AMLODIPINE AFFECTS PLASMA ANGIOTENSINOGEN LEVEL : INDIRECT EVIDENCE

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ABSTRACT: The renin-angiotensin system acts to regulate body fluid volume and blood pressure. The circulating renin substrate, angiotensinogen, secreted mainly from the liver is affected by various hormones. Calcium ions thus far, have not been reported to affect the plasma level of angiotensinogen. Nevertheless, since we have previously reported that amlodipine, a selective vascular calcium channel blocker, could raise plasma prorenin and renin activities in Malaysian hypertensive patients, the aim of the present study is therefore to investigate the effect of amlodipine on the plasma angiotensinogen level. An open single-blind study was performed on male and female hypertensives (32 - 60 years) without other complications. Following a washout period, the patients were prescribed amlodipine either 5 or 10 mg daily, for 2 - 3 months. Blood samples were collected before and after treatment with the obtained plasmas analysed for plasma renin concentration (PRC) and plasma renin activity (PRA) using direct and indirect renin radioimmunoassay methods, respectively. Data obtained showed that amlodipine treatment significantly ($p<0.05$) increased the PRC and the PRA of the patients. In addition, the PRA was highly correlated to the PRC before ($r = 0.62$, $p<0.001$) and after ($r = 0.68$, $p<0.001$) the amlodipine treatment. The finding that the PRA rose in parallel with the rise in PRC indicated that angiotensinogen was not limiting, and that amlodipine treatment may raise the plasma angiotensinogen level. *(JUMMEC 1997 2(1): 31-34)*

KEYWORDS: Hypertension, amlodipine, renin, angiotensinogen, angiotensin I, direct renin assay and indirect renin assay.

Introduction

The renin-angiotensin system (RAS) plays a key role in the regulation of blood pressure and body fluid volume. Renin, an aspartic protease secreted mainly by the kidneys, is known to act on only one substrate, angiotensinogen, a 55 - 60 kDa glycoprotein, producing angiotensin (Ang) I which is subsequently converted by the angiotensin converting enzyme (ACE) to the vasoactive peptide, Ang II. Renin is secreted by the kidneys via a regulated pathway, while its precursor, prorenin, via a constitutive pathway by renal and extrarenal tissues (1). Angiotensinogen is secreted solely by a constitutive pathway and thus the regulation of the production is at the level of its synthesis (2,3). The main source for circulating angiotensinogen is the liver; although, various other tissues such as the brain, kidney, adrenal, ovary, adipose tissue and the vascular wall have also been implicated (4,5,6). Hormones such as estrogens (3,7,8), testosterone (9), glucocorticoids (3,10), and thyroid hormone (3) have been reported to affect the production of angiotensinogen, in addition to Ang II which has been shown to stimulate angiotensinogen synthesis (2,11). Calcium ions, however, appear to have no effect on angiotensinogen secretion (2,11).

Although calcium antagonists are now increasingly being prescribed for the control of hypertension (12), effects of such treatment and the consequent altered intracellular calcium concentration on the RAS are relatively not well studied. Nevertheless, we have previously reported that a vascular selective calcium channel blocker, amlodipine, can alter the plasma profiles of prorenin, renin and aldosterone in hypertensive patients (13, 14). Since factors that affect plasma renin level may also affect the secretion of angiotensinogen, the purpose of our present study is then to investigate the effect of amlodipine on the plasma level of angiotensinogen.

Materials and Methods

Patients

Male (n=11) and female (n=9) essential hypertensive patients aged between 32 and 60 years, with a mean age of 49.8 ± 1.6 years, and mean systolic (SBP) and

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diastolic blood pressures (DBP) of 153 ± 1.9 and 101 ± 0.9 mmHg respectively, and with no renal or liver disease were recruited from the Polyclinic of the University Hospital for an open, single-blind study. Consent was obtained from each patient after the purpose and nature of the study were fully explained. Approval was also obtained from the Medical Centre Research Committee and the University Hospital Ethics Committee.

The patients underwent a washout period of 2 weeks to clear the effects of previously administered anti-hypertensive therapies and subsequently were prescribed only amlodipine (Pfizer Malaysia) either 5 or 10 mg once daily, depending on their sensitivity or severity, for a period of 2-3 months. The end-point was a normal blood pressure reading.

