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Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine

(cardiomegaly/vasculopathy/albuminuria/glomerulosclerosis)

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ABSTRACT **Human aging is impacted severely by cardiovascular disease and significantly but less overtly by renal dysfunction. Advanced glycation endproducts (AGEs) have been linked to tissue damage in diabetes and aging, and the AGE inhibitor aminoguanidine (AG) has been shown to inhibit renal and vascular pathology in diabetic animals. In the present study, the effects of AG on aging-related renal and vascular changes and AGE accumulation were studied in nondiabetic female Sprague–Dawley (S-D) and Fischer 344 (F344) rats treated with AG (0.1% in drinking water) for 18 mo. Significant increases in the AGE content in aged cardiac ($P < 0.05$), aortic ($P < 0.005$), and renal ($P < 0.05$) tissues were prevented by AG treatment ($P < 0.05$ for each tissue). A marked age-linked vasodilatory impairment in response to acetylcholine and nitroglycerine was prevented by AG treatment ($P < 0.005$), as was an age-related cardiac hypertrophy evident in both strains ($P < 0.05$). While creatinine clearance was unaffected by aging in these studies, the AGE/creatinine clearance ratio declined 3-fold in old rats vs. young rats (S-D, $P < 0.05$; F344, $P < 0.01$), while it declined significantly less in AG-treated old rats ($P < 0.05$). In S-D but not in F344 rats, a significant ($P < 0.05$) age-linked 24% nephron loss was completely prevented by AG treatment, and glomerular sclerosis was markedly suppressed ($P < 0.01$). Age-related albuminuria and proteinuria were markedly inhibited by AG in both strains (S-D, $P < 0.01$; F344, $P < 0.01$). These data suggest that early interference with AGE accumulation by AG treatment may impart significant protection against the progressive cardiovascular and renal decline afflicting the last decades of life.**

Older individuals exhibit an increased incidence of cardiovascular disease and reduced renal function compared to young persons (1–6). With aging, physical and structural changes in the arterial wall are associated with alterations in elasticity (3, 7) leading to decreased aortic compliance, increased peripheral vascular resistance, and decreased renal blood flow (3, 6). With or without clinically evident hypertension, left ventricular hypertrophy is present in the older heart (3). Nephrosclerosis is also common, although frequently of a clinically silent course. Late in life, glomerular filtration rate decreases, the kidney's ability to produce a concentrated urine declines, tubular reabsorption and water homeostasis become impaired, and albuminuria becomes a common manifestation (8–11). Similarly, most strains of rats, especially males, are susceptible to nephrosclerosis with age (12), while in females renal decline is less severe (12, 13).

Advanced glycation endproducts (AGEs), the late products of the modification of proteins, lipids, and nucleic acids by reducing sugars, have been shown to accumulate slowly in vascular and renal tissues with age, and at a more rapid rate in diabetes (14, 15). Mounting *in vitro* and *in vivo* evidence indicates that AGEs can exert adverse physicochemical and biological effects (14, 15), negatively influencing vascular elasticity and NO·-mediated vasodilatory responsiveness (16, 17), as well as basement membrane and extracellular matrix composition and expansion in the kidney (18, 19). The AGE inhibitor aminoguanidine (AG) has been reported to reduce tissue AGE accumulation and to significantly inhibit several vascular and renal manifestations of experimental diabetes (20, 21) or AGE administration (17, 19). We now present evidence indicating that early interference with AGE accumulation by AG protects against adult onset vascular and renal impairment in the rat.

MATERIALS AND METHODS

Animal Studies. Six-month-old female Sprague–Dawley (S-D) and Fischer 344 (F344) rats ($n = 30$ per group; Charles River Breeding Laboratories, Wilmington, MA) were fed regular rat chow ad libidum during these studies, which were conducted in accordance with The Picower Institute Laboratory Animal Use and Care Committee guidelines. Five animals of each strain were evaluated and sacrificed at the beginning of the study for baseline values as described below. Half of the remaining rats of each strain were placed on oral AG-HCl treatment (0.1% or 9 mM in drinking water) up to the age of 24 mo, while the others were used as untreated controls. Body weight measurements and blood samples were obtained at baseline and at 4-mo intervals from all animals. Vasodilatory responses were assessed in separate subgroups of three animals from each strain at 4-mo intervals (see below). At the end of the study, the surviving animals were sacrificed by exposure to CO_2. Hearts, kidneys, and aortas were removed rapidly, weighed, and processed for microscopy (see below) and for AGE content by an AGE ELISA (22). Serum and urine samples were also assayed for AGEs.

