The effects of imidazole on pulmonary damage induced by bleomycin*

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Bleomycin may produce diffuse pulmonary damage. Our objective was to evaluate the effect of imidazole, a thromboxane-synthetase inhibitor, on pulmonary damage induced by endotracheal instillation of bleomycin in rats
Bleomycin 1 U/100 g body weight produced diffuse pulmonary damage and increased number of inflammatory cells after 3 days, hemorrhage and focal fibrosis after 7 days, and diffuse fibrosis and pneumocyte hyperplasia after 14 to 30 days. Imidazole 5 mg/100 g body weight, given intraperitoneally 30 min before bleomycin, decreased the 3rd day lesions without altering the histopathology in subsequent periods. Imidazole reduced ($p < 0.05$) the increases in cell number (3rd and 14th days) as well as in proteins in bronchoalveolar lavage (3rd day), without modifying the increase in phospholipids observed in rats treated with bleomycin.
We conclude that imidazole decreases initial bleomycin-induced pulmonary damage, but it does not interfere with fibrosis and late development of epithelial hyperplasia.

Key words: *bleomycin, bronchoalveolar lavage, histopathology, imidazole, pulmonary damage.*

INTRODUCTION

Bleomycin is an antineoplastic glycopeptide compound (15). Its mechanism of action on tumor cells implies DNA degradation, which requires oxygen and metallic ions (Fe) by producing a Fe^{+3}-bleomycin complex with DNA (6-10). In the presence of reduction agents, the Fe^{+3}-bleomycin complex is transformed to Fe^{+2}-bleomycin which combines with oxygen to form an "activated bleomycin", which in turn exercises its effect on tumor cells. Nevertheless, the innermost action mechanisms of activated bleomycin are still unknown (5).

Although bleomycin exhibits minimal myelosuppressive and immunosuppressive activities, it may induce lung damage and pulmonary fibrosis in human patients. Endotracheally instilled bleomycin produces an early pulmonary response of exudative type (24 hours), which is characterized by edema, hyaline membrane and increased number of cells (polymorphonuclear neutrophils, eosinophils) in pulmonary tissue and bronchoalveolar lavage. The late pulmonary response (14 days) is of proliferative type, with an increased number of macrophages and development of pulmonary fibrosis (8, 14), which persists 60

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days post-instillation (8). This pulmonary response has also been identified in patients treated with bleomycin, whose initial pneumonitis was followed by interstitial fibrosis (1, 6, 10).

With the purpose of modifying bleomycin-induced pulmonary damage, anti-inflammatory agents have been experimentally used. The administration of indomethacin, a prostaglandin synthesis inhibitor, did not diminish pulmonary vascular permeability (14 days) nor the pulmonary fibrosis, as evidenced by the increase of pulmonary collagen observed 60 days after bleomycin instillation. Another inhibitor of arachidonic acid's metabolism is imidazole, which inhibits thromboxane-synthetase (2). Imidazole pretreatment was able to diminish pulmonary edema in another experimental model of diffuse pulmonary damage, produced by iv administration of free fatty acids in the rabbit (12). Therefore, this study was intended to search if imidazole pretreatment was able to diminish the pulmonary damage produced by the endotracheal instillation of bleomycin in the rat.

MATERIALS AND METHODS

Seventy adult male Sprague-Dawley rats weighing 203.5 ± 7.7 g (x ± SD) were used. These rats were divided into four experimental series receiving the following treatments:

a) Bleomycin: 1 U of bleomycin per 100 g of body weight, dissolved in 0.3 ml of 0.9% NaCl (saline), was instilled in the tracheal lumen (n = 17).
b) Bleomycin + imidazole: 30 min before bleomycin instillation, imidazole 5 mg/100 g was administered intraperitoneally (n = 19).
c) Imidazole + saline: Imidazole 5 mg/100 g was administered i p 30 min before the intratracheal instillation of saline 0.3 ml per 100 g of body weight (n = 16).
d) Control: 0.3 ml of saline per 100 g of body weight was instilled in the tracheal lumen (n = 18).

Bleomycin was obtained from Farmitalia, Carlo Erba, and imidazole from Sigma Chemical Co.

The experimental procedures utilized have already been described in a previous report (8). In summary, after the rats were anaesthetized with ether and killed by exsanguination, the lungs were removed. The right lungs were used for obtaining bronchoalveolar lavage (BAL) fluid, and the left lungs for histopathology, lesions being evaluated by a preestablished scoring system, applied without knowing the treatment series of the rat under examination.

The right lung was degassed and underwent BAL with saline, at total lung capacity 4 times and at 4°C. The BAL fluid was subjected to total and differential cell count, and determinations of proteins (11) and total phospholipids content (3, 4).

