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COMPARISON OF THE EFFECTS OF AMINOGUANIDINE AND L-CARNITINE TREATMENTS ON PERIPHERAL NEURAL RESPONSES IN ALLOXAN-DIABETIC RATS

ALLOKSAN-DİYABETİK SIÇANLARDA AMİNOGUANİDİN VE L-KARNİTİN TEDAVİLERİNİN PERİFERAL NÖRAL CEVAPLARA ETKİLERİNİN KARŞILAŞTIRILMASI

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The effects of aminoguanidine (AG) and L-carnitine (LC) on nerve conduction velocity (NCV) and neural levels of malondialdehyde (MDA), a product of lipid peroxidation, were compared in alloxan-diabetic rats. AG and LC were given to diabetic rats starting from the 3rd week after the induction of diabetes and lasting for 4 weeks. NCV was measured in the caudal nerves once weekly during the treatments. Diabetes caused deficits in NCV ($p<0.05$ vs non-diabetic control rats). AG and LC improved NCV similarly ($p<0.05$ for either treatment vs untreated diabetic rats). Diabetes caused elevation in neural MDA levels ($p<0.05$ vs non-diabetic group) which was prevented by both AG and LC ($p<0.05$ vs untreated diabetic rats, respectively). Weight and the glucose levels were not influenced by the treatments. Our results suggest that improvements in NCV during 1 month of treatment with AG and LC are comparable in alloxan-diabetic rats. Our results also suggest that the beneficial effects of both AG and LC on diabetic neuropathy are not associated with the regulation of glycemia, but may be related with prevention of lipid peroxidation.

Aminoguanidin (AG) ve L-karnitin (LK)'in sinir iletim hızına (SİH) ve bir lipid peroksidasyon ürünü olan nöral malondialdehid (MDA) seviyelerine etkileri alloksan-diyabetik sıçanlarda karşılaştırıldı. Diyabetik hayvanlara, diyabet indüksiyonundan 3 hafta sonra başlanarak 4 hafta boyunca AG ve LK verildi. SİH, kaudal sinirden stimulasyon ve kayıt yapılmak suretiyle, tedaviler boyunca haftada bir kez ölçüldü. Diyabet SİH'da defisitlere neden oldu ($p<0.05$, sırasıyla diyabetik olmayan kontrollere göre). AG ve LK, SİH'nı benzer biçimde düzelttiler ($p<0.05$, her iki tedavi için tedavi edilmeyen diyabetiklere göre). Diyabet nöral MDA seviyelerinde artışa yol açtı ($p<0.05$, diyabetik olmayan kontrollere göre), ki bu etki hem AG hem de LK tedavisi ile düzeldi ($p<0.05$, her iki tedavi için tedavi edilmeyen diyabetiklere göre). Tedaviler ağırlık ve glisemi seviyelerinde değişiklik oluşturmadı. Bizim sonuçlarımıza göre, alloksan-diyabetik sıçanlarda bir ay süreyle AG ve LK uygulamarı, SİH'nı benzer şekilde düzeltmektedir. Bizim sonuçlarımız göstermektedir ki, AG ve LK'in diyabetik nöropatideki faydalı etkileri gliseminin düzenlenmesi ile ilişkili değil, ancak lipid peroksidasyonunun engellenmesi ile ilişkili olabilir.

Keywords: *Diabetic neuropathy; Nerve conduction velocity; Aminoguanition velocity; Aminoguanidine L-carnitine; Rat; Alloxan*

Anahtar kelimeler:*Diyabetik nöropati; sinir iletim hızı; aminoguanidin; L-karnitin; Sıçan; alloksan*

Introduction

Diabetic neuropathy is a progressive disorder that causes functional and structural alterations of peripheral, autonomic and central nervous system. Although several pathogenetic hypotheses have been put forward, the underlying cause of diabetic neuropathy still remains unclear. Excessive flux of glucose through polyolpathway (1,2), increased glycation of structural poteins (3,4), ischemic hypoxic insults (5,6) as well as decreased endoneural levels of acetyl-L-

carnitine (ALC) and L-carnitine (LC) (7,8) may be implicated in the causation of diabetic neuropathy. Recent evidence suggests that oxidative injury may be ultimate factor of aggression to the diabetic nerve (9-12). Several factors promote oxidative stress in diabetes, including nerve-ischemia-reperfusion (13, 14), increased free radical production caused by autooxidation reactions of sugars with proteins and unsaturated lipids (12), and impairment of tissue antioxidant protection systems (15,16). Three is increasing evidence that antioxidant treatment prevents nerve dysfunction in experimental diabetes (17-20).

