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Effect of alpha-lipoic acid supplementation on blood pressure, renal oxidant-antioxidant status and renal damage in spontaneously hypertensive rats

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ABSTRACT

Objective: To investigate the effect of alpha-lipoic acid (ALA) supplementation on systolic blood pressure (SBP), renal oxidant-antioxidant status and renal damage in spontaneously hypertensive rats (SHR) and SHR administered with N$^{\omega}$-nitro-*L*-arginine methyl ester (*L*-NAME).

Methods: Male rats were divided into four groups (SHR, SHR+ALA, SHR+*L*-NAME, SHR+ALA+*L*-NAME). The respective group of rats was administered with ALA (100 mg/kg/day) from age 4 weeks to 28 weeks and *L*-NAME (25 mg/kg/day) from age 16 weeks to 28 weeks. SBP was measured every two weeks and twenty four hour urine was collected at 4 weeks, 16 weeks and 28 weeks for estimation of protein, creatinine and *N*-acetyl- β -*D*-glucosaminidase. At the end of 28 weeks, rats were sacrificed and blood and kidneys collected for assessment of blood creatinine, kidney thiobarbituric acid reactive substances, protein carbonyls, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, glutathione disulfide, glutathione, total antioxidant status and nitric oxide as well as histopathological examination.

Results: ALA supplementation significantly reduced SBP of SHR and SHR+*L*-NAME rats when compared to their respective non-supplemented groups. Renal oxidant status markers including thiobarbituric acid reactive substances and protein carbonyls were significantly reduced on SHR and SHR+*L*-NAME rats supplemented with ALA at 28 weeks as well as ALA supplementation significantly increased renal antioxidants including superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione and glutathione/glutathione disulfide ratio at 28 weeks. No significant change in nitric oxide levels was observed between the ALA supplemented and non-supplemented groups. Renal dysfunction was ameliorated on ALA supplementation as evidenced by significant reduction in urine protein levels, *N*-acetyl- β -*D*-glucosaminidase activity and significant increase of creatinine clearance in SHR and SHR+*L*-NAME at 28 weeks. Renal histopathological examination showed that ALA supplementation prevented vascular damage in SHR and ameliorated glomerular damage in SHR+*L*-NAME at 28 weeks.

Conclusions: ALA has hypotensive and renoprotective effects on both SHR and SHR+*L*-NAME, which could be due to its ability to ameliorate oxidative stress in the kidneys.

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1. Introduction

Hypertension has emerged as a global public health problem due to its high prevalence[1]. If not adequately controlled, hypertension can cause damage to various organs resulting in serious health problems including renal failure, premature mortality and disability[2]. Primary or essential hypertension accounts for about 95% of all cases of hypertension in which the exact cause(s) or mechanisms involved in its pathogenesis have not been elucidated[3]. However, the kidney is strongly believed to play a key role in the development of hypertension and at the same time a target of hypertension-induced damage[4].

Oxidative stress, where there is increased levels of molecular oxygen or reactive oxygen species (ROS) when compared to antioxidant levels, can lead to tissue injury and dysfunction[5]. Reports have implicated free radical mediated oxidative damage in the pathogenesis and progression of hypertension including organ damage[6-9]. However, these studies have not been comprehensive enough as they did not examine the development of hypertension in a detailed time course manner in relation to all the important antioxidants and related metabolites. These studies also did not focus much on the involvement of the kidney. Studies have indicated that mobilization of antioxidants occurs in response to oxidative stress which reflects a dynamic process whereby dietary antioxidant supplementation might exert a significant influence[10]. In relation to this, research on the effect of antioxidant supplementation on hypertension has been undertaken, but the results obtained are conflicting as some studies showed that supplementation was beneficial[11-14] whereas in others it was not[15-17]. Most of these studies also did not look extensively into the role and biochemical mechanisms of oxidative stress as well as the antioxidant defense systems involved in the kidney.

Alpha-lipoic acid (ALA) is a water and fat soluble natural antioxidant widely distributed in plants and animals in both cellular membranes and cytosol[18,19]. Due to its antioxidant property, ALA has been researched as a potential therapeutic agent for many chronic diseases including hypertension where oxidative stress is believed to play a role[20]. Some studies have also indicated that ALA supplementation has a hypotensive effect in addition to its antioxidant effect[21,22]. However, these studies were of a short duration and did not examine in detail the effect of ALA supplementation on the oxidant-antioxidant status throughout the period from pre-hypertension until end-organ damage. In relation to this, studies on the kidneys are also limited.

The spontaneously hypertensive rat (SHR) is extensively used as a research model of human essential hypertension[23]. As kidney damage occurs at a much later stage in the SHR, researchers developed the SHR+ N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME) hypertensive model whereby the hypertensive state is greatly accelerated so that organ damage including the kidneys occurs much earlier[24]. This study was undertaken to investigate the effect of ALA supplementation on systolic blood pressure (SBP), renal oxidant-antioxidant system and renal damage of SHR and *L*-NAME treated SHR rats.

