Original Article

The Effects of Antihypertensive Agents on the Survival Rate of Polycystic Kidney Disease in Han:SPRD Rats

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disorder in humans. Hypertension is one of the major complications, and its control might affect the renal survival and disease mortality. Suitable antihypertensive agents have been discussed based on clinical and animal studies, but no definitive conclusion has been reached. Generally, therefore, all antihypertensives are indiscriminately treated as if providing the same level of blood pressure control. In this study, the blood pressure control of two antihypertensives was investigated using a rat model of ADPKD in humans. Twenty-four male Hannover-Sprague Dawley (Han:SPRD) rats were divided into three groups: a group receiving amlodipine (6 mg/day), a group receiving benazepril (6 mg/day) and an untreated control group. Blood pressure, body weight, and urinary protein excretion were regularly measured up to week 52. Amlodipine and benazepril significantly decreased blood pressure and urinary protein excretion to the same degree. Moreover, a remarkably prolonged survival rate was observed in both groups (at week 52, the survival rate was 25% in controls, 50% in the amlodipine group, and 50% in the benazepril group). Examination at autopsy revealed that enlarged cysts were prevalent in the renal tissue of both experimental all three groups, suggesting that the cystic disease had reached the end-stage in all the animals. In conclusion, both amlodipine and benazepril significantly improved blood pressure control, urinary protein excretion, and survival rate, possibly due to their enhancement of renal survival. (*Hypertens Res* 2002; 25: 939–943)

Key Words: autosomal dominant polycystic kidney disease, hypertension, complication

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disorder in humans. Hypertension is one of the major complications in ADPKD, and its control is considered crucial to attenuate the progression of renal dysfunction (1). The efficacy of various antihypertensive agents in protecting renal function has been well investigated in humans and animals. Angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers (CCBs) have demonstrated renal protective effects in many

studies (2, 3), but their mechanisms remain unclear. In addition to their antihypertensive effects, these drugs have been shown to exert a number of organoprotective effects in humans and animals (4–6). In regard to ADPKD, Chapman *et al.* reported a series of studies showing the efficacy of ACE inhibitors and the importance of the renin-angiotensin system in ameliorating controlling hypertension in ADPKD (7, 8). Due to restrictions in their study design, however, they were unable to investigate either the renal survival or disease mortality. Therefore, the aim of the present study was to investigate the efficacy of antihypertensives in Hannover-Sprague Dawley (Han:SPRD) rats, an animal model of

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Fig. 1. Changes in systolic blood pressure measured by the tail cuff method. Systolic blood pressure increased after week 8 in all groups. There was no significant difference throughout the study between the two groups that are received each drugs from the same week. * $p < 0.05$ vs. the control group.

Fig. 2. Changes in 24-h urinary protein excretion. Urinary protein excretion increased after week 4 in all groups. There were significant differences between the control group and treated groups 2 and 3 at weeks 12 and 44. No significant difference was seen between the two treated groups. * $p < 0.01$ vs. the control group.

ADPKD in humans.

Materials and Methods

Twenty-four male Han:SPRD rats were derived from heterozygous breedings as described previously (9). Rats were kept in individual metabolic cages throughout the study, and were initially fed a normal diet (15 g/day, 0.38% sodium, 0.97% potassium, 25.1% protein; Nippon Clea, Tokyo, Japan). The animals were given a week to become acclimated to their cages. Then, the 8-week-old rats were randomly distributed into three groups. The control group (group 1; $n = 8$) was fed a diet without any drugs. The amlodipine group (group 2; $n = 8$) received 6 mg/day of amlodipine besilate (Pfizer Pharmaceuticals Inc., Tokyo, Japan) mixed in their diet. And the benazepril group (group 3; $n = 8$) received 6 mg/day of benazepril hydrochloride (Novartis Pharmaceuticals Inc., Tokyo, Japan) mixed in their diet. All groups were allowed drinking water *ad libitum*. Body weight, systolic blood pressure (measured by the tail cuff method (10); KN210, Natsume Seisakusyo Co., Ltd., Tokyo, Japan), and 24-h urinary protein excretion (measured by Lowry's method (11)) were measured before the study and at weeks 4, 8, 12, 20, 28, 36, 44, and 52. At week 52, rats were decapitated and blood was collected to measure the serum levels of total protein, albumin, creatinine, and urea nitrogen using an automatic analyzer (FDC-3500; Fuji Film Co., Tokyo, Japan). In addition, the kidneys of all rats were removed (the kidneys were also removed from all rats who died before 52 weeks). The kidneys were fixed in 10% formaldehyde overnight, embedded in paraffin, cut into 3 μ m-thick sections, and stained with periodic acid Schiff's reagent. They

