Toxic effects of oxygen on cultured alveolar epithelial cells, lung fibroblasts and alveolar macrophages


ABSTRACT: Exposure to hyperoxia results in endothelial necrosis followed by type II cell proliferation. This suggests that type II cells are resistant to hyperoxia. Oxygen-induced lung injury may result from an overproduction of oxygen metabolites normally scavenged by antioxidants such as superoxide dismutase (SOD), glutathione peroxidase, catalase and reduced glutathione (GSH). Therefore, resistance of type II cells to hyperoxia may be linked to high antioxidant activities. To test this hypothesis we compared in vitro the effects of a 24 h exposure period to 95% O₂ on cultured type II cells, lung fibroblasts and alveolar macrophages isolated from rats. We show that type II cells, when compared with other cell types, are highly sensitive to hyperoxia as shown by increased lactate dehydrogenase (LDH) release, decreased deoxyribose nucleic acid (DNA) and protein content of Petri dishes and decreased thymidine incorporation into DNA. Synthesis of dipalmitoylphosphatidylcholine was also significantly reduced. Antioxidant enzyme activities as well as glutathione content were not higher in type II cells than in other cell types. However, hyperoxia results in a decreased SOD activity and glutathione content in type II cells which was not observed in fibroblasts. We conclude that adaptive changes in SOD and glutathione metabolism could be important defence mechanisms in cells exposed to hyperoxia.

In most species, exposure to normobaric hyperoxia results in lung injury. Morphometric studies show in rats an early necrosis of endothelial cells followed by a proliferation of alveolar type II cells and fibroblasts [1,2]. These data suggest that type II cells and fibroblasts are resistant to oxygen toxicity. A substantial number of studies have shown in various species that alveolar macrophages are altered early in the course of in vivo or in vitro hyperoxic exposure [3–5]. Antioxidant defences such as superoxide dismutase, glutathione peroxidase, catalase and glutathione may protect, at least partly, the cells against oxidant stress. This is in keeping with the biochemical mechanisms leading to oxygen-induced lung injury. Cell damage may result from intracellular oxygen-derived free radicals overproduction [6] or from toxic products release by phagocytes such as polymorphonuclear neutrophils [7]. Under these circumstances, oxygen metabolites production exceeds the capacity of the cellular antioxidant defence mechanisms [8]. It follows that the sensitivity of specific cell types to oxygen injury could be related in part to activities of endogenous cellular antioxidant enzymes.

For a better understanding of the factors affecting cell injury, we used an in vitro model as a critical approach of the direct mechanisms of cell damage induced by oxidant stress. This contrasts with in vivo studies where the toxic effects also result from complex cellular interactions.

In this study we compared the activities of antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase, and total glutathione content in cultured alveolar type II cells, alveolar macrophages and fibroblasts isolated from rat lung. We also investigated in these three cultured cell types, the effects of a 24 h exposure period to 95% O₂ on these antioxidant substances, on some indexes of cytotoxicity as well as on the incorporation of 3H-palmitate into dipalmitoylphosphatidylcholine, an index of function of cultured type II cells.

Service de Pneumologie, Hôpital Saint-Antoine, 184 rue du Fg Saint Antoine 75012, Paris.

* Service de Biochimie B, CHU Saint-Antoine, 12 rue de Chaligny, F-75571 Paris, Cedex 12.

** Laboratoire d'histophysiologie des processus de défense, CHU Henri Mondor, 8 rue du Général Sarrail, F-94000 Créteil, France.

Correspondence : B. Housset, Service de Pneumologie, Hôpital Saint-Antoine, 184 rue du Fg Saint Antoine 75012, Paris.

