Effects of valsartan with or without benazepril on blood pressure, angiotensin II, and endoxin in patients with essential hypertension

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ABSTRACT

AIM: To evaluate the effects of valsartan (Val) with or without benazepril (Ben) on blood pressure and plasma levels of angiotensin (Ang II) and digoxin-immunoreactive factors (endoxin) in patients with essential hypertension. **METHODS:** Ninety patients with essential hypertension were randomly divided into 3 groups (*n*=30 per group): Ben group (Ben 10 mg/d, *po)*; Val group (Val 80 mg/d, *po)*; combination drug therapy group (Val 80 mg/d+Ben 10 mg/d, *po)*; all patients were treated for 12 weeks. Age and sex-matched 20 normal subjects were served as control group. **RESULTS:** The levels of plasma endoxin and Ang II in patients with essential hypertension were remarkably higher than those in normal subjects. The levels of plasma Ang II and endoxin were all obvious positive correlation with systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Ang II: *r*=0.5151, 0.7978; endoxin: *r*= 0.4706, 0.7274, respectively). Within 6 weeks of drug intervene, SBP and DBP were remarkably decreased in 3 groups. After 6 weeks, SBP and DBP were continuously decreased in Ben group and Val+Ben group, but not in Val group. Level of plasma Ang II was remarkably decreased as SBP and DBP decreased in Ben group and Val+Ben group; level of plasma Ang II was remarkably increased in Val group. **CONCLUSION:** Val with or without Ben remarkably decreased SBP and DBP in patients with essential hypertension within 6 weeks. Antihypertensive efficacy was weakened after long-term use of Val alone. The antihypertensive effect of Val+Ben group was the most remarkable among 3 groups and could avoid the side effects of high plasma Ang II.

INTRODUCTION

Renin-angiotensin-aldosterone system (RAAS) plays a key role in maintaining normal blood pressure, liquids, and electrolyte balance. Benazepril (Ben) is an angiotensin converting enzyme inhibitor (ACEI) and has excellent antihypertensive effect by blocking angiotensin I transformed into angiotensin II (Ang II). Ben is extensively used to treat hypertension[1,2], but has no effect on Ang II formed *via* chymase[3,4]. Valsartan (Val) is an Ang II type 1 receptor (AT$_1$R) antagonist and plays an antihypertensive effect by blocking the binding of Ang II to AT$_1$R[5,6]. However, level of plasma Ang II was remarkably increased when Val was used to treat hypertension[7]. High concentration of Ang II may com-

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petitively bind to AT_1R to weaken the long-term curative effect of Val. This effect may present in patients who take long-term AT_1R antagonist when drug dose was decreased or stopped because of some causes. Meanwhile, high concentration of Ang II may stimulate secretion of digoxin-immunoreactive factors (endoxin) in adrenal gland *via* AT_2 receptor[8,9]. The latter has been certified to take part in pathogenesis of hypertension[10,11]. Because ACEI and AT_1R antagonists all affected RAAS, Val was used alone or combined with Ben to evaluate their effects on blood pressure and levels of plasma Ang II and endoxin in patients with essential hypertension in this research.

MATERIALS AND METHODS

Reagents Val and Ben were provided by Beijing NOVARTIS Pharmaceutical Ltd, China. Radioimmunoassay (RIA) kits for determining plasma Ang II were purchased from Beijing Northern Biological Technological Institute (China). RIA kit for endoxin was purchased from Radioimmune Institute of Tongji University (China).

Patients One hundred and eight patients were admitted to the study. All patients came from outpatient clinics or were hospitalized in Yijishan hospital (patients were followed after they left hospital), and had no concomitant illness. All patients gave written consent to participate in the trials, and received formal approval from an Institutional Review Board and Ethical Review Board in hospital. Ninety patients met the diagnosis standard of hypertension[12] were chosen to enter into this research after placebo was used for 2 weeks, and had not taken any antihypertensive drugs in the past 4 weeks (ie 4 weeks wash-out phase and 2 weeks placebo run-in phase). They were randomly divided into 3 groups which showed no significant difference among the background data (*P*>0.05, Tab 1).

Drugs were taken orally for 12 weeks. Drug dose in each group was not adjusted in experimental stage unless there were remarkable side effects. The systolic blood pressure (SBP) and the diastolic blood pressure (DBP) in all patients were measured in seated position by a special clinician at 9:00-10:00 am before treatment, and 1, 2, 4, 6, 8, and 12 weeks after treatment. The blood pressures were measured twice and the average value was calculated. Meanwhile, side effects such as hypotension, edema, cough, headache, and fatigue were recorded. The target level of SBP was set

Tab 1. Background demographics of hypertensive patients and normal subjects.

