RESEARCH ARTICLE

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Effects of Fisetin and Alpha Lipoic Acid on Diabetic Neuropathic Pain*: In Vivo* Behavioral Study in Mice Model *

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Objective: To determine the effects of fisetin and alpha lipoic acid (ALA) on diabetic neuropathic pain development and their acute and chronic effects on established neuropathic pain in vivo, in an experimental mice model.

Materials and Methods: Type I diabetes mellitus was induced by streptozotocin (STZ, 150 mg/kg, i.p.) in adult male BALB/C mice. After confirmation of diabetes, animals were divided into two main groups: acute and chronic. The chronic group had three subgroups; control (no treatment), fisetin (10 mg/kg, i.p.) and ALA (10 mg/kg) groups (n=9 for each). Fisetin and ALA treatment was started at 3th week following STZ injection and applied weekly for the next 5 weeks. Pain threshold, as measured by paw withdrawal latency in response to heat stimulus, was measured by plantar analgesiometer at baseline and just before the consecutive injection time points. The acute group had further subgroups including control, fisetin (3 and 10 mg/kg), ALA (50 and 100 mg/kg) and combination group (Fisetin 5 mg/kg and ALA 10 mg/kg). In acute tests pain threshold measurements were performed at 10, 30, 60 and 90 minutes.

Results: Chronic treatment with ALA and fisetin prevented neuropathic hyperalgesia development. Acute application of ALA provided dose and time dependent analgesic effect. Acute fisetin application did not provide analgesia but in combination it contributed analgesic actions of ALA.

Conclusion: Both chronic ALA and fisetin treatment effectively prevented neuropathic pain development. Acute test on established neuropathic pain revealed that ALA was more effective than fisetin in ameloriating hypoalgesia in this study.

Key words: *Pain, diabetic neuropathy, fisetin, alpha lipoic acid, oxidative stress*

Fisetin ve Alfa Lipoik Asidin Diyabetik Nöropatik Ağrı Üzerine Etkileri: Fare Modelinde In Vivo Davranışsal Çalışma

Amaç: Fisetin ve alfa lipoik asidin (ALA) deneysel fare modelinde in vivo oluşturulmuş diyabetik nöropatik ağrı gelişimi üzerindeki akut ve kronik etkilerini belirlemek.

Gereç ve Yöntem: Yetişkin erkek BALB / C farelerinde streptozotosin (STZ, 150 mg/kg, i.p.) ile Tip I diabetes mellitus oluşturuldu. Diyabet oluşumundan sonra hayvanlar akut ve kronik olarak iki ana gruba ayrıldı. Kronik grup, kontrol, fisetin (10 mg/kg, n=9) ve ALA (10 mg/kg, n=9) olmak üzere 3 subgruba ayrıldı. Kronik gruplarda Fisetin ve ALA tedavisine STZ enjeksiyonunu izleyen 3. haftada başlandı ve sonraki 5 hafta boyunca haftalık olarak uygulandı. Plantar Analjezimetre kullanılarak termal uyarıya bağlı pençe çekme süreleri başlangıçta ve ardışık enjeksiyon zaman noktalarından hemen önce ölçüldü. Akut grup kontrol, fisetin 3 mg/kg, fisetin 10 mg / kg, ALA 50 mg/kg, ALA 100 mg/kg ve kombinasyon grubu (Fisetin 5 mg/kg+ALA 10 mg/kg) olmak üzere 5 alt grupa ayrıldı. Akut testlerde ağrı eşiği ölçümleri 8. haftanın sonunda 10, 30, 60 ve 90. dakikalarda yapıldı.

Bulgular: ALA ve fisetin ile yapılan kronik tedavi nöropatik hiperaljezi gelişimini önledi. ALA'nın akut uygulaması doz ve zamana bağlı analjezik etki sağlamıştır. Akut fisetin uygulaması analjezi sağlamadı, ancak kombinasyon halinde ALA'nın analjezik etkilerine katkıda bulundu.

