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ORIGINAL ARTICLE

Dexpanthenol therapy reduces lung damage in a hyperoxic lung injury in neonatal rats

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Abstract

Objective: Dexpanthenol (Dxp) plays a major role in cellular defense and in repair systems against oxidative stress and inflammatory response and it has not yet been evaluated in treatment of bronchopulmonary dysplasia (BPD). We tested the hypothesis that proposes whether Dxp decreases the severity of lung injury in an animal model of BPD.
Methods: Forty rat pups were divided into four groups: control, control + Dxp, hyperoxia and hyperoxia + Dxp. All animals were processed for lung histology and tissue analysis. The degree of lung inflammation, oxidative and antioxidant capacity was assessed from lung homogenates.
Results: Lung injury score and alveol diameter increased in the hyperoxia group ($p < 0.001$). Median level of malondialdehyde, total oxidant status and oxidative stress indexes was significantly higher in the hyperoxia group compared to the other groups. The median superoxide dismutase activity in the hyperoxia group was notably less than those of control + Dxp and hyperoxia + Dxp groups ($p < 0.01$). Similarly, lung catalase, glutathione (GSH) peroxidase and reduced GSH activities in the hyperoxia group were significantly lower than other groups. Furthermore, the hyperoxia + Dxp group had lower tumor necrosis factor-α and interleukin-1β median levels compared to the hyperoxia group ($p = 0.007$).
Conclusion: Dxp treatment results in less emphysematous change as well as decrease in inflammation and oxidative stress markers in an animal model of BPD.

Keywords

Bronchopulmonary dysplasia, chronic lung disease, dexpanthenol, hyperoxia, hyperoxic lung injury, pantothenic acid

History

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Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease mostly affecting preterm infants with serious morbidity and mortality rates. Surfactant therapy, antenatal steroids and incremental improvements in perinatal care have all modified the pattern of injury and allowed survival of even more immature infants. However, there is still no specific treatment for BPD [1,2].

The etiology of the BPD is likely to be multifactorial. Although the strongest association is made with preterm birth, other factors such as prenatal and postnatal infection and inflammation, oxygen toxicity with decreased host antioxidant defenses and mechanical ventilation also contribute to the pathogenesis of BPD [2–4]. Oxygen causes tissue injury through a formation of highly reactive and destructive radicals like hydroxyl radicals and through peroxidation of membrane lipids. Premature infants are deficient in antioxidant enzyme systems at birth, and oxidative stress plays a major role in the development of BPD; therefore, antioxidant therapies are thought to improve lung morphology. The immaturity of the intracellular enzymatic anti-oxidant defense, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), makes these infants highly susceptible to oxidative injury [5,6].

Today, it is evident that there is a close relationship between hyperoxia and inflammation. Hyperoxia is a powerful proinflammatory stimulus, and its role in the pathogenesis of BPD has been reviewed in up-to-date studies [7,8]. Hyperoxia may increase reactive oxygen species (ROS) and initiate lung inflammation via multiple mechanisms including activation of transcription factors, signal transduction and gene expression of proinflammatory mediators [9,10]. The development of BPD in preterm infants is associated with increases in proinflammatory cytokines such as interferon-γ, tumor necrosis factor-α (TNF-α), interleukin-6, interleukin-8, interleukin-10, interleukin-1β (IL-1β) and alveolar macrophage [8,11,12].

Dexpanthenol (Dxp) is an alcoholic analog of pantothenic acid (PA), and it is oxidized to PA within the tissues.

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PA protects against cell damage produced by oxygen free radicals. It is well established that PA and its derivatives increase the level of reduced glutathione, Coenzyme A and adenosine-5′-triphosphate synthesis within the cell [13–15]. All these items play a major role in cellular defense and in the repair systems against oxidative stress and inflammatory response [16,17]. Moreover, experimentally important beneficial antioxidant effects of Dxp therapy have been reported in relation to some disorders such as testicular torsion, pulmonary fibrosis, renal and caustic esophageal injury, menopause, cardidiovascular damage, chemical cystitis injury of bladder and ischemia–reperfusion injury of heart [18–25].

