The Blood Vessel, Linchpin of Diabetic Lesions

Gérard E. Plante, Jude Alfred, and Mouna Chakir

The morbidity and mortality associated with diabetes mellitus are essentially related to the vascular lesions that develop over time in this condition. Both the macrocirculation and microcirculation are involved, and as a consequence, vital organs such as the brain, retina, heart, and kidney and the limbs become damaged. Because microalbuminuria represents the earliest and probably most sensitive indication of endothelial dysfunction in diabetes mellitus, the results of pharmacologic intervention with angiotensin-converting enzyme inhibitors, which treat glomerular hypertension were the first indication of potential beneficial effects in reducing diabetic nephroplasty. The nature of endothelial dysfunction related to diabetes is probably not homogeneous, since microcirculation networks are affected at different periods and with variable intensity. This appears to be the case for the aorta, the heart, segments of the digestive tract, the skin, and the skeletal muscle, the largest consumer of insulin. Although the aorta and large arteries contain a small portion of the total blood volume, their distribution of blood flow (pulse pressure) to peripheral organs may affect endothelial function in the microcirculation. Changes in the structure of conduit arteries, partly responsible for the alteration in compliance characteristics, could well be related to the way these arteries are fed by the vasa vasorum system. This report describes a new in vitro approach to examine capillary permeability in normal and alloxan-induced diabetic rabbits. Preliminary results indicate that the size of terminal arterioles of the vasa vasorum (increased diameter) and the capillary permeability to albumin (markedly enhanced) in this specialized network are profoundly affected in the thoracic aorta obtained from diabetic animals. Albumin extravasation into the interstitial fluid compartment of the aorta is likely to lead to structural and physicochemical changes: in fact, removal of interstitial macromolecules via lymphatic drainage is poor in the blood vessel wall of large arteries. This experimental approach is likely to be useful in the exploration of medications affecting the structure and function of conduit vessels.

THE BLOOD VESSEL should be regarded as the primary target organ in diabetes mellitus, as well as in arterial hypertension, and likely several other systemic diseases. In the diabetic patient, both the macrocirculation and microcirculation are affected, and as a consequence, a number of vital organs, which contributes to the morbidity and mortality of this common condition.[1,2] Several decades after the discovery of insulin, diabetes mellitus remains a major challenge for the physician because full protection of the blood vessel has not yet been achieved. Restoration of coronary or renal blood flow by balloon vascular interventions certainly contributes to functional restoration of these vital organs. Hemodialysis and kidney transplantation are also responsible for prolonging the life of diabetic patients.[3] However, peripheral vascular complications requiring amputation or progressive retinopathy or neuropathy impact the quality of life of these patients, despite the fact that they live much longer nowadays. The definition of "vital organ" may be quite different for the scientist and the patient. Since humans are an ambulatory animal species, the foot is a critical vital organ.

The pathophysiological relationships between large conduit vessel, resistance artery, and capillary dysfunction have not been clearly established in diabetes mellitus. Moreover, the venous component of the vasculature, particularly the postcapillary venules that play a critical role in establishing Starling forces in the microcirculation,[4] has been almost completely neglected by investigators. An alteration in large conduit artery compliance due to interstitial remodeling (collagen to elastin ratio), disturbances in resistance artery reactivity (early vasodilatation and enhanced flow, and late increased contractility and hypertension), and enhanced plasma extravasation due to capillary dysfunction have been described by several groups in experimental models of diabetes mellitus,[4-7] but no unified pathophysiological view has been proposed. As a consequence, the therapeutic approach to counteract diabetic vasculopathy remains empirical, targeting either the polyols,[8] the advanced glycation end products,[9] or the renin-angiotensin system,[10] with limited if not relatively poor success.