were examined under a light microscope connected to a CCD camera (HC-2500; Fuji Film Co.). Ten, randomly selected non-overlapping images at 100 $\times$ magnification were scanned as graphic image files for each kidney sample. Each image was analyzed quantitatively with image analysis software (MacScope; Mitani Corp., Chiba, Japan). In each image, the total area of the expanding collecting ducts was measured and its percentage of the whole image area was calculated. Individual expanding collecting duct percentages were obtained as the average of 10 images in each section (12). To control for changes in the collecting ducts, 3 newly prepared rats were maintained and treated in the same way, then decapitated at week 8. All data are expressed as the means $\pm$ SEM. Analysis of variance (ANOVA) was employed to determine the significance of any difference among the groups. Comparisons among the groups were made using Scheffé's method, and values of $p < 0.05$ were considered to indicate statistical significance (13). For analyzing differences in the life survival, all rats were included in the Kaplan-Meier analysis, and observations were censored if the animals died before the day of evaluation. The significance of differences among the groups was analyzed by the log-rank test.

Results

Body Weight

In the control group (group 1), body weight changed from 440 ± 25 g to 468 ± 36 g, in the amlodipine-treated (group 2) it changed from 438 ± 35 g to 461 ± 28 g, and in the benazepril-treated group (group 3) it changed from 446 ± 19 g

Table 1. Laboratory Data

	Total protein (g/dl)	Serum albumin (g/dl)	Urea nitrogen (mg/dl)	Serum creatinine (mg/dl)
Control (<i>n</i> = 3)	5.5 ± 0.4	2.9 ± 0.1	33 ± 5	0.8 ± 0.1
Amlodipine (<i>n</i> = 5)	5.8 ± 0.3	3.2 ± 0.4	26 ± 2	0.7 ± 0.1
Benazepril (<i>n</i> = 4)	6.2 ± 0.8	3.5 ± 0.8	28 ± 1	0.7 ± 0.3

Fig. 3. Typical image of a kidney section at week 52 (A). Significant cyst expansion was observed. This image was scanned through a CCD camera, and analyzed in B. The area of cyst expansion was calculated as 61.5% of the whole area in this image.

to 466 ± 33 g. There were no significant differences in body weight among the three groups.

Blood Pressure

The systolic blood pressure was increased in group 1 from 100 ± 8 to 161 ± 6 mmHg at week 52 (the peak value was 168 ± 10 mmHg at week 36). In groups 2 and 3, systolic blood pressure did not show any significant increase. At weeks 28, 36, and 44, the blood pressures of the two treated groups were significantly lower than that of the control group ($p < 0.05$) (Fig. 1).

Urinary Protein Excretion

The urinary protein excretion of group 1 was increased from a baseline value of 76.7 ± 7.2 mg/day to 228.3 ± 25.4 mg/day at week 12. In contrast, the amlodipine- and benazepril-treated groups increased only slightly from their baseline values to 62.3 ± 6.5 and 68.6 ± 8.2 mg/day (Fig. 2). The control group showed significantly higher protein excretion at weeks 12 and 44 ($p < 0.01$). For the protein determination (Lowry's method), the coefficient of intraassay variation in our laboratory was 8.8%.

Biochemistry

The biochemical measurements are given in Table 1. Serum samples were obtained only from rats that survived until week 52, with the exception of one rat in the amlodipine-treated group. Serum creatinine increased in all three groups from 0.38 ± 0.08 mg/dl (week 8, $n = 3$). Because of the small number of groups, it was considered inappropriate to analyze their data in detail; generally, however, there were no significant differences in biochemical parameters among the three groups.

Cystic Expansion of Tubules in Renal Tissue

Figure 3A shows a typical kidney section image, and its analysis is shown in Fig. 3B. The area of cystic expansion was calculated as 61.5% of the whole area in this image. At the start of the study, the percentage of tubular lumen area to total image area was $6.4 \pm 1.3\%$ ($n = 3$). This percentage was significantly increased to $54.6 \pm 3.4\%$ in the control group, to $53.3 \pm 3.8\%$ in the amlodipine group, and to $57.1 \pm 3.2\%$ in the benazepril group at dead. There was no significant difference among the groups (Fig. 4). In addition, no major changes in the glomerulus area were observed in any of the groups.

Life Survival

At week 52, the survival rates were 25% in the control group, 50% in the amlodipine group, and 50% in the benazepril group. As shown in Fig. 5, the survival was remarkably improved in groups 2 and 3 compared to that in the control group (log-rank test; χ^2 value = 12.302, $df = 3$, $p = 0.0064$).