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Cell isolation and culture

Type II epithelial cells (PII). Male Wistar rats (200–250 g) were anaeasthetized with ether. Then 500 U/kg of heparin was injected i.p. The trachea was cannulated, the vena cava was cut and the lungs were perfused via the pulmonary artery with 20 ml of sterile saline and then excised. To remove some of the macrophages, the lungs were lavaged four times via the trachea with a solution containing 140 mM NaCl, 5 mM KCl, 2.5 mM sodium phosphate, 10 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 6 mM glucose, 0.2 mM EGTA (ethylene glycolis(B-amino ethyl ether), N-tetraacetic acid), pH 7.4. Then 10 ml of a 0.25% trypsin solution ( Worthington Biochemical Corp., Freehold, New Jersey) was injected for a 30 min incubation period at 37°C. Reaction was stopped by adding 10 ml DMEM (Dulbecco’s Modified Eagle Medium containing 4500 mg glucose l–1; Gibco Limited, Paisley, Scotland) and 20% FBS (Fetal Bovine Serum; Gibco). The lungs were minced in the presence of 2 μg/ml DNAase I (Sigma, St Louis, MO) and then shaken for 5 min. Lung minces and cell suspension were filtered through a nylon gauze (100 μ). Cells were centrifuged at 200 × g for 5 min. The resulting pellet was resuspended in DMEM supplemented with penicillin (50 U·ml–1) and streptomycin (50 μg·ml–1) and cells were incubated for one hour in 100 mm diameter Petri dishes. Then supernatant was collected and centrifuged at 200 × g for 5 min. Cells were resuspended in DMEM 10% FBS and added at a density of 2 × 10⁶ cells per 2 ml medium in 35 mm diameter Petri dishes. Plates were incubated for 24 h at 37°C in a 5% CO₂ - air atmosphere. Type II cells were identified on their morphological appearance under optic and electron microscopy.

Macrophages. Cells were obtained by bronchoalveolar lavage as described above. The pooled lavage fluid was centrifuged at 200 × g for 5 min and the pellet was resuspended in DMEM. The cells were plated in 35 mm culture dishes at a density of 10⁶ cells per dish and incubated for one hour at 37°C to allow macrophages to adhere. After removal of medium and of non adhering cells, alveolar macrophages were incubated in DMEM containing 10% FBS.

Fibroblasts. Lung fibroblasts were isolated from minced lung which was incubated in 0.25% trypsin. After digestion for one hour at 37°C, minces were incubated in DMEM 10% FBS. Fibroblasts were passed at least 3 times before use and were cultured till the twentieth passage.

Exposure to normobaric oxygen

Culture dishes from the same pool of cells were divided into two groups. One group was incubated for 24 h in a 95% O₂ - 5% CO₂ incubator. Oxygen concentration was monitored using a Toptronic oxymeter (Bloblock, France) and was consistently found to be over 90% until the end of the exposure period. The other group was maintained under control conditions (5% CO₂ - air).

Thymidine incorporation into DNA

Cells were incubated for 24 h with 1 μCi·ml⁻¹ of tritiated thymidine (45 Ci·mmol⁻¹, CEA, Saclay). At the end of the exposure period, cells were washed twice, scraped in saline and sonicated. One volume of cell homogenate was diluted with two volumes of 15% trichloracetic acid (TCA). After centrifugation (4°C, 5000 × g for 10 min) the pellet was suspended in 10% TCA and sonicated. Radioactivity was counted in a scintillation counter (Delta 300, Searle). As the efficiency was constant, results were expressed in cpm. Incorporation of labelled thymidine into DNA was expressed as the ratio of the radioactivity found in TCA insoluble extract to the total radioactivity added to the Petri dish. This ratio was reported to the cell mass and therefore expressed per μg DNA. In some type II cells cultures autoradiography was performed with a pulse of 10 μCi·ml⁻¹ ³H-thymidine for 4 h in order to identify, after a tannic acid coloration, the cell types taking up radioactivity [9]. For each experiment, 400 cells were counted to obtain the percentage of cells with a labelled nucleus (labelling index).

