Imaging Lung Physiology

Role of glutathione in lung retention of $^{99m}$Tc-hexamethylpropyleneamine oxime in two unique rat models of hyperoxic lung injury

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Audi SH, Roerig DL, Haworth ST, Clough AV. Role of glutathione in lung retention of $^{99m}$Tc-hexamethylpropyleneamine oxime in two unique rat models of hyperoxic lung injury. J Appl Physiol 113: 658–665, 2012. First published May 24, 2012; doi:10.1152/japplphysiol.00441.2012.—Rat exposure to 60% oxygen (O2) for 7 days (hyper-60) or to >95% O2 for 2 days followed by 24 h in room air (hyper-95R) confers susceptibility or tolerance, respectively, of the otherwise lethal effects of subsequent exposure to 100% O2. The objective of this study was to determine if lung retention of the radiopharmaceutical agent technetium-labeled-hexamethylpropyleneamine oxime (HMPAO) is differentially altered in hyper-60 and hyper-95R rats. Tissue retention of HMPAO is dependent on intracellular content of the antioxidant GSH and mitochondrial function. HMPAO was injected intravenously in anesthetized rats, and planar images were acquired. We investigated the role of GSH in the lung retention of HMPAO by pretreating rats with the GSH-depleting agent diethyl maleate (DEM) prior to imaging. We also measured GSH content and activities of mitochondrial complexes I and IV in lung homogenate. The lung retention of HMPAO increased by ∼50% and ∼250% in hyper-60 and hyper-95R rats, respectively, compared with retention in rats exposed to room air (normoxic). DEM decreased retention in normoxic (∼26%) and hyper-95R (∼56%) rats compared with retention in the absence of DEM. GSH content increased by 19% and 40% in hyper-60 and hyper-95R lung homogenate compared with normoxic lung homogenate. Complex I activity decreased by ∼50% in hyper-60 and hyper-95R lung homogenate compared with activity in normoxic lung homogenate. However, complex IV activity was increased by 32% in hyper-95R lung homogenate only. Furthermore, we identified correlations between the GSH content in lung homogenate and the DEM-sensitive fraction of HMPAO retention and between the complex IV/complex I activity ratio and the DEM-insensitive fraction of HMPAO retention. These results suggest that an increase in the GSH-dependent component of the lung retention of HMPAO may be a marker of tolerance to sustained exposure to hyperoxia.

ventilation with oxygen (O2) at high concentrations (hyperoxia) is a necessary and effective initial treatment of hypoxemia or low blood partial pressure O2 in adult and pediatric patients with acute lung injury, such as acute respiratory distress syndrome (51, 56). While improving O2 delivery to vital organs, sustained exposure to O2 at high fractions (>50%) impairs lung function (16, 24, 31, 49). There is ample evidence that the increased rate of formation of reactive O2 species (ROS) plays a key role in the pathogenesis of O2 toxicity and that this injury is exacerbated by a subsequent inflammatory response (10, 11, 25, 71).

Presently, there is no clinical means for detecting early or subclinical lung oxidant injury secondary to hyperoxia, i.e., before injury can be detected by computed tomography (CT) scans. Because patients requiring high O2 therapy come with widely differing tolerances of hyperoxia, real-time information stratifying patients based on degree of lung O2 toxicity is needed. Thus our long-term goal is to develop a clinical means for early detection of high O2 lung injury or tolerance in critically ill patients.

Several animal models have been developed to evaluate the time course, severity, and pathophysiological mechanisms of lung O2 toxicity (15, 24, 26, 53, 70). The rat model is unique in that when adult rats are exposed to >95% O2 environment for 2 days followed by a 24-h “rest period” in room air (hyper-95R), they acquire tolerance of the otherwise lethal effects of 100% O2, in that if transferred to a 100% O2 environment, they survive for prolonged periods (26). Conversely, rats exposed to 60% O2 for 7 days (hyper-60) become more susceptible to 100% O2, as evidenced by a decrease in their subsequent survival time in a 100% O2 environment (14, 36).