In this study, we hypothesized that Dxp prophylaxis may be effective in the treatment of hyperoxic lung injury in rats by improving its antioxidant and anti-inflammatory effect. To this end, we designed a study to evaluate the efficacy of Dxp prophylaxis in an experimental model of BPD. This model was selected because neonatal rats are born at the saccular stage of lung development, which is comparable to premature infants born in the 24th to 28th weeks of gestational age [26]. The parameters adapted in the study were lung histology, pro-inflammatory cytokines, oxidative stress markers and antioxidant enzyme activities from lung homogenates.

Materials and methods

Animal model and treatment

Our study was approved by the Animal Research Ethical Committee of Inonu University (Malatya, Turkey) and we followed the National Institute of Health Guide (Washington, DC) for the Care and Use of Laboratory Animals.

Timed pregnant Wistar rats were kept in a 12-h dark/light cycle and fed a standard rat chow (Special Diet Services, Witham, Essex, UK) *ad libitum*. Breeding pairs were allowed access for 1 h on the day female rats showed very specific sexual behaviors such as lordosis, hopping and air-flapping. Pups were delivered spontaneously at full gestation and only pups delivered during a specified 24-h period were used. The pups were randomly distributed to the dams to achieve 10 pups per group. Totally 40 pups were derived from four dams. All pups were left with their dams to breastfeed freely. Experimental treatments began on postnatal day 1 and lasted on postnatal day 10, with the date of birth being day 1.

Forty rat pups were randomly divided into four groups. The pups in one of the groups ($n = 10$) were kept in room air; these pups served as the control group. The second group ($n = 10$) was also kept in room air yet the pups in this group were treated with Dxp (control + Dxp group). The third group ($n = 10$) was exposed to hyperoxia (hyperoxia group) and the fourth group ($n = 10$) was exposed to hyperoxia and treated with Dxp (hyperoxia + Dxp group). The pups exposed to hyperoxia were kept in transparent Plexiglas chambers in which the oxygen concentration (>95%) was monitored continuously with an oxygen sensor; humidity was maintained above 80%, and CO_2 was removed by soda lime absorption. The oxygen concentration was kept at >95% using a flow of 2.5 l/min. The control group was also kept in a similar chamber [27]. Weight, signs of disease and mortality were checked daily.

All pups were weighed each morning and received daily intraperitoneal injections of either Dxp or placebo (normal saline) from day 1 of life to day 10. The second and fourth groups of pups received injections of Dxp (Bepanthene ampul®, 500 mg, Bayer Corp., Istanbul, Turkey) at a dose of 500 mg/kg body weight, whereas the pups in the control and hyperoxia groups were given normal saline (0.2 mL) by intraperitoneal injection.

Nursing mothers were rotated between the litters and were exposed to room air and hyperoxia every 24 h to prevent damage to lungs. Injections were administered in the morning while changing bedding, water and chow. All animals were kept in the same room with a light/dark cycle of 12 h. On postnatal day 10, the animals from each group were killed.

Tissue preparation

To avoid post-mortem fibrin deposition in the lungs, we injected heparin intraperitoneally (100 units; Mustafa Nevzat, Istanbul, Turkey). After 5 min, pups were exsanguinated by transection of the abdominal blood vessels. The thoracic cavity was opened and the lungs were removed. The removed parts were then snap-frozen in liquid nitrogen and stored at −80 °C until their assessment for biochemical examination. For the histological studies, the trachea was cannulated and the lungs were fixed *in situ* via the trachea cannula with buffered formaldehyde (4% paraformaldehyde in PBS; pH 7.4) at 25 cm H_2O (2.4 kPa) pressure for 5 min.

Biochemical analysis

After adding phosphate buffer (pH 7.4), the frozen tissues were homogenized on an ice cube using a homogenizer. We used the supernatant for the entire assay. The protein content of the tissue homogenates was measured as described by Lowry et al. [28] with bovine serum albumin regarded as the standard. The malondialdehyde (MDA) contents of homogenates were determined spectrophotometrically [29] by measuring the presence of thiobarbituric acid reactive substances. The amount of lipid peroxides was considered as thiobarbituric acid reactive substances of lipid peroxidation. SOD activity was assayed using Sun et al.'s nitroblue tetrazolium method [30]. The CAT activity was determined according to Aebi's method [31]. The GPx activity was measured using the method described by Paglia and Valentine [32]. For the analysis of reduced glutathione (GSH) content in lung tissue as non-protein sulfhydryls, we followed a previously described method [33]. The index levels of tissue total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress indexes (OSI) levels were all assayed [34] using commercial assay kits (Rel Assay Diagnostics, Gaziantep, Turkey). Lung homogenate TNF-α and IL-1β concentration was measured in duplicates with a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China).

