ORIGINAL ARTICLE

EFFECT OF L-ARGININE THERAPY ON PLASMA NO_2 AND NO_3 LEVELS, AND BLOOD PRESSURE IN UREMIC RABBITS

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Background: Normal kidney function is regulated by Nitric oxide (NO) and Superoxide (O_2^-) in the body, and consequently controls blood pressure. Nitric Oxide promotes natriuresis and diuresis, and therefore results in reduction of blood pressure. The objective of this study was to determine the effect of L-arginine supplementation on blood pressure, urinary protein, nitrite and nitrate in addition to blood urea, serum creatinine and creatinine clearance in uremic rabbits. **Methods**: This study was carried out in the Department of Biochemistry Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. A total of 48 rabbits were included in the study. Twenty-four of the rabbits on surgical intervention were prepared as uremic and so became hypertensive as well. Two groups were uremic, one group was given L-arginine and the other was remained untreated. Systolic and diastolic blood pressure was measured on week 0, week 2, week 4, and week 6, while blood and urine was collected on week 0 and week 6. **Results:** On supplementation with L-arginine to uremic rabbits systolic and diastolic blood pressures were decreased significantly. Nitrite/nitrate and urinary protein were corrected to some extent while blood urea and serum creatinine were unaffected. **Conclusion:** L-arginine has a beneficial role as blood pressure lowering agent in uremic rabbits. It corrects NO_2/NO_3 plasma level and proteinuria which is indicator of renal failure.

Keywords: Renal failure, uremia, L-arginine, hypertention, NO_2/NO_3

INTRODUCTION

Uremia is defined as increased blood urea level due to reversible or irreversible and long-standing loss of renal function causing ill-health. Due to impair excretion concentration of asymmetric dimethyl L-arginine (ADMA) and other L-arginine analogue increases in CRF patients and consequently nitric oxide synthase is inhibited with the resultant decreased synthesis of nitric oxide. As L-arginine is the precursor of NO, so L-arginine supplementation improves the situation because the substrate for the enzyme increases. Patients with end-stage chronic renal failure are treated principally by renal dialysis and transplant.[1] In chronic renal failure (CRF) there is endothelial dysfunction, accelerated atherosclerosis and a high incidence of thromboembolic complications. In this context, cardiovascular disease is the main cause of mortality in CRF patients.[2] Normal Kidney function is regulated by NO and superoxide (O_2^-) in the body, and consequently controls blood pressure. Nitric oxide promotes natriuresis and diuresis, and therefore results in reduction of blood pressure. Superoxide promotes salt and water retention, and thus favours increases in blood pressure. Changes in natriuresis and urinary volume caused by these two reactive oxygen species can be due to alterations in renal haemodynamics or ion transport along the nephron. In the thick ascending limb, NO inhibits NaCl reabsorption.[3] In contrast, O_2^- stimulates it. Perturbations in production or degradation of NO or increase in O_2^- can lead to hypertension.[4]

Nitric oxide (NO) is a widespread signalling molecule involved in numerous biological functions, such as regulation of vascular tone, control of thrombosis, the interaction of platelets and leukocytes with the vessel wall.[5] It is also involved in smooth muscles relaxation.[6] In chronic renal failure, NO release appears to be impaired. This has been attributed in part to the accumulation in plasma of endogenous inhibitors of the enzyme, nitric oxide synthase (NOS) and enhanced production of oxygen free radicals, which rapidly inactivate NO. It is produced *in vivo* from L-arginine, which is converted to nitrite and nitrate that can be converted back to NO. Nitric oxide is produced in the body from its physiological precursor L-arginine, which is a semi-essential amino acid for most mammals and is required during periods of growth, severe stress and injury.[6] The availability of L-arginine in circulating blood cells and the vasculature modulate the rates of NO biosynthesis.[7] Structurally L-arginine is referred as α-amino δ-guanidino valeric acid.[8]

The impaired endothelial function in CRF is attributable to the competitive inhibition of NO synthase by a structural analogue of L-arginine, i.e., asymmetric dimethyl arginine (ADMA) and possibly elevated the systemic blood pressure. The NO is

thought to be involved in relaxation of smooth muscle and vasodilation.[9]

MATERIAL AND METHODS

This study was an experimental Randomized Control study carried out in the Department of Biochemistry Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre Karachi; in collaboration with the Animal House Dow University of Health Sciences, Karachi.

A total of 48 rabbits were included in the study. Twenty-four of the rabbits on surgical intervention were prepared as uremic and so became hypertensive as well. All the subjects were divided in four groups. Two groups were uremic, one group was given L-arginine and the other was remained untreated. Similarly two groups were control; one was given L-arginine supplementation while the other was remained untreated.

Systolic and diastolic blood pressures were measured on week 0, week 2, week 4 and week 6 while blood and urine was collected on week 0 and week 6. NOx (Total nitric oxide, nitrite and nitrate) was determined by Nitrate/Nitrite Colorimetric Assay Kit. Serum creatinine and urinary creatinine was measured by Jaffe's method. Blood urea was determined by manual enzymatic kit (RANDOX Laboratories Ltd). Urinary protein was determined manually by trichloroacetic acid method.

