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EXPERIMENTALS STUDIES ON THE EFFECT OF HYPERBARIC OXYGEN ON PSEUDOMONAS AERUGINOSA IN VITRO

Małgorzata Wolańska

Sanitary and Hygienic Laboratory of the Navy in Gdynia

ABSTRACT

The aim of the study was to determine the effect of hyperbaric oxygen on the growth and metabolism of Pseudomonas aeruginosa in vitro. The experiments were carried out on agar media and blood culture plates. Bacterial culture plates were exposed to hyperbaric oxygen under varying conditions of pressure and exposure time. Significant inhibition of bacterial growth and proteolytic activity was observed. Hyperbaric-treated pathogens also showed less virulence after the infection of experimental animals.
Keywords: oxygen, hyperbaric conditions, aerobic bacteria, relatively anaerobic bacteria.

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INTRODUCTION

Interest in the effects of hyperbaric oxygen on microorganisms most often concerns anaerobic bacteria, as hyperbaric oxygen therapy has been used as one of the methods to treat some forms of infections caused by these bacteria [1,2,3,4,5].

Previous results of work on relative anaerobes and absolute aerobes suggest that elevated oxygen pressure also has an inhibitory effect on the growth of these bacteria [6,7,8,9.10,11]. The changes that were observed in the bacteria studied concerned not only growth, but also appearance, metabolism, ability to produce venoms and pigments and sensitivity to antibiotics [9,10,12,13,14].

Works by individual authors [6,7,10,15].on the effects of hyperbaric oxygen therapy on *Pseudomonas aeruginosa in vitro* and on the applicability of this method in the treatment of infections caused by these bacteria are scarce and their results are inconclusive.

However, *Pseudomonas* infections are occurring with an increasing frequency, mainly intra-hospital, and are often life-threatening, especially in neonatal, resuscitation, urology and surgical wards, in all those patients whose physiologically normal defence mechanisms are impaired. The number of these infections is growing rapidly and etiotropic treatment options are limited, often unreliable.

As *Pseudomonas aeruginosa* infections represent one of the biggest epidemiological and clinical problems today, it was decided to study the effect of hyperbaric oxygen on these bacteria *in vitro*, in order to provide a basis for the possible future use of this therapeutic method to combat infections caused by these bacteria.

MATERIAL AND METHODOLOGY

A strain of *Pseudomonas aeruginosa* isolated from urine, exhibiting high proteolytic activity and high virulence for mice, was used for the study. Studies were conducted using solid media (plain agar and agar plate with 5% preserved human blood). A surface culture technique was used. In the first stage of the study, plates with inoculated material were exposed to oxygen at 2, 3 and 4 ata for 4, 8 and 16 hours, and in the second stage the physiological and biochemical properties of the strains were studied in comparison with the control sample. The latter consisted of plates with material stored at atmospheric pressure and plates treated with 1 ata 0_2 for 4, 8 and 16 hours.

The study was conducted in an oxygen pressure chamber constructed for animal experiments. A total of 1,800 platelets were exposed to hyperbaric oxygen. After the plates were removed from the chamber, they were incubated for 24 hours at 37°C and biochemical, physiological characteristics, as well as LD_{50} for mice and antibiotic resistance were determined. The range of tests included:

- Dye assay [16],
- Determination of growth and motility in the presence of 1% TTC on originally modified Selenka's substrate,
- Observation of growth at 22 and 42° according to Hayens [17],
- Growth in the presence of 6% sodium chloride [18],
- Growth in the presence of 0.1% cetrimide using Brown and Lowbury's method modified by Muszyński [17],
- Cytochrome oxidase production by the Kovacs method [16],
- Oxygen degradation of glucose [18],
- Production of arginine dihydrolase according to Phillips [18],
- Decomposition of gelatine,
- Catalase production,
- Determination of proteolytic activity according to Schmidt et al.[20],
- Determination of haemolysin activity according to Liu [17],
- LD_{50} determination for mice using Reed and Muonch's method [16],
- Determination of antibiotic resistance by serial dilution method on Grove and Rendall medium [16].

The results obtained were subjected to statistical analysis, determining the degree of significance of the results at the confidence level of alpha = 0.01 and alpha = 0.05 and using the Student's t-test. The relationship between the characteristics was presented in the form of a simple regression equation, which was calculated using the least squares method.

