

Effect of 0.25 ppm Ozone exposure on pulmonary damage induced by bleomycin

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ABSTRACT

To study the effect of ozone in a chronically damaged lung, we used a bleomycin (BLM) induced pulmonary fibrosis model. Both endotracheal instillation of BLM and O₃ exposure both produce lung inflammation and fibrosis. Oxidative stress would be a common mechanism of damage for both BLM and O₃. Our aim was to assess lung injury induced by 5 and 60 days of intermittent exposure to 0.25 ppm O₃ in rats with bleomycin-induced pulmonary fibrosis. Thirty-day-old Sprague Dawley rats were endotracheally instilled with BLM (1 U/100 g body weight) and, 30 days later, exposed to 0.25 ppm O₃ (0.25 ppm 4 h per day, 5 days a week). Histopathology controls were instilled with saline and breathing room air. Histopathological evaluation of lungs was done 5 and 60 days after O₃ exposure. BLM-induced lung damage did not change after 60 days of intermittent O₃ exposure. Five days of O₃ exposure increased the mean score of BLM-induced pulmonary inflammation and fibrosis (p=0.06). Frequency of bronchopneumonia increased from 1/7 to 6/6 (p <0.001), suggesting that a short-term exposure to O₃ in a previously damaged lung might be a risk factor for developing further lung injury.

Key terms: bleomycin, histopathology, lung damage, ozone exposure.

INTRODUCTION

Because individuals with chronic lung disease are a high-risk group for the effects of air pollutants (Bascom, 1996; Oyarzún et al., 1998), experimental animal models of pulmonary emphysema (Yokoyama et al., 1987) and airway inflammation (Last et al., 2004) have been used to study the effects of ozone (O₃). Another suitable model may be bleomycin-induced pulmonary fibrosis (F-BLM) in rats (Dussaubat et al., 1995, Cooper 2000, Borzone et al., 2001). Bleomycin (BLM) is an antineoplastic glycopeptidic whose cytotoxic effect is related to DNA binding and cleaving (Umezawa, 1974). BLM generates reactive oxygen species (Cooper et al., 1986) that induce DNA strand breaks (Zhang et al., 1997). Endotracheally instilled BLM produces lung inflammation within 24

hours followed by a patchy pulmonary fibrosis 14 days after exposure.

Several pharmacological agents can reduce F-BLM in rats and mice, among others indomethacin (Thrall et al., 1979), prednisone, allopurinol, and cyclosporin A + superoxide dismutase (Oyarzún et al., 1995); antioxidants superoxide dismutase (Tamagawa et al., 2000) and N-acetyl-L-cysteine (Jamieson et al., 1987); and the redox-active protein thioredoxin (Hoshino et al., 2003). O₃ exposure in rats also produces pulmonary damage: inflammation and fibrosis, through an oxidative stress. The interaction of O₂ species with essential biomolecules can lead to changes in their structure and function, if these prooxidants are not adequately balanced by a similar rate of cellular antioxidants, which constitutes the molecular basis of oxidative stress phenomenon (Fernández et al., 1996).

Our aim was to assess lung injury induced by intermittent exposure to O₃ in rats with F-BLM. We try to test the hypothesis that chronically injured lung might show more damage than healthy lung as a response to O₃ exposure depending on time exposure.

MATERIAL AND METHODS

Sprague-Dawley rats, 30 days old, weighting 110 g in average, received under anesthesia (ether and 2% lidocaine subcutaneous), a single intratracheal dose of 1 U/100 g body weight of BLM (Pharmacia-Upjohn). Control rats received intratracheal saline sterile solution (0.9 % sodium chloride: 0.2 ml/ 100 g body weight) instead of BLM. Thirty days later, rats were exposed in a chamber to 0.25 ppm O₃, 4 h per day during 5 days a week in two different periods 5 or 60 days. An O₃ generator (Ozocav ZT 21, Interozone SA) was used as O₃ supplier of the chamber and a monitor (Anseros, Werk, Tübingen, Germany) allowed us to measure O₃ concentration of the air chamber at 20 to 22°C and a relative humidity of 50 to 54%.

