Effect of hyperbaric oxygen therapy in experimental subcutaneous and pulmonary infections due to *Pseudomonas aeruginosa*

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Luongo C, Imperatore F, Materia MG, Mangoni G, Marmo M, Baroni A, Catalanotti P, Rossi F, Filippelli A. Effect of hyperbaric oxygen therapy in experimental subcutaneous and pulmonary infections due to *Pseudomonas aeruginosa*. Undersea Hyper Med 1999; 26(1):21–25. About 80% of nosocomial infections are caused by aerobic bacteria. *Pseudomonas aeruginosa* is a Gram-negative bacterium belonging to the Pseudomonadaceae family. *P. aeruginosa* is responsible for 6–22% of all hospital infections. The aim of this study was to evaluate the efficacy of hyperbaric oxygen (HBO₃) therapy (2 atm abs × 55 min · day⁻¹) alone for 8 days and combined with antibiotic chemotherapy (amikacin 15 mg · kg⁻¹ · day⁻¹ for 8 days by intraperitoneal route) in rats infected subcutaneously and via the pulmonary route. In the rats infected by *P. aeruginosa*, HBO₃ induced a reduction in mortality and morbidity with bacteria eradication in blood culture, bronchial aspirate, and skin biopsies when compared to control. These effects were increased by the use of amikacin, an antibiotic used for the treatment of sensitive Gram-negative bacteria.

hyperbaric oxygen, amikacin, pulmonary and subcutaneous infections, Pseudomonas aeruginosa

About 80% of nosocomial infections are caused by aerobic bacteria (1). *Pseudomonas aeruginosa* is a Gram-negative, opportunistic, motile, aerobic bacterium belonging to the Pseudomonadaceae family (2). More recently, it has been demonstrated that this microorganism is responsible for 6–22% of all intensive care unit infections (3). Recently, the incidence of these infections has increased due to the use of new invasive diagnostic or therapeutic procedures and to the increasing number of immunodeficient patients (4). *P. aeruginosa* has also been isolated in pulmonary, kidney, and skin infections (5).

In the literature, it has been demonstrated that hyperbaric oxygen (HBO₃) therapy has bacteriostatic and bactericidal activity (6). HBO₃ therapy is commonly used in the treatment of lower extremity infections in patients with diabetes mellitus, peripheral vascular diseases or both (7). The efficacy of HBO₃ alone or in combination with specific antimicrobial agents in several experimental animal models has been evaluated (8). The aim of this study was to experimentally evaluate the efficacy of HBO₃ therapy without and with antibiotic chemotherapy (amikacin) in rats infected by *P. aeruginosa* subcutaneously and via the pulmonary route.

**MATERIALS AND METHODS**

**Animals**: Wistar male rats (Morini, Milan, Italy) weighing 250–300 g were used. The animals were housed in compliance with good laboratory practice for the protection of experimentally used animals (constant temperature: 21° ± 1°C; relative humidity: 60%; regular alternation light-dark: light 0700–1900 water and food, ad libitum).

**Bacterial stock**: *P. aeruginosa* was isolated in broth culture at 37°C from a cystic fibrosis patient's expectoration at our institution, identified by conventional methods and biochemically confirmed by miniature system (APE 20 Biomerieux, Marcy l'Etoile, France). The broth culture was washed in 0.9% NaCl solution and suspended again in 0.9% NaCl solution to exhibit an optic density of about 0.400 nm [5 × 10⁶ colony-forming unit (CFU) · 100 μl⁻¹].

**Subcutaneous infection induction**: 100 μl of broth culture of *P. aeruginosa* (5 × 10⁶ CFU · 100 μl⁻¹) were inoculated subcutaneously into the paw dorso-lateral zone, after trichotomy and disinfection. After 30 h, a gangrenous ecihyma with ecchymosis and opalescent and serous-hemorrhagic blisters was observed in the paw (Fig. 1). The infection was confirmed by two biopsies (Fig. 2). Only

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infected rats were used for the experimental study.