	Ben	Val	Ben+Val	Normal
Male	18	16	19	11
Female	12	14	11	9
Age/a	48±7	50±7	49±8	48±7
Range	36-63	36-61	36-63	36-62
Stage II[1]	16	19	17	
Stage III[2]	14	11	13	
SBP/mmHg	159±9	159±9	161±7	122±5
DBP/mmHg	101±5	100±5	99±4	80±3

[1] Stage II: SBP 160-179 mmHg and (or) DBP 100-109 mmHg; [2] Stage III: SBP≥180 mmHg and (or) DBP≥110 mmHg. Group Ben: benazepril 10 mg/d; group Val: valsartan 80 mg/d; Ben+Val group: Ben 10 mg/d+Val 80 mg/d.

as ≤140 mmHg and (or) the target of DBP ≤85 mmHg[13]. Age and sex matched twenty subjects who had normal blood pressure, blood sugar, liver, and renal function were served as normal control group.

Estimating concentration of endoxin and Ang II The concentrations of plasma endoxin and Ang II were respectively estimated in 20 normal subjects and all patients with hypertension before drug treatment, 4, 8, and 12 weeks after drug intervene with radioimmunoassay. Blood samples were collected from brachial vein at 7:00-8:00 am in seated position. The blood was put into anticoagulation test tubes, which had cooled aprotinin, and centrifuged at 1200×*g,* 4 °C for 10 min. The supernatant was preserved at -20 °C to detect endoxin and Ang II.

Statistic analysis All data were expressed as mean±SD and analyzed using ANOVA followed by *t*-test. Interrelation of two factors was adopted with simple beeline correlation analysis. *P*<0.05 was considered to be statistically significant.

RESULTS

Three, two, and four patients were respectively rejected in group Ben, group Val, and Val+Ben group in 3-4 weeks because of severe cough or myosalgia.

Effects of drug intervene on systolic blood pressure (SBP) in patients with essential hypertension SBP were all remarkably decreased after drug intervene in the 3 groups. SBP were all progressively decreased in group Ben and Val+Ben group as drug

intervene. The effect was more remarkable in Val+Ben group. Antihypertensive effect reached maximum at week 6 in group Val and after this, its antihypertensive effect was gradually weakened. To SBP, the rate that the blood pressure met the target level was respectively 74 % (20/27), 61 % (17/28), and 85 % (22/26) in group Ben, group Val, and combination drug therapy group in 12 weeks (Tab 2).

Effects of drug intervene on diastolic blood pressure (DBP) in patients with essential hypertension DBP were all remarkably decreased after drug intervene in 3 groups. DBP were all progressively decreased in group Ben and combination drug therapy group as drug intervene and antihypertensive effect was more remarkable in combination drug therapy group. Antihypertensive effect reached maximum at week 6 in group Val and after this, its antihypertensive effect was gradually weakened. To DBP, the rate that the blood pressure met the target level was respectively 67 % (18/27), 54 % (15/28), and 85 % (22/26) in group Ben, group Val, and combination drug therapy group in 12 weeks (Tab 3).

Effects of drug intervene on level of plasma Ang II in patients with essential hypertension The level of plasma Ang II in patients with essential hypertension was higher than that in normal subjects, and was positive correlation with SBP and DBP in pretreatment ($r=0.5151$, 0.7978, respectively, $P<0.01$). The level of plasma Ang II was remarkably decreased after drug intervene in group Ben and combination drug therapy group and it was more remarkably decreased in group Ben, but it was remarkably increased after drug intervene in group Val (Tab 4).

Effects of drug intervene on level of plasma endoxin in patients with essential hypertension The level of plasma endoxin in patients with essential hypertension was higher than that in normal subjects, and was positive correlation with SBP and DBP in pretreatment ($r=0.4706$, 0.7274, respectively, $P<0.01$). The level of plasma endoxin was remarkably decreased after drug intervene in group Ben and combination drug therapy group. The level of plasma endoxin was remarkably decreased at week 4 in group Val and after this, the level of plasma endoxin resumed to level of pretreatment. Level of plasma endoxin was also significantly positive correlation with SBP and DBP after treatment, respectively ($r=0.4564$, 0.7831, in 4 weeks; $r=0.6906$, 0.8366 in 8 weeks; $r=0.6143$, 0.8126 in 12 weeks; respectively, $P<0.01$) (Tab 5).