2. Materials and methods

2.1. Animals and experimental protocols

Male SHR rats obtained from the Animal Research and Service Centre were used for the study. The experimental protocols used in this study were approved by the Animal Ethics and Welfare Committee (USM/PPSP/Ethics Com./2006[180.3(6)], 9 October 2006).

SHR were divided into 4 different groups of six rats each, including untreated SHR (SHR), SHR supplemented with ALA from age 4 weeks to 28 weeks (SHR+ALA), SHR administered with *L*-NAME from age 16 weeks to 28 weeks (SHR+*L*-NAME) and SHR supplemented with ALA from 4 weeks to 28 weeks and *L*-NAME from age 16 weeks to 28 weeks (SHR+ALA+*L*-NAME). Each rat was housed in individual cage in standard controlled environment.

2.2. ALA and L-NAME administration

After acclimatization of the rats in the cages, the average daily water intake of rats was determined. ALA (Sigma, USA) was supplemented to rats at a dose of 100 mg/kg BW/day. The ALA was dissolved in 5 % (w/v) potassium hydroxide (KOH) in saline and given in a 1 mL dose by gastric gavage[25]. Control rats were given a 1 mL dose of 5 % (w/v) KOH in saline only. *L*-NAME at a dose of 25 mg/kg BW/day was administered in the daily drinking water[26].

2.3. Blood pressure measurement

SBP was measured every two weeks in conscious rats during the experimental period by the non-invasive (indirect) blood pressure tail plethysmography method, using an automated cuff-inflator pulse detection system (Model 6R22931, IITC Life Science, USA).

2.4. Specimen collection and processing

One to two days before 4 weeks, 16 weeks and 28 weeks of age, the rats were placed in metabolic cages for collection of 24 hour urine. Collected urine samples were stored at -80 ℃ until analysis. Rats were sacrificed at the end of 28 weeks. Blood samples were collected in plain tubes, allowed to clot, centrifuged to obtain serum and then stored at -80 ℃ until analysis. Kidneys were rapidly removed, washed in saline, decapsulated and blot-dried before being used for histopathology examination and homogenate preparation.

2.5. Preparation of kidney homogenates

A weighed amount of kidney tissue was homogenized to make 10% homogenates (w/v) in ice cold (0-4 ℃) 0.05 M sodium phosphate buffer pH 7.4, using an ice-chilled glass homogenizing vessel in a homogenizer fitted with Teflon pestle (Glass-Col, USA) at 900 rpm. The homogenates were centrifuged in a refrigerated centrifuge at 1 000 ×*g* at 4 ℃ for 10 min to remove nuclei and debris[27]. The supernatant obtained was used for biochemical assays. Homogenates were kept frozen at -80 ℃ until analysis for the other assays.

2.6. Histopathology examination

Routine histopathology procedures were followed whereby kidney sections were stained with haematoxylin/eosin (HE) for microscopic examination to assess any glomerular, tubular and vascular changes[28].

2.7. Biochemical assays

Biochemical assays were performed on the -80 ℃ stored samples within 3 days of collection and biochemical indices were determined at 4, 16 and 28 weeks. Protein concentration of urine and kidney homogenates were determined by spectrophotometry using the Micro TP kit (Wako Pure Chemicals, Japan)[29]. Serum and urine creatinine were determined by spectrophotometry using a commercial reagent kit (Randox Laboratories, Crumlin, UK). Creatinine clearance was calculated from these data and 24 hour urine volumes. *N*-acetyl- β -*D*-glucosaminidase (NAG) activity in urine was determined by spectrophotometry using the NAG (Roche, Switzerland) commercial kit[30]. Total antioxidant status (TAS) was determined by a spectrophotometric method[31]. Lipid peroxidation was determined as thiobarbituric acid reactive substances (TBARS) by a spectrophotometric method[32]. TBARS assay was carried out on the day of sacrifice. Protein carbonyls (PCO) levels were determined by a spectrophotometric method[33]. Superoxide dismutase (SOD) activity was assayed by spectrophotometry based on the autoxidation of epinephrine[34]. Catalase (CAT) activity was assayed by spectrophotometry based on the enzyme-catalyzed decomposition of hydrogen peroxide and assay of the remaining hydrogen peroxide[35]. Glutathione peroxidase (GPx) activity was determined by spectrophotometry based on the oxidation of glutathione by hydrogen peroxide and measurement of the decrease in concentration of reduced nicotinamide adenine dinucleotide phosphate (NADPH)[34]. Glutathione reductase (GR) activity was assayed by spectrophotometry using glutathione disulfide (GSSG) as a substrate coupled to oxidation of NADPH[36]. Glutathione S-transferase (GST) activity was assayed by spectrophotometry based on the rate of glutathione conjugation to the substrate 1-chloro-2,4-dinitrobenzene (CDNB)[37]. Glutathione (GSH) and GSSG levels were measured by spectrophotometry using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as substrate[38]. GSH to GSSG ratio was calculated from these values. Nitric oxide (NO) was determined using the commercial Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemicals, USA) which measures the sum of both nitrate and nitrite (denoted as NOx) which is the best index of total NO production[39].

