Perspectives in Diabetes

Pathogenesis of Diabetic Retinopathy

RONALD L. ENGERMAN

Diabetic retinopathy involves anatomic changes in retinal vessels and neuroglia. The pathogenetic mechanism responsible for retinopathy is imperfectly understood, but much of the mechanism is apparently reproduced by experimental diabetes in animals and by chronic elevation of blood galactose in nondiabetic animals. The evidence that retinopathy is a consequence of excessive blood sugars and their sequelae is consistent with a demonstrated inhibition of retinopathy by strict glycemic control in diabetic dogs. However, retinopathy in the dog model has shown a tendency to resist intervention by strict control. Biochemical and pathophysiological sequelae of hyperglycemia possibly critical to the development of retinopathy in humans and animal models are being studied in many laboratories. Retinopathy occurs in experimental galactosemia in the absence of the renal hypertrophy, mesangial expansion, and glomerular obliteration typical of diabetes in humans and dogs, implying that retinopathy and nephropathy differ appreciably in pathogenesis. *Diabetes* 38:1203–206, 1989

Diabetic retinopathy is often divided, for purposes of discussion, into two stages, a vasoproliferative stage and a preceding "background" stage during which microaneurysms, occluded vessels, and other abnormalities occur within the substance of the retina. Proliferative retinopathy, in which new vessels and connective tissue grow on the surface of the retina or optic nerve head and into the vitreous, seldom occurs until many years after the background lesions have appeared and is believed to be a consequence of retinal damage during the preceding background stage. Improvements in photocoagulation therapy and other methods for surgical management of retinopathy have, in recent years, significantly reduced the risk of blindness from the proliferative disorder. By then, of course, the retina has already been seriously damaged, and the surgical treatment itself is not without additional risk. Unfortunately, a reliable means for preventing retinopathy or arresting its progression at an earlier stage in diabetic patients has yet to be demonstrated.

From the Department of Ophthalmology, University of Wisconsin, Madison, Wisconsin.

Address correspondence and reprint requests to Dr. Ronald L. Engerman, Department of Ophthalmology, University of Wisconsin, 1300 University Avenue, Madison, WI 53706.

Received for publication 24 May 1989 and accepted 6 June 1989.

EARLY MICROVASCULAR LESIONS

The vascular lesions that are identified with the onset of retinopathy include the formation of saccular capillary aneurysms, disappearance of pericytes from capillaries having endothelial cells, nonperfusion and obliteration of capillaries and small arterioles, gradual thickening of vascular basement membrane, and associated changes such as vessel leakage, exudate, and hemorrhage. The sequence in which these early lesions appear is difficult to establish precisely, partly because the abnormalities show a patchy distribution in the retina, and subtle early changes tend to become confounded with an occasional aneurysm, pericyte ghost, or obliterated capillary that may be found in apparently normal eyes from nondiabetic humans or dogs. In experimentally diabetic dogs, the loss of pericytes and/or endothelial cells from retinal capillaries seems to begin usually before the first one or two aneurysms form. Studies of autopsy eyes from diabetic patients have led to a similar conclusion (1). Pericyte loss first becomes evident histologically as an excessive prevalence of degenerate pericytes and pericyte ghosts, the latter being a pocket formerly occupied by the cell within the basement membrane of a viable capillary. Endothelial cells also degenerate in diabetes, but because no ghost or trace is left behind and proliferative changes eventually occur in the endothelium, subtle early changes in the endothelial cell population are more difficult to define.

Pericyte loss. The histological finding of capillaries with endothelial cells but few or no pericytes is typical of diabetic retinopathy and is reportedly characteristic only of the retina. In diabetic patients with retinopathy, pericyte ghosts have

been found to be rare or absent from capillaries of the optic nerve and cerebral cortex but numerous in the retinal capillaries, vessels normally similar in many respects (2,3). Microaneurysms show a similar predilection for the retina. The finding that the pericyte population is greatly altered by diabetes in the retina and not in the optic nerve and cerebral cortex has been interpreted as evidence that the disappearance of retinal pericytes might be due to local disorders within the retina (possibly hemodynamic) rather than to the pericyte's metabolic response to systemic abnormalities such as hyperglycemia. Degeneration of pericytes and entire capillaries probably occurs in many body regions in diabetes (4), but the high frequency of pericyte ghosts and pericyte-depleted capillaries in the retina, and their rarity in the optic nerve and brain, remains to be explained.