The concept that tissue uptake and retention of radiolabeled biomarkers change as a consequence of disease or injury forms the basis of nuclear medicine imaging, in which single-photon emitters, such as technetium ($^{99m}$Tc), are used for both conventional planar imaging and single-photon emission CT (SPECT) (38). Studies have shown that the lung capillary endothelium is an early, primary target of O2 toxicity and is a potential site at which biochemical adaptation to high O2 occurs, including up-regulation of the GSH redox cycle (15, 29, 45). This and the fact that the lung capillary endothelium has a large surface area in direct apposition with blood-borne compounds suggest that imaging of radiolabeled biomarkers can serve as noninvasive probes for detecting early lung oxidant injury in subjects requiring high O2 therapy (17, 33).

$^{99m}$Tc-labeled hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) is a clinical cerebral blood flow biomarker commonly used in SPECT (39, 46, 47). It is a neutral complex that exists in two forms—an oxidized lipophilic form and a reduced
hydrophilic form (46, 55, 62). Tissue uptake of HMPAO from the vascular region is determined by its rate of diffusion across the plasma membrane, which in turn is determined by the lipophilicity and concentration gradient of HMPAO across the plasma membrane (2, 46). Within the tissue, HMPAO can either diffuse back to the vascular region or is reduced to its hydrophilic, nondiffusible form and hence, retained within the tissue (2, 46). Conversion of HMPAO from the lipophilic to the nondiffusible hydrophilic form and thus its cellular retention is dependent on intracellular GSH content and other factors involving mitochondrial function (1, 30, 64). Altered HMPAO lung retention has been reported in subclinical lung injury due to chemotherapy, irradiation lung injury, lupus erythematosus, diabetes, volatile anesthetics, and inhalation and smoking injuries in the absence of significant perfusion impairment or X-ray abnormalities (13, 34, 37, 41, 43, 58, 61, 65). The objective of this study was to determine whether lung retention of HMPAO is differentially altered in rats exposed to hyper-60 or to hyper-95R and the contribution of GSH to HMPAO retention in these unique hyperoxia models. The results will be important in determining the use of HMPAO for providing prognostic information regarding the presence of high O2 lung injury or tolerance in individual hosts with differing susceptibilities to this disorder.

**METHODS**

**Materials.** HMPAO (Ceretec) was purchased from GE Healthcare (Arlington Heights, IL) and 99mTc-macroaggregated albumin (99mTc-MAA; particle sizes, 20–40 μm) from Cardinal Health (Wauwatosa, WI). Diethyl maleate (DEM) and other reagent-grade chemicals were purchased from Sigma Chemical (St. Louis, MO).

**Animals.** For normoxic (control) lung studies, adult male Sprague-Dawley rats (Charles River; 300–325 g) were exposed to room air. For the hyperoxic lung studies, age-matched rats were housed in a Plexiglass chamber maintained at hyper-60 or hyper-95R. The total gas flow was 3.5 l/min, and the carbon chamber dioxide (CO2) was maintained at <0.5%. The temperature within the chamber was 20–22ºC. Every other day, the rats were weighed, and their cage, food, water, and CO2 absorbent were changed. All rats were kept on a 12:12-h light-dark cycle. A total of 15 normoxic, 20 hyper-60, and 19 hyper-95R rats were studied. The protocol was approved by the Institutional Animal Care and Use Committees of the Zablocki Veterans Affairs Medical Center and Marquette University (Milwaukee, WI). For the hyper-60 and hyper-95R rats, the imaging and other studies described below were conducted immediately following the exposure period.

