The Aldose Reductase Controversy

ROBERT N. FRANK

The first complication to be attributed to this mechanism was the acute sugar cataract that develops early in the course of diabetes in rats and some other species (4). Similar cataracts develop even more rapidly in animals fed a diet containing a high percentage (usually 30–50% by weight) of galactose. The explanation for this is that galactitol, the sugar alcohol of galactose, cannot be reduced by sorbitol dehydrogenase, the second enzyme of the pathway, and therefore accumulates to much higher levels in susceptible tissues than does sorbitol (5). Both diabetic and galactosemic cataracts in rats can be delayed, if not prevented, by aldose reductase inhibitors (ARIs), a strong piece of evidence supporting this mechanism of sugar cataract formation. Experimental galactosemia also has been widely used as an experimental surrogate for diabetes for studying the role of the sorbitol pathway in other complications of diabetes in laboratory animals. Although there is little doubt that aldose reductase is responsible for acute cataracts in diabetic and galactosemic laboratory animals, its role in cataract formation in diabetic humans is less clear. Perhaps it is important in the rare, rapidly progressive lens opacities that occur in young people with insulin-dependent diabetes mellitus (IDDM), but the only evidence to support this contention is the speed of development of these cataracts, and their morphological similarity to those that occur acutely in diabetic laboratory animals. The cataracts that occur in adults with diabetes look no different anatomically than those in nondiabetic individuals. These cataracts in adult humans with diabetes do contain an excess of sorbitol (6), but probably not enough to produce the osmotic damage that causes the acute lens opacities in diabetic or galactosemic rats (4,5).

The second complication of diabetes for which the sorbitol pathway has been thought to play a pathogenetic role is neuropathy. Sorbitol accumulates in peripheral nerves of diabetic animals (7), as does galactitol in the nerves of animals fed an excess of galactose (8). The motor nerve conduction velocity in such animals is sub-
 substantially slowed (8,9), as it is in motor nerves of diabetic humans (10). The reduced motor nerve conduction velocity can be at least partially corrected by a wide variety of ARIs (8,11). Whether this improvement is attributable to the decrease in tissue hexitol content or for other reasons, such as increased tissue myo-inositol concentration or sodium-potassium activated ATPase activity (12) may be arguable. Regardless, improvement in several parameters relating to motor, sensory, and autonomic neuropathy in diabetic human subjects who received drug, by comparison with those receiving placebo, has been reported in several clinical trials of ARIs (13–16). Still other studies have concluded that no symptomatic improvement provided by ARIs could be demonstrated over the treatment period (17–20). Although the clinical trials with positive results cannot be considered definitive because of their relatively small size, the negative results of other studies can be explained in some cases by their short duration (much less than one year in most cases), and in others by the fact that the drug under consideration was ponalrestat (Statll), which is now generally considered a highly ineffective ARI.

Finally, there is controversy in the investigation of the role of the sorbitol pathway in diabetic neuropathy, as has also occurred in the study of diabetic retinopathy, resulting from experiments described by Engerman et al. (21). They measured motor nerve conduction velocities and erythrocyte and nerve polyol levels in dogs made diabetic by alloxan and maintained in poor blood glucose control for 5 years and in nondiabetic dogs fed a 30% galactose diet for the same period. Erythrocyte polyol levels over the duration of the study were substantially higher in the galactosemic animals than in the diabetic ones (reflecting the inability of sorbitol dehydrogenase to oxidize galactitol), and nerve polyol levels over an initial 2- to 4-month period were at least as great in the galactosemic as in the diabetic dogs. (Presumably, nerve polyol levels in the galactosemic and in the diabetic animals remained elevated throughout the duration of the study, but levels near the study's end were not reported.) However, although motor nerve conduction velocity declined progressively in the diabetic dogs, no decline was observed in the galactosemic animals.

