EXPERIMENTAL STUDY

The influence of hyperoxia on cough reflex intensity in guinea pigs treated with bleomycin

Brozmanova M, Hanacek J, Tatar M, Szepe P

Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia. **brozmanova@jfmed.uniba.sk**

Abstract

Inhalation of high concentration of oxygen produces a lung injury in men and experimental animals. In our previous experiment we have found suppression of cough reflex in healthy guinea pigs after an exposure to 100 % O_2 for 60 hours. This study was designed to find the effect of hyperoxia on cough reflex in guinea pigs with lungs damaged by bleomycin. We used 48 animals (300–400 g) in two separated experiments. 32 of them were intratracheally injected with 1.5 mg bleomycin (Bleocin, Nippon Kayaku Co., Ltd., Tokyo, Japan) for induction of lung damage according to the method described by Parizada et al (20). 16 animals were given saline, only (control). Animals of experimental group were divided into two subgroups according to the lapse of time from bleomycin application. 13 days after bleomycin application animals of the 1st subgroup (16) were exposed to 100 % O_2 (8) or to room air (8) for 48 h. Similarly, 20 days after bleomycin application guinea pigs of the 2nd subgroup (16) were exposed to 100 % O_2 (8) or air (8), respectively. Cough was provoked in conscious animals placed in bodyplethysmograph box by inhalation of citric acid aerosol (0.3 mol/L) before, then 13 or 20 days after bleomycin application, and finally at the end of 48-h exposition to 100 % O_2 (air). The number of coughs was counted from airflow trace recorded by pneumotachograph. Cough was also induced by mechanical stimulation of laryngopharyngeal (LPh) and tracheobronchial (TBr) region in anaesthetized animals (Urethane, 1.1 g/kg, i.p.) just after the end of oxygen exposition and was evaluated from the interpleural pressure record. The results have shown a tendency to inhibition of citric acid cough reflex in animals 13 days treated with bleomycin and exposed to 100 % O_2, and significant decrease in citric acid induced cough in animals 20 days treated with bleomycin and exposed to 100 % O_2. Significant changes were present in cough intensity induced by mechanical stimulation of TBr region of the guinea pigs airway treated with bleomycin and exposed to oxygen, too. ***(Tab. 1, Fig. 3, Ref. 29.)***
Key words: hyperoxia, bleomycin, citric acid cough, mechanically induced cough.

Long-term oxygen therapy has a vital role in surviving in pulmonary critically ill patients such as chronic obstructive pulmonary disease, diffuse interstitial lung disease, cystic fibrosis or pulmonary neoplasia. The goal of oxygen therapy is to correct hypoxemia and prevent tissue hypoxia, thus increasing survival and improving quality of life (6, 24). On the other hand we should not forget that oxygen breathing is not only a medical therapy that can be lifesaving, but it can be also dangerous. Pulmonary normobaric oxygen toxicity is well described in many animal species (8, 18). Oxidative stress, through the generation of excess reactive oxygen species, is thought to play the major role in this condition (18). In animals or patients with previous lung injury it is more difficult to delineate whether pathologic pulmonary lesions might result from hyperoxia or primary lung insult (5).

There is also a considerable evidence that oxidative stress and reactive oxygen species play a key role in the pathogenesis of chronic inflammatory airways diseases (2), immune-mediated tissue injury and bleomycin induced lung fibrosis (13).

Bleomycin is an antibiotic and antineoplastic agent and thus represents a group of glycopeptides produced by Streptomyces verticullis that are used as chemotherapy in treatment of cancer. The use of bleomycin as an antineoplastic drug is, however, limi-

Department of Pathophysiology and Department of Pathology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Address for correspondence: M. Brozmanova, RND, PhD, Dept of Pathophysiology JLF UK, Sklabinska 26, SK-037 53 Martin, Slovakia. Phone: +421.43.4238213, Fax: +421.43.4134807

Tab. 1. Experimental protocol.

