Pharmacological Inhibition of Diabetic Retinopathy
Aminoguanidine and Aspirin

Timothy S. Kern1 and Ronald L. Engerman2

Effects of aminoguanidine and aspirin on the development of retinopathy have been examined in 5-year studies of diabetic dogs. Either agent was administered daily in doses of 20–25 mg·kg⁻¹·day⁻¹. Because severity of hyperglycemia greatly influences development of the retinopathy, special effort was devoted to maintaining comparable glycemia in experimental and control groups. The retinal vasculature was isolated by the trypsin digest method, and retinopathy was assessed by light microscopy. Diabetes for 5 years resulted, as expected, in saccular capillary aneurysms, pericyte ghosts, acellular capillaries, retinal hemorrhages, and other lesions. Administration of aminoguanidine essentially prevented the retinopathy, significantly inhibiting the development of retinal microaneurysms, acellular capillaries, and pericyte ghosts compared with diabetic controls. Aspirin significantly inhibited the development of retinal hemorrhages and acellular capillaries over the 5 years of study, but had less effect on other lesions. Although diabetes resulted in significantly increased levels of advanced glycation end products (AGEs) (namely, pentosidine in tail collagen and aorta, and Hb-AGE), aminoguanidine had no significant influence on these parameters of glycation. Nitration of a retinal protein was significantly increased in diabetes and inhibited by aminoguanidine. The biochemical mechanism by which aminoguanidine has inhibited retinopathy thus is not clear. Aminoguanidine (but not aspirin) inhibited a diabetes-induced defect in ulnar nerve conduction velocity, but neither agent was found to influence kidney structure or albumen excretion. Diabetes 50:1636–1642, 2001

Intensive control of glycemia has been found to inhibit the development of retinopathy in diabetic dogs and patients (1,2). Nevertheless, it remains difficult or impossible for many patients to achieve and maintain the optimal level of metabolic control. If the pathogenesis of the retinopathy were better understood, new therapies might be feasible by which retinopathy could be inhibited, even if control of glycemia were less than perfect. The finding that diabetic-like retinopathy can be produced in nondiabetic animals by feeding them a galactose-rich diet (3–8) has provided strong evidence that elevated hexose concentration is itself sufficient to initiate the retinopathy. Unfortunately, it has remained unclear which of the many biochemical sequelae of hyperglycemia contribute more directly to development of the retinopathy.

Several biochemical sequelae of hyperglycemia have attracted particular attention as possible causes of the retinopathy, and methods to inhibit these biochemical defects continue to be identified. Chronic aspirin consumption was reported many years ago to be associated with protection from diabetic retinopathy, raising a possibility that alteration of prostaglandin production might influence the development of retinopathy. The effectiveness of aspirin in clinical trials has been controversial, however, showing a statistically significant (although modest) inhibitory effect of the drug on retinopathy in one clinical trial (9), but having no significant beneficial effect in another larger clinical trial (10). Another drug, aminoguanidine, was shown to inhibit many sequelae of advanced glycation end product (AGE) formation (11–13) and was observed to have beneficial effects on a number of diabetes-induced alterations of tissue function and structure (14–20). Moreover, aminoguanidine was found by Hammes et al. (21,22) and later also by Kern and Kowluru (23) to inhibit the development of some retinal lesions in diabetic rats. In our experience, diabetic rats develop the early stages of diabetic retinopathy, but do not reproducibly develop microaneurysms and advanced lesions of the retinopathy (7).

Dogs that develop diabetes either spontaneously or experimentally develop a retinopathy that is morphologically indistinguishable from that which is characteristic of diabetic patients (24,25). In the present study, we examined the effects of aminoguanidine and aspirin on the development of retinopathy in diabetic dogs over a 5-year interval. Unlike in clinical trials, where many patients have some retinopathy at the onset of the study, drug administration in our dog studies was initiated at the time of diabetes onset.