The first studies to suggest a possible role of the sorbitol pathway in diabetic retinopathy described retinal capillary basement membrane thickening in galactosemic rats, similar to that which occurs in retinal and other capillaries in diabetic humans, which was prevented by an ARI (22,23). However, the exact relationship of microvascular basement membrane thickening to the later lesions of retinopathy or other complications of diabetes is unclear. Of much greater relevance was the report that nondiabetic dogs fed a diet containing 30% galactose for at least 4 years demonstrate a loss of the pericytes of the retinal capillaries (pericyte ghosts) and develop capillary microaneurysms typical of human and canine diabetic retinopathy (24). This finding has been confirmed by Kador et al. (25,26), but controversy resulted after their claim (25,26) that ARIs prevent, or at least delay, the development of the diabetic-like lesions. Recently, Engerman and Kem (27) have reported that in dogs made diabetic by alloxan and maintained for 60 months thereafter, and in dogs maintained for 42 or 60 months on a 30% galactose diet, the ARI sorbinil did not prevent the development of retinal capillary microaneurysms or pericyte ghosts, or the thickening of capillary basement membranes. Similar results were reported by Robinson et al. (28,29) and in rats fed a diet containing 50% galactose for 26 months, whereas Kem and Engerman, in a study that has thus far appeared only in abstract form (30), concur with the pericyte loss and capillary microaneursms in rats receiving this diet, but deny the formation of microaneurysms or the effect of an ARI in preventing them. Yet another puzzling result reported by these investigators (31), which has also not yet appeared in a full-length publication, is the finding that sorbinil maintains a near-normal motor nerve conduction velocity, but does not prevent retinopathy after 5 years of alloxan-induced diabetes in dogs compared with diabetic animals dosed with placebo. Finally, the only controlled clinical trial of an ARI for human diabetic retinopathy (32) found no benefit of sorbinil treatment in preventing progression of retinopathy over a 30- to 48-month follow-up period in a group of 406 IDDM subjects.

Despite the contradictory results of at least some of these studies, I believe that several conclusions can be drawn. 1) The preponderance of experimental and clinical evidence supports the role of aldose reductase in the pathogenesis of several complications of diabetes. These include, in particular, diabetic neuropathy, for which evidence is provided by a number of clinical studies in humans and experimental studies in animals. Although negative results in human clinical trials using ARIs and in animal studies opposing the hypothesis have been reported, they can either be explained by the brief duration of the clinical trial or the ineffectiveness of the inhibitor, or are directly contradicted by other studies, albeit in different species (compare, e.g., 8 and 21). A possible problem with the clinical studies in diabetic neuropathy is that they deal with surrogate measurements, such as nerve conduction velocity or electron microscopic morphometry, rather than with actual clinical evaluation of neurological function or symptomatology. However, there is at least some reason to believe that these surrogates are directly related to clinically meaningful end points (16,33), which are, unfortunately, very difficult to quantitate precisely or require too long a study duration to be evaluated practically. (The DCCT protocol did formulate an operational definition of diabetic neuropathy, combining both clinical and laboratory findings, that permitted meaningful evaluation of the development and progression of this complication in a very large-scale and long-term clinical trial [11].)

2) The apparently differing results of studies of ARIs in galactosemic dogs and rats are harder to unravel, but may be related to differences in the degree of enzyme inhibition achieved by these groups of investigators. This is most clearly evident in the published results on retinopathy in galactosemic dogs, because both Kador et al. (29) and Engerman and Kem (27,34) have provided data on erythrocyte galactitol levels in their animals. The former investigators used the
ARI sorbinil at a minimal dose of ~40 mg·kg⁻¹·day⁻¹, varying upward to ~80 mg·kg⁻¹·day⁻¹ in three daily doses, with other groups of dogs also dosed with the ARI M79175, reported to be substantially more potent than sorbinil. Kem and Engerman (27) used sorbinil 60–80 mg·kg⁻¹·day⁻¹ in two doses. Kador et al. (26) described erythrocyte galactitol levels to be reduced by 80% in the sorbinil-treated animals, but by >95% in the sorbinil-M79175 or high-dose M79175 groups, which, they claimed, had the most substantial inhibition of retinopathy. Kador et al. do not report the erythrocyte galactitol level in normal dogs, and in addition they give only the percentage of reduction by ARls but not the absolute levels in any group of animals. Finally, nowhere do they report retinal levels of this sugar alcohol. Kem and Engerman claimed a 91% reduction in erythrocyte galactitol by the sorbinil dose they used, but this galactitol level was still threefold greater than in the erythrocytes of nongalactosemic dogs.