Phases	Steps	Timing and procedures	No of animals	Obtained parameters
1st	1	**3 days before BL or saline administration** - Induction of citric acid cough (1st cough challenge)	48	Initial number of citric acid coughs
	2	**BL or saline administration** - Experimental group: application of BL - Control group: application of saline	48 32 16	
2nd	3	**1st experiment** - 13 days after BL or saline application - Induction of citric acid cough (2nd cough challenge) - Creation of 3 groups of animals: A group: BL+O_2 B group: BL+air C group: saline+air (sham) - Exposure of A group to 100% O_2 for 48 h in glass cages - Exposure of B group to air for 48 h in glass cages - Exposure of B group to air in animal care facility	24 24 24 8 8 8	 Number of citric acid coughs 13 days after BL or saline administration Breathing rate Breathing rate
	4	**Immediately after exposure to 100% O_2 or air was finished** - Induction of citric acid cough (3rd cough challenge) - Mechanical stimulation of LPh mucosa - Mechanical stimulation of TBr mucosa	 24 24 24	 Number of citric acid coughs Number of mechanically induced coughs
3rd	5	**Killing of animals, taken samples of trachea, bronchi and lungs for histological examination**	24	Results of histological assessment
2nd	6	**2nd experiment** - 20 days after BL or saline application - Induction of citric acid cough (2nd cough challenge) - Creation of 3 groups of animals: A group: BL+O_2 B group: BL+air C group: saline+air (sham) - Exposure of A, B, C group to O_2/air as in 1st experiment	24 24 24 8 8 8	 Number of citric acid coughs 20 days after BL or saline administration Breathing rate in A and B group
	7	**Immediately after exposure to 100% O_2 or air was finished** - Induction of citric acid cough (3rd cough challenge) - Mechanical stimulation of LPh mucosa - Mechanical stimulation of TBr mucosa	 24 24 24	 Number of citric acid coughs Number of mechanically induced coughs
3rd	8	**Killing of animals, taken samples of trachea, bronchi and lungs for histological examination**	24	Visual and manual assessment of lungs

ted because it produces a dose dependent pneumonitis, which often progresses to interstitial pulmonary fibrosis in humans. The precise mechanisms of this lung injury, however, are not yet understood in details. The lung injury induced by bleomycin in animals is a well-characterized morfological and biochemical model of human pulmonary fibrosis (10, 29). The lung injury in this model is progressive, characterized by an initial alveolitis with edema and recruitment of inflammatory cells (eosinophills, neutrophills and lymphocytes) into the air spaces, followed by fibroblast proliferation during the second phase after agent administration (10, 29).

There is the unresolved question whether overproduction of reactive oxygen species induced by inhalation of higher oxygen concentration is able to potentiate primary tissue damage originating from primary lung pathologic changes (e.g. infectious, toxic) in men and animals.

We know almost nothing up to now about the influence of long-term oxygen breathing on the defensive respiratory reflexes. The results of our previous experiment have thrown first light to this problem (16). We have shown suppression of cough reflex in healthy guinea pigs exposed to 100 % oxygen for 60 hrs. The inhibitory effect of long-term oxygen breathing on cough reflex has occurred in our other experiment in guinea pigs with allergic airway inflammation (3), too.

Another experimental evidence suggests that cough reflex has become significantly changed in animals with airway inflammation (15) as well as in patients suffering from different airway and lung diseases (7, 11, 21, 28).

On the basis of the available information we supposed that combination of lung tissue injury and lung exposition to high oxygen concentration (two damaging factors enhancing reactive oxygen generation) should probably potentiate the damaging effect to lung tissues including nerve endings localized in airway mucosa, which are responsible for the production and modulation of cough.

We tested the mentioned hypothesis by presented experiments.

Methods

Animals

The study was performed on 48 adult female Trik guinea pigs weighing 300–400 g. They were divided into two separated

experiments according the lapse of time (13 or 20 days) from bleomycin (BL) injection. In each experiment animals were divided into three groups. The animals of A group (8 animals) were treated with bleomycin and exposed to 100 % oxygen ($BL+O_2$), the animals of B group (8 animals) were treated with bleomycin and exposed to room air (BL+air) and 8 animals of C group were treated with saline and exposed to room air (sham). Except during the time of experimental procedures the animals were kept in the animal care facility with food and water ad libitum and with standard air conditioning system.

Experimental protocol is illustrated on the Table 1.

Model of bleomycin-induced lung injury

In the first phase of our experiments we applied bleomycin to 32 animals using the method described by Parizada et al (20). Animals were injected intratracheally with a single dose of 1.5 mg bleomycin in 0.5 ml saline (Bleocin, Nippon Kayaku Co., Ltd., Tokyo, Japan). Bleomycin solution was prepared immediately before the administration and was administered as a single dose directly into the trachea under light anesthesia with 5 % Narkamon (Spofa, 150 mg/kg, i.p.). The trachea was exposed via a small cervical skin incision and punctured with a needle for administration of bleomycin. Then we carried out a simple suture and a wound toilet with the standard procedures.

