—Regular Article—

Alpha Lipoic Acid Treatment Improved Endothelium-dependent Relaxation in Diabetic Rat Aorta

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The aim of this study was to ascertain the effects of α-lipoic acid (ALA) treatment on relaxant responses of acetylcholine (ACh) and isoprenaline (ISO) in aortic rings precontracted with serotonin (5-HT, 10^{-6} M) obtained from streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in the rats by 50 mg/kg streptozotocin (STZ) *via* an intraperitoneal injection. Rat body and aorta weights were measured. The isometric tension to ACh (10^{-9}–3×10^{-6} M) and ISO (10^{-9}–10^{-4} M) of 5-HT-precontracted diabetic and non-diabetic rat (control), diabetic-ALA-treated, and ALA-treated aortas, in organ baths were recorded. Six weeks after STZ treatment blood glucose was elevated compared to control rats. In aortic rings from diabetic rats ACh and ISO-induced relaxations were impaired whereas endothelium-independent relaxation to sodium nitroprusside (SNP) was unaffected. ALA (100 mg/kg/day) treatment for 5 weeks enhanced ACh and ISO-induced relaxation in diabetic aortas. This recovering effect was *via* NO because prevented by incubating the vessels with N^G-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor). It may be assumed that ALA treatment *in vivo*, can protect against impaired vascular responsiveness in STZ-induced diabetic rats.

Key words——vascular smooth muscle; streptozotocin-induced diabetes; alpha lipoic acid; vasodilatation

INTRODUCTION

There is close relationship between chronic hyperglycaemia and the onset/progression of the vascular complications of diabetes.[1] In diabetes, the arterial wall often becomes diffusely damaged by a pathogenic process termed diabetic arteriopathy, which is mediated *via* pathways/mechanisms that modify its structure/function. These processes combine to cause a pathological state named 'arteriosclerosis' that damages large and medium-sized arteries, which display increased stiffness, hardness, loss of elasticity and diffuse wall thickening.[2] Several lines of evidence suggest that endothelial dysfunction could play a key role in the development of both macro-and microangiopathy in diabetes patients and in animal models of diabetes.[3,4] Conflicting results have been obtained from studies that investigated the endothelial regulation of vascular smooth muscle function in experimental diabetes: decreased, unchanged and increased responses to acetycholine (ACh) in aortic ring preparations from diabetic rats have been reported.[3,5–7] In addition, reduced endothelium-dependent relaxation to ACh was reported in rat mesenteric resistance arteries.[8]

It was found that hyperglycemia induces reactive oxygen species (ROS) production mediating endothelial dysfunction.[9] Alpha-lipoic acid (ALA) is a nutritional dithiol compound and an essential cofactor in oxidative metabolism in the mitocondria.[10] ALA acts with its reduced form, dihydrolipoate, as a potent antioxidant to scavenge free radicals, chelate metal ions, and recycle antioxidants.[11] Therapeutic approaches using ALA with definite effects in both the prevention and treatment of diabetes-induced oxidative stress have been reported in rat kidney[12] and enteric nerves of the rat ileum.[13]

To our knowledge, however, there is no information available of effects of ALA on impaired vascular reactivity of diabetic rats. The purpose of this study was to investigate the effects of ALA treatment on vascular reactivity in streptozotocin (STZ)-induced diabetic rats. Although there are several studies in rat aorta, currently, there is insufficient information. Moreover, hyperglycemia and insulin resistance were reported to have important roles in the pathogenesis of macrovascular complications. Therefore, aorta is used in this study.

