Role of Blood Flow and Impaired Autoregulation in the Pathogenesis of Diabetic Retinopathy

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Several mechanisms are implicated in the pathogenesis of diabetic retinopathy. They include biochemical, hemodynamic, and hormonal factors, all of which have an important role in the development of diabetic retinopathy. These factors are not independent of each other, but rather they interact and together are responsible for the well-known lesions of vascular occlusion, microaneurysms, hemorrhages' hard exudates, and eventually new vessel formation. Diabetes 44:603–607, 1995

In the pathogenesis of diabetic nephropathy, hyperperfusion and raised intravascular pressure have been shown to be essential to the development and progression of lesions (1). In retinal circulation, hyperperfusion early in the disease has been suggested (2), but it was only with the introduction of laser Doppler velocimetry (LDV) by Riva et al. (3) that it became possible to measure retinal blood flow in larger vessels accurately, reproducibly, and noninvasively. That increased blood flow may be of importance in diabetic retinopathy is suggested by conditions that are associated with its progression—poor diabetes control, hypertension, pregnancy, and autonomic neuropathy—all of which are characterized by increased blood flow. Other conditions, such as raised intraocular pressure, good diabetes control, and moderate stenosis of the carotid artery, which reduce or normalize retinal blood flow, have a protective effect. These observations led to the hypothesis that increased retinal blood flow, made worse by impaired autoregulation, is of pathogenic importance in the development of diabetic retinopathy.

Evidence for Increased Blood Flow in Diabetes

Animal studies. The hallmark of diabetes is elevated blood glucose levels. High blood glucose is associated with increased retinal blood flow. This has been demonstrated experimentally in nondiabetic animals. Atherton et al. (4) were the first to show that in cats an intravenous bolus of glucose, which increased blood glucose level to a mean of 26 mmol/l, significantly increased blood flow. This was maintained for a long period of time with infusion of glucose. Equimolar mannitol raised blood flow much less, and the increase was not maintained even when infusion was continued (4). Similarly, in minipigs, using LDV, a tremendous increase in retinal blood flow was recorded (from 12 to 60 μl/min) when glucose was elevated from the normal of ~6 to 22 mmol/l. This increase in blood flow was present irrespective of whether the rise in glucose was the result of bolus injection or a gradual rise by slow infusion (5). Perhaps more unexpectedly, in minipigs, high glucose levels were associated not only with increased blood flow but also with hypoglycemia (6). In experimental diabetes, when blood glucose was elevated, retinal blood flow was shown to be increased by both Tilton et al. (7), using microspheres as a measure of blood flow, and Cringle et al. (8), using hydrogen clearance polarography. The problem with diabetic animals is that it is not clear whether it is the diabetic state or the high glucose per se that is responsible for the increased blood flow.

Animal experiments are important because animals are more easily manipulated than are humans. Indeed, in nondiabetic patients, it is difficult to raise the blood glucose level >11 mmol/l without infusing somatostatin together with the glucose. This causes too many side effects for valid experiments (P. Chahal, E.M.K., unpublished observations). Patient studies. In non-insulin-dependent diabetic patients with poor control, Grunwald et al. (9) found increased blood flow. This was decreased when the high glucose levels were corrected. In a larger study of insulin-dependent diabetes mellitus (IDDM) patients with mild diabetic retinopathy, improvement of control resulted in deterioration of retinopathy in patients in whom blood flow had not decreased by 5 days after institution of good control (10). Indeed, mild background retinopathy in Grunwald et al.'s (11) studies was generally associated with increased blood flow.

To test the relationship between diabetic retinopathy and retinal blood flow, Patel et al. (12) studied 100 patients, 12 of whom were nondiabetic control subjects. In this study, those without retinopathy had blood flow similar to that in nondiabetic control subjects, while blood flow increased stepwise with increasing severity of retinopathy by 33.2% in those with background retinopathy and by 50.1% in those with proliferative retinopathy. Increased blood flow with increasing severity of retinopathy was also found by Feke et al. (13), who studied the pulsatility of retinal vessels (systolic velocity/diastolic velocity). During pregnancy, retinal blood flow increased significantly in those diabetic patients whose ret-
inopathy appeared for the first time during the pregnancy or in those whose retinopathy deteriorated during the period of the pregnancy (14).