The control group of animals (16) was given an intratracheal injection of 0.5 ml saline, only (sham experiment) in the same condition as described above.

Exposure to oxygen/air

Thirteen days (1st experiment) or 20 days (2nd experiment) after bleomycin administration we continued with the second phase of the experiment. Animals of experimental groups were exposed to 100 % oxygen for 48 hrs continuously ($BL+O_2$) and animals of B group were exposed to room air (BL+air).

The exposures of animals to oxygen or air were performed in individual glass metabolic cages. Oxygen concentration was periodically monitored by oxygen analyser (Permolyt 3, Veb Junkalor) and found to be ~100 %. Other biophysical parameters of the cage environment were maintained at the following level: temperature 23–25 °C, humidity 60–70 %, concentration of CO_2 ~0.2 vol% (Capnograph) and concentration of O_2 in cages with ambient air ~21 %.

Control group of animals (sham experiment) was maintained in the animal care facility and freely breathed an ambient air.

Chemically-induced cough

Unanaesthetized animals were individually placed into a bodyplethysmograph box (type 855, Hugo Sachs Elektronic, Germany). To expose an animal to the aerosol, the head chamber was connected to a jet nebulizer (Pari Provocation Test I, Pari Starneberg, Germany manufacturers specification: output of aerosol 5 l/min, particle mass median diameter 1.2 μm). Cough challenge was performed using citric acid aerosol as a tussigen (Lachema, 0.3 mol/L) for 2 min. The cough was identified on the basis of plethysmograph airflow changes and measured using pneumotachograph (Godart, Germany) with Fleish head (Gould Godart Statham BV, type 18515, No 1) connected to the head chamber of a bodyplethysmograph box. The airflow was directly registered with the moving pen recorder (Multiscriptor Hellige 21) and in PC using analog-to digital converter. We counted the number of coughs from the airflow trace on the basis of sudden enhancement of expiratory airflow during 2 min of exposition and 1 min after the end of exposure and according a typical sound accompanied each cough.

Cough reflex was provoked in all animals involved in experiment 3 days before treatment with bleomycin (1st cough challenge) and the obtained data were regarded as initial cough parameters. The next provocation of cough was performed 13 (1st experiment) or 20 days (2nd experiment) after bleomycin injection (2nd cough challenge) and final cough challenge was performed after 48 hrs of exposition to 100 % O_2 or ambient air, respectively (3rd cough challenge). The number of cough expiratory efforts was used to quantify the intensity of cough reflex.

Mechanically-induced cough

Cough was also induced by mechanical stimulation of laryngopharyngeal (LPh) and tracheobronchial (TBr) mucosa using nylon fibre during 5 seconds in anaesthetized animals (Urethane, 1.1 g/kg, i.p.) after tracheostomy just after the end of exposure to hyperoxia and chemically induced cough. The number of coughs was then counted from the trace of interpleural pressure recorded by an electromanometer (Multiscriptor Hellige 21) using interpleural cannula.

Other measured parameters

During the exposure to hyperoxia or air, the respiratory rate in all animals was counted by visual observation of chest movements at two-hour intervals except for night hours. At the end of the experiment, anesthetized animals were killed by the sectioning of the aorta, and samples of trachea, bronchi and lungs were removed, fixed in 10 % formalin solution and subsequently embedded in paraffin. A transverse section was cut and stained with hematoxylin and eosin. The histopathological assessment was performed by light microscopy. The visual appearance of lung was examined and manual investigation of lung consistency was done.

Statistical analysis

The number of coughs is presented as the median and interquartile range. The data of respiratory rate are expressed as mean values ±S.E.M. Statistical analysis was performed using Kruskal Wallis One Way Analysis and Friedman test. Other used statistical tests include multiple range tests using Statgraphics version 5.0 programme. The $p<0.05$ value was considered as significant.

Results

The effects of 100 % O_2 breathing on cough reflex intensity and other parameters in guinea pigs treated with bleomycin for 13 days