Sonuç: ALA ve fisetinin nöropati yerleşmeden olan kronik kullanımı, nöropatik ağrı gelişimini etkili bir şekilde önlemiştir. Yerleşik nöropatik ağrı üzerinde yapılan akut testlerde ise hipoaljezide ALA'nın fisetin'den daha etkili olduğu ortaya konulmuştur. Nöropati yerleşmeden kullanılacak antioksidatif tedavi diyabetik nöropatide etiyopatogenetik tedavi için ümit verici olabilir.

Anahtar Kelimeler: *Ağrı, diyabetik nöropati, fisetin, alfa lipoik asit, oksidatif stres*

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Introduction

Diabetic neuropathy (DNP) is a common and important complication of diabetes mellitus which deteriorates effects on quality of life of diabetic patients. Its prevalence varies between 10 to 90% depending on whether the study is population or hospital-based, as well as on the differences in the methods that are used for establishing neuropathy (1).

The pathogenesis of DNP has not been fully understood and there is still no effective, ideal treatment. The mechanisms underlying the development and

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maintenance of DNP involve multiple biochemical and structural alterations in the peripheral and the central nervous systems (2, 3). Currently, principally symptomatic treatment is given for diabetic neuropathy. The drugs used for this purpose alleviates pain, whereas they have no activity on the disease course, which should be more advantageous treatment approach. It is hopeful that treatment modalities directly targeting specific mechanisms involved in pathogenesis of DNP have a higher potential for the treatment of neuropathy.

Although there are species difference and limitations of translational capacities, experimental studies on animal models of DNP are promising and they have actually contributed to the current state of success in this field. The use of rodents as a painful neuropathy model allows evaluation of both the electrical and neurochemical activities of the nervous system, as well as behavioral responses to sensory stimulants (4-6). Among the type 1 and type 2 rat models the pathophysiological features and related complications, Zucker Diabetic Fatty (ZDF) rats, Diabetes-Prone BB (BBDP), diabetes mellitus **rat** model (***LEW.1AR1***/Ztm-***iddm***) rats, Goto-Kakizaki rats, and chemically induced models are widely used (7). Streptozotocine (STZ)-induced pancreas damage is the most commonly used model of type 1 diabetes and also utilized in the current study.

Oxidative stress plays a central role in the pathogenesis of diabetic complications. Caused by free radicals in diabetic neuropathy, oxidative stress leads to endoneural hypoxia and nerve dysfunction. Another mechanism that is held responsible in the pathogenesis is protein glycosylation, and increased protein glycosylation in diabetes is known to be among the primary causes of diabetic complications (8).

Alpha lipoic acid (ALA) is a potent radical scavenger and a part of the endogenous antioxidative protection system. Studies have shown that ALA increased neural blood flow and improved decreased levels of glutathione (GSH), a major marker of oxidative stress (9). Fisetin, a flavonoid derivative, protects nerve cell cultures from toxic stimuli such as ischemia and oxidative stress. In addition to its direct antioxidant capacity, fisetin also increases the levels of GSH, which is the major intracellular antioxidant (10).

There are studies involving investigation of antinociceptive effects of antioxidants including fisetin against diabetic neuropathic pain in animal models (11-13). But information whether combination of fisetin with ALA would provide better result is lacking. The aim of this study was to investigate effects of fisetin and ALA on neuropathic pain in diabetic mice.

Materials and Methods

All experiments were performed in accordance with the guidelines for animal experiments in Karadeniz Technical University (OMU-HADYEK, Decision number: 2011/56, Date: 31.10.2011). Fortyeight male BALB/C mice aged 8 weeks with a mean weight 25 g (25±5) were maintained in an animal room with controlled temperature (22–25°C) and humidity (45%–65%) under a 12-h light /dark cycle (light on 06:00 to 18:00). Mice had free access to standard food and water.