RESEARCH DESIGN AND METHODS

Young adult dogs (1.5–2.5 years old) were randomly assigned to be made alloxan diabetic or to remain as normal untreated (control) animals. Diabetes was induced in fasted dogs by injection of alloxan monohydrate intravenously (50–60 mg/kg); after 4–6 weeks of hyperglycemia, to ensure that the animals were comparably insulin deficient, the diabetic animals were randomly divided among three groups: control (n = 8), aspirin treated (n = 8), and aminoguanidine treated (n = 9). Aspirin (20 mg·kg⁻¹·day⁻¹ in tablets) was given twice each day (8:00 a.m. and 6:00 p.m.), 1 h before insulin and a standard dry diet (Purina Lab Canine Diet No. 5,006; Ralston Purina, St. Louis, MO). Aminoguanidine (20 mg·kg⁻¹·day⁻¹ in tablets) was administered each day at a dose of 20 mg·kg⁻¹·day⁻¹. The finding that diabetic-like retinopathy can be produced in nondiabetic animals by feeding them a galactose-rich diet (3–8) has provided strong evidence that elevated hexose concentration is itself sufficient to initiate the retinopathy. Unfortunately, it has remained unclear which of the many biochemical sequelae of hyperglycemia contribute more directly to development of the retinopathy.

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AGE, advanced glycation end product; ETDRS, Early Treatment of Diabetic Retinopathy Study; HPLC, high-performance liquid chromatography; INOS, inducible isoform of nitric oxide synthase.
morning as described above. The dose of aspirin was chosen to approximate that administered chronically to humans (per kilogram of body weight) and per its ability to inhibit platelet aggregation in dogs, and was judged to be the maximum chronic dose tolerated by dogs (administration of a higher dose of 40 mg·kg⁻¹·day⁻¹ to one dog resulted in stomach ulcerations). The selected dose of aspirin was similar per kilogram of body weight to doses administered chronically to humans. The dose of aminoguanidine was selected in consultation with Alteon, Inc., based on concentrations needed to inhibit protein crosslinking secondary to nonenzymatic glycinolysis. Insulin doses were reviewed twice daily to prevent body weight loss and maintain urinary excretion of glucose within a range of 2–4 g·day⁻¹·kg⁻¹ body wt⁻¹. Because severity of hyperglycemia greatly influences development of the retinopathy, special effort was devoted to maintaining glycemic control in the experimental setting.

Data are n or means ± SD.

table 1 Glycemia, platelet aggregation, and tissue levels of AGEs during alloxan diabetes of 5 years’ duration

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HbA₁ (%)</th>
<th>Blood glucose (fasting; mg/dl)</th>
<th>Glocosuria (g·day⁻¹·kg⁻¹ body wt⁻¹)</th>
<th>Platelet aggregation (% of max)</th>
<th>Pentosidine (Aorta) (U/mg protein)</th>
<th>Tail collagen (pmol/mg protein)</th>
<th>Protein-bound AGE (Aorta-AGE) (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diabetes</td>
<td>8</td>
<td>5.8 ± 0.2</td>
<td>70 ± 5</td>
<td>0 ± 0</td>
<td>70 ± 23</td>
<td>19 ± 3</td>
<td>6.2 ± 1.8</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>7.7 ± 0.4</td>
<td>160 ± 37</td>
<td>2.9 ± 0.3</td>
<td>72 ± 17</td>
<td>26 ± 4</td>
<td>10.4 ± 2.9</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>+ Aminoguanidine</td>
<td>9</td>
<td>7.7 ± 0.4</td>
<td>148 ± 29</td>
<td>3.0 ± 0.5</td>
<td>24 ± 2</td>
<td>9.9 ± 3.2</td>
<td>2.5 ± 0.4</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>+ Aspirin</td>
<td>8</td>
<td>7.8 ± 0.2</td>
<td>161 ± 53</td>
<td>2.7 ± 0.4</td>
<td>29 ± 8</td>
<td>11.2 ± 1.5</td>
<td>29 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Data are n or means ± SD.
Administration of aminoguanidine significantly inhibited the development of retinopathy over the 5 years of study (Table 2). Retinal microaneurysms, acellular capillaries, and pericyte ghosts in aminoguanidine-treated animals were found to be significantly less numerous than in diabetic controls ($P < 0.001, P < 0.001,$ and $P < 0.005,$ respectively) and not different from nondiabetic animals. The severity of sudanophilia likewise was reduced ($P < 0.001),$ and retinal dot and blot hemorrhages were observed in only one of the nine aminoguanidine-treated diabetic animals, compared with five of the eight diabetic controls ($P = 0.051).$