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MATERIALS AND METHODS

Animals Male Wistar rats (6–8 weeks) used were divided randomly into four groups of 6 animals each. All experiments were carried out with the approval of Local Animal Use Ethical Committee of Selçuk University. Diabetes was induced by a single injection of STZ (50 mg/kg, i.p.) that was prepared in a 0.1 M citrate buffer solution, pH 4.5. Plasma glucose levels were determined from tail vein blood samples (Acura Ac 1018) two days after STZ administration. Rats with blood glucose concentration of 300 mg/dl or more were considered diabetic. The experimental groups included control, ALA-treated (100 mg/kg/d, i.p., for 5 weeks), diabetic, and diabetic-ALA-treated. Nondiabetic rats were injected with saline only. After diabetes was verified ALA (100 mg/kg/d) was given for 5 weeks in the diabetic-ALA-treated group. Body weights of rats were measured in all groups before and for 6 weeks after diabetes induction. All rats were kept under identical conditions for 6 weeks with free access to food and water before the experiments were conducted.

Isolation of Aortic Rings and Vascular Reactivity Studies The descending thoracic aorta was quickly isolated, cleaned and sectioned into 3-to 4-mm-long rings. The rings were then placed in organ baths containing Krebs-Henseleit solution (KHS, mM: NaCl 119, KCl 4.70, $MgSO_4$ 1.50, KH_2PO_4 1.20, $CaCl_2$ 2.50, $NaHCO_3$ 25, Glucose 11), which were thermoregulated at 37°C and aerated (95% O_2 and 5% CO_2). Changes in isometric tension were recorded by a force-displacement transducer (Grass FT04, Grass Instruments Co, W. Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D, Grass Instrument Co). The rings were equilibrated for 60 min under a resting tension of 1 g before experiments began. After equilibration, the rings were contracted with 5-HT (10^{-6} M) as described by Dursun *et al.*[14] At the peak of contraction, a cumulative concentration-response curve for ACh (10^{-9}–3×10^{-6} M) was obtained on each ring. The same procedure was determined with ISO (10^{-9}–10^{-4} M), in these rings at the beginning of the experiments endothelial cell integrity was assessed when a single addition of ACh (10^{-6} M) caused relaxation of aortic segments precontracted with 5-HT. Then the preparations were washed. Only one agent was tested in each preparation.

To determine an endothelium-independent vasodilatation SNP (10^{-10}–3×10^{-5} M) was added in aortic rings contracted with 5-HT.

Statistical Analyses Relaxation to ACh and ISO were expressed as a percentage decrease of the 5-HT-induced contraction. Data are presented as group means±S.E.M. Maximal responses and IC_{50} values for curves were compared by using Student's t test. Statistical significance was set at $p<0.05$.

Materials All chemicals used in experiments were obtained from Sigma Chemical (St. Louis, MO, USA).

RESULTS

General Characteristics of Animals The initial and final body weights and blood glucose levels for all animals of the four treatment groups are shown in Table 1. There was no difference in the starting weights in the animals from the four groups but the diabetic rats lost significantly high weight than the control rats. ALA treatment did not significantly affect the final weight of either control or diabetic rats. All of the STZ-treated rats exhibited significantly elevated blood glucose levels and ALA did not affect those levels in either control or diabetic rats.

Vascular Relaxation Cumulative addition of ACh (10^{-9}–3×10^{-6} M) and ISO (10^{-9}–10^{-4} M) to the isolated organ bath resulted in concentration-dependent relaxations of aortic rings precontracted with 5-HT (10^{-6} M) in all groups. The maximum relaxation to ACh and ISO was significantly reduced in diabetic rats in comparison to the control rats. Treatment with ALA (100 mg/kg/d) for 5 weeks in control rats did not affect responses to ACh or ISO. However, in aortic rings from diabetic rats treated with ALA, the maximum relaxation to both ACh and

Table 1. Body Weight and Blood Glucose Levels of Control of ALA-treated, Diabetic and Diabetic-ALA-treated Rats

	Body weight (g)		Blood glucose level (mg/dl)
	Initial	After 6 weeks	
Control	273.2±5.0	317.3±2.1	102.3±3.3
ALA	271.3±4.3	318.4±3.8	105.4±2.6
DM	275.2±6.2	152.2±5.9[a]	439.2±4.0[a]
DM+ALA	270.1±2.8	160.3±3.6[a]	389.1±2.1[a]

Values are mean±S.E.M. of six animals. [a] $p<0.05$ compared to control group.