After 7 days of adaptation to pain measurement setting, diabetes was induced by intraperitoneal administration of STZ (150 mg/kg) (streptozotocine, Sigma, Deisenhofen, Germany) dissolved in 0.1 mol/L citrate buffer (pH 4.5). One week after STZ injection, blood samples were collected from tail vein after 8-10 hours fasting and all animals with plasma glucose level ≥ 250 mg/dL were considered as diabetic and included in the study. Plasma glucose levels were determined with a commercially available kit Medisense Optium Xceed (Abbott, USA).

Grouping and Procedure: Type 1 diabetes mellitus was chemically-induced by single injection of STZ (150 mg/kg, i.p.) in adult male BALB/C mice. After confirmation of diabetes (>250 mg/dL); animals were divided into two main groups: acute and chronic. The chronic group had three subgroups; control (vehicle treatment), fisetin (10 mg/kg, i.p.) and ALA (10 mg/kg) groups (n=9 for each). Fisetin and ALA treatment was started at 3th week following STZ injection and applied weekly for the next 5 weeks. Pain threshold, as measured by paw withdrawal latency in response to heat stimulus, was measured by plantar analgesiometer at baseline and just before the consecutive injection time points. The acute group had further subgroups including control, fisetin 3 mg/kg, fisetin 10 mg/kg, ALA 50 mg/kg, ALA 100 mg/kg and combination group (Fisetin 5 mg/kg + ALA 10 mg/kg). Following induction of diabetes and neuropathy (at week 8 of the study) in the five treatment groups except the control group, the drug at the specified doses was administered intraperitoneally after pain threshold measurements. Pain threshold measurements were repeated 10, 30, 60 and 90 minutes after drug administration. For the control group, DMSO corresponding to the fisetin volume was injected at week 8, and final pain threshold measurement was performed.

Following adaptation to pain measurement system in the laboratory, group's baseline body weights, baseline pain thresholds, weekly live weight measurements and fasting blood glucose measurements were repeated.

Fisetin (Sigma Chemical Co., Germany) was prepared dissolving in pure olive oil that included 2% DMSO in solution at 37ºC until dissolution. It was administered intraperitoneally at a volume of 0.4 mL. Alpha lipoic acid (Sigma Chemical Co., Germany) solution was initially prepared in ethanol, with the remaining consisting normal saline, with an injection volume of 0.4 mL.

In the second set of experiments fisetin and ALA was injected weekly, first injection being performed on the day of STZ injection and pain threshold

measurements were performed 24 hour after weekly injections of test agents. The effects of ALA and fisetin was tested for 8 week, the duration is well-established to be hyperaljezia period of diabetes-induced neuropathy.

Thermal Plantar Analgesiameter Test: Thermal paw withdrawal test is an indirect indicator of acute thermal hyperalgesia. As part of this study, paw withdrawal times depending on thermal stimuli (thermal nociceptive threshold) was determined using a plantar analgesiameter with a size that is fit for mice (MAY PWAM 0903 Plantar Test, Ankara, Turkey). In mice, the time to paw withdrawal response to heat stimuli from an infra red light source focused on the hind paw. For this purpose, the mice were placed in Plexiglas boxes (30x15x15 cm). Radian light source that can be controlled and moveable underneath the glass surface was focused on the midpoint of the rear paws of the mice, and the time the mice withdrew their paws as a response to the heat induced by light was measured. The temperature was about 53ºC. With an automated timer system, the time was measured by spontaneous interruption of light at the exact time when the mice withdrew their paws, with a 0.1 sec sensitivity. The intensity with which normal mice (euglycemic control) would withdraw their paws after approximately 10 seconds of radian heat exposure was established by performing pre-testing with this assembly and the cut-off time was set to 10 seconds to prevent tissue damage. Tests were performed between 9.00 and 12.00 a.m. and care was taken to ensure silence during the performance of the tests. Experimental animals were transferred to the experiment room at least 15 minutes before the measurement. The experiments were performed in a room that was different from the one that the animal colonies were held. Pain threshold measurements were repeated eight weeks after baseline pain threshold measurements.

Statistical Method: SPSS 15 for Windows software package was used for statistical evaluation. The results were presented as mean ± standard deviation. Data are normalized to their respective baseline values. Inter-group comparisons pre- and post-treatment were performed using one-way variance analysis followed by Tukey's b test, while the differences between different time points were evaluated using the non-parametric Wilcoxon test. *p* values < 0.05 were considered statistically significant in all analyses.

