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The investigation of effect of alpha lipoic acid against damage on neonatal rat lung to maternal tobacco smoke exposure

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ABSTRACT

This study was carried out to determine the changes in the lungs of the rat pups exposed to tobacco smoke during pregnancy period and to investigate the protective effects of alpha lipoic acid, which is administered during pregnancy, on these changes.

Spraque-Dawley female rats were divided into four groups: control, tobacco smoke (TS), tobacco smoke + alpha lipoic acid (TS + ALA) and alpha lipoic acid (ALA). The rats in control group were untreated. Rats were exposed to TS twice a day for one hour starting from eight weeks before mating and during pregnancy. 20 mg / kg of ALA was administered to rats. On 7th and 21st days 7 of the pups from each group were decapitated. Histological, morphometric, biochemical and quantitative real-time RT-PCR analyzes were performed.

Histopathological and biochemical changes were observed in TS group. While a significant decrease was observed both in SP-A and VEGF immunoreactivities and mRNA levels, caspase-3 immunoreactivity and TUNEL positive cells were increased in TS group.

It is suggested that prenatal TS exposure leads to morphological and histopathological changes on lung development by causing oxidative damage in lungs of neonatal rats and the maternal use of ALA can provide a limited protective effect on the neonatal lung development against this oxidative stress originating from TS. Although pregnant women are increasingly aware on health risks of smoking, environmental tobacco smoke exposure is still a widespread problem. For this reason, it is thought that this damage can be partially reduced by some antioxidant supplements in pregnancy.

1. Introduction

Exposure to tobacco smoke during the prenatal period affects multiple organ system developments in neonatals such as nervous, respiratory and cardiovascular systems [1]. The harmful chemicals in all forms of tobacco smoke, such as nicotine and carbon monoxide (CO), rapidly pass the placental barrier and accumulate overdose in the fetal compartment [2]. It is known that these chemicals cause oxidation by increasing the production of superoxide anion from free radicals, and these oxidants cause damage to alveolar-capillary membranes. Therefore, it is thought that the underlying mechanism of damage caused by tobacco smoke on the lungs is due to oxidants [3,4]. Surfactant protein-A (SP-A) is a surface tension reducing apoprotein which is very important in regulation of the surfactant concentration [5,6]. Alterations in surfactant insufficiency or composition are one of the findings associated with inadequate lung development in the prenatal and neonatal period [7]. Vascular endothelial growth factor (VEGF) plays a key role in lung development and damage, regulating endothelial cell proliferation, mitogenesis, migration, differentiation, vascular permeability, mobilization of endothelial cell precursors [8].

Programmed cell deaths, or apoptosis, of lung cells play an essential role in normal development of the respiratory system [9,10]. Caspase-3 is involved in characteristic morphological changes in apoptotic cells, such as chromatin condensation, DNA fragmentation, and membrane budding [11]. Thus, Caspase-3 is an important predictor of the change in apoptosis levels in the lungs.

Alpha lipoic acid (ALA) is a non-enzymatic, natural, metabolic antioxidant [12]. Potential antioxidant function is achieved by passing

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two sulfur atoms in the structure through oxidation-reduction reactions [13].

This study was fulfill to detect the histological, immunohistochemical and biochemical changes in the lungs of the rat pups exposed to tobacco smoke during gestation period, and to research the protective effects of ALA, which is administered during pregnancy, on these changes.

2. Materials-methods

2.1. Ethical approval

All experimental procedures including animals were confirmed by the local ethics committee of Firat University (07.10.2015, 2015/17-159).

2.2. Experimental design

This study was conducted at the Experimental Research Unit of Firat University (FUDAM). Rats were fed with standard rat chow and tap water freely available. 12-h light-dark cycles, 21 ± 1 °C standard temperature and humidity conditions were provided. In the study, six-weeks old 28 Spraque-Dawley female rats weighing 160 ± 10 g were used. Animals were group housed from the start of the experiment until one week before the birth and then singly housed until the end of the experiment. The minimum number of animals required for the statistical evaluation of the difference between the groups in the obtained data was preferred. Rats were randomly divided into four groups: control, tobacco smoke (TS), tobacco smoke + alpha lipoic acid (TS + ALA) and alpha lipoic acid (ALA) (n = 7). The rats in the control group were untreated. Rats in the TS and TS + ALA groups were exposed to tobacco smoke twice a day for one hour starting from eight weeks before mating and during gestation period. 20 mg / kg of ALA (cat: 29,862 Lot: 002241-20161019, DL-α-Lipoic acid, Chem-Impex Int'l Inc, USA) was administered via oral gavage to the ALA and TS + ALA groups [14]. A glass cage was planned specifically for exposing the rats to tobacco smoke. The smoke of 10 g tobacco was put in the glass cage via air pump (AP-001 Aquarium Air Pump Xilong, China). Once after the birth, all administrations were stopped. On the 7th and 21 st days, 7 of the pups from each group were decapitated under xylazine (5 mg/kg)–ketamine (50 mg/kg) anesthesia. The lungs were removed rapidly and utilized for morphometric, histological, biochemical and quantitative real-time polymerase chain reaction (qRT-PCR) analyses as described below.