We believe that damage to any target organ results from defective delivery of vital substrates from the blood to the cellular mass and reduced microcirculation removal of toxic metabolites resulting from cellular activity for elimination by the excretory organs.[11] Since the largest fraction of the blood volume is contained in capillaries and postcapillary venues, it is evident that these are the sites where alterations in fluid and solute movement, including macromolecules, are likely to affect the interstitial space both in size and in physicochemical characteristics, with this fluid volume occupying a strategic position between microcirculation networks and the cellular mass that is different from one organ to the other.[12] Because there is evidence that the large conduit vessels play an important role in the pathophysiology of diabetes mellitus, not only upstream toward the left ventricle but also downstream toward the microcirculation networks,[13] we recently undertook studies on the microcirculation network of the thoracic aorta, particularly the capillary permeability within the vasa vasorum system, in the alloxan-induced model of diabetes mellitus in the rabbit.

MATERIALS AND METHODS

Forty-six rabbits weighing between 1.6 and 2.0 kg were used in the experiments. Thirty were administered alloxan (25 mg/kg intravenously) and evaluated weekly for blood glucose, which reached steady "diabetic" values (20 to 30 mmol/L) between 2 and 4 weeks later. Twenty-two of these survived as stable "diabetic" animals. Sixteen were sham-injected with normal saline, remained normoglycemic, and

From the Departments of Medicine (Nephrology) and Pharmacology, University of Sherbrooke, Sherbrooke, Quebec, Canada.

Submitted August 20, 1998; accepted August 20, 1998.

Address reprint requests to Gérard E. Plante, MD, PhD, 3001 12th Ave N, Sherbrooke, Quebec J1H 5N4, Canada.

0026-0495/99/4803-0024$10.00/0

survived. All rabbits were killed, and the thoracic aorta was carefully removed. Precautions were taken to avoid injury to the fat tissue around the aorta, irrigated by a significant number of vasa vasorum branches.

The 10-cm segment of the thoracic aorta was placed in a thermoregulated (37°C) organ bath, where all intercostal arteries but one were carefully ligated at approximately 2 mm from their origin under binocular microscopic observation (×10). Vasa vasorum branches originate from intercostal arteries in the thoracic segment of this major conduit vessel.[14] The one remaining intercostal artery, usually in the middle portion of the thoracic aorta, was canulated with a PE10 tube connected to a microsyringe containing a normal Krebs solution, which was used for eventual injection of test substances. The two terminal portions of the thoracic aorta were also canulated with appropriate PE tubing (6 to 8 mm). Continuous peristaltic perfusion with a normal Krebs solution containing albumin-bound Evans blue at a concentration (4 mg/dL) far below the albumin saturation point[15] was started at pulsatile pressure averaging 100/70 mm Hg.

Direct observation of the vasa vasorum branches, shown as thin blue lines, as well as baseline Evans blue capillary leakage, seen as pale blue patches, was made and color photographs were taken. Changes in the size of blood vessels and Evans blue leakage patterns were measured from these photographs, which are magnified 10 times. New sites of Evans blue leakage as a function of time could also be measured. To maintain validity, an experimental preparation had to meet the following criteria: five identical measurements of arteriolar caliber and extravasation patches in two different fields containing at least three vasa vasorum arteriolar branches. Sixty-five percent of the thoracic aortae obtained from control normoglycemic rabbits and 50% obtained from diabetic rabbits were considered valid for vascular parameter measurements. Time-control experiments showed that both the size of arterioles and the size and number of capillary leakage sites remained constant over periods of 60 to 90 minutes.

RESULTS

The results of these measurements are summarized in Table 1. The diameter of first-order branches of the vasa vasorum was significantly larger in diabetic animals versus the normoglycemic controls ($P < .01$). However, the most impressive changes were found in the microcirculation networks. Not only did the number of extravasation sites increase significantly ($P < .01$), but the diameter of those sites was markedly enhanced ($P < .001$), to the point that in some spots coalescence of the blue patches occurred, resulting in massive areas of Evans blue–bound albumin.