Results obtained were subjected to either Student's *t* tests for unpaired samples or analysis of variance (ANOVA), followed by Newmann-Keuls tests (16). A value of $p < 0.05$ was considered as significantly different.

RESULTS AND DISCUSSION

Rat growth, as evaluated by the weight curve, was inhibited by 18.5% on day 3rd by the administration of bleomycin. This effect was prevented through the previous administration of imidazole.

Bleomycin produced a mild degree of peribronchial edema. However, the principal damage was located in the alveoli: hyaline membrane, inflammatory reaction, alveolar hemorrhage, hypertrophy and hyperplasia of type II alveolar cells, associated to damage and destruction of alveolar type I cells, and interstitial fibrosis.

Pulmonary histopathology showed that imidazole decreased alveolar and interstitial edema on the 3rd day of bleomycin instillation, as well as prevented the development of hyaline membrane (Table I, Fig 1). Furthermore, lower amounts of alveolar polymorphonuclear cells and macrophages in rats pretreated with imidazole were also observed. In general, the rats receiving imidazole and bleomycin showed less pulmonary damage than those that received only bleomycin. Nevertheless, the previous administration of imidazole did

TABLE I

Histopathological assessment of the effect of imidazole on bleomycin-induced lung damage

HISTOPATHOLOGY	Controls	B	B + I	B	B + I	B	B + I
n	6	4	6	4	6	7	6
days	3-30	3		14		30	
Alveolar edema	0	2 ± 0.4*	1.3 ± 0.1	0	0.16 ± 0.1	1.7 ± 0.1	0
Hyaline membrane	0	2 ± 0.1*	0	0	0	0	0
Alveolar hemorrhage	0.1 ± 0.01	0	0.5 ± 0.1*	2 ± 0.2*	0.25 ± 0.1	0.22 ± 0.1	0.43 ± 0.1
Interstitial edema	0	3 ± 0.2*	1.3 ± 0.1	0	0	0	0.7 ± 0.2
PMN cell in AS	0.1 ± 0.01	2 ± 0.2*	1.5 ± 0.2#	1 ± 0.2*	0	0	0.7 ± 0.1*
Macrophages in AS	1 ± 0.2	2 ± 0.4*	1.1 ± 0.1	2 ± 0.2*	0.8 ± 0.2	0.43 ± 0.1#	1.1 ± 0.1*
Pulmonary fibrosis	0	0	0	2 ± 0.2	2.3 ± 0.1	1.4 ± 0.01	2 ± 0.1

Score: 0 = absent; **1** = slight; **2** = mild; **3** = intense; **4** = very intense.
AS, alveolar spaces; **B**, bleomycin; **I**, imidazole, **PMN**, polymorphonuclear (cells).
Means ± SEM's. * $p < 0.05$ incomparison to control and treated rats, at the same day. # $p < 0.05$ in comparison to control. ANOVA followed by Newmann-Keuls test.

not prevent subsequent bleomycin-induced interstitial fibrosis (14 and 30 days) (Table I and Fig 2).

The administration of bleomycin significantly increased cell count in the BAL fluid on post instillation days 3rd and 14th,

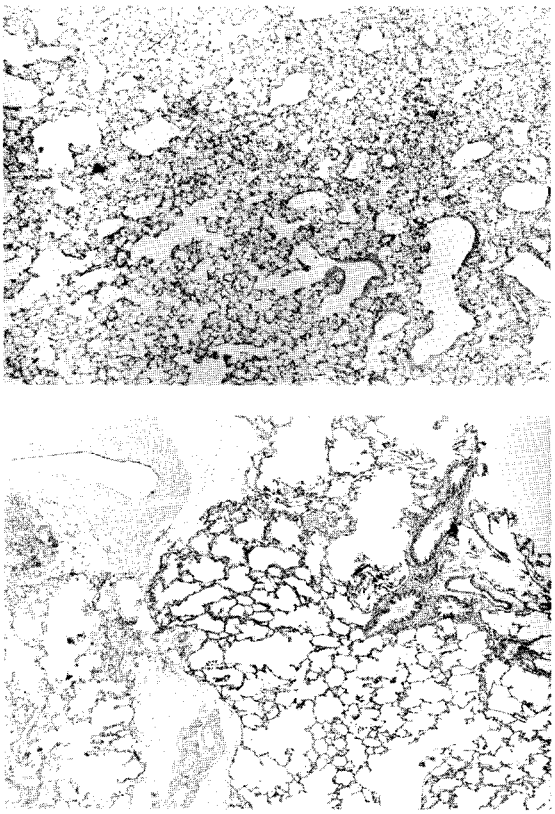

Fig 1. A. Lung histopathology of a rat after 3 days treatment with bleomycin. Hyaline membrane, alveolar edema and inflammatory cells are evident. **B.** Lung histopathology of a rat after 3 days treatment with imidazole and bleomycin. Alveolar spaces are free of edema and cells, and hyaline membrane is absent. (Hematoxylin-eosin, x10).

