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Long term effects of amlodipine on organ damage, stroke and life span in stroke prone spontaneously hypertensive rats

Makoto Suzuki [a], Kyozo Yamanaka [a], Hiroshi Nabata [b] and Masakatsu Tachibana [b]

[a] Pharmacology Laboratory and [b] Safety Evaluation Laboratory, New Product Development Center, Pfizer Pharmaceuticals Inc , 5-2 Taketoyo-Cho, Chita-Gun, Aichi 470-23, Japan

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The long term effects of amlodipine, a new long acting Ca^{2+} channel antagonist on organ damage, stroke and life span, were examined in stroke prone spontaneously hypertensive rats (SHRSPs) Blood pressure of the SHRSPs increased over the first 16 weeks and reached a stable level of about 250 mmHg in controls and about 200 mmHg in the amlodipine treated group At 15 weeks after starting amlodipine treatment, all control SHRSPs exhibited varying degrees of myocardial fibrosis, proliferative and/or necrotic vasculitis and glomerular lesions, whereas only a few animals in the amlodipine group showed slight lesions The average life span of animals was estimated to be 43 3 weeks and 71 1 weeks for control and amlodipine groups, respectively, which suggested a 1 6-fold prolongation of their life span by amlodipine treatment These results indicate that the long term treatment of amlodipine suppresses the incidence of organ damage and stroke in SHRSPs and prolongs their life span

Amlodipine, Spontaneously hypertensive rats, stroke prone (SHRSPs), Cardiac hypertrophy, Renal lesion, Stroke, Life span

1. Introduction

Amlodipine is a new long acting Ca^{2+} channel antagonist of the 1,4-dihydropyridine class, with highly selective preference for the vascular system (Burges et al , 1987, 1989, Matlib et al , 1988; Yamanaka et al , 1991b) It produces a stable lowering of blood pressure in hypertensive animals and man without changes in heart rate (Burges et al., 1988, Julius, 1988, Yamanaka et al , 1991a), and exerts cardioprotective effects during ischemia with increasing coronary blood flow (Gross et al., 1989, Hoff et al., 1989, Nayler, 1989), thus indicating a potential clinical use in the therapy of hypertension and angina pectoris.

Hypertension is well known to be aggravated by the incidence of vascular and organ damage such as cardiac hypertrophy, atherosclerosis and nephrosclerosis (Saxena and Man In't Veld, 1991, Struyker Boudier et al., 1990) Therefore, ideal antihypertensive agents are believed to be those capable of preventing or improving such complications, as well as lowering blood pressure.

In this respect, long term treatment with amlodipine has been shown to attenuate cardiac hypertrophy and aortic collagen synthesis in spontaneously hypertensive rats (SHR) (Chichester and Rodgers, 1987, Nayler, 1988). It also prevents the development of sclerotic calcinosis of the mesenteric artery in Dhal-S rats (Fleckenstein et al , 1988, 1989), at a high dose regimen that maintains blood pressure in the near-normal range Furthermore, amlodipine slows aortic atherogenesis in cholesterol fed rabbits and alters low density lipoprotein metabolism by human skin fibroblasts (Nayler and Gu, 1990, Paoletti and Bernini, 1990). The evidence would seem to indicate that amlodipine has potential benefits in antihypertensive therapy in terms of cardiovascular protection

The present study was undertaken to assess whether long term treatment with amlodipine affects the incidence of organ damage, stroke and life span of stroke prone spontaneously hypertensive rats (SHRSPs) even at a daily dose which leaves the animals hypertensive This strain is the well accepted model that exhibits complications as severe hypertension progresses, similar to those encountered in human essential hypertension

Correspondence to K Yamanaka, Pharmacology Laboratory, New Product Development Center, Pfizer Pharmaceuticals Inc , 5-2 Taketoyo-Cho, Chita-Gun, Aichi 470-23, Japan Tel 81-569-72-6816, Fax 81-569-73-1064

2. Materials and methods

2 1 *Amlodipine treatment of animals*

Stroke prone spontaneously hypertensive rats (SHRSPs) were kindly donated by Prof Okamoto (Kinki University, Japan) and bred in our laboratory Each SHRSP was kept in an individual cage in a colony room with a 12 h day/night cycle at constant temperature (23 ± 2°C) and humidity (55 ± 5%), and given ad lib access to powder diet (Oriental Yeast Co , MF) and tap water

