Perspectives in Diabetes

The Aldose Reductase Controversy

ROBERT N. FRANK

Next to the debate as to whether chronic hyperglycemia is the primary cause of the late vascular complications of diabetes, the longest running controversy among researchers and clinicians studying this disease is the role of the sorbitol pathway, particularly its initial enzymatic step, the reduction of glucose to sorbitol by aldose reductase in the pathogenesis of these complications. Because the outcome of the Diabetes Control and Complications Trial (DCCT) (1) appears to have effectively resolved the first issue, the aldose reductase controversy now takes center stage. The sorbitol pathway hypothesis is not the only mechanism for the pathogenesis of the complications of diabetes to have achieved serious consideration. Among others, for example, nonenzymatic glycation of proteins (2) is a plausible, if still largely untested, possibility. But over the more than 30 years since the British biochemist, Ruth van Heyningen, first demonstrated an excess of sorbitol in the lenses of diabetic rats (3), a substantial accumulation of evidence has supported the sorbitol pathway as the principal actor in at least some of the late complications of diabetes in humans and experimental animals. This view is not unchallenged, however. In this discussion, I will summarize some of the evidence supporting the role of aldose reductase in producing several of the late complications of diabetes, but also point out those contradictory findings that render the sorbitol pathway hypothesis controversial.

From the Kresge Eye Institute of Wayne State University School of Medicine, Detroit, Michigan.

Address correspondence and reprint requests to Dr. Robert N. Frank, The Kresge Eye Institute, Wayne State University School of Medicine, 4717 St. Antoine Boulevard, Detroit, MI.

Received for publication 12 November 1993 and accepted in revised form 6 December 1993.

DCCT, Diabetes Control and Complications Trial; ARI, aldose reductase inhibitor; IDDM, insulin-dependent diabetes mellitus.

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-

stantially 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 (Statil), 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. Similarly, Robison et al. (28,29) have reported diabetic-like lesions (pericyte loss, acellular capillaries, microaneurysms) in rats fed a diet containing 50% galactose for 26 months, whereas Kern and Engerman, in a study that has thus far appeared only in abstract form (30), concur with the pericyte loss and acellular capillaries 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 [1].) *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. (26) 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 ~60 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 Engemman (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 ARIs 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 Kern and Engerman (30), evaluated rats that had a comparable degree of aldose reductase inhibition. Whereas it is reasonable 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 ARIs 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 ARIs (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 osmolytes, 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 ARIs 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

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes. *N Engl J Med* 329:977–86, 1993
2. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end