Aminoguanidine significantly inhibited the development of acellular capillaries, retinal hemorrhages, and capillary sudanophilia over the 5 years of study ($P < 0.001, P < 0.002,$ and $P < 0.05,$ respectively). The effect of aspirin on the number of microaneurysms and pericyte ghosts was equivocal, achieving statistical significance by Fisher’s test only for the worst eye, and not when using other statistical tests. **Biochemical effects of aminoguanidine.** Diabetes resulted in significantly increased levels of pentosidine in tail collagen and aorta ($P < 0.005$ and $0.005$) and of protein-bound AGE on hemoglobin and aortic protein ($P < 0.05$ and $< 0.01,$ respectively; Table 1). Administration of aminoguanidine had no significant influence on any of these parameters. Western blots of retinal protein (from dogs studied 2 years) stained with antibody against the AGE, imidazolone, revealed numerous bands, but in only one of those bands (~50 kDa) did stain intensity tend to be increased in diabetes and seem also inhibited by aminoguanidine. The density of immunostain of this imidazolone, revealed numerous bands, but in only one of the nine aminoguanidine-treated diabetic animals, compared with five of the eight diabetic controls ($P = 0.051).$

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**RESULTS**

HbA1 and plasma and urinary glucose became significantly elevated above normal in all diabetic animals ($P < 0.01),$ confirming the expected chronic elevation of blood hexose (Table 1). Plasma cholesterol and triglycerides became elevated during diabetes ($197 \pm 32$ and $44 \pm 8$ mg/dl, respectively, for nondiabetic animals and $234 \pm 32$ and $66 \pm 26$ for diabetic animals). As intended, the severity of hyperglycemia in our diabetic animals was not significantly different between the diabetic control group and either the aspirin- or aminoguanidine groups, as can be seen from the values for HbA1, plasma glucose, and 24-h glucoseuria (all $P > 0.4$). Neither aminoguanidine nor aspirin had any effect on the diabetes-induced increase in plasma cholesterol or triglycerides. Intake of aminoguanidine and aspirin averaged $22$ and $28$ mg/kg body wt, respectively, throughout the study. Plasma levels of aminoguanidine rose to $9.6 \pm 2.9$ μg/ml $3$ h after drug administration from a low of $0.7 \pm 0.4$ μg/ml after an overnight fast. Plasma levels of salicylate were more stable, being $4.3 \pm 1.4$ mg/dl after an overnight fast and $5.8 \pm 1.2$ mg/dl $1$ h after aspirin consumption. These plasma levels are consistent with lev-

**TABLE 2**

Inhibition of retinopathy by aminoguanidine or aspirin

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Duration (months)</th>
<th>Aneurysms per eye</th>
<th>Pericyte ghosts</th>
<th>Acellular capillaries</th>
<th>Sudanophilia*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(count)</td>
<td>(per 1,000 cells)</td>
<td>(per mm² retina)</td>
<td></td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>60</td>
<td>1.9 ± 1.4</td>
<td>1.2 ± 0.6</td>
<td>6.1 ± 3.4</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>+ Aminoguanidine</td>
<td>9</td>
<td>60</td>
<td>25.1 ± 20.6</td>
<td>4.6 ± 2.2</td>
<td>17.7 ± 12.7</td>
<td>13.1 ± 7.9</td>
</tr>
<tr>
<td>+ Aspirin</td>
<td>8</td>
<td>60</td>
<td>11.6 ± 17.1</td>
<td>2.8 ± 2.1</td>
<td>10.2 ± 6.2</td>
<td>5.2 ± 1.2</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>60</td>
<td>3.3 ± 1.9</td>
<td>1.7 ± 0.7</td>
<td>7.0 ± 2.6</td>
<td>5.1 ± 1.4</td>
</tr>
<tr>
<td>+ Aminoguanidine</td>
<td>9</td>
<td>60</td>
<td>2.6 ± 0.9</td>
<td>2.3 ± 0.8</td>
<td>6.4 ± 5</td>
<td>163 ± 148</td>
</tr>
<tr>
<td>+ Aspirin</td>
<td>8</td>
<td>60</td>
<td>2.4 ± 0.7</td>
<td>2.8 ± 1.0</td>
<td>56 ± 5</td>
<td>43 ± 36</td>
</tr>
</tbody>
</table>

Data are n or means ± SD. *n = 6 per group.