32 male SHRSPs (initial age of 5 weeks) were used in this experiment and divided into two groups for control and amlodipine treatment The animals of the latter group were given a daily dose of 3 mg/kg amlodipine as an admixture with powder diet, throughout the experimental period until death or killing The dose of amlodipine was selected on the basis of its effective dose (2 3 mg/kg) producing reduction of blood pressure by 30 mmHg in SHR (Yamanaka et al , 1991a) The amount of amlodipine added to the diet was corrected every week based on the estimation of food consumption and body weight of the animals 11 rats in each group were used to assess survival rate and systolic blood pressure and fed until death An additional five rats in each group were killed at 15 weeks after beginning amlodipine treatment and used for the histopathological assessment of organs and hematological assays

2 2 *Blood pressure measurement*

Systolic blood pressure (SBP) and heart rate of SHRSPs were measured once a week for the first 10 weeks and thereafter once every 2 weeks, by a tail-cuff method using an electrosphygmomanometer (Muromachi, MK-1000) Before each measurement, the rats were warmed for 6–12 min in a box kept at 37°C to assist measurement of the SBPs

2 3 *Histopathological and hematological assays*

At 15 weeks treatment with amlodipine, blood samples were collected from the abdominal aorta of five rats in both control and treated groups under ether anesthesia, for the assay of blood parameters (blood urea nitrogen, creatinine, total cholesterol, triglyceride, phospholipid and non-esterified fatty acid) Furthermore, several organs (brain, heart, kidney and mesenteric arteries) were excised from these animals and prepared for histopathological assessment by conventional methods of staining with hematoxylin-eosin, azan-Mallory, elastica-Van Gieson and periodic acid-Schiff (for kidney)

2 4 *Data analysis*

The significance of difference between treated and non-treated groups was tested using unpaired Student's t test, Mann-Whitney's U test and Fischer's χ^2 test The ages at which 50% of the animals died were calculated with the least square method

3. Results

3 1 *Development of hypertension*

The initial systolic blood pressure (SBP) of SHRSPs did not differ statistically significantly between control and amlodipine groups, corresponding SBPs were 138 ± 4 mmHg and 141 ± 4 mmHg, respectively The SBPs of these animals increased rapidly with time and reached the steady state at about 16 weeks of amlodipine treatment (fig 1) The amlodipine group exhibited significantly lower SBPs than the control group at 4 weeks of treatment and the difference between the two groups became more marked during subsequent treatment Consequently, the steady state level of SBPs in the treated group was 196 ± 2 mmHg and about 50 mmHg lower than that of control animals (251 ± 4 mmHg) These levels then remained constant in both groups until the animals died (throughout the experiment, fig 1 only shows the data until the end of 30 weeks) This indicates that the treated animals were still hypertensive

The animals in these groups showed similar initial mean values and time courses of gain in body weight throughout the experiment

TABLE 1

Hematological parameters in SHRSPs with or without 15 weeks of treatment with amlodipine

Each value is the mean ± S E

	BUN (mg/dl)	CRE (mg/dl)	T-cho (mg/dl)	TG (mg/dl)	PL (mg/dl)	NEFA (mEq/l)
Control (n = 5)	32 1 ± 1 40	0 95 ± 0 05	64 6 ± 3 56	62 2 ± 8 30	83 4 ± 3 66	0 81 ± 0 08
Amlodipine (n = 5)	21 4 ± 0 64 *	0 81 ± 0 04 *	56 4 ± 1 75	48 6 ± 1 50	74 2 ± 1 83	0 77 ± 0 04

*, P < 0 05 by Mann-Whitney U test, BUN blood urea nitrogen, CRE creatinine, T-cho total cholesterol, TG triglyceride, PL phospholipid, NEFA non-esterified fatty acid