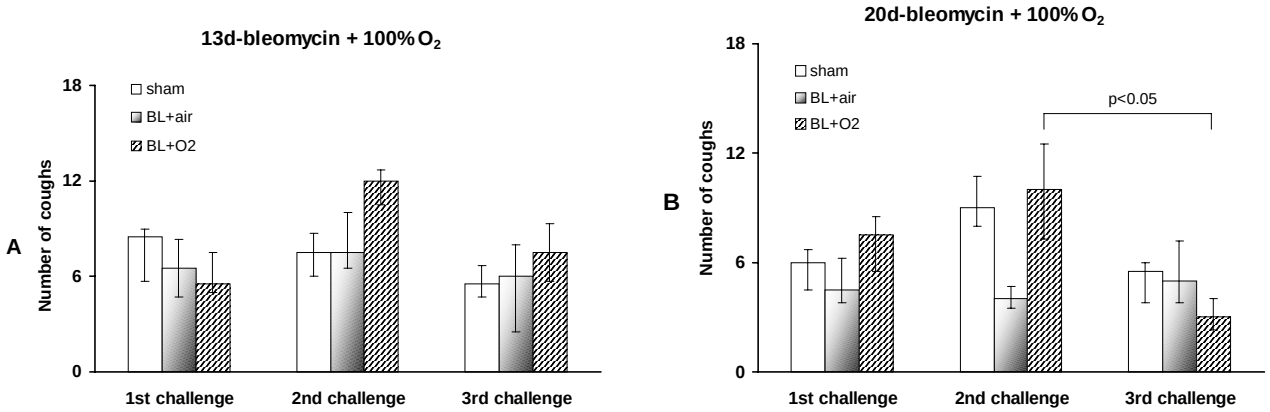

Fig. 1. Changes in citric acid cough in guinea pigs treated with bleomycin and exposed to 100 % O_2 (BL+O_2), treated with bleomycin and exposed to room air (BL+air) and control sham animals at the onset of experiment, before bleomycin treatment (1st challenge), 13 (A) or 20 (B) days treated with bleomycin (2nd challenge) and at 48 hrs of exposition to 100 % O_2/air (3rd challenge). Median and interquartile ranges. BL — bleomycin.

We found out no significant quantitative changes in chemically-induced cough among three groups of animals at the 1st, 2nd and 3rd cough challenges (Fig. 1A).

However, only in C group of animals (BL+O_2) we observed a tendency to increase the intensity in citric acid-induced cough during the 2nd cough challenge compared to its initial value at the 1st cough challenge [12 (5–16) vs 5.5 (4–8); p=0.48]. In the same group of animals 100 % O_2 breathing for 48 h did not significantly influence the citric acid-induced cough, but there was a mild tendency of decrease in the total number of coughs during the 3rd cough challenge comparing to the result obtaining at the 2nd cough challenge [7.5 (4–11) vs 12 (5–16); p=0.56].

Using the same groups of animals we did not find any significant differences in the number of coughs induced by mechanical stimulation of LPh mucosa among animals treated with BL and exposed to oxygen (BL+O_2) or animals treated with BL and exposed to room air (BL+air) or control (sham) group of animals (Fig. 2A). In cotrast to LPh-induced cough we found a tendency of decrease in mechanical-induced cough from TBr mucosa in experimental group (BL+O_2) compared to animals treated with BL and breathed ambient air (BL+air) and significant decrease comparing with control (sham) group [2 (1–4) vs 4 (2.5–6); p<0.05].

The respiratory rate showed a significant fall down in the experimental group of animals (BL+O_2) from the 26th hour of exposure to 100 % O_2 to the end of experiment (p<0.05) when compared with control animals breathing air (BL+air) (Fig. 3A).

The histological examination of samples taken from the trachea, bronchi and the lungs of the experimental group (BL+O_2) revealed pathological changes including chronic inflammation of tracheal and bronchial mucosa, emphysema and lung inflammation with aggregates of lymphocytes and hyperplasia of vessels. The convincing fibrotic changes in the lung were present in three animals, only. The intensity and quality of morphological changes in animals treated with BL and breathed air (BL+air) were very similar.

The effects of 100 % O_2 breathing on cough reflex intensity and other parameters in guinea pigs treated with bleomycin for 20 days

The values of cough reflex intensity obtained at the 1st, 2nd and 3rd cough challenges inside of groups have shown no significant differences similarly as in the first experiment. Although some differences in citric acid-induced cough at the 2nd cough challenge occurred between B (BL+air) and C (BL+O2) group of animals treated with BL for 20 days however without any significance (Fig. 1B). Finally, we determined that 100 % oxygen breathing for 48 hrs has significantly decreased citric acid cough intensity in guinea pigs treated with BL for 20 days (BL+O_2) [3 (1.5–5) vs 10 (4.5–15.5); p<0.05].

Similar results as in the 1st experiment were found in mechanically-induced cough from LPh and TBr mucosa (Fig. 2B). No significant differences occurred among three groups of animals in LPh-induced cough. We determined significant decrease in the mechanically-induced cough from TBr region in the experimental group (BL+O_2) compared with animals treated with BL and breathing ambient air (BL+air) [2 (2–4) vs 4 (3–4); p<0.05] and compared with control (sham) group of animals [2 (2–4) vs 5 (4–7); p<0.01].