Histological evaluation

The lung tissues that were fixed in 10% formalin for 24 h were embedded in paraffin. Tissue sections were cut

Table 1. Comparison of biochemical evaluations for each group.

Groups	Control (*n*:10)	Control + Dxp (*n*:10)	Hyperoxia (*n*:8)	Hyperoxia + Dxp (*n*:8)
MDA, nmol/g protein	25 (13.6–35.5)	26.6 (18.2–38)	40.7 (33.4–50.3)[a,b]	30.8 (22.1–38.7)[c]
SOD, U/g protein	2.7 (1.4–4.6)	3.1 (1.5–4.3)	1.8 (1.2–2.3)[b]	2.8 (2–3.7)[c]
CAT, k/g protein	12.7 (7–16.7)	12.1 (6.1–19)	7.1 (4.1–9.8)[a,b]	12.8 (7.7–17.9)[c]
GPx, U/g protein	720 (420–1087)	597 (333–1072)	303 (207–520)[a,b]	529 (210–981)
GSH, μmol/g protein	16.5 (13.5–26.5)	20.4 (16.8–26.8)	13.5 (9.1–15)[a,b]	15.2 (12.9–31.3)[c]
TOS, μmol H_2O_2/L	8.2 (5.6–10)	8.9 (5.1–11.1)	11.9 (9–15.1)[a,b]	6.8 (6.2–11)[c]
TAC, mmol Trolox Eq/L	1.3 (0.9–1.4)	1.3 (1.1–1.4)	1.4 (1.1–1.5)	1.3 (1.2–1.3)
OSI, arbitrary unit	5.9 (4.3–8.9)	7.3 (3.9–9.5)	9.4 (6–10.2)[a]	5.2 (4.4–8.1)[c]
TNF-α, μg/g protein	2.6 (1.5–3.7)	1.7 (0.9–5.6)	4.7 (2.6–5.4)[a,b]	2.3 (1.4–4.6)[c]
IL-1β, ng/g protein	20.1 (11–27.5)	14.2 (6.8–41.3)	32.6 (20.9–47.5)[a,b]	19.5 (11.8–25)[c]

Dxp, dexpanthenol; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; TOS, total oxidant status; TAC, total antioxidant capacity; OSI, oxidative stress indexes; TNF-α, tumor necrosis factor α; IL-1β, interleukins 1β.
[a]Significantly different from the control group.
[b]Significantly different from the control + Dxp group.
[c]Significantly different from the BPD group.

at 5 μm, mounted on slides, stained with hematoxylin–eosin for general lung structure, periodic acid schiff to identify alveolar macrophage and with Masson's trichrom for connective tissues. Lung injury was scored each for hemorrhage, enlarged alveoli and increase in connective tissue in paranchyma's on a scale of 0–3: 0 for normal lungs, 1 for 25% injury involvement, 2 for 26–75% injury involvement and, 3 for 75% injury involvement. We evaluated 10 areas for each slide. In addition, 100 randomly selected alveoli from each of the groups were evaluated at a magnification of 40×. Two different (vertical and horizontal) diameters of each alveolus were measured to identify an average diameter. Alveolar macrophages were counted in the 10 microscopic under 40× objective magnification using Leica Q Win Image Analysis System (Leica Micros Imaging Solution Ltd., Cambridge, UK). All the sections were examined using a Leica DFC280 light microscope.

Statistical analysis

Statistical analyses were carried out using SPSS for Microsoft Windows (Chicago, IL). The data provided in this study are expressed in medians (minimum to maximum). We analyzed the differences between the groups using the Kruskal–Wallis test. The post hoc comparisons among the groups with significant values were evaluated using the Bonferroni-corrected Mann–Whitney *U* tests. The statistical significance was defined as $p < 0.05$.

Results

Throughout the tests, we lost two rats in each of the hyperoxia group and hyperoxia + Dxp group. At birth, on postnatal day 1, median body weight of the rat pups was 5.1 (4.8–6) g. At the end of the study, on postnatal day 10, the median weight of rats in the room air-exposed groups [control group 21.1 (18.2–25.1) g and control + Dxp 20.5 (17–24.3) g] was significantly higher than the hyperoxia-exposed groups [hyperoxia group 11.9 (9.2–14.2) g and hyperoxia + Dxp group 16.6 (14.9–18.2) g] ($p < 0.001$). Similarly, the median weight of rats in hyperoxia + Dxp group was significantly higher than in the hyperoxia group ($p < 0.001$).