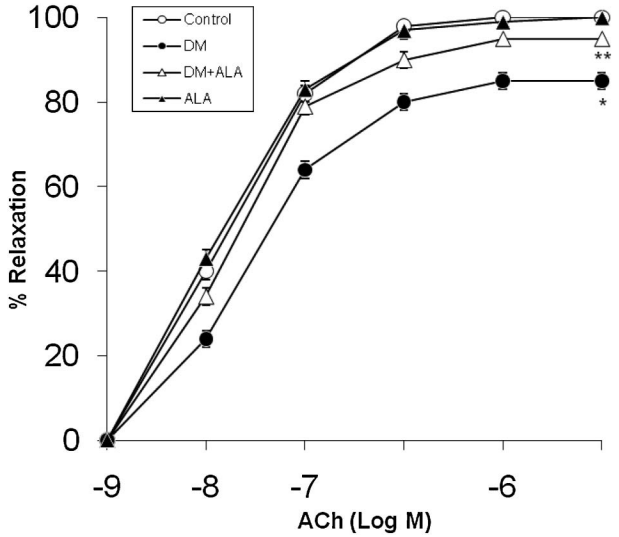

Fig. 1. Acetycholine (ACh) Concentration-response Curves in Serotonin (5-HT, 10^{-6} M)-precontracted Rat Aorting Rings

Diabetes decreased relaxation responses to ACh, and treatment with alpha lipoic acid (ALA) prevented this effect. $^{*}p<0.05$, compared to control (○); $^{**}p<0.05$, compared to diabetic (●). Each point is the mean±S.E.M. of six experiments.

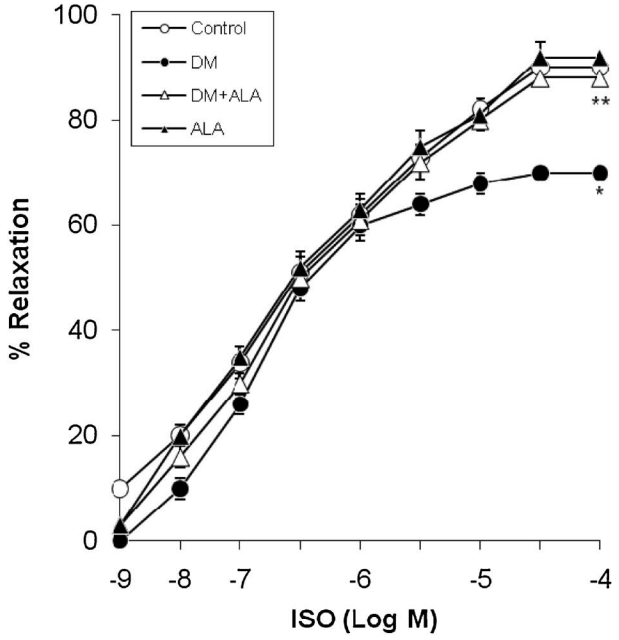

Fig. 2. Isoprenaline (ISO) Concentration-response Curves in Serotonin (5-HT, 10^{-6} M)-precontracted Rat Aorting Rings

Diabetes decreased relaxation responses to ISO, and treatment with alpha lipoic acid (ALA) prevented this effect. $^{*}p<0.05$, compared to control (○); $^{**}p<0.05$, compared to diabetic (●). Each point is the mean±S.E.M. of six experiments.