**Imaging studies.** 99mTc-HMPAO was prepared according to kit directions, whereas 99mTc-MAA was obtained in its labeled form. Rats were anesthetized with pentobarbital sodium (40–50 mg/kg body wt ip), the trachea was clamped, and heparin (0.7 IU/g body wt) was injected into the right ventricle (29). The pulmonary artery and the trachea were cannulated, and the pulmonary venous outflow was accessed via a cannula in the left atrium. The lungs and heart were removed from the chest and attached to a ventilation and perfusion system. The perfusate flow was set at 10 ml/min, and the lung was ventilated (15% O2, 6% CO2, 6% N2, 40 breaths/min) with end-inspiratory and end-expiratory pressures of −6 and 3 mmHg, respectively. The pulmonary arterial pressure was referenced to atmospheric pressure at the level of the left atrium. The venous effluent pressure was atmospheric pressure. At the end of some of the experiments, the lungs were weighed and then dried (60ºC) to a constant weight for determination of lung dry weight.

**Isolated Lung Preparation.** Mitochondrial dysfunction is a cardinal feature of hyperoxic lung injury (4, 7, 8, 23) and has been shown to alter HMPAO retention in other organs (1, 30). Thus the activities of mitochondrial complexes I and IV were determined as described previously (29). Briefly, lungs were isolated and washed free of blood with perfusate containing (in mM) 4.7 KCl, 2.5 CaCl2, 1.19 MgSO4, 2.5 KH2PO4, 118 NaCl, 25 NaHCO3, 5.5 glucose, and 2.5% Ficoll. Lungs were then removed from the perfusion system, weighed, minced, and homogenized with buffer (pH 7.2) containing (in mM) 225 mannitol, 75 sucrose, 5.3-[N-morpholino]propanesulfonic acid, 20 ethylene glycol-bis(b-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 2% fatty acid-free BSA, and 0.02 ml/ml protease inhibitor cocktail set III (Calbiochem, La Jolla, CA), using a Polytron tissue homogenizer. Lung homogenates were centrifuged at 1,500 g for 5 min at 4ºC, and the resulting supernatants were centrifuged again at 13,000 g for 30 min at 4ºC to obtain a crude mitochondrial fraction (P2). The P2 fractions were washed twice by resuspension in 8 ml ice-cold homogenization buffer without BSA and then centrifuged (13,000 g for 20 min at 4ºC). The final P2 fractions were resuspended in 1 ml BSA-free homogenization buffer. Complex I (NADH dehydrogenase) activity (nmol NADH oxidized-min−1·mg−1 protein) was determined as the difference between the rates of NADH oxidation in the presence and absence of rotenone over the linear portion of the reaction progress curve, as we have described previously (4, 29). Mitochondrial complex IV (cytochrome c oxidase) activity was measured as described by Storrie and Madden (63) using ferrocyanochrome c as the substrate. The protein concentrations were determined colorimetrically as described previously (3, 4).
GSH content. Lungs were isolated and washed free of blood with buffer containing (in mM) 10 HEPES, 5 glucose, and 5% dextran (~67,000 MW), pH 7.4. Lung tissue was dissected free from large airways and connective tissue and weighed. The tissue was then placed into 10 vol (per lung wet weight) of 4°C sulfosalicylic acid (5%), minced, and homogenized as above. The homogenate was centrifuged (10,000 g) at 4°C for 20 min, and the supernatant was used to determine GSH and GSSG lung content, as described by Owens and Belcher (50) and modified by Tietze (67) and Griffith (32).

Statistical evaluation of data. Statistical comparisons were carried out using ANOVA followed by Tukey’s test, with \( P < 0.05 \) as the criterion for statistical significance. ANOVA as well as linear regression analysis were carried out using SigmaStat (Systat Software, San Jose, CA).

RESULTS

Rats exposed to hyper-60 gained body weight steadily at a rate that is virtually the same as age-matched normoxic rats (29). On the other hand, rats exposed to hyper-95R did not gain body weight over the exposure period. These results are consistent with those from previous studies (26, 29).

