Arch Bronconeumol. 2013;49(1):15–21

ARCHIVOS DE BRONCONEUMOLOGIA

www.archbronconeumol.org

Original Article

Protective Effects of *Nigella sativa* Oil in Hyperoxia-Induced Lung Injury☆

Cuneyt Tayman,[a,*] Ferhat Cekmez,[a] Ilker Mustafa Kafa,[b] Fuat Emre Canpolat,[a] Merih Cetinkaya,[a] Alparslan Tonbul,[c] Sema Uysal,[d] Turan Tunc,[a] S. Umit Sarici[a]

[a] *Department of Neonatology, GATA Military School of Medicine, Ankara, Turkey*
[b] *Department of Anatomy, Uludag University Faculty of Medicine, Bursa, Turkey*
[c] *Department of Neonatology, Fatih University Faculty of Medicine, Ankara, Turkey*
[d] *Department of Biochemistry, Fatih University Faculty of Medicine, Ankara, Turkey*

ARTICLE INFO

Article history:
Received 19 December 2011
Accepted 3 March 2012
Available online 8 December 2012

Keywords:
Bronchopulmonary dysplasia
Oxygen-induced lung injury
Preterm infants
Nigella sativa oil
Protection
Rat

ABSTRACT

Background: Oxygen-induced lung injury is believed to lead to the development of bronchopulmonary dysplasia in premature infants. We have evaluated the beneficial effects of *Nigella sativa* oil (NSO) on rats with hyperoxia-induced lung injury.
Methods: Thirty newborn Sprague-Dawley rats were randomly divided into 3 groups as hyperoxia (95% O_2), hyperoxia+NSO and control (21% O_2). Pups in the hyperoxia+NSO group were administered intraperitoneal NSO at a dose of 4 ml/kg daily during the study period. Histopathologic, immunochemical, and biochemical evaluations (superoxide dismutase [SOD], glutathione peroxidase [GSH-Px], malonaldehyde [MDA] and myeloperoxidase [MPO]) were performed.
Results: In the histopathologic and immunochemical evaluation, severity of lung damage was significantly lower in the hyperoxia+NOS group (P<.05). Tissue GSH-Px and SOD levels were significantly preserved, and MDA, MPO levels were significantly lower in the hyperoxia+NSO group (P<.05).
Conclusion: NSO significantly reduced the severity of lung damage due to hyperoxia.

Efectos protectores del aceite de *Nigella sativa* en la lesión pulmonar inducida por hiperoxia

Palabras clave:
Displasia broncopulmonar
Lesión pulmonar inducida por oxígeno
Recién nacidos pretérmino
Aceite de *Nigella sativa*
Protección
Rata

RESUMEN

Antecedentes: Se cree que la lesión pulmonar inducida por el oxígeno conduce al desarrollo de una displasia broncopulmonar en los recién nacidos prematuros. Hemos evaluado los efectos favorables del aceite de *Nigella sativa* (NSO) en ratas con lesión pulmonar inducida por hiperoxia.
Métodos: Se utilizaron 30 ratas Sprague-Dawley recién nacidas a las que se dividió aleatoriamente en 3 grupos para aplicarles hiperoxia (O_2 al 95%), hiperoxia + NSO o el grupo de control (O_2 al 21%). A las crías del grupo de hiperoxia + NSO se les administró NSO a una dosis de 4 ml/kg al día por vía intraperitoneal durante el periodo de estudio. Se realizó una evaluación histopatológica, inmunoquímica y bioquímica (superóxido dismutasa [SOD], glutatión peroxidasa [GSH-Px], malonilaldehído [MDA] y mieloperoxidasa [MPO]).
Resultados: En la evaluación histopatológica e inmunoquímica, la gravedad de la lesión pulmonar fue significativamente inferior en el grupo de hiperoxia + NOS ($p < 0,05$). Los niveles tisulares de GSH-Px y SOD se mantuvieron significativamente preservados, y los niveles de MDA y MPO fueron significativamente inferiores en el grupo de hiperoxia + NSO ($p < 0,05$).
Conclusión: El NSO redujo significativamente la gravedad de la lesión pulmonar debida a la hiperoxia.

☆ Please cite this article as: Tayman C, et al. Efectos protectores del aceite de *Nigella sativa* en la lesión pulmonar inducida por hiperoxia. Arch Bronconeumol. 2012;49:15–21.
* Corresponding author.
E-mail address: ctayman22@gmail.com (C. Tayman).

1579-2129/$ – see front matter

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic pulmonary disease seen in premature newborns who required mechanical ventilation and oxygen therapy due to acute respiratory distress.[1,2] Although its etiopathogenesis is not completely understood, it is believed to be multifactorial, influenced by prematurity, oxidative lung lesion and inflammation, like chorioamnionitis, hyperoxia, infection, respirator-induced injury and many other adverse stimuli. The lesion alters the normal growth and maturation of the lungs caused by deteriorated alveolar and vascular growth, leading to the abnormal lung structure of BPD.[2–4] It was reported for the first time more than 40 years ago, but the nature of BPD has been modified with the increasing use of prenatal corticosteroids, surfactant therapy, new ventilatory strategies and other treatments.[5,6] Nonetheless, BPD continues to be an important cause of neonatal morbidity and mortality, as improved perinatal care has increased the survival rate of extremely premature newborns (<28 weeks of gestation).[2,4,7] These children are at high risk for having long-term lung injury due to the significant deterioration in lung function produced by the prolonged use of respirators during neonatal intensive care unit stays. After hospital discharge, these children may also have frequent hospitalizations, recurring respiratory exacerbations, intolerance to exercise and other signs of late cardiorespiratory disease, in addition to adverse neurodevelopmental evolution.[4] Clinical and laboratory studies suggest that early intervention strategies that directly preserve survival and lung cell function should prevent the appearance of BPD.[3,7] However, we still do not have treatments that can effectively attenuate lung lesions and promote lung growth in order to reduce the incidence and severity of BPD. As a consequence, it is necessary to identify new treatment options for the prevention of severe pulmonary injury in the course of BPD.