*Technique inducing pulmonary infection:* Animals were anesthetized with 30 mg · kg⁻¹ of sodium thiopental by intraperitoneal route; 100 μl of broth culture of *P. aeruginosa* (5 × 10⁶ CFU · 100 μl⁻¹) were then inoculated by endotracheal instillation through a polyethylene tube into the trachea. The tracheal tube was rapidly removed and the animals were put in single cages. Infection was confirmed by an endobronchial washing after 24 h.

*Analysis of blood and infected tissues:* After induction of infection in all experimental groups, a blood culture was performed on 0.5 ml of arterial blood from the femoral artery. The blood culture was repeated at Day 8 after starting of antibiotic chemotherapy and/or HBO₂ therapy. Day 8 was considered the last day of experimental study. Also, at the beginning and at the end of the study a subcutaneous biopsy fragment culture test was obtained from the subcutaneous tissues of infected rats. A bronchial aspirate culture test was obtained on an endobronchial washing with 1 ml of 0.9% of NaCl solution 24 h after the induction of lung infection and at the end of the study.

*Tissue bacterial count:* Tissue fragments (skin biopsy and bronchial aspirate) were homogenized in peptone (Difco Laboratories, Detroit, MI) 0.1% and then diluted in the same medium. One milliliter of each dilution was then incorporated in plate count (Difco) and incubated for 48 h at 37°C. Bacterial count was stopped at values of 10⁶ CFU · ml⁻¹ bronchial aspirate and 10⁸ CFU · mg⁻¹ tissue. These values are considered significant infections (9).

*Treatment:* Eight groups of animals were studied (10 rats for each group): group 1, subcutaneous infection by *P. aeruginosa*; group 2, pulmonary infection by *P. aeruginosa*; group 3, subcutaneous infection by *P. aeruginosa* treated with amikacin; group 4, pulmonary infection by *P. a-
eruginosa* treated with amikacin; group 5, subcutaneous infection by *P. aeruginosa* treated with HBO₂; group 6, pulmonary infection by *P. aeruginosa* treated with HBO₂; group 7, subcutaneous infection by *P. aeruginosa* treated with amikacin and HBO₂; and group 8, pulmonary infection by *P. aeruginosa* treated with amikacin and HBO₂.

Hyperbaric oxygen treatment was given starting 24 h after induction in a cylindrical steel chamber (40 cm diameter × 65 cm length, Galeazi La Speza, Italy) with thick glass windows allowing for direct observation of animals during treatment. Before pressurization, 100% medical oxygen was flushed through the chamber for 5 min to displace room air. Oxygen pressure was then increased at constant rate to reach a pressure of 2 atm abs in 4 min. Animals were treated for 55 min under compression. The chamber was constantly ventilated at a rate of 4 liter · min⁻¹ to avoid carbon dioxide accumulation during pressurization. Gases were analyzed and O₂ concentration was >99% (Taylor Servomex 0A272 Oxygen Analyzer, Naples, Italy) and carbon dioxide below 0.2% (Medical Gas Analyzer LB-2, model 240M, Beckman, USA). Animals were exposed to HBO₂ daily for 8 days.

Antibiotic therapy was carried out with amikacin (Bristol Meyers Squibb, Rome, Italy) 15 mg · kg⁻¹ · day⁻¹ for 8 days by intraperitoneal route, administered starting 24 h after induction of infection.

*Statistical analysis:* Statistical analysis was carried out by Snedecor and Cochran Fisher's test (Fisher's Exact Test) (10). Values with a P < 0.05 were considered significant.

**RESULTS**

*Subcutaneous infection:* In control subcutaneously infected rats a 10% mortality rate was observed by Day 5 (Table 1). The persistence of *P. aeruginosa* in blood
culture and in biopsy tissues was observed in 100% of the surviving animals (Table 2).

The treatment with amikacin did not reduce the incidence of mortality in subcutaneously infected rats, but it induced a significant reduction of persistence of \textit{P. aeruginosa} in blood culture and biopsy tissues (Tables 1 and 2). In subcutaneously infected rats, HBO\textsubscript{2} therapy alone reduced mortality (Table 1). Moreover, a reduction of \textit{P. aeruginosa} in blood culture and in biopsy tissues was observed.