Results

In all 5 groups (except control) diabetes was induced by STZ injection. Fasting blood glucose levels measured from the tail vein after 8 weeks were > 250 mg /dl, which were found to be statistically significantly higher compared to baseline glucose levels measured before STZ injection (P<0.05, Table 1)

In the acute treatment groups, the mean latency of normalized pain threshold was siginificantly increased from 1.00±0 sec (n= 40) at baseline to 1.30±0.14 sec (n= 34, P<0.05) at 8 weeks respectively (This mean was calculated by pooling data from all groups for acute protocols since at 8th week none of these groups had received any treatment). In chronic treatment groups; weekly administration of fisetin prevented hypoalgesia development in diabetic mice. The mean latency of normalized pain threshold was 1.00±0 sec (n= 9) at baseline and 0.92±0.18 sec (n= 6), 0.98±0.18 sec (n= 6), 1.01±0.21 sec (n= 6), 0.95±0.20 sec (n= 6), 1.01±0.24 sec (n= 6) and 1.32±0.34 sec (n= 5) at 3, 4, 5, 6, 7 and 8 weeks of chronic ALA (10 mg/kg/week) treatment, repectively (Figure 1A, please note that some animals are lost during the chronic period and number of animals are indicated by respective "n" numbers). The mean latency of normalized pain threshold was 1.00±0 sec (n= 9) at baseline and 1.22±0.14 sec (n= 5), 1.00±0.13 sec (n= 5), 1.01±0.19 sec (n= 5), 1.12±0.18 sec (n= 5), 1.25±0.27 sec (n= 5) and 1.12±0.32 sec (n= 5) at 3, 4, 5, 6, 7 and 8 weeks of chronic fisetin (10 mg/kg/week) treatment, respectively (Figure 1B, please note that some animals are lost during the chronic period and number of animals are indicated by respective "n" numbers).

In the second sets of experiments, acute effects of fisetin and ALA was tested on pain thresholds after 8 weeks of diabetes induction. The mean latency of normalized pain threshold was 1.00±0 sec (n= 8) at baseline and 0.99±0.08 sec (n= 8), 0.86±0.12 sec (n= 8), 0.91±0.20 sec (n= 8) and 0.91±0.31 sec (n= 8) after 10, 30, 60, and 90 minutes of vehicle administration, respectively (Figure 2A).

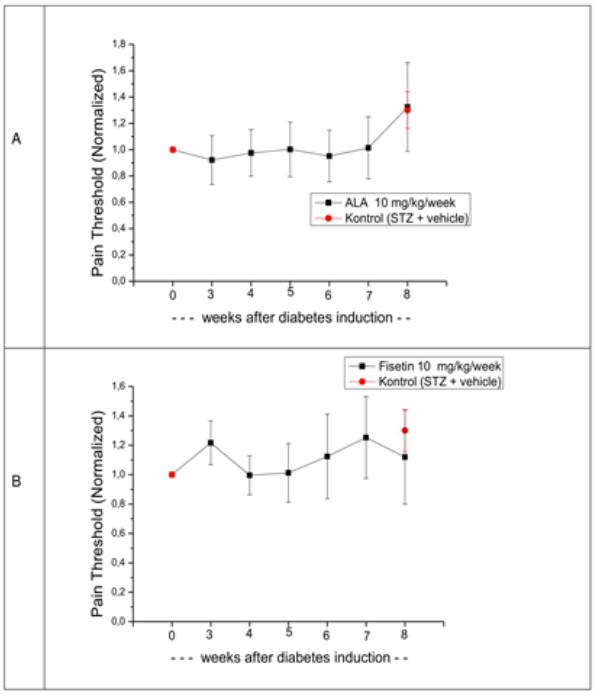

Figure 1. Effects of chronic administration of fisetin and alpha lipoic acid on diabetic neuropathic pain development profile.