NO·-Dependent Vasodilatory Responses. Animals were anesthetized with sodium pentobarbital (Nembutal) (50 mg/kg, i.p.) and cannulated as described (16). Blood pressure dose–response curves in response to acetylcholine (ACh) and nitroglycerine (NTG) were generated by injecting drugs directly into the jugular vein at a constant vol of 1 ml/kg as described (16, 17). Duplicate measurements for each drug dose were obtained for each animal. AG treatment alone was not noted to influence baseline arterial blood pressure or heart rate.

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Abbreviations: AGE, advanced glycation endproduct; AG, aminoguanidine; GBM, glomerular basement membrane; ACh, acetylcholine; NTG, nitroglycerine.

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Urinalyses. Urine samples (24 hr) from all animals were obtained for 3 consecutive days every 4 mo as well as before sacrifice for total creatinine (Stanbio Laboratory, San Antonio), total protein (Bio-Rad), albumin levels (by an anti-rat albumin antibody-based ELISA (19), and urinary AGE levels by ELISA (22).

Light and Electron Microscopy. After perfusion with 1% glutaraldehyde in Millonig buffer, kidneys were cut into 2-mm-thick slices. One-third of the slices were selected without bias (19, 23, 24), stored in 10% buffered formalin, and embedded in Historesin (Leica). From several of the remaining kidney slices, small blocks (1-mm cubes) were cut and processed for routine electron microscopy. The remaining slices were dehydrated and embedded in paraffin. Sections cut from the paraffin blocks were stained using periodic acid/Schiff reagent (PAS) and evaluated as described (19, 23, 24) in a blinded fashion for total and segmental glomerular sclerosis. The tissue embedded in Historesin was exhaustively sectioned. A known fraction of these sections were saved, stained with PAS, and used to count the total number of glomeruli per kidney using the fractionator-dissector technique (23, 25) and to measure glomerular volume (24). From the tissue processed for electron microscopy, three glomeruli were selected without bias. Electron micrographs obtained from the glomeruli were used to obtain estimates of glomerular basement membrane (GBM) width and volume fractions of mesangium and its components per glomerulus (26).

Statistical Analyses. Comparisons between groups were generally carried out by a two-way ANOVA or *t* test. Comparisons from measurements that were not normally distributed (mesangial volume and glomerulosclerosis) were completed by the Mann–Whitney *U* test.

RESULTS

Both strains of rats gained weight normally throughout the study, as was reflected in similar increases in kidney and heart weights of the untreated groups. AG-treated rats did not differ in body or kidney weight from untreated controls; however, the significant age-associated increase (by 40–50%) in heart weight in both rat strains (S-D, $P < 0.05$; F344, $P < 0.05$) was not observed in the corresponding AG-treated groups (Table 1). Mortality did not differ significantly between treated and untreated groups during the study, which was terminated at 24 mo.

As expected, aging resulted in a significant increase in the AGE content of heart, aorta, and kidney tissue of each rat strain over the younger animals' baseline values ($P < 0.05$ for each tissue) (Table 2). Treatment with AG resulted in lower tissue AGE levels in all three tissues ($P < 0.05$) in the old S-D rats. Kidney and heart AGE levels were also lower in AG-treated old F344 rats; in the heart, these values reached statistical significance ($P < 0.05$, old vs. old + AG). Age-associated increases in AGE levels were also noted in sera from aged animals of either strain and were lower in those treated with AG (Table 2).