When the biopsy, carried out at the end of study, showed the presence of \textit{P. aeruginosa}, the quantitative culture revealed 10\textsuperscript{8} CFU \cdot mg\textsuperscript{-1} of tissue.

In the group of rats subcutaneously infected, the treatment with amikacin associated with HBO\textsubscript{2} produced 0% of mortality (Table 1). Also, blood culture and biopsy tissues of subcutaneously infected rats did not reveal the presence of \textit{P. aeruginosa} in this group (Table 2).

\textbf{Pulmonary infection:} In control rats infected via the pulmonary route, 80% mortality rate was observed by Day 5 (Table 3). In the surviving animals the persistence of \textit{P. aeruginosa} in blood culture and in bronchoalveolar washing was 100% (Table 4).

The treatment with amikacin significantly reduced the incidence of mortality in rats infected via the pulmonary route. This last effect was due to a decrease of persistence of \textit{P. aeruginosa} in blood culture and bronchoalveolar washing (Tables 3 and 4).

In rats infected via the pulmonary route, HBO\textsubscript{2} alone significantly reduced mortality (Table 3) and a reduction of \textit{P. aeruginosa} in blood culture and in bronchoalveolar washing was observed. In endobronchial washing showing the persistence of \textit{P. aeruginosa} at the end of study, the quantitative culture revealed 10\textsuperscript{8} CFU \cdot ml\textsuperscript{-1}.

In the group of rats infected via the pulmonary route, the treatment with amikacin associated with HBO\textsubscript{2} produced 0% mortality (Table 3). Also, blood culture and bronchoalveolar washing of rats infected via the pulmonary route did not reveal the presence of \textit{P. aeruginosa} in this group (Table 4).

\textbf{DISCUSSION}

It has been demonstrated that the interaction between HBO\textsubscript{2} and antimicrobial agents has important implications for the therapy of infections, because when combined they deeply affect the bacteriostatic and bactericidal activity of certain antimicrobial agents against specific microorganisms (11).

Increased O\textsubscript{2} tension can induce changes in host tissues that may influence metabolism and/or activation of certain antimicrobial agents (11). Moreover, increased O\textsubscript{2} tension can also induce metabolic or genetic responses in microorganisms that may alter the microorganisms's susceptibility to antimicrobial agents (11). Finally, increased O\textsubscript{2} tension may alter pharmacokinetics of antimicrobial agents by affecting central hemodynamics and/or regional blood flow. The former two hypothe-

\begin{table}
\centering
\caption{Mortality in Rats Infected via the Subcutaneous Route}
\begin{tabular}{|c|c|c|}
\hline
Group, \( n = 10 \) & \multicolumn{2}{c|}{Mortality} \\
& \( n \) & Percent \\
\hline
1, control & 1 & 10 \\
3, amikacin & 1 & 10 \\
5, HBO\textsubscript{2} & 0 & 0 \\
7, HBO\textsubscript{2} + amikacin & 0 & 0 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Mortality in Rats Infected via the Pulmonary Route}
\begin{tabular}{|c|c|c|c|}
\hline
Group, \( n = 10 \) & \multicolumn{3}{c|}{Mortality} \\
& \( n \) & Percent & Day of Mortality \\
\hline
2, control & 8 & 80 & 2.4 \pm 0.6\textsuperscript{a} \\
4, amikacin & 2 & 20\textsuperscript{b} & 2 \\
6, HBO\textsubscript{2} & 1 & 10\textsuperscript{b} & 2 \\
8, HBO\textsubscript{2} + amikacin & 0 & 0 & -- \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Persistence of \textit{P. aeruginosa} in Blood Culture and Persistence and Quantification in Subcutaneous Biopsy Tissues in Rats Infected via the Subcutaneous Route}
\begin{tabular}{|c|c|c|}
\hline
Group & Blood Culture & Biopsy Tissues \\
& \( n \) & Persistence of \textit{P. aeruginosa}, \% & Biopsy Tissues \\
& & & CFU \cdot mg\textsuperscript{-1} of tissue \\
\hline
1, control; \( n = 9 \) & 9 & 100 & -- \\
3, amikacin; \( n = 9 \) & 2 & 22.2\textsuperscript{a} & -- \\
5, HBO\textsubscript{2}; \( n = 10 \) & 1 & 10\textsuperscript{b} & -- \\
7, HBO\textsubscript{2} + amikacin; \( n = 16 \) & 0 & 0 & -- \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}All tests were done on Day 8 (the end of study). \textsuperscript{b}\( P < 0.05 \). \textsuperscript{c}\( P < 0.01 \) vs. control.
Table 4: Persistence of *P. aeruginosa* in Blood Culture and Persistence and Quantitative Number in Bronchial Aspirate in Rats Infected via the Pulmonary Route