There is little direct evidence from which to determine whether pericyte loss is a cause of clinically significant retinal disorders, e.g., microaneurysm formation or capillary closure, as has at times been proposed, or is merely a symptom of the underlying biochemical or vascular disorder. However, endothelial cell proliferation in vitro has been reported to become inhibited when cocultured in the presence of pericytes and thus conceivably might be influenced in vivo by pericyte loss (5).

Capillary closure. Nonperfusion and obliteration of small blood vessels is a characteristic feature of diabetic retinopathy in humans and dogs and seemingly begins at an early stage of retinopathy. Retinal neovascularization invariably seems to be preceded by such vessel closure and is believed to be one of its consequences. Closure of retinal blood vessels is seen histologically as an increased prevalence of acellular capillaries, capillary basement membrane tubes lacking endothelial cells, and pericyte nuclei. The acellular capillaries first seem to occur singly or in small groups scattered about the retina and later are found also in larger clusters often associated with atrophic (hypocellular and acellular) arterioles.

Several possible mechanisms for vessel closure in diabetes have been postulated, including vessel compression by swollen extravascular cells, changes in blood (e.g., excessive platelet or erythrocyte aggregation, microthrombi, or subnormal fibrinolysis), and changes in the vessel wall and its cells. Attempts to demonstrate possible mechanisms of occlusion have been inconclusive.

Histological evidence of apparent occlusion of retinal capillaries by platelet aggregates has occasionally been reported in diabetic animals (6,7). The observations are consistent with earlier reports that some diabetic patients show platelet hypersensitivity to aggregating agents and may possess a plasma factor capable of enhancing the aggregation of platelets from nondiabetic subjects. However, other workers have found platelet aggregation to be not abnormal in diabetic patients or to be greater than normal only in those showing rapidly deteriorating retinopathy. Interest in the possible role of platelet aggregation in retinopathy has prompted a randomized clinical trial of aggregation inhibitors that has recently been completed (8). In that study, diabetic patients given aspirin with or without dipyridamole were found to develop significantly fewer microaneurysms than placebo-treated patients. It remains to be determined whether the results are reproducible and attributable to a pharmacological effect of aspirin other than on platelet aggregation.

A plasma factor such as that said to enhance platelet aggregation in diabetic patients appears to be absent in alloxan-induced diabetic dogs, notwithstanding the development of retinopathy in these animals. Moreover, platelets from the animals show no sign of excessive aggregation on exposure to ADP or collagen in vitro (9). Animal studies suggest that retinopathy is capable of developing in the absence of the extraordinary platelet aggregation that may occur in diabetic patients. Other hemorheological abnormalities may be associated more closely with retinopathy (10,11).

Retinal ischemia and new vessel formation, although traceable to vessel closure, are also associated with an apparent failure of the atrophic vessel network to reendothelialize and resume function. By electron microscopy, the basement membrane tube that remains after disappearance of the endothelial cells and pericytes is often seen to be filled with glial cell processes (Fig. 1). These structures have also been encountered by other workers and correspond with the so-called acellular capillaries seen in trypsin digest specimens of the retina. The glial ingrowth appears to be a reparative phenomenon not specific for diabetes. Presumably, neuroglia invade the lumen at sites of capillary cell loss. There is no good evidence that the ingrowth of glial processes precedes and contributes to capillary closure, but the ingrowth would be expected to at least interfere with endothelial regrowth and vessel reperfusion and thereby to contribute to ischemia and neovascularization. A role for extravascular cells in the elaboration of angiogenic factors or inhibitors is well known, but the possibility that the occlusion of retinal vessels and/or their recanalization is determined in part by neuroglia has received little attention.

HYPERGLYCEMIA

The relationship between retinopathy and the metabolic manifestations of diabetes is a matter of continuing controversy. Despite intensive efforts, studies in patients have not established whether the development or progression of diabetic retinopathy can be effectively inhibited by improved glycemic control (12). Valuable perspective on the problem has been gained in recent years through study of animal models of retinopathy.

Saccular capillary aneurysms and other retinal lesions typical of diabetes in patients are known to develop in dogs with spontaneous or experimentally induced diabetes, and in this animal model the development of retinopathy was found to be inhibited by good glycemic control, at least if good control was begun promptly 1–2 mo after the onset of diabetes (13). Dogs randomly assigned to good glycemic control developed significantly fewer retinal microaneurysms, acellular capillaries, and pericyte ghosts than dogs randomized to poor glycemic control, and only the latter group showed retinal hemorrhages, vessel sudanophilia, and apparent intraretinal neovascularization.

