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ROLE OF OXYGEN FREE RADICALS IN RESPIRATORY DISTRESS INDUCED BY ARACHIDONIC ACID IN THE RAT

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The purpose of our present study is the possible implication of oxygen free radicals in the respiratory distress induced in rats by intravenous administration of arachidonic acid (20 mg/kg). The respiratory frequency was measured and plasma TXB2 concentration was assayed by RIA from blood withdrawn 1 min after arachidonic acid administration. The substances studied were: SOD, catalase, mannitol, DMSO, BHT, imidazole. All the drugs, except imidazole, significantly protect the rats from the respiratory distress induced by arachidonic acid. SOD, catalase, BHT and imidazole inhibit whereas mannitol and DMSO increase the plasma levels of TXB2. We suggest that oxygen free radicals generated in the respiratory burst induced by arachidonic acid are mainly responsible for the consequent respiratory distress.

KEY WORDS: Arachidonic acid, respiratory distress, oxygen free radicals, thromboxane, superoxide dismutase, catalase, hydroxyl radical scavengers.

INTRODUCTION

Intravenous administration of arachidonic acid is an experimental model of thrombosis. Initially proposed in the rabbit,[1-5] it has been extended to the mouse[6] the rat[7,8] and other species.[9-11]

The rat has the significant advantage that the metabolism of arachidonic acid in its lung is closer to that in the human lung.[12,13]

The respiratory alterations and death induced by arachidonic acid has been explained implicating the cyclooxygenase metabolite, thromboxane, a potent platelet aggregating and vasoconstrictor agent.[3-5,8,14] In this paper our proposal has been the study of a possible implication of oxygen free radicals in respiratory distress induced in rats by arachidonic acid. This proposal is in line with the suggestion of Kontos that the actions initially attributed to the production of arachidonic acid metabolites could be due to oxygen free radicals.[15] Our results agree with this suggestion.

MATERIALS AND METHODS

Respiratory Distress

Sprague-Dawley rats (200-250 g) were anesthetized with ethyluretane (1 gr/kg ip). Fifteen minutes later arachidonic acid (20 mg/kg) was injected in the femoral vein at

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a constant rate (1.1 ml/min). The control group was injected with 1% bicarbonate solution, the solvent for arachidonic acid.

The substances studied were: Superoxide dismutase (SOD) (40 mg/kg ip), Catalase (40 mg/kg iv), Mannitol (400 mg/kg ip), Dimethyl sulphoxide (DMSO) (5 ml/kg ip), Butylated hydroxytoluene (BHT) (350 mg/kg oral) and Imidazole (20 mg/kg ip). SOD, DMSO and BHT were administered 60 min, imidazole 30 min and catalase and mannitol 10 min before anesthesia.

The respiratory frequency was measured with two needle electrodes implanted in each side of the thorax which were connected to an impedance pneumograph and recorded.

The arachidonic acid solution (5 mg/ml) was prepared from a Sigma vial (99% pure) in a 1% sodium carbonate solution and stored at nitrogen atmosphere at −20°C, for no longer than two months. Using a spectrophotometric technique[16] we found no peroxidation during this period of time.

The results were expressed in percentages for each animal, with 100% (41.86 ± 3.6 breaths/minute) representing the respiratory rhythm measured before administration of arachidonic acid.

TXB2 Assay

Determinations of TXB2 according to Powell and Carey and Haworth.[17,18] The experimental set up was as previously described, but blood was withdrawn from the abdominal aorta 1 min after arachidonic acid administration. The blood was collected in plastic tubes containing 90 μl of 2% EDTA and 10 μl of 0.02 M indomethacine to minimize the formation of eicosanoids *in vitro*. Plasma was harvested within 30 min by centrifugation at 1200 × g for 10 minutes. The plasma was acidified with ClH 1N to pH 3–3.5 and passed through Sep-pak C18 (Waters Millipore) and eluted according to Powell.[17] The collected samples were stored at −20°C until the RIA (Seragen) was done.

The results were expressed in absolute values (pg/ml plasma); and in percentages in relation to the control group.

Statistical Analysis

Student's t-test was used for statistical comparisons.

RESULTS

The enzymes SOD and catalase, the hydroxyl radical scavengers mannitol and DMSO, and the antioxidant BHT significantly protect from respiratory distress induced in rats by arachidonic acid. Imidazole induces a certain but not significant protection (Figures 1 and 2).

SOD, catalase, BHT and imidazole inhibit TXB2 production whereas mannitol and DMSO significantly increase the plasma levels of TXB2 (Table I).