RESULTS OF STUDIES

Findings from these studies show that some of the traits tested using hyperbaric oxygen therapy undergo significant changes. Differences between the test strain and the control strain concerned growth, dye production capacity, proteolytic activity, and virulence for mice. Most of these changes occurred after oxygen pressures of 3 and 4 ata O_2 were applied to the bacteria for 16-hour exposures. A pressure of 3 and 4 ata O_2 at 16-hour exposures was found to result in a loss of pyocyanin and fluorescein dye production capacity and a lack of haemolysis of cells on a plate with 5% human preserved blood.

The effect of hyperbaric oxygen on bacterial growth was already marked at 2 ata O_2 after 16-hour exposures, followed by a significant decrease in bacterial numbers. At pressures of 3 and 4 ata O_2, a significant decrease in the number of bacteria was already marked after an 8-hour exposure. In the group that was subjected to 1 ata O_2 pressure, a significant increase in the number of bacteria was observed after 4-hour exposures.

The study also found an effect of hyperbaric oxygen on the ability of *Pseudomonas aeruginosa* to produce gelatine. Oxygen pressures of 2 ata O_2 at 16-hour exposures resulted in statistically significant differences between the test and control strains. At pressures of 3 and 4 ata O_2, these differences were also present after 16-hour exposures. Based on the results, it can be said that increasing oxygen pressure values were accompanied by a decrease in gelatine activity, as manifested by a reduced gelatine digestion zone. It was noted that the correlation of the characteristics was straightforward. A regression equation was calculated for the results obtained, and the straight line for this equation is shown in Fig. 1.

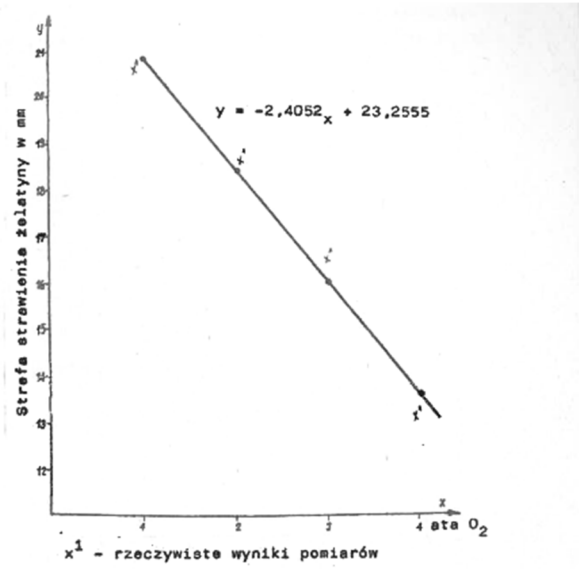

Fig. 1 Dependence of the activity of *Pseudomonas aeruginosa* on oxygen pressure (16-hour exposures). (OY – gelatine digestion zone; OX – actual measurements).

Similar results were obtained when examining the effect of oxygen hyperbaria on the proteolytic activity of *Pseudomonas aeruginosa*. Statistically significant differences occurred at pressures of 2, 3 and 4 ata O_2, but also only after 16-hour exposures. Proteolytic activity was found to decrease with increasing pressure. A simple regression equation is shown in Fig. 2.

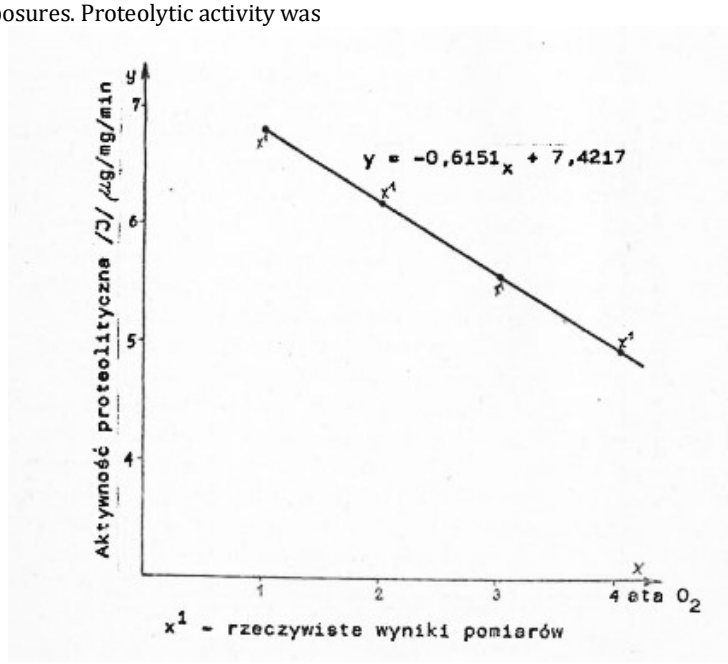

Fig. 2 Dependence of proteolytic activity *Pseudomonas aeruginosa* on oxygen pressure (16-hour exposures). (OY - proteolytic activity, OX – actual measurements).