ISO was significantly increased in comparison to the response in aorta from untreated diabetic rats (Figs. 1 and 2, Table 2). This recovering effect was prevented

Table 2. pD_2 Values for ACh-and ISO-induced Relaxations of Aortic Rings from Rats

	ACh		ISO	
	pD_2	E_{max}	pD_2	E_{max}
Control	7.74±0.09	100±2.70	6.52±0.97	90±3.10
ALA	7.80±0.06	100±4.4	6.54±0.08	92±2.3
DM	7.40±0.08	85±3.8[a]	6.40±0.13	70±4.2[a]
DM+ALA	7.52±0.11	95±5.6[b]	6.49±0.98	88±3.5[b]

Each value is derived from six experiments. Data are means±S.E.M. [a] $p<0.05$ compared to control. [b] $p<0.05$ compared to diabetic.

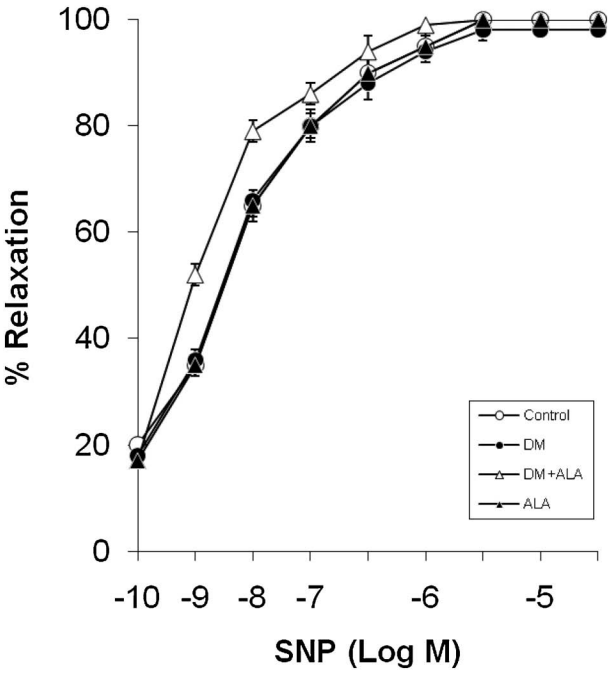

Fig. 3. Sodium Nitroprusside (SNP) Concentration-response Curves in Srotonin (5-HT, 10^{-6} M)-precontracted Rat Aorting Rings

Neither diabetes nor treatment with alpha lipoic acid (ALA) affected relaxation responses to SNP. Each point is the mean±S.E.M. of six experiments.

by L-NAME; a well-known NO synthesis inhibitor (Max relaxation to ISO was 69±3.2). In aortic rings from diabetic rats the maximum relaxation to SNP (10^{-10}–3×10^{-5} M); an endothelium-independent relaxing agent, was not different to that observed in control rats. ALA treatment did not affect responses to SNP in control rats but increased the sensitivity in diabetic rats (Fig. 3).

DISCUSSION

This study demonstrated that STZ-induced diabetes impaired endothelium-dependent relaxation to

agonists such as ACh in rat aorta. The present study was the first to report the protective effects of ALA treatment on vascular bed of STZ-induced diabetic rats. We have shown that 5 weeks ALA treatment did not affect blood glucose and body weight but reverted vascular reactivity alteration in diabetic rats. Diabetic rats gained no weight during the experiments, whereas nondiabetic control rats increased in weight. Less weight of diabetic rats cannot be explained by less food and fluid intake, but by impaired lipid and carbonhydrate metabolism.

In many vascular beds, ACh stimulates production and release of EDRFs, including NO, prostacyclin and EDHF, in vascular endothelial cells and thereby relaxes vascular smooth muscle in an endothelium-dependent manner. It is reported that the ACh-induced relaxation response was endothelium-dependent and NO-mediated.[15–18] The effects of diabetes on the vascular responsiveness of the rat aorta have been widely studied but there are conflicting results. Although some researchers asserted that the response to ACh increases in diabetes,[8,19] the results of the present study, in accordance with others,[20–22] revealed that diabetes decreased the responses to ACh in maximum relaxation but not the sensitivity. The discrepancies could be due to differences between diabetic models and/or the duration of the diabetes as mentioned by Pieper.[23] The results of this study revealed that the endothelium-dependent relaxant response was reduced in aortas from STZ-induced diabetic rats. Although some researchers asserted that the sensitivity to ACh decreased in diabetes,[24,25] the results of this research, in accordance with those of many previous ones,[21,25,26] revealed that diabetes decreased the responses to ACh in maximum relaxation but not the sensitivity.