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Culture</th>
<th>Bronchial Aspirate</th>
<th>Bronchial Aspirate</th>
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<tbody>
<tr>
<td></td>
<td>Persistence of <em>P. aeruginosa</em>, n</td>
<td>Persistence of <em>P. aeruginosa</em>, n</td>
<td>CFU <em>·mL&lt;sup&gt;-1&lt;/sup&gt;</em> Bronchial Aspirate</td>
</tr>
<tr>
<td>2, control; n = 2</td>
<td>2</td>
<td>2</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>4, amikacin; n = 8</td>
<td>4</td>
<td>2</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>6, HBO&lt;sub&gt;2&lt;/sub&gt;; n = 9</td>
<td>2</td>
<td>2</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>8, HBO&lt;sub&gt;2&lt;/sub&gt; + amikacin; n = 10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

*All tests were done on Day 8 (end of study).<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 vs. control, <sup>c</sup>P < 0.05 vs. amikacin.*

...res are quantitatively more important in the development of the antimicrobial activity of specific agents (11).

In particular, it is possible to suppose that the increase of superoxide (toxic radicals) induced by HBO<sub>2</sub> is toxic to anaerobic and aerobic bacteria that are devoid of enzymes (i.e., superoxide dismutase), which are able to neutralize these radicals (12).

Our results show that HBO<sub>2</sub> has antimicrobial activity in experimental subcutaneous and via pulmonary route infections caused by *P. aeruginosa*. In fact, HBO<sub>2</sub> induced 100% survival in rats infected via the subcutaneous route, and 80% survival in rats infected via the pulmonary route. Moreover, HBO<sub>2</sub> treatment caused a significant decrease of *P. aeruginosa* in blood culture, in bronchial aspirate, and in subcutaneous biopsy tissues.

Our results also show that HBO<sub>2</sub> induces an increase in the therapeutic effects of amikacin, a selective drug for *P. aeruginosa* infections. In fact, in the rats infected via the subcutaneous and pulmonary route, HBO<sub>2</sub>, combined with amikacin improved survival rate to 100%; these effects were associated with the absence of *P. aeruginosa* in blood culture, bronchial aspirate, and subcutaneous biopsy tissues at the end of treatment.

Several authors have observed that O₂ tension significantly influences the activity of some aminoglycosides (amikacin, gentamicin, netilmicin). Two mechanisms that might explain this interaction have been proposed (12). The first one involves the uptake of the aminoglycosides into the bacteria. Uptake begins with the diffusion of aminoglycosides through the cell wall into the periplasmic space of Gram-negative bacteria. The antimicrobial agent is then transported into the cytoplasm by a low affinity carrier(s) in the bacterial cytoplasmic membrane; this transport is ATP dependent and therefore requires the high concentration of ATP that only aerobic metabolism can produce. Therefore O₂, influencing the potential of cytoplasmic membrane, may affect the rate of transport of aminoglycosides.

The second mechanism by which oxygen may potentiate the activity of aminoglycosides is the prolongation of antibiotic effect. This latter hypothesis is not clearly understood. One possibility is that O₂ induces an oxidative stress that bacteria fail to adapt to for the presence of protein synthesis inhibition caused by the aminoglycoside (13). In normal conditions, procarvocytes rapidly adapt to oxidative stress induced by reactive oxygen species (13).

Our data showed that HBO<sub>2</sub> alone or combined with a specific antibiotic therapy is able to promote resolution of infections caused by *P. aeruginosa*.

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REFERENCES