Blood Collection

Blood samples were obtained from patients before treatment with amlodipine and 2 -3 months after. To avoid postural effects on the RAS, during each collection time the patients were allowed to rest for about 30 minutes before blood was collected into tubes containing disodium ethylenediaminetetra-acetic acid (Na_2-EDTA; Sigma Chemical Co, St. Louis, MO, USA) and kept on ice. The plasmas obtained were subsequently aliquoted and stored at -70°C until analysed.

Assays and data analysis

The plasmas were assayed for plasma renin concentration (PRC) and renin activity (PRA). The PRC was obtained using a direct renin radioimmunoassay (RIA) (Active renin, Nicholls Institute Diagnostics, CA, USA). The PRA was estimated by an indirect assay technique which involved the incubation of each plasma sample at 4°C and 37°C for 30 min (15). The samples were subsequently assayed for Ang I by RIA (Renin RIAbead, Abbott Laboratories, USA). The difference between the Ang I values obtained from samples incubated at 4°C and 37°C, corrected per hour, would give the amount of Ang I generated per hour by the action of renin on the angiotensinogen present in the plasma.

Data were analysed for statistical significance by Student's paired t-test and Kendall Tau correlation analysis. The Student's paired t-test was used when a direct comparison of parameters measured, before and after amlodipine treatment, was made. The Kendall-Tau correlation analysis was performed when correlating the PRA with the PRC.

Results

Treatment of essential hypertensive patients with amlodipine significantly ($p<0.001$) lowered the SBP from 153 ± 1.91 to 134 ± 1.44, the DBP from 101 ± 0.90 to 87.2 ± 0.99 and consequently reduced the mean arterial blood pressure from 118 ± 1.04 to 102 ± 0.92 mmHg (figure not shown). Amlodipine significantly raised their PRC ($p<0.05$) from 7.62 ± 0.63 to 13.10 ± 2.62 pg/ml (Figure 1, left panel) as well as their PRA ($p < 0.05$) from 0.30 ± 0.08 to 1.22 ± 0.42 ng/ml/hr (Figure 1, right panel). The Kendall Tau correlation analysis showed that the PRA was highly correlated with the PRC before (Figure 2, top graph, $r=0.62$, $p<0.001$) and after (Figure 2, lower graph, $r=0.68$, $p<0.001$) being prescribed with amlodipine.

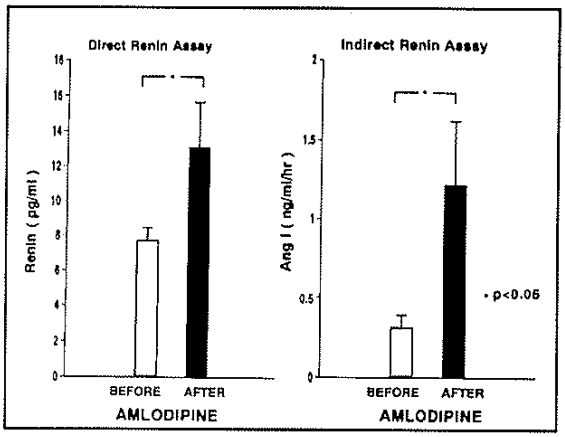

Figure 1. The plasma renin concentration as obtained using the direct renin assay (left panel) and plasma renin activity as obtained using the indirect renin assay (right panel) of hypertensive patients before and after treatment with amlodipine.

Discussion

This report provides evidence that treatment with amlodipine in hypertensive patients may increase their plasma angiotensinogen level, an hitherto unknown effect. As previously reported, amlodipine treatment was also found to significantly lower the systolic and diastolic blood pressures of the hypertensive patients (14).

