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Effects of sevoflurane and/or nitrous oxide on bacterial growth in in vitro culture conditions

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Abstract
The aim of this study was to determine the role of sevoflurane and/or nitrous oxide on bacterial growth under conditions in vitro similar to those of clinical practice. We assessed these effects on *Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, and *Staphylococcus aureus* growth. Bacterial inoculums were prepared from reference strains in nutritive broth. Airtight chambers were filled with bacterial suspensions. Each strain was studied with and without exposure to sevoflurane and/or nitrous oxide at baseline, after 1 and 3 h. Serial dilutions and agar plates were made and the colonies were counted. *P. aeruginosa* were grown after exposure to the nitrous oxide alone (2.8×10^3 colony-forming units/ml; CFU ml^{-1}) after 3 h according to the control ($P < 0.05$). *A. lwoffii* were grown after exposure to the nitrous oxide and sevoflurane with nitrous oxide (8.7×10^3 and 8.0×10^3 CFU ml^{-1}) ($P < 0.05$), respectively. There were no changes in *S. aureus* growth in controls and anesthesia groups. We conclude that the effects of anesthetic agents on bacterial growth may change owing to the type of anesthetic and microorganism.

Key words Anesthetics · Inhalation · Sevoflurane · Nitrous oxide · Bacterial growth

The effects of inhalational anesthetics on bacterial growth have shown contradictory results in previous studies. In some of these studies it has been stated that inhalational anesthetics have no direct effects on bacterial growth [1,2]. Conversely, it has been reported in some other studies that some inhalational anesthetics are bactericidal [3,4]. The effects of nitrous oxide on bacterial growth have not yet been clearly described. The effects of sevoflurane also have not yet been investigated. The growth of *Acinetobacter* species, *Staphylococcus* species, and gram-negative rods in anesthetic circuits were determined in a previous bacteriologic study [5].

The aim of this study was to evaluate the effect of clinically used concentrations of sevoflurane and/or nitrous oxide on the growth rate of three different pathogenic bacteria: *Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, and *Staphylococcus aureus* in cultures under conditions similar to those in clinical practice.

We examined bacterial growth in an anesthetic circuit, in which a ventilator operating in the anesthetic circuit (S/5; Datex-ohmeda, Helsinki, Finland) was set for a patient weighing 70 kg: tidal volume, 700 ml; inspired O_2, 40%; breath frequency, 12 per min. The mean pressure in the circuit was kept at 10 cmH_2O. Sevoflurane, at a minimal alveolar concentration (MAC) of 1.5, and/or nitrous oxide 60% was circulated in oxygen. A 2.5-l anesthetic bag was used to represent the respiratory system. Three airtight glass chambers were set up in series on the inspiratory limb. Each chamber contained 200 ml of bacterial suspension. Disposable heat-moisture exchangers and antibacterial filters (Thermovent HEPA; Portex, London, UK) were fixed to both sides of each chamber. The chambers were placed and fixed in a water bath at 37°C. There was a double aperture in the chambers for the circulation of gases (Fig. 1). Anesthetics were monitored at the last chamber exit (Diascope Anesthetic; Artema, Albertslund, Denmark). Tubes, filters, and chambers were replaced with new ones at the beginning of each experiment. A new setting was used for each combination of bacteria and gases.

The following microbiological method was used. Reference strains of *P. aeruginosa* (ATCC 27853), *A. lwoffii* (ATCC 19002), and *S. aureus* (ATCC 25923) were subcultured from stock cultures and incubated for 24 h. Paired sets of each bacteria, containing 10^3 colony-forming units/ml (CFU ml^{-1}) in 200 ml of nutrient broth (DIFCO; Detroit, MI, USA), were prepared. The strains were randomly assigned to the sevoflurane, nitrous

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Table 1. Growth of *P. aeruginosa*, *A. lwoffii*, and *S. aureus* after exposure to sevoflurane and/or nitrous oxide