2.8. Statistical analysis

Data were analyzed by One-Way ANOVA with *post hoc* Tukey test using Statistical Package for the Social Science (SPSS) software version 20. Significant level was set at $P < 0.05$. Data are expressed as mean and standard error mean (mean ± SEM) for six animals in each group.

3. Results

3.1. SBP

Figure 1 shows the time course changes in SBP of SHR, SHR+*L*-NAME, SHR+ALA and SHR+ALA+*L*-NAME rats. ALA supplementation significantly reduced SBP in SHR rats from age 8 weeks onwards until 28 weeks ($P < 0.01$) when compared to untreated SHR. However, the SBP of the ALA supplemented rats was still in the hypertensive range (> 140 mm Hg) from the age of 16 weeks onwards. Moreover, ALA supplementation significantly decreased the SBP of SHR+*L*-NAME rats when compared to non-supplemented SHR+*L*-NAME rats ($P < 0.01$). SHR+ALA+*L*-NAME rats had significantly higher SBP than SHR+ALA rats from 26 weeks of age until 28 weeks.

3.2. Renal SOD, CAT, GPx, GR and GST activities

The renal SOD, CAT, GPx, GR and GST activities are shown in Table 1. ALA supplementation significantly increased the SOD activity in SHR+ALA rats at 28 weeks of age when compared to non-supplemented SHR rats ($P < 0.05$). The supplementation also significantly increased the SOD activity in SHR+ALA+*L*-NAME rats at 28 weeks of age when compared to non-supplemented SHR+*L*-NAME rats ($P < 0.01$). SHR+ALA+*L*-NAME rats at 28 weeks had significantly increased CAT activity when compared to age-matched SHR+*L*-NAME rats ($P < 0.001$). GPx activity was significantly lower in SHR+ALA rats at 16 and 28 weeks compared to age-matched SHR rats ($P < 0.001$) while higher in SHR+ALA+*L*-NAME rats at 28 weeks compared to SHR+*L*-NAME rats ($P < 0.001$). Moreover, GST activity was significantly higher in SHR+ALA+*L*-NAME rats at 28 weeks of age when compared to age-matched SHR+*L*-NAME rats ($P < 0.001$). As for GR, there was

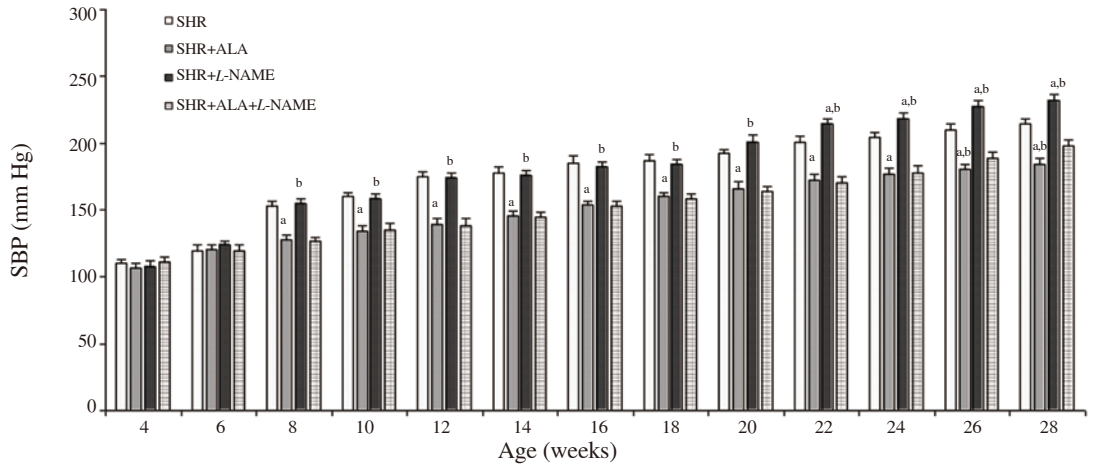

Figure 1. SBP in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats. [a]$P < 0.01$, compared with SHR. [b]$P < 0.01$, compared with SHR+ALA+*L*-NAME. SBP: systolic blood pressure. ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester.