2.3. Histological evaluation

Lung tissue samples were fixed in 10% buffered formaldehyde, and then embedded in paraffin. Samples were cut at 5 µm thicknesses and stained with hematoxylin and eosin (H&E), Periodic Acid Schiff (PAS) and Masson's Trichrome staining and examined under light microscope (NovelN-800 M, Ningbo, China). Severity of lung injury was semi-quantitatively assesed with the following changes: increased inflammatory cell, hemorrhagic areas, edema, interalveolar septal thickening, decrease in alveolar numbers, degeneration of some bronchi and bronchial epithelium, epithelial cells that were fallen into the lumen and hyaline membrane formation. Histoscores were given as 0 = absent, 1 = weak, 2 = moderate and 3 = strong for each parameter.

2.4. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

ApopTagPlus Peroxidase in situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA) was used for determination of apoptotic cells. In the evaluation of TUNEL staining, cells with blue nuclei were normal, while brown nuclei belonged to apoptotic cells. At least 1000 cells were counted on each field. Apoptotic index was calculated as a ratio of the TUNEL positive cell number to the total cell number (normal + apoptotic cells).

2.5. Immunohistochemical evaluation

Avidin-biotin-peroxidase complex method was used to define SP-A, VEGF, Caspase-3 immunreactivities (RB-9031-P lot No: 9031P1505 A, Polyclonal Anti-VEGF Antibody, Thermo Scientific, SC-13977 lot No: G2314, Polyclonal Anti-SP-A Antibody, Santa Cruz Biotechonology, PA5-16335 lot No: QD2017719, Polyclonal Anti-Caspase-3 Antibody, Thermo Scientific) in lung tissue. The immunohistochemical histoscore was calculated with immunoreactivity prevalence (0.1: < 25%, 0.4: 26–50%, 0.6: 51–75%, 0.9: 76–100 %) and severity (0: no, +0.5: very little, +1: little, +2: medium, +3: severe). (Histoscore = prevalence × severity).

2.6. Biochemical evaluation of lung tissue

Malondialdehyde (MDA) concentrations were assessed according to a modified method of Placer et al. [15]. Glutathione (GSH) levels and superoxide dismutase (SOD) activities were measured respectively using the methods of Beutler and Sun et al. [16,17].

2.7. Calculation of relative lung weight

After decapitation, the lung tissues of pups were removed from peripheral fat tissues and then calculated the relative lung weights (Relative lung weight = (absolute lung weight (g) / body weight) x100).

2.7. Morphometric analysis

The radial alveolar count (RAC) method was performed strictly according to the method of Cooney and Thurlbeck [18]. According to this method, a linear line was drawn from respiratory bronchiole to the closest connective tissue septum. The number of alveoli cut by this line was then counted. Ten such counts were done from each pup lung.

2.8. qRT-PCR analysis

For Total RNA isolation from lung tissues, TRIzol reagent was used (Invitrogen, Carlsbad, CA). Random primed cDNAs were produced by reverse-transcription of total RNA samples by using High Capacity RNA to cDNA Synthesis kit (Invitrogen, Carlsbad, CA). A real-time PCR analysis was applied with the ABI Prism 7500 Fast Real Time PCR Instrument (Applied Biosystems, CA) using Tag Man Master Mix (Applied Biosystems, CA). Each value was standardized to the levels of GAPDH. The samples were quantified for SP-A (Rn-04338808_g1, Thermo Scientific) and VEGF (Rn01511610_m1, Thermo Scientific), utilizing the comparative Ct (ΔΔCt) method [19].

2.9. Statistical analysis

Statistical analyses were implemented by SPSS 22.0 (Statistical Package for Social Sciences) software. The values were presented as the means ± standard deviation. The probability values (p) less than 0.05 were accepted as statistically significant. Statistical differences of the multiple groups with normal distribution were measured by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

3. Results

3.1. Body weight and relative lung weight values

It was shown that tobacco smoke exposure and ALA administration led to body weight changes. Significant decrease was observed in the