Biochemical findings

Median levels of lung oxidative stress markers (MDA, TOS and OSI) were significantly higher in the hyperoxia group compared to the other groups. The SOD activity of the hyporexia group was notably less than those of the control + Dxp and hyperoxia + Dxp groups ($p = 0.004$ and $p = 0.005$, respectively). Lung homogenate CAT, GPx and GSH activities in the hyperoxia groups were significantly lower in comparison to the other groups. Furthermore, the hyperoxia + Dxp group had lower median levels of TNF-α and IL-1β in lung homogenates than the hyperoxia group ($p = 0.007$ and $p = 0.007$, respectively). There were no statistically differences among four groups according to TAC levels (Table 1).

Histopathological findings

The lungs of the rats in the control group showed normal lung structure and there were no lesions (Figure 1A). The group treated with Dxp was similar to that of the control group in terms of lung structure (Figure 1B). No sign of connective tissue deposition was observed with Masson's trichrom staining methods in control and Dxp groups (Figure 1C and D). However, considerable histological changes such as hemorrhage of the parenchyma and enlarged alveoli were observed in lung tissues in the hyperoxia group (Figure 1E). There were no statistically significant changes with regards to connective tissue deposition in the hyperoxia group when compared with the others group ($p > 0.05$) (Figure 1F). However, the lung lesions in hyperoxia + Dxp group were significantly lower compared with the hyperoxia group ($p < 0.001$). In the hyperoxia + Dxp group, lesions were not completely ameliorated and degenerative changes such as hemorrhage and enlarged alveoli were still present (Figure 1G). However, the view of connective tissue around the bronchiol in this group was similiar to that of the hyperoxia group (Figure 1H).

We have observed prominently increased median alveol diameters in the hyperoxia group compared to the control groups ($p < 0.001$). However, the median alveol diameters statistically decreased in the hyperoxia + Dxp group in comparison to the hyperoxia group ($p < 0.001$).