Wet weight, dry weights, and wet/dry weight ratios of lungs from hyper-60 rats were not different from those of normoxic lungs (Table 1) (29). Rat exposure to hyper-95R increased lung wet and dry weight by 17% and 12%, respectively, with no effect on wet/dry weight ratio compared with normoxic lungs (Table 1), consistent with results from a study by Frank et al. (26). There was no difference in the isolated lung perfusion pressure at 10 ml/min perfusate flow among the three groups of lungs.

Typical HMPAO images from a normoxic, hyper-60, and hyper-95R rat acquired 10 min postinjection of HMPAO are shown in Fig. 1. The lung ROI was identified from the subsequent injection of \( ^{99m} \text{Tc-MAA} \), of which >95% lodges in the pulmonary capillaries in proportion to flow. Figure 1 shows lung and background HMPAO time-activity curves obtained from the time sequence of images of these same rats. Minimal HMPAO recirculation is observed, suggesting that most HMPAO lung uptake and retention occur during its first pass through the lungs (Fig. 1). This is consistent with the previously reported, relatively fast blood clearance rate; fast first-pass uptake; and prolonged retention (2, 46, 65) of HMPAO. The ratio of lung and background HMPAO activities (lung-to-background ratio) at steady state, a commonly used measure of lung tissue retention, increased significantly by ~25% in hyper-60 and ~250% in hyper-95R rats compared with normoxic rats (Fig. 2).

The role of tissue GSH content in the lung retention of HMPAO was investigated by pretreating rats with the GSH-depleting agent DEM prior to imaging (55). DEM decreased lung tissue GSH content (in lung tissue homogenate) by 96%, 84%, and 91% in normoxic, hyper-60, and hyper-95R rats, respectively (Table 2), consistent with results from previous studies (9, 19). Lung treatment with DEM decreased HMPAO lung-to-background ratio in normoxic and hyper-95R lungs by 26% and 56%, respectively, compared with ratios in the absence of DEM (Fig. 2). In hyper-60 lungs, the ratio in the presence vs. absence of DEM was not different (\( P = 0.125 \)). In addition, the ratios in the presence of DEM were higher in hyper-60 (46%) and hyper-95 (108%) rats than in normoxic rats. These results demonstrate that additional (DEM-insensitive) factors are involved in mediating HMPAO lung retention and that these factors are altered in both hyper-60 and hyper-95R rats.

Table 1. Lung wet weights, dry weights, and wet/dry weight ratio

<table>
<thead>
<tr>
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<th>Wet Weight (g)</th>
<th>Dry Weight (g)</th>
<th>Wet/Dry Ratio</th>
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<tbody>
<tr>
<td>Normoxic</td>
<td>1.16 ± 0.02</td>
<td>0.227 ± 0.005</td>
<td>5.11 ± 0.05</td>
</tr>
<tr>
<td>Hyper-60</td>
<td>1.24 ± 0.03</td>
<td>0.242 ± 0.004</td>
<td>5.13 ± 0.07</td>
</tr>
<tr>
<td>Hyper-95R</td>
<td>1.37 ± 0.04*</td>
<td>0.254 ± 0.008*</td>
<td>5.24 ± 0.08</td>
</tr>
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</table>

Values are mean ± SE. For lung wet weights, \( n = 13, 8, \) and 12 for control (normoxic), exposure to 60% oxygen (O\(_2\)) for 7 days (hyper-60), and exposure to >95% O\(_2\) for 2 days followed by 24 h in room air (hyper-95R) rats, respectively. For lung dry weights and wet/dry ratio, \( n = 9, 8, \) and 7 for normoxic, hyper-60, and hyper-95R, respectively. *Significantly different from normoxic values (\( P < 0.05 \), ANOVA).

