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Effects of different antihypertensive treatments on morphologic progression of diabetic nephropathy in uninephrectomized dogs

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Effects of different antihypertensive treatments on morphologic progression of diabetic nephropathy in uninephrectomized dogs. We previously reported the renal hemodynamic effects of different antihypertensive regimens in uninephrectomized, alloxan-induced, diabetic (DM) beagle dogs following one year of treatment. Dogs were prospectively randomized to one of five groups ($N = 26$): nondiabetic controls, Group I; dogs with DM on no antihypertensive drugs, Group II; dogs on a converting enzyme inhibitor, lisinopril (L), Group III; dogs on a calcium antagonist, TA3090 (diltiazem-like), Group IV; and dogs on a combination of each drug, in reduced doses, Group V . The current paper extends our previous studies by describing the morphologic changes that occurred within each group of dogs studied. More than 100 glomeruli from the renal cortex of each dog were evaluated for increases in mesangial volume fraction (V_v), glomerulosclerosis (GS) and arteriolar hyalinosis. The interstitium was also evaluated for associated changes. Increases in V_v were attenuated in all treated groups (0.28 ± 0.04, DM alone versus 0.16 ± 0.05 L; 0.21 ± 0.07, TA-3090; 0.19 ± 0.06 $\mu m^2/\mu m^2$, L + TA 3090; $P < 0.05$) compared to untreated DM. An attenuated increase in V_v also correlated with a blunted rise in proteinuria in Groups III ($r = 0.79$) and V ($r = 0.81$) but not Group IV ($r = 0.29$). Development of focal GS was blunted in all treated groups; however, global GS was fourfold greater in Group IV compared to untreated DM. The degree of interstitial fibrosis also correlated with the degree of global GS. These data support the concept that both a converting enzyme inhibitor and heart rate lowering calcium antagonist attenuate morphologic progression of diabetic renal disease. When used alone, however, the calcium antagonist increases development of global GS, an effect that appears to be independent of blood pressure control.

Diabetic nephropathy is a major cause of end-stage renal disease in the United States [1, 2]. Reduction of both elevated arterial pressure and albuminuria, which portends this disease process, attenuates progression of renal dysfunction and reduces mortality [3–7]. A maintained reduction in arterial pressure, however, has not been shown to stop progression of diabetic renal disease [5, 8]. Thus, elevation in arterial pressure is not the only factor that contributes to renal demise from diabetes.

Numerous studies document that morphologic changes occur in the kidneys of diabetic patients prior to any clinical findings of microalbuminuria or hypertension [9–11]. Specifically, these studies demonstrate an increase in the matrix component of the mesangium as an early morphologic manifestation of diabetic nephropathy. They further illustrate that focal glomerulosclerosis (GS) occurs significantly after the presence of these mesangial changes. Unfortunately, focal GS is not an early finding of this disease in humans. Focal GS, however, is the earliest morphologic change in the most common animal model studied, the diabetic rat [12–16]. This is due to a failure of the matrix component of the mesangium to continuously expand beyond that which occurs in the very early stages of the disease. Diabetic beagle dogs, however, may be a better model to study this aspect of glomerular disease. These dogs manifest continuous increases in the matrix component of the mesangial volume fraction for at least five years after the induction of diabetes [17–18].

Recent studies in diabetic rats have compared the effects of various antihypertensive agents on morphologic changes associated with disease progression. In general, these studies demonstrate an attenuation in both focal and global GS with most agents [12–14]. Moreover, these investigations document that converting enzyme inhibitors have the greatest effect on attenuating progression to GS. Unlike other antihypertensive agents, most studies in either diabetic or hypertensive rats with dihydropyridine calcium antagonists (nifedipine-like) fail to show any effect on the development of focal GS [14, 19–22]. In addition, these agents have been associated with an increased development of global GS [19, 21]. Moreover, there are no studies in an appropriate animal model that have examined the effects of heart-rate lowering calcium antagonists or converting enzyme inhibitors on expansion of the matrix component of the mesangium. One recent study in diabetic, normotensive rats evaluated the effects of both therapy with three drugs (diuretic, hydralazine, reserpine) and converting enzyme inhibition on mesangial expansion [23]. These investigators found no significant effect of either therapeutic regimen on the expansion of mesangial volume. They attributed this lack of effect to poor glycemic control rather than changes in arterial pressure.

We recently conducted a randomized, prospective study in unilaterally nephrectomized, alloxan-induced diabetic beagle dogs that assessed the renal hemodynamic effects of a converting enzyme inhibitor, lisinopril, or heart-rate-lowering calcium antagonist, TA 3090 (diltiazem-like), alone and in combination

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following one year of treatment [24, 25]. The specific methodology for this renal hemodynamic study has been published elsewhere [25]. To summarize the salient features of this study: intraarterial blood pressures were assessed weekly to achieve a goal mean arterial pressure of < 106 mm Hg. Thereafter, blood pressures were assessed monthly. Fasting blood glucose values were evaluated daily. No significant differences were present in any of the treated groups for either blood pressure control or fasting blood glucose values over the duration of the study. The kidneys of all animals, except those on combination therapy, were micropunctured at one year. There was a significant increase in both glomerular capillary pressure and volume in the untreated diabetic group compared to controls. Treatment with lisinopril reduced both the glomerular capillary pressure and volume. TA 3090, however, only reduced glomerular volume. Lastly, the increase in urinary protein excretion, measured by a spectro-photometric method, was significantly blunted by both agents. The greatest effect on the rise in proteinuria, however, was seen in the group that received both lisinopril and TA 3090.