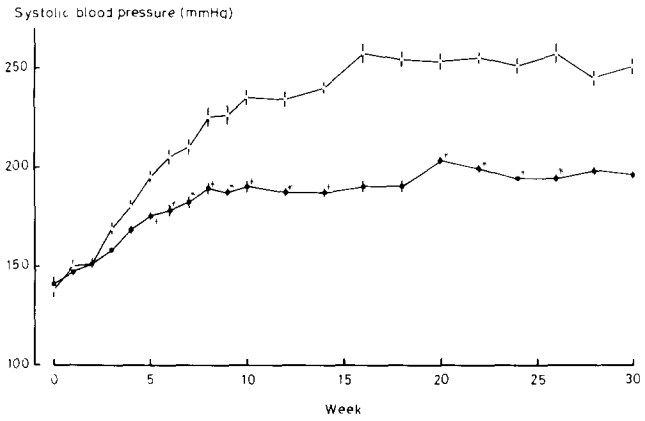

Fig 1 Development of hypertension in SHRSPs with or without amlodipine treatment Amlodipine treatment was started at the age of 5 weeks Each point is the mean ± S E of 11 animals *, significantly different from control ($P < 0.05$), ○, Control group, ●, amlodipine (3 mg/kg/day) group

3 2 *Hematological change*

The SHRSPs treated with amlodipine for 15 weeks showed significantly lower values of blood urea nitrogen and creatinine, by about 33% and 15%, respectively, compared to the value of control SHRSPs (table 1) However, no significant changes were observed in the levels of total cholesterol, triglyceride, phospholipid or non-esterified fatty acid in the treated group

3 3 *Histopathological changes in organs*

Amlodipine treatment of SHRSPs for 15 weeks produced small but significant decreases (by about 9–19%) in the ratios of wet weights of heart, lung, liver, spleen and brain to the body weights of animals, compared to those ratios from control animals (table 2). In other organs (kidney, adrenals and brain), no significant differences in the ratios of weights were observed between the control and amlodipine groups

Histopathological examination revealed significant lesions of heart and kidneys in all control animals (five SHRSPs) at 15 weeks These changes were characterized by myocardial fibrosis (fig. 2) and proliferative vasculitis with fibrinoid necrosis (fig 2), glomerular lesion, tubular atrophy, interstitial fibrosis, interstitial cell infiltration and proteinaceous cast in tubuli (table 3)

However, similar changes in heart and kidney occurred in only one animal of the amlodine group and the extent of the lesion was only slight compared to the control animals (table 3)

Additionally, two control animals, but not amlodipine treated animals, exhibited hemorrhage or thrombosis in the brain, although no notable lesions were present in the mesenteric arteries of either control or amlodipine groups (table 3)

3 4 *Survival rate*

All control groups of SHRSPs (11 animals) died 34–46 weeks after the start of the study, whereas the amlodipine treated animals (11 animals) all died after 53–79 weeks (fig. 3) Therefore, the ages at which 50% of the animals died were estimated to be 43 3 weeks (95% confidence limit 42 4–44 2 weeks) and 71 1 weeks (95% confidence limit 68 7–73 5 weeks) for the control and amlodipine treated animals, respectively, indicating about a 1 6-fold prolongation of life span of SHRSPs by long-term treatment of amlodipine Furthermore, the main cause of death seemed to be stroke in the control group, autopsy revealed an apparent cerebral hemorrhage in eight of 11 rats, with or without subarachnoid effusion In the amlodipine group, however, an apparent hemorrhage suggesting stroke was not found in any of the rats

4. Discussion

Long term treatment with amlodipine at 3 mg/kg/day reduced the rapid development of severe hypertension in SHRSPs and maintained a steady state blood pressure of around 200 mmHg which was still hypertensive when compared to SHRs (Okamoto and Aoki, 1963) The SHRSPs (20 weeks of age) receiving amlodipine for 15 weeks showed a lower heart weight suggesting suppression of cardiac hypertrophy as in the case of SHR reported by Nayler (1988) Furthermore, all (five) control SHRSPs exhibited histopathological changes in myocardial fibrosis and renal changes such as proliferative vasculitis, fibrinoid necrosis, glomerular lesion and tubular atrophy, whereas only one of five treated SHRSPs showed similar but only slight changes