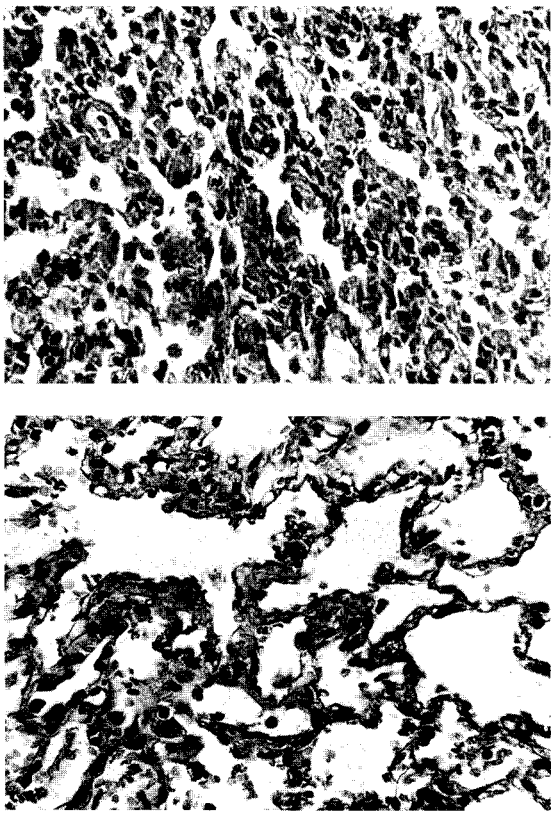

Fig 2. A. Interstitial lung fibrosis of a rat after 14 days treatment with bleomycin. **B.** Similar intensity of interstitial lung fibrosis in a rat after 14 days treatment with imidazole and bleomycin. (Van Giesson, x400). After 30 days of treatment with either bleomycin or bleomycin plus imidazole, the appearance of pulmonary fibrosis was not modified.

TABLE II

Total number of cells in bronchoalveolar lavage fluid from rats treated with bleomycin and imidazole

Series	3rd day	14th day	30th day
Bleomycin	10.8 ± 1.3* (5)	20.4 ± 4.5* (6)	3.0 ± 0.3 (6)
Bleomycin + Imidazole	2.4 ± 0.4 (7)	2.8 ± 0.7 (6)	2.6 ± 0.4 (6)
Imidazole + Saline	1.6 ± 0.1 (5)	1.5 ± 0.5 (5)	3.3. ± 0.4 (6)
Saline	3.3 ± 1.2 (6)	4.9 ± 0.5 (6)	4.9 ± 1.2 (6)

Means ± SEM's. () = n. Number of cells x 10^5/ml of BAL fluid.
* $p < 0.05$ in comparison to the other series.
ANOVA followed by Newmann-Keuls test.

with relation to the control group. The administration of imidazole prevented this effect of bleomycin (Table II). Not only did the previous administration of imidazole inhibit the cell count increase in BAL fluid observed on days 3rd and 14th, but it also modified the cell type distribution of the BAL fluid, decreasing the proportion of polymorphonuclear neutrophils from 60.2% to 34% at the 3rd day and from 30.5% to 1.3% at the 14th day post instillation, respectively (Table III). On the other hand, macrophages reached their normal proportion on day 14th, in contrast to what instillation of bleomycin alone produced. These modifications in cell type composition of the BAL fluid coincide with the histopathology, suggesting that bleomycin-

TABLE III

Differential cell count from bronchoalveolar lavage fluid from rats treated with bleomycin and imidazole

	PMN cells (%)		Macrophages (%)		Lymphocytes (%)	
days	3rd	14th	3rd	14th	3rd	14th
Bleomycin	60.2 ± 0.4* (5)	30.5 ± 5.2* (6)	16.0 ± 2.9 (5)	25.5 ± 4.1 (6)	24.0 ± 2.6* (5)	42.5 ± 7.3* (6)
Bleomycin + Imidazole	34.0 ± 5.8# (7)	1.3 ± 0.8 (7)	41.1 ± 8.1 & (7)	94.3 ± 1.9* (7)	25.0 ± 3.1* (7)	4.3 ± 1.4 (7)
Saline + Imidazole	9.4 ± 1.7 (5)	0.4 ± 0.2 (5)	76.8 ± 7.2* (5)	96.6 ± 1.0* (5)	13.8 ± 6.1 (5)	3.0 ± 1.1 (5)
Saline	8.3 ± 1.6 (6)	6.4 ± 1.9 (6)	85.0 ± 2.9* (6)	86.3 ± 3.7* (6)	6.7 ± 1.7 (6)	7.3 ± 1.9 (6)

