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Negative pressure produced by bacteria: A possible cause of negative middle ear pressure in ears with otitis media

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Conclusions: It is suggested that oxygen consumption by bacteria could be a cause of the negative middle ear pressure in ears with otitis media.

Objective: To determine whether oxygen consumption and production of carbon dioxide by bacteria could be a cause of negative pressure in ears with otitis media.

Methods: Hermetically-sealed tubes (2ml of volume) containing 1 ml of culture media with *Streptococcus pneumoniae* leaving 1 ml of airspace were connected to a micro-pressure sensor, and were maintained at 37 degree centigrade in a water bath. Chronological changes of air pressure in the tube were monitored for 15 hours (bacterial group), and were compared with those in the tubes containing culture media only (controls). Also, partial pressures of oxygen and carbon dioxide in the culture media of those bottles were measured.

Results: The pressure of the bacterial group were significantly lower than that of controls (bacterial group; -94.6±92mmH_2O, control group; 4.6±11.1mmH_2O, 2 way-ANOVA, $P<0.001$). The partial pressures of oxygen were lower (bacterial group; -86.0±22.1mmHg, control group; -34.6±7.93mmHg, 2 way-ANOVA, $P<0.0001$) and those of carbon dioxide were significantly higher in the bacterial group than in the control group (bacterial group; 11.8±3.8mmHg, control group; -0.23±0.41mmHg, 2 way-ANOVA, $P<0.0001$).

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Key words: Bacteria, negative middle ear pressure, otitis media, oxygen consumption

Introduction

Otitis media with effusion (OME) is one of the most common ear diseases seen in children. It is well known that most of ears with OME have negative middle-ear (ME) pressures, manifesting retraction of the tympanic membrane, which is considered the first step of developing retraction-type serious ear diseases such as cholesteatoma and adhesive otitis media [1, 2]. Although many causes of the negative ME pressure, such as 'hydrops ex vacuo' theory [3], sniffing [4], clearance of effusion [5], and Toynbee's phenomenon [6] have been reported, it is true that there are many cases of which negative ME pressure cannot be explained by those factors.

We previously reported that consumption of oxygen by bacteria in the ME during suffering from otitis media (OM) may possibly be one of the causes of the development of negative ME pressure by measuring the pressure in the hermetically-sealed tubes containing bacteria and culture media [7]. This time, in addition to the total ME pressure, we examined the change of the partial pressures of the oxygen and carbon dioxide to clarify the mechanism of the production of the negative pressure by bacteria.

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Materials and methods

Bacteria

We used the clinical isolates of a bacterial strain of *Streptococcus Pneumoniae (S. pneumoniae)* obtained from the Department of Laboratory Medicine, Nagasaki University Hospital.

Liquid culture media

The product used was Muller-Hinton broth (Disco, USA), to which 0.8 % Strepto-Heamo supplement (Eiken Chemical Co. Ltd, Japan) was added.

Experiment procedures

Hermetically-sealed bottles (2ml Eppendorf (EP) tubes) containing 1ml of 10^7-10^8 cfu/ml of *S. pneumoniae* leaving 1ml airspace (10 samples, bacterial group) being maintained at the temperature of 37 degree centigrade in an incubator were connected to a micro-pressure sensor (Mikrotip; Millar Instrument, TX, USA) (Figure 1). Before starting the pressure monitoring, we kept the circuit open for approximately 30 min for adjustment of the temperature of the media and for stabilization of the sensor. The air pressure in the EP tube was monitored for 15 hrs (bacteria group). The chronological changes of the pressure were compared with those of the control group (only culture media without *S. pneumoniae,* 10 samples). We also measured partial pressures of oxygen and carbon dioxide of these culture media with bacteria every several hours (i-STAT 300F FUSO pharmaceutical Industries, Ltd.). Since those partial pressures in the culture media have to be measured by sampling the media, the tubes were opened for the measurement. However, once the tube was opened, the total pressure of the airspace in the tube cannot be monitored after that. This is why we prepared 8 bottles of samples of the same condition, and monitored them. One of the 8 samples was used for monitoring only the total pressure without opening it through 15 hours, and the remaining 7 bottles were opened at 0, 1, 2, 3, 4, 5, and 15 hours after starting monitoring.

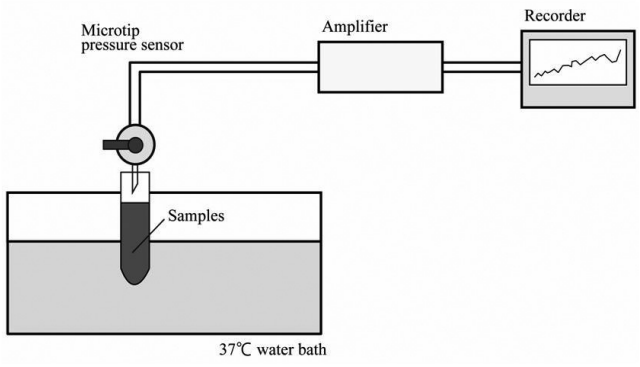

Fig. 1 Block diagram of the experiment. Samples were hermetically sealed and maintained at 37oC in the bath, and the pressure was continuously monitored by a micro pressure sensor.

