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Accumulation of Circulating Macrophages in Lungs of Guinea Pigs Exposed to Hyperoxia

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Summary

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) key enzymes in alveolar macrophages regulating levels of superoxide anion and hydrogen peroxide, respectively, were observed to fluctuate in response to FIO_2 of 50 and 85% for 18 to 90 hr. At the lower oxygen tension, SOD rose two-fold and GPx decreased significantly by 18 hr and throughout the exposure periods compared to a delayed increase in SOD activity which was not sustained beyond 66 hr of exposure and a sustained rise in GPx to an FIO_2 of 85%. Peritoneal macrophages containing lower SOD activity and greater GPx activity than resident alveolar macrophages upon injection into the circulation resulted in a 10-fold accumulation in the lungs during exposure of animals to FIO_2 of 85% but not at 50%. This study indicates that brief exposure to FIO_2 of 85% but not 50% resulted in alterations of the vascular integrity of the lungs resulting in the accumulation of circulating macrophages to the alveolar macrophage pool. The delayed rise in SOD activity and the sustained increase in GPx activity in alveolar macrophages from animals exposed to FIO_2 of 85% could in part be related to this influx of circulating macrophages with enzymatic characteristics observed for peritoneal macrophages.

Speculation

Toxic concentrations of oxygen not only affect the metabolism and enzyme responses of resident alveolar macrophages but incite the early influx of circulating macrophages into the lung favoring the accumulation of superoxide anion which could propagate the inflammatory reaction.

Pulmonary alveolar macrophages (AM) are equipped with a variety of enzymes to dispose of potentially damaging reduced oxygen by-products which are generated during hyperoxic conditions (14, 15). Two of these by-products, superoxide anion and hydrogen peroxide (H_2O_2), have been shown to be toxic to the elaborating phagocytic cells themselves (2, 3) as well as to adjacent tissues (6, 13, 21). Superoxide anion is converted to H_2O_2, in large part, by superoxide dismutase (SOD) located in both the cytosol and mitochondria (8, 12, 22).

H_2O_2 in turn is metabolized to H_2O by glutathione peroxidase (GPx) and catalase. Changes in the level of activities of these enzymes, especially SOD, in response to hyperoxia have been seen both in prokaryotic and eukaryotic cells (9, 17, 18) including the AM (15). In the AM, the rate of rise of SOD is inversely linked to the concentration of inspired oxygen (FIO_2) (10, 15). Furthermore, the release of O_2 by AM phagocytizing opsonized zymosan is attenuated in AM exposed to hyperoxia (16). In this study, we have evaluated the cellular basis for the attenuated rise in SOD activity and the disparate response in GPx activity when animals were exposed to fractional inspired oxygen (FIO_2) of 50% compared to 85%.

MATERIALS AND METHODS

Male guinea pigs (250 to 300 g) were exposed to room air or to atmospheres of either 50 or 85% oxygen for 18 to 90 hr. The animals were maintained in plastic chambers at a temperature of 24°C. An atmosphere of 50 or 85% FIO_2 was maintained by flowing humidified mixtures of oxygen and air through a Bennett AO-1 air-oxygen mixer at a flow rate of 8 liters/min. The oxygen concentration was monitored with a Beckman oxygen monitor model M12 (Beckman Instruments). Guinea pig AM were isolated by lung lavage (14, 16). The alveolar macrophage cell suspensions were further purified on a Ficoll-Hypaque gradient (5) which achieved a purification of 95% viable macrophages as determined by exclusion of 0.2% trypan blue. Guinea pig peritoneal macrophages were obtained as previously described (14). Cell preparations were suspended in Krebs-Ringer phosphate (pH 7.4) at a cell concentration of 5×10^6/ml before sonication.

Cell suspensions were then sonicated at 4°C for 1 min with a sonifier cell disruptor model W140 (Branson Power Corporation) at an output setting of 3 which disrupted greater than 99% of the cells. These sonicates were assayed for SOD (in the presence of 1 mM sodium azide to inhibit cytochrome *c* reduction), catalase, and glutathione peroxidase activities as previously described (15). All sonicates were assayed for protein concentration by the method of Lowry *et al.* (11).