Aminoguanidine (AG) inhibits the formation of advanced glycation end products (21-23), it may act as an aldose reductase inhibitor (24,25) and it has also been suggested to be an antioxidant and an inhibitor of nitric oxide synthase (26, 27). Experimental studies have demonstrated structural and functional improvements in peripheral nerve of AG-treated diabetic rats (27,28). ALC is an endogenous substance similar in structure to acetylcholine and is involved in uptake and oxidation of long-chain fatty acids in mitochondria (29). ALC enhances the activity of antioxidant factors such as reduced glutathione, and protects the cell against lipid peroxidation (free radical scavenging effect)(30). A beneficial effect of ALC or LC on nerve function has also been demonstrated in animal diabetes(8, 31-33). A recent study (34) has reported that ALC has a role in the treatment of symptomatic diabetic neuropathy. However, the use of LC therapy for human diabetic neuropathy is not approved yet.

Nerve conduction velocity (NCV) has been shown to be sensitive and reliable indicator of neuropathy in experimental studies (35). No study on the comparison of AG and LC has been reported previously. In this study, we compared the effects of two compounds on peripheral nerves using NCV to evaluate their possible future therapeutic use.We also determined neural levels of malondialdehyde (MDA), a product of lipid peroxidation, in order to explore possible mechanisms by which these two compounds exerted their effects.

Materials and Methods

Male Wistar rats (breeding colony of Medical Pharmacology Department, Gülhane School of Medicine, Ankara, Turkey), 8-10 weeks of age, weighing 150-200 g were randomised into control or diabetic rats. Diabetes was induced in rats (n=17) by single injection of alloxan (75 mg/kg body weight) via tail vein, after an overnight fast. Animals with plasma glucose levels, measured by Glucostix(Ames, UK), greater than 15 mmol/L at 4 days after alloxan injection were included in the diabetic group. Beginning at 3rd week after the induction, the diabetic rats received no treatment in the 1st group (DC, n=7), aminoguanidine bicarbonate (AG; 25 mg. kg body weight^{-1}.day s.c.; Sigma, St. Louis, MO, USA) in the 2nd group (D-AG, n=5) and L-carnitine hydrochloride (LC; 200 mg.kg body weight^{-1}.day i.p.; Carnitine; Sigma Tau, Rome, Italy) in the 3rd group (D-LC, n=5), for 4 weeks. As mentioned above age-matched control non-diabetic rats served as the 4th group (NC, n=6). All rats were maintained under standard housing conditions with normal rat chow and water available ad libitum during the study. Plasma glucose levels and body weights were monitored at weekly intervals throughout the treatment period.

Electrophysiological studies

After induction of anaesthesia by intramuscular injection of ketamine hydrochloride (Ketalar, 50 mg.kg $^{-}$body weight, Parke-Davis, Morris Plains, NJ, USA) nerve conduction velocity (NCV) was measured with a DISA 1500 EMG Equipment (DISA Electronic A/S, Skovlunde, Denmark). This equipment has a four channel display monitor (15H01 monitor) with large rectangular screen. The examination was performed in a warm room (22-24°C). The rat tail was immersed in a temperature controlled oilbath (37±5°C), rectal temperature was moni-tored by an electronic thermometer and body tem-perature was maintained at 37.5 C with a heating lamp.

For NCV measurement, stainless steel needle electrodes were used for stimulating and recording

(needle size:0.75x25 mm, needle size gauge: 22x 1, Dantec Electronic A/S, Skovlunde, Denmark). The stimulating and revording electrodes were placed subcutaneously proximal and distal area in the tail of rat, respectively (35). The electrical stimulation was obtained by a single square wave of 100 us with voltage sufficient to evoke a supramaximal response. The NCV was amplified using a band pass filter of 20-2000 Hz. The distance between the stimulating and revording electrodes, measured by callipers, was divided by the measured latencies to calculate the NCV values.

NCV was periodically performed once a week during 1 month of treatment period.

MDA Measurement

Blood samples (3-5 ml) were taken by intracardiac route from the animals at 4th week of therapy for biochemical analysis. Soon afterward, the animals were exsanguinated. The sciatic nerve trunk, between sciatic notch and its bifurcation at the knee, was carefully removed for MDA measurement. The nerve samples were frozen in liquid N_2 and stored at -70°C until subsequent analysis (for less than 4 weeks).