RESULTS

Table-1 shows the comparison of systolic and diastolic blood pressures of all four groups with the control without L-arginine and uremic without L-arginine at week 6. The mean systolic and diastolic blood pressures of uremic with L-arginine vs control without L-arginine were increased highly significantly ($p<0.01$). In control groups there were no significant differences. The systolic and diastolic blood pressures of uremic with L-arginine vs uremic without L-arginine were decreased highly significantly ($p<0.01$).

Table-2 shows the comparison of all groups for serum creatinine and blood urea at week 6. When mean of serum creatinine for uremic with L-arginine was compared with that of the control without L-arginine the former was increased highly significantly ($p<0.01$). In control groups there were no significant differences. Similarly in uremic groups there were also no significant differences. When mean value of blood urea for uremic with L-arginine was compared with that of the control without L-arginine the former was increased highly significantly ($p<0.01$), while in control groups there was no significant difference. Similarly in uremic groups there was also no significant difference.

Table-3 shows comparison of control and uremic groups for NO_2/NO_3 and urinary protein at week 6. When mean of NO_2/NO_3 for uremic with L-arginine was compared with that of the control without L-arginine the former was decreased highly significantly ($p<0.01$). In control groups there was no significant difference. Similarly when mean values of NO_2/NO_3 for uremic with L-arginine and uremic without L-arginine were compared the former was increased highly significantly ($p<0.01$). When the mean value of urinary protein for uremic with L-arginine was compared to that of control without L-arginine the value for uremic with L-arginine was increased highly significantly ($p<0.01$). In control groups there was no significant difference. The comparison of these values for uremic with L-arginine and uremic without L-arginine showed that the former was decreased highly significantly ($p<0.01$).

Table-1: Systolic and diastolic BP in groups at week 6

Groups	Systolic BP (mmHg)	Diastolic BP (mmHg)
Uremic +L-a	158.33±1.88*‡	96.25±1.05*‡
Uremic	193.33±3.49*	117.92±1.99*
Control +L-a	113.92±2.21	76.33±1.32
Control	120.33±1.88	82.92±1.30

*$p<0.01$ vs control, ‡$p<0.01$ vs uremic

Table-2: Serum creatinine and blood urea in groups at week 6

Groups	S. Creatinine (mg/dl)	B. Urea (mg/dl)
Uremic +L-arginine	2.06±0.05*	85.50±1.29*
Uremic	2.03±0.07*	81.58±1.90*
Control +L-arginine	0.95±0.04	36.00±1.12
Control	0.97±0.04	35.92±1.18

*$p<0.01$ vs control

Table-3: Comparison of control and uremic groups for NO_2/NO_3 and urinary protein at week 6

Subjects	NO_2/NO_3 (mg/dl)	Urinary protein (mg/dl)
Uremic + L-a	19.18±0.05*‡	62.33±2.17*‡
Uremic	8.93±0.57*	129.58±8.46*
Control + L-a	38.95±0.47	24.17±0.47
Control	27.93±0.39	24.08±0.47

*$p<0.01$ vs control, ‡$p<0.01$ vs uremic

DISCUSSION

Uremia and hypertension is becoming an increasingly important public health care problem. Chronic disorders worldwide and secondary forms of hypertension are found in 5–10% of the hypertensive population, of which most can be linked to renal disease. In humans, as well as in experimental models of salt-sensitive hypertension, there is a growing body of evidence pointing at a close relationship between nitric oxide deficiency and development of

hypertension. The precursor for nitric oxide is the semi essential amino acid, the L-arginine.

In this study, we found that supplementation with L-arginine (1%) blunted the rise in systolic and diastolic blood pressure highly significantly in rabbits with reduced renal mass but this effect of L-arginine supplementation was not significant in control group. Carlstrom *et al*[10], also found the same results. We also observed that the supplementation of L-arginine corrected to some extent the nitrite/nitrate serum level in uremic rabbits. Baylis described that NO production is reduced in renal disease and corrected by L-arginine supplementation.[11]

We found that the supplementation with L-arginine in uremic rabbits improves proteinuria this may be due to the improvement in NO release which prevents the development and progression of glomerulosclerosis and in this way reduces the glomerular hyperfiltration and proteinuria. The reduction in proteinuria and the possible mechanism is also described by Yannick.[12] In this study we have also observed that L-arginine supplementation in uremic rabbits have no significant effect on serum creatinine and blood urea. This fact is also reported by Yannick *et al*.[12]

CONCLUSION

Oral administration of L-arginine has a beneficial role in uremic rabbits. It is blood pressure lowering agent. It corrects NO_2/NO_3 level the metabolites of NO and also corrects the proteinuria in uremic conditions. L-arginine has no significant effects on serum creatinine and blood urea. Further work on humans is suggested.

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