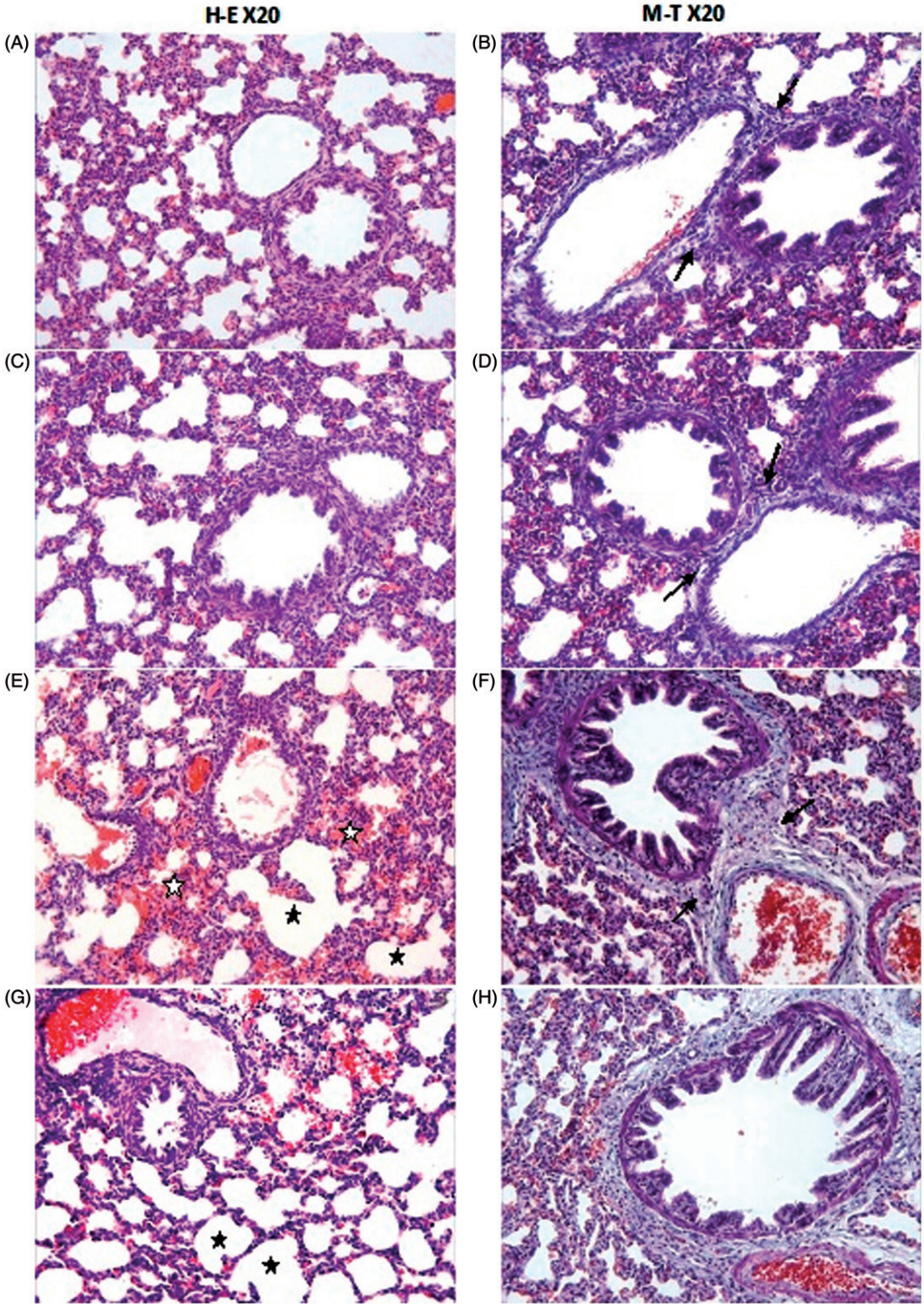

Figure 1. Control (A and B) and control + Dxp (C and D) groups. (A and C) show normal alveoli and interstitial tissue; (B and D) show the view of connective tissues (indicated by blue staining; arrows). In the hyperoxia group, (E) hemorrhage of the parenchyma (white asterisk), marking the enlargement of alveolar walls (black asterisk) and (F) bundles of collagens can be identified around the blood vessels and bronchiol (arrows). (G) Hemorrhage (arrows) and enlarged alveoli (asterisk) are still present in the hyperoxia + Dxp group. (H) The view of connective tissues around the bronchiol is similiar to that of the other groups.

Another notable finding in the hyperoxia group was the increased number of alveolar macrophage compared to the control and control + Dxp groups (Figure 2A–C). However, the amount of alveolar macrophage has significantly decreased in the hyperoxia + Dxp group [2 (0–12)] in contrast to the macrophage in the hyperoxia group [3 (0–16)] ($p < 0.001$) (Figure 2D).

The results of semiquantitative histological scores and the number of alveolar macrophage in all groups are shown in Table 2.

