EFFECT OF NITROUS OXIDE ON THE GROWTH OF ESCHERICHIA COLI IN CULTURE CONDITIONS: A PRELIMINARY STUDY

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SUMMARY

Purpose: *The aim of this study was to determine the role of nitrous oxide (N_2O) in the bacterial growth in vitro.* ***Methods:*** *We assessed the effects of N_2O on Escherichia coli (E.coli) growth in a liquid nutrient broth under conditions similar to those in clinical practice, using an anaesthetic circuit. Bacterial inoculums were prepared from reference strains with exponential growth of 10^3 colony forming units (CFU) ml^{-1}. Sterile Petri dishes were filled with 10 ml suspension of an E. coli strain and incubated at 37°C. Each strain was studied with or without exposure to 50% N_2O, using an airtight glass chamber in different exposure times (baseline,1 and 3 h). For each time, a control point was obtained. Serial dilutions and agar plates were made and developed colonies were counted by using the standardized microbiological methods.* ***Results:*** *E. coli was grown on nutrient agar after exposure to a mixture of N_2O-oxygen, and to air alone as the control group. Although there were no significant differences between the N_2O and the control group, the significant increases in bacterial growth were observed at the first and third hours in both groups ($p < 0.001$).* ***Conclusion:*** *We concluded that N_2O did not alter the growth of E.coli in vitro.*

Key Words: *Anaesthetics, Gases; Nitrous Oxide, Anti-Bacterial Effect, Bacterial Growth.*

INTRODUCTION

Postoperative infection is still a considerable problem for anaesthetized patients, as well as for the operating room personnel. There are many important factors in the development of postoperative infections, such as the size of the inoculum, the type of anaesthetic circuit, the effectiveness of sterilization, intubation and mechanical ventilation; but the role of anaesthetic agents have not yet been clearly defined (1). The direct effect of inhalational anaesthetics on bacterial growth has given contradictory results in previous studies. On the one hand, inhalational anaesthetics have specific immunological effects (2, 3) and direct effects on bacterial growth (4-6). On the other hand, many studies have reported that some inhalational anaesthetics are bactericidal (7-9). Although the inhibitory effect of some inhalational anaesthetics on human neutrophils is reversible, it is surprising that this effect is not reversible when these agents are combined with nitrous oxide (7, 10).

Nitrous oxide (N_2O) is a non-irritating and colourless gas and has a pleasant odor. It is obtained by heating ammonium nitrate to 240°C ($NH_4NO_3 \rightarrow 2H_2O+N_2O$). N_2O was introduced into medicine as an analgesic and sedative during painful procedures like the extraction of teeth in 1844 by American dentist Horace Wells. In 1881, Klicowitsch introduced N_2O as an analgesic during childbirth (11). Since 1971, it has been used as an analgesic for emergency medical purposes in ambulances. Today, in many countries, N_2O itself is an obvious ingredient in general anaesthesia and the use of N_2O as an analgesic during childbirth has been established all over the world. It is also being used during the cryosurgery in gynaecology. Even though N_2O has been used for a long period of time, its effects on bacterial growth have not yet been fully described.

The aim of this preliminary study was to evaluate the role of clinically used concentrations of N_2O on the growth rate of a gram-negative bacteria (E.coli) in cultures under conditions similar to those of clinical practice.

MATERIALS AND METHODS

Anaesthetic circuit

A ventilator (Boyle 2000 ohmeda- 7700, Finland) operating in anaesthetic circuit was set for a patient weighing 70 kg: tidal volume 700 ml, inspired O_2 (FiO_2) 50%, breath frequency 12 per minute. The mean pressure was kept at 10 cm H_2O in the circuit. N_2O at 50% was circulated with oxygen in the anaesthetic circuit. An anaesthetic bag of 2.5 l was used to represent the respiratory system. Three airtight glass chambers were set up on the inspiratory limb. Each chamber contained 10 ml of bacterial suspension in exponential growth. The disposable heat-moisture exchangers and antibacterial filters (Thermovent HEPA, Portex, United Kingdom) were fixed to both sides of each chamber to prevent contamination of other traps and that of the ventilator. The chambers were placed and fixed in a water bath thermostatically kept at 37°C .

There was a double aperture in the chambers for the circulation of gas. Oxygen and N_2O concentrations were monitored at the last chamber exit, using a gas chromatograph (Diascope Anesthetic-Artema, Denmark) to determine the concentrations of oxygen and N_2O.