Is the blood flow crucial? In the development of diabetic nephropathy, it is not so much the increased blood flow but rather an increase in perfusion pressure that is thought to be of importance. Is an increase in perfusion pressure also important in diabetic retinopathy? If so, is this factor of greatest importance, or is the blood flow? In their initial study on IDDM patients, Grunwald et al. (10) found that, although all of their patients had blood pressure in the normal range, both the mean blood pressure and perfusion pressure were significantly higher in those five patients whose blood flow did not decrease at 5 days and whose retinopathy showed deterioration at 6 months. There is considerable evidence that an increase in blood pressure is associated with increased prevalence of retinopathy (15-17). Importantly, Klein et al. (18), in the Wisconsin study, found that systolic blood pressure was a significant predictor of the incidence of diabetic retinopathy, while diastolic blood pressure was an important predictor of progression.

In diabetic retinopathy, perfusion pressure may be of special importance because the normal autoregulatory response of vessels is impaired.

EVIDENCE FOR ABNORMAL AUTOREGULATION IN DIABETES

Before we discuss the abnormalities, the role of autoregulation in the retinal circulation must be clarified.

Blood is distributed to the tissues by the pumping action of the heart. Its distribution to the local vascular beds is controlled by central and local mechanisms through alterations in peripheral resistance. The central mechanism is through the autonomic nervous system, while the local mechanism is autoregulation. In its strictest sense, autoregulation, as defined by Johnson (19), is the ability of the vessels to keep blood flow constant in the face of changes in perfusion pressure. Guyton and Coleman (20), believing in a wider role for autoregulation, thought that autoregulation is the ability of the vessels to control blood flow to meet the metabolic demands of the tissues. In different tissues, the major stimulus for autoregulation is different. Thus, in the kidneys, alteration in electrolyte balance is of paramount importance, while the retinal oxygen supply is a major stimulus for blood flow changes and is used for the study of autoregulation.

Why is autoregulation of special importance in the retina? While the ophthalmic artery has rich autonomic innervation, the retinal vessels themselves have no known functioning autonomic nerve supply. The control of the blood flow through the retina, therefore, relies heavily on autoregulation.

Flow in the retinal vessels is controlled by the relationship expressed by Murray’s law (because blood is a non-Newtonian fluid) (21). This law states that:

\[ Q = (P_a - P_b) \times \frac{\pi r^4}{8\eta l} \]

where \( Q \) = flow, \( P_a - P_b \) = pressure difference between the two ends of the tube, \( r \) = radius of the tube, \( \eta \) = viscosity, and \( l \) = length of the tube.

Perfusion pressure in retinal circulation is calculated by subtracting the intraocular pressure from two-thirds of the mean arterial pressure (MAP) (22). In normal retinal circulation, autoregulation keeps blood flow relatively constant, by constriction of the resistance vessels, until the MAP is raised -40% above baseline (23). At that level, it breaks down, and this is when blood flow increases. This gives rise to a marked increase in shear stress, and in hypertensive patients, this results in hypertensive retinopathy.