Results

The pressures in the most of the bacterial group showed progressive decrease during 15 hours' monitoring (-94.6 ± 92mmH_2O), while those of control group showed almost no change; the difference was statistically significant (4.6 ± 11.1mmH_2O, control group, P<0.001、2way-ANOVA, Fig.2). The partial pressures of oxygen gradually decreased compared with control group. The decrease was rapid during the first one hour, and the pressure kept on slowly decreasing until 5 hours, but became the steady state afterwards in the bacterial group, while those of control group showed only a slight change; the difference between them was statistically significant (-86.0 ± 22.1mmHg, control group; -34.6 ± 7.93mmHg, 2 way-ANOVA, P<0.0001, Fig.3, 4). The partial pressures of the carbon dioxide increased significantly more in the bacterial group than in the control group 5 hours after start of the measurement (8 bottles, 11.8 ± 3.8mmHg, control group; -0.23 ± 0.41mmHg, 2way-ANOVA, P<0.001, Fig 5, 6).

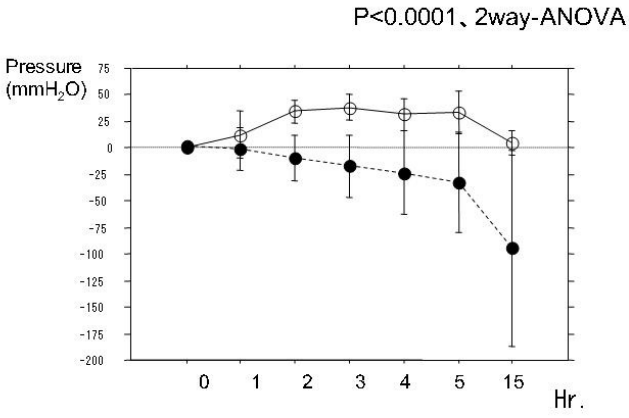

Fig. 2 Chronological changes in the mean pressure of the bacterial and control groups. It gradually decreased in the bacterial group in comparison with control group.

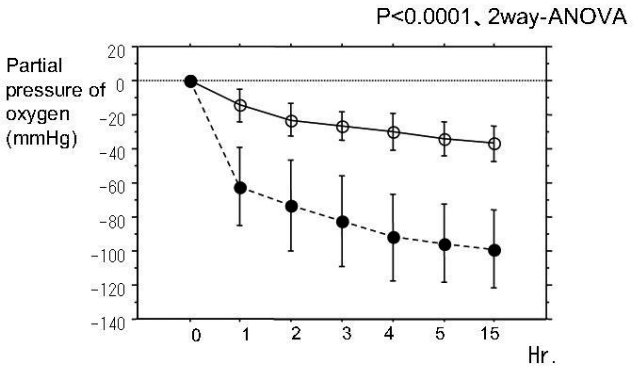

Fig.3 Chronological change of the partial pressure of the oxygen in culture media. Red circle: bacterial group. Black circle: Control group.

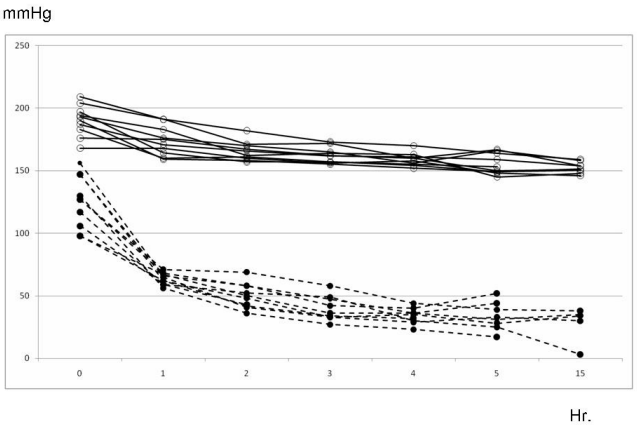

Fig.4 The difference in mean of the change of the partial pressure from the beginning of the oxygen in culture media. The decrease in the partial pressure was significantly greater in bacterial group than in the control group.

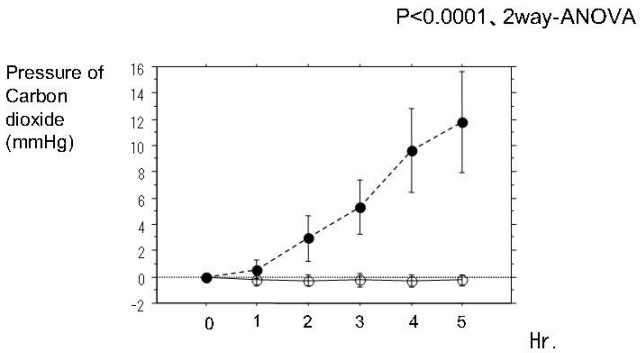

Fig.5 Chronological change of the partial pressure of the carbon dioxide in culture media. Red circle: bacterial group. Black circle: control group.