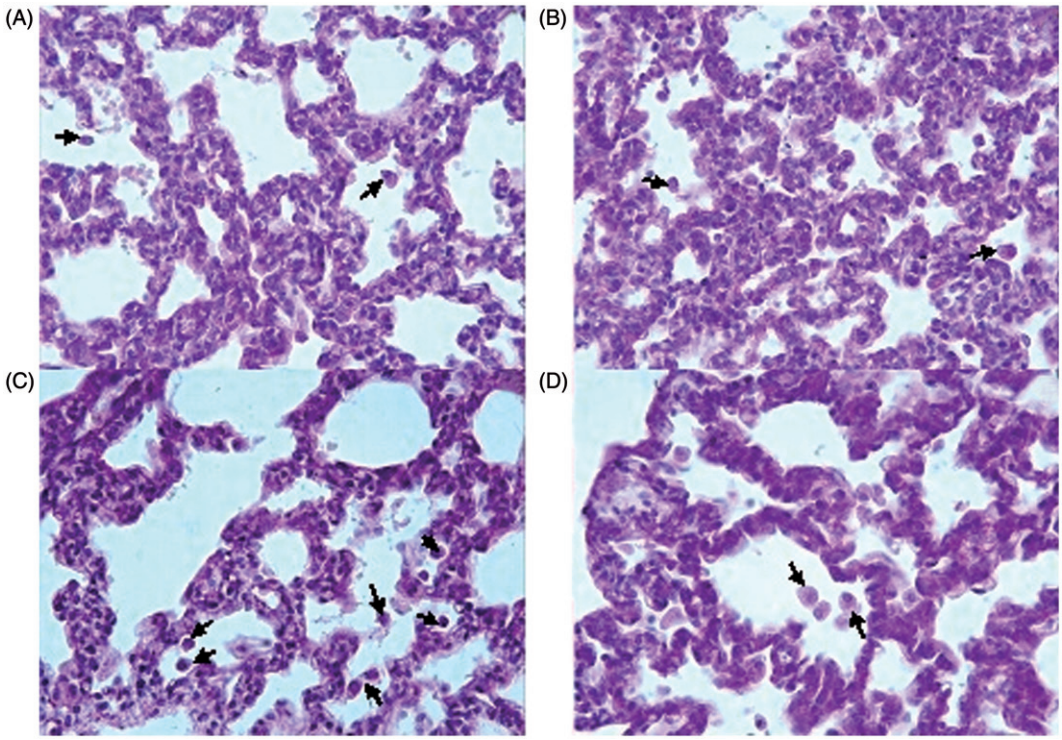

Figure 2. Note the alveolar macrophages seen as alveolar lumen in the control (A) and control + Dxp (B) groups (arrows). Numerous alveolar macrophages are observed in the hyperoxia group (C) (arrows). The number of alveolar macrophages in the hyperoxia + Dxp group (D) has decreased compared to the hyperoxia group (arrows); periodic acid schiff: ×40.

Table 2. The results of semiquantitative histological assessment and the number of alveolar macrophage in all groups.

Parameters	Control	Control + Dxp	Hyperoxia	Hyperoxia + Dxp
Hemorrhage	0 (0–1)	0 (0–1)	1 (0–3)[a]	0 (0–2)[b]
Enlarged alveoli	0 (0–1)	0 (0–1)	1 (0–3)[a]	0 (0–2)[b]
Increase in connective tissues	0 (0–0)	0 (0–0)	0 (0–1)[c]	0 (0–0)[c,d]
Alveol diameter, μm	48 (28–64)	43 (24–61)	59 (41–88)[a]	51 (36–78)[b]
Number of alveolar macrophage	0.5 (0–7)	0 (0–8)	3 (0–16)[a]	2 (0–12)[b]

Data were presented as median (min–max).
[a]Significant increase ($p < 0.001$) versus control.
[b]Significant decrease ($p < 0.001$) versus hyperoxia.
[c]Insignificant change ($p > 0.05$) versus control.
[d]Insignificant change ($p > 0.05$) versus hyperoxia.

Discussion

This study is the first to describe the effects of Dxp in an experimental BPD model. Throughout the study, the hyperoxic neonatal rats have demonstrated a significant improvement in terms of lung development in terms of histopathological evaluation, increased antioxidant enzyme activities (SOD, CAT, GPx, GSH), decreased lipid peroxidation and oxidative stress (MDA, TOS, OSI), and decreased inflammatory cytokines (IL-1β, TNF-α) as they were treated with Dxp.

BPD was first associated with oxygen toxicity more than 40 years ago [35]. The damage to the lung caused by oxygen toxicity appears to be mediated by ROS that are produced during the univalent reduction of molecular oxygen. Oxygen toxicity is caused by an imbalance between the production of ROS and the ability to detoxify ROS with the help of antioxidants. ROS, including superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radical, are produced by both the cellular metabolisms of molecular oxygen and the activation of neutrophils and macrophages exposed to inflammatory mediators. ROS cause oxidative stress by apoptosis and oxidation of lipids, proteins and DNA. They may also function as signaling molecules that mediate biological responses. Evidence suggests that the presence of an oxidant (ROS)-antioxidant (SOD, GPx, GSH) imbalance in lungs results in inevitable risk of BPD [36–38].

Dxp is oxidized enzymatically to PA, which is widely distributed in tissues. PA protects tissues against cell damage caused by ROS [14,15,39]. It has been demonstrated that PA supports cellular antioxidant systems, including GSH, GPx, SOD, CAT and other enzymatic reactions that prepare the host for encountering physiopathological conditions mediated by ROS [14,15,39]. GSH and GPX are the major defense systems against lipid peroxidation and oxidative stress [39].