A representative view of the vasa vasorum system obtained from a normal rabbit and a diabetic animal is illustrated in Fig 1. Small arterioles are indicated (arrows) in the normal aortic segment, and baseline extravasation patches of Evans blue are shown (n = 7). There was no major change in the size or in the number and diameter of Evans blue patches over the 30-minute observation period. The vasa vasorum arteriolar system and the pattern of Evans blue extravasation patches observed in the thoracic aorta obtained from a 4-week diabetic rabbit differ markedly, as illustrated in the bottom portion of the Figure. The arteriolar diameter measured in the diabetic animal (arrows) is approximately threefold the diameter obtained in normoglycemic control rabbits. In addition, not only is the number of extravasation patches higher (n = 12) in diabetic versus control rabbits, but several of these patches, especially in the lower left and middle right areas of the photographs, spread out with time, producing large coalescence regions that almost cover the entire external surface of the blood vessel.

Table 1. Morphologic Characteristics of the Vasa Vasorum System in Thoracic Aorta Obtained From Normal and Diabetic Rabbits

Characteristic	Control	Diabetic
First-order arteriole diameter (μm)	120 ± 12	236 ± 37
Evans blue extravasation spots		
Number (per cm²)	3 ± 1.2	7 ± 2.4
Diameter (mm)	4 ± 0.6	12 ± 1.7

NOTE. Results are the mean ± SEM.

DISCUSSION

The present report introduces four presentations to describe the mechanisms involved in the development of diabetic vascular complications, excluding the kidney, which has already been described. The reduction in the progression of renal glomerular failure documented in microalbuminuric diabetic patients treated with angiotensin-converting enzyme inhibitors[16,17] may open new avenues in the management of other vascular complications encountered in this disease. The positive effect of angiotensin-converting enzyme inhibition in remodeling the media of extrarenal resistance arteries, in particular by reducing smooth muscle hypertrophy, thereby improving blood flow to other vital organs such as the retina, confirms the key role of resistance arteries in the development of diabetic vascular morbidity. These observations should by no means minimize the other important segments of the vasculature such as the large conduit arteries and the capillaries of microcirculation networks.

The aorta represents an excellent model for the simultaneous study of macrocirculatory and microcirculatory events in health and disease. In fact, this organ is the prototype of conduit arteries, and is fed by a special microcirculatory network, the vasa vasorum system. Therefore, the relationship between alterations in the microcirculation and structural-functional changes in the aortic wall can be best studied in the experimental model described in this report. The physiology and pathophysiology of the vasa vasorum system have been poorly studied, yet the anatomy of this specialized circulatory system, including the aortic wall,[18,19] has been relatively well described.

The results presented herein confirm the fact that a major component of diabetic complications includes endothelial dysfunction characterized by enhanced plasma extravasation in different organs.[4-7] The thoracic aorta is another autonomous and vital organ in which the microcirculation was abnormal, showing similar endothelial dysfunction characterized by large areas of albumin extravasation. These findings represent an original contribution to the pathophysiology of diabetic vasculopathy. The arteriolar component of the vasa vasorum system, representing the precapillary site of the adventitial microcirculation, was dilated in the diabetic animals, as previously described for the afferent arteriole in the glomerular network.[8] This phenomenon can contribute to plasma extravasation by increasing local capillary hydrostatic pressure. In addition, the permeability characteristics of endothelial cells in capillaries and postcapillary venules are also affected by the disease process through cell-cell dysjunction mechanisms,[20] the structural basis for macromolecular leakage. Together, these abnormalities lead to interstitial accumulation of albumin. Because of the poorly