Incorporation of palmitic acid and identification of labelled lipids

Alveolar type II cells were incubated with DMEM 10% FBS containing 2 μCi·ml⁻¹ tritiated palmitic acid (27.5 Ci·mmol⁻¹, New England Nuclear, Boston, MA). Cells were exposed to hyperoxic conditions or maintained under control conditions for 24 h. Cells were scraped and sonicated. Lactate dehydrogenase (LDH), protein and DNA were measured in cell homogenate. Radioactivity was counted in cells and media. Lipids were extracted according to the method of BLISH and DYER [10]. The lipid extract from cell homogenate was evaporated to dryness and separated in two aliquots before resolution by thin layer chromatography following the method described by GILFILLAN et al. [11]. A lipid extract from whole rat lung was added as a standard to these aliquots before migration. The lipids were visualized by exposure to iodine vapour. In the first aliquot, the spots corresponding to the different lipid classes were scraped into scintillation vials and counted. In the second aliquot only phosphatidylcholine (PC) was scraped and extracted by the method of BLISH and DYER [10]. ¹⁴C-dipalmitoylphosphatidylcholine (DPPC) was added as an internal standard and DPPC was separated by the method of GILFILLAN et al. [11]. Incorporation of palmitate in DPPC was expressed as the percentage of radioactivity incorporated in PC or total phospholipids. In three experiments, to assess changes in the specific activity of
palmitate incorporation into phospholipids, total phosphorus was also measured by the method of Fisk and Subbarow [12] with some modifications.

Chemical analysis

Superoxide dismutase from bovine red cells, horse heart cytochrome c type III, xanthine, xanthine oxidase, reduced glutathione, glutathione reductase type III, NADPH tetra sodium salt type III, β-NADH disodium salt grade III, pyruvic acid, calf thymus DNA, 3,5- diaminobenzoic acid and bovine serum albumin were purchased from Sigma. Hydrogen peroxide 30% was obtained from Merck. LDH activity was measured by the method of Bergmeyer et al. [13]. DNA was assessed by a fluorimetric assay [14]. Protein determination was made according to Peterson [15]. Glutathione peroxidase activity was determined using H_2O_2 as substrate according to the method of Paglia and Valentine [16]. Superoxide dismutase activity (SOD) was measured by the ferricytochrome c assay described by McCord and Fridovich [17] at pH 10 with and without 10^-3 M KCN to have an estimation of both the total SOD and the KCN-independent SOD. Catalase activity was assayed following the method of Luck [18]. Total glutathione was determined according to the assay described by Sies and Akerboom [19].

Morphologic analysis

Rat alveolar type II cells were processed directly on tissue culture dishes. The monolayers were washed with cacodylate buffer at pH 7.4, and then fixed with freshly prepared Hirsch and Fedorko mixture [20] for 30 min at room temperature. The monolayers were again washed twice with cacodylate buffer (pH 7.4) overnight as described by Mason [21]. Then they were washed twice and stored in cacodylate buffer. For light microscopy, the polychrome stain developed by Mason [21], or the May-Grünwald-Giemsa were used. For electron microscopy, the monolayers were dehydrated with a series of graded ethanols, infiltrated in graded epon 812 and ethanol and embedded in epon 812. Sections were stained with 4% aqueous solution of uranyl acetate. They were observed under a Philips EM 300 microscope at 60 kv.

Data analysis

Student's paired t-test was used to compare the effect of control and hyperoxic conditions. Analysis of variance was used to compare the effects of hyperoxia on the three cell types cultures. Results are expressed as means±SEM.

### Table 1. Antioxidant activities in cultured type II cells, lung fibroblasts and alveolar macrophages isolated from rat lungs. Effects of a 24 h exposure period to control (air-5% CO₂) or hyperoxic conditions (95% O₂-5% CO₂)

<table>
<thead>
<tr>
<th></th>
<th>SOD</th>
<th>GP</th>
<th>CAT x 10⁴</th>
<th>GSH</th>
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<tbody>
<tr>
<td>PII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.059±0.005</td>
<td>0.271±0.004</td>
<td>3.41±0.38</td>
<td>9.2±0.1</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>0.033±0.006*</td>
<td>0.221±0.032</td>
<td>3.37±0.33</td>
<td>6.9±0.1**</td>
</tr>
<tr>
<td>AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.053±0.005</td>
<td>0.453±0.090*</td>
<td>4.91±0.80*</td>
<td>9.8±1.3</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>0.050±0.010</td>
<td>0.570±0.140</td>
<td>4.39±0.71</td>
<td>6.6±1.3**</td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.026±0.002*</td>
<td>0.151±0.020*</td>
<td>2.07±0.33*</td>
<td>1.7±1.2*</td>
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<td>Hyperoxia</td>
<td>0.025±0.001</td>
<td>0.099±0.020</td>
<td>1.45±0.15</td>
<td>1.5±0.7</td>
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### Units/µg DNA