Experimental protocols

1.- Five days O₃ exposure: rats were separated in 4 different series: a) saline breathing room air (n= 6); b) saline + ozone (n=6); c) BLM breathing room air (n=7); and d) BLM + ozone (n = 6). Thirty-five days after intratracheal instillation of either BLM or saline and 20 hours after completing the last O₃ exposure, the rats were killed by abdominal aorta exsanguination under deep ether anesthesia.

2.- Sixty days of O₃ exposure: rats were divided in the same 4 different series described above: a) n= 7 ; b) n=7 ; c) n=5; and d) n = 7. Rats were killed as previously described 90 days after instilling them with BLM or saline solution.

Histopathological analysis

Lungs were removed, inflated, and fixed under 20 cm H₂O of airway pressure with

10% buffered formalin. Histological sections stained with hematoxylin-eosin and Masson's trichrome were examined by a pathologist (SG) under light microscopy without knowing the treatment series. To assess intensity of inflammation and fibrosis, we used a score system: 0 absent, 1 mild, 2 moderate, 3 intense, and 4 very intense. Emphysema's score (0 to 9) resulted from adding the following features: dilatation and enlargement of air spaces (0 to 3; being 0 absent, 1 mild, 2 moderate, and 3 intense); thickness and fragmentation of alveolar septa (0 to 3); and interstitial fibrosis (0 to 3).

The histopathological scores were compared by using Mann-Whitney's, Kruskal-Wallis' and Newmann-Keuls' tests. Other specific lung lesions were analyzed by using χ^2 test. A p value ≤ 0.05 was considered as statistically significant.

RESULTS

Short-term (5 days) ozone exposure

In rats breathing room air, BLM produced a very mild fibrosis and a mild inflammatory cells infiltration (Fig 1a). Emphysema was absent, and only one of the 7 rats presented bronchopneumonia (Table I).

In the series of rats treated with BLM + O₃, emphysema was absent, focal lung interstitial fibrosis was mild, and although the inflammatory reaction was mild to moderate, they were not significant different as compared to the scores of rats treated with BLM + air (p=0.06; Mann-Whitney's test, Table I and Fig 1b). Bronchopneumonia was present in all the rats of this series. In two rats, bronchopneumonia was hemorrhagic (Fig 1c), and in another one, a suppurative necrotizing granuloma also was observed. Detachment of bronchiolar epithelium was additionally observed in 4 out of 6 rats of the series (Table I, Fig 1d).

Detachment of bronchiolar epithelium was observed in all the rats instilled with saline solution and exposed to O₃, however pulmonary inflammation, fibrosis or emphysema were absent (Table I).