PMN, polymorphonuclear (cells).
Means ± SEM's. ( ) = n.
* $p < 0.05$ in comparison to the other series.
# $p < 0.05$ in comparison to controls.
& $p < 0.05$ in comparison to bleomycin-treated group.
ANOVA followed by Newmann-Keuls tests

TABLE IV

Protein and total phospholipid contents in bronchoalveolar lavage fluid from rats treated with bleomycin and imidazole

	Protein (mg/100 g b wt)			Phospholipid (mg/100 g b wt)		
days	3rd	14th	30th	3rd	14th	30th
Bleomycin	15.6 ± 1.3* (5)	5.0 ± 0.6 (6)	6.2 ± 0.5 (6)	1.1 ± 0.1 (5)	1.5 ± 0.2* (5)	1.5 ± 0.1* (6)
Bleomycin + Imidazole	12 ± 2.1# (7)	9.9 ± 2.4* (6)	5.9 ± 0.9 (6)	0.8 ± 0.1 (7)	1.5 ± 0.2* (6)	1.4 ± 0.2* (7)
Imidazole + Saline	6.2 ± 0.6 (5)	5.3 ± 1.3 (5)	4.8 ± 0.6 (5)	0.7 ± 0.1 (5)	0.5 ± 0.1 (5)	0.8 ± 0.03 (6)
Saline	4.2 ± 0.7 (6)	4.1 ± 0.4 (6)	4.7 ± 0.4 (5)	1 ± 0.1 (6)	0.8 ± 0.07 (6)	(6)

Means ± SEM's. ( ) = n.
* $p < 0.05$ in comparison to the other series.
# $p < 0.05$ in comparison to controls.
ANOVA followed by Newmann-Keuls tests.

induced pulmonary inflammation was reduced by the previous administration of imidazole.

The total protein content in BAL fluid increased significantly 3 days after bleomycin instillation, in relation to the control group (Table IV). The rats receiving imidazole previous to bleomycin had a lower protein increase on the 3rd day, which however remained elevated until day 14th.

The total phospholipid content of the BAL fluid in rats treated with bleomycin was increased by 90% ($p < 0.05$) on days 14th and 30th, in relation to their respective controls and their initial level (Table IV). Previous administration of imidazole did not prevent this bleomycin effect from occurring. The results obtained, as far as pulmonary histopathology and alterations of BAL fluid (cell, protein and phospholipid contents) after bleomycin treatment, were similar to those previously reported (8, 9, 13, 14).

The fact that administration of imidazole previous to bleomycin decreased protein content at the 3rd day is in accordance with the decrease in inflammatory reactions seen in the histopathology on the 3rd day, which could indicate less initial pulmonary damage and decreased protein passage to the air spaces. The protein increase observed on day 14th in the series treated with both imidazole and bleomycin would suggest that imidazole pretreatment only partially protects from the initial bleomycin-induced pulmonary damage. This hypothesis is supported by the fact that the total phospholipid increase was not prevented and that the degree of fibrosis in the animals treated with bleomycin alone or with bleomycin and imidazole were similar on days 14th and 30th. However, administration of bleomycin alone produced an initial phase of higher intensity, as reflected by the more pronounced alveolar edema and leukocyte infiltrates, followed later by fibroblastic proliferations (7).

We administered only one dose of imidazole 30 minutes before bleomycin because of our previous observations in which imidazole pretreatment was able to diminish pulmonary edema produced by iv administration of free fatty acids in the rabbit (12). Because imidazole has a short life time, we can not discard that repeated imidazole administration could have had a better protective effect on bleomycin-induced pulmonary damage than a single dose.

The reduction of the initial bleomycin-induced lung damage by imidazole could be due to inhibition of thromboxane synthesis, which suggests the participation of thromboxane. Nevertheless, imidazole could have other effects besides inhibition of

thromboxane-synthetase. Measurement of thromboxane levels and administration of other thromboxane-synthetase inhibitors (Dazoxiben, for example) should confirm this hypothesis (12).

Finally, our results suggest that development of an intense initial pulmonary edema may not be an absolutely necessary stage for the later development of bleomycin-induced pulmonary fibrosis.

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