<table>
<thead>
<tr>
<th></th>
<th>SOD</th>
<th>GP</th>
<th>CAT x 10⁴</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
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<td>PII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.99±0.29</td>
<td>10.37±2.38</td>
<td>114±15</td>
<td>419±52</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>1.07±0.16</td>
<td>8.49±1.99</td>
<td>112±10</td>
<td>298±34**</td>
</tr>
<tr>
<td>AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.70±0.22</td>
<td>13.20±2.93</td>
<td>150±21*</td>
<td>559±67</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>1.41±0.17</td>
<td>17.45±3.01*</td>
<td>125±22</td>
<td>347±87**</td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.63±0.20</td>
<td>9.72±1.68</td>
<td>109±17</td>
<td>75±4*</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>2.00±0.07</td>
<td>7.81±1.30</td>
<td>111±11</td>
<td>73±4</td>
</tr>
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</table>

*: significantly different from type II cells (p<0.05); **: significantly different from control conditions (p<0.05). Results are expressed as mean±SEM n=7. SOD: superoxide dismutase; GP: glutathione peroxidase; CAT: catalase; GSH: glutathione; PII: type II pneumocytes; LF: lung fibroblasts; AM: alveolar macrophages.
Results

Antioxidant enzyme activities

As shown in table 1, antioxidant enzyme activities expressed per mg protein were different in the three cell types. The highest glutathione peroxidase and catalase activities were found in alveolar macrophages. Cultured fibroblasts had a significantly lower activity for the three enzymes tested. When the results were expressed in Units·mg⁻¹ DNA, enzyme activities were not significantly different in fibroblasts, type II cells and macrophages except for a higher level of catalase in alveolar macrophages. Total glutathione content was significantly lower (p<0.01) in fibroblasts when expressed per µg DNA as well as per mg protein. The KCN-independent form of SOD was similar in type II cells and fibroblasts when expressed as the percentage of total SOD (45±10 and 44±9 respectively).

Cell morphology

In control cultures, light microscopy shows that type II cells formed either islands or confluent monolayers of polygonal cells, which represent 85–95% of cells. Other cells were scattered ciliated cells (0–3%) and macrophages (2–15%). Most of polygonal cells (50–85%) had characteristic tannic acid stained myelinic inclusion bodies (fig. 1). Large cells with few lamellar granules were more frequent 48 and 72 h after plating. Binucleated as well as dividing cells were rarely seen. Under electron microscopy the appearance of polygonal cells was similar in many respects to that seen in type II cells: they exhibited numerous membrane bound inclusions containing concentric lamellae with scattered vesicular components, numerous mitochondria, evident Golgi apparatus, free ribosomes and smooth endoplasmic reticulum, microvilli and occasional tight or gap junctions (fig. 2). The abluminal surface was anchored to a basal lamina. Occasionally endoplasmic reticulum could be seen dilated with balloonized mitochondria. Exposure to hyperoxia for 24 h was associated under light microscopy, with a decreased number of cells. Cells formed islands or, sometimes, clusters of few cells. There was no other significant difference. Under electron microscopy, size of cells and appearance of nucleus, lamellar bodies and mitochondria were not clearly modified. Myelinic inclusion bodies had characteristic lamellar structure. Balloonized mitochondria were not evidently more frequent than in control cells.

Effects of hyperoxia on indexes of cytotoxicity

Cytolysis, as assessed by the percent release of LDH activity into the medium, was significantly increased by oxygen in cultured type II cells (p<0.001) and alveolar macrophages (p<0.05). This contrasts with an unchanged release of LDH in cultures of fibroblasts (fig. 3). In parallel to LDH release, protein and DNA were significantly reduced in cultured pneumocytes. No significant change in DNA and protein content was noted in alveolar macrophages. In fibroblast cultures, DNA content was significantly reduced (p<0.05) without change in protein content (figs 4 and 5).

Fig. 1. – Light micrograph of monolayers of rat alveolar type II epithelial cells cultured for 2 days. The large dark granules are the lamellar bodies (x 400).
Fig. 2. - Electron micrograph of a rat alveolar type II epithelial cell cultured for 2 days. Cytoplasm contains characteristic lamellar bodies. (*×10,080).