This paper extends our previous results by focusing on morphologic changes that occurred in the kidney of each dog following one year of treatment. The principal objective of this study was to evaluate the changes in mesangial volume fraction and degree of focal and global GS that developed within each group of dogs in the presence and absence of arterial pressure reduction with different classes of antihypertensive drugs. In addition, we also assessed tubulointerstitial changes, a morphologic parameter that has been suggested to antedate the development of mesangial volume expansion in this disease. The results of these analyses form the basis of this report.

Methods

General

The methods discussed in this section apply solely to evaluations of renal tissues and related procedures. Light microscopic and ultrastructural quantitative analysis were performed on kidney samples from 26 adult beagle dogs with a mean weight of 13.0 ± 0.3 kg. All dogs underwent right nephrectomy. Diabetes mellitus was induced in 20 dogs by intravenous injection of alloxan, at a dose of 60 mg/kg body weight. The left renal artery was clamped throughout the infusion period, and for an additional five minutes to prevent acute tubular necrosis from alloxan [23]. All dogs were fed a diet which contained 15.4% protein and 0.21% sodium (Hill's Pet Products, Topeka, Kansas, USA). A control group of uninephrectomized, nondiabetic dogs, Group 1 (N = 6), were studied at the same time and in a similar manner to the other groups. All diabetic dogs received daily subcutaneous insulin therapy throughout the one year duration of the study. Group II (N = 6) received insulin alone; Group III (N = 5) received the converting enzyme inhibitor (CEI), lisinopril, orally; Group IV (N = 5) received oral administration of the calcium antagonist TA 3090, and Group V (N = 4) received the combination of a reduced daily oral dose of both lisinopril and TA3090. Insulin dosage was adjusted to achieve a goal plasma glucose concentration between 350 to 450 mg/dl immediately prior to the subsequent insulin injection. Similarly, the once daily dosages of antihypertensive agents were adjusted to maintain the mean arterial pressure between 95 and 105 mm Hg.

Histologic specimens and tissue preparation

At the end of one year, the dogs were sacrificed to obtain samples of the renal cortex. Tissue specimens for light microscopy were fixed in 4% buffered formalin mixed with 2% gluteraldehyde (Trump's fixative) for paraffin processing. A single two to three μm thick section, stained with periodic acid Schiff (PAS), was available for light microscopy on each case. At least 100 glomeruli were present in each section. Several 0.1 mm cubes of renal cortex also fixed in Trump's fixative were processed by standard electron microscopy techniques with the addition of in-block staining with 2% uranyl acetate prior to dehydration. The processed samples were embedded in spur. One μm sections thick were stained with toluidine blue to select adequate representative tissue samples for ultrastructural stereologic examination. The most centrally located glomerulus in the block was chosen for thin sections. If three glomeruli could not be identified in three separate blocks, blocks with more than one glomerulus were then selected. For each case, two to three glomeruli were analyzed. Thin sections were collected on formvar coated, single-slot grids, then stained with 2% uranyl acetate and lead citrate. Sections were examined and photographed by a JEOL 2000EX electron microscope (Tokyo, Japan). Additionally, sections from random kidneys removed at the inception of the study from each dog were prepared in a similar manner. These sections were used to determine the normal range of mesangial volume in these dogs.

Light microscopy

Sections were divided by two perpendicular lines into four quadrants to facilitate glomerular counting. Based on the location and distribution of sclerosis within the glomerular tuft, three patterns of GS were identified and analyzed. The first pattern was hilar sclerosis [26]. This was characterized by expansion of the matrix component of the mesangium into the vascular pole and the hilar region of the glomerular tuft with frequent sclerosis and hyalinosis of the periglomerular arterioles (Fig. 1A). The second pattern was focal segmental GS that involved the peripheral segments of the glomerular tuft resulting in collapse and occlusion of the peripheral capillaries, and adhesion to Bowman's capsule (Fig. 1B). The third pattern was diffuse GS where the entire glomerular tuft was sclerosed (Fig. 2).

Determination of tubulointerstitial changes

The extent of interstitial fibrosis and tubular atrophy was determined semiquantitatively by examining the PAS stained sections of renal cortex previously divided by two perpendicular lines into four quadrants. The degree of tubular changes and interstitial fibrosis was graded from 0 to 3+ (0 = no changes; 1+ = changes affecting <20% of the sample; 2+= changes affecting 20 to 40% of the sample; 3+ = changes affecting > 40% or the sample). All renal biopsies were analyzed by the same renal pathologist who was unaware of what treatment each dog received.