Table 1. Renal SOD, CAT, GPx, GR and GST activity in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats (U/mg protein).

Groups	SHR	ALA	*L*-NAME	ALA+ *L*-NAME
SOD				
4 W	76.33 ± 2.78	-	-	-
16 W	84.33 ± 4.06	79.00 ± 1.33	-	-
28 W	64.16 ± 1.81	76.96 ± 1.24*	55.77 ± 2.18	80.05 ± 0.15^{++}
CAT				
4 W	902.83 ± 14.37	-	-	-
16 W	1034.50 ± 13.86	1031.13 ± 20.92	-	-
28 W	1105.33 ± 39.52	1146.97 ± 12.30	677.67 ± 16.33	914.46 ± 6.70^{+++}
GPx				
4 W	1261.63 ± 58.78	-	-	-
16 W	1606.83 ± 39.05	1147.96 ± 16.24***	-	-
28 W	1733.51 ± 50.43	1137.12 ± 18.66***	789.94 ± 26.73	945.26 ± 13.11^{+++}
GR				
4 W	303.79 ± 9.71	-	-	-
16 W	262.82 ± 4.40	311.29 ± 8.36	-	-
28 W	273.31 ± 11.89	304.62 ± 9.44	215.15 ± 12.94	225.20 ± 6.57
GST				
4 W	126.77 ± 5.93	-	-	-
16 W	181.62 ± 16.43	151.23 ± 8.38	-	-
28 W	191.87 ± 9.42	167.25 ± 5.25	95.23 ± 5.45	122.08 ± 4.84^{+++}

Values are expressed as mean ± SEM (n=6 per group). W = age in weeks; ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; GST: glutathione S-transferase. $^{*}P < 0.05$, $^{***}P < 0.001$: SHR+ALA compared to age-matched SHR. $^{++}P < 0.01$, $^{+++}P < 0.001$: SHR+ALA+*L*-NAME compared to age-matched SHR+ *L*-NAME, '–' indicates no analysis was performed because intervention had not commenced yet /just commenced at this time-point.

no significant difference between SHR+ALA and SHR groups as well as SHR+ALA+*L*-NAME and SHR+*L*-NAME groups at the end of the experiment.

3.3. Renal GSH, GSSG and GSH/GSSG ratio values

The renal GSH, GSSG and GSH/GSSG ratio values are shown in Table 2. ALA supplementation significantly increased the GSH levels in SHR+ALA and SHR+ALA+*L*-NAME rats at 28 weeks when compared to non-supplemented SHR and SHR+*L*-NAME rats, respectively ($P < 0.01$). GSSG levels in ALA supplemented SHR+ALA and SHR+ALA+*L*-NAME rats were significantly lower at 28 weeks when compared to the respective non-supplemented rat group ($P < 0.001$). Meanwhile, ALA supplementation also significantly increased the GSH/GSSG ratio values in SHR+ALA and SHR+ALA+*L*-NAME rats at 28 weeks when compared to the respective non-supplemented rat group ($P < 0.001$ and $P < 0.05$, respectively).

3.4. Renal TAS and NOx levels

The renal TAS and NOx levels are shown in Table 3. SHR+ALA rats at 28 weeks had significantly increased TAS levels when compared to age-matched SHR+ALA+*L*-NAME rats ($P < 0.001$). There was no significant change in NOx values between the supplemented and non-supplemented rat groups.

Table 2. Renal GSH, GSSG and GSH/GSSG ratio values in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats.

Groups	SHR	ALA	*L*-NAME	ALA+ *L*-NAME
GSH (nM/mg protein)				
4 W	58.37 ± 1.46	-	-	-
16 W	50.25 ± 1.32	56.11 ± 0.92	-	-
28 W	38.99 ± 2.17	50.02 ± 0.77**	30.94 ± 1.68	42.41 ± 0.62^{++}
GSSG (nM/mg protein)				
4 W	1.63 ± 0.02	-	-	-
16 W	2.17 ± 0.07	2.30 ± 0.01	-	-
28 W	5.50 ± 0.19	2.89 ± 0.12***	10.10 ± 0.08	6.92 ± 0.10^{+++}
GSH/GSSG ratio				
4 W	35.80 ± 0.94	-	-	-
16 W	23.19 ± 0.54	24.38 ± 0.41	-	-
28 W	7.18 ± 0.41	17.48 ± 0.85***	3.07 ± 0.18	6.12 ± 0.08^{+}

Values are expressed as mean ± SEM. (n=6 per group). W = age in weeks; ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester; GSH: glutathione; GSSG: glutathione disulfide. $^{**}P < 0.01$, $^{***}P < 0.001$: SHR+ ALA compared to age-matched SHR. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$: SHR+ ALA + *L*-NAME compared to age-matched SHR+*L*-NAME. '–' indicates no analysis was performed because intervention had not commenced yet /just commenced at this time-point.