Almost the same effects of BL and hyperoxia as in the 1st experiment were occurred in monitoring of respiratory rate during oxygen exposure. We observed significant fall down in respiratory rate in experimental group (BL+O_2) exposed to 100 % O_2 from the 24th hour to the end of exposure (48 h) comparing with controls (BL+air) (Fig. 3).

Discussion

Most experiments using BL-induced lung injury are regarded to morphometric determination with dose- and time-dependent effects. As compared with histologic studies, relatively little is known about airway defensive mechanisms in this condition.

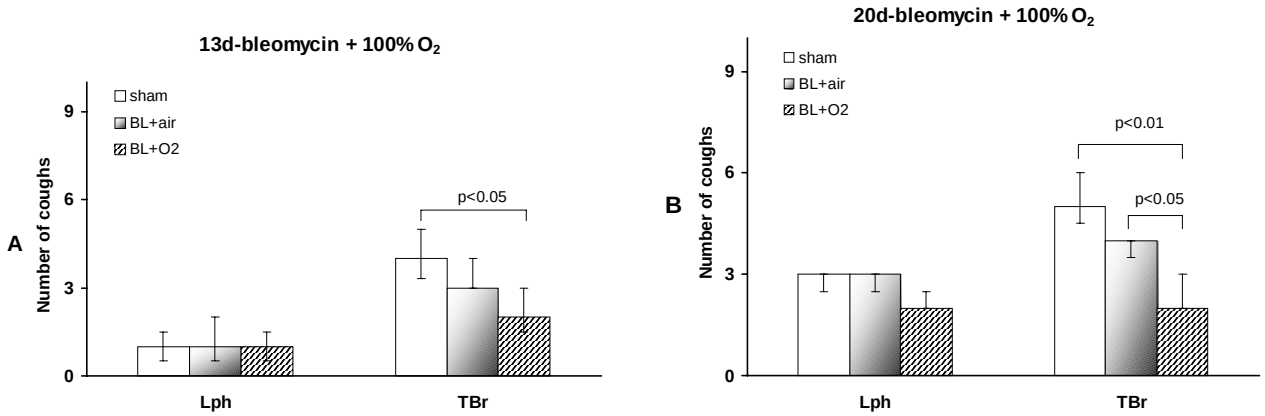

Fig. 2. Changes in mechanically induced cough in guinea pigs 13 (A) or 20 (B) days treated with bleomycin and 100 % O_2 (BL+O_2), treated with bleomycin and exposed to room air (BL+air) and control sham animals. Median and interquartile ranges. BL — bleomycin, LPh — laryngopharyngeal cough, TBr — tracheobronchial cough.

Hakkinen and co-workers (14) reported the increased risk of pulmonary fibrosis when BL as the anti-cancer drug is given together with oxygen therapy. One of the adverse reactions of bleomycin administration in humans is cough (26). Therefore one of the purposes of our study was to examine the cough reflex reactivity in BL-induced lung injury. It is known from our previous experiments (3, 16) that pure oxygen breathing leads to inhibition of cough reflex. We wonder what changes of cough reflex will be present when oxygen and BL influence the lung at the same time.

It is generally considered that idiopathic pulmonary fibrosis (IPF) begins with an inflammatory phase that progresses to fibrosis. Thereafter the pathophysiology of BL-induced pneumonia is considered to consist of two phases. First is the early inflammatory phase characterized by cellular infiltration into the interstitium and alveolar spaces. The second is the subsequent late fibrotic phase. Reactive oxygen species and proteases generated by inflammatory cells are considered to injure the lung tissue, and excessive fibrosis occurs as a reparative process (13, 27). Although no single animal model completely mimics IPF, the instillation of BL into the airways of animals, with or without oxygen exposure, leads to an acute lung injury and patchy alveolitis that progress to a chronic inflammatory state characterized by the development of pulmonary fibrosis (17). Intratracheal instillation of BL in rodents is often used as an animal model of interstitial pulmonary fibrosis as it resembles that seen in humans (12). As a model of lung damage, we used BL-induced lung inflammation described by Parizada et al (20). Most of investigators reported that the 13th–14th day from the instillation of BL seems to be a crucial time since it histologically corresponds to the proliferation of type II pneumocytes (12, 13, 22). Therefore the 1st experiment was performed on the 13th day of treating the guinea pigs with BL.