Fig. 1. Right: HMPAO images from control (normoxic), exposure to 60% oxygen (O\(_2\)) for 7 days (hyper-60), or exposure to >95% O\(_2\) for 2 days followed by 24 h in room air (hyper-95R) rats. Dotted lines trace the lung based on macroaggregated albumin injection. Left: Time-activity curves from lung (•) and background (○) regions of interest of images from corresponding normoxic, hyper-60, and hyper-95R rats. LU, lung; BG, background.
For instance, Serrano-Mollar et al. (57) reported rat lung tissue glutathione content of 2.22 ± 0.12 (SE) μmol/(g lung wet weight) in the GSH form and 22.12 ± 2.49 μmol/(g lung wet weight) in the GSSG form and a GSH:GSSG ratio of 103.7 ± 8.7. These values are consistent with those from the present study for normoxic lungs (Table 2) when expressed per gram of lung wet weight (~2.0 μmol/(g lung wet weight) in the GSH form and ~15 μmol/(g lung wet weight) in the GSSG form).

Rat exposure to hyper-60 and hyper-95R increased lung tissue glutathione content by 19% and 40%, respectively, consistent with a potential role for GSH in the elevated lung HMPAO retention in these groups of rats (46, 64). For each group of rats, the HMPAO lung-to-background ratio at steady state from normoxic, hyper-60, and hyper-95R rats, respectively. With DEM treatment, n = 11, 3, and 3 for normoxic, hyper-60, and hyper-95R rats, respectively. * and †Different from normoxic, without or with DEM treatment, respectively. #Difference between without and with DEM for a given group of rats (P < 0.05, ANOVA).

To begin to determine the role of mitochondrial dysfunction in the GSH-independent (i.e., DEM-insensitive) lung retention of HMPAO, we evaluated the effect of hyper-60 and hyper-95R on the activities of complexes I and IV in lung tissue homogenates. Exposure to hyper-60 and hyper-95R decreased complex I activity by ~50% compared with activity in normoxic lungs (Table 2). However, complex IV activity was increased by 32% in hyper-95R only (Table 3). Figure 4 shows the correlation between the DEM-insensitive fraction of HMPAO retention (Fig. 3) and the complex IV/complex I activity ratio, which is an index of imbalance between mitochondrial respiratory complexes (44).

### DISCUSSION

The results of this study demonstrate that rat exposure to hyper-60 and hyper-95R increased the lung retention of HMPAO compared with normoxic lungs, but the increase was substantially larger in hyper-95R rats than in hyper-60 rats. The results without and with DEM treatment prior to imaging suggest that GSH-dependent process(es) contribute more to the lung retention of HMPAO in hyper-95R rats than in normoxic or hyper-60 rats. This observation is consistent with the larger increase in the GSH content in the lung homogenate of hyper-60 and hyper-95R rats compared with activity in normoxic lungs, but the increase was substantially larger in hyper-95R rats than in hyper-60 rats. The results without and with DEM treatment prior to imaging suggest that GSH-dependent process(es) contribute more to the lung retention of HMPAO in hyper-95R rats than in normoxic or hyper-60 rats. This observation is consistent with the larger increase in the GSH content in the lung homogenate of hyper-60 and hyper-95R rats compared with activity in normoxic lungs, but the increase was substantially larger in hyper-95R rats than in hyper-60 rats.