DISCUSSION

Our results show that the enzymes and scavengers assayed, all related to oxygen free radical generation, protect against respiratory distress induced by arachidonic acid,

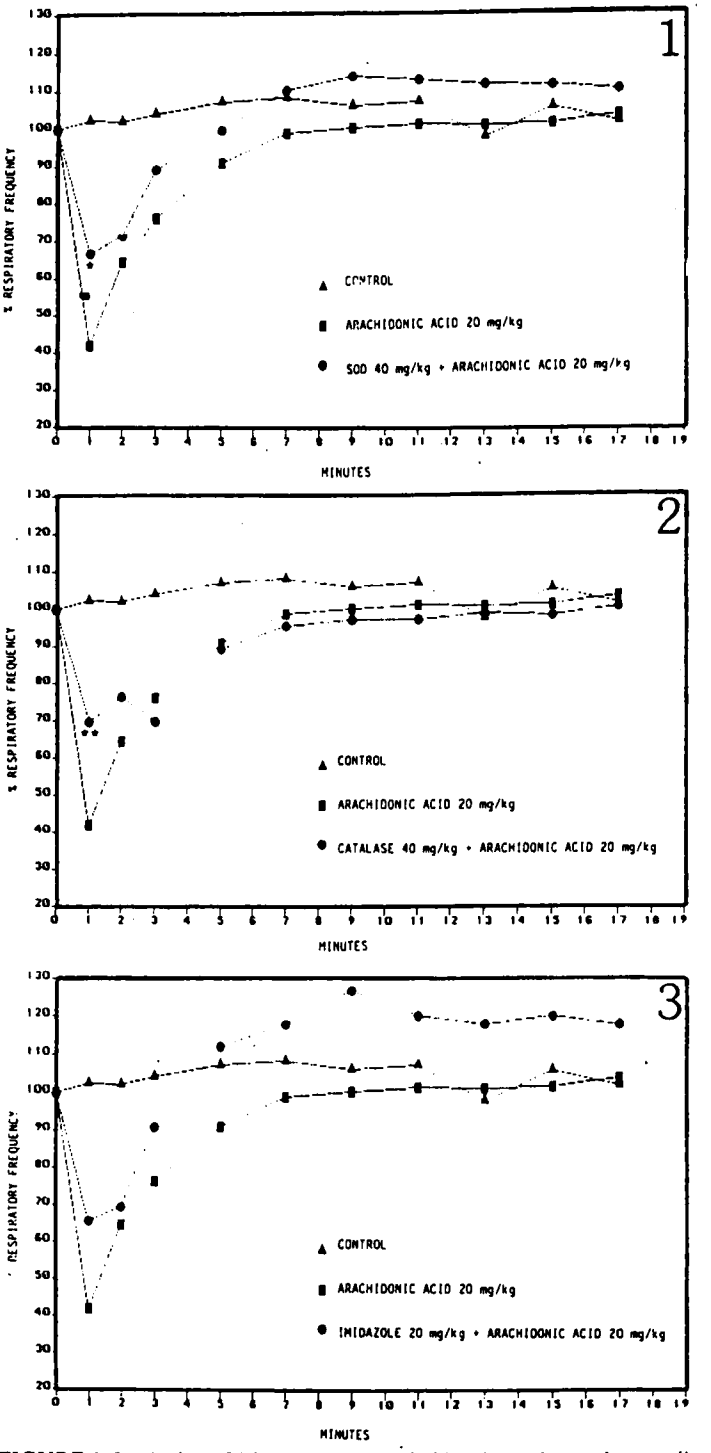

FIGURE 1-3 Action of SOD, catalase and imidazole on the respiratory distress induced by arachidonic acid.