Fig. 3. - LDH release expressed as the percentage of total LDH activity (cells + medium) released in the medium of cultured type II cells (PIL), lung fibroblasts (LF) and alveolar macrophages (AM). Effects of a 24 h exposure period to control (air-5% CO₂) or hyperoxic conditions (95% O₂-5% CO₂). Mean±SEM. Control; Hyperoxia. Control vs Hyperoxia, *: p<0.01; **: p<0.001.

Incorporation of ³H-thymidine into DNA was significantly lower in type II cells (9,100±862 cpm·µg⁻¹ DNA) when compared to fibroblasts (53,865±5,764 cpm·µg⁻¹ DNA) (p<0.001). Hyperoxia resulted in a significant decrease of thymidine incorporation into type II cells (56±7% of control value; p<0.01) and into fibroblasts (90±5% of control values; p<0.05). However, this decrease was significantly more pronounced in type II cells (p<0.01). Incorporation of ³H-thymidine in AM was negligible (data not shown). Autoradiography showed that radioactivity was incorporated in epithelial cells (fig. 6). The labelling index was 15.6±1.9 under control conditions and was significantly decreased by hyperoxia (2.3±0.5; p<0.001).
Fig. 4. - DNA content in cultures of type II cells (P11), lung fibroblasts (LF) and alveolar macrophages (AM). Effects of a 24 h exposure period to control (air–5% CO₂) or hyperoxic conditions (95% O₂–5% CO₂). Mean±SEM. ■: Control; □□□□: Hypoxia. Control vs Hypoxia, *: p<0.05.

Fig. 5. - Protein content in cultures of type II cells (P11), lung fibroblasts (LF) and alveolar macrophages (AM). Effects of a 24 h exposure period to control (air–5% CO₂) or hyperoxic conditions (95% O₂–5% CO₂). Mean±SEM. ■: Control; □□□□: Hypoxia. Control vs Hypoxia, *: p<0.05.

The incorporation of ³H-palmitate into phospholipids was reduced by 17±3% when expressed per µg of phospholipid phosphorus (p<0.05). The pattern of palmitate distribution among phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol was unchanged (fig. 7). However, labelled DPPC was slightly but significantly reduced when expressed as percent of labelled phospholipids (p<0.05) or percent of labelled phosphatidylcholine (p<0.01) (fig. 8).
Fig. 6. - Autoradiography of monolayers of rat alveolar type II epithelial cells cultured for 2 days after a pulse of 10 Ci/ml $^{3}$H-thymidine for 4 h. Arrows indicate labelled nuclei of epithelial cells. (Light micrograph; × 200).

Fig. 7. - Classes of phospholipids labelled with $^{3}$H-palmitate for 24 h. Results are expressed as percentages of total labelled phospholipids. Effects of a 24 h exposure period to control (air-5% CO$_2$) or hyperoxic conditions (95% O$_2$-5% CO$_2$). Means±SEM. PC: phosphatidylcholine; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol. ■: Control; □: Hyperoxia.

Effects of hyperoxia on antioxidant substances

Exposure to hyperoxia for 24 h was associated with changes in antioxidant enzyme activities (table 1). Total superoxide dismutase activity was significantly reduced in type II cells but remained unchanged in other cell types. The percentage of the cyanide-insensitive SOD was also significantly reduced in type II cells (38±7% of total SOD; $p<0.05$) but was not significantly changed in fibroblasts despite a tendency to increase (51±4% of total...
OXYGEN TOXICITY IN CULTURED LUNG CELLS

SOD). Catalase activity was not significantly affected by hyperoxia except for a decrease in fibroblasts (p<0.05). The only change observed in glutathione peroxidase activity was a significant increase in alveolar macrophages exposed to hyperoxia (p<0.05). A slight but not significant decrease in this enzyme activity was noted in type II cells as well as in fibroblasts. Total glutathione content was unchanged in fibroblasts but significantly decreased in type II cells (p<0.05) and macrophages (p<0.05).

Discussion

Oxygen-induced lung injury is a well studied model of acute and chronic lung damage which is of clinical interest because of oxygen’s contributory role in the aetiology of acute respiratory distress syndrome [22] and bronchopulmonary dysplasia in neonates [23]. Changes in whole lung metabolism associated with this damage can be better understood if biochemical responses of individual lung cell types are known.