Similarly, the retinal galactitol level was reduced 96% by sorbinil, but also was threefold greater than in retinas of nongalactosemic animals. Robison et al. (28,29) have not published galactitol levels in any tissues of their galactosemic rats, which makes it difficult to determine whether studies that report negative results, such as that of Kem and Engerman (30), evaluated rats that had a comparable degree of aldose reductase inhibition. Whereas it is reason- able to suppose that the different results reported by these groups of investigators are attributable to differing degrees of aldose reductase inhibition in their experiments, the lack of data on this point in the papers of Kador et al. and Robison et al. make their results difficult to reproduce or to compare with other studies on the subject.

It should be evident from the above that a requirement for future studies of the efficacy of ARls must be the careful measurement of erythrocyte and, where possible, tissue levels of polyols in the experimental and control groups. Arguments have been made about the importance of maintaining prolonged high blood (and, by implication, tissue) levels of ARls (25,26) to refute the negative results of studies in dogs (27) in which only sorbinil (which has a relatively short plasma half-life in dogs) was used. One might similarly argue that the reduced nerve conduction velocity produced in dogs by diabetes but not by galactosemia (21) is somehow related to the prolonged and relatively stable hyperglycemia in the alloxan-induced diabetic animal, whereas experimental galactosemia, which is produced only by diet, may fluctuate considerably. However, these arguments become irrelevant if investigators report erythrocyte and tissue polyol levels, because the slow (sorbitol) or virtually absent (galactitol) metabolism of these substances imply that their levels should show relatively little temporal fluctuation even over the course of a lengthy experiment, although there may be wide diurnal shifts in the blood and tissue levels of aldoses. 3) Experiments that show differing effects of diabetes and of galactosemia on nerve conduction velocity (21) and differing responses to aldose reductase inhibition of the abnormalities produced in peripheral nerve and in retinal vessels by diabetes (31) also may be explained without refuting the aldose reductase theory of the complications of diabetes. Aside from the pronounced hyperosmolarity by which polyols produce cataracts in the lenses of diabetic or galactosemic animals, the mechanisms by which these agents might produce other diabetic, or diabetic-like, complications are speculative. As osmoles, sorbitol and galactitol are equal. Hence, the polyol that reaches the higher concentration faster will be the more cataractogenic. But sorbitol and galactitol may not show identical behavior at equal concentrations for other possible mechanisms, particularly those that involve biochemical pathways. This may explain the different effects reported for diabetes and galactosemia on nerve conduction velocity in dogs (21). The critical pathways may differ in activity in the same tissue in different species, or in different tissues in the same species, accounting for the contrasting effects of galactosemia on nerve conduction velocity in dogs and rats (8,21), and of ARls on peripheral nerves and retinal vessels of alloxan-induced diabetic dogs (31).

4) As I have discussed elsewhere (35), the Sorbinil-Retinopathy Trial (32) may have produced negative results because of the reduced dose of the drug (because of unanticipated toxic reactions at higher doses) and, therefore, likely insufficient enzyme inhibition, and/or because of insufficient duration of the trial. An important lesson from the DCCT is that the complications of diabetes develop slowly, and especially in the early stages of these complications, statistically significant differences between treatment groups in a clinical trial may not become evident for years. Randomized controlled clinical trials of large scale and long duration are clearly necessary to evaluate definitively the results of new treatments designed to prevent the development or progression of early retinopathy. But these studies are excruciatingly difficult to design, time-consuming, and forbiddingly expensive to run. The financial risks are great. Currently, Wyeth Ayerst’s tolrestat is the only ARI undergoing such very-large-scale clinical trials for neuropathy, retinopathy, and nephropathy. (In the interest of space, I have not discussed nephropathy in this article.) Should these trials fail, the reason may not be that the sorbitol hypothesis is incorrect but only that the inhibitor is insufficiently effective. Yet in such a scenario, will anyone be willing to incur the enormous risks of yet another clinical trial, even should a drug be developed that completely inhibits aldose reductase in tissues at doses that are not toxic to humans even over very prolonged periods of administration?

ACKNOWLEDGMENTS

Studies from my own laboratory have been supported by Grants RO1-EY-01857 and RO1-EY-02566 from the National Eye Institute, National Institutes of Health; and by a departmental unrestricted grant from Research to Prevent Blindness, New York.

REFERENCES

2. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end...