Side effects Three patients and two patients had severe cough in group Ben and in combination drug therapy group, respectively. Two patients had myosalgia in Val group and in combination drug therapy group, respectively. There were no remarkable other side effects during drug intervene. Hepatic function, renal function, and electrolyte were all normal.

Tab 2. Effects of drug intervene on SBP (mmHg) in patients with essential hypertension. Mean±SD. [b]$P<0.05$, [c]$P<0.01$ *vs* pretreatment. [e]$P<0.05$, [f]$P<0.01$ *vs* group Ben. [i]$P<0.01$ *vs* group Val.

Group	Pretreatment	1	2	4	6	8	12 weeks
Ben	159±9 (*n*=30)	155±9 (*n*=30)	153±9[b] (*n*=30)	150±7[c] (*n*=27)	147±5[c] (*n*=27)	142±5[c] (*n*=27)	140±6[c] (*n*=27)
Val	159±9 (*n*=30)	154±9[b] (*n*=30)	150±7[c] (*n*=30)	144±6[cf] (*n*=28)	140±5[cf] (*n*=28)	146±7[cf] (*n*=28)	148±9[cf] (*n*=28)
Ben+Val	161±7 (*n*=30)	156±7[c] (*n*=30)	151±6[c] (*n*=30)	146±7[ce] (*n*=26)	140±5[cf] (*n*=26)	136±7[cfi] (*n*=26)	135±6[cfi] (*n*=26)

Tab 3. Effects of drug intervene on DBP (mmHg) in patients with essential hypertension. Mean±SD. [b]$P<0.05$, [c]$P<0.01$ *vs* pretreatment. [e]$P<0.05$, [f]$P<0.01$ *vs* group Ben. [h]$P<0.05$, [i]$P<0.01$ *vs* group Val.

Group	Pretreatment	1	2	4	6	8	12/weeks
Ben	101±5 (*n*=30)	99±4 (*n*=30)	96±4[c] (*n*=30)	91±3[c] (*n*=27)	89±3[c] (*n*=27)	88±3[c] (*n*=27)	86±4[c] (*n*=27)
Val	100±5 (*n*=30)	97±5[b] (*n*=30)	94±5[c] (*n*=30)	88±5[ce] (*n*=28)	86±5[ce] (*n*=28)	89±7[c] (*n*=28)	91±7[cf] (*n*=28)
Ben+Val	99±4 (*n*=30)	97±4 (*n*=30)	93±3[c] (*n*=30)	85±4[cfh] (*n*=26)	82±3[cfi] (*n*=26)	79±3[cfi] (*n*=26)	77±3[cfi] (*n*=26)

Tab 4. Effects of drug intervene on level of plasma Ang II (ng/L) in patients with essential hypertension. Mean±SD. ^cP<0.01 vs normal group. Let me use proper format.

Tab 4. Effects of drug intervene on level of plasma Ang II (ng/L) in patients with essential hypertension. Mean±SD. $^c P<0.01$ vs normal group. $^f P<0.01$ vs pretreatment. $^i P<0.01$ vs group Val. $^k P<0.05$, $^l P<0.01$ vs group Ben.

Group	Pretraetment	4	8	12 weeks
Normal	23.7±2.4 (*n*=20)			
Ben	35±4^c (*n*=30)	27±5^{fi} (*n*=27)	22.8±2.9^{fi} (*n*=27)	22±4^{fi} (*n*=27)
Val	34±4^c (*n*=30)	84±12^f (*n*=28)	101±9^f (*n*=28)	116±12^f (*n*=28)
Val+Ben	34±3^c (*n*=30)	32±3^{ik} (*n*=26)	26±5^{fik} (*n*=26)	29±7^{fil} (*n*=26)

Tab 5. Effects of drug intervene on level of plasma endoxin (ng/L) in patients with essential hypertension. Mean±SD. $^c P<0.01$ vs normal group. $^e P<0.05$, $^f P<0.01$ vs pretreatment. $^h P<0.05$, $^i P<0.01$ vs group Val. $^k P<0.05$ vs group Ben.