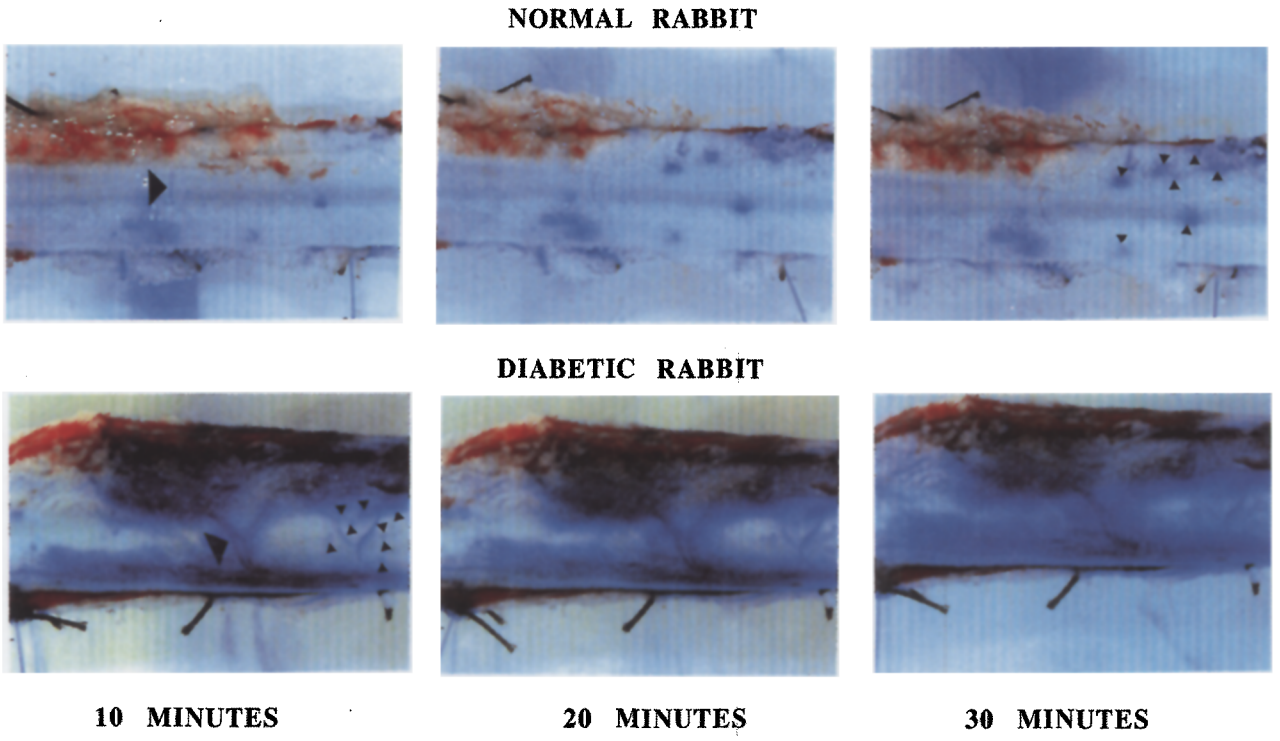

Fig 1. Representative photographs of the vasa vasorum system from a normal rabbit and a 4-week-old alloxan-induced diabetic rabbit. Values correspond to the periods following Evans blue injection via the ligated intercostal artery (PE tubing appears in the right and left bottom quadrants for the normal and diabetic animals, respectively).

developed lymphatic system in the aortic wall,[18] albumin and likely other macromolecular material become trapped in the interstitial space, changing the geometry and physicochemical properties of this strategic fluid compartment.[11,21,22]

It is conceivable that these pathophysiologic events contribute to the remodeling process of the aortic wall, leading to alterations in the dynamic properties of this large conduit vessel.[13] It is also possible that interstitial trapping of albumin impairs the clearance of macromolecules that normally travel from the subluminal area through the media of large arteries.[23] A similar dysfunction of macromolecular clearance through the wall of conduit vessels has been advocated in the atherogenesis process.[24] Exploration of the eventual actions of pharmacologic agents on the vasa vasorum system in experimental diabetes mellitus will certainly be of potential clinical interest with respect to target organ protection.