Injection of 3 mg/kg or/ 10 mg/kg fisetin did not cause any significant change in pain threshold of diabetic mice. The mean latency of normalized pain threshold was 1.00±0 sec (n= 8) at baseline and 1.00±0.18 sec (n= 8) / 0.95±0.24 sec (n= 7), 0.95±0.22 sec (n= 8) / 1.07±0.15 sec (n= 7), 1.03±0.23 sec (n= 8) /0.90±0.13 sec (n= 7) and 0.98±0.26 sec (n= 8) / 0.93±0.18 sec (n= 7) after 10, 30, 60, and 90 minutes of vehicle administration, respectively (Figure 2C&D).

Injection of 50 mg/kg or 100 mg/kg ALA caused decrease in pain threshold of diabetic mice. The mean latency of normalized pain threshold was 1.00 ± 0 sec (n= 8) at baseline and 0.81±0.06 sec (n= 6, P<0.03)/ 0.79±0.19 sec (n= 6, P<0.01), 0.88±0.17 sec (n= 7, P<0.02) / 0.76±0.11 sec (n= 6, P<0.01), 0.79±0.18 sec (n= 6, P<0.02) / 0.70±0.05 sec (n= 6, P<0.001) and 0.86±0.13 sec (n= 6) / 0.75±0.06 sec (n= 6) after 10, 30, 60, and 90 minutes of 50 mg/kg /100 mg/kg ALA administration, respectively (Figure 2D&E). The effects of 100 mg/kg ALA was also tested 4 hour after injection and its significant analgesic effect was found to be persistent (0.68±0.16 sec n= 6, P<0.0001).

The mean latency of normalized pain threshold was 1.00±0 sec (n= 8) at baseline and 0.82±0.12 sec (n= 7, P<0.02), 0.77±0.09 sec (n= 7), 0.78±0.13 sec (n= 7, P<0.02) and 0.88±0.23 sec (n= 7) after 10, 30, 60, and 90 minutes of combination of fisetin (5 mg/kg) plus ALA (10 mg/kg) administration, respectively (Figure 2B).

