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Antihypertensive and Antioxidant Action of Amlodipine and Vitamin C in Patients of Essential Hypertension

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Summary **The etiology of essential hypertension includes increased oxidative stress. The role of antihypertensive drug amlodipine as an antioxidant and the benefit of addition of vitamin C, an antioxidant to antihypertensive therapy were studied. Forty male patients of essential hypertension were randomly divided into two groups and treated with 5 mg amlodipine. In addition one group also received 1000 mg vitamin C (as two 500 mg tablets) once daily for three months. Although blood pressure decreased in both groups, the systolic blood pressure in patients given vitamin C was less (126.4 ± 7.47) compared to the other group (130.9 ± 7.27). A decrease in malondialdehyde, an increase in erythrocyte sodium-potassium adenosine triphosphatase (Na^+ K^+ ATPase) and an increase in the superoxide dismutase levels were observed in both groups. The increase in SOD was statistically more in the patients given vitamin C in addition to amlodipine (0.1717 ± 0.0150 compared to 0.152 ± 0.0219 units/100 ml assay). In spite of the known antihypertensive, antioxidant activity, similarity in correcting endothelial dysfunction independently, giving the two drugs together and early introduction of vitamin C perhaps decreases oxidative stress and augments the antioxidant status. This may prevent further vascular damage due to oxidative stress, leading to a better prognosis in essential hypertension patients.**

Key Words**: amlodipine, antioxidant, essential hypertension, vitamin C**

Introduction

The pathophysiology of essential hypertension may involve one or more abnormalities in cardiovascular homeostatic mechanisms including endothelial dysfunction [*1*]. The endothelial dysfunction is related to a defect in vasodilator nitric oxide (NO) synthesis. There may be an elevation of reactive oxygen species (ROS) like superoxide anion, which inactivate the NO. Normally compounds like tocopherol and ascorbic acid, the endogenous antioxidant mechanism involving enzymes like catalase, superoxide dismutase (SOD) and glutathione scavenge and regulate the superoxide formed. This protective mechanism may be defective in essential hypertension patients. [*2–4*]. Free radicals and organic peroxides also decrease erythrocyte Na^+ K^+ ATPase levels by affecting the pump activity and antioxidants may prevent this inhibition. The changes in erythrocyte membrane reflect similar changes occurring in the vascular smooth

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muscle leading to increase in vascular tone via a mechanism involving calcium transport [*5*, *6*]. Hence the balance between ROS and the antioxidant may play a role in hypertension [*7*, *8*]. This imbalance along with the ATPase activity reverts back to normal after the control of hypertension with drugs [*9*, *10*]. In a recent review, antioxidant therapy has been suggested to reduce oxidative stress in human hypertension and end stage renal disease [*11*].

Antihypertensive and antianginal drugs may also exert antioxidant and cytoprotective effect against free radical mediated vascular injury [*12*]. Amlodipine is a long acting calcium antagonist with vascular selectivity. Its dihydropyridine ring reduces. The free radicals to nonreactive forms [*13*]. Amlodipine decreases plasma levels of vasoconstrictor, thromboxane A, elevates vasodilators like prostacyclin, NO, increases aortic tissue cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) [*14*]. It increases NO production in pulmonary circulation in patients of essential hypertension, probably through a mechanism involving bradykinin [*15*]. It is known to up regulate the activity of SOD [*16*]. Animal studies with cholesterol fed rabbits showed that amlodipine reduced aortic cholesterol accumulation, blood and aortic lipid peroxidation, malondialdehyde (MDA) levels, increased SOD in blood and aortic tissue, suppressed catalase and consumption of vitamin E [*17*].

Vitamin C or ascorbic acid is an antioxidant that may also decrease the blood pressure (BP) and influence the endothelial dysfunction. The mechanism probably involves scavenging of intracellular superoxide, activation of either smooth muscle guanylyl cyclase or endothelial NO synthetase, release of NO from tissue S- nitrosothiols and Snitrosoalbumin which reaches the smooth muscle in vascular wall, direct reduction of nitrite to NO, a decrease in low density lipoprotein (LDL) oxidation [*18*]. However the exact dose response relationship, effect on both systolic and diastolic BP and mechanism of link between BP control and antioxidant activity is still controversial [*19*, *20*].