REFERENCES

1. Klein R, Klein BE, Moss SE: Epidemiology of proliferative diabetic retinopathy. Diabetes Care 15:1875, 1992

2. Bojestig M, Arnqvist HJ, Hermansson G, et al: Declining incidence of nephropathy in insulin-dependent diabetes mellitus. N Engl J Med 330:15-18, 1994

3. Arbus GS, Fenton SSA, Jeffery JR, et al: Le Registre Canadien des Insuffisances et Transplantations d' Organes. Rapport de la Fondation Canadienne du Rein. Montreal, Canada, National Kidney Foundation, 1987

4. Chakir M, Regoli D, Plante GE: B1 Bradykinin Receptor Blockade May Prevent Diabetic Vascular Morbidity. Amsterdam, The Netherlands, European Dialysis and Transplantation Association, 1996.

5. Chakir M, d'Orleans-Juste P, Plante GE: Neutral endopeptidase inhibition, a new approach in the exploration of diabetic vasculopathy. Eur J Pharmacol 285:11-18, 1995

6. Chakir M, Plante GE: Endothelial cell dysfunction in diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 54:45-51, 1996

7. Parving HH, Rossing N, Sander W: Increased metabolic turnover rate and transcapillary escape of albumin in long-term juvenile diabetics. Scand J Clin Invest 35:59-64, 1975

8. Bank N, Coco M, Aynedjian HS: Galactose feeding causes glomerular hyperfusion: Prevention by aldose reductase inhibition. Am J Physiol 256:F994-F999, 1989

9. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 87:432-458, 1991

10. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int 19:410-417, 1981

11. Plante GE, Chakir M, Lehoux S, et al: Disorders of body fluid balance: A new look into the mechanisms of disease. Can J Cardiol 11:788-802, 1995

12. Wigg H, DeCarlo M, Sibley L, et al: Interstitial exclusion of albumin in rat tissues measured by a continuous infusion method. Am J Physiol 263:H1222-H1233, 1992

13. Asmar RG, Pannier B, Santoni JP, et al: Reversion of cardiac hypertrophy and reduced arterial compliance after converting enzyme inhibition in essential hypertension. Circulation 78:941-950, 1988

14. Woerner CA: *Vasa vasorum* of arteries, their demonstration and distribution, in Lansing AI (ed): The Arterial Wall. Baltimore, MD, Williams & Wilkins, 1959, pp 1-14

15. Lehoux S, Plante GE, Sirois MG, et al: Phosphoramidon blocks big-endothelin-1 but not endothelin-1 enhancement of vascular permeability in the rat. Br J Pharmacol 107:996-1000, 1992

16. Mathiesen ER, Hommel E, Giese J, et al: Efficacy of captopril in postponing nephropathy in normotensive insulin dependent diabetic patients with microalbuminuria. BMJ 303:81-84, 1991

17. Mogensen CE: Long-term antihypertensive treatment inhibits progression of diabetic nephropathy. BMJ 285:685-689, 1982

18. Lang J: Über die vaskularization der wand und des einbaugewebes mittelgrossergefässe des unterschenkels. Z Anat Entwicklungsgeschichte 122:482-488, 1961

19. Schöenenberger F, Müller A: Überdie vaskularization der rinderaortenwand. Helvet Physiol Pharmacol 18:136-142, 1960

20. Wysolmerski RB, Lagunoff D: Involvement of myosin light-chain kinase in endothelial cell retraction. Proc Natl Acad Sci USA 87:16-22, 1990

21. Aukland K, Nicolaysen G: Interstitial fluid volume: Local regulatory mechanisms. Physiol Rev 61:556-643, 1981

22. Comper WD, Laurent TC: Physiological function of connective tissue polysaccharides. Physiol Rev 58:255-315, 1978

23. Caro CG, Lever MJ: Factors influencing arterial wall transport. Biorheology 21:197-202, 1984

24. Colton CK, Friedman S, Wilson DE, et al: Ultrafiltration of lipoproteins through a synthetic membrane. Implications for the filtration theory of atherosclerosis. J Clin Invest 51:2472-2477, 1972