SOD and CAT are the antioxidant enzyme components of the defense mechanism against ROS activities [20]. In our study, we found out that the median SOD activity in the hyperoxia group was clearly less than those in the control + Dxp and hyperoxia + Dxp groups. We also found that tissue CAT, GPx and GSH activities in the hyperoxia group were significantly lower than those of the control and control + Dxp groups, whereas these activities manifested a tendency to increase in rats receiving the Dxp therapy. Thus, through an enhanced antioxidant enzyme activity, Dxp may be the reason for a possible beneficial effect in reducing tissue damage.

PA significantly attenuates serum lipid peroxidation [39]. At this point, MDA, the last product of lipid breakdown caused by oxidative stress, is considered to be a good indicator of free radical-induced lipid peroxidation. In our study, tissue MDA levels has significantly increased in the hyperoxia group and while it has decreased in the treatment group. In a testicular ischemia–reperfusion study, Etensel et al. found Dxp to be useful in reducing lipid peroxidation, which translates into protection against the sequences of oxidative stress that may amplify the inflammatory response [18]. Correspondingly, we could not identify any significant differences between the median TOS and OSI levels, both of which have given us clues about the oxidant status and oxidative stress, in the control, control + Dxp and hyperoxia + Dxp groups ($p > 0.05$), while the low values for the same parameter in the hyperoxia group were quite revealing ($p < 0.05$). TAC levels, however, were similar in all the groups. Recently, Sandal et al. reported that TOS and OSI indexes after the hydrocortisone treatment were significantly lower than those before the treatment with hydrocortisone [40]. Our results demonstrated the protective role of Dxp in BPD development through its inhibitory action on oxygen-derived free radicals due to the increasing cellular level of CoA [16].

Both hyperoxia and inflammation act via identical receptors such as Toll-like receptor 4 and nuclear factor kappa B (NF-κB). NF-κB regulates the cellular response for inflammatory and oxidant stress [41]. The inflammatory and oxidant stress-induced activation of NF-κB impair branching morphogenesis in the developing lung [42,43]. In addition to this, IL-1β and TNF-α activation of NF-κB disrupts normal expression of fibroblast growth factor-10 in fetal lung mesenchyme and inhibits lung morphogenesis [43]. Our study has demonstrated that pups in the control and control + Dxp groups had lower median levels of TNF-α, IL-1β in lung homogenate along with fewer alveolar macrophages than those pups in the hyperoxia group. More importantly, we have observed that median levels of TNF-α, IL-1β in lung homogenate and the number of alveolar macrophage significantly decreased in the rats after the Dxp treatment. In our opinion, the inhibitory effect of Dxp on these three parameters was a consequence of the stimulation of the antioxidant defenses induced by the Dxp therapy as well as the inhibition of the NF-κB activation and ROS.

Prolonged oxidative stress arrests alveolar development in premature infants. Its histology is characterized by hemorrhage and irregularly shaped, enlarged and saccular-like air spaces surrounded by thickened septae [44]. In addition, larger alveol diameter values correspond to greater disruption of normal alveolar growth. In our study, the histologic evaluation of the four groups showed that the median lung injury scores (hemorrhage, enlarged alveoli) and alveol diameters of the hyperoxia groups were notably greater than those of the control group. Hence, adding Dxp has resulted in less emphysematous injury in the hyperoxia plus Dxp group compared with the hyperoxia group. We suggest that antioxidant and anti-inflammatory activities of Dxp have the potential to give substantial results in the treatment of ongoing alveolarization.

There were, however, a number of limitations to our study. First, it should stated that the amount of oxygen used in our experimental BPD model was actually considerably higher than the amount necessary for the clinical development of BPD in neonatals. Second, our model has eleminated other possible risk factors (such as mechanical ventilation, infection and immaturity) and has solely depended on large amount of oxygen in the creation of BPD. Lastly, and correspondingly, this hinders the possibility of identifying the relationship between Dxp, the basic means of our treatment, and other risk factors.

Conclusion

This study is the first report to demonstrate the beneficial effects of Dxp on alveolarization, lung oxidative stress and inflammation in neonatal rats with hyperoxic lung injury. This study emphasises Dxp's antioxidant and anti-inflammatory potential in the treatment of BPD in premature infants. However, further studies are needed to elucidate the underlying mechanisms and beneficial effects of Dxp therapy on BPD.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The authors are also grateful to Inonu University Department of Scientific Research Projects, for it was financial support.

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