TABLE 2

Ratios of organ weights to body weight in SHRSPs with or without 15 weeks treatment with amlodipine

The ratios of organ weight to body weight were expressed as organ wet weight (mg) per 100 g body weight Each value is the mean ± S E

	Heart	Lung	Liver ($\times 10^3$)	Kidneys	Spleen	Adrenals	Brain
Control (n = 5)	521 4 ± 11 1	431 6 ± 9 1	3 161 ± 0 033	802 6 ± 15 7	275 9 ± 10 0	18 8 ± 1 5	665 8 ± 25 6
Amlodipine (n = 5)	433 1 ± 5 9 *	385 6 ± 5 8 *	3 033 ± 0 032 *	787 8 ± 7 5	223 8 ± 5 0 *	15 3 ± 0 5	605 7 ± 14 8 *

*, $P < 0.05$ by Mann-Whitney U test

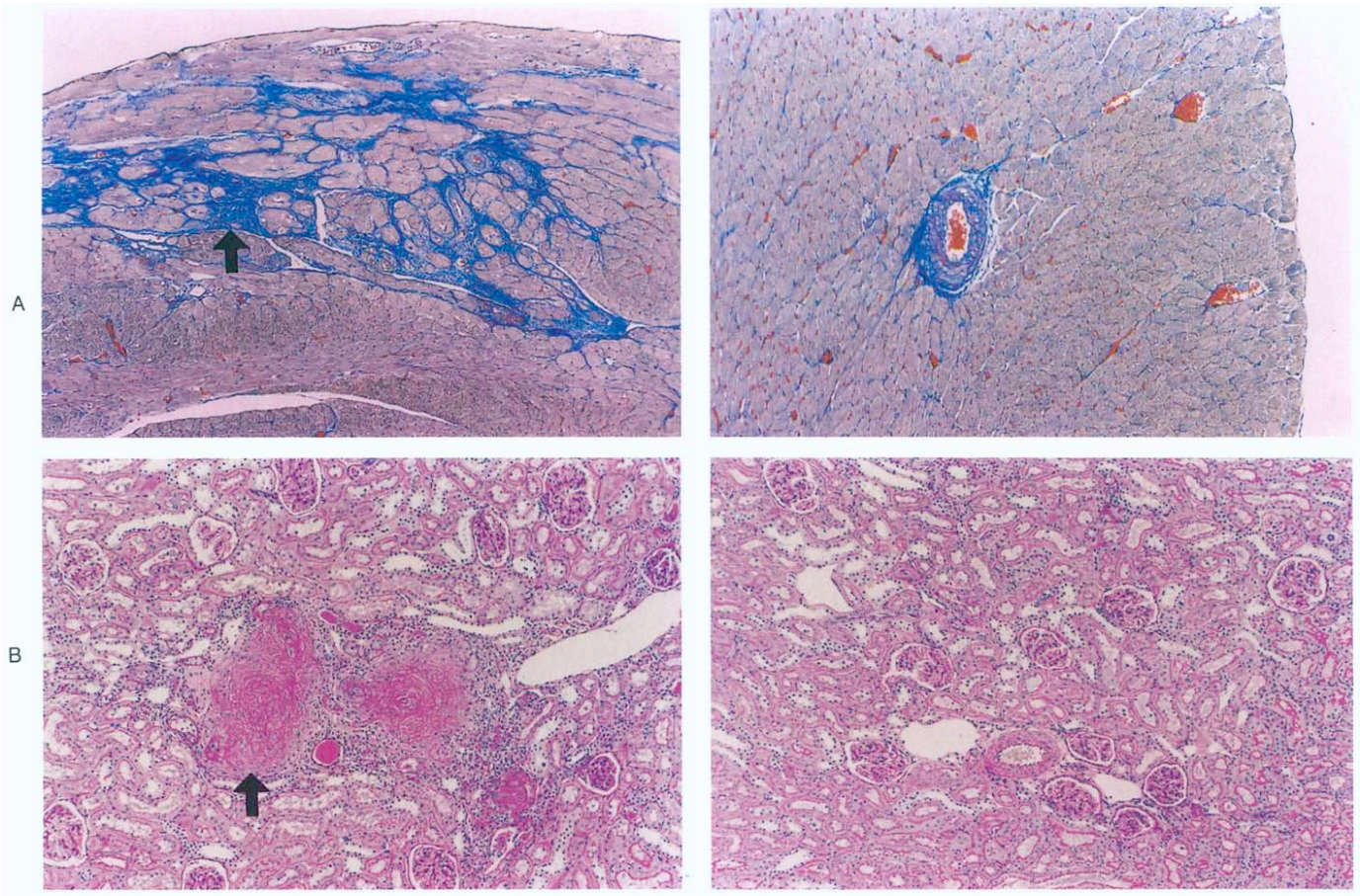

Fig 2 Typical histopathological appearance of myocardium and kidney in SHRSPs with or without 15 weeks of treatment with amlodipine (×100) Amlodipine treatment was started at the age of 5 weeks, 15 weeks later the animals were killed Myocardium and kidneys of SHRSPs were stained with Azan-Mallory and periodic acid-Schiff respectively A myocardium, left control animal (myocardial fibrosis is indicated by arrow), right treated animal (normal appearance) B kidney, left control animal (fibrinoid necrosis with proliferative vasculitis is indicated by arrow), right treated animal (normal appearance)

TABLE 3

Histopathological assessment of several organs in SHRSPs with or without 15 weeks treatment with amlodipine

Histopathological changes	Control	Amlodipine
Heart		
Myocardial fibrosis	+5 [a]/5 [b]	+1/5 *
Kidney		
Fibrinoid necrosis	+ +5/5	±1/5 *
Proliferative vasculitis	+ +5/5	±1/5 *
Glomerular lesion	+ +5/5	0/5 *
Tubular atrophy	+ +5/5	0/5 *
Interstitial fibrosis	+ +5/5	0/5 *
Interstitial cell infiltration	+ +5/5	±1/5 *
Proteinaceous cast in tubuli	+ +5/5	±1/5 *
Brain		