### Table 2. GSH and GSSG content of lung homogenate

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<tr>
<th></th>
<th>GSH (μmol/g Dry Wt)</th>
<th>GSSG (μmol/g Dry Wt)</th>
<th>GSH (μmol/g Dry Wt + DEM)</th>
<th>GSSG (μmol/g Dry Wt + DEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic</td>
<td>10.15 ± 0.56</td>
<td>0.074 ± 0.020</td>
<td>0.39 ± 0.22</td>
<td>ND</td>
</tr>
<tr>
<td>Hyper-60</td>
<td>12.07 ± 0.46*</td>
<td>0.108 ± 0.011</td>
<td>1.89 ± 0.26*</td>
<td>0.019 ± 0.002</td>
</tr>
<tr>
<td>Hyper-95R</td>
<td>14.17 ± 0.46*</td>
<td>0.130 ± 0.033</td>
<td>1.34 ± 0.26*</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are mean ± SE. For GSH content in the absence of diethyl maleate (DEM), n = 9, 6, and 5 for normoxic, hyper-60, and hyper-95R lungs, respectively. For GSSG content in the absence of DEM, n = 6, 4, and 4 for normoxic, hyper-60, and hyper-95R lungs, respectively. For GSH content in DEM-treated rats, n = 11, 3, and 3 for normoxic, hyper-60, and hyper-95R lungs, respectively. For GSSG content in DEM-treated rats, n = 11, 3, and 3 for normoxic, hyper-60, and hyper-95R lungs, respectively. ND, not detectable. GSSG content was lower than the detection limit of the assay. * and †Value significantly different from the corresponding normoxic value and hyper-60, respectively (P < 0.05, ANOVA).
Table 3. Mitochondrial complex I and complex IV activity measured in P2 fractions of lung homogenate

<table>
<thead>
<tr>
<th></th>
<th>Complex I Activity (nmol.min⁻¹.mg⁻¹ protein)</th>
<th>Complex IV Activity (nmol.min⁻¹.mg⁻¹ protein)</th>
<th>Complex IV/Complex I Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic</td>
<td>40.82 ± 2.21</td>
<td>231.8 ± 3.0</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Hyper-60</td>
<td>20.10 ± 2.61*</td>
<td>174.0 ± 17.9*</td>
<td>9.5 ± 1.9</td>
</tr>
<tr>
<td>Hyper-95R</td>
<td>19.94 ± 1.52*</td>
<td>304.9 ± 10.3*†</td>
<td>15.6 ± 1.3*†</td>
</tr>
</tbody>
</table>

Values are mean ± SE. n = 4, 5, and 5 for normoxic, hyper-60, and hyper-95R lungs, respectively. * and †Value significantly different from the corresponding normoxic value and hyper-60, respectively (P < 0.05, ANOVA).

95R rats than of hyper-60 rats compared with normoxic rats (Table 2). Furthermore, the results show that for all three conditions, a significant fraction of the lung retention of HMPAO is DEM insensitive, demonstrating that other (GSH-independent) factors determine the lung retention of HMPAO. Additional data suggest a role for mitochondrial function in the DEM-insensitive fraction of the lung retention of HMPAO.

Lung tissue retention of HMPAO is determined by its rate of diffusion across the plasma membrane and its rate of intracellular conversion to the reduced, nondiffusible form (46). Assuming HMPAO has freely permeating access to lung tissue on passage through the pulmonary circulation, then its tissue retention is determined by its rate of intracellular reduction to the nondiffusible form. This assumption is consistent with the fact that HMPAO is highly lipophilic with an octanol-water partition of ~83 (as compared with 0.006 for the reduced form) (2) and with the use of HMPAO as a clinical perfusion marker (2, 39, 46, 47).

Several studies have evaluated the role of GSH in the tissue retention of HMPAO in various organs, including the lung, using GSH-depleting agents such as DEM (5, 22, 55, 62). Sasaki et al. (55) reported that mouse treatment with DEM (0.55 g/kg ip) decreased the lung retention of HMPAO by 21%, prompting them to suggest a role for GSH in this retention. This result is consistent with the effect of DEM on the lung retention of HMPAO in normoxic rats in the present study. On the other hand, El-Shirbiny et al. (22) showed that rat treatment with DEM (0.68 g/kg ip) had no significant effect on the lung retention of HMPAO, leading them to conclude that this retention is not related to lung tissue GSH content. This result could be in part due to the dose of DEM used (0.68 g/kg vs. 1.0 g/kg in the present study), which decreased the GSH content in lung homogenate by only 60% compared with >90% in the present study and previous studies (9, 19).