products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–21, 1982
3. Van Heyningen R: Formation of polyols by the lens of the rat with 'sugar' cataract. *Nature* 184:194–95, 1959
4. Kinoshita JH: Mechanisms initiating cataract formation. *Invest Ophthalmol Vis Sci* 13:713–24, 1974
5. Kinoshita JH: Cataracts in galactosemia. *Invest Ophthalmol Vis Sci* 4:786–89, 1965
6. Varma SD, Schocket SS, Richards RD: Implications of aldose reductase in cataracts in human diabetes. *Invest Ophthalmol Vis Sci* 18:237–41, 1979
7. Gabbay KH, Merola LO, Field RA: Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science* 151:209–10, 1966
8. Gabbay KH, Snider JJ: Nerve conduction defect in galactose-fed rats. *Diabetes* 21:295–300, 1972
9. Gabbay KH: Role of sorbitol pathway in neuropathy. In *Vascular and Neurological Changes in Early Diabetes.* Camerini-Davalos RA, Cole HS, Eds. New York, Academic, 1972, p. 417–24
10. Gregersen G: Variations in motor conduction velocity produced by acute changes ofthe metabolic state in diabetic patients. *Diabetologia* 4:273–77, 1968
11. Sarges R, Oates PJ: Aldose reductase inhibitors: recent developments. *Progr Drug Res* 40:99–161, 1993
12. Greene DA, Lattimer SA, Sima AAF: Sorbitol, phosphoinositides, and sodiumpotassium-ATPase in the pathogenesis of diabetic complications. *N Engl J Med* 316:599–606, 1987
13. Judzewitsch RG, Jaspan JB, Polonsky KS, Weinberg CR, Halter JB, Halar E, Pfeifer MA, Vukadinovic C, Bernstein L, Schneider M, Liang K-Y, Gabbay KH, Rubenstein AH, Porte D Jr: Aldose reductase inhibition improves nerve conduction velocity in diabetic patients. *N Engl J Med* 308:119–25, 1983
14. Giugliano D, Marfella R, Quatraro A, De Rosa N, Salvatore T, Cozzolino D, Ceriello A, Torella A: Tolrestat for mild diabetic neuropathy: a 52-week, randomized, placebo-controlled trial. *Ann Intern Med* 118:7–11, 1993
15. van Gerven JMA: Further clinical studies with an aldose reductase inhibitor in diabetic neuropathy. *Diabetic Med* 10 (Suppl. 2):35S–38S, 1993
16. Sima AAF, Prashar A, Nathaniel V, Bril V, Werb MR, Greene DA: Overt diabetic neuropathy: repair of axo-glial dysjunction and axonal atrophy by aldose reductase inhibition and its correlation to improvement in nerve conduction velocity. *Diabetic Med* 10:115–21, 1993
17. Fagius J, Brattber A, Jameson S, Berne C: Limited benefit of treatment of diabetic polyneuropathy with an aldose reductase inhibitor: a 24-week controlled trial. *Diabetologia* 28:323–29, 1985
18. Lehtinen JM, Hyvonen SK, Uusitpa M, Pahakainen E, Halonen T, Kilpelainen H: The effect of sorbinil treatment on red cell sorbitol levels and clinical and electrophysiologic parameters of diabetic neuropathy. *J Neurol* 223:174–77, 1986
19. Lewin IG, O'Brien IAD, Morgan MH, Corrall RJM: Clinical and neurophysiological studies with the aldose reductase inhibitor, sorbinil, in symptomatic diabetic neuropathy. *Diabetologia* 26:445–48, 1984
20. Krentz AJ, Honigsberger L, Ellis SH, Harman M, Nattrass M: A 12-month randomized controlled study of the aldose reductase inhibitor ponalrestat in patients with chronic symptomatic diabetic neuropathy. *Diabetic Med* 9:463–68, 1992
21. Engerman RL, Kern TS, Larson ME: Nerve conduction velocity in dogs is reduced by diabetes and not by galactosemia. *Metabolism* 39:638–40, 1990
22. Frank RN, Keirn RJ, Kennedy A, Frank KW: Galactose-induced retinal capillary basement membrane thickening: prevention by Sorbinil. *Invest Ophthalmol Vis Sci* 24:1519–24, 1983
23. Robison WG Jr, Kador PF, Kinoshita JH: Retinal capillaries: basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. *Science* 221:1177–79, 1983
24. Engerman RL, Kern TS: Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 33:97–100, 1984
25. Kador PF, Akagi Y, Terubayashi H, Wyman M, Kinoshita JH: Prevention of pericyte ghost formation in retinal capillaries of galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol* 106:1099–1102, 1988
26. Kador PF, Akagi Y, Takahashi Y, Ikebe H, Wyman M, Kinoshita JH: Prevention of retinal vessel changes associated with diabetic retinopathy in galactose-fed dogs by aldose reductase inhibitors. *Arch Ophthalmol* 108:1301–309, 1990
27. Engerman RL, Kern TS: Aldose reductase inhibition fails to prevent retinopathy in diabetic and galactosemic dogs. *Diabetes* 42:820–25, 1993
28. Robison WG Jr, Nagata M, Laver N, Hohman TC, Kinoshita JH: Diabetic-like retinopathy in rats prevented with an aldose reductase inhibitor. *Invest Ophthalmol Vis Sci* 30:2285–92, 1989
29. Robison WG Jr, Tillis T, Laver N, Kinoshita JH: Diabetes-related histopathologies of the rat retina prevented with an aldose reductase inhibitor. *Exp Eye Res* 50:355–66, 1990
30. Kern TS, Engerman RL: Galactose-induced retinal lesions in rats and effects of aldose reductase inhibition (Abstract). *Diabetes* 41 (Suppl. 1):20A, 1992
31. Engerman RL, Kern TS, Larson ME: Aldose reductase inhibition benefits neuropathy but not retinopathy in diabetic dogs (Abstract). *Diabetologia* 35 (Suppl. 1):A45, 1992
32. Sorbinil Retinopathy Trial Research Group: A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. *Arch Ophthalmol* 108:1234–44, 1990
33. Sima AAF, Bril V, Nathaniel V, McEwen TAJ, Brown MB, Lattimer SA, Greene DA: Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy. *N Engl J Med* 319:548–55, 1988
34. Kern TS, Engerman RL: Retinal polyol and *myo*-inositol in galactosemic dogs given an aldose-reductase inhibitor. *Invest Ophthalmol Vis Sci* 32:3175–77, 1991
35. Frank RN: Aldose reductase inhibition: the chemical key to the control of diabetic retinopathy? *Arch Ophthalmol* 108:1229–31, 1990