A study to assess the benefit of using both amlodipine and vitamin C was done in hypertension patients. Change in forearm blood flow after acetylcholine infusion, measured by venous occlusion plethysmography was used to study the endothelial function. Vitamin C (24 mg/min) for 10 minutes acutely and amlodipine (5–10 mg/day) for 2 months chronically improved the endothelial function while a NO synthase inhibitor blunted the improvement. Intraarterial infusion of vitamin C, in amlodipine treated hypertensive patients did not further increase forearm vasodilatation in response to acetylcholine. It was suggested that since both increase the bioavailability of NO, perhaps there is no advantage of giving them together [*21*].

Thus our study was undertaken with the aim to evaluate, the blood pressure and the oxidative, antioxidant status using levels of malondialdehyde, erythrocyte Na^+ K^+ ATPase and superoxide dismutase levels respectively, in patients of essential hypertension. We also wanted to see whether 5 mg of amlodipine alone or when given with 1000 mg ascorbic acid could influence these variables. Since both drugs are known to have antihypertensive and antioxidative properties as well, we wanted to assess the additional benefit of giving them together for correction and prevention of further endothelial damage.

Material and Methods

Enrolment

The study was conducted in forty male patients of essential hypertension in the age group, 30–50 years. They were recently diagnosed after a series of recordings with a systolic blood pressure (SBP) between140–180 mmHg and a diastolic blood pressure (DBP) between 90–110 mmHg. This range was chosen to increase the availability of patients. During screening they were advised to improve their lifestyle by increasing physical activity (walking 3–4 times a week for 40 minutes) and restrict salt (avoid the use of extra topping of salt). Baseline investigations of blood sugar, urea, haemoglobin, serum electrolytes, creatinine, uric acid, urine examination for albumin, sugar, microscopy and specific gravity, blood urea nitrogen, electrocardiogram (ECG), X-ray abdomen and ultrasonography of kidneys was done.

Exclusion criteria

Patients with history, lab finding suggestive of secondary hypertension, hyperlipidemia or any chronic disease were excluded. Similarly those who had habits of smoking, alcohol, substance abuse, receiving any alternate traditional forms of therapy like ayurvedic and homeopathic medicines, using diet supplements containing vitamins (especially vitamin C) and requiring more than 5 mg amlodipine for blood pressure control were excluded. The patients were advised to restrict the use aspirin and nonsteroidal anti-inflammatory drugs.

No strict dietary or lifestyle modifications other than previously mentioned were used in order to ensure compliance. However one patients started yogic exercises and two underwent dietary restrictions. They were not discouraged but their data is not included.

Experimental protocol

Each patient served as his own control. They were randomly divided into groups and were either given amlodipine 5 mg alone (Aml) or amlodipine 5 mg with 1000 mg (500 mg tablet, two tablets once daily with meals) of vitamin C (Aml + Vit C) with the goal to normalize the BP. The dose of amlodipine was initiated in order to find

the lowest effective dose for successful early treatment and long-term compliance. This dose of vitamin C was selected because previous literature has reported that high physiological/pharmacological doses of ascorbate are required for antioxidant reactions in hypertension and long term moderate doses of 500 mg a day have not proved useful as antihypertensive treatment in many studies [*22–24*]. Baseline height, weight and BP (after adequate rest) were taken. A consent form was filled. Blood was collected for estimation of biochemical parameters. Thereafter, weekly evaluation of BP between 10.00am to 12 noon was done.

After three months of therapy, weight, BP and biochemical investigations were repeated for comparison. Weekly dispensing of medicines, counting the medicines consumed, questioning about daily consumption of medicines, associated lapses and reinforcement ensured compliance.

Biochemical investigation

Blood was collected in the morning hours; venous blood from the anticubital vein was drawn into a clean dry disposable sterile syringe under all aseptic precautions. The blood was immediately transferred into two clean and dry test tubes, one with and the other without the anticoagulant. The tube with anticoagulant was centrifuged at 1500 g for 5 minutes to separate the red blood cells (RBC) from the plasma. The plasma transferred to a clean and dry test tube. The serum in test tube with coagulated blood was transferred after centrifugation into another dry test-tube. Care was taken to prevent haemolysis.