Fig. 4. Cystic expansion of the tubules after sacrifice. The percentages of the area of the tubular lumen to the area of the whole image were not significantly different among groups. In each group, the kidneys from 8 rats were analyzed.

Discussion

In the present study, amlodipine and benazepril exerted similar beneficial effects on blood pressure, urinary protein excretion, and survival.

The Han:SPRD rat model was established in 1989 (14), and has been well investigated as a model of renal dysfunction due to genetic polycystic kidney disease. Although many factors, including ACE inhibitors, angiotensin II receptor antagonists, sodium restriction (15, 16), soy and protein (17), had been reported to be renoprotective in these rats, our study is the first to confirm the renal protective effect of CCBs. In this study, the effects of amlodipine on blood pressure, urinary protein, and survival were largely identical to those of benazepril. This result supports the findings of our previous study in patients with ADPKD (18).

Neither amlodipine nor benazepril showed any significant effect on cystic expansion in this study, in contrast to the study of Ogborn *et al.*, which showed that cilazapril significantly reduced the cystic volume (19). There are two possible reasons for this discrepancy. One is the difference in study period. While their study had an observation period of 5 months, our tissue evaluation was carried out over 12 months. The progression of renal dysfunction over the final 7 months might have lessened the effects of antihypertensives. In spite of the antihypertensive treatments, cystic expansion reached the end-stage during our longer study period, even in the surviving rats. Both antihypertensives improved the survival rate, possibly as a result of an improvement in renal survival, since all the deaths were caused by the end-stage cystic kidney. Because of the small number of rats in each group, the statistical power of the present results is somewhat limited. Nevertheless, these findings would seem to in-

Fig. 5. Survival was remarkably improved in groups 2 and 3 compared to that in the control group (group 1). The black rectangle, circle, and triangle represent groups 1 (control), 2 (amlodipine), and 3 (benazepril), respectively.

dicate that antihypertensive treatment with amlodipine or benazepril likely retards the cystic expansion in Han:SPRD rats. The second possible cause of the difference in results between our study and that of Ogborn *et al.* is the difference in the route of excretion (19). The metabolism of ACE inhibitors *in vivo* can be mainly divided into two categories, renal excretion and liver metabolism. Ninety percent of cilazapril is usually excreted through urine, and thus may accumulate in rats with injured kidneys. For this reason, it is possible that the effects of ACE inhibitors may also have been enhanced in the study of Ogborn *et al.* (19). For patients with renal dysfunction, the dose of renal excretion-type ACE inhibitors must be decreased to avoid adverse effects. On the other hand, liver metabolism-type ACE inhibitors, such as benazepril, do not require a dose reduction. The former studies concerning ADPKD and ACE inhibitors have not addressed this problem.

Both antihypertensive treatments in the present study reduced the levels of proteinuria, in agreement with previous studies (20, 21). The mechanism of the effects on reducing proteinuria of antihypertensives (ACE inhibitors, CCBs, and α -blocker) (6, 22) were explained mainly by reducing blood pressure, but both ACE inhibitors and CCBs are reported that they have the effects which are not due to lowering the blood pressure (2, 3). Although the blood pressures in groups 2 and 3 were increased slightly even after week 12, the effect on proteinuria was observed throughout the study, which is suggesting that it was related to effects other than the reduction of blood pressure. For some time now, both ACE inhibitors and CCBs have been considered appropriate antihypertensive agents for patients with ADPKD, but which should be the drug of choice has remained uncertain. Chapman *et al.* reported a series of studies showing the efficacy of ACE inhibitors, and the importance of the renin-angiotensin

system on hypertension in ADPKD (7, 8). At the same time, we suggested that CCBs and ACE inhibitors had nearly identical renoprotective effects in patients with this disease (18). In addition, Ecder *et al.* recently showed that both ACE inhibitors and CCBs offered similar, long-term protection of the renal function in patients with ADPKD (23). Therefore, with respect to kidney function, the question changes from "Which agent is best?" to "How much blood pressure reduction is appropriate?"

Finally, several limitations of the present study bear mention. Because the protocol was too long to evaluate the effects of antihypertensives, and the total mortality was unexpectedly high, the cause of death, the effects of antihypertensives, and the cyst expansion were not considered as independent factors. It is therefore possible to analyze these data from many other aspects.

In conclusion, ACE inhibitors and CCBs had almost identical effects on blood pressure control, urinary protein excretion, and survival in Han:SPRD rats. Further studies will be needed to more precisely clarify the effects of antihypertensive agents on other factors.

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