Abnormal autoregulation in diabetes in response to pressure changes. Hypertension has been suggested as a risk factor for diabetic retinopathy (15-17). Previously, we put forward the hypothesis that this was because in diabetics, especially when the control is poor, blood flow is increased (24). This may prevent the full expression of the normal autoregulatory adaptation of the vessels to increased perfusion pressure: vasoconstriction. Vessels will be unable to constrict adequately and, therefore, blood flow will increase even when blood pressure rise is only mild (24). To prove this hypothesis, we (24) studied normotensive control subjects and diabetic patients when their blood glucose was in the normal range (<10 mmol/l) and again when their blood glucose was elevated to >15 mmol/l. We raised the blood pressure by tyramine infusion and measured blood flow when the MAP rose to 15, 30, and 40% above baseline. Retinal blood flow did not change in nondiabetic control subjects significantly until MAP rose by 40%. In patients with well-controlled diabetes, there was a significant increase in blood flow of >20% at a 30% increase in MAP; in the same patients, when blood glucose was >15 mmol/l, blood flow was already increased by >25% when the MAP rose 15% above baseline and was increased by >100% when the pressure was raised by 40% (Fig. 1). This increase in flow was partly due to the fact that the normal vascular constriction was absent in diabetic patients, especially when glucose levels were elevated.

Abnormal autoregulation in response to oxygen breathing (oxygen reactivity). Oxygen is a powerful vasoconstrictor in retinal circulation and is used as a test for autoregulatory ability in response to changing metabolic needs. Oxygen reactivity is expressed as a percentage.

\[ \text{Oxygen reactivity} \% = \frac{\text{Difference in RBF after } O_2 \times 100}{\text{Baseline RBF}} \]

where RBF = retinal blood flow.

Using 100% oxygen, Grunwald et al. (25) found an oxygen reactivity of 61% in a normal control population; in diabetic patients, this was reduced 53% in those who had no retinopathy and to 38% in those with background retinopathy. The interesting finding in Grunwald et al.’s work (26) was that photocoagulation restored oxygen reactivity to the level of diabetic patients without retinopathy. When we examined oxygen reactivity in the patients at Hammersmith Hospital, 60% oxygen was used as the stimulus. In normal control subjects, this resulted in a 41% oxygen reactivity. In patients with background retinopathy, oxygen reactivity was reduced to 32% when the glucose was >10 mmol/l and reduced further to 21% in the same patients when their blood glucose levels were high (27). This again demonstrates that the diabetic retina is not able to control blood flow adequately.

The hypothesis that hyperglycemia specifically impairs oxygen reactivity is further evidenced by the work of Ernst et al. (28). In dogs, 100% oxygen breathing resulted in a 36.6 mmHg increase in preretinal oxygen tension but a greater increase of 63.5 mmHg when the animals were hyperglyce-
FIG 1. Percentage increase in volume flow with rise in MAP. Baseline blood flow taken as zero. NC, normal control subject; D/Lo, diabetic patient with low blood glucose; D/Hi = diabetic patient with high blood glucose.

The explanation was that the normal response of the retinal circulation in preventing hyperoxygenation is impaired by hyperglycemia. Retinal oxygen concentration is an essential stimulus for circulatory responses, since preretinal oxygen tension remained constant when the retinal perfusion was manipulated within the limits of autoregulation: reducing perfusion pressure from 150 to 50 mmHg (29). Retinal oxygen tension appears to be the homeostatic trigger for changes in retinal blood flow.

Hypertension and hyperglycemia appear to work in concert in diabetic patients in impairing autoregulation. In nondiabetic patients, hypertension impaired oxygen reactivity, which remained reduced even when blood pressure was controlled. In diabetic patients, hypertension further impaired autoregulation (compared with normotensive diabetic patients); this again was much more marked when the glucose was elevated. Controlling the blood pressure without controlling the glucose made little difference in oxygen reactivity. However, when both blood pressure and blood glucose were controlled, this improved nearly to the level in the normotensive diabetic patients (27).

The studies suggest that in diabetes, especially in the presence of hyperglycemia, blood flow is increased, and normal control of blood flow is lost. An increase in perfusion pressure will worsen this, and if marked, a torrential increase in blood flow is the result.

WHY IS INCREASED BLOOD FLOW DETRIMENTAL TO DIABETIC RETINOPTHY?