Measurement of erythrocyte Na^+ K^+ ATPase [*25*]

The methodology involved preparation of RBC ghosts, estimation of proteins in the ghosts, ATP assay and estimation of inorganic phosphate.

After the plasma was decanted and the buffy coat was carefully removed, the packed RBCs were washed with 0.9% NaCl. RBC ghosts were prepared using Tris HCl 10 mM and EDTA 1 mM solution. They were then centrifuged at 15000 g for 5 minutes at 4°C in a refrigerated centrifuge. The procedure was repeated thrice and the supernatant decanted to collect the packed ghosts.

The proteins in the RBC ghosts were estimated by reacting them with alkaline copper tartarate and then with Folins reagent. ATP assay was done with magnesium chloride, potassium and sodium chloride, Tris HCl and disodium ATP. 20 mM. ATPase activity is the difference in the amount of inorganic phosphate (Pi) liberated by 0.5 ml membrane with and without K^+(KCl). Ultimately the Na^+ K^+ ATPase activity was estimated as μ moles of Pi/mg membrane protein/h.

Measurement of serum malondialdehyde [*26*]

Thiobarbituric acid (TBA) reacts similarly with lipid peroxide and MDA to give a florescent compound. Tetramethoxy propane converts quantitatively to MDA during the reaction, is reacted with TBA. Relative florescence intensity of the product was measured by flurometry at 515 nm excitation and 553 emissions. This was compared with the standards and the lipid peroxide levels were expressed in terms of the MDA.

Measurement of serum superoxide dismutase [*27*]

Freshly prepared 2.6 mM pyrogallol in 10 mM HCl, 30 mM EDTA, tris buffer and sample were mixed. A lag of 1.5 minutes allowed for a steady state of pyrogallol to be reached. The rate of increase in absorbance at 420 nM was measured spectrophotometrically. This increase is prevented by SOD as it inhibits auto oxidation of pyrogallol. One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of auto oxidation of pyrogallol per 3 ml of assay containing 0.1 ml of serum. The results are expressed as units/100 ml of assay mixture.

Statistical analysis

The mean and standard deviation of the data was calculated. The comparison between the baseline and the follow-up of the two groups was compared by the paired t test. The comparison between the baselines, follow-up of one group with the other was done with the unpaired t test. Similarly body weight, body mass index, SOD levels, MDA, erythrocyte Na^+ K^+ ATPase was compared with the students t test. A $p<0.05$ was considered significant. Pearson's one and two tailed test was used for correlation between BP (systolic, diastolic) and MDA level.

Results

There was no difference in the baseline weight, body mass index, systolic and diastolic BP between the two groups as seen in Table 1. No significant change in weight and body mass index was observed at the end of three months of therapy $p = 1.00$, 0.94 respectively in group (Aml), $p = 1.00$, 1.00 respectively in group (Aml + Vit C). Table 1 shows significant decrease in the systolic and the diastolic BP in the two groups at the end of three months. Also a significant difference in the two, follow-up systolic but not the diastolic BP was now observed as measured by the unpaired one tailed t test. ($p = 0.0285$).

The biochemical values are indicated in the Table 2. The serum malondialdehyde decreased, superoxide dismutase and Na^+ K^+ ATPase activity increased significantly after three months. The superoxide dismutase level was higher in the (Aml + Vit C) group after treatment ($p = 0.029$). The correlations between MDA level and BP were not significant.

