Orijinal Araştırma

Effects of Alpha Lipoic Acid on Limb Lengths in Neonatal Rats Exposed to Maternal Tobacco Smoke

Maternal Tütün Dumanına Maruz Kalan Yavru Sıçanlarda Ekstremite Uzunlukları Üzerine Alfa Lipoik Asitin Etkileri

Ramazan Fazıl Akkoç[1], Elif Erdem[2], Nalan Kaya[3], Gonca Ozan[4], Durrin Özlem Dabak[3], İbrahim Enver Ozan[3]

[1]Department of Anatomy, Faculty of Medicine, Firat University, Elazıg, Turkey
[2]Department of Nursing, School of Health Sciences, Artuklu University, Mardin, Turkey
[3]Department of Histology and Embriology, Faculty of Medicine, Firat University, Elazıg, Turkey
[4]Department of Biochemistry, Faculty of Veterinary Medicine, Firat University, Elazıg, Turkey

Özet

Maternal tütün dumanı maruziyetinin yenidoğan iskelet sisteminde gelişim geriliğine neden olduğu bilinmektedir. Alfa lipoik asit (ALA), osteoblastın kemik oluşum mekanizmasını destekler. Bu çalışmada maternal tütün dumanına maruz kalan yavru sıçanların uzun kemikleri, boy ve kuyruk uzunlukları üzerine ALA'nın etkilerinin araştırılması amaçlandı. Sıçanlar dört gruba ayrıldı: 1) kontrol, 2) tütün dumanı, 3) tütün dumanı + ALA, 4) ALA. Grup 2 ve 3'te sıçanlar çiftleşme öncesi 8 hafta ve gebelikleri boyunca günde 2 kez 1'er saat tütün dumanına maruz bırakıldı. Ayrıca Grup 3'e 20 mg/kg dozunda ALA oral gavaj yoluyla verildi. Grup 4'e sadece ALA uygulandı. Doğumdan sonra 21. günde tüm gruplardaki yavru sıçanların boy ve kuyruk uzunlukları, dekapitasyondan sonra ise ekstremite uzun kemiklerinin boyları ölçüldü. Grup 2'de yapılan tüm morfometrik ölçümlerde grup 1'e kıyasla anlamlı derecede azalma, grup 3' teki bütün ölçümlerde ise grup 2'ye göre anlamlı derecede artış bulundu ($p<0.001$). Maternal tütün dumanı maruziyeti ile yavru sıçanların uzun kemik, boy ve kuyruk uzunluklarında oluşan gerilemeye karşı ALA'nın koruyucu bir etki gösterdiği tespit edildi.
Anahtar Kelimeler: Alfa Lipoik Asit, Kemik, Morfometri, Sıçan, Tütün Dumanı.

Abstract

Maternal tobacco smoke exposure is known to cause development retardation in the skeletal system of the newborn. Alpha lipoic acid (ALA) supports the bone formation mechanism of osteoblast. The present study aimed to investigate the effect of ALA on the long bone lengths, heights and tail lengths of the rat pups exposed to maternal tobacco smoke. The rats were divided into four groups: 1) control, 2) tobacco smoke, 3) tobacco smoke +ALA, 4) ALA. The rats in Group 2 and 3 were exposed to tobacco smoke twice a day for an hour for eight weeks before copulation and during their pregnancies. Also, ALA at the dose of 20 mg/kg was administered via oral gavage route to Group 3. Only ALA was administered to Group 4. On the postpartum day 21, the heights and tail lengths of the rat pups in all groups were measured, and after decapitation, the lengths of the long extremity bones were measured. A significant decline was noted in all morphometric measurements of Group 2 compared to Group 1 while a significant increase was found in all measurements of Group 3 compared to Group 2 ($p<0.001$). A protective effect of ALA against the regression occurring in the long bone, height and tail lengths of rat pups upon maternal tobacco smoke exposure was noted.
Keywords: Alpha Lipoic Acid, Bone, Morphometry, Rat, Tobacco Smoke

Introduction

Tobacco is a plant that contains approximately 4,000 chemicals in it. Among these chemicals, there are also substances which are quite harmful and addictive, such as nicotine (1). Smoking is one of the main preventable causes of illness and death (2). More than 5 million people die of cigarette smoking worldwide every year (3).