Mean arterial blood pressure levels did not change significantly as animals aged, nor did they change in response to AG treatment (Table 1). Because AGEs have been shown to impair NO·-dependent responses and AG has been demonstrated to prevent this hyporesponsiveness (16, 17), we sought to determine the effects of AG treatment on the course of the age-related NO· vasodilatory dysfunction. Mean blood pressure responses to increasing doses of either ACh or NTG in animals of either the S-D or the F344 strain were already markedly impaired by the age of 12 mo (data not shown) and became progressively worse with increasing age compared to the brisk relaxation exhibited by the young, 6-mo-old controls (old vs. young S-D, $P < 0.001$; F344, $P < 0.005$) (Fig. 1). However, the 24-mo-old rats that were treated with AG retained dilatory responses that were comparable to those of the younger controls (old vs. old + AG: S-D, $P < 0.002$; F344, $P < 0.005$) (Fig. 1). Similar results were obtained in a study of AG-treated, 24-mo-old Lewis rats (data not shown).

In our studies, values for renal creatinine clearance did not diminish with age in either strain, even when corrected for kidney weight changes; they were in fact higher than baseline at the end of the study. This change was less or absent in rats treated with AG (Table 3). In contrast, renal AGE clearance, based on 24-hr urinary excretion of AGEs, decreased with age in both rat strains and significantly in the F344 rat (Table 3) ($P < 0.005$). When adjusted for the corresponding changes in creatinine clearance rates (AGE/creatinine clearance ratio), a significant reduction in AGE clearance with age was noted (S-D, $P < 0.05$; F344, $P < 0.01$) (Table 3). In old AG-treated rats, AGE clearance appeared higher compared to old untreated rats and, when adjusted for creatinine clearance, it showed less of a decline compared with the old untreated rats.

A considerable increase in total proteinuria was noted in both strains of rats with advancing age (Fig. 2 *A* and *B*). In contrast, over the 18 mo of treatment, AG-treated S-D rats

Table 1. Systemic parameters: Effects of aging and AG

Rat strain	MBP, mmHg	BW, g	HW, g	KW, g	S_{Cr}, mg/dl	U_{Cr}, mg per 24 hr
S-D						
Young (5)	119 ± 10	317 ± 16	1.1 ± 0.2	1.17 ± 0.04	0.74 ± 0.5	12.8 ± 1.4
Old (6)	120 ± 7	548 ± 31	1.4 ± 0.1	1.54 ± 0.07	0.50 ± 0.03	15.6 ± 0.6
Old + AG (6)	98 ± 8	463 ± 82	1.1 ± 0.09	1.55 ± 0.13	0.55 ± 0.09	12.0 ± 1.3
P value						
Old vs. young	NS	<0.005	<0.05	<0.01	NS	NS
Old vs. old + AG	NS	NS	<0.05	NS	NS	NS
F344						
Young (5)	135 ± 10	169 ± 3	0.5 ± 0.2	0.60 ± 0.02	1.1 ± 0.08	4.2 ± 0.2
Old (7)	115 ± 14	249 ± 7	0.9 ± 0.05	0.98 ± 0.03	0.70 ± 0.05	9.0 ± 0.6
Old + AG (6)	145 ± 18	237 ± 10	0.7 ± 0.05	0.99 ± 0.02	0.65 ± 0.02	6.1 ± 0.4
P value						
Old vs. young	NS	<0.005	<0.05	<0.005	NS	<0.01
Old vs. old + AG	NS	NS	<0.05	NS	NS	<0.01

Female S-D and F344 rats were divided into three groups: untreated, 6 mo old (young), untreated 24 mo old (old), and 24 mo old, treated with AG (0.1% in drinking water) starting at 6 mo of age (old + AG). All values were obtained at or just prior to sacrifice. MBP, mean blood pressure; BW, body weight; HW, heart weight; KW, kidney weight; S_{Cr}, serum creatinine; U_{Cr}, urinary creatinine; NS, no statistical significance. Numbers in parentheses, animals per group. Data are expressed as means ± SEM.