The dog model offered an exceptional opportunity to assess the extent to which the progression of incipient retinopathy might be arrested. Our intention was to compare the efficacy of intervention with that of prevention by use of

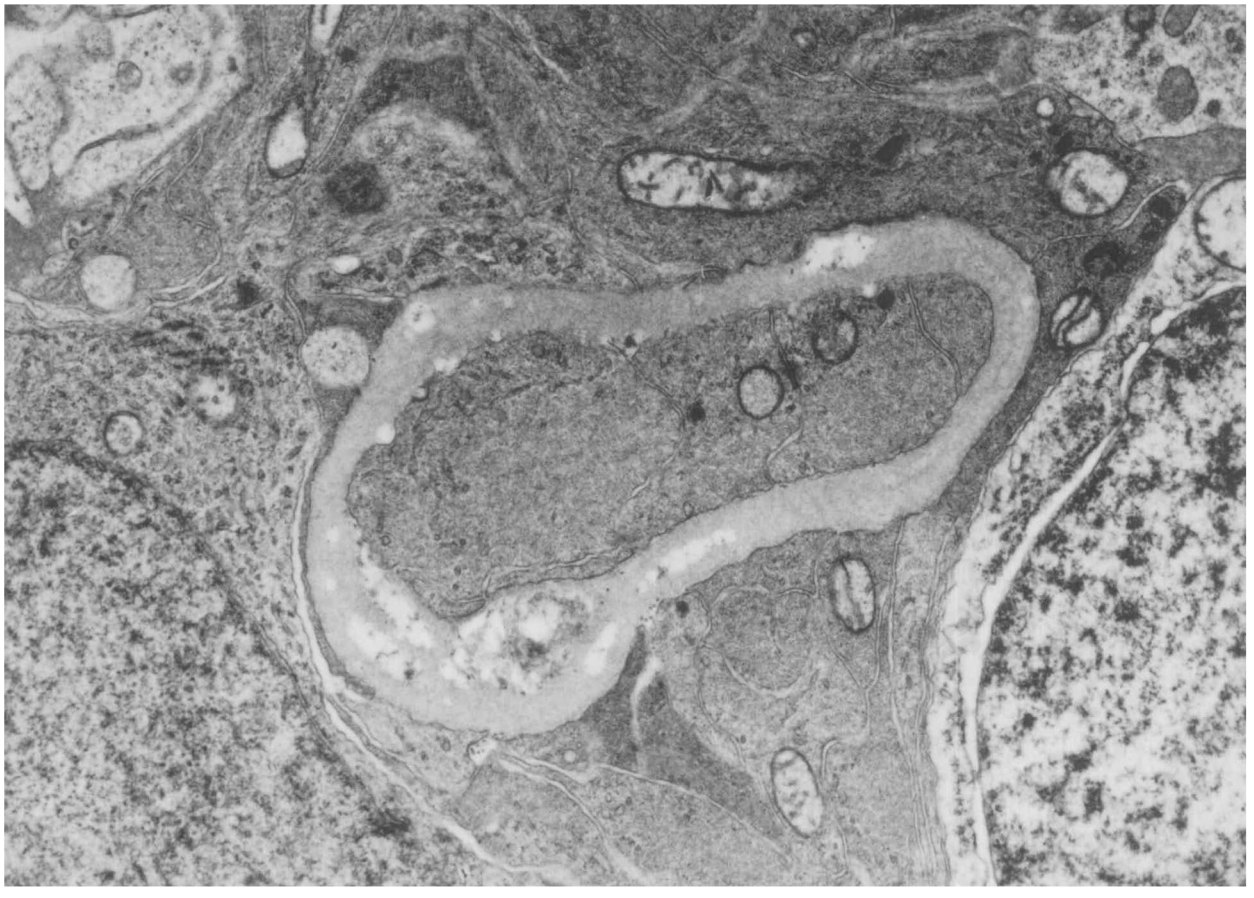

FIG. 1. Degenerate retinal capillary obliterated by glial cell processes in diabetic dog. Endothelial cells and pericytes have disappeared from capillary, and residual basement membrane contains large vacuoles and debris in place of pericyte processes. Former lumen of such atrophic capillaries is commonly filled by multiple glial processes resembling astrocytes, Müller cells (as in this case), or both. ×17,100.

a medical treatment (strict glycemic control) capable of inhibiting the onset of retinopathy in the dog.