The DEM-sensitive fraction of the lung retention of HMPAO (Fig. 2) may underestimate the contribution of lung tissue GSH to HMPAO retention. For instance, GSH depletion in other organs would be expected to decrease the retention of HMPAO by these organs and hence, increase the plasma concentration of the lipophilic HMPAO that is available for lung uptake. In addition, DEM would be expected to deplete plasma GSH content and hence, preserve a larger fraction of the injected HMPAO dose in the lipophilic diffusible form. Furthermore, Neirinckx et al. (46) suggested that GSH-independent factors, which may not be important to the overall lung retention of HMPAO in the absence of DEM, may contribute more in the presence of DEM, increasing the availability of HMPAO for these processes. Thus the DEM-sensitive fraction provides a lower bound on the GSH-dependent fraction of the lung retention of HMPAO.

For hyper-95R rats, the ~40% increase in the GSH content of lung homogenate is much lower than the approximate sevenfold increase in the DEM-sensitive fraction of the lung retention of HMPAO compared with normoxic lungs. One possible reason for this difference could be that the GSH content reported in this study is the average GSH content of all lung cells. Although the results of this study do not provide information regarding the specific types of lung cells contributing to the lung retention of HMPAO, previous studies have suggested that the lung retention of HMPAO is limited to endothelial cells (41, 59, 65), which account for ~50% of lung cells and are in direct contact with blood (15). Previous studies have also demonstrated that different cell types have different GSH content, and oxidant stress has different effects on the GSH content of these cells (20, 21). For instance, Denke et al. (20) demonstrated that exposure of endothelial cells to hyperoxia (85% O2 for 48 h) increased GSH content by 85%. On the other hand, neutrophil GSH content is not sensitive to oxidant injury (21). Thus depending on the GSH content of the various lung cells and how the GSH content of these cells change in response to exposure to hyperoxia, the GSH content in lung homogenate measured in this study may overestimate or underestimate the effect of hyperoxia on the GSH content of pulmonary capillary endothelial cells.

Another possible reason for the difference between the effect of rat exposure to hyper-95R on GSH in lung homogenate and the DEM-sensitive fraction of the lung retention of HMPAO could be the increase in lung mitochondrial complex IV activity in hyper-95R compared with normoxic lungs. This increase could decrease ROS production at complexes I and III since the higher the respiration rate, the less time the electrons are...
delayed at critical leakage sites such as complexes I and III (12, 42). Since GSH is a key scavenger of hydrogen peroxide (H₂O₂), a decrease in ROS production in hyper-95R lungs would increase the fraction of GSH tissue content that is available for HMPAO reduction in hyper-95R lungs compared with normoxic lungs (66).

Reduced thioredoxin is an effective reductant of disulfides in proteins and peptides, such as GSSG and peroxiredoxins, which catalyze the reduction of H₂O₂ using reduced thioredoxin as an electron donor (48, 69). Thus reduced thioredoxin could contribute to the DEM-sensitive component of HMPAO lung tissue retention through its effect on GSSG and H₂O₂. As such, an increase in the reduced thioredoxin content of lung tissue of hyper-95R rats could also contribute to the difference between the effect of rat exposure to hyper-95R on GSH in lung homogenate and the DEM-sensitive fraction of the lung retention of HMPAO discussed above. Additional studies would be needed to evaluate the effect of rat exposure to hyper-60 and hyper-95R on the lung thioredoxin system (68, 69).

Several GSH-independent factors have been suggested to contribute to the tissue retention of HMPAO, including vascular permeability, mitochondrial dysfunction, and endothelial amine metabolism dysfunction (1, 30, 41, 60, 65). Shih et al. (60) demonstrated an increase in the lung uptake of HMPAO in smokers and suggested that this increase may be due in part to increased vascular permeability. Although in the present study, vascular permeability was not measured, the results show that neither hyper-60 nor hyper-95R had an effect on lung wet/dry weight ratio as an index of vascular permeability.