The hemodynamic interface between blood flow and the vessel is at the endothelium. It is here that the potential for damage by abnormal hemodynamics exists. The foremost factor to consider is the effect of shear stress—the viscous drag that exists between the laminae of blood flow closest to the vessel wall and the vessel wall itself. The force tangential to the vessel wall is known as the shear stress.

\[ \text{Wall shear stress} = \frac{4\eta Q}{\pi r^2} \]

where \( \eta \) = viscosity, \( Q \) = blood flow, and \( r \) = radius of vessel.

FIG 2. Fluorescein angiogram of right macular area showing area of nonperfusion. Arrows point to dilated capillaries.
Increased shear stress in large vessels like the aorta results in increased permeability of the vessel wall to proteins and lipids and is thought to be of importance in atheroma formation (30). In experimental animals, the vessels tend to remodel themselves to maintain a constant shear stress (31). Increased shear stress has many effects, which are harmful in the context of diabetic retinopathy. Thus, it induces the stimulation of DNA in endothelial cells to increase their turnover (32), and it also increases both proliferation and migration of endothelial cells (33).

In vitro increased shear stress has an effect on both vasodilators and constrictors. The production of the vasoconstrictor endothelin I is reduced (34). A high glucose level has a similar effect. Even when endothelin is present, high glucose will inhibit its action in pericytes, where endothelin is crucial in protein kinase C production (35). High glucose, by its direct toxic effect on pericytes (36), will also inhibit contraction of small vessels, impairing autoregulation and worsening the vicious circle set up initially by high glucose levels. At the same time, increased shear stress increases the production of vasodilators, such as prostacyclins and nitric oxide (37), with the increased vasodilation allowing an additional increase in flow and impairment of autoregulation.

Increased shear stress increases matrix production by the endothelial cells; this may be of importance in one of the hallmarks of diabetic microangiopathy, basement membrane thickening (35,38). Tissue-type plasminogen activator secretion is increased (30), which may lead to the increased thrombotic tendency in diabetic microcirculation. Once vessels are occluded, hypoxia will cause dilatation of neighboring vessels (Fig. 2) and increased secretion of, and responsiveness to, growth factors (40), leading to new vessel growth. Vasoactive factors, such as hypoxia, will dilate the remaining vessels, and the increased blood flow through them will further compromise the retinal circulation. Whether the effects of hypoxia could be present without vascular occlusion has not been clearly established. Williamson et al. (41) believe that increased flux through the polyol pathway, induced by high glucose, causes an imbalance between pyruvate and lactate levels, thereby resulting in pseudohypoxia, which could also increase blood flow and be a direct cause of glucose-induced hyperperfusion in diabetes.

Whether increased blood flow alone or in addition to increased intracapillary pressure is responsible for the damage to the capillary wall has not been clearly established. If postcapillary resistance is increased, tangential pressure on the vessel wall would increase; this could be one of the reasons for increased permeability seen in diabetes, although breakdown of the tight junctions between endothelial cells is probably a late phenomenon (42). Evidence for increased intracapillary pressure comes from the work of Nehls and Drenckhahn (43), which suggests a heterogeneity of pericytes in the microcirculation (postcapillary venules). They noted that pericytes near the venular end of capillaries contain both myosin and smooth muscle cell actin. Muscle cell actin is absent in other parts of the capillaries. Thus, an increase in intracapillary pressure could arise from preferential action of these pericytes.

In conclusion, a working model for the pathogenesis of diabetic retinopathy can be set up (Fig. 3). Hyperperfusion, or increased blood flow, is initiated by high glucose levels but made worse by high blood pressure and impaired autoregulation. The high glucose level has a direct deleterious effect on both pericytes and endothelial cells, and the increased blood flow results in a marked increase in shear stress. The effects of this increase are further damage to the vessel wall, occlusion of some vessels, hypoxia, and ischemia, resulting in proliferative retinopathy.

Much remains to be discovered about the interaction of the individual factors and the underlying cellular and biochemical changes. But the early steps in the evolution of diabetic retinopathy are now closer to being defined and, eventually, effectively prevented.

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