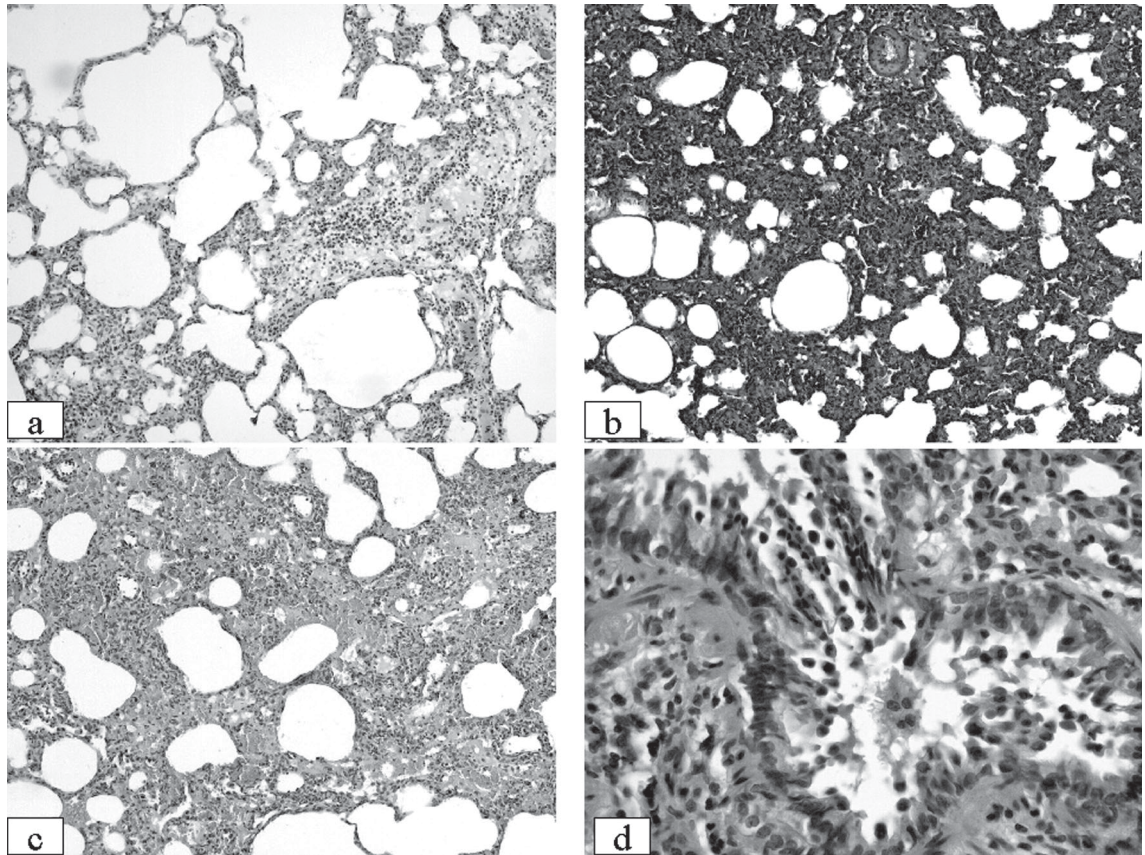


Figure 1. Histopathology in short-term experiments, series BLM + O₃; a) Lung inflammatory reaction (H-E x 80); b) Focal lung fibrosis (Masson's trichromic stain x 80); c) Hemorrhagic bronchopneumonia (H-E x 80); d) Detachment of bronchiolar epithelium (H-E x 200).

Long-term (60 days) ozone exposure

In rats breathing room air, BLM produced a intense focal fibrosis and a moderate inflammation. Emphysema score was very low and was not statistically significantly different as compared to controls (Table II).

In rats treated with BLM + O₃, lung interstitial fibrosis and inflammation had an average score similar to that found in rats treated with BLM + air (Table II). Frequency of bronchopneumonia was similar in BLM + air and BLM + O₃ series (Table II).

Rats exposed to O₃ and instilled with saline exhibit a similar average score of inflammation and fibrosis as controls breathing air that was significantly lower than the average scores of inflammation and fibrosis found in the series treated with BLM

breathing O₃ or room air (Table II). Bronchopneumonia was present only in one out of 7 rats of the series instilled with saline and subjected to O₃ exposure (Table II).

DISCUSSION

Short-term ozone exposure

The histology of lung injury after 35 days of BLM administration was not different from previously published data for similar periods (Cooper et al., 1986; Snider et al., 1978; Thrall et al., 1979). The main effect of 5 days of O₃ exposure in F-BLM was to increase the frequency of bronchopneumonia from 1/7 (14%) to 6/6 (100%), suggesting that acute exposure to O₃ in a previously damaged lung might enhance a further

pulmonary injury. The reduction of alveolar phagocytosis (Canning et al., 1991) and the increase in transmucosal permeability (Bhalla, 1999) induced by O₃ exposure may help to explain this effect.

Ozone exposure after instilling saline did not induce pulmonary inflammation or fibrosis or emphysema. However, detachment of bronchiolar epithelium was detected in most of the rats exposed to O₃ and instilled with saline (6/6) or with BLM (4/6) (Table I). In long-term exposure to O₃, all the rats with O₃, BLM or BLM+O₃ presented this lesion, meanwhile its frequency in controls was 2/5 (Table II).