Table 2. Levels of AGEs in rat tissues, serum, and urine

Strain	Heart, units/mg	Aorta, units/μg	Kidney, units/mg	Serum, units/ml	Urine, units/24 hr
S-D					
Young (5)	5.5 ± 2	12 ± 2	84 ± 14	39 ± 7	336 ± 92
Old (6)	12.0 ± 5*†	40 ± 1**†	124 ± 26*†	66 ± 16*	342 ± 86
Old + AG (6)	9.5 ± 4	18 ± 1	93 ± 18	50 ± 12	389 ± 44
F344					
Young (5)	1.25 ± 0.3	10 ± 2	41 ± 6	58 ± 23	230 ± 45
Old (7)	4.05 ± 0.7*†	40 ± 3*	55 ± 12*	90 ± 20*†	217 ± 16
Old + AG (6)	3.36 ± 0.7	40 ± 2	48 ± 8	69 ± 18	208 ± 36

Female S-D and F344 rats were divided into three groups: untreated, 6 mo old (young), untreated 24 mo old (old), and 24 mo old, treated with AG (0.1% in drinking water) starting at 6 mo of age (old + AG). All values were obtained at or just prior to sacrifice. AGEs were measured by ELISA (22). Numbers in parentheses indicate number of animals per group. Data are expressed as means ± SEM. Old vs. young: *, $P < 0.05$; **, $P < 0.005$. Old vs. old + AG: †, $P < 0.05$; ††, $P < 0.005$.

exhibited virtually no increase in proteinuria above the 6-mo baseline value (Fig. 2*A*), while in F344 AG-treated rats, urinary protein excretion was half that of untreated rats ($P < 0.04$) (Fig. 2*B*). Similarly, the marked age-associated albuminuria observed in older rats was suppressed in those treated with AG (Fig. 2*B*). Even when values were expressed as renal albumin clearance rate and adjusted for kidney weight, the effect of AG treatment was highly significant (S-D, $P < 0.005$; F344, $P < 0.01$) (Table 3).

In concert with protein and albumin losses, morphologic and morphometric changes pointed to ongoing glomerular injury, which was more severe in the S-D than the F344 strain (Table 4). A striking age-related fall in total glomerular number in the S-D strain (by 24%, $P < 0.04$) was accompanied by significant increases in glomerular sclerosis ($P < 0.02$) and glomerular volumes ($P < 0.05$) (Table 4). In contrast, there was no glomerular unit loss in age-matched S-D rats treated with AG (Table 4). Furthermore, AG-treated S-D rats exhibited fewer sclerotic glomeruli ($P < 0.01$). AG had no apparent effect on mean glomerular volume, on GBM width (Table 4), or on volume fractions of the mesangium and its components (data not shown).

By comparison, young F344 rat kidneys exhibited a substantially lower glomerular number at baseline and no further decline with age (Table 4). Despite a modest increase in the percentage of sclerotic glomeruli ($P < 0.05$), there were no age-associated changes in GBM width or in glomerular volumes. Consequently, there were no significant structural effects associated with AG treatment in this strain.

Of note, compared to untreated older animals, which typically exhibited cataracts, unkempt fur, reduced locomotor activity, hindlimb weakness suggesting peripheral neuropathy, and a lethargic disposition, age- and weight-matched AG-treated animals appeared alert, active, well groomed, and without visible lenticular opacities or coat pigmentation (Fig. 3).

DISCUSSION

In this report, we provide evidence of an interrelationship between two key manifestations of physiological aging in the rat, cardiovascular and renal decline, and the spontaneous age-associated biochemical process termed advanced glycation thought to contribute to progressive tissue damage and organ failure. AG, an inhibitor of advanced glycation, was used in this study as a reagent known to interfere with advanced glycation (20) as well as to intervene in glycation-mediated pathogenicity (14, 15, 20, 21).

Adult life in humans is almost universally accompanied by cardiovascular decline, most frequently manifested as hypertension or atherosclerosis (1–4), and contributes to >50% of all deaths in the adult population (27). Renal decline in humans occurs slowly, and in the last decades of life overall kidney function remains adequate for survival in the absence of other diseases (5, 6). A typically modest decrease in creatinine clearance is exceeded by definitive changes in renal plasma flow, protein leakage, tubular absorption, and concentrating ability, such that nearly one-third of all aged persons are thought to be afflicted by significant renal disease (5, 6, 8, 9, 10).