For MDA concentration measurement, the nerve samples were first homogenised in phosphate buffered saline (pH7.4) by a sonic dismembrator (Fischer Model 300, Artek Systems Corp., NY, USA) and the analysis was performed as described by Ohkawa et al. (36) by a spectrofluorometer (Perkin Elmer LS 50B, Buckinghamshire, UK). Neural tissue protein was determined as described by Lowry et al. (37).

Statistical Analysis

All data are presented as means±SEM. For statistical comparisons among the experimental groups, one way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to assign differences to individual between-groups comparisons when overall significance ($p<0.05$) was attained. Paired or unpaired Student's t test was used to compare the values from two groups when necessary and p level< 0.05 was considered significant.

Results and Discussion

Animals had similar body weights and plasma glucose levels before the induction of diabetes ($p>0.05$, DC vs. NC groups, t test, respectively). After the induction, the glycemia level in diabetic group (19.16±0.84 mmol/dl) was significantly greater than that in NC group (6.69±0.43 mmol/dl) ($p<0.01$, t test). The body weights of these diabetic animals did not increase during this early post-induction period (1 week after injection of alloxan) ($p>0.05$ before vs. after induction, t test) whereas, NC group showed significant increase in body weight during the same period ($p<0.05$ before vs. after induction, t test)(Table 1).

Table 1.Body weights and plasma glucose levels in control and diabetic rats before and after the induction of diabetes

	Control (n=6)		Diabetic (n=18)	
	Pre-induction	Post-induction	Pre-induction	Post-induction
Weight(g)	103.21±4.96	126.83±6.14[a]	115.34±7.87	115.27±5.81
Plasma glucose (mmol/lt)	6.87±0.76	6.69±0.43	6.45±0.49	19.16±0.84 [b]

Data (mean±SEM) were analysed by the Students' t test;
a : $p<0.05$ vs. pre-induction values of control group;
b : $p<0.05$ vs. pre-induction values of diabetic group.

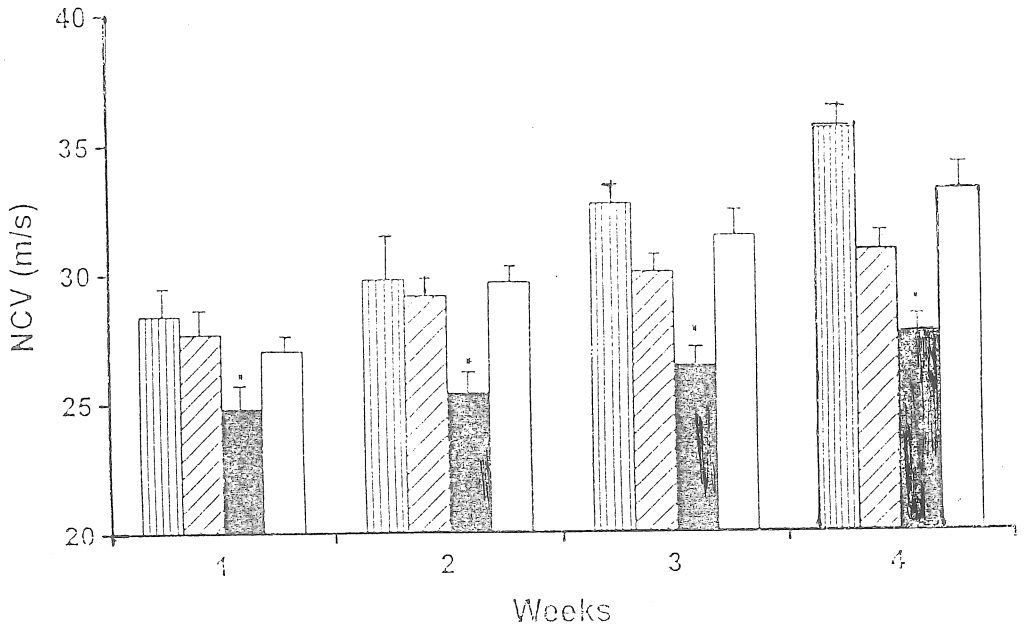

Fig.1. NCV values in alloxan-diabetic-control (■), -aminoguanidine (▣), -L-carnitine(▨) and normal control (□) groups of rats. Data (mean±SEM) were analysed by ANOVA and between-group differences for each variable were tested using Duncan's multiple range test; *p<0.05 vs normal-control, diabetic-aminoguanidine treated and diabetic-L-carnitine treated groups.