In a few studies, antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and catalase, have been measured. From studies in isolated type II cells and alveolar macrophages [24, 25], it appears that the levels of antioxidant enzymes are roughly similar in both cell types despite a higher level of mitochondrial SOD activity in alveolar macrophages contrasting with a higher value of cytoplasmic SOD activity in type II cells. These results are in agreement with those of the present study where the level of antioxidant enzymes is not significantly higher in type II cells. Furthermore, it should be outlined that some authors have estimated, on both morphometric and biochemical measurements performed on whole lung and isolated type II cells, the relative contribution of type II cell antioxidant enzyme activities to activities of whole lung. They found that this contribution was similar to the DNA contribution of type II cells to whole lungs except for mitochondrial SOD [25]. This enzyme specific activity was lower in type II cells than in whole lung homogenate. These data demonstrate that alveolar type II cells are not enriched in antioxidant enzyme activities when compared with alveolar macrophages, fibroblasts and whole lung homogenate. Therefore, the levels of antioxidant enzymes cannot account for the relative resistance of type II cells to oxygen toxicity in vivo.

Type II cells are considered to be an oxidant-resistant lung cell type on the basis of morphological [1] and of biochemical studies performed on type II cells isolated from oxygen-exposed rats [24, 25]. Higher levels of antioxidant enzyme activities were noted in these cells when compared with cells isolated from air exposed rats. However the sensitivity to oxygen was not studied in vitro. Our results clearly show that isolated type II cells are highly sensitive to 95% O₂ for 24 h as assessed by increased LDH release and decreased thymidine incorporation into DNA. Several reports have shown that cultured alveolar type II cells isolated from adult rats do not go through the replication cycle [26]. However non proliferating type II cells are able to synthetize DNA from thymidine [26]. Our results suggest that this step is highly sensitive to oxygen toxicity in vitro. This was confirmed by a decreased labelling index. Autoradiography also clearly shows that thymidine incorporation was not due to contaminating cells such as fibroblasts. The decrease in palmitate incorporation into phospholipids

Fig. 8. – Dipalmitoylphosphatidyl choline expressed as the percentage of total labelled phospholipids and of labelled phosphatidylcholine in cultured type II cells. Effects of a 24 h exposure period to control (air–5% CO₂) or hyperoxic conditions (95% O₂–5% CO₂). Mean±SEM. PL: total labelled phospholipids; PC: phosphatidylcholine. ■: Control; □: Hyperoxia. Control vs Hyperoxia, *: p<0.05; **: p<0.01.
was associated with a decreased proportion of labelled DPPC related to labelled phospholipids and labelled phosphatidylcholine. Despite the fact that the magnitude of observed changes was low and therefore of questionable physiological relevance, these results strongly support the hypothesis that hyperoxia impairs the synthesis of surfactant phospholipids as suggested by studies on oxygen-exposed animals [27, 28]. This is in contrast with the increased surfactant synthesis reported in animals exposed to sublethal concentrations of oxygen (85%) [29] which is thought to be an adaptive rather than a toxic response to hyperoxia.

Fibroblasts are more resistant than type II cells to hyperoxia, though they have the lowest levels of antioxidant enzymes activities and the lowest content in total glutathione. These results argue again for the absence of a tight relationship between levels of antioxidant substances and sensitivity to oxygen poisoning. However this does not exclude a protective role of high enzyme levels as shown by experiments using catalase entrapped liposomes [30]. Under hyperoxic conditions, total glutathione content was unchanged, contrasting with the significant decrease observed in macrophages and alveolar type II cells. As glutathione is known as one of the most important antioxidant defences, this may contribute to fibroblast resistance against oxygen toxicity. The metabolic mechanism involved remains unknown. On the other hand it should be noted that the peculiar sensitivity of cultured type II cells to hyperoxia was associated with a significant decrease in total and cyanide-insensitive SOD activity, a change that was not observed in other cell types. Therefore SOD metabolism may be of importance against oxygen injury. Forman and Fisher [24] have reported no change in mitochondrial SOD activity of isolated type II cells exposed for 24 h to 100% O₂. However, total SOD activity was not measured and cytotoxic effects were not assessed. These results contrast with the increased SOD activity that has been reported in type II cells isolated from rats exposed to 100% O₂ for 2 days [24] or 85% O₂ for 7 days [25]. This suggests either a metabolic change due to the isolation procedure leading to decreased inducibility of SOD activity, or some cellular interactions that, in vivo, stimulate the induction of SOD in type II cells. An adjacent and potentially interactive cell is the alveolar macrophage which is thought to play a major role in oxygen-induced lung damage [31].