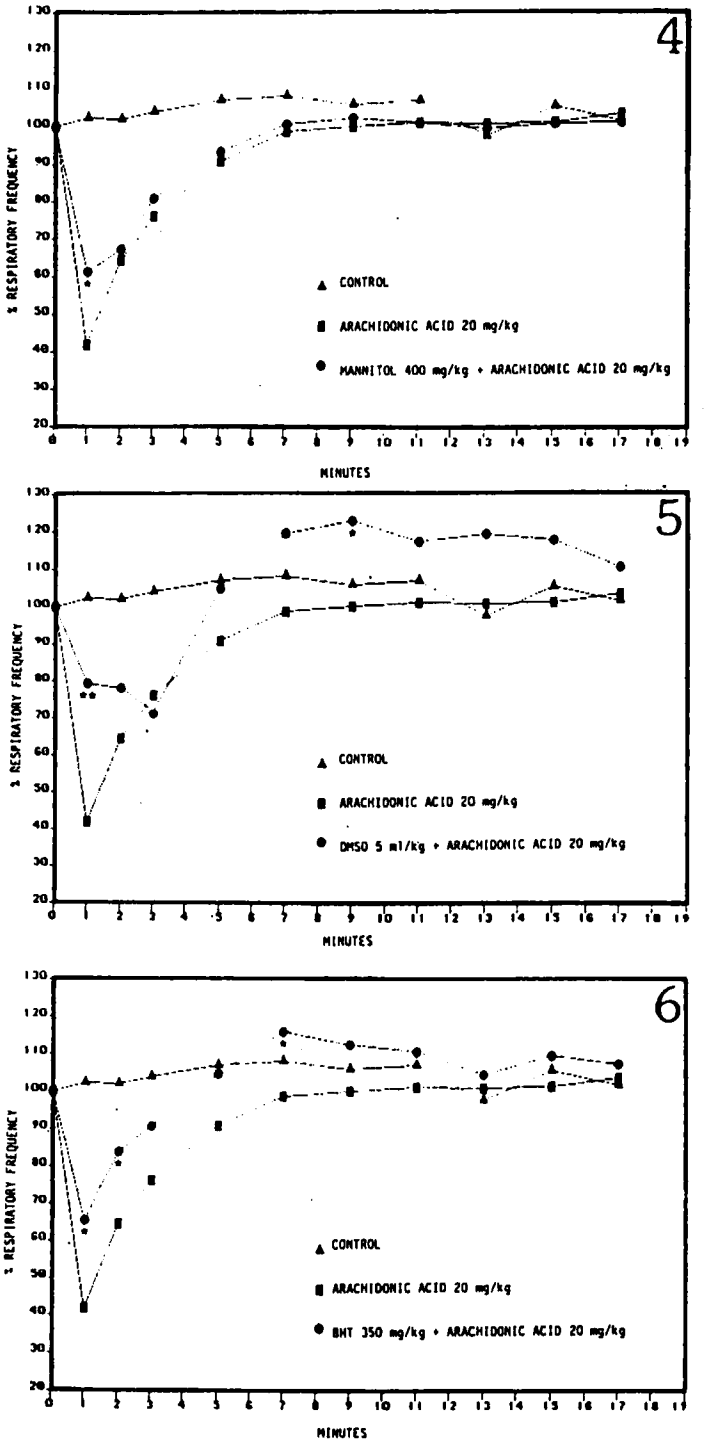

FIGURE 4–6 Action of mannitol, DMSO and BHT on the respiratory distress induced by arachidonic acid.

TABLE I
Thromboxane B2 concentration in plasma after different treatments.

DRUGS	DOSE (mg/kg)	CONCENTRATION (pg/ml plasma)	%	P
Control	–	1220.0 ± 107.6	100	–
SOD	40	584.0 ± 91.7	47.9 ± 7.5	0.01
Catalase	40	920.2 ± 62.0	75.4 ± 5.1	0.01
Mannitol	400	7271.7 ± 922.2	596.0 ± 75.6	0.001
DMSO	5	1978.8 ± 90.0	162.2 ± 7.4	0.001
BHT	350	182.8 ± 40.7	15.0 ± 3.3	0.001
Imidazole	20	710.6 ± 105.8	58.2 ± 8.7	0.01

suggesting a direct relationship between oxygen free radical production and arachidonic acid administration.

Arachidonic acid is seen in macrophages, the main source of arachidonic acid metabolites and oxygen free radicals in the lung,[19-22] as the second messenger active in the stimulus response coupling leading to superoxide production.[23,24]

There is no molecular explanation for the way in which arachidonic acid induces the respiratory burst. One of the mechanisms, the simplest one, could be a direct effect of the fatty acid on NADPH oxidase, but an arachidonic acid direct activation of proteinkinase C or of phospholipase C has also been proposed.[24-26]

Cis-unsaturated fatty acids, like arachidonic acid, may also release superoxide ion by intercalating into and disordering gel like regions of the membrane.[27]

Thromboxane has been implicated in the sudden death and respiratory distress induced by arachidonic acid.[3-5,8,14] Our results show that there is no direct relationship between protection against respiratory distress and inhibition of thromboxane synthesis. Hydroxyl radical scavengers and imidazole are two examples. Hydroxyl radical scavengers protect against respiratory distress without a parallel thromboxane inhibition and imidazole inhibits thromboxane synthesis without a significant repsiratory distress protection.

Our data agree with the proposition that arachidonic acid metabolism is not an obligatory step in triggering the respiratory burst.[28]

In the cyclooxygenase pathway, hydroxyl radicals are generated in the endoperoxide synthesis. These hydroxyl radicals deactivate both the peroxidase and cyclooxygenase enzymes. Our results suggest that hydroxyl radical scavengers prevent the oxygen free radical induced deactivation.[29,30]

In summary our results show that the arachidonic acid administered stimulates, in the alveolar macrophages, the membrane located NADPH oxidase system and the cyclooxygenase enzyme system. The first event induces the respiratory burst with the production of toxic oxygen free radicals. The second explains the TXB2 synthesis. Both events are independent. The oxygen free radical generation is the main factor responsible for arachidonic acid induced respiratory distress.

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