Determination of mesangial volume fraction

Photomontages of the glomerular profiles were made by taking a series of micrographs using previously described techniques [27, 28]. The micrographs were printed at a final

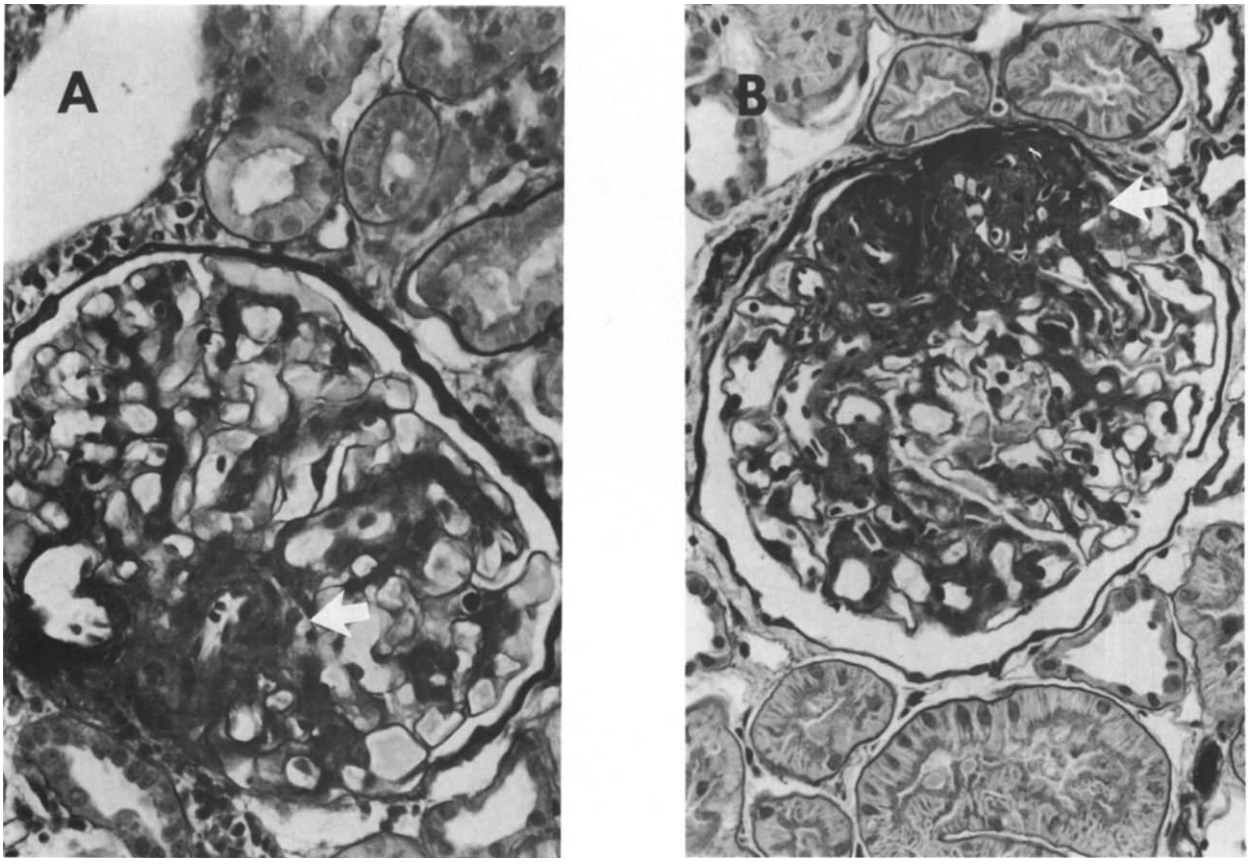

Fig. 1A. *Glomerulus from an untreated diabetic dog (group II) showing moderate sclerosis of the glomerular arterioles and the mesangium at the glomerular hilum.* **B.** Glomerulus from a diabetic dog treated with TA 3090 illustrates the segmental enlargement of the mesangial volume, obliteration of the peripheral capillary loops and capsular adhesion. (PAS; original magnification × 200).

magnification of × 12,000. Magnification was monitored by using a carbon calibrated grid (1,134 lines/mm). An average of 60 micrographs was needed to reconstruct a single glomerulus. The glomerular profile was delineated by the least convex polygon, whose points connected the epithelial side of the basement membrane of the most distal capillary loops. A square lattice grid was then placed over the photomontage for point counting. The grid was composed of evenly spaced fine and coarse points at a 1:4 ratio, (Fig. 3). The distance between each coarse point was 6 mm, and between each fine point was 3 mm. The following equation was used to calculate the fractional mesangial volume (V_v):

$$V_v = EP_f/EP_c \times 4$$

where EP_f is the sum of fine points falling on the mesangial areas, and EP_c is the sum of coarse points falling within the polygon outlining the glomerular profile.

Specific components of the mesangium such as cells and matrix were not evaluated separately or quantitated to assess their contribution to the mesangial volume fraction. We assumed, however, that the accepted convention by light microscopy, established in normal renal tissue, of ≤ 3 cells per mesangial area was valid [29]. Moreover, the renal biopsy slides of each dog were reviewed at the end of the one year study. We did not find more than three cells per mesangial area in any of the specimens examined. Therefore, since this was within the normal range, any expansion of the mesangial volume fraction was assumed to be due to increased matrix production.

Statistical analysis

All data are expressed as mean ± standard error of the mean. Differences between groups for expansion of the mesangial volume fraction were assessed using a Student's *t*-test for independent groups. In addition, differences in fractional mesangial volume among all five groups at one year were tested using an analysis of variance with Bonferroni's method for comparing multiple groups [30]. Since we reviewed a large number of glomeruli from each renal cortex (>50) we did not do an arcsine transformation. Additionally, estimates of the degree of interstitial fibrosis and GS in the kidneys of the dogs studied at one year versus those initially removed at the beginning of the study were assessed by a Mann-Whitney test for nonparametric data. Lastly, we used multiple regression analysis to compare slopes of the lines that demonstrate the relationship between changes in urinary protein excretion and mesangial volume fraction. Statistical significance was designated at a level of $P < 0.05$ for all analyses.