The results of our 1st experiment showed no significant differences in citric acid induced cough among three followed groups of animals before, after BL treatment or oxygen exposure. Taken together these data, only C group of animals (BL+O_2) pointed to the tendency to increase in chemically induced cough after BL treatment and mild tendency to decrease after exposure to 100 % O_2 for 48 hrs.

A mechanical stimulation of LPh mucosa did not lead to significant differences in cough intensity between animals treated with BL alone or animals treated with BL and exposed to hyperoxia. However mechanical stimulation of TBr region in animals treated with BL and hyperoxia produced a significant decrease in intensity of cough comparing with the control (sham) group.

As opposed to literature data about increased sensitivity of the cough reflex in patients with interstitial lung disease (9, 19, 21), we found no significant changes in cough response in guinea pigs after 13 or 20 days of treatment with BL. Some differences in citric acid cough between groups of animals after BL treatment during the 2nd cough challenge are seen in Figs 1A,B which demonstrate a high individual cough variability in guinea pigs. Although patients with cryptogenic fibrosing alveolitis (CFA) often reported cough, the underlying mechanisms are not clear. Doherty and co-workers in their paper (9) reported a marked increase in the cough reflex intensity using capsaicin in patients with CFA. This increase could probably be related to the reduction of lung compliance leading to the sensitisation of rapidly adapting receptors, other mechanical changes, or to the destruction of pulmonary C fibres secondary to interstitial inflammation (9).

According to histological findings of trachea, bronchi and lungs, experimental animals have shown chronic inflammation with cells infiltration. Because the convincing fibrotic changes in the lungs were only present in three animals and there were no significant changes in citric acid cough in animals treated with BL combined with hyperoxia, we decided to prolong the time of treatment with BL from 13 days to 20 days.

Comparing with the 1st experiment, the data obtained from the 2nd experiment have shown a significant decrease in citric

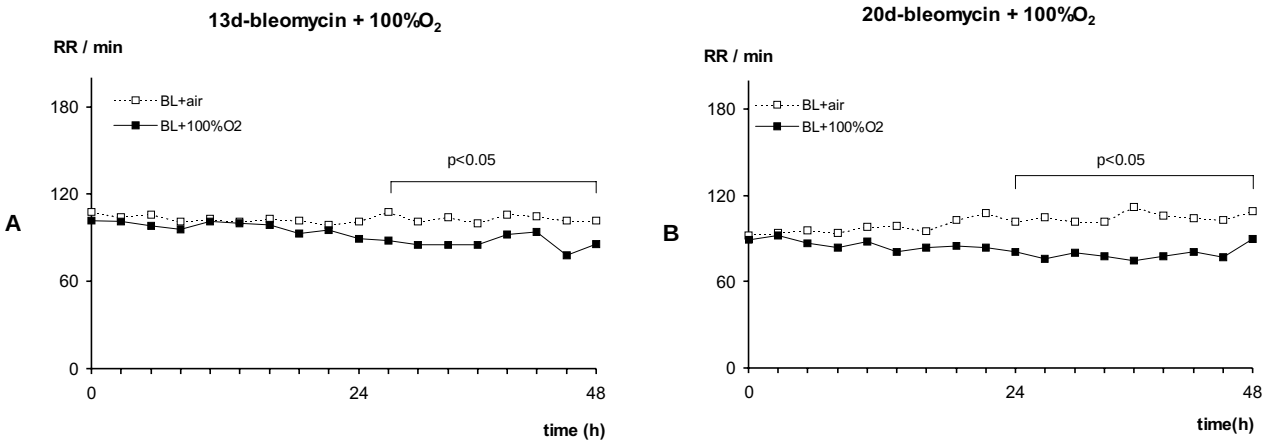

Fig. 3. The effects of exposure to 100 % O_2 for 48 hrs on respiratory rate (RR/min) in guinea pigs 13 (A) or 20 (B) days treated with bleomycin monitored in two-hour interval except night hours comparing with control animals (BL+air). BL — bleomycin

acid cough intensity in guinea pigs treated with bleomycin for 20 days and exposed to 100 % O_2 for 48 hrs.

BL alone or in combination with hyperoxia has had an inhibitory effect on tracheobronchial cough in anaesthetized animals. Laryngopharyngeal cough was not influenced by BL or hyperoxia likewise as in the 1st experiment. These results strongly suggest that BL and 100 % O_2 rather influence lower than upper airways. This effect is supported by Putman and co-workers (23) who reported different levels of antioxidants in upper and lower airways. It is generally known that BL affects dominantly lower airways. The increased level of oxidants interacts primarily with the epithelial lining fluid, a thin layer covering the epithelial cells of the lung that contain surfactant and antioxidants. In the upper airways this layer is thick and contains high level of antioxidants. Therefore oxidant injury in this area is rare and more common in the lower airways where the epithelial lining fluid is thin and contain fewer antioxidants.