Diabetic dogs were randomly assigned to poor glycemic control for 60 mo, good glycemic control for 60 mo, or poor control for 30 mo followed by good control for 30 mo. As expected based on previous work, retinal microaneurysms and other lesions developed during 60 mo of poor control and were clearly inhibited if good control was begun promptly (1–2 mo) after onset of diabetes. In the group assigned to good control after 30 mo of poor control, the inhibitory effect of good control on retinopathy was decidedly impaired. Retinopathy, which was absent or at an incipient stage during 30 mo of poor control, first began to appear and progressed significantly during the following 30 mo, notwithstanding good glycemic control. Good glycemic control thus proved capable of inhibiting the development of retinopathy but was found to be much less effective if postponed until many months after the onset of diabetes (14). The tendency for retinopathy to resist arrest is not readily explained but may account in part for the often disappointing results of clinical trials aimed at arresting the progression of retinopathy in diabetic patients.

Much remains to be learned about the mechanism by which retinal vascular disease in these animals is elicited and inhibited. On improvement of glycemic control with insulin in humans or dogs, numerous metabolic disorders involving lipids, proteins, and other substances are corrected in addition to carbohydrate, and the role of hyperglycemia itself tends to be blurred. New light on the importance of the blood sugars concentration has been provided by studies of animals in which hyperglycemia was achieved by elevating the blood level of D-galactose, an isomer of glucose.

Microaneurysms, pericyte ghosts, nonperfused capillaries, and other retinal lesions typical of diabetes in humans and dogs have been found to develop in nondiabetic dogs made galactosemic by a diet containing 30% D-galactose (15). Unlike diabetes, retinopathy develops in experimental galactosemia in the absence of subnormal serum insulin or elevated levels of blood glucose, nonesterified fatty acids, branched-chain amino acids, and other substances. The evidence suggests that excessive blood aldohexose itself is capable of initiating the retinopathy.

Comparison of diabetic patients with both the diabetic and galactosemic dog models might help discern which of the many biochemical and pathophysiological sequelae of hyperglycemia are unlikely to be important to the development of retinopathy. Biochemical disorders not common to both animal models and humans seem unlikely to play a critical role in the pathogenesis of retinopathy. Interest in the biochemical sequelae of hyperglycemia has become centered on the role of excessive aldose reductase activity and polyol production, excessive nonenzymatic glycosylation of proteins and nucleic acids, and impaired *myo*-inositol and phosphoinositide metabolism. The significance of excessive

polyol production in the retina is the subject of long-term studies in diabetic patients and animal models.

A postulate that retinopathy is a result of excessive polyol-pathway activity is consistent with evidence that aldose reductase or aldose reductase–like activity occurs in retinal vessels and extravascular cells and in endothelial cells and pericytes cultured from retinal vessels. Some of the functional disorders observed in hyperglycemic animals can be prevented by aldose reductase inhibitors (16). Administration of aldose reductase inhibitors reportedly inhibits basement membrane thickening in retinal vessels of rats with galactosemia or diabetes and has been said to inhibit pericyte loss in galactosemic dogs (17), although such studies neglected to establish that the inhibition of lesions occurred without reduction of hyperglycemia. In galactosemic dogs in our laboratory, tissue polyol production has been suppressed similarly by administration of high doses of an aldose reductase inhibitor, but no significant inhibition of retinopathy has been apparent through the first 42 mo of treatment. Observations are to continue through 60 mo of treatment, but the preliminary results indicate that marked suppression of polyol production and accumulation may have little influence on retinopathy (18). Whether the development of retinopathy can be inhibited by inhibition of the polyol pathway has yet to be established.