Table 3. Renal TAS and NOx levels in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats.

Groups	SHR	ALA	*L*-NAME	ALA+ *L*-NAME
TAS (μM/mg protein)				
4 W	2.50 ± 0.04	-	-	-
16 W	3.49 ± 0.07	3.37 ± 0.08	-	-
28 W	3.64 ± 0.11	3.54 ± 0.06***	2.46 ± 0.05	2.76 ± 0.09
NOx (nM/mg protein)				
4 W	4.97 ± 0.21	-	-	-
16 W	5.37 ± 0.16	5.35 ± 0.08	-	-
28 W	4.81 ± 0.14	5.10 ± 0.06	4.06 ± 0.09	4.32 ± 0.80

Values are expressed as mean ± SEM. (n=6 per group). W = age in weeks; ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester; NO: nitric oxide; TAS: total antioxidant status. $^{***}P < 0.001$: SHR+ALA compared to age-matched SHR+ALA+*L*-NAME. '–' indicates no analysis was performed because intervention had not commenced yet /just commenced at this time-point.

3.5. Renal TBARS and PCO levels

The renal TBARS and PCO levels are shown in Table 4. ALA supplementation had significantly reduced TBARS levels ($P < 0.001$) at 28 weeks compared to age-matched SHR and SHR+*L*-NAME rats, respectively. PCO level was significantly reduced in SHR+ALA and SHR+ALA+*L*-NAME rats ($P < 0.001$) at 28 weeks in comparison with their respecive non-supplemented group.

3.6. Urine protein, NAG and creatinine clearance values

Results of renal damage parameters are presented in Table 5. Urine protein levels were significantly reduced in SHR+ALA and SHR+ALA+*L*-NAME rats at 28 weeks in comparison with respective non-supplemented group ($P < 0.001$). Creatinine clearance was significantly increased in SHR+ALA and SHR+ALA+*L*-NAME rats at 28 weeks ($P < 0.001$). Moreover, urine NAG activity was significantly reduced in SHR+ ALA rats at 28 weeks when compared to age-matched SHR rats and SHR+ ALA+*L*-NAME rats ($P < 0.001$). ALA supplementation also significantly decreased urine NAG activity in SHR+ALA+*L*-NAME rats at 28 weeks compared to age-matched untreated SHR+*L*-NAME rats ($P < 0.001$).

3.7. Renal histopathological examination

The renal histopathological examination results of ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats are shown in Figure 2. ALA supplementation prevented the vascular damage in SHR rats at 28 weeks. In SHR+ALA+*L*-NAME rats at 28 weeks, ALA supplementation was able to ameliorate the glomerular damage. However, the degree of vascular and tubulo-interstitial damage was still the same as in non-supplemented SHR+*L*-NAME rats.

Table 4. Renal TBARS and PCO levels in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats.

Groups	SHR	ALA	*L*-NAME	ALA+ *L*-NAME
TBARS (μM/mg protein)				
4 W	1.87 ± 0.05	-	-	-
16 W	2.25 ± 0.12	2.24 ± 0.02	-	-
28 W	2.76 ± 0.04	1.93 ± 0.02***	3.27 ± 0.09	2.20 ± 0.03^{+++}
PCO (nM/mg protein)				
4 W	0.71 ± 0.01	-	-	-
16 W	0.84 ± 0.01	0.73 ± 0.01	-	-
28 W	1.02 ± 0.01	0.80 ± 0.01***	1.34 ± 0.09	0.94 ± 0.06^{+++}

Values are expressed as mean ± SEM (n=6 per group). W = age in weeks; ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester; TBARS: thiobarbituric acid reactive substances; PCO: protein carbonyls. $^{***}P < 0.001$: SHR+ALA compared to age-matched SHR. $^{+++}P < 0.001$: SHR+ALA+*L*-NAME compared to age-matched SHR+*L*-NAME. '–' indicates no analysis was performed because intervention had not commenced yet /just commenced at this time-point.

Table 5. Urine protein, creatinine clearance and urine NAG values in ALA supplemented and non-supplemented SHR and SHR+*L*-NAME rats.