An important step was to test whether and to what extent the virulence of *Pseudomonas aeruginosa* treated with hyperbaric oxygen changes. By determining the LD_{50} for mice, it was shown that virulence decreases at 16-h exposures from a value of 1.8 x 10^7 cells for the control strain to 13 x 10^7 cells for the strain treated with 4 ata O_2

These results are presented in Fig. 3.

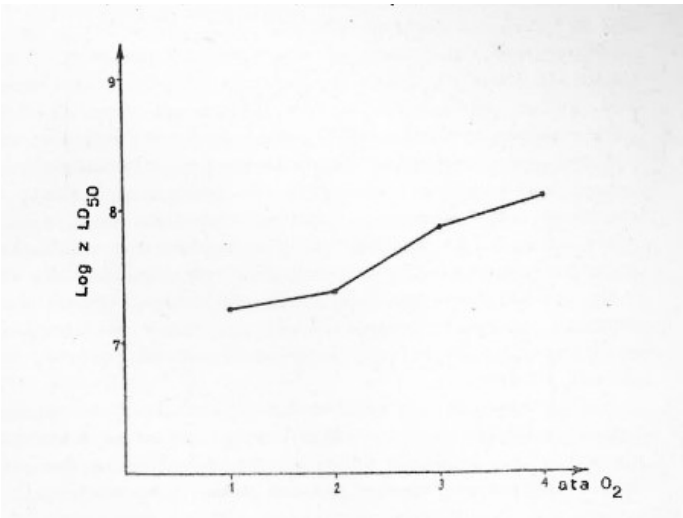

Fig. 3. Impact of oxygen pressure on LD *Pseudomonas aeruginosa* for mice (16-hour exposure).

The decrease in the virulence of the strain is probably linked to a decrease in its proteolytic activity, since the roles of these enzymes in the virulence of *Pseudomonas aeruginosa* strains, according to recent work, appear to be unquestionable [17,21].

It would be necessary to examine the mechanism and processes triggered by the increased oxygen pressure leading to the observed changes. Discussion around this issue is far from easy as no attempts to explain this mechanism in bacteria have been reported in the available literature. There are a number of theories explaining toxic effects of oxygen on human and animal body [22]. Apparently, similar mechanisms could explain such effects of oxygen on bacteria. The reduction in proteolytic enzyme activity could be explained by the impact of pressurised oxygen on the sulfhydryl groups, as they are particularly sensitive to oxygen [22]. Oxygen causes the blockade of the -SH groups of enzymes, so it acts as their inhibitor. Most proteolytic enzymes contain -SH groups in their active centres. It may be that the attachment of the inhibitor resulted in a conformational change of the protein [23], resulting in a decrease in the activity of the enzyme. This would result in a reduced number of oligopeptides entering the bacterial cell, which are formed specifically by proteolytic enzymes. It appears that the metabolic activity of the cell may be undermined because of this, and at the same time the energy balance necessary for, among other things, cell growth would be reduced.

Reduced cellular metabolism and insufficient energy storage may also have been the reason for the inhibition of pigment excretion, as the mechanism of excretion outside the cell comes at the expense of consuming a certain amount of energy.

CONCLUSIONS

1 Under hyperbaric conditions oxygen exhibited bacteriostatic effects on *Pseudomonas aeruginosa* bacilli *in vitro*.
2 A decrease in proteolytic activity of *Pseudomonas aeruginosa* was found for hyperbaric oxygen.
3 At higher oxygen pressures, a lack of pyocyanin and fluoresceine production was noted.
4 *Pseudomonas aeruginosa* bacilli exposed to oxygen under hyperbaric conditions showed a lower degree of virulence for mice.
5 The effect of hyperbaric oxygen on *Pseudomonas aeruginosa in vitro* was dependent on oxygen pressure and the duration of oxygen exposure.

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Małgorzata Wolańska
Laboratorium sanitarno-higieniczne Marynarki Wojennej w Gdyni