Untreated diabetic rats showed a significant reduction in NCV ($p<0.05$ vs. NC group at any week during 1 month of follow-up). In D-AG and D-LC groups, significant improvement in NCV was noted during the treatments ($p<0.05$ vs DC groups, respectively). This improvement was constant in time and comparable from 3 to 7 weeks of diabetes. Additionally, the NCV's in treatment groups were similar with those in NC group during the observation period. NCV in NC group increased progressively during the whole observation period whereas NCV in DC group also tended to increase spontaneously (Fig.1).

During the treatment period, diabetic rats showed elevated blood glucose levels that were unaffected by AG or LC (Fig. 2). Weight also was not influenced by the treatments (Fig.3).

The present work indicates that treatment with AG and LC can improve peripheral neuropathy in experimental diabetes. These beneficial effects of both treatments occurred in the absence of any changes in the severity of diabetes, as indicated by body weight loss and glycemia levels. We have first shown that LC will improve abnormalities of peripheral nerve in alloxan-diabetic rat.

In our study, we measured peripheral NCV in the caudal nerve, which contains motor and sensorial fibers. Our results showed that AG and LC have beneficial effects on peripheral NCV. A previous study, performed by using the same technique, has shown the beneficial effect of ALC (32). A beneficial effect of ALC on nerve function has also been previously demonstrated in animal models of diabetes (8,31-33). However, we used LC

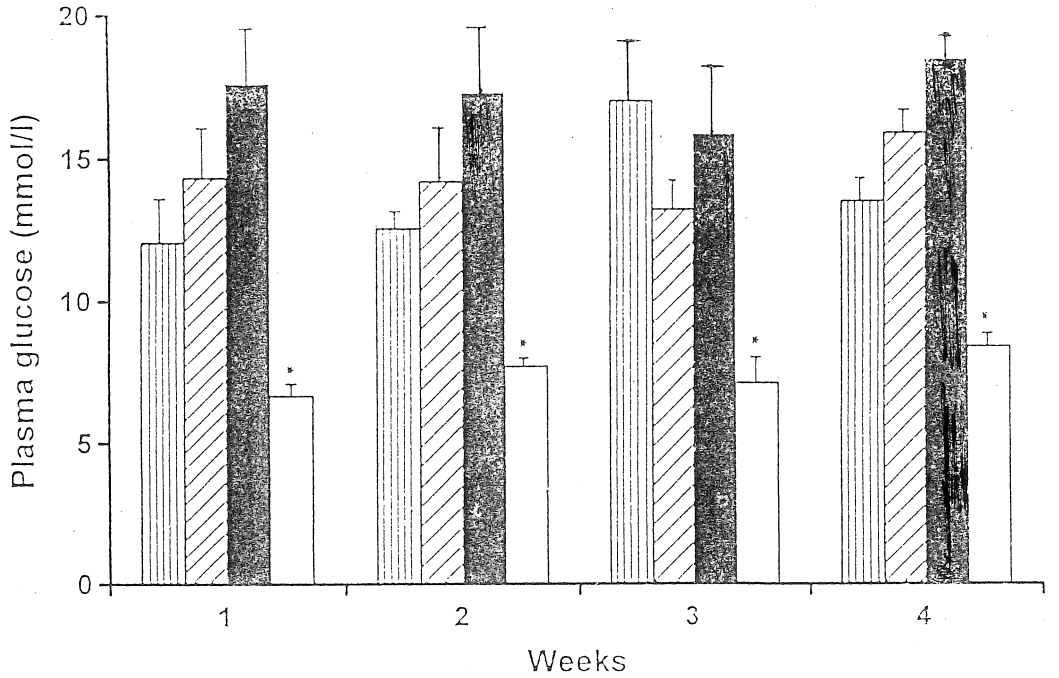

Fig.2. Plasma glucose levels in alloxan-diabetic-control (■), -aminoguanidine (▥), -L-carnitine (▨) and normal control (□) groups of rats. Data (mean±SEM) were analysed by ANOVA and between-group differences for each variable were tested using Duncan's multiple range test; *$p<0.05$ vs diabetic-control, diabetic-aminoguanidine treated and diabetic-L-carnitine treated groups.

instead of ALC and found similar beneficial effects.

Previous experimental studies with AG (27, 28, 38) were performed by using either caudal, tibial or schiatic nerves and these studies demonstrated the beneficial effects of this compound on NCV in experimental diabetes. The bene-ficial effects of AG may be derived from inhibiting the formation of advanced glycation end products (21-23), aldose reductase (24,25), diamine oxidase (39) and nitric oxide synthase (26,27). It has also been reported that AG reacts with the reactive aldehyde products of lipid peroxidation and prevents apolipoprotein B lysine modification, resulting in inhibition of macrophage uptake of oxidatively damaged low density lipoprotein (40).