Statistics. Experimental groups were compared statistically using the non-parametric Kruskal-Wallis test followed by Mann-Whitney U tests. Analysis of variance followed by Fisher’s least significant difference tests gave similar conclusions. Results are expressed as means ± SD.

**TABLE 3**

Effect of aminoguanidine and aspirin on kidney and nerve

<table>
<thead>
<tr>
<th></th>
<th>Kidney weight (g/kg initial body wt)</th>
<th>Glomerular volume (μm³ × 10⁶)</th>
<th>Silver-stained mesangium + matrix (% of glomerulus)</th>
<th>Albumin excretion (mg/24 h)</th>
<th>Nerve conduction velocity (year 5) (m/sec) % of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.7 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>52 ± 12</td>
<td>6 ± 5</td>
<td>64 ± 1</td>
</tr>
<tr>
<td>+ Aminoguanidine</td>
<td>3.2 ± 0.2</td>
<td>2.6 ± 0.9</td>
<td>60 ± 4</td>
<td>70 ± 116</td>
<td>58 ± 2</td>
</tr>
<tr>
<td>+ Aspirin</td>
<td>3.4 ± 0.4</td>
<td>2.3 ± 0.8</td>
<td>64 ± 5</td>
<td>163 ± 148</td>
<td>60 ± 2</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<tr>
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<td>2.4 ± 0.7</td>
<td>56 ± 5</td>
<td>43 ± 36</td>
<td>58 ± 3</td>
</tr>
</tbody>
</table>

Data are means ± SD.
bands, but in only one of those bands (≈80 kDa) was stain intensity increased in diabetes and was stain intensity inhibited by aminoguanidine. The increase in stain intensity of this band in diabetes (440 ± 35 arbitrary units in those with diabetes vs. 279 ± 42 arbitrary units in those without diabetes) and inhibition of staining by aminoguanidine (309 ± 25) both were statistically significant (P < 0.05). No other protein bands were observed to have greater than normal immunostain in samples from the diabetic animals or inhibition of immunostain intensity in samples from aminoguanidine-treated diabetic animals.

**Biochemical effects of aspirin.** As we discovered in previous studies (31), diabetic dogs showed no abnormality of collagen-induced platelet aggregation. Nevertheless, aspirin treatment significantly inhibited collagen-induced platelet aggregation (P < 0.001), even 14 h after the previous dose of drug (the longest interval between administration of aspirin). Plasma levels of 6-keto F1α and thromboxane B2 tended to be elevated in diabetic animals, but the results did not achieve statistical significance. Aspirin significantly reduced the diabetes-induced rise in prostaglandins only for plasma 6-keto F1α (data not shown). In short-term studies, release of prostaglandins from the retina tended to be increased (6-keto F1α: 2.4 ± 0.8 ng · mg⁻¹ · min⁻¹ vs. 5.9 ± 4.0 for nondiabetic and diabetic, respectively, and thromboxane: 13 ± 5 ng · mg⁻¹ · min⁻¹ vs. 20 ± 4 for nondiabetic and diabetic, respectively), although the small sample sizes preclude statistical comparison. Aspirin consumption inhibited production of these prostaglandins by retinas of diabetic animals by >90% (0.4 ± 0.1 and 0.5 ± 0.1, respectively).

**Drug side effects.** No evidence of gross anatomical pathology was observed at autopsy in either the aminoguanidine- or aspirin-treated group. No tumors were noted in any animals, and aspirin-treated animals had no evidence of stomach hemorrhages or ulcerations. One aminoguanidine-treated dog developed cyclical insulin resistance in association with going into heat, but no clear relationship with aminoguanidine was evident.

**Kidney.** Moderate glycemic control in the diabetic animals resulted in statistically significant, although modest, changes in renal structure, including nephromegaly and glomerular enlargement with increased absolute volume of matrix/basement membrane. The fractional area of the silver-stained matrix/basement membrane in these diabetic animals in moderate glycemic control tended to be greater than normal (51 ± 12 and 60 ± 3% for normal and control diabetic animals, respectively), but the increase did not achieve statistical significance in this small sample. Albumin excretion progressed with duration of diabetes, becoming statistically greater than normal during the fifth year of moderate glycemic control (P < 0.01). Neither aspirin nor aminoguanidine had a significantly beneficial effect on albumin excretion in diabetic dogs (P = 0.68 and 0.11, respectively).