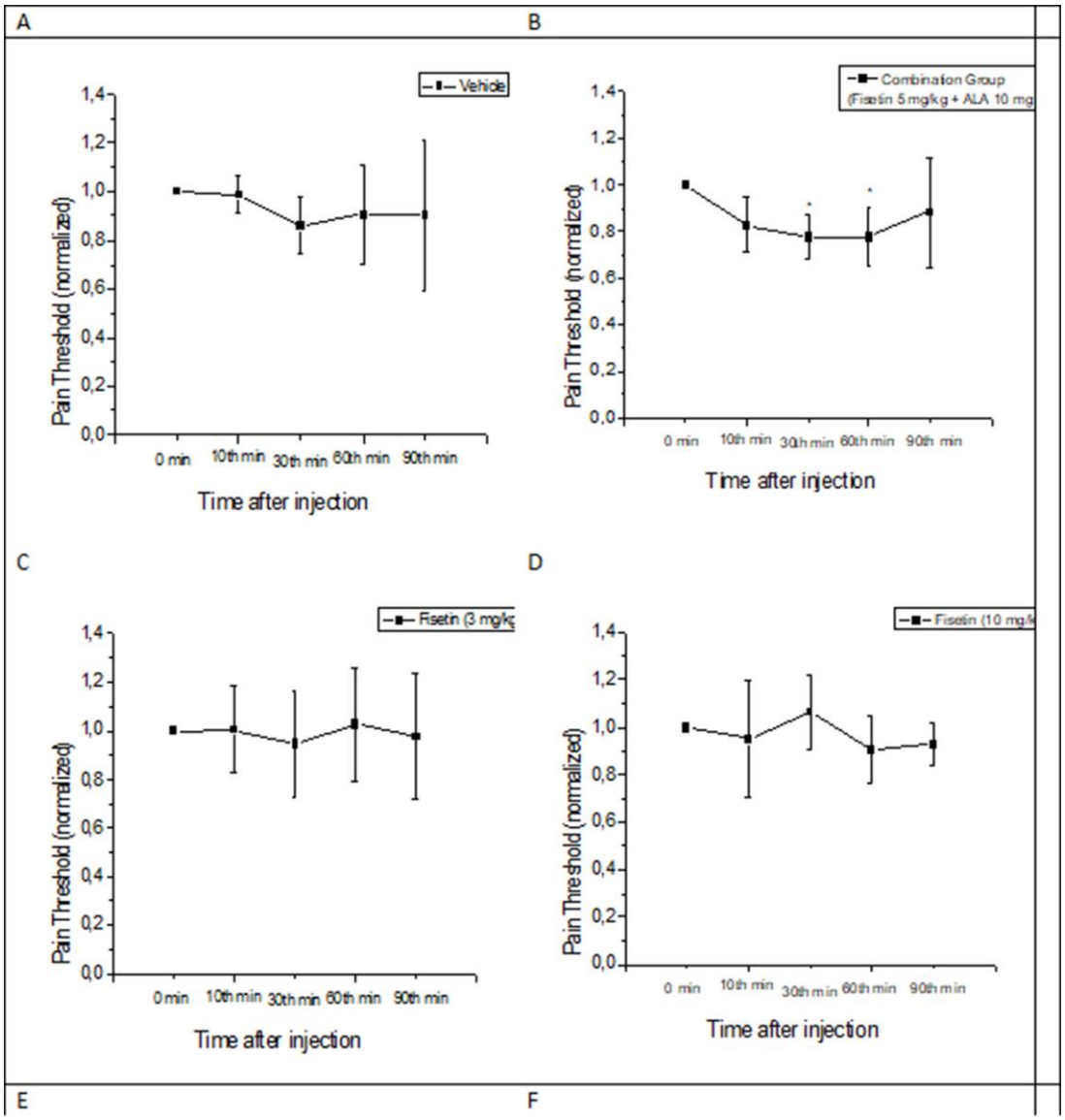

Figure 2. Effects of acute administration of fisetin and alpha lipoic acid on diabetic neuropathic pain threshold values

Table 1. Blood glucose values at baseline and 8-weeks after diabetes induction

	Mean Fasting Blood Glucose* (mg/dL)	n	SD	P
Acute groups				
Control	118	8	26.7	
Fisetin 3 mg/kg				
Prediabetes	130.3	8	33.7	
Postdiabetes	263.2	8	110.1	0.013
Fisetin 10 mg/kg				
Prediabetes	149.2	7	18.5	
Postdiabetes	327.8	7	114.3	0.005
ALA 50 mg/kg				
Prediabetes	168.3	6	39.2	
Postdiabetes	267.5	6	146.2	0.004
ALA 100 mg/kg				
Prediabetes	145.3	6	20.3	
Postdiabetes	336	6	94.7	0.004
Combination group				
Prediabetes	128.8	7	18.8	
Postdiabetes	293.2	7	69.1	0.001
Chronic groups				
Fisetin 10 mg/kg				
Prediabetes	136.2	9	32.5	
Postdiabetes	289.7	5	58.9	0.03
ALA 10 mg/kg				
Prediabetes	127.8	9	32.5	
Postdiabetes	319.4	5	66.3	0.002

* Blood samples were collected from tail vein after 8-10 hours fasting

Discussion

The results of this experimental study documents that fisetin and ALA, alone and in combination provides protective and analgesic effect against diabetes associated neuropathic pain. The effects of fisetin in this model has novel aspects as no chronic effect of this agent has been examined in the literature. ALA results are consistent with those previously reported (12).

In the present study we tested acute effects of fisetin and ALA, alone and in combination. For the chronic group, lower doses of the agents are used.