Neural MDA levels (nmol/100 mg protein) in DC group (40.98±2.03) were significantly higher than those in NC group (18.55±1.25). When compared to levels in DC group, both AG and LC depressed MDA levels significantly (24.50±1.61, 25.72±1.72; $p<0.05$ vs. DC group, respectively).

The present work also suggests that both of the treatments may decrease oxidative stress as assessed by neural thiobarbituric acid reactive substances (TBARS), as MDA, after four weeks in alloxan-diabetic rats. Although MDA may give conflicting results (16), it increases in the early period of STZ-induced diabetes (16), and it has been reported to decrease by antioxidant agents such as butylated hydroxytoluene (41).

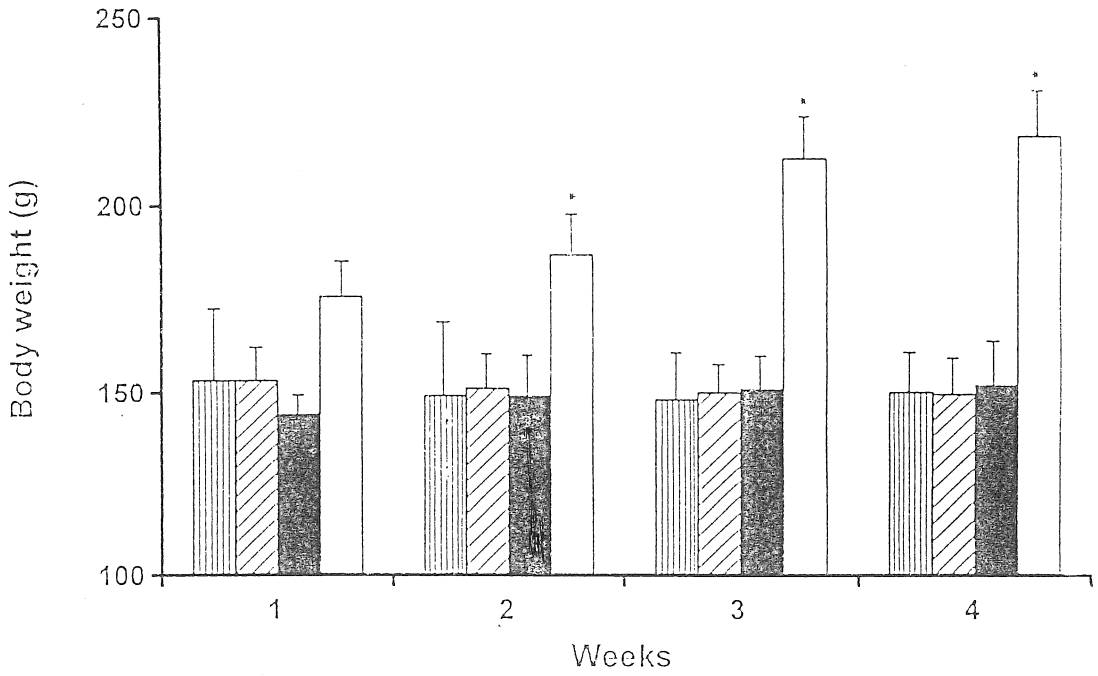

Fig.3. Body weights in alloxan-diabetic-control (■), -aminoguanidine (▥), -L-carnitine (▨) and normal control (□) groups of rats. Data (mean±SEM) were analysed by ANOVA and between-group differences for each variable were tested using Duncan's multiple range test; *p<0.05 vs diabetic-control, diabetic-aminoguanidine treated and diabetic-L-carnitine treated groups.

Recently, it has been suggested that diabetic complications may be partly attributed to oxidative stress (9-12). Raised levels of lipid peroxidation products as a result of oxidative stress have been reported both in human (42) and STZ-diabetic rats (16,43) and this is the first report that neural levels of MDA rise in alloxan-induced diabetic rats. Although the exact mechanisms of alloxan and STZ -induced diabetes are not well defined, and in fact may be different, the present study indicates that neuropathic changes emerging after induction with alloxan may share common characteristics with STZ - induced diabetes such as raised levels of lipid peroxidation products (16).

In this study, NCV increased with age in control and diabetic rats. This improvement with age may be due to maturity (44,45), since the animals were only 8-10 weeks old at the begininig of our study.

The results of the present study show that both AG and LC treatments produced a marked impovement of the periheral nerve function in alloxan-diabetic rats; moreover, the finding that the neural levels of MDA was also reduced led us to suggest that the beneficial effects of the two compounds might be related to

reduced oxidative stress in the nerve.

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