It has been reported that cigarette increases the risk of osteoporosis by affecting the endocrine system leading to a decrease in oestrogen and parathyroid hormones and an increase in cortisol and adrenal androgens (4). Studies have reported that smoking reduces vitamin D levels and intestinal calcium absorption required for bone that smoking increases osteoclast activity by escalating oxidative stress and induces osteocyte apoptosis (5, 6). Exposure to tobacco smoke during pregnancy leads to short stature, head and chest circumference in the neonatal due to retarded development of the skeletal system (7, 8).

Alpha lipoic acid (ALA), a branched-chain alpha-keto acid, is a naturally occurring co-enzyme in the mitochondrial multienzyme complexes catalyzing the oxidative decarboxylation of alpha-keto acids such as pyruvate and alpha-ketoglutarate (9). ALA, which is a natural antioxidant found in some foods and synthesized in trace amounts in the body, functions as scavenging for many reactive

oxygen species (ROS) such as hydroxyl, superoxide, and peroxyl radicals (10, 11).

It has been shown that alpha lipoic acid limits the activities of osteoclasts and osteoblasts promote bone formation mechanism (12-15). Also, ALA was suggested to be quite useful for the prevention of abortion and premature birth, and that ALA supplement was safe for living organisms even at high doses (16). In this study, it is aimed to investigate the effects of ALA on the anterior-posterior extremity long bones, length and tail lengths in pups exposed to maternal tobacco smoke.

Materials and method

Test animals

Twenty-eight 6-week-old Sprague-Dawley female rats obtained from Firat University Experimental Research Center with the permission of the Ethical Committee of Animal Experiments at Firat University are tested in this study.

Experimental design

The rats are divided into four groups. Group 1: control group, group 2: the tobacco smoke exposed group, group 3: tobacco smoke exposed + ALA applied group, group 4: ALA applied group.

In tobacco smoke exposed groups, for eight weeks before mating, rats were exposed to tobacco smoke for 2 times in a day lasting 1 hour in a specially designed glass cage. For rats that get pregnant, tobacco smoke exposure continues during pregnancy period. In ALA-treated groups, 20 mg/kg dose of ALA (cat: 29862 Lot: 002241-20161019, DL-α-Lipoic acid, Chem-Impex Int'l Inc, USA) dissolved in normal saline was given to the rats every other day for 8 weeks before mating and during the pregnancy period by oral gavage (17).

Creation of a pregnancy

From the beginning of the experiment to the end of the 8th week, tobacco smoke exposure and ALA administration were ensured. At the end of the eighth week, all female rats were mated with male rats that attained healthy sexual maturity. The date of observation of sperm in the vaginal smear following the mating was accepted as the embryonic 0 day.

Morphometric measurements

On the postnatal 21st day, pups were taken assuring that each group has seven. Length (frontal bone-anus) and tail (anus-tail end) measures of pups in all groups were made. Later, the pups were sacrificed by decapitation method under anaesthesia with ketamine and xylazine (5 mg / kg-50 mg / kg) (n=7).

After the skin, muscle and organs of the rat pups were removed as much as possible from the bones, the bones subjected to maceration were cleaned and dried. Then, the lengths of the right and left anterior extremity long bones (humerus, radius, ulna) and the right and left posterior extremity long bones (femur, fibula, tibia) were taken and measured. Measurements were made by two individuals unaware of each other and of which group the bone was measured (blinding). Averages of the results were obtained. Morphometric measurements are made using digital callipers with a resolution of 0.01 mm.