DNP is an important complication which has a high morbidity and mortality and deteriorates the quality of life of diabetic patients. Sensorial motor polyneuropathy is the most common type, also impairing quality of life especially due to diabetic foot ulcers. The use of rodents as a painful neuropathy model allows evaluation of both the electrical and neurochemical activities of the nervous system, as well as behavioral responses to sensory stimulants. The time the animal withdraws extremities upon exposing the tail or paw to heat is used as the method to determine hyperalgesia and hypoalgesia. Pain threshold responses contribute to obtaining indirect information on diabetic neuropathic pain. Hyperalgesia development in mice in which diabetes was induced with STZ starts on day 8 following STZ injection and continues for at least 4 weeks (14). STZ results in an increase in the action potential of C fibers in diabetic mice and induces impairment in pain threshold responses. There are several published studies measuring the changes in pain threshold in experimentally-induced animal models of diabetes. Thermal and mechanical hyperalgesia develop in experiments which induce short-term diabetes, whereas thermal and mechanical hypoalgesia are observed in those that induce long-term diabetes (15, 16). Slowing down in nerve conduction in rats occur 6 to 9 weeks following diabetes induction (16, 17). Time-dependent thermal pain threshold changes observed in experimental studies also reflect diabetic nephropathy symptoms observed in humans. Long-standing diabetes results in losses in peripheral nerves and an increase in nerve conduction rate (18). Plantar analgesiameter is a method to measure nerve conduction rate experimentally in animals. This is a thermal acute pain model to determine pain threshold by measuring the time of response the animal gives to heat stimuli (19). The duration of this study is 8 weeks, and statistically significant prolongation compared to pre-diabetes values, thus hypoalgesia, was seen with

latencies in pain thresholds measured by plantar analgesiameter 8 weeks after diabetes induction.

Hyperglycemia is the main cause of physiological, neurochemical and behavioral changes in painful neuropathy of rodents. Glycemic control is the most effective method in the treatment of diabetic neuropathy. The Diabetes Control and Complications Trial (DCCT) indisputably demonstrated the importance of glycemic control in the development of neuropathy in type 1 diabetic individuals. With strict glycemic control, the risk of developing neuropathy is reduced by about 64% over 5-year follow-up (20). United Kingdom Prospective Diabetes Study (UKPDS), the largest and longest study on type 2 diabetes mellitus, demonstrated that blood glucose regulation corrected vibration perception (21). Glycemic control reduces the risk of neuropathy but it is not a definitive method of treatment. Therefore, many alternative medicinal products were introduced to market in attempt to find a solution to neuropathy. The pathogenesis of DNP has not been fully understood and there is still no effective, ideal treatment. Currently, principally symptomatic treatment is given for diabetic neuropathy. The drugs used for this purpose alleviates pain, whereas they have no activity on the disease course, which is the main treatment target.

Alpha lipoic acid is a potent radical scavenger and a part of the endogenous antioxidative protection system. Oxidative stress plays a central role in the pathogenesis of diabetic complications. Caused by free radicals in diabetic neuropathy, oxidative stress leads to endoneural hypoxia and nerve dysfunction. Hyperglycemia-induced oxidative stress induces programmed cell death of nerves. The role of oxidative stress in nerve damage has been studied in experimental diabetes and in diabetic subjects. An experimental DNP model induced by STZ demonstrated that ALA improved neural blood flow and improved decreased levels of GSH, one of the most important markers of oxidative stress (9). ALADIN-1, the first prospective, placebo controlled, randomized, double-blind study with alpha lipoic acid demonstrated that intravenously administered ALA decreased symptom scores and was well tolerated (22). Similarly, SYDNEY-1 study demonstrated that ALA infusion given 5 times a week for three weeks resulted in clinically significant improvement in scales that assess disorders and deficits associated with diabetic neuropathy, including nerve conduction rate and neuropathic disorder (23).