Table 1. Demographic profile of hypertensive patients in the beginning of the study

Parameter	Aml (n = 20)	Aml + Vit C (n = 20)	*p* value
Age (years)	38.05 ± 7.51	36.57 ± 7.94	0.54
Sex	Male	Male	
Food habits	Vegetarian	Vegetarian	
Occupation	office desk work	office desk work	
Height (cm)	170.30 ± 6.81	169.90 ± 5.58	0.84
Weight (kg)	75.8 ± 12.1	76.48 ± 8.86	0.18
BMI (kg/m^2)	26.02 ± 3.09	26.56 ± 3.53	0.60
Baseline Systolic BP (mmHg)	158.2 ± 8.48	160.0 ± 8.67	0.25
Baseline Diastolic BP (mmHg)	97.10 ± 3.97	95.14 ± 3.77	0.06
Follow-up Systolic BP (mmHg)	130.9 ± 7.27[a]	126.4 ± 7.47[ab]	0.02
Follow-up Diastolic BP (mmHg)	82.7 ± 5.28[a]	81.24 ± 4.54[a]	0.17

Values are Mean ± standard deviation. *p* value denotes the difference between the two groups of hypertension patients. $p<0.05$ is significant.

[a] Indicates significant difference between baseline and follow-up using paired *t* test.

[b] Indicates significant difference between follow-up of the two groups using one tailed unpaired *t* test. df = 38.

Table 2. Changes in the biochemical profile (Baseline and follow up) in the hypertensive patients with three months of treatment

Variable	Group	Baseline	Follow up
Malondialdehyde nmol/ml	Aml	2.07 ± 0.22	1.07 ± 0.18[a]
Malondialdehyde nmol/ml	Aml + Vit C	1.95 ± 0.36	1.13 ± 0.35[a]
Superoxide dismutase Units/100 ml assay	Aml	0.0704 ± 0.0155	0.1582 ± 0.0219[ab]
Superoxide dismutase Units/100 ml assay	Aml + Vit C	0.0706 ± 0.0127	0.1717 ± 0.0150[ab]
RBC Na^+ K^+ ATPase µmolPi/mgprt RBCmemb/h	Aml	0.15680 ± 0.0025	0.16905 ± 0.00590[a]
RBC Na^+ K^+ ATPase µmolPi/mgprt RBCmemb/h	Aml + Vit C	0.15648 ± 0.00262	0.16886 ± 0.00360[a]

Values are Mean ± standard deviation. n = 20 for each group.

[a] Significant difference between baseline and follow up (after 3 months treatment) ($p<0.001$).

[b] Significant difference between follow up of both groups ($p = 0.029$; *t* test df = 38).

Discussion

The blood pressure of our hypertensive patients decreased when amlodipine 5 mg was given alone and along with 1000 mg vitamin C. Thus this therapy using a single anti-hypertensive drug with lifestyle changes proved beneficial in both stage 1 and 2 hypertensive patients [*28*]. Various mechanisms of amlodipine's action may contribute to the decrease in BP [*14*, *29*].

Although we cannot comment about the antihypertensive action of vitamin C independently, the post-treatment systolic BP was less when vitamin C was also given along with amlodipine. Most of the studies related to vitamin C and hypertension deal with therapeutic effect, serum levels, dietary levels, and vasodilatory response to drugs to assess endothelial function. Although many studies are favourable but there is no consensus of opinion about the therapeutic dose, duration of effect and treatment. Some studies suggest that vitamin C decreases systolic BP, in doses 2000 mg bolus followed by 500 mg once daily or 500 mg twice daily for a period of 4–6 weeks in hypertensive patients [*20*, *30*]. In another study intravenous administration of ascorbic acid rapidly reduced BP in hypertensive patients. A decrease of both systolic as well as diastolic BP in a group of borderline hypertensives has been reported [*31–33*]. The relationship between dietary, serum levels of vitamin C and BP was studied in healthy men who were given a diet deficient in vitamin C for a month. Those with a greater decrease in vitamin C levels had a higher BP [*34*]. Intrarterial administration of vitamin C is reported to improve vasodilatory response directly but not following amlodipine therapy in hypertensive patients [*21*]. Also combinations of high oral dose of antioxidants, zinc sulphate, vitamin C, alpha tocopherol and beta-carotene, when compared with placebo treatment have shown a decrease in systolic BP in patients receiving antihypertensive treatment [*35*]. The mechanism by