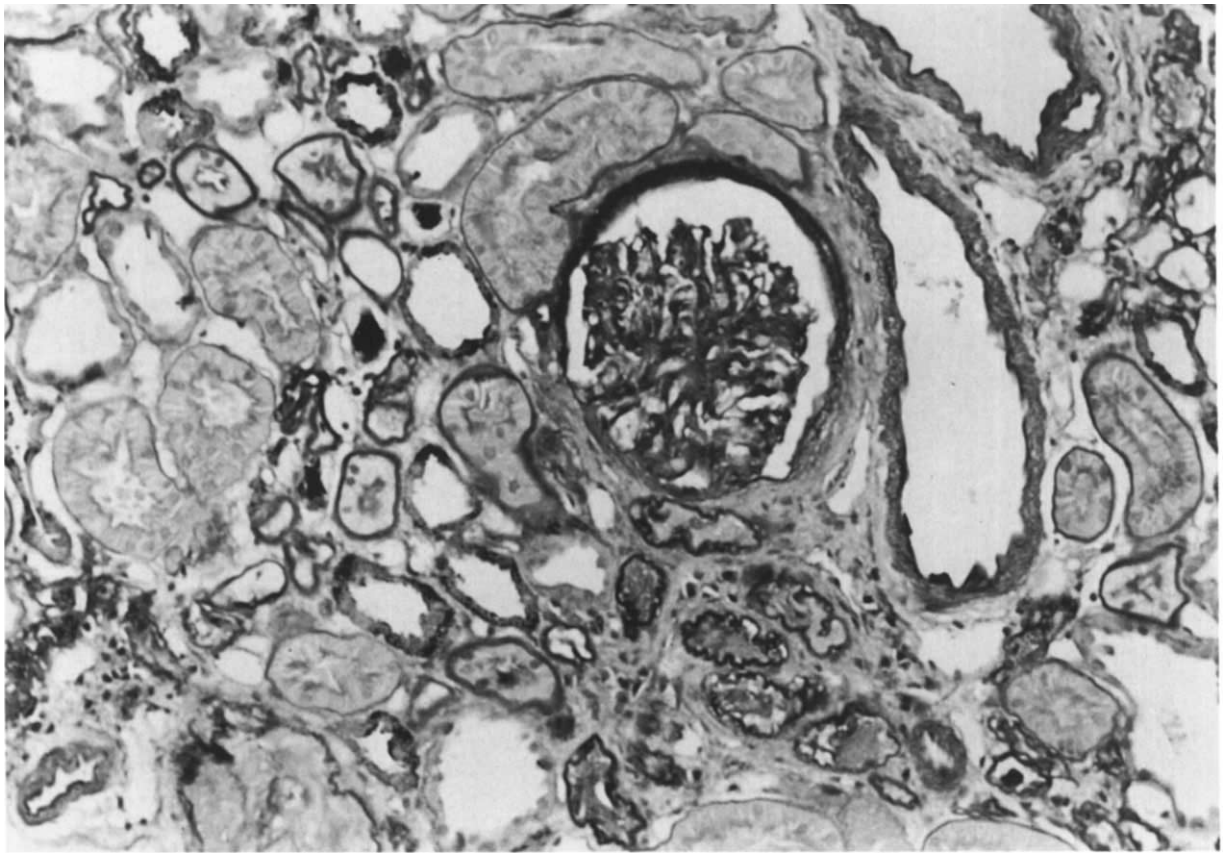

Fig. 2. *Globally sclerosed glomerulus demonstrating glomerular tuft shrinkage with mesangial volume increase and capillary obliteration.* Note the surrounding interstitial fibrosis and tubular atrophy (PAS; original magnification × 100).

Results

Light microscopy

Untreated diabetic dogs (Group II) demonstrated a substantial increase in the mean percentage of glomeruli with hilar or focal GS compared to the control (Group I) animals (Figs. 4 and 5). Sclerosis of the glomerular hilum including the most proximal portion of the mesangium and the vascular pole of the glomerular tuft was more frequently seen in the diabetic dogs compared to control uninephrectomized animals (9.5 ± 0.3%, Group II vs. 2.5 ± 0.2%, Group I; $P < 0.05$). The amount of focal GS among the untreated diabetic group was substantially higher than the control group (4.6 + 0.3%, Group II vs. 0.2 + 0.1%, Group I; $P < 0.05$). Moreover, there was a good correlation between the amount of proteinuria present and degree of focal GS at one year ($r = 0.63$, Group I; $r = 0.92$, Group II; $r = 0.86$, Group III; $r = 0.81$, Group IV; $r = 0.72$, Group V).

The effects of various antihypertensive drug treatments on progression of focal, hilar and global GS are depicted in Figures 4 through 6. While all treated groups manifested a marked reduction in the amount of focal GS, the extent of the lesion remained greater when compared to control dogs (Fig. 5). Furthermore, combination therapy did not provide any greater benefit on slowing progression of focal or hilar GS over either agent alone, (Figs. 4 through 6). Lastly, a marked increase in global GS was noted only among diabetic dogs that received TA3090 alone. This increase in global GS, however, was not seen in the combined therapy group (Fig. 6).