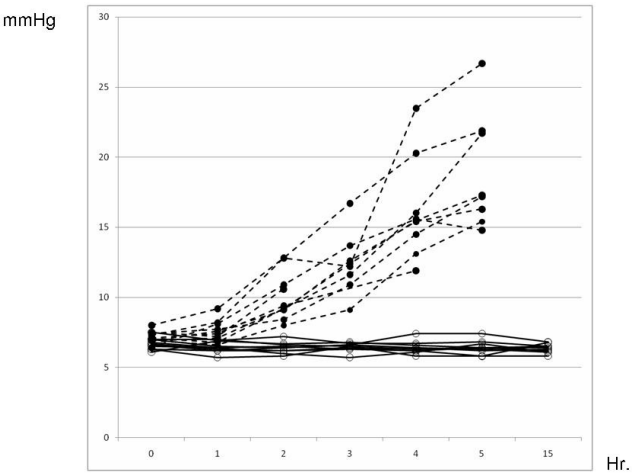

Fig.6 The difference in mean of the change of the partial pressure from the beginning of the carbon dioxide in culture media. The bacterial group showed significantly increase whereas the control group showed almost no change.

Discussion

'Hydrops ex vacuo' theory, where ME gas is absorbed and consists of negative pressure to make ME effusion when the Eustachian tube (ET) is obstructed, had been a reasonable explanation of the cause of negative ME pressure in ears with OM. However, it has become well known that ME pressure may not become progressively negative even if ET function was abolished [8-10]. It is at least partly because the gas exchange through the ME mucosa plays an important role in the ME pressure regulation as well as the ET.

We previously reported that the pressures in the bottles containing *S. pneumoniae*, one of the most common pathogens causing OM, was observed to decrease progressively; it showed a clear contrast to those in the bottles without bacteria [7]. This suggested that bacterial infection in the ME could be one of the causes of the negative ME pressure through metabolism of bacteria. However, we could not have its sufficient evidence, because the measurements of the partial pressure of oxygen done in our previous study were only at the start and at the end of the experiment, and those measurements were not done on all but only on a few samples. Furthermore, the partial pressure of carbon dioxide was not measured in any samples in the previous study.

This time, the air pressure of the bottles decreased as in the previous report [7], accompanying the decrease in the partial pressure of oxygen and the increase in that of the carbon dioxide. These results seem to support our hypothesis that oxygen consumption by bacteria could be one of the causes of negative ME pressure in patients with OM. The

reason why we could measure the partial pressure of carbon dioxide only 8 out of 10 samples in the bacterial group is unknown, but is speculated to be due to technical limitation of the measuring apparatus during measuring in the changing environment of the culture medium such as pH or bacterial concentration, etc., because those troubles happened only in the bacterial group.

S. pneumonia, an aerotolerant anaerobe, is an organism that creates ATP by aerobic respiration in the environment with rich oxygen or by anaerobic respiration in the environment with a low level of oxygen [11], and it suggests it can make ATP in environments of various oxygen concentrations [12]. At first, oxygen in the culture media was consumed rapidly and carbon dioxide increased as the result of consumption of oxygen by *S. pneumoniae*. The rate of dissolution of gases into the culture media from the air in sealed bottles depends on the solubility coefficient of each gas. It was thought that the time spent in the passive diffusion in the airspace of the bottle should be longer than the change of partial pressures within the media, because the former is a slow passive movement while the latter is an active change produced by metabolism of *S. pneumoniae*. This may account for the difference in the course of the change in the partial pressures between oxygen and carbon dioxide in this experiment.

The bacteria in the culture media is considered to increase according to the growth curve, and the ratio of the increase depends on the environment. In this study, the negative pressure was about -94.6mmH_2O on an average. We may speculate that the pressure decrease would be more profound in the ME, because nutrients and oxygen would be supplied abundantly in the living human bodies from the blood supply under the mucous membrane.

Furthermore, in the control group, the pressures increased for several hours from the start of the measurement in some bottles, while the partial pressure of the oxygen decreased during several hours. A possible reason of those changes is that more oxygen might have been solved into in the culture media during preparing the samples than in the normal state, for example, due to bubbling during pipetting, and after the start of the measurement the excessive oxygen within the culture medium may have gradually moved into the air within the bottle so that its partial pressure may be equilibrated between in the culture medium and the airspace. This may have caused the increase in the air pressure within the bottle and the decrease in the partial pressure of oxygen in the culture medium. On the other hand, the partial pressure of the carbon dioxide did not change throughout the measurements in the control groups. It may be because the partial pressure of carbon dioxide in the air was too low to cause the change of its partial pressure during preparing such as by pipetting, and was also too low to cause the partial pressure change in the fluid or in the air pressure.

Conclusions

From the present results, the decrease in the oxygen and increase in the carbon dioxide by the bacterial metabolism could be one of the causes of the production of negative ME pressure in ears with OM.

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