which antioxidant vitamin C improves endothelial function in essential hypertension is probably by directly scavenging free radicals within the vasculature. This restores the nitric oxide level improving endothelium dependant vasodilatation [*36*]. Increase in NO synthase, increase delivery of NO from plasma, reduction of nitrite to NO and stimulation of guanylate cyclase by NO may be additional mechanisms involved. [*18*, *37*, *38*]. However other authors feel that vitamin C has very little or negligible effect on BP [*19*, *23*]. Thus there are inconsistent reports by different authors, but our results have shown a lower value (4 mmHg decrease) of systolic BP, suggesting a beneficial effect of adding vitamin C to therapy with amlodipine. However this change is significant only by the one tailed and not the two-tailed unpaired t test. There is need for extensive trials of longer duration if the relevance of this BP lowering effect of vitamin C is to be seen in the clinical or public health perspective. Till such a time, ethical reasons may not permit its long-term use as a single drug regime in hypertension.

The MDA levels decreased significantly in both the groups. This decrease in MDA could be either a result of BP reduction or antioxidant property of amlodipine. These results are in agreement with various other studies that have shown MDA levels to decrease after amlodipine administration [*17*, *39*]. Amlodipine directly reduces several free radical species [*40*, *41*]. Other studies however have suggested no effect on circulating indices of oxidative stress [*42*]. In experiments with spontaneously hypertensive rats and normotensive rats, with or without amlodipine treatment, no significant difference in total antioxidant capacity was observed [*43*]. Vitamin C is a well-known antioxidant that scavenges extra free radicals and whose levels inversely correlate with those of lipid peroxides [*30*, *44*]. In a recent study, six months of treatment with 600 mg per day of ascorbic acid has reported a similar decrease [*45*]. However since the decrease in MDA levels in our patients was not different in the two groups, we presume that the addition of vitamin C was not particularly helpful in this respect.

A low level of SOD is reported in hypertensive patients. This decrease may be associated with a decrease in NO levels, reinforcing that SOD is helpful in the release of NO [*46*, *47*]. Amlodipine has been reported to increase SOD, prevent the decrease in glutathione, and the consumption of vitamin E, but has no effect on the catalase activity [*12*, *17*]. SOD levels may increase due to up regulation and decreased consumption. Increase in SOD activity, with more NO bioavailability may be one of the mechanisms contributing to the antioxidant properties of antihypertensives ramipril and losartan [*48*]. Similarly SOD levels were lower in hypertensive patients compared to controls but increased after 24 weeks of acebutolol therapy [*49*]. SOD levels increased in both groups of our hypertensive patients following treatment, more where vitamin C was given. However had we normalized the SOD values to serum proteins, it could have helped in better quantification of the increase, but we can definitely state that vitamin C in therapy augments the increase in SOD levels.

The Na^+ K^+ ATPase activity is inhibited by oxidative stress and hydroperoxides. It is susceptible to free radical induced lipid peroxidation. A lower level compared to control is reported in diabetic hypertensives. Hence the increase in the activity is a sign of reduction in the oxidative stress [*50*]. An increase is reported more with angiotensin converting enzyme (ACE) inhibitors compared to calcium channel blockers and also in treatment with enapril and captopril [*10*, *51*, *52*]. The increased level in our patients following amlodipine therapy supports the finding of MDA decrease, indicating a reduction in oxidative stress on the RBC membrane. As the values of both groups were not statistically different vitamin C probably had no effect on this enzyme.

Conclusion

This study has thus shown that amlodipine therapy decreased blood pressure, and improved the status of oxidative stress as shown by a decrease in MDA and increase in Na^+ K^+ ATPase and SOD levels in essential hypertension patients. It therefore has antioxidative action in addition to the antihypertensive action. Following treatment with amlodipine and 1000 mg of vitamin C, in addition to the abovementioned benefits another group of hypertensive patients had a lower systolic BP and higher SOD level.

Estimation of plasma ascorbic acid, NO production, a complete dietary analysis to study contribution of key nutrients like sodium and potassium in the diet and a longer follow up may be required to further ensure and state the mechanism of benefit of that extra 1000 mg of vitamin C in the pathophysiology of essential hypertension. However it can be definitely stated that adding vitamin C to amlodipine therapy can further increase SOD, an enzyme of the endogenous antioxidant mechanism which may help to reduce and prevent further endothelial damage due to oxidative stress in essential hypertension.

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