Groups	SHR	ALA	*L*-NAME	ALA+*L*-NAME
Urine protein (mg/24 hour)				
4 W	1.010±0.201	-	-	-
16 W	6.210±0.329	5.830±0.120	-	-
28 W	13.592±0.232	9.812±0.074$^{***\Delta\Delta\Delta}$	70.774±0.972	12.295±1.213^{+++}
Creatinine clearance (mL/min)				
4 W	0.845±0.053	-	-	-
16 W	1.630±0.061	1.610±0.012	-	-
28 W	1.282±0.072	1.539±0.009$^{***\Delta\Delta\Delta}$	0.919±0.071	1.373±0.024^{+++}
NAG (mU /mmol creatinine)				
4 W	1.204±0.006	-	-	-
16 W	1.981±0.009	1.884±0.013	-	-
28 W	3.490±0.029	2.283±0.025$^{***\Delta\Delta\Delta}$	5.322±0.033	3.132±0.053^{+++}

Values are expressed as mean ± SEM (n=6 per group). W = age in weeks; ALA: alpha-lipoic acid; SHR: spontaneously hypertensive rat; *L*-NAME: N$^{\omega}$-nitro-*L*-arginine methyl ester; NAG: *N*-acetyl- β -D-glucosaminidase. $^{***}P < 0.001$: SHR+ ALA compared to age-matched SHR. $^{+++}P < 0.001$: SHR+ ALA+*L*-NAME compared to age-matched SHR+*L*-NAME. $^{\Delta\Delta\Delta}P < 0.001$: SHR+ALA compared to age-matched SHR+ALA+*L*-NAME. '–' indicates no analysis was performed because intervention had not commenced yet/just commenced at this time-point.