Tubulointerstitial disease

The tubulointerstitial changes consisted of atrophic renal tubules with thickened, wrinkled basement membranes and simplified epithelium embedded in a fibrotic interstitium that often contained plasma cells (Fig. 2). These were not prominent findings, however, among untreated diabetic dogs (0.6 ± 0.3, controls vs. 0.9 ± 0.1, untreated DM; $P = 0.14$). The most extensive tubulointerstitial disease was noted among dogs with the greatest amounts of global GS. Specifically, dogs treated with TA 3090 alone had almost twice as much interstitial fibrosis compared to untreated DM dogs (0.9 ± 0.1, untreated DM vs. 1.7 ± 0.4, TA 3090 alone; $P < 0.04$). No significant differences in interstitial fibrosis were noted between the groups that received lisinopril and nondiabetic controls (0.6 ± 0.2, Group III or 0.74 ± 0.2, Group V vs. 0.6 ± 0.3, control).

Mesangial volume fraction

The mean value of the mesangial volume fraction for the non-diabetic (control) dogs was 0.18 ± 0.01 $\mu m^2/\mu m^2$ following one year of observation. As expected, induction of diabetes

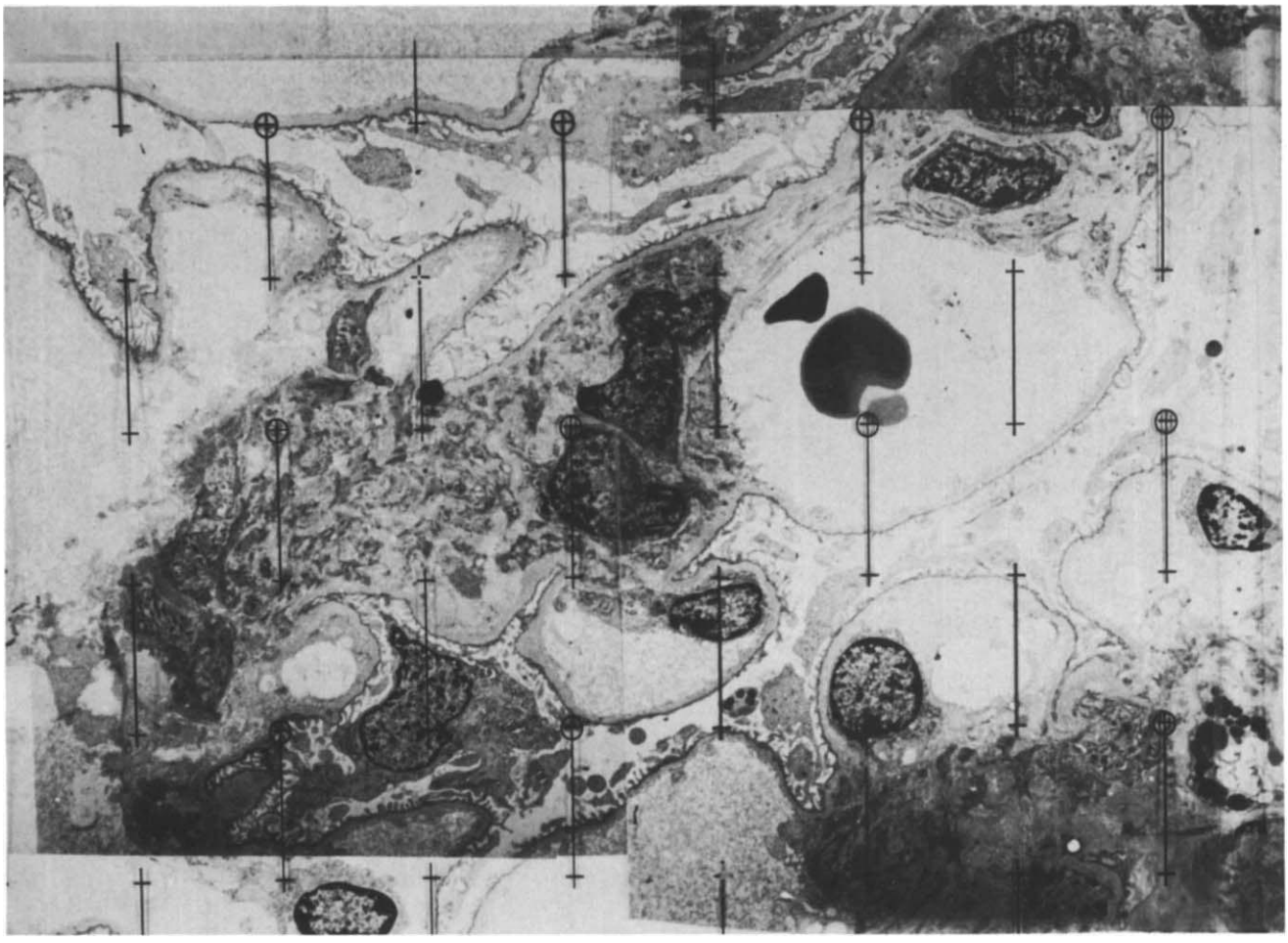

Fig. 3. *Electronmicroscopy photomicrographs taped together to reconstruct the glomerulus.* A grid was superimposed to calculate the mesangial density (V_v).