To investigate the possible influx of blood mononuclear cells into the lung after hyperoxic exposure for 18 hr, guinea pig peritoneal exudate macrophages were elicited after intraperitoneal injection of 1.2% sodium caseinate. Forty-eight to 72 hr later, the monocytes were harvested from the peritoneal cavity and purified on a Ficoll-Hypaque gradient. Cells washed free of the Ficoll were resuspended in autologous plasma to provide a concentration of 1×10^7 monocytes per ml and were judged to be 95% free of contaminating cells and to be 95% viable as determined by Wright's stain smears and trypan blue exclusion, respectively. The macrophages were incubated with 200μCi ^{51}Cr-labeled sodium chromate (^{51} Cr) at 37°C for 30 min, washed free of unbound ^{51}Cr, and resuspended in autologous plasma to yield a specific activity of approximately 25,000 to 30,000 dpm/10^7 cells (4). Subsequently, 5×10^6 ^{51}Cr-labeled cells were injected by the intracardiac route and after 18 hr of exposing guinea pigs to room air or to atmospheres of FIO_2 of either 50 or 85%, macrophages were harvested from the lungs by bronchial lavage. The cells were then counted for ^{51}Cr activity on a gamma counter.

RESULTS

SOD AND GPx ACTIVITIES IN AM AFTER EXPOSURE OF GUINEA PIGS TO 50% AND 85% FIO_2

As noted in Table 1, total SOD activities of AM obtained from guinea pigs exposed to FIO_2 of 50% increased two-fold by 18 hr after exposure and remained significantly elevated throughout the entire 90-hr exposure period. In contrast, cells obtained from

animals exposed to FIO_2 of 85% demonstrated increased SOD activity of 1½-fold by 18 hr and achieved a two-fold increase by 66 hr, which was not sustained beyond that time point. GPx activity decreased significantly by 18 hr and remained depressed throughout the study period in the AM of animals exposed to FIO_2 (50%). On the other hand, the GPx activity rose significantly by 42 hr and remained elevated in cells obtained for animals exposed to FIO_2 of 85%. Catalase activity remained constant throughout the 90 hr of exposure to an FIO_2 of 50% and also remained constant for the initial 42 hr of exposure to an FIO_2 of 85% and then fell.

To determine whether the delayed increase in SOD activity and the increase in GPx activity may represent either a population of more youthful AM or an influx of mononuclear phagocytes from the circulation, peritoneal macrophages were radiolabeled with ^{51}Cr and injected into the circulation. These cells were selected because blood monocytes were not obtainable for purification in sufficient numbers for this study and because their myeloperoxidase activity is similar to blood monocytes (1). The immigration of circulating radiolabeled peritoneal macrophages into the lungs of guinea pigs exposed for 18 hr to FIO_2 of either 50 or 85% was monitored. As noted in Table 2, exposure of animals to FIO_2 of 85% resulted in a 10-fold increase in circulating ^{51}Cr-labeled macrophages into the alveolar macrophage pool compared to the responses observed in animals exposed to room air or to an FIO_2 of 50%. Thus, the alterations in SOD activity observed at FIO_2 of 50% is unlikely the result of an influx of circulating macrophages, whereas, the alterations of enzyme activities of AM from animals exposed to an FIO_2 of 85% may, in part, reflect the influx of inflammatory macrophages containing lower total SOD, but higher GPx activity (Table 3).