**Nerve.** Conduction velocity of ulnar nerve decreased with duration of diabetes, and the decrease was statistically significant during the fifth year of moderate glycemic control (P < 0.001). Aspirin had no apparent effect on the conduction velocity. Aminoguanidine had a modest beneficial effect (P = 0.04), the diabetes-induced decrease in conduction velocity in year five being reduced by about half.

**DISCUSSION**

Improved glycemic control has been the only systemic therapy shown to date to inhibit the onset and/or early progression of diabetic retinopathy (1,2). Aminoguanidine treatment apparently is capable also of exerting beneficial effects on retinopathy, and results in an essentially total inhibition of the development of retinopathy in diabetic dogs despite continued hyperglycemia. The present results in dogs indicate that aspirin also inhibited the development of the retinopathy, although possibly to a lesser extent.
degree than aminoguanidine. The dramatic effect of aminoguanidine in diabetic dogs is particularly impressive if compared with the total lack of benefit of aldose reductase inhibition observed previously by us in diabetic dogs and by others in clinical trials of patients with diabetes (6,37,38). Aminoguanidine has been found previously to inhibit the development of acellular capillaries and other lesions in the retina also in diabetic rats (21–23).

Both aminoguanidine and aspirin were found to significantly inhibit the development of acellular capillaries in diabetes. An acellular capillary consists of the remnant basement membrane skeleton of a once-functional capillary from which all capillary cells have disappeared. Acellular capillaries are of interest because they are seen to be not perfused (39). Thus, increased numbers of acellular capillaries in diabetes likely are causally related to the development of retinal ischemia and clinically significant retinal neovascularization. Inhibition of the development of acellular capillaries by agents such as aminoguanidine and aspirin can be expected to inhibit the development of retinal ischemia and neovascularization.

Aminoguanidine was viewed originally as an inhibitor of sequelae of AGE formation (11). Hammes et al. (21,22) observed that the drug inhibited retinal pathology in their diabetic rats, and concluded that the drug did so by inhibiting formation of AGEs because they found retinal arterioles of drug-treated animals had less in situ fluorescence at wavelengths characteristic of AGEs than did untreated diabetic animals. These wavelengths, however, are not specific for AGEs, and likely include also a variety of oxidation products. We have found that the inhibition of retinal lesions by aminoguanidine in diabetic rats occurs without systemic reductions in parameters of AGEs, such as Hb-AGE (mainly carboxymethyl lysine), retinal pentosidine, and tail collagen fluorescence and pentosidine (23). In the present study, aminoguanidine had no significantly beneficial effect on systemic AGEs (such as Hb-AGE and pentosidine). The therapy tended to inhibit accumulation of retinal imidazolone, which is derived from the AGE, 3-dexyglucosone (30), but the effect was not statistically significant. Whether or not the therapy had a more dramatic effect on other retinal AGEs remains to be investigated. Aminoguanidine has been reported to inhibit biochemical processes other than those merely related to AGE formation, including activities of semicarbazide-sensitive amine oxidase and the inducible isozyme of nitric oxide synthase (iNOS), as well as diabetes-induced oxidative stress in the retina and other tissues (15,17,40–45). In the present study, diabetes increased the amount of nitration of especially one retinal protein (presumably secondary to formation of peroxynitrite from nitric oxide), and aminoguanidine inhibited this nitration (consistent with aminoguanidine-mediated inhibition of iNOS). Nevertheless, determination of the biochemical mechanism by which aminoguanidine has inhibited retinopathy will require additional study.

Kern et al. (46) have found aminoguanidine to inhibit a diabetes-induced increase in apoptosis of retinal capillary cells. Rapid advances in understanding of the biochemical basis for apoptosis offer a valuable bridge between diabetes-induced alterations in metabolism and the histopathology characteristic of diabetic retinopathy. It seems likely that the inhibition of apoptosis by aminoguanidine contributes to the drug’s inhibition of acellular capillaries and pericyte loss.