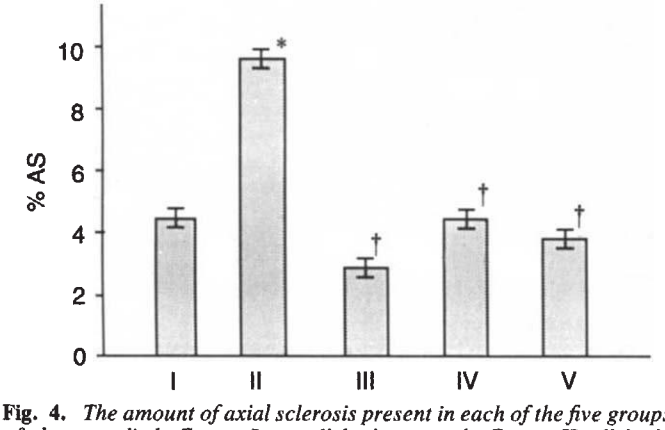

Fig. 4. *The amount of axial sclerosis present in each of the five groups of dogs studied.* Group I, nondiabetic control; Group II, diabetic untreated; Group III, diabetic + lisinopril; Group IV, diabetic + TA 3090; Group V, diabetic + lisinopril/TA3090, each in reduced doses. *$P < 0.05$ compared to all groups; †$P < 0.05$ compared to Group II.

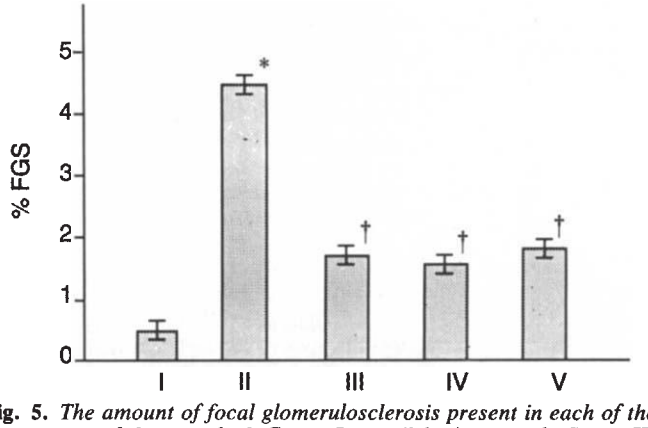

Fig. 5. *The amount of focal glomerulosclerosis present in each of the five groups of dogs studied.* Group I, nondiabetic control; Group II, diabetic untreated; Group III, diabetic + lisinopril; Group IV, diabetic + TA 3090; Group V, diabetic + lisinopril/TA3090, each in reduced doses. *$P < 0.05$ compared to all Groups; †$P < 0.05$ compared to Group II.

resulted in significant expansion of the mesangial volume fraction as the mean V_v in Group II (0.28 ± 0.04 $\mu m^2/\mu m^2$) was approximately 60% greater than in group I ($P < 0.05$). Figure 7 is a summary of the changes in the mesangial volume fraction associated with different antihypertensive drug interventions at similar levels of arterial pressure reduction. The attenuated increase in mesangial volume fraction observed between lisinopril and TA 3090 at one year was not statistically different (42 ±

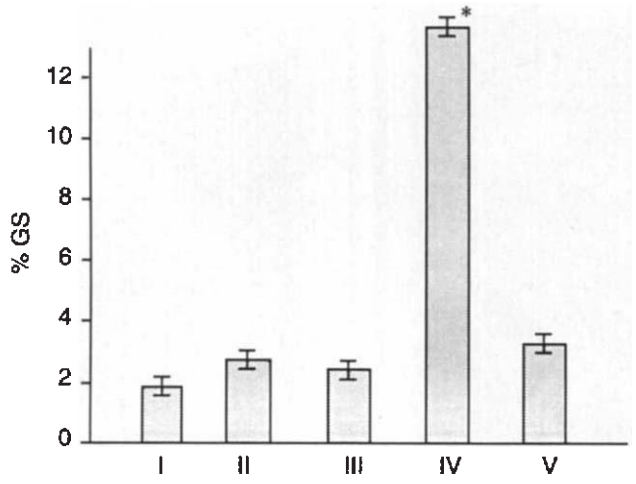

Fig. 6. *The amount of global glomerulosclerosis present in each of the five groups of dogs studied.* Group I, nondiabetic control; Group II, diabetic untreated; Group III, diabetic + lisinopril; Group IV, diabetic + TA 3090; Group V, diabetic + lisinopril/TA3090, each in reduced doses. *$P < 0.05$ compared to any of the other groups.

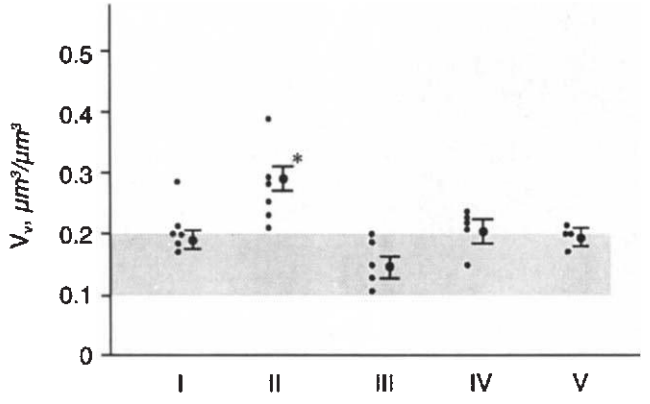

Fig. 7. *The effects of different antihypertensive treatments, diabetes alone and normal aging on mesangial volume expansion in unilaterally nephrectomized diabetic dogs.* Each dot represents a single dog. Each group was compared to each other at the same time point, that is, one year. Mean ± standard error of the mean for each group is presented next to the individual dots for each group. The shaded area represents the normal range for mesangial volume. Group I, nondiabetic control; Group II, diabetic untreated; Group III, diabetic + lisinopril; Group IV, diabetic + TA 3090; Group V, diabetic + lisinopril/TA3090, each in reduced doses. *$P < 0.05$ compared to any of the other groups.