DISCUSSION

The ready access to AM by bronchial lavage provides a means for assessing enzymatic and functional consequences of hyperoxia

Table 1. *SOD, GPx, and catalase activities in AM obtained from guinea pigs during hyperoxia*[1]

FIO_2 (%)	Hr of exposure	*n*	SOD (%)	*n*	GPx (%)	*n*	Catalase (%)
50	0	11[2]	100	5	100	5	100
	18	9	205 ± 6[3, 4]	6	76.1 ± 4.6[4]	6	98.4
	42	9	191 ± 5[4]	6	65.1 ± 4.6[4]	6	103.7
	66	8	189 ± 3[4]	12	48.6 ± 2.8[4]	12	97.5
	90	9	188 ± 3[4]	12	58.7 ± 2.8[4]	12	97.6
85	0	12	100	5	100	5	100
	18	14	156 ± 12[4]	7	114 ± 9[4]	7	95.8
	42	15	169 ± 6[4]	4	147 ± 12[4]	4	92.7
	66	15	194 ± 6[4]	9	185 ± 16[4]	9	87.3
	90	16	106 ± 12	9	186 ± 18[4]	9	54.8

[1] Activities expressed as % of zero time. See "Materials and Methods" for details. Control SOD GPx and catalase absolute values were 16.8 ± 0.7, and 11.6, and 245 ± 23 units/mg protein, respectively.

[2] Numbers in parentheses, number of individual animals studied.

[3] Mean ± S.E.

Table 2. *Immigration of ^{51}Cr-labeled sodium chromate macrophages into the lung of guinea pigs after 18 hr of hyperoxic exposure*

Exposure	cpm/10^6 cells
Air	41.9 ± 12′
50% O_2	39.4 ± 14
85% O_2	414 ± 10^2

[1] Mean ± S.E. of three separate experiments with five animals for each group.

[2] Significantly different from other values; $P < 0.05$.

Table 3. *Comparison of SOD and GPx activities in AM and peritoneal macrophages*

	SOD (units/mg protein)	GPx (units/mg/min)
AM	16.8 ± 0.7[1]	11.6 ± 0.7
Peritoneal macrophage	12.60 ± 0.16	23.7 ± 2.3

[1] Mean ± S.E. of three separate experiments with five animals for each group.

in at least one type of lung cell. In the guinea pig model, SOD activity rises by two-fold during the first 18 hr of exposure of animals to FIO_2 of 50% and remains elevated throughout 90 hr of exposure, whereas a lethal concentration of 85% resulted in a retarded rise of SOD activity which could not be sustained at 90 hr. Others have failed to observe increases in SOD activity of alveolar macrophages in adult murine models (7) but have observed changes in neonatal cells (19).

GPx, another enzyme which catalyzes the oxidation of reduced glutathione by hydrogen peroxide was also previously found to be responsive to the effect of hyperoxia (15). At lower oxygen concentrations, GPx activity fell but at the higher concentrations it rose. On the other hand, catalase remained relatively constant at the lower and higher concentrations until the late exposure periods when it fell (15). In an attempt to account for the variable effect of FIO_2 of 85% in provoking as much rise in SOD activity coupled with a more rapid increase in GPx, we considered that the higher oxygen tension might induce early inflammatory changes in the lung leading to substantial immigration of circulating mononuclear phagocytes into lung (20). Peritoneal macrophages labeled with ^{51}Cr when infused intravenously accumulated in large amounts in the lung of animals exposed to FIO_2 of 85% compared to animals exposed to FIO_2 of 50% or room air. There was a 10-fold enrichment of labeled cells in the bronchial lavages obtained from animals at 18 hr of exposure to FIO_2 of 85% compared to FIO_2 of 50% room air. These studies indicate that the higher oxygen tension incites an accelerated influx of mononuclear phagocytes from the circulation into the lungs (20). The delayed rise in SOD activity and the increase in GPx in the cells obtained by bronchial lavage at these early time points may reflect contamination of the pool of AM with circulating monocytes. Blood monocytes resemble the peritoneal monocyte in myeloperoxidase activity and energy metabolism (7) and probably contain similar levels of SOD and GPx.

CONCLUSION

The blunted rise in SOD activity and the prompt sustained rise in GPx activity in AM from guinea pigs exposed to FIO_2 of 85% is in part due to an influx of inflammatory blood mononuclear cells containing lower SOD and higher GPx activity than AM. At less toxic FIO_2 of 50%, the SOD activity reaches higher levels by 18 hr, and the GPx activity falls in the AM, and these fluctuations are not associated with an influx of blood monocytes and reflects an intrinsic response of the AM to hyperoxic stress.

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