As in most rat strains, the two strains of rats used in this study, although free of obvious cardiovascular disease, are susceptible to age-related renal disease (12, 13, 28). In particular, S-D rats are subject to an earlier-onset, and more rapidly progressive, renal disease, compared to F344, which exhibit a milder decline (28). Furthermore, a marked dimorphism in renal response to senescence protects female rats from overt functional and structural deterioration until later in life (12, 13). To better depict the milder course of human aging, and to allow for "muted" cardiovascular dysfunction to be dissected

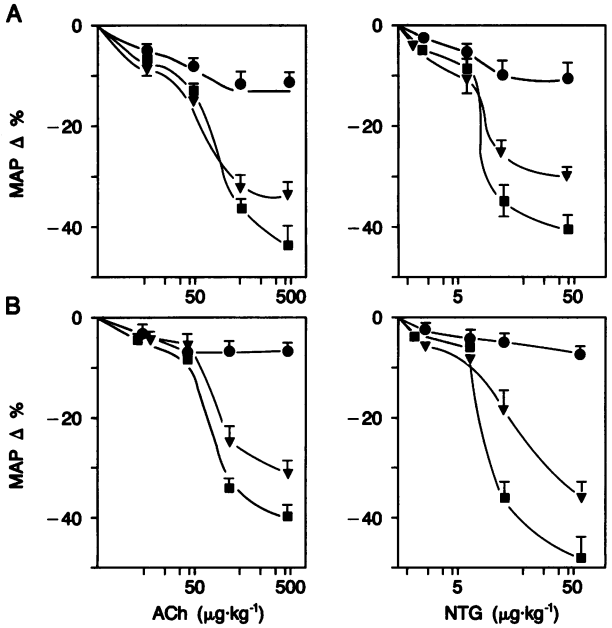

FIG. 1. Vasodilatory impairment in older rats is suppressed by AG. Female S-D (*A*) and F344 (*B*) rats were divided into three groups: untreated, 6 mo old (■), untreated 24 mo old (●), or 24 mo old, treated with AG (0.1% in drinking water) (▼). Rats were tested prior to sacrifice for dilatory responses to increasing doses of ACh or NTG. MAP, mean arterial pressure. Data represent means ± SEM obtained from five or six rats. S-D: old vs. young, $P < 0.001$; old vs. old + AG, $P < 0.005$. F344: old vs. young, $P < 0.001$; old vs. old + AG, $P < 0.05$, for either ACh or NTG.

Table 3. Effects of aging and AG on renal function

Rat strain	Creat. Cl ($\times 10^{-5}$)	Alb. Cl ($\times 10^{-9}$)	AGE Cl ($\times 10^{-6}$)	AGE Cl/Creat. Cl ($\times 10^{-3}$)
S-D				
Young (5)	109 ± 9	0*	5.5 ± 1.8	0.05
Old (6)	135 ± 46	29 ± 9	2.9 ± 2.0	0.02
Old + AG (6)	109 ± 26	4 ± 2	4.1 ± 3.3	0.04
P value				
Old vs. young	NS	<0.001	NS	<0.05
Old vs. old + AG	NS	<0.005	NS	<0.03
F344				
Young (5)	62 ± 4.5	0*	4.2 ± 0.9	0.07
Old (7)	93 ± 30	43 ± 1	1.8 ± 0.2	0.02
Old + AG (6)	70 ± 8	22 ± 4	2.2 ± 0.4	0.03
P value				
Old vs. young	NS	<0.001	<0.004	<0.01
Old vs. old + AG	<0.04	<0.01	NS	<0.05

Female S-D and F344 rats were divided into three groups: untreated, 6 mo old (young), untreated 24 mo old (old), and 24 mo old, treated with AG (0.1% in drinking water) starting at 6 mo of age (old + AG). All values were obtained at or just prior to sacrifice. Renal clearance of creatinine (Creat. Cl), albumin (Alb. Cl), and AGE (AGE Cl) is expressed in ml per min per mg of kidney weight.

from the more overwhelming renal disorder in this species, the female gender was selected for study.