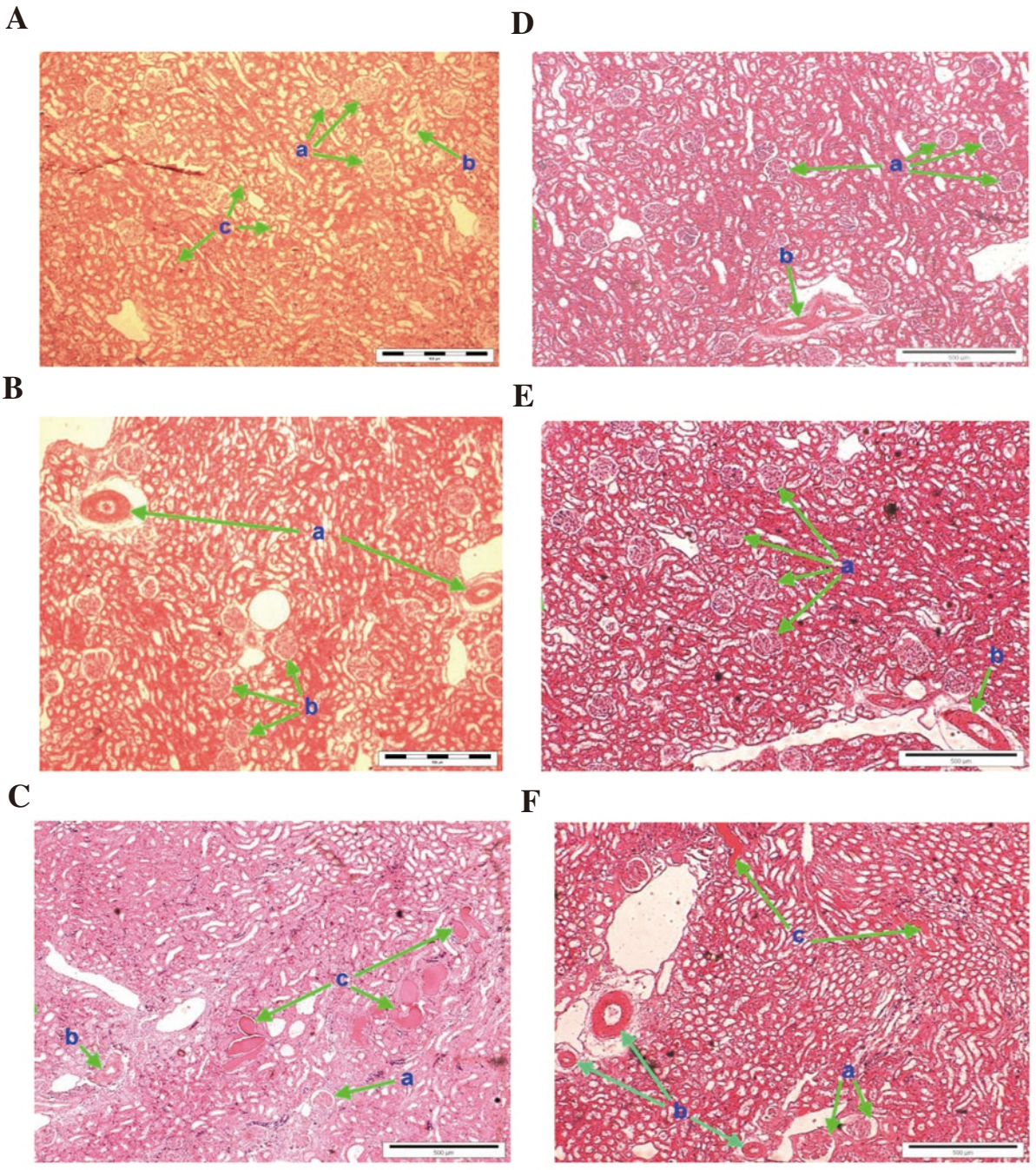

Figure 2. Kidney section of alpha-lipoic acid (ALA) supplemented and non-supplemented rat groups. (A) Spontaneously hypertensive rats (SHR) group at 16 weeks shows normal histological features (a-glomerulus, b-blood vessel, c-tubules), (B) SHR group at 28 weeks shows mild medial hypertrophy of blood vessel, (C) SHR+ N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME) at 28 weeks shows damaged glomerulus (a), blood vessel hypertrophy (b) and tubular vacuoles (c), (D) SHR+ALA group at 16 weeks shows normal histological features (a – glomerulus, b – blood vessel), (E) SHR+ALA group at 28 weeks shows normal histological features (a – glomerulus, b – blood vessel) (F) SHR+ALA+ *L*-NAME group at 28 weeks shows slightly damaged glomeruli (a), blood vessel hypertrophy (b) and tubular casts/vacuoles (c). (H&E staining, ×40 magnification, scale bar = 500 μm).

4. Discussion

The ALA dose used in this study is similar to what has been used by other researchers to produce effective hypotensive and antioxidant effects in hypertensive rats[40–42]. In this study, *L*-NAME inhibition in SHR which was commenced at the age of 16 weeks and continued for 12 weeks until 28 weeks, resulted in significant and extensive nephropathy at age of 28 weeks. *L*-NAME inhibition was not started at an earlier age as it was found that enhancing hypertension rapidly at a younger age affected the survival rate of the rats. The study time points of 4 weeks, 16 weeks and 28 weeks were selected so as to observe the changes from pre-hypertension to established hypertension and finally the period where hypertensive kidney damage was caused. The study time points of 16 and 28 weeks were also selected as our previous research showed that this age period had greater increase in blood pressure and antioxidant changes[43].

The results of this study revealed SHR+ALA rats were found to have significantly lower SBP than untreated SHR from week 8 onwards until the end of the study at week 28. The SBP of the SHR+ALA rats was in the normotensive range until week 14, after which it began to rise gradually until it reached 184 mm Hg at age of 28 weeks. This value, even though in the hypertensive range, was still about 14% lower than untreated SHR. SHR+ALA+*L*-NAME rats also had significantly lower SBP than SHR+*L*-NAME rats even though the values were still in the hypertensive range from week 16 until week 28. Overall, results indicated that ALA has hypotensive property whereby it was able to reduce SBP in SHR and SHR+*L*-NAME rats. This finding is supported by other studies which also found ALA prevented hypertension or reduced blood pressure in SHR and other hypertensive rat models[21,40,44,45].

In this study, ALA was only able to delay or partially prevent the development of hypertension in SHR and *L*-NAME treated SHR rats. This could be because the dosage used was not high enough to sustain continuous reduction of blood pressure. Dose-dependency for the beneficial effect of ALA in hypertension is supported by some studies[40,46]. Other factors including mode and duration of supplementation as well as age could also have affected the results. In addition, it is also possible that the hypotensive effect of ALA is limited to a certain extent of pathophysiological changes in the hypertensive state, beyond which it is ineffective.

Urine protein levels were reduced about 28% in SHR+ALA rats at 28 weeks when compared to age-matched untreated SHR rats. In 28 weeks old SHR+ALA +*L*-NAME rats, the proteinuria reduction was about 82% when compared to the age-matched non-supplemented rat group. These results indicated that ALA has antiproteinuric property in these hypertensive animal groups. This antiproteinuric property of ALA has also been found in other studies on hypertensive animal models[40,44]. ALA supplementation also improved the reduced creatinine clearance observed in non-supplemented hypertensive rats. Creatinine clearance at 28 weeks was found to be significantly higher in SHR+ALA and SHR+ALA+*L*-NAME rats when compared to age-matched SHR and SHR+*L*-NAME rats, respectively. Overall, results of this study on the urine protein and creatinine clearance showed that ALA supplementation provides renoprotection in terms of glomerular function. Results obtained also showed that ALA supplementation provided renoprotection in terms of tubular function as urinary NAG activity was significantly lower at 28 weeks in SHR+ALA and SHR+ALA+*L*-NAME rats. Reduced urinary NAG activity of ALA agrees with the findings of other studies[40,47].

Histopathological results showed that in the 28 weeks old SHR+ALA group, the vascular damage was completely prevented whereas the glomerular lesions were reduced in SHR+ALA+*L*-NAME rats. However, the vascular and tubulo-interstitial lesions in the SHR+ALA+*L*-NAME group were still present to the same extent. Other studies have also found ALA significantly reduced renal histopathological changes in SHR[21] and other experimental hypertensive models[44].