In alveolar macrophages, the effect of oxygen was mild as shown by unchanged DNA and protein content of Petri dishes. However, LDH release which is a more sensitive index of cellular injury, was significantly increased despite an increased glutathione peroxidase activity. Harada et al. [5] have also shown, in rabbit alveolar macrophages, that 72 h exposure to 95% O₂ results in a significant increase in LDH release into the culture medium. Some authors have suggested that alveolar macrophages may play a pivotal role in oxygen-induced lung injury by releasing factors that recruit and activate damaging neutrophils [7, 31]. Interactions between alveolar macrophages and type II cells are also suggested by some reports. It has been shown that rat bronchoalveolar fluid [32] as well as substances released by alveolar macrophages [33] increase type II cells DNA synthesis under normoxic conditions. Other cellular interactions may modulate the toxic effects of oxygen on type II cells: Tanswell et al. [34] have reported that conditioned medium from fibroblasts exposed to hyperoxia modifies phospholipid metabolism in cultured type II cells. The nature of the products released by alveolar macrophages or fibroblasts is still unknown.

Beside metabolic changes that may be induced by isolation and/or culture conditions [35], discrepancies between results obtained in vivo and in vitro under hyperoxic conditions may be related to intercellular relationships that are lost in vitro. Fibroblasts and macrophages may be the source of factors that stimulate type II cell proliferation and increase the resistance of this cell type to hyperoxia. This hypothesis is currently under investigation.

References


Young SL, Crapo JD, Kremers SA, Brunley GW. - Pulmonary surfactant lipid production in oxygen-exposed rat lungs. Lab Invest, 1982, 46, 570-576.


Effets toxiques de l'oxygène sur les cellules epithéliales alvéolaires en culture, les fibroblastes pulmonaires et les macrophages alvéolaires. B. Houset, I. Hurbain, J. Mastiha, A. Logal, M.T. Chaumette-Demaugre, H. Karam, J.P. Berre. RÉSUMÉ: L'exposition à l'hyperoxygène entraîne une nécrose endothéliale suivie d'une prolifération des cellules de type II. Ceci suggère que les cellules de type II résistent à l'hyperoxygène. Les lésions pulmonaires induites par l'oxygène peuvent provenir d'une hyperproduction de métabolites d'oxygène normalement détruits par des anti-oxydants comme la superoxyde dismutase (SOD), la glutathion peroxydase, la catalase et le glutathion réduit (GSH). Dès lors la résistance des cellules de type II à l'hyperoxygène peut être liée à des activités anti-oxydantes importantes. Pour tester cette hypothèse, nous avons comparé in vitro les effets d'une exposition de 24 heures à 95% d'oxygène sur les cellules de type II en culture, les fibroblastes pulmonaires, et les macrophages alvéolaires isolés chez le rat. Nous démontrons que les cellules de type II, par comparaison avec les autres types cellulaires, sont particulièrement sensibles à l'hyperoxygène, ainsi qu'en témoigne une augmentation de la libération de LDH, d'une diminution de DNA et du contenu protéique des boîtes de Petri, et d'une diminution de l'incorporation de la thymidine dans la DNA. La synthèse de dipalmitoylphosphatidylcholine est également réduite de manière significative. Les activités enzymatiques anti-oxydantes, aussi bien que le contenu en glutathion, ne sont pas plus élevés dans les cellules de type II que dans les autres types cellulaires. Toutefois, l'hyperoxygène entraîne une diminution de l'activité SOD et du contenu en glutathion des cellules de type II, que l'on n'observe pas au niveau de fibroblastes. Ainsi les modifications d'adaptation du métabolisme de la SOD et du glutathion, pourraient être des mécanismes de défense importants dans les cellules exposées à l'hyperoxygène. Eur Respir J, 1991, 4, 1066-1075.