Our study, similar to some other studies,[27–29] also showed that the diabetes reduced the maximum relaxation to ISO. In literature there are a number of contrary reports, showing increased relaxations and sensitivity of diabetic aorta to ISO[30] or no significant effect on ISO-induced relaxations.[31] Recently, it has also been reported that β-adrenoceptors induce vascular smooth muscle relaxation by acting through the NO-cGMP pathway and, when that is disrupted by endothelium removal or the presence of an NO synthase inhibitor, the cAMP pathway in smooth muscle is used.[32]

To the best of our knowledge, there is no previous data on the effects of ALA on ACh- and ISO-induced relaxations in rat aorta. It is reported that ALA is a potent antioxidant for the protection of the vascular wall against oxidative injury associated with diabetes mellitus.[33] In the present study, treatment with ALA in control rats did not affect responses to ACh and ISO. The reduction in ACh- and ISO-induced responses were recovered partially by ALA treatment in diabetic rat aorta. This recovering effect was prevented by L-NAME; a well-known NO synthesis inhibitor, It is reported that lipoic acid may modulate endogenous nitric oxide bioavailability.[34] Furthermore, Heinisch *et al.*[35] demonstrated that ALA improves vascular endothelial function in patients with type 2 diabetes. As we know, vascular deterioration is one of the complicating features of human and experimental diabetes and hyperglycaemia is the primary cause of diabetic micro and macrovascular complications.[36,37] Furthermore, diabetes is believed to cause endothelial dysfunction, abnormal vascular reactivity, and hypertension.[19] In the rat arteries, NO bioavailability was expressed as the increase in contractile responses to phenylephrine in the presence of L-NAME.[38] Singhania *et al.*[39] reported that in diabetes mellitus, the endothelial dysfunction is characterized by the impairment of vasorelaxation. Our results demonstrated that ALA has potential effect in preventing diabetes-induced endothelial dysfunction which is characterized by the impairment of ACh-activated vasorelaxation. Similar findings were reported by Wongeakin *et al.*[40] for another antioxidant curcumin and its analog; tetrahydrocurcumin on diabetic rat mesenteric artery.

We all know that SNP acts *via* direct stimulation of vascular smooth muscle cells independently of an intact endothelium. SNP and NO share a final common pathway to produce vasodilation. Some of the previous studies claimed that the NO donor SNP induced decreased endothelium-independent relaxation responses in diabetes.[41] However, many others have shown that diabetes had no effect on SNP responses.[22,42,43] In this study, the responses to SNP were not different between control and diabetic rat aortas. The ALA-treatment restored the endothelium-dependent response to ACh but did not affect the endothelium-independent response to SNP. Therefore, the potential mechanisms by which ALA restored the endothelial-dependent relaxation in the aortic rings of diabetic rats are probably related to its

antioxidant activity as reported by other investigators.[20] For example, ALA and its reduced form dihydrolipoic acid (DHLA), which are essential for reactions catalyzed by dehydrogenases in the mitochondria, react with superoxide and hydroxyl radicals, hypochlorous acid, peroxoyl radicals and singlet oxygen.[44] Furthermore, we observed that 5 weeks of treatment with ALA increased the sensitivity to the NO donor SNP in diabetic rats but not in the control group. A possible explanation for these findings is that chronic treatment with ALA additionally increases smooth muscle sensitivity to NO in diabetic rats. But we have no explanation why a similar effect was not observed in control rats.

In conclusion, the antioxidant agent ALA indicates that it has therapeutic and preventive potential for vascular complications of diabetes. Further investigation in this direction is warranted.

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