Consistent with earlier reports (14, 15), advanced glycation products were detected in a variety of young tissues and increased significantly as the animals aged. The age-associated increases in serum AGEs observed in the absence of hyperglycemia constitute a novel finding and could reflect either increased AGE protein turnover activity or impaired AGE clearance due to renal dysfunction. The latter notion has received support from studies in patients with renal disease in which serum AGE levels correlated inversely with kidney function (29, 30). In view of the evidence available in the present study, pointing to early impairment of renal clearance for AGE protein degradation products, the rising tissue and serum AGE levels may reflect second generation AGEs formed from "reactive" glycation intermediates that are not cleared by the kidney (30).

As shown previously by many investigators, rat aging was accompanied by increases in total body, kidney, and heart weight. Unlike the former two, which were not influenced by AG treatment, heart weight remained close to baseline in AG-treated groups, suggesting a distinct mechanistic link between age-related cardiac hypertrophy and the consequences of AGE accumulation. Cardiac hypertrophy in older humans is attributed generally to increased mechanical load (3, 7, 31). Even in normotensive elderly subjects, cardiac hypertrophy accompanies increased arterial stiffness, an increase in aortic impedance, and an increase in peripheral vascular resistance (3, 7, 32). In view of the present data on lower tissue AGE content in the heart and aorta of treated animals, the effects of AG on cardiac hypertrophy may be due in part to the overall reduction of AGE crosslinking and preservation of elastic properties of aortic and heart tissues. Another plausible explanation for the protective effects rendered by chronic AG treatment is the nearly complete preservation of normal NO· dependent blood pressure responses, an effect noticeable as early as at 12 mo of age (or after 6 mo of treatment) (data not shown). Similar results were obtained from AGE-treated older Lewis rats (data not shown). Age-associated vascular unresponsiveness to vasodilators has been previously attributed to reduced nitric oxide production, or sensitivity to NO· action, as a function of age (8, 33, 34). Based on evidence that AGEs

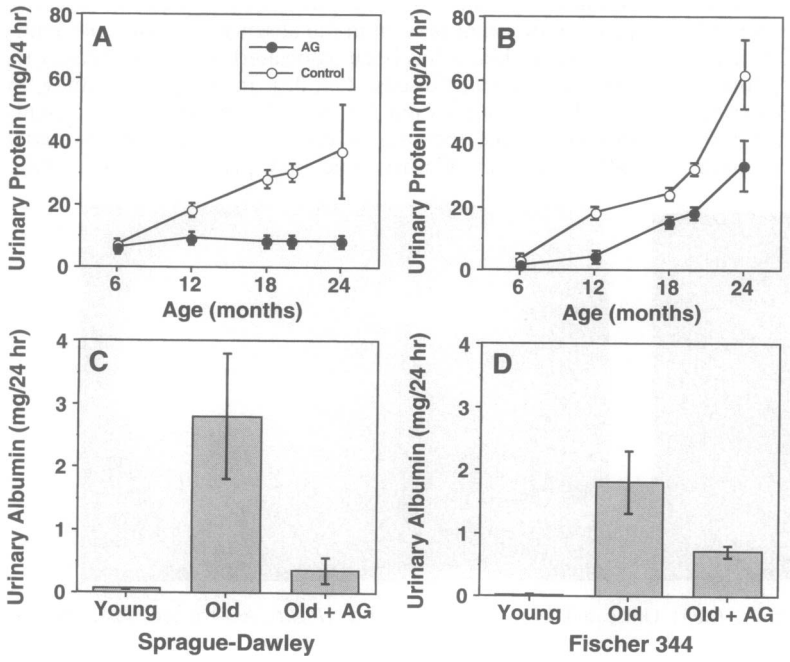

FIG. 2. Age-related urinary protein (*A* and *B*) and albumin loss (*C* and *D*) in the rat is suppressed by AG. Total urinary protein concentration was determined in 24-hr urine collected every 4 mo over an 18-mo period from S-D (*A*) or F344 (*B*) rats treated with AG (0.1% in drinking water) (●) or untreated, age-matched controls (○). Albuminuria was determined at baseline and at the end of the study in S-D (*C*) or F344 (*D*) rats. Data are expressed as means ± SEM. Comparisons (old vs. young; old vs. old + AG) of all experimental groups of both strains were significant at $P < 0.05$ ($n = 5$–7 rats per group).