4%, L vs. 28 ± 9%, TA 3090; $P = 0.15$). Moreover, the combination of TA 3090 + lisinopril did not blunt the expansion of the mesangial volume fraction to an extent greater than either agent alone (36 ± 5%, combined vs. 42 ± 4%, L; $P = 0.24$ or 28 ± 9%, TA 3090; $P = 0.17$). Note that increases were ascribed in the mesangial volume fraction to be the result of increases in the matrix and not the cellular component of the mesangium. This is based on the fact that no increased numbers of cells per mesangial area were found in our dog glomeruli. Moreover, additional studies in the literature support this observation [31].

The relationship between associated changes in proteinuria and mesangial volume fraction is illustrated in Figure 8. A strong correlation between a blunted rise in proteinuria and a

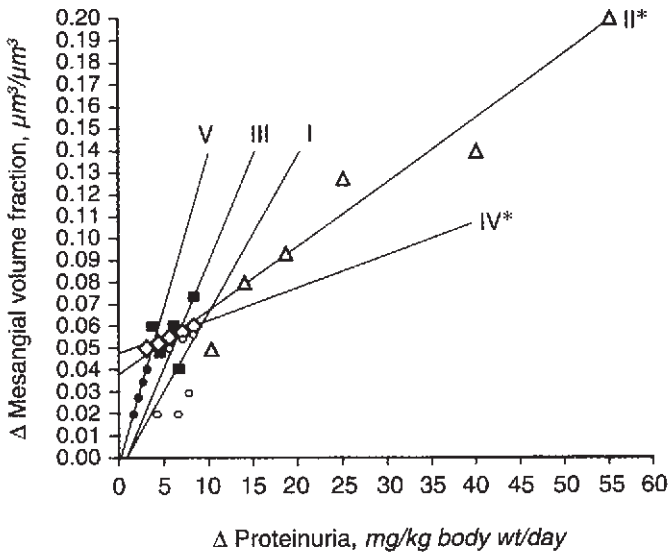

Fig. 8. *The predictive value of attenuating the rise in proteinuria on increases in fractional mesangial volume in the unilaterally nephrectomized diabetic dog.* Group I, nondiabetic control; Group II, diabetic untreated; Group III, diabetic + lisinopril; Group IV, diabetic + TA 3090; Group V, diabetic + lisinopril/TA3090, each in reduced doses. *$P < 0.002$ slope different from Group III; *$P < 0.001$ slope different from Group V; *$P < 0.004$ slope different from Group I; *$P < 0.05$ slope different from Group IV. Symbols are: (○) Group I; (△) Group II; (■) Group III; (◇) Group IV: (●) Group V. Linear regression equations: Group I, 0.98x + 0.172; Group II, 0.96x + 0.153; Group III, 0.79x + 0.099; Group IV, 0.88x + 0.192; Group V, 0.78x + 0.15.

reduced expansion in the fractional mesangial volume was found in Groups I through III and V ($r = 0.61$, Group I; $r = 0.97$, Group II; $r = 0.79$, Group III and $r = 0.82$, Group V). The weakest correlation was present in Group IV ($r = 0.29$). Moreover, a comparison of the slopes between individual groups demonstrated a significant difference between untreated diabetic animals and Groups I, III and V but not IV, (Fig. 8). This difference, however, should be viewed in the context of relatively small numbers of dogs completed in each group as well as a large scatter among the data sets. Moreover, the slopes between Groups I, III and V ranged in P values between $<0.08 > 0.06$.

Discussion

This study extends our previous findings with three new observations. First, chronic administration of a converting enzyme inhibitor or a nondihydropyridine calcium antagonist, either alone or in combination, attenuates development of focal GS and expansion of mesangial volume. Second, treatment with a nondihyropyridine calcium antagonist alone results in a marked increase in both global GS and a secondary increase in parenchymal scarring. Moreover, these increases in global GS appear to be independent of sustained reductions in arterial pressure or proteinuria. This is evidenced by a lack of global GS in either of the two groups that received lisinopril as well as the untreated diabetic group that remained hypertensive. Lastly, only the groups that received the converting enzyme inhibitor had a strong correlation between a blunted rise in proteinuria and attenuated expansion of the mesangial volume fraction.

Previous animal studies with dihydropyridine calcium antagonists in either hypertensive or diabetic rats demonstrate an

increased development of both focal and global GS [14, 19–22]. Our data in diabetic dogs extend these findings by demonstrating that a nondihydropyridine calcium antagonist can also increase global GS. Moreover, this increase in global GS was not related to between group differences in either arterial or intraglomerular pressures or blood glucose control [25]. Interestingly, the augmented development of global GS was attenuated when the calcium antagonist was combined with a converting enzyme inhibitor. The mechanism of this effect was not studied; however, it is probably not related to an elevation in intraglomerular pressure. This is supported by the fact that untreated diabetic dogs did not manifest an increase in global GS, yet had similar levels of intraglomerular pressures when compared to dogs treated with the calcium antagonist alone [25].