As mentioned above we used two different methods to elicite cough including chemical and mechanical tussigenic stimulants under different conditions. While chemically induced cough was evoked in awake animals, mechanical stimulation was performed on anaesthetized ones. In our experiment the mechanical stimulus-inducing cough was applied to the mucosa of larynx and pharynx or trachea and large bronchi, whilst citric acid aerosol affected the whole surface of airways. Our results suggest that bleomycin-induced inflammation, and 100 % oxygen affect sensory afferent nerve endings localized rather in small than in large airways. We determined altered cough response that may be due to changes in the sensitivity of the afferent neural pathway involved in cough control.

Although histological examination was not done in the 2nd experiment we assumed that fibrotic changes highly probably were present there. Our assumption comes from functional and macroscopic changes in experimental animals. With respect to functional lung impairment there were observed changed breathing patterns with the shortening of inspirium and prolonged expirium or strenuous or exhausting breathing. According to macroscopic changes the lungs of experimental animals were smaller with rigid consistency when compared with animals from the 1st experiment.

Normobaric oxygen toxicity is well described in human and many animal species (4, 5, 8, 18). The toxic threshold of oxygen (concentration and length of exposure) in human and animals is still under debate. In patients with previous lung injury, this threshold is even more difficult to delineate as pathologic pulmonary lesions might result from hyperoxia or primary lung insult. Reactive oxygen species play a key role in the pathophysiology of oxygen toxicity (5, 8, 18). The lung toxicity of BL is associated with an increased sequestration of neutrophils, increased lipid peroxidation, increased lung hydroxylase activity and increases pulmonary vascular permeability. These findings are consistent with the earlier findings and support the hypothesis that generation of reactive oxygen species is involved in BL-induced lung toxicity (12, 14).

When monitoring the respiratory rate (RR) in guinea pigs during the exposure to 100 % O_2 we observed a significant decrease in RR from the 24th hour of exposure to the end of exposure. These results are in accordance with our previous papers (3, 16).

More recently, it was shown that oxygen might aggravate BL-induced lung damage. For example continuous exposure to 40 % oxygen significantly shortens the median survival of BL-treated mice from 8 to 4 wk (14). The similar effect was found in hamster after treatment with BL and exposure to 70 % oxygen for 3 days (25).

This observation becomes even more important in view of studies that have described the possible hazards of oxygen administration to patients after treatment with BL (1). The conclusion was drawn that BL therapy must be considered as a potential hazards in patients subsequently requiring general anaesthesia or any form of oxygen therapy. A high incidence of postoperative respiratory failure was also seen in a series of patients who underwent surgery after cancer treatment with BL (1).

In conclusion, we have found that bleomycin alone does not change intensity of chemically induced cough in guinea pigs, but combination of bleomycin and exposition to 100 % O_2 lead to significant decrease in citric acid and tracheobronchial cough intensity, as well. This finding might reflect a functional change in airway afferent nerve endings mediating cough induced by inflammatory processes.

References

1. Allen SC, Riddel GS, Butchart EG. The possible hazards of oxygen administration to patients after treatment with bleomycin. Anaesthesia 1981; 36: 60—63.

2. Barnes PJ. Reactive oxygen species in asthma. Europ Resp Rev 2000; 73: 240—243.

3. Brozmanová M, Hanáček J, Tatár M, Strapková A, Szépe P. Effects of hyperoxia and allergic airway inflammation on cough reflex intensity in guinea pigs. Physiol Res 2002; 51: 529—536.

4. Capellier G, Zhang Z, Maheu MF, Pointet H, Racadot E, Kantelip B, Regnard J, Barale F. Nasal mucosa inflammation induced by oxygen administration in humans. Acta Anaesthesiol Scand 1997; 41: 1011—1016.

5. Capellier G, Maupoil V, Boussat S, Laurent E, Neidhardt A. Oxygen toxicity and tolerance. Minerva Anestesiol 1999; 65: 388—392.

6. Chen B, Guber A, Marom Z. Advance long-term oxygen therapy. RT International 1995: 55—60.

7. Choudry NB, Fuller RW. Sensitivity of the cough reflex in patients with chronic cough. Europ Resp J 1992; 5: 296—300.

8. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: a review. Pharmacol Rev 1971; 23(2): 37—132.