Statistical Analysis

SPSS 22.0 (Statistical Package for Social Sciences) package statistical program is used to evaluate the data. The data is presented as mean ± standard deviation, and $p < 0.05$ values are considered significant. The data were analyzed by the Kolmogorov-Smirnov test to evaluate if the data had normal distribution before the analysis was passed, and the data showed normal distribution. One-Way Analysis of Variance (ANOVA) is used to test differences between multiple groups with normal distribution.

Results

The tail length, length, and morphometric measurement values of the anterior-posterior extremity long bones of rat pups are given as the arithmetic mean ± standard deviation (Table 1, 2). The mean values of the right and left measurement values of each bone is used in the statistical analysis of the morphometric measurements of the anterior and posterior extremity bones.

In morphometric measurements made in both male and female pups; it is founded out that tail length, length, and the lengths of the anterior extremity long bones (humerus, radius, ulna) and the posterior extremity long bones (femur, fibula, tibia) decreased significantly in the tobacco smoke exposed group (group 2) compared to the control group (group 1) ($p < 0.001$). In the group with tobacco smoke + ALA (group 3), there is a statistically significant increase in all measurements compared to the group of tobacco (group 2) ($p < 0.001$). Although there is an increase in the measurements in the ALA-only group (group 4) compared to the control group, it is not statistically insignificant ($p > 0.05$).

Table 1. Morphometric measurement values of tail, length and anterior-posterior extremity long bones of female rat group.

	Group 1 [Control]	Group 2 [tobacco smoke]	Group 3 [tobacco smoke+ALA]	Group 4 [ALA]
Tail length [cm]	6.74±0.02	6.28±0.01 [a]	6.63±0.03 [a]	6.76±0.01 [b]
Height [cm]	7.96±0.02	7.43±0.02 [a]	7.84±0.02 [a]	7.99±0.02 [b]
Humerus [mm]	13.77±0.04	12.71±0.05 [a]	13.38±0.02 [a]	13.89±0.02 [b]
Radius [mm]	11.95±0.05	11.03±0.05 [a]	11.64±0.03 [a]	12.07±0.02 [b]
Ulna [mm]	15.78±0.06	14.34±0.06 [a]	15.43±0.04 [a]	15.91±0.05 [b]
Femur [mm]	15.22±0.07	13.96±0.04 [a]	14.97±0.04 [a]	15.37±0.05 [b]
Fibula [mm]	15.42±0.05	14.20±0.04 [a]	14.82±0.19 [a]	15.57±0.05 [b]
Tibia [mm]	19.10±0.05	17.45±0.07 [a]	18.76±0.03 [a]	19.12±0.17 [b]

Values are given as mean ± standard error.
[a]Compared to the other groups [p <0.05].
[b]Compared to groups 2 and 3 [p <0.05].

Table 2. Morphometric measurement values of tail, length and anterior-posterior extremity long bones of male rat group.

	Group 1 [Control]	Group 2 [tobacco smoke]	Group 3 [tobacco smoke+ ALA]	Group 4 [ALA]
Tail length [cm]	6.94±0.02	6.42±0.02 [a]	6.81±0.02 [a]	6.97±0.02 [b]
Height [cm]	8.22±0.02	7.65±0.02 [a]	8.07±0.01 [a]	8.26±0.02 [b]
Humerus [mm]	14.08±0.05	13.00±0.07 [a]	13.76±0.05 [a]	14.24±0.05 [b]
Radius [mm]	12.19±0.04	11.20±0.15 [a]	11.86±0.04 [a]	12.32±0.04 [b]
Ulna [mm]	16.06±0.04	14.76±0.04 [a]	15.58±0.9[a]	16.27±0.03 [b]
Femur [mm]	15.57±0.05	14.31±0.07 [a]	15.10±0.07 [a]	15.74±0.05 [b]
Fibula [mm]	15.71±0.04	14.44±0.05 [a]	15.34±0.03 [a]	15.90±0.05 [b]
Tibia [mm]	19.50±0.04	18.03±0.07 [a]	19.10±0.09 [a]	19.75±0.06 [b]

Values are given as mean ± standard error.
[a]Compared to the other groups [p<0.05].
[b]Compared to groups 2 and 3 [p<0.05].