Increases in global GS may also relate to increases in arteriolar hyalinosis and concomitant expansion of the mesangial volume fraction. Studies in diabetic patients demonstrate that development of global GS correlates with the amount of arteriolar hyalinosis present [32]. Our results support the association between increases in arteriolar hyalinosis and expansion of mesangial volume but not global GS. Moreover, there was a disproportionate increase in the development of global GS in the group treated with the nondihydropyridine calcium antagonist alone. This increased development of global GS was in contradistinction to the development of both mesangial volume expansion and arteriolar hyalinosis which was distinctly blunted. This suggests a mechanism other than capillary occlusion, secondary to progressive mesangial volume expansion, may be responsible for the ultimate development of global GS. One possible explanation for this phenomenon is periodic or intermittent hypotension that may have occurred during the one year follow-up period of the study. The frequency of hypotensive episodes, that is, systolic pressure < 90 mm Hg, during the follow-up period was greatest in the dogs that received TA 3090 alone. Prolonged periods of hypotension could have resulted in some degree of ischemic injury to this group of animals. The use of continuous arterial pressure monitoring may provide a clue to the etiology of this problem. Recent animal studies document the existence of such technology and its usefulness in addressing this issue [33].

Previous studies with dihydropyridine calcium antagonists demonstrated no abatement in the progression of focal GS in animal models of diabetes [14, 19, 22]. Conversely, studies with nondihydropyridine calcium antagonists illustrate an attenuated progression of this process [34, 35]. Our data confirm the observations with nondihydropyridine calcium antagonists. Moreover, our studies extend these findings by demonstrating an attenuated increase in expansion of mesangial volume fraction with a calcium antagonist or converting enzyme inhibitor, either alone or combined. The mechanism for this slowed morphologic progression of diabetic nephropathy may relate to direct effects of these antihypertensive agents on extracellular matrix proteins. Specifically, both converting enzyme inhibitors and the nondihydropyridine calcium antagonist, diltiazem, prevent the reduced synthesis of various matrix proteins such as heparan sulfate, glucoaminoglycan and others in animal models of diabetes [36, 37]. Additionally, converting enzyme inhibitors, through their effects on angiotensin II, may interact with other growth factors, such as endothelin, to effect deposition of various matrix proteins [38]. Thus, these agents may ameliorate the morphologic progression of diabetic nephropathy separate from, but perhaps interrelated to, their intrarenal hemodynamic effects.

The presence of hyperglycemia is known to increase deposition of extracellular matrix by mesangial cells [39]. Moreover, morphometric data from the kidneys of diabetic dogs and rats indicate that restoration of euglycemia can prevent expansion of the matrix component of the mesangium and, consequently, retard progression of diabetic glomerulopathy [40, 41]. Studies in human subjects with diabetes also support these observations [42–45]. Interestingly, all our dogs had very poor blood glucose control and yet those whose blood pressures were reduced to < 106 mm Hg did not develop significant increases in mesangial volume or focal GS. Thus, in contradistinction to previous reports [23], these data support the concept that blood pressure reduction plays a very important role in the preservation of renal function and that both converting enzyme inhibitors and nondihydropyridine calcium antagonists may have effects on progression of diabetic nephropathy independent of blood glucose control.

The relationship between increases in proteinuria and expansion of mesangial volume are well documented in both rodent and canine models of diabetes. Moreover, a recent study by Steffes et al demonstrates a strong correlation between concomitant increases in both the matrix component of the mesangium and albuminuria among type I diabetic patients [31]. Our data support these observations. Moreover, we demonstrated that dogs taking a converting enzyme inhibitor had a persistently small amount of proteinuria which correlated with both a blunted expansion of mesangial volume and development of focal GS.

Tubulointerstitial fibrosis is a common lesion in advanced diabetic nephropathy and is indicative of irreversible renal injury [10, 46–49]. Studies involving a rat model of immune injury demonstrate that tubulointerstitial disease precedes development of GS [50, 51]. A possible etiologic factor in our study relates directly to the alloxan used to make the dogs diabetic [24]. However, this would not account for the clear predominance of tubulointerstitial fibrosis observed only in the group that received single agent treatment with TA 3090. Interestingly, this was also the only group to manifest a significant increase in global GS. Unfortunately, we did not do serial biopsies and thus cannot comment on the time course or association of this process to GS.

In summary, our results indicate that the morphologic events associated with diabetic nephropathy are slowed by treatment with either a converting enzyme inhibitor or heart-rate-lowering calcium antagonist, administered either alone or together. When used alone, however, the calcium antagonist reduced mesangial volume expansion but increased the amount of global GS and tubulointerstitial fibrosis. The co-administration of each agent, however, prevented this occurrence. The mechanism underlying the genesis of global GS in this model is unclear but may relate to periodic or persistent hypotension and warrants further study.

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Reprint requests to George L. Bakris, M.D., Rush University Hypertension Research Center, Department of Preventive Medicine, Rush Medical Center, Suite 117, 1725 West Harrison St., Chicago, Illinois 60612, USA.

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