9. Doherty MJ, Mister R, Pearson MG, Calverley PMA. Capsaicin induced cough in cryptogenic fibrosing alveolitis. Thorax 2000; 55: 1028—1032.

10. Dolhnikoff M, Mauad T, Ludwig MS. Extracellular matrix and oscillatory mechanics of rat lung parenchyma in bleomycin-induced fibrosis. Amer J Resp Crit Care Med 1999; 160: 1750—1757.

11. Fujimura M, Sakamoto S, Kamio Y, Matsuda T. Cough receptor sensitivity and bronchial responsiveness in normal and asthmatic subjects. Europ Resp J 1992; 5: 291—295.

12. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor on bleomycin induced accumulation of lung collagen in mice. Thorax 1993; 48: 959—966.

13. Hagiwara S, Ishii Y, Kitamura S. Aerosolized administration of N-acetylcysteine attenuates lung fibrosis induced by bleomycin in mice. Amer J Resp Crit Care Med 2000; 162: 225—231.

14. Hakkinen PJ, Whiteley JW, Witschi HR. Hyperoxia, but not thoracic X-irradiation, potentiates bleomycin- and cyclophosphamide-induced lung damage in mice. Amer Rev Resp Dis 1982; 126: 281—285.

15. Hanáček J. Changes in the intensity of cough in non-anesthetized cats with inflammation of the respiratory organs induced by croton oil (in Slovak). Bratisl Lek Listy 1986; 85: 444—450.

16. Hanacek J, Brozmanova M, Tatar M, Karcolova D, Strapkova A, Szepe P. Effect of long term high oxygen concentration breathing on defensive respiratory reflex in guinea pigs. Pulm Pharmacol 1996; 9 (5/6): 367—368.

17. Hirose N, Lynch DA, Cherniack RM, Doherty DE. Correlation between high resolution computed tomography and tissue morphometry of the lung in bleomycin-induced pulmonary fibrosis in the rabbit. Amer Rev Resp Dis 1993; 147: 730—738.

18. Klein J. Normobaric pulmonary oxygen toxicity. Anesth Analg 1990; 70: 195—207.

19. Laloo UG, Lim S, Dubois R, Barnes PJ. Increased sensitivity of the cough reflex in progressive systemic-sclerosis patients with interstitial lung disease. Europ Resp J 1998; 11(3): 702—705.

20. Parizada B, Werber MM, Nimrod A. Protective effects of human recombinant MnSOD in adjuvant arthritis and bleomycin-induced lung fibrosis. Free Rad Res Comms 1991; 15(5): 297—301.

21. Péčová R, Tatár M, Rozborilová E, Buchancová J. Citlivosť kašľového reflexu u pacientov s respiračnými ochoreniami. Stud Pneumol Phthiseol 1999; 59(3): 115—119.

22. Pozzi E, Salmona M, Masturzo P, Genghini M, Scelsi M, Spialtini L, Luisetti M. Role of alveolar phospholipids in bleomycin-induced pulmonary fibrosis in the rat. Respiration 1987; 51 (1): 23—32.

23. Putman E, van Golde LM, Haagsman HP. Toxic oxidant species and their impact on the pulmonary surfactant system. Lung 1997; 175: 75—103.

24. Tarpy SP, Celli BR. Long-term oxygen therapy. The new England journal of Medicine 1995; 14: 710—714.

25. Toledo CH, Ross WE, Hood CI, Block ER. Potentiation of bleomycin toxicity by oxygen. Cancer Treat Rep 1982; 66: 359—362.

26. Umezawa H. J Antibiotics 1968; 21: 379.

27. Venketasen N, Ebihara T, Roughley PJ, Ludwig MS. Alteration in large and small proteoglycans in bleomycin-induced pulmonary fibrosis in rats. Amer J Resp Crit Care Med 2000; 161: 2066—2073.

28. Wong CH and Morice AH. Cough threshold in patients with chronic obstructive pulmonary disease. Thorax 1999; 54: 62—64.

29. Yasui H, Gabazza EC, Tamaki S, Kobayashi T, Hataji O, Yuda H, Shimizu S, Suzuki K, Adachi Y, Taguchi O. Intratracheal administration of activated protein C inhibits bleomycin-induced lung fibrosis in the mouse. Amer J Resp Crit Care Med 2001; 163: 1660—1668.

Received January 20, 2004.
Accepted January 31, 2004.