Previous studies have suggested a role for mitochondrial redox state and bioenergetics in the cellular retention of HMPAO (1, 27, 30). Gardner et al. (30) demonstrated a negative correlation between the brain uptake of HMPAO and the activity of the Krebs cycle enzyme citrate synthase as an index of mitochondrial function in depressed patients. With the use of mouse brain homogenate, Fujibayashi et al. (27) demonstrated that ~68% of HMPAO was found in the mitochondrial fraction compared with 27% in the cytosolic fraction. Ahn et al. (1) demonstrated that mitochondrial inhibitors and uncouplers decreased HMPAO tissue-to-suspending-medium ratio in rat brain cerebral cortex slices. They postulated that these inhibitors may decrease mitochondrial ATP, which is needed for the synthesis of GSH and its maintenance in the reduced form and hence, account for the decrease in HMPAO tissue-to-medium ratio (1). This suggests that the GSH-dependent fraction of lung HMPAO retention is sensitive to a decrease in mitochondrial ATP synthesis.

The results of the present study demonstrate that both hyper-60 and hyper-95R resulted in ~50% depression in complex I activity. This depression has been suggested to play a key role in lung O₂ toxicity by compromising mitochondrial ATP synthesis (29, 52). Thus according to Ahn et al. (1), this depression in complex I activity would be expected to decrease lung retention of HMPAO in hyper-60 and hyper-95R lungs compared with normoxic lungs. However, HMPAO retention was higher in both hyper-60 and hyper-95R lungs. Additional studies would be needed to evaluate the effect of this depression in complex I activity on mitochondrial ATP synthesis in hyper-60 and hyper-95R lungs.

The mitochondrial complex IV/complex I ratio has been used as an index of imbalance between mitochondrial respiratory complexes and has been shown to correlate inversely with protein carbonyl content (as a measure of mitochondrial protein oxidation) in mouse brain synaptic mitochondria (44). The results of the present study demonstrate a positive correlation between the ratio of complex IV/complex I and the DEM-insensitive fraction of HMPAO lung retention. This supports the possibility that altered mitochondrial function and HMPAO retention are causally related. Additional studies would be needed to evaluate the effect of these changes in complexes I and IV activity on mitochondrial function and in turn, on the lung retention of HMPAO.

In this study, we opted to use in vivo planar imaging with a single modular gamma camera. The advantage of this approach over full three-dimensional SPECT is that the equipment required is readily available and affordable, and the experimental methods and analysis are straightforward. Moreover, hyperoxic lung injury is thought to be a relatively homogeneous injury throughout the lung so that SPECT would not be expected to provide any additional information about total HMPAO retention within the lung. Also, in this study, images were acquired at one frame/s during the 1st min to visualize the dynamics of the uptake and retention and then at one frame/min out to 15 min when steady state is attained. Although here, we used only the steady-state portion of the time-activity curves, the earlier portion of the curves provide kinetic information for future pharmacokinetic modeling of HMPAO intended to characterize more fully the mechanisms involved in its lung uptake and retention.

In conclusion, the results suggest a role for GSH in HMPAO lung retention and that hyper-95R treatment resulted in a larger HMPAO retention than hyper-60, predominantly due to an increase in the DEM-sensitive component, consistent with a larger increase in GSH in hyper-95R lungs than hyper-60 lungs compared with normoxic lungs. Because patients requiring high O₂ come with differing tolerances of hyperoxia, information that permits clinicians to determine which patients are developing high O₂ lung injury or tolerance is valuable. The results suggest that the ability of lung tissue to increase GSH content as measured by the GSH-dependent component of the lung retention of HMPAO may be indicative of a patient’s ability to tolerate sustained exposure to hyperoxia.
REFERENCES


Effect of Hyperoxia on Lung HMPAO Retention in Vivo • Audi SH et al.