In rats, aminoguanidine has been reported to have beneficial effects also on diabetes-induced complications in kidney and nerve (14–20). Aminoguanidine did have a modest beneficial effect on nerve conduction velocity in our diabetic dogs, although the results barely achieved statistical significance in this small sample. Aspirin’s effect on the development of diabetic complications, in contrast, has been studied little in laboratory animals and chiefly in humans. In the present study of diabetic dogs, neither aspirin nor aminoguanidine had a significant effect on any of the parameters of renal structure and function examined. Dogs in the present experiment intentionally were kept “moderately” insulin deficient, so the severity of renal disease is less than that reported previously by us for dogs in poor glycemic control (47,48) and consists largely of hypertrophic changes. The difference in conclusions reached between previous rat studies and the present dog study might be due to species differences, or to the great difference in duration of the experiments in the two species. Nevertheless, the ability of aminoguanidine to inhibit diabetic retinopathy in dogs while having little or no effect on renal disease in diabetes is consistent with our previous evidence that the pathogenesis of the retinopathy differs appreciably from that of nephropathy (49).

Aspirin, like aminoguanidine, significantly inhibited retinal hemorrhage and the formation of acellular capillaries, but less effectively diminished the frequency of microaneurysms and pericyte ghosts. A lower than expected severity of retinopathy in diabetic subjects with arthritis led to a suggestion many years ago that aspirin might be a potentially effective therapy against diabetic retinopathy (50,51), but prospective clinical trials to assess this possibility have yielded contradictory results. Aspirin treatment resulted in a statistically significant (although weak) inhibition of the mean yearly increase in number of microaneurysms in the DAMAD trial (9), whereas no beneficial effect was observed on any aspect of retinopathy in the Early Treatment of Diabetic Retinopathy Study (ETDRS) trial (10). Differences in design of the two studies may have contributed to the divergent conclusions, but two differences in particular seem especially important. Patients in the DAMAD study had only little retinopathy at onset of the trial, whereas patients in the ETDRS had a more advanced stage of retinopathy (mild to severe nonproliferative or early proliferative retinopathy). Thus, the lack of effect of aspirin in the ETDRS might be attributable to the greater severity of retinopathy, especially since animal (27,52) and clinical (2) studies have shown that retinopathy, once initiated, tends to resist arrest. Moreover, patients in the DAMAD study received more aspirin than those in the ETDRS (990 vs. 660 mg/day). Aspirin did not make retinal hemorrhages worse in diabetic patients (10) or in diabetic dogs; in the present studies, it actually inhibited their development.

Our study of aspirin in dogs differed from the clinical studies in humans in at least two respects. Aspirin was administered to the diabetic dogs from the onset of diabetes; in the clinical trials with human subjects, however, diabetes had persisted for extended periods before aspirin
therapy was initiated. In addition, the histological methods we used to detect retinal lesions are more sensitive than methods available clinically, allowing us to detect lesions on individual capillaries and at an earlier stage than is feasible clinically. The animal and human studies, taken together, are consistent with a hypothesis that aspirin therapy is more effective if initiated early in diabetes rather than later. Aspirin is capable of altering prostaglandin-mediated processes (via cyclooxygenase) and inflammatory processes independently of one another, but the dose of aspirin used in our rodent studies was sufficiently high (when expressed relative to body weight) to inhibit presumably both of these processes. Whether aspirin at a different dose might result in a more complete inhibition of the retinopathy remains to be learned.

Aminoguanidine and aspirin, in addition to the independent effects, also share activities that might constitute a mechanism in common for their observed beneficial effects on retinopathy. Both aspirin and aminoguanidine reportedly can alter blood flow and vessel permeability (15,53,54), inhibit nonenzymatic glycation or its sequelae (55–58), and inhibit oxidative stress (44,45,59,60). The identification of multiple therapies by which retinopathy can be inhibited can be expected to reveal the various biochemical and physiological steps responsible for the retinopathy, and at which the retinopathy might be best inhibited. One mechanism for the inhibition of vaso-occlusion by aspirin might be via inhibiting formation of the microthrombi reported recently in diabetic retinopathy.

ACKNOWLEDGMENTS

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