In our study, statistically significant latency was observed at all time points following administration of the drug compared to pre-treatment pain threshold values in both ALA groups, with the effect being more pronounced in the group treated with ALA 100 mg. Based on this result, it can be concluded that ALA corrected hypoalgesia in both groups with a more marked effect in the higher dose group. Hyperglycemia eventually leads to oxidative stress and reactive oxygen radicals (ROS) accumulation by interacting with a variety of pathways. The organism remains unaffected by free radicals as long as the oxidative balance is maintained. Studies showing the relationship between diabetes and its complications and reactive oxygen species emphasize that tissue damage increased free radical production and altered antioxidant defense system (24, 25). Flavonoids were historically characterized on the basis of their antioxidant and free radical scavenging effects. Fisetin, a flavonoid derivative, protects nerve cell cultures from toxic stimuli such as ischemia and oxidative stress. Previous studies have shown that fisetin had several properties including anticancer, antiangiogenic, neuroprotective and antioxidation properties (26-28). In addition to its direct antioxidant capacity, fisetin also increases the levels of GSH, the major intracellular antioxidant. Glycation of macromolecules by reactive dicarbonyl and α-oxoaldehyde methylglyoxal (MG) is responsible for diabetic complications. Glutathione is a cofactor that is necessary for glyoxalase 1, the rate-limiting enzyme in eliminating MG. Fisetin also ensures maintenance of mitochondrial functions in the presence of oxidative stress. It further has anti-inflammatory activity against microglial cells and inhibits lipooxygenase. A study in diabetes-induced Akita mice demonstrated that orally administered fisetin reduces renal damage and protects the mice from anxiety-associated behaviors (29). The same study also showed that fisetin reduces the levels of oxidative stress markers thiobarbituric acid products (TBARS) and osteopontin. The effects of fisetin, with known oxidative effects, on pain threshold were examined in a DNP model because oxidative stress has an important role in the pathogenesis of diabetic neuropathy. Intraperitoneal fisetin at protocol-defined doses were administered to the mice with diabetic neuropathy, which was achieved through the study protocol. In the group treated with fisetin 3 mg, no significant changes were observed in pain threshold latencies measured 10, 30, 60 and 90 minutes after DNP was induced. In the group treated with fisetin 10 mg, on the other hand, shortened latencies, though not statistically significant compared to pre-treatment pain threshold latency values, were observed especially after 60 and 90 minutes. A study that induced experimental DNP investigated the effect of GSH in preventing and treating DNP. The study showed that the role of GSH in preventing DNP was only partial, and that it was ineffective in correcting sensory nerve conductions late after neuropathy has settled (30). In our study, late fisetin administration when hypoalgesia has developed and DNP has settled may explain the inefficacy on pain threshold. Oshawa et al. demonstrated that administration of acetyl-L-carnitine in diabetic neuropathic mice, in which hypoalgesia was induced, reduced pain thresholds and improved hypoalgesia to a statistically significant extent when it was given both with chronic administration before hypoalgesia development and at week 7, the time of onset of hypoalgesia (17). An other study showed that use of preventive low dose of fisetin for two weeks delayed the development of neuropathic hyperalgesia and allodynia in diabetic mice (11). Similar to this study,

the observed effectiveness of ALA on hypoalgesia that define the late period of DNP may be due to the involvement of several different mechanisms in the pathogenesis, including those which have been described and those that await elucidation. In a mouse model of neuropathic pain fisetin can correct thermal hyperalgesia when administred orally and chronically. This action is time dependent since it is sensitive to thermal stimuli but not mechanical stimuli and is present following chronic rather than acute fisetin treatment (12). In our study fisetin was administred after hypoalgesia occurence and administration period was very short. The acute administration of fisetin may explain its ineffectiveness.

Although we did not measure mortality, some animals were lost due to very high blood glucose levels and no standart therapy (no insulin being given). But, the level of mortality was lower in ALA received group, which indicates an advantage of this agent which is already in clinical use as adjuvant.

In conclusion, neuropathic pain is a heterogeneous condition with different causes and mechanisms. Various treatments have been used in DNP but a prototype drug or a method has not yet been found. New treatment protocols will be discovered once the pathogenetic mechanisms has been elucidated. It should be borne in mind that treatment efficacy observed in experimental diabetes may be different from that seen in human, and that the effect of diabetes on nervous system in humans is more extensive, chronic and serious. Therefore, novel pharmacological agents and new treatment methods that target the pathogenesis of DNP are needed.

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