The *Nigella sativa* (NS) plant, commonly known as "black seed", is used as a medicinal plant for the treatment of many diseases.[8] In clinical and experimental studies, several therapeutic effects of NS and its extracts have been reported in respiratory diseases.[9–12] In NS extracts, many enriched bioactive molecules have been identified, both in fixed oil as well as essential oil. It has been demonstrated that much of the biological activity of NS is due to thymoquinone, which is the main component of the essential oil and fixed oil.[8] It has been observed that the volatile oil contains 18.4%–24% thymoquinone and also 46% monoterpenes (e.g. p-cymene, a-pinene).[13] Several papers have identified that the extracts of NS have anti-inflammatory and antimicrobial properties (against several germs) as well as antioxidant properties due to its activity of free-radical elimination.[14] In addition, its inhibitory action of human neutrophil elastase has been determined[15] along with its immunomodulation and cytoprotection activity in many systems of the body with a low grade of toxicity in clinical and experimental studies.[8,16] However, the effects of NS oil (NSO) on lungs that have undergone hyperoxic injury have not yet been researched. The objective of this study was therefore to investigate the favorable effects of NSO on the lungs of rat pups with BPD induced by hyperoxia.

Material and Methods

Experimental Design

The study was approved by the Ethics Committee for animal experimentation studies at the GATA Military School of Medicine (Ankara, Turkey). National Research Council guidelines were followed for the care and use of laboratory animals. Four pregnant Sprague-Dawley rats were used, which spontaneously gave birth. Afterwards, all the pups were grouped, randomly distributed and then returned to the mother rats. Thirty newborn rats were used and distributed into the 3 following groups: control, hyperoxia and hyperoxia with NSO treatment. The pups in the hyperoxia group were exposed to 95% O_2, while the pups of the control group breathed room air with 21% O_2. The mothers were rotated between the litters exposed to hyperoxia and room air every 24 h in order to avoid oxygen toxicity in them. Continuous exposure to 95% O_2 was reached in a Plexiglas chamber (70 cm×60 cm×30 cm) with the use of a continuous flow system. The level of oxygen in the interior of the Plexiglas chamber was continuously monitored with a Ceramatec oxygen analyzer (MAXO2) in order to maintain O_2 saturation ≥95%. The experimental pups of the control group (room air with 21% O_2 for 10 days+placebo) and those with hyperoxia alone (95% O_2 for 10 days+placebo) were administered intraperitoneal saline solution (4 ml/kg), while the pups in the hyperoxia+NSO group (95% O_2 for 10 days+NSO) were administered NSO (Origo [100% natural *N. sativa* seed oil], Gaziantep, Turkey) at a dose of 4 ml/kg[17] once a day with a microsyringe, intraperitoneally, from the first until the last day of the study. Before being administered, the NSO was sterilized by filtration with Bexen 0.2 μm filters (Oiarso S. Coop., Guipuzkoa, Spain). It has been demonstrated that the different compositions present in NSO act synergically, and this suggests the importance of using the complete oil of the seeds in studies. Thus, in this study we have used whole oil.[8] The pups were weighed daily on a scale with a sensitivity of 0.01 g, and the weights were recorded.

Histopathologic, Morphologic and Immunochemical Analyses of the Lungs

All the pups were sacrificed on the 10th day of the study under deep anesthesia with intraperitoneal ketamine/xylazine (100–10 mg/kg). The thorax was opened, the lungs extracted and set with 4% paraformaldehyde perfusion (PFA) buffered with 0.1 M phosphate (PBS). During perfusion, constant inflation pressure was maintained at 5 cm H_2O by means of a tracheal catheter. When the perfusion was completed, the trachea was ligated with a surgical suture and the lungs incubated in a 4% solution of cold PFA–PBS, with ice for 4–5 h. After this incubation, the PFA–PBS solution was replaced with two quick changes of cold PBS to eliminate outer residue. Then, the lungs were transferred to a solution of 30% PBS/sucrose that was sterile and filtered, and they were maintained at 4 °C until total equilibrium. The lungs were included in paraffin and 5 μm slices were made from the paraffin blocks. Slices were selected using a systematic randomized sampling procedure, and they were mounted on slides coated with poly-L-lysine (Histobond adhesive slides, Marienfeld, Germany). The slides were stained with standard techniques for hematoxylin-eosin and Masson's trichrome for the histopathologic exam and with the ABC technique for the of lamellar body membrane protein.

For the immunohistochemical detection of lamellar body membrane protein, the slices were rehydrated and then treated with 13% hydrogen peroxide for 30 min. Afterwards, the blocks underwent a non-specific block with goat serum for 30 min and then were incubated with primary antibodies against membrane protein of lamellar bodies (1:100, Chemicon, AB3623-rabbit, USA) for a night at 4 °C, followed by a treatment with biotinylated anti-rabbit secondary antibody (1:200, Vector Laboratories, Peterborough, United Kingdom) for 30 min at room temperature. After the treatment with the avidin–biotin complex, 3,3′-diaminobenzidine was used (DAB; Vector Laboratories, Peterborough, United Kingdom) to reveal the color. Negative control preparations were also used,