Table 4. Effects of aging and AG on renal structure

Rat strain	Glom./K	GBM	GS, %	Glom. vol. ($\times 10^{-6}$ μm^3)
S-D				
Young (5)	31500 ± 3100	N/A	0.2 ± 0.1	0.65 ± 0.04
Old (6)	23800 ± 6100	373 ± 22	11 ± 7	1.17 ± 0.08
Old + AG (4)	32100 ± 5900	369 ± 18	4 ± 2	0.99 ± 0.06
P value				
Old vs. young	<0.04	N/A	<0.02	<0.05
Old vs. old + AG	=0.07	N/A	<0.01	NS
F344				
Young (5)	19700 ± 3200	N/A	0.6 ± 0.4	0.43 ± 0.02
Old (7)	17500 ± 3500	376 ± 42	6.0 ± 6.0	1.04 ± 0.05
Old + AG (8)	17600 ± 2900	357 ± 33	9.0 ± 9.0	1.03 ± 0.07
P value				
Old vs. young	NS	N/A	<0.05	<0.05
Old vs. old + AG	NS	NS	NS	NS

Female S-D and F344 rats were divided into three groups: untreated, 6 mo old (young), untreated 24 mo old (old), and 24 mo old, treated with AG (0.1% in drinking water) starting at 6 mo of age (old + AG). Glom./K, no. of glomeruli per kidney; GS, glomerulosclerosis; Gl. vol., glomerular volume. Data are expressed as means ± SD. Numbers in parentheses represent animals per group. N/A, not available; NS, not significant.

interfere chemically with NO· action (16), age-associated dilatory impairment could also be attributed to NO· quenching by vascular tissue AGEs. A similar mechanism has been suggested from animal studies using experimental diabetes or AGE administration to normal animals, in which defective vasodilatory responses were effectively inhibited by AG (16, 17). Throughout this study, there was no evidence of an age-related rising arterial blood pressure, despite documented vasodilatory dysfunction and cardiac hypertrophy. This is consistent with human aging where significant, mainly left ventricular hypertrophy frequently coexists with an apparently normal blood pressure level (3, 32). Whichever the precise mechanism, by maintaining normal NO·-dependent vasodilatory capacity or by interfering early with AGE-mediated crosslinking, AG treatment may have protected against rising mechanical stress on the cardiovascular system, increased vascular resistance, and cardiac hypertrophy.

The effects of aging on renal function in humans include, among others, a reduction in creatinine clearance, alterations in renal vasculature, changes in autonomic control by vascular adrenoreceptors, and arterial baroreflex, changes in the renin angiotensin system, and in the action of endothelin, a potent vasoconstrictor (35). NO·-mediated renal responses are reportedly blunted in the aging rat (36). The maintenance of a normal NO·-dependent dilatory reserve in the systemic vasculature by AG may provide a basis to speculate significant opposing action to renal vasoconstrictive forces, resulting in a lowering of renal vascular resistance adequate enough to maintain normal renal blood flow. The latter would be instrumental in slowing glomerular sclerosis and other responses to acute and chronic insults in susceptible individuals.

Despite an apparently enhanced creatinine clearance in the older female rats, functional impairment was evident by the significant reduction in urinary clearance of AGEs. In fact, AGE clearance in both strains of older animals was markedly reduced, even in light of the increased total tissue AGE accumulation, compared to younger animals. The selective decline in AGE clearance was even greater when compared to creatinine clearance, which normally is also influenced by increasing body weight in the rat. In human studies, levels of circulating low molecular weight AGE-rich substances correlated inversely with creatinine clearance such that significant elevations in serum AGEs corresponded with overt reductions in creatinine glomerular filtration rate in end-stage renal disease (29, 30). Although treatment with AG protected only partially against AGE clearance dysfunction in the present studies, further information is needed to better define both the significance of the selective defect in AGE clearance with age and the drug effect.