The RAS regulates both blood pressure and body fluid volume. Renin, the renal aspartic protease is known to act only upon the glycoprotein, angiotensinogen, with a consequent production of Ang I which is subsequently converted to Ang II by ACE. The liver is thought to be the main source for circulating angiotensinogen and is the most widely studied. However, the presence of mRNA for angiotensinogen reported in various other tissues such as the brain, kidneys, adrenals, ovary, the vascular wall and adipose tissue (4,5,6) implicates extrahepatic sources for circulating angiotensinogen. Since angiotensinogen is secreted via a constitutive pathway, any changes of angiotensinogen production would therefore be at the level of its synthesis (2,3). Several hormones that affect transcriptional processes such as estrogens (3,7,8), testosterone (9), glucocorticoids (3,10)

and thyroid hormone (3) have been shown to affect the production of angiotensinogen. Angiotensin II has also been shown to stimulate angiotensinogen production (2,11).

The production of Ang I and therefore Ang II is limited by the availability of angiotensinogen within the circulation for renin to act upon and consequently, the more substrate there is, the more Ang I that is produced (16,17). Changes in plasma angiotensinogen level have

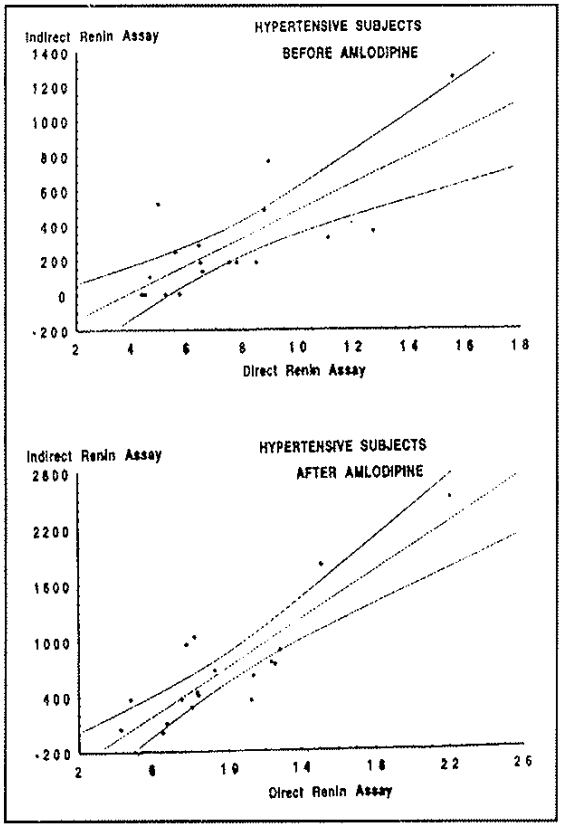

Figure 2. The Kendall Tau correlation analysis of plasma renin activity (PRA, indirect renin assay) with plasma renin concentration (PRC, direct renin assay) in hypertensive patients before (top) and after amlodipine treatment (bottom). The PRA is highly correlated with the PRC before (top graph, $r = 0.62$, $p<0.001$) and also after (bottom graph, $r = 0.68$, $p<0.001$) amlodipine administration.

been reported to be accompanied by an inversely proportional concentration in renin (18). This implied that a high plasma renin concentration would convert more of the circulating angiotensinogen to Ang I, and thereby lowering the plasma concentration of angiotensinogen and vice-versa. Since the production rate of Ang I is dependent on the level of plasma angiotensinogen, conditions which raise plasma renin concentration resulting in an increase in the measurement of plasma Ang I as determined by PRA, could be due either to a concomitant increase in angiotensinogen level or that the renin substrate is present in abundance. If the angiotensinogen level present in the plasma was not increased in parallel with the renin concentration, the substrate may be limited and thus, one would expect that the rate of Ang I production would remain unchanged rather than increased. Our present study showed that the treatment of hypertensive patients with the vascular selective calcium channel-blocker, amlodipine, significantly raised the PRC (Figure 1, left panel, $p< 0.05$) with a concomitant increase of the PRA of these patients (Figure 1, right panel, $p<0.05$) thus, suggesting an elevation of angiotensinogen level. These changes in PRC and PRA were highly correlated both before (Figure 2, top graph; $r = 0.62$, $p<0.001$) and after (Figure 2, lower graph; $r = 0.68$, $p<0.001$) the amlodipine treatment. The finding that such treatment resulted in an increase in PRA in parallel with the rise in PRC in the hypertensive patients indicated that the level of plasma angiotensinogen was not rate limiting, and may be raised by amlodipine administration. However, the possibility that the plasma angiotensinogen of these patients was present in abundance prior to the amlodipine treatment remains to be elucidated.