Group	Pretreatment	4	8	12 weeks
Normal	0.28±0.05 (*n*=20)			
Ben	0.38±0.05^c (*n*=30)	0.31±0.04^f (*n*=27)	0.28±0.05th (*n*=27)	0.26±0.05^{fi} (*n*=27)
Val	0.35±0.04^c (*n*=30)	0.30±0.06^e (*n*=28)	0.32±0.07 (*n*=28)	0.36±0.11 (*n*=28)
Val+Ben	0.35±0.05^c (*n*=30)	0.26±0.04^{fhk} (*n*=26)	0.24±0.03^{fi} (*n*=26)	0.25±0.04^{fi} (*n*=26)

DISCUSSION

Ang II has effects such as contracting vascular smooth cell, increasing aldosterone secretion, and promoting vascular and myocardial cell growth by binding to $AT_1R^{[14]}$. ACEI makes antihypertensive effects, regression of myocardial hypertrophy by inhibiting ACE activities and decreasing Ang II level in plasma and tissue[1], but does not affect Ang II formed *via* chymase[3,4].

AT_1R antagonist makes an antihypertensive effect by inhibiting of Ang II binding to AT_1R. Some researches showed that levels of Ang II in plasma and myocardial tissue were significantly increased after AT_1R antagonist was used. It was thought that high concentration of Ang II, when binding to AT_2 receptor, could induce cell apoptosis and was helpful to regression of myocardial hypertrophy when AT_1R was blocked[15,16].

Some researches had showed that the rate that the blood pressure met the target was only 40 %-50 % in those that single drug such as ACEI or AT_1R antagonist was used. However, antihypertensive efficacy was only slight higher and side effects were remarkably increased when dose of drug was increased[13,17]. Therefore, antihypertensive efficacy of combination drug therapy was superior to the manner of adjusting dose of single drug. So, it is important to search for optimal treatment manner to improve long-term prognosis in hypertensive patients.

Our study found that Val with or without Ben had all significant effect on SBP and DBP in patients with essential hypertension in a short time. Antihypertensive efficacy was weakened after long-term use of Val alone. Antihypertensive effect was most remarkable in combination drug therapy group. Our data also showed that after drug intervene, the level of plasma Ang II was significantly risen in group Val, decreased in group Ben and in combination drug therapy group. These results showed that Val may stimulate secretion and synthesis of Ang II. These also may be one of causes for that blood pressure was not effectively controlled as drug was used long-term in group Val.

Endoxin is an inhibitor of Na^+-K^+-ATPase. Circulating levels of endoxin depend upon the adrenal cortex and metabolic events preceding and following pregnenolone formation are involved in endoxin biosynthesis. Laredo *et al*[8-10] found that Ang II stimulated the secretions of endoxin from bovine adrenocortical cells. The secretion of endoxin was activated maximally by the AT_2R agonist CGP42112. The AT_2R antagonist PD123319 blocked the effects of Ang II on secretion of endoxin. These results demonstrated that AT_2R stimulated secretion of endoxin from bovine adreno-

cortical cells.

The studies had shown that endoxin was an endogenous medium of digitalis receptor and could remarkably inhibit Na^+-K^+-ATPase activity in cell membrane, had competitive displacing activity against ^{3}H-ouabain binding to the enzyme, inhibitory activity for ^{86}Rb uptake into intact human erythrocytes, cross-reactivity with anti-digoxin antibody. Therefore, it was a Na^+ pump inhibitor. Na^+ pump in cell membrane maintained intracellular ionic concentration and controlled membrane potential. Its inhibition by endoxin enhanced the intracellular Na^+ concentration. This in turn activated the Na^+-Ca^{2+} exchange mechanism, which induced intracellular Ca^{2+} increase, membrane depolarization, and noradrenaline release from perivascular adrenergic nerve endings. In addition, endoxin stimulated endothelin-1 (ET-1) secretion in a dose-dependent manner from cultured endothelial cells[11]. These were its mechanisms which promoted vasoconstriction, positive inotropic effect, and enhanced renal tubular sodium excretion. It was considered to play a causative role in pathogenesis such as hypertension.

Endoxin took part in processing of hypertensive pathophysiology[10, 11]. Our research found that level of plasma endoxin in patients with essential hypertension was more higher than that in normal subjects. These results showed that endoxin took part in the mechanism of hypertension. Our research also found that the level of plasma endoxin was remarkably decreased after drug intervene in group Ben and combination drug therapy group, but it had no remarkable change after drug intervene in group Val. It showed that higher Ang II induced by Val might stimulate secretion of endoxin and lessened antihypertensive effect of Val.

Our research certified that combination drug therapy had good quality that antihypertensive effect was excellent, the influence of circulating RAAS balance was slight. Combination drug therapy decreased high level of plasma Ang II and endoxin caused by AT_1 receptor antagonist alone and therefore could avoid side effect caused by high level of Ang II and endoxin.

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