Similar lesion was shown in rats exposed to 3 ppm O₃ for 4 hours (Plopper et al., 1973) and in macaques exposed to 0.15 or 0.30 ppm O₃ 8 h/day for 60 or 90 days (Harkema et al., 1993). Oxygen free radicals induced by high oxygen concentrations or by O₃ might be involved in the mechanism causing this change. Hyperplasia of bronchiolar associated lymphatic tissue seems to be more related to bleomycin than to O₃ exposure effects, because it was present in most of the rats treated with BLM + air or O₃, being less frequent in controls series (saline + air or saline + O₃).

TABLE I

Histopathological lesions found in the lungs of rats treated with bleomycin and subjected to short-term 0.25 ppm ozone exposure

	Saline + room air (n = 6)	Saline + ozone (n = 6)	BLM + room air (n = 7)	BLM + ozone (n = 6)	p
Inflammation score (0-4)	0	0	1.1	1.67	0.06 Mann-Whitney's test #
Fibrosis score (0-4)	0	0	0.14	1	0.06 Mann-Whitney's test #
Emphysema score (0-9)	0	0	0	0	n.s.
Detachment of bronchiolar epithelium *	0/6	6/6	1/7	4/6	0.001 χ^2
Broncho-pneumonia *	0/6	0/6	1/7	6/6	<0.001 χ^2

* number of cases / n # [BLM + air] vs [BLM + O₃]

TABLE II

Histopathological lesions found in the lungs of rats treated with bleomycin and subjected to long-term 0.25 ppm ozone exposure

	Saline + room air (n = 7)	Saline + ozone (n = 7)	BLM + room air (n = 5)	BLM + ozone (n = 7)	P
Inflammation score (0-4)	1.1	1.1	1.8	2.1	0.06 Kruskal - Wallis
Fibrosis score (0-4)	1.3	0.7	3	2.6	0.05 Kruskal - Wallis
Emphysema score (0-9)	1	1	0.6	0.9	n.s. Kruskal - Wallis
Detachment of bronchiolar epithelium *	2/5	7/7	5/5	7/7	<0.0001 χ^2
Broncho-pneumonia *	0/7	1/7	2/5	2/7	n.s. χ^2

* number of cases / n

The lack of inflammatory and fibrotic effect of O₃ in our short-term experiments may be explained by the development of tolerance to O₃. Such phenomenon has been described in intermittent exposure to O₃ as it was in our design. The mechanism involved in the induction of lung inflammation by O₃ exposure implies a number of chemotactic factors, cellular mediators, and cell surface-association molecules (Bhalla, 1999). Among these agents, interleukines 6 and 10 have been involved in the attenuation of lung response detected in intermittent exposure to O₃ (McKinney et al., 1998; Reinhart et al., 1999).

Long-term ozone exposure

A more intense lung fibrosis and inflammation was observed after 90 days than after 35 days of administering BLM. Once instilled, the mechanisms activated by BLM seem to be continuously acting. In addition, following BLM instillation, there was an increase of the bronchopneumonia frequency from 14% (1/7) at 35 days to 40% (2/5) at 90 days.

Sixty days of O₃ exposure in rats with BLM-induced pulmonary damage did not further increased either the intensity of pulmonary damage or the frequency of bronchopneumonia. Similarly, a lack of synergism between O₃ exposure and previous lung damage has been reported in rats with elastase-induced pulmonary emphysema (Yokoyama et al., 1987). Sequence of O₃ exposure could be determinant. In fact, when mice with ovoalbumin-induced airway inflammation also are exposed to 0.2 to 0.5 ppm of O₃, an additive response was obtained only by a simultaneous exposure to ovoalbumin and O₃, otherwise O₃ appears to antagonize the specific inflammatory response to ovoalbumin (Last et al., 2004).

In conclusion, synergism between bleomycin- and ozone-induced pulmonary damage in long-term experiments, as found in the short-term experiments in relation to bronchopneumonia, was not observed.

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