Thus, we conclude that amlodipine treatment not only raised the PRC but may also increase the plasma angiotensinogen level. To the best of our knowledge, this finding has yet to be reported elsewhere.

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References

1 Osmond DH, Sealey JE and McKenzie JK. Activation and function of prorenin: different viewpoints. Can J Physiol Pharmacol 1991; 69: 1308-14.

2 Klett C, Muller F, Gierschik P and Hackenthal E. Ang II stimulates angiotensinogen synthesis in hepatocytes by a pertussis toxin-sensitive mechanism. FEBS Lett 1989; 259: 301-4.

3 Clauser E, Bounik J, Coezy E, Corvol P and Menard J. Synthesis and release of immunoreactive angiotensinogen by rat liver slices. Endocrinology 1983; 112: 1188-93.

4 Campbell DJ and Habener, JF. Angiotensinogen gene is expressed and differently regulated in multiple tissues of the rat. J Clin Invest 1986; 78: 31-9.

5 Deschepper CF and Hong-Brown LQ. Hormonal regulation of the angiotensinogen gene in liver and other tissues. In: Raizada MK, Phillips MI and Sumners C, Eds. Cellular and molecular biology of the renin-angiotensin system. Florida: CRC Press; 1993; 149-63.

6 Deschepper CF. Angiotensinogen: hormonal regulation

and relative importance in the generation of angiotensin II. Kidney Int 1994; 46: 1561-63.

7 Helmer OM and Griffith RS. The effect of administration of estrogens on the renin-substrate content of rat plasma. Endocrinology 1952; 51: 421-6.

8 Menard J and Catt KJ. Effects of estrogen treatment on plasma renin parameters in the rat. Endocrinology 1973; 92: 1382-8.

9 Ellison KE, Ingelfinger JR, Pivor M. and Dzau VJ. Androgen regulation of rat renal angiotensinogen messenger RNA expression. J Clin Invest 1989; 83: 1941-5.

10 Brasier AR, Li J and Copland A. Transcription factors modulating angiotensinogen gene expression in hepatocytes. Kidney Int 1994; 46: 1564-6.

11 Klett C, Bader M, Ganten D and Hackenthal E. Mechanism by which angiotensin II stabilizes messenger RNA for angiotensinogen. Hypertension 1994; 23 (suppl I): I-120-I-125.

12 Buhler FR. Introduction: Once-a-day calcium antagonist for antianginal and antihypertensive therapy. J Cardiovasc Pharmacol 1988; 12(suppl 7): S1-S2.

13 Ismail R, Lam SK and Chua CT. Alteration of prorenin-renin profile in Malaysian hypertensives after amlodipine treatment. Fed of Pacific Rim Hypertension Societies, Melbourne, Australia March 20-24, 1994.

14 Lam SK, Ismail R, Chua CT and Chong YS. The influence of amlodipine on the plasma prorenin-renin profile and aldosterone concentration in patients with hypertension. In: Vinson GP, Anderson DC, Eds. Adrenal glands, vascular system and hypertension. England: J Endocrinology Ltd; 1996; 259-65.

15 Osmond DH, MacFadzean PA and Scaiff KD. Renin activity by radioimmunoassay: the plasma incubation step. Clin Biochem 1975; 7:52-63.

16 McDonald WJ, Cohen EL, Lucas CP and Conn JW. Renin-renin substrate kinetic constants in the plasma of normal and estrogen-treated humans. J Clin Endocrinol Metab 1977; 45:1297-304.

17 Skinner SL, Lumbers ER and Symonds EM. Alterations by oral contraceptives of normal menstrual changes in plasma renin activity, concentration and substrate. Clin Sci 1969; 36: 67-76.

18 Gahnem F, Camargo MJF, von Lutterotti N, Laragh JH and Sealey JE. Angiotensinogen depletion by high renin levels in hypertensive rats: no evidence for tonic stimulation of angiotensinogen by angiotensin II. J Hypertens 1995; 13: 91-6.