Microbiological method

A reference E. coli strain (ATTC 35218) was isolated from stock cultures and incubated for 24 h. A paired set of each bacteria containing 10^3 CFU ml^{-1} in 200 ml of nutrient broth (DIFCO, USA) was prepared. The strains were randomly assigned to the N_2O group and the control group. While one of these pairs was incubated at normal atmospheric conditions, the other was subjected to the flow of 50% N_2O- 50% oxygen mixture. Bacterial growth of each strain was measured by the samples taken at baseline (T_0), after 1 h (T_1) and after 3 h (T_2). For each time, a control point was obtained with bacteria incubated in air. Three samples were removed from each plate at each sampling period. Five serial ten-fold dilutions were prepared from each sample in sterile saline for each time point (T_0,T_1 and T_2). Three samples of 100 ml, removed from each dilution, were plated onto nutrient agar media in standard sterile Petri dishes (diameter of 9 cm) at each sampling time. All incubations were performed at 37°C for 18-24 hours. The determination of the number of viable bacterias was made by counting CFUs on the plates yielding colonies between 50-300 on a box.

Statistical analysis

For each measurement, a median of the number of bacteria was calculated in order to enable us to establish a correlation between bacterial growth and sampling times. Mauchly's test was used to analyse the interaction between time and group. For each defined sample, the N_2O and control groups were compared with each other by repeated measures of two-way ANOVA.

RESULTS

The growth of E. coli has been shown in Table 1. The bacterial growth for the control group confirmed that the organisms were in the log-phase of growth. Compared with initial values, a significant increase in bacterial growth was observed in the first hour of exposure in both

Table - 1 : Effect of N_2O on the growth of E.coli.

Time	Bacterial cell growth after exposure to (median) 50% N_2O	Air alone
T_0	10^3	10^3
	10^3	10^3
	10^3	10^3
	(10^3)	(10^3)
T_1	$2.0x10^3$	$2.5x10^3$
	$2.0x10^3$	$2.0x10^3$
	$3.0x10^3$	$2.5x10^3$
	($2.0x10^3$)*	($2.5x10^3$)*
T_2	$3.5x10^3$	$4.5x10^3$
	$4.0 x10^3$	$4.5x10^3$
	$4.5x10^3$	$4.0x10^3$

*: $p<0.05$ (comparison with T_0)

+: $p<0.05$ (comparison between groups)

Results are expressed in colony forming units (C.F.U.)

To = baseline, T_1 = after 1 h, and T_2 = after 3 h with or without N_2O.

groups. This effect persisted at the third hour of exposure ($p<0.001$). There were no significant differences between the N_2O group and the control group.

DISCUSSION

The main finding of this study is that exposure to N_2O does not alter E.coli growth under experimental conditions similar to those in clinical practice. During this study the fresh gas was a mixture of oxygen and N_2O delivered at a concentration rate equivalent to 50%, which is a normal clinical concentration. The contact between the gas and the bacteria was performed in liquid at 37°C, which corresponds to clinical conditions and gives optimal conditions for bacterial growth. The studied bacterial inoculums, dosed to CFU ml^{-1}, were in exponential growth. Soda lime was not used in the circuit because of its controversial bactericidal effects (12,13). E. coli was chosen as the experimental species because it was one of the most commonly isolated agent of gram-negative rod bacteremias. The liquid nutrient broth was selected due to its high protein content, which closely approximates bronchial or alveolar fluid content. The large interface was provided between gas and liquid broth, in which a large surface area is suitable for gas exchange. Under these conditions, we observed the effect of N_2O on the bacterial growth at a clinically relevant concentration. The observed relationship between the time course exposure and the change of bacterial growth in this study was the same in the N_2O group and the control group.

Welch (8) reported that enflurane was able to inhibit microbicidal activity of human polymorphonuclear leukocytes against E.coli and that this inhibition was temporal and did not alter in combination with 70% N_2O. Furthermore, no effects on bactericidal activity were observed with 70% N_2O alone. Welch and Zaccari (10) have reported that 80% N_2O did not inhibit the microbicidal oxidative activity of human neutrophils, nor did it show an additive inhibition when combined with halothane. Although N_2O concentrations, time-course exposure and experimental conditions used in these studies were different from those in our study, our findings were similar to those of two above-mentioned studies relevant to N_2O.

N_2O, which that is a compressed gas, is often used to obtain the low temperatures needed for the cryosurgery in gynaecology. Due to this low temperature, optimal conditions for bacterial growth in the area of cryosurgery cannot be provided. As a consequence of this, during the cryosurgery in gynaecology, infection is not a considerable problem in the tissue to which cryosurgery is applied. However, even with a proper scavenging unit, leaks which would increase the level of pure N_2O in the work area may occur. There are still unanswered questions. We neither measured the possible effects of oxygen nor studied the other pathogenic bacterias in this experimental study.

Under the simulated clinical conditions, we were not able to find any experimental and statistically significant differences in the growth

of E. coli between with or without N_2O groups. We concluded that N_2O did not alter the growth of E.coli in vitro, but whether this effect is clinically important or not remains to be determined. This preliminary study has been continuing to investigate the effects of other anaesthetic gases and oxygen on the growth of other pathogen bacterias.

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