Discussion

There are high reactive oxygen radicals in tobacco and these radicals lead to oxidative stresses by destroying the oxidant-antioxidant balance in tissues (18). There is evidence that oxidative stress increases the activities of osteoclasts (19). For example, studies conducted in humans and rodents show that endogenous oxidative stress markers and bone density are negatively correlated, and antioxidant applications such as vitamin C decreases osteoporosis (20, 21).

ROS, hydrogen peroxide, and superoxide anions have been reported to enhance osteoclast activity in the study carried out on bone surface of the rats and in new-born bone organ cultures (22). Many substances in tobacco smoke have been shown to have a direct toxic effect on bone cells (6). There are many studies showing that cigarette smoking increases osteoporosis and fracture risk (23, 24).

It has been determined that there is a shortening in humerus, ulna, femur and tibia bones of human foetuses exposed to tobacco smoke during pregnancy (25).
It has been reported that femur ossification of rat foetuses exposed to tobacco smoke is delayed (26). In another study, it is determined that nicotine applied during pregnancy and lactation causes decrease in femur lengths in new-born rat bones (27). In this study, the morphometric measurements made in the tobacco smoke exposed group (Group 2) are found to be significantly lower than the control group (group 1), supporting previous studies. Alpha lipoic acid has been shown to limit osteoclast activity thanks to its ROS scavenging ability in osteoclast genesis models based upon inflammation (12, 13). It has also been reported that alpha lipoic acid reduces the expression of these genes by targeting genes encoding ROS-producing enzymes in the femur (28). In addition, it has been shown that osteoblasts promote bone formation by regenerating low molecular weight antioxidants such as glutathione (14, 15).

ALA, which was administered as treatment following rat femur fracture model created by Aydin et al. (29), was reported to support bone healing. In the study by Mainini et al. (30), 23 of 44 postmenopausal women were treated with oral ALA supplement in order to investigate the effects of ALA on the bone mineral density of osteopenic postmenopausal women and the

remaining 21 women were followed up as the control group. At the end of the study, they suggested that the bone mineral densities of women who took ALA supplement were higher than that of the women in the control group and that oral ALA supplement may reduce bone loss in osteopenic postmenopausal women. In the report of Polat et al. (13), ALA was indicated to have a protective effect on both elderly and postmenopausal osteoporosis in the elderly and inflammation-mediated osteoporosis rat models.

The fact that the morphometric measurements made in tobacco + ALA group (group 3) are found out to be statistically significantly higher compared to the tobacco group (group 2) is in compliance with the above mentioned mechanisms.

In another study conducted in Italy in 2017 (16), in order to investigate the safety of ALA supplement in pregnant women, 600 mg of ALA supplement was given orally every day for at least seven weeks to 610 pregnant women during their pregnancies. Finally, the study reported that it did not have any side effect for mothers and newborns and that the use of ALA during pregnancy was safe.

As a result, it has been shown that ALA has a protective effect against the decrease in length, tail length, and anterior-posterior extremity long bones in the rat pups due to the maternal tobacco smoke exposure. With further and more extensive studies in the future, we believe that, for a healthy skeletal systems of new generations, alpha lipoic acid can be used in pregnancies using tobacco products or exposing them for a healthy skeletal systems of new generations.

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Sorumlu Yazar
Ramazan Fazıl AKKOÇ
Department of Anatomy, Faculty of Medicine, Firat University, ELAZIG, TURKEY
E-Mail: ramazan_fazil@hotmail.com
fakkoc@ firat.edu.tr