Hemorrhage	+ +1/5	0/5
Thrombosis	+1/5	0/5
Mesenteric artery	0/5	0/5

[a] Number of animals with histopathological changes [b] Total number of animals examined Changes minimum(±), mild(+), moderate (++) * $P < 0.05$ by Fischer's χ^2 test

in heart and kidney Such protection from renal change was suggested by decreased serum levels of urea nitrogen and creatinine in the treated animals Similar suppression of the levels of blood urea nitrogen and creatinine in SHRSPs was reported with treatment of angiotensin converting enzyme inhibitors (Kawakami et al , 1992) and combined β and α adrenoceptor blocking agent (Izumi et al , 1985), which significantly reduced blood pressure and protected against cardiovascular and renal damage In the control, but not the amlodipine group, cerebral hemorrhage and thrombosis were observed These results indicate that amlodipine is effective in reducing the incidence of cardiovascular and renal morbidity in SHRSPs.

The average life span of male animals was estimated in this study to be 43 3 weeks for control SHRSPs and is in consistent with a report from Okamoto et al (1974) In the amlodipine treated SHRSPs, it was 71 1 weeks, suggesting an approximately 1 6-fold prolongation of their life span This finding extended the evi-

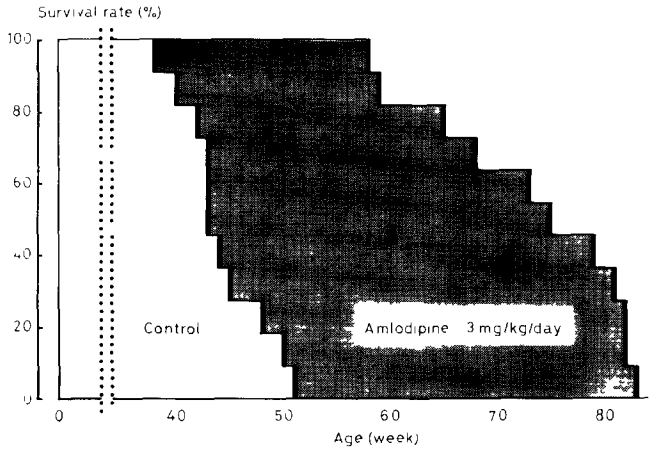

Fig 3 Survival rate of SHRSPs with or without amlodipine treatment Amlodipine treatment was started at the age of 5 weeks

dence provided by Nayler and Gu (1990) that after 30 weeks of treatment, survival rates of SHRSPs were 23% and 89% in control and amlodipine groups, respectively In the present study, the cause of death of control, but not treated animals, seemed to be stroke