OTHER COMPLICATIONS

The development of retinopathy is associated, in diabetic animals and humans, with the development of renal lesions and thickening of epithelial and vascular basement membranes, abnormalities believed to be a consequence of inadequate glycemic control based on animal studies. Although the lesions produced by experimental galactosemia in the retina and basement membrane closely resemble those of diabetes, the kidney is an exception and retains a surprisingly normal appearance in long-term experimental galactosemia despite the development of retinopathy (19). Detailed morphometric analysis of kidney tissue has shown that glomeruli in galactosemia remain free of the hypertrophy, mesangial expansion, and glomerular obliteration typical of diabetic dogs and humans (20). Capillary basement membrane in the glomerulus, like that in the retina, nonetheless becomes significantly thickened in both galactosemia and diabetes. The observed dissociation of retinopathy from glomerulopathy in experimental galactosemia suggests that these complications differ appreciably in pathogenesis. Comparison of pathophysiological and biochemical abnormalities in diabetic patients and available animal models should help clarify the significance of the apparent difference.

ACKNOWLEDGMENTS

I thank Drs. T.S. Kern and M.D. Davis for helpful comments on this manuscript and M. Garment and M. Larson for conscientious technical assistance.

This work was supported in part by Public Health Service Research Grant EY-00300 from the National Eye Institute and by a research grant from the Juvenile Diabetes Foundation International.

REFERENCES

1. Kuwabara T, Cogan DG: Retinal vascular patterns. VI. Mural cells of retinal capillaries. *Arch Ophthalmol* 69:492–502, 1963
2. De Oliveira F: Pericytes in diabetic retinopathy. *Br J Ophthalmol* 50:134–43, 1966
3. Addison DJ, Garner A, Ashton N: Degeneration of intramural pericytes in diabetic retinopathy. *Br Med J* 1:264–66, 1970
4. Tilton RG, Faller AM, Burkhardt JK, Hoffmann PL, Kilo C, Williamson JR: Pericyte degeneration and acellular capillaries are increased in the feet of human diabetic patients. *Diabetologia* 28:895–900, 1985
5. Orlidge A, D'Amore PA: Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. *J Cell Biol* 105:1455–62, 1987
6. Sima A, Garcia-Salinas R, Basu P: The BB Wistar rat: an experimental model for the study of diabetic retinopathy. *Metabolism* 32 (Suppl. 1): 136–40, 1983
7. Ishibashi T, Tanaka K, Taniguchi Y: Platelet aggregation and coagulation in the pathogenesis of diabetic retinopathy in rats. *Diabetes* 30:601–606, 1981
8. The DAMAD Study Group: Effect of aspirin alone and aspirin plus dipyridamole in early diabetic retinopathy: a multicenter randomized controlled clinical trial. *Diabetes* 38:491–98, 1989
9. Kern TS, Engerman RL: Platelet aggregation in experimental diabetes and experimental galactosemia. *Diabetes* 33:846–50, 1984
10. Kern TS, Romang TC, Engerman RL: Erythrocyte filterability in alloxan diabetes and in experimental galactosemia. *Clin Hemorheol* 6:395–404, 1986
11. Kern TS, Engerman RL, Peterson CM: Elevated blood viscosity in alloxan-diabetic dogs and experimentally galactosemic dogs. *J Diabetic Complications*. In press
12. Brinchmann-Hansen O, Dahl-Jorgensen K, Hanssen KF, Sandvik L: The response of diabetic retinopathy to 41 months of multiple insulin injections, insulin pumps, and conventional insulin therapy. *Arch Ophthalmol* 106:1242–46, 1988
13. Engerman R, Bloodworth JMB, Nelson S: Relationship of microvascular disease in diabetes to metabolic control. *Diabetes* 26:760–69, 1977
14. Engerman RL, Kern TS: Progression of incipient diabetic retinopathy during good glycemic control. *Diabetes* 36:808–12, 1987
15. Engerman RL, Kern TS: Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* 33:97–100, 1984
16. Tilton RG, Chang K, Weigel C, Eades D, Sherman WR, Kilo C, Williamson JR: Increased ocular blood flow and ^{125}I-albumin permeation in galactose-fed rats: inhibition by Sorbinil. *Invest Ophthalmol Visual Sci* 29:861–68, 1988
17. 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* 106:1099–102, 1988
18. Engerman RL, Kern TS: Determinants of experimental diabetic retinopathy. In *Diabetes: Proc Congr Int Diabetes Fed, 13th*. Larkins RG, Ed. Amsterdam, Elsevier, 1989, p. 221–24
19. Kern TS, Engerman RL: Kidney morphology in experimental hyperglycemia. *Diabetes* 36:244–49, 1987
20. Engerman RL, Kern TS: Hyperglycemia and development of glomerular pathology: diabetes compared with galactosemia. *Kidney Int* 36:41–45, 1989