Results indicated that ALA only gave partial renoprotection as the renal damage was ameliorated but not totally prevented. This was more obvious in the *L*-NAME treated hypertensive rats. This could be because the dose used was not high enough and/or the duration of supplementation was not long enough. In addition to inadequate dose and duration, the renoprotective property of ALA is possibly affected by the hypertensive state of the animals based on the observation of this study where comparison between the hypertensive treatment groups (SHR+ALA and SHR+ALA+*L*-NAME) showed that the more hypertensive SHR+ALA+*L*-NAME rats had greater renal damage. This suggests that the renoprotective property of ALA is affected by the hypertensive state of the animal *i.e.* its renoprotective property that is dependent on its blood pressure lowering capability.

ALA supplementation reduced renal TBARS in 28 weeks old SHR+ALA and SHR+ALA+*L*-NAME rats when compared to the age-matched non-supplemented rat groups, respectively, suggesting that ALA is able to prevent lipid peroxidation in renal tissue of hypertensive rats. Moreover, the supplementation reduced renal PCO levels in hypertensive rats, which proves ALA was able to reduce oxidative damage to proteins. ALA possibly reduced protein oxidation by reducing lipid peroxidation whereby the byproducts such as malondialdehyde and hydroxynonenal are not available to cause protein oxidation[48].

ALA supplementation was also found to increase renal GSH and GSH/GSSG ratio while reducing GSSG levels in hypertensive rats treated with ALA. This finding is consistent with that of another study which showed ALA increased GSH and/or GSH/GSSG ratio in kidney of Ang II-induced hypertensive rats[44]. The results of the present study suggest that ALA increased GSH levels through its indirect antioxidant actions whereby it regenerated GSH by reducing oxidized GSH (*i.e.* GSSG) to GSH as well as by increasing intracellular GSH levels. ALA increases intracellular GSH levels by being able to continuously supply cysteine for its formation[49]. This action of ALA is important as the availability of cysteine inside a cell determines its rate of GSH synthesis[50].

ALA supplementation also affected the activity of the antioxidant enzymes in the kidney. Renal SOD activity was increased in treated rats. Decreased SOD levels have generally been observed in hypertension, possibly due to inactivation by ROS[51]. Based on this, it is likely that ALA was able to restore SOD activity through its direct action of scavenging ROS such as superoxide. Similar effect of ALA has been reported in kidney of salt-induced hypertensive rats[45].

Depressed renal CAT activity in SHR+ALA+*L*-NAME rats at 28 weeks was also improved. This finding is in agreement with another study on hypertensive streptozotocin-diabetic rats which showed ALA supplementation increased CAT activity in aorta[52]. The increased CAT activity observed in this study may be due to upregulation of CAT enzyme by the increased production of its substrate H_2O_2 by the action of improved SOD activity on ALA supplementation.

ALA also reduced the increased renal GPx activity in SHR+ ALA rats at 16 weeks and 28 weeks when compared to age-matched non-ALA supplemented SHR. This reduction could be due to decrease of H_2O_2 levels by ALA action, leading to lesser requirement of GPx to decompose H_2O_2. As such, this reduction is probably a normalization of antioxidant enzymes that were elevated earlier as a compensatory mechanism to overcome metabolic changes in hypertension. In 28 weeks old SHR+ALA+*L*-NAME rats, ALA was found to significantly increase GPx and GST activities when compared to age-matched non-ALA supplemented SHR+*L*-NAME rats,

demonstrating ALA supplementation ameliorates the hypertensive state, resulting in an increase in the depressed activities of GPx and GST. Another study has also shown ALA supplementation enhanced GPx activity in hypertensive rats[21]. Meanwhile, ALA supplementation was unable to improve significantly the depressed GR activity in SHR+ALA+*L*-NAME rats. This is probably due to the reduced levels of GSH in this group of animals as GSH depletion has been shown to cause a substantial decrease in GR activity[53]. In addition, increased free radicals have also been suggested to cause inhibition of GR activity[54]. Increase in renal GSH and GSH/GSSG ratio as well as normalization of antioxidant enzymes suggest that ALA is able to increase the renal antioxidant capacity.

Non-significant increase in renal NOx levels was found in 28 weeks old SHR+ALA and SHR+ALA+*L*-NAME rats when compared to the respective age-matched non-supplemented rats. Thus, although the dose of 100 mg/kg BW of ALA supplementation improves the antioxidant system, the dose may not be sufficient to significantly improve the renal NOx level.

In conclusion, ALA has hypotensive and renoprotective properties that are associated with its ability to reduce renal oxidative stress during the progression of hypertension and development of renal damage. However, these beneficial effects of ALA decline at later advanced stages of hypertension.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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