It has been shown that there is a close correlation between the incidence of stroke and the severity of hypertension (more than 180 mmHg) and/or the initial rate of rise of blood pressure, the faster the blood pressure of rats reaches 230 mmHg, the earlier the animals die with stroke (Okamoto et al , 1974) Accordingly, the prevention of stroke leading to the prolongation of life span would seem to be the result of significant suppression by amlodipine of rapid acceleration in hypertension and maintenance of blood pressure reduction to around 200 mmHg.

A similar explanation may be true for the attenuation by amlodipine of histopathological changes in heart and kidney, because structural and functional alterations of these organs have been described in relation to hypertension The development of hypertension in SHRs is well known to be associated with early adaptive metabolic changes within the cardiovascular system even at ages of 4–5 weeks (Yamabe and Lovenberg, 1974, Ooshima et al , 1974) These changes are represented by enhanced syntheses of collagen and non-collagenous proteins, subsequently, such changes cause the development of adaptive structural changes (Folkow et al , 1973) such as arteriosclerosis and hypertrophy or proliferation of the arterial medial layer at the ages of 12–16 weeks Glomerulosclerosis in SHR is suggested to be due to an elevation in glomerular pressure with the elevation in systemic pressure, and also additional non-hemodynamic factors (Kimura et al , 1991) Thus, the suppression of myocardial fibrosis and proliferative vasculitis in the kidney and protection from glomerular lesion might be the result of hypotensive action of amlodipine and also the effect on metabolic changes It might also stem from the mode of action on non-hemodynamic factors, since our observations were obtained at a dose regimen leaving SHRSPs hypertensive In support of this, decreased aortic collagen synthesis and content has been demonstrated in amlodipine treated SHRs (Chichester and Rodgers, 1987).

It has been shown that structural alterations of vessels in SHR or SHRSP are a consequence of increased blood pressure (Mulvany, 1987), and long term administration of antihypertensives (Ca^{2+} channel antagonist and angiotensin converting enzyme inhibitor) suppresses the incidence of organ damage with a lowering of blood pressure in SHR (Mulvany, 1991) Interestingly, nimodipine, a Ca^{2+} channel antagonist prevents vascular damage and stroke without affecting blood pressure in SHRSPs, since nimodipine inhibits the calcium overload of vessels (Kazda et al , 1987) Amlodipine also has an inhibitory effect on vascular calcium accumulation in salt loaded Dahl-S rats (Fleckenstein et al , 1989) Accordingly to a certain extent, the protection against cardiovascular and renal damage observed in our study may result from the calcium entry blocking effect of amlodipine However, it still remains unclear to what extent the lowering of blood pressure contributes to organ damage Therefore, in order to clarify this point, further studies are necessary at a dose regimen which does not lower blood pressure in SHRSPs

Nevertheless, the observed changes in cardiovascular and renal systems are major risk factors for aggravating severe and sustained hypertension through a vicious cycle of vascular tone, structural changes and blood pressure Our observations in the present study suggest that amlodipine could delay or interrupt the development of such a cycle This possibility may be further supported by additional evidence that amlodipine attenuates atherogenesis in cholesterol fed rabbits and alters low density lipoprotein metabolism (increases binding and internalization in human skin fibroblast) (Paoletti and Bernini, 1990) These effects would be preferable for preventing aggravation of vascular damage and the accompanying progression of severe hypertension leading to stroke

In conclusion, the present study indicates that long term treatment with amlodipine prevents the development of hypertension, cardiovascular and renal damage, and stroke in SHRSPs, thereby prolonging their life span These findings support the idea of the potential clinical use of this new Ca^{2+} channel antagonist in terms of reduced cardiovascular morbidity and mortality, although the quantitative relationship between the blood pressure lowering effect of amlodipine and its effects on organ damage is still unclear

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