Most dramatic, however, was the amelioration of albuminuria and proteinuria as a result of AG treatment, especially prominent in the older S-D rats. Although in humans frank proteinuria is not present in the absence of glomerular injury, microalbuminuria has been associated with an increased risk for cardiovascular disease (37, 38). In the present study, the coordinate suppression by AG of this marker of vascular disease, of the increase in cardiac size, and of the loss in NO·-dependent dilatory tone may provide a novel unified

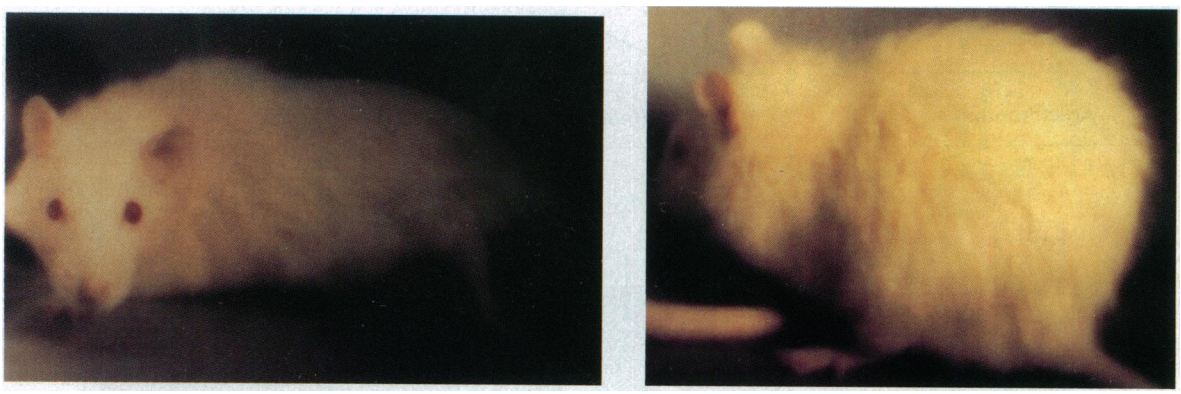

FIG. 3. Representative F344 rats at the end of the study. (*Left*) Untreated 24-mo-old female rat. (*Right*) AG-treated, sex-, age-, and weight-matched rat.

mechanistic explanation for this association. The effect of AG on glomerular permeability, as reflected in protein and albumin loss, was noticeable within the first 6 mo of treatment, as was the effect on vasodilatory potential. The present data cannot distinguish between onset of either renal injury or systemic vascular dysfunction. Further studies can be expected to establish whether renal injury due to local accumulation of reactive AGEs may precede and promote systemic vascular dysfunction. At present, this is merely hinted at by the higher kidney tissue AGE content at baseline compared to other young tissues. It is likely that early exposure of the renal apparatus to the constant passage of catabolic products containing reactive AGEs leads to early modification of renal tissues and possibly to renal injury. Such early AGE deposition in the kidney may compromise renal function in ways that remain undetectable for extended periods of time and may later materialize as systemic vascular dysfunction.

Consistent with numerous previous studies, a typically vascular glomerular sclerosis was also found in the present study. Of particular interest was the significant fall in glomerular number in the aging S-D rat (by 24%) compared to young animals. Even more intriguing was the complete protection rendered by AG against glomerular loss. In F344 rats, however, there were no significant changes in glomerular number, while less striking structural changes were influenced to a lesser extent by AG treatment. These data are consistent with evidence suggesting that, although both strains are likely to develop glomerular disease, the S-D strain is particularly susceptible to injurious factors (28). The precise nature of such toxic factors in age-dependent glomerular damage has not been defined. However, the data suggest that an increased genetically determined tendency of this strain to glomerular injury may be exacerbated by the mounting AGE load filtered through the kidneys as a function of time. This, together with the progressive systemic vascular dysfunction, may precipitate earlier and more severe glomerular damage in susceptible strains—thus, the selective nephron loss in S-D. Lower tissue AGE burden together with significant relief from AGE-related toxicity afforded by AG administration appears to preserve an altogether more satisfactory level of cardiovascular and renal function, as evidenced by the generally healthier appearance of old animals treated by AG compared to untreated age- and weight-matched controls. Although not an aim of this report, the essential question of whether this intervention may ultimately improve survival must be addressed in future studies.

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