,10] In the present study the glucose 6-phosphate content of the lens is even higher than that reported previously, and there is evidence that the period of maximum accumulation of the phosphorylated intermediates, glucose 6-phosphate, fructose 6-phosphate, and glycerol 3-phosphate, and of sorbitol occurs 1 wk after induction of diabetes; coincident with the massive increase in these products was a sharp fall in lens glutathione content.[10]

The present results show that neither glucose nor glucose 6-phosphate accumulation was altered by Sorbinil treatment; it could be anticipated, therefore, that the nonenzymatic glycosylation of lens crystallins would continue and that this facet of damage to the lens would not be significantly changed by Sorbinil treatment alone.

The changes in diabetic rat lens glycerol 3-phosphate after Sorbinil treatment throw some light on the second question posed in this study, namely the source of the accumulated lens glycerol 3-phosphate in diabetes. There are two potential precursors and routes for glycerol 3-phosphate formation in lens: one is from dihydroxyacetone phosphate via the NAD-linked glycerol 3-phosphate dehydrogenase, the other via glyceraldehyde 3-phosphate and aldose reductase using NADPH from the pentose phosphate pathway as the reductant (see Figure 1). It has been shown that glyceraldehyde 3-phosphate is an excellent substrate for the aldose reductase reaction.[16] The 53% fall in the glycerol 3-phosphate content of diabetic rat lens after Sorbinil treatment may be interpreted as showing that the aldose reductase route plays a highly significant role in the formation of this intermediate, and that there is an approximately equal contribution from the two potential routes of synthesis.

The increment in glycerol 3-phosphate content that is observed in the presence of Sorbinil may be ascribed to the reduction of dihydroxyacetone phosphate by NADH generated by the residual activity of the aldose reductase pathway during the conversion of sorbitol to fructose (see Figure 1). In this context it is interesting to note that, calculated on the basis of the differences between the metabolite content of the lenses from diabetic rats treated with Sorbinil (control values in Table 1), there is an apparent, if fortuitous, stoichiometry between the increment of fructose (0.3 μmol/g) and the remaining increment of glycerol 3-phosphate (0.26 μmol/g) in the lenses of diabetic rats treated with Sorbinil.

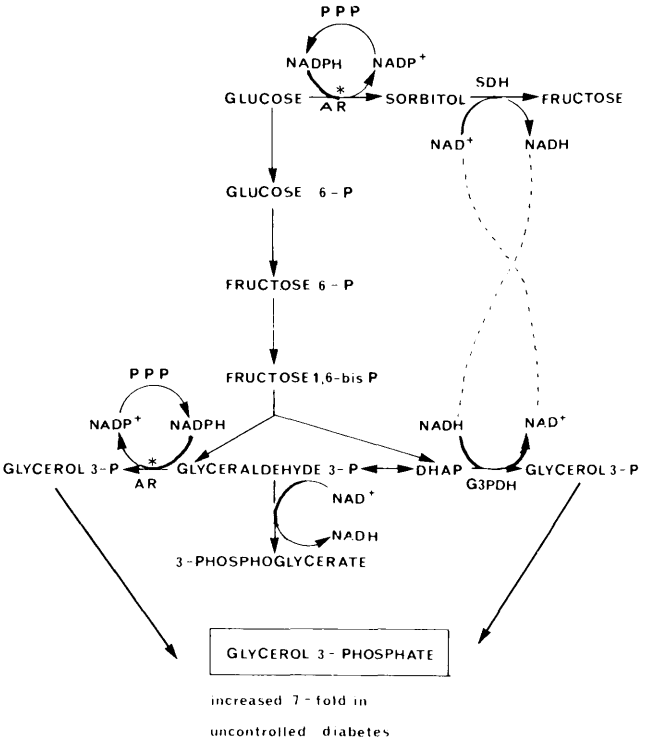

FIGURE 1. The proposed effect of aldose reductase inhibitor (Sorbinil) in the regulation of routes of glycerol 3-phosphate in the diabetic rat lens. The interrelationship among metabolic routes: glucose to sorbitol via aldose reductase (AR); sorbitol to fructose via sorbitol dehydrogenase (SDH); the pentose phosphate pathway (PPP) and the two potential routes for glycerol 3-phosphate formation (1) via dihydroxyacetone phosphate (DHAP), NADH, and glycerol 3-phosphate dehydrogenase (G3PDH) and (2) via glyceraldehyde 3-phosphate, NADPH, and aldose reductase (AR). The sites of action of Sorbinil on aldose reductase are shown by asterisks.

Perhaps the most striking observation illustrated in Table 1 is the finding that glutathione is sustained at a normal level in the diabetic rat lens treated with Sorbinil. In view of the many reports on the role of glutathione in the lens and the importance of the loss of this peptide in cataractogenesis, whether from radiation, chemical induction, or aging,[11-13] this aspect of the present study appears to merit further investigation. It is clear from Table 1 that the rat lens glutathione has decreased to approximately 60% of the normal value within 1 wk of induction of diabetes, at a time when the lens ATP is sustained at a normal level. This fall is possibly due to leakage of the peptide that may have occurred as a result of osmotic damage, a hypothesis strengthened by the finding that normal values of glutathione were obtained in diabetic rat lenses from the Sorbinil-treated group.

The present results show that, in addition to the prevention of osmotic damage via inhibition of the sorbitol pathway,

Sorbinil restores the changed level of glutathione and glycerol 3-phosphate found in the diabetic lens. These changes, and the attendant alterations in the cellular redox state, could play a significant part in the role of aldose reductase inhibitors as agents retarding the development of cataracts in diabetes.

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