	Control	Sevoflurane	Control	Nitrous oxide	Control	Sevoflurane with nitrous oxide
P. aeruginosa						
T_0 ($n = 3$)	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
T_1 ($n = 3$)	$1.2 \times 10^{3\#}$	$1.7 \times 10^{3\#}$	$1.2 \times 10^{3\#}$	$1.3 \times 10^{3\#}$	$1.6 \times 10^{3\#}$	$1.4 \times 10^{3\#}$
T_2 ($n = 3$)	$1.9 \times 10^{3\#}$	$2.3 \times 10^{3\#}$	$1.3 \times 10^{3\#}$	$2.8 \times 10^{3}*{:}^{\#}$	$1.8 \times 10^{3\#}$	$1.6 \times 10^{3\#}$
A. lwoffii						
T_0 ($n = 3$)	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
T_1 ($n = 3$)	$2.9 \times 10^{3\#}$	$2.4 \times 10^{3\#}$	$1.2 \times 10^{3\#}$	$2.0 \times 10^{3\#}$	$1.3 \times 10^{3\#}$	$2.0 \times 10^{3\#}$
T_2 ($n = 3$)	$4.1 \times 10^{3\#}$	$4.4 \times 10^{3\#}$	$2.3 \times 10^{3\#}$	$8.7 \times 10^{3}*{:}^{\#}$	$2.1 \times 10^{3\#}$	$8.0 \times 10^{3}*{:}^{\#}$
S. aureus						
T_0 ($n = 3$)	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
T_1 ($n = 3$)	1.1×10^3	1.0×10^3	1.0×10^3	0.9×10^3	1.0×10^3	0.9×10^3
T_2 ($n = 3$)	1.0×10^3	1.1×10^3	1.0×10^3	1.1×10^3	1.0×10^3	1.1×10^3

Values are expressed in exponential colony-forming units per ml (median)
$^*P < 0.05$ (compared with control); $^{\#}P < 0.05$ (compared with T_0)
T_0, baseline; T_1, after 1 h; T_2, after 3 h with or without gases

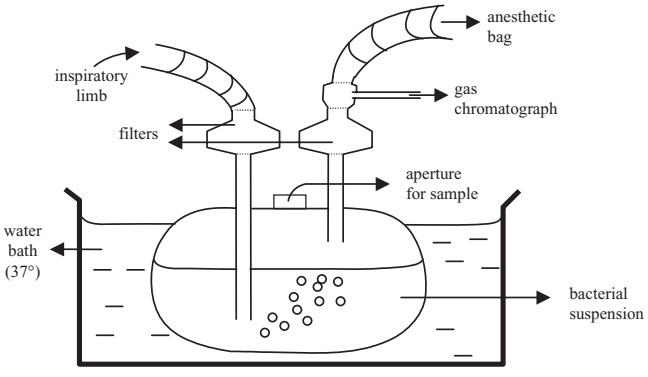

Fig. 1. Model of glass chamber

oxide, and sevoflurane with nitrous oxide groups. One of the pairs was subjected to a flow of sevoflurane 3% (sevoflurane), or nitrous oxide 60% (nitrous oxide), or sevoflurane 3% with nitrous oxide 60% (sevoflurane with nitrous oxide) in oxygen. An oxygen concentration of 40% and temperature of 37°C were provided in all control and study groups, using the same system. The bacterial growth of each strain was measured by samples taken at baseline (T_0), and after 1 (T_1) and 3 h (T_2). For each time, a control was obtained with bacteria incubated at 37°C. Three samples were removed from each chamber at each sampling period. Five serial tenfold dilutions in sterile saline were prepared from each sample for each time point (T_0, T_1, and T_2). Three samples, each of 100 µl, removed from each dilution, were plated onto nutrient agar media in standard sterile Petri dishes at each sampling time. All incubations were performed at 37°C for 24 h. The estimation of the number of viable bacteria for each time point was made by counting colonies on plates yielding between 50 to 300 colonies on a box.

For the statistical analysis, all values were corrected exponentially in such a way that the initial values would be 1.0×10^3. For each measurement, a median of the number of bacteria was calculated from three samples. A repeated-measures two-way analysis of variance (ANOVA) test was used in the comparisons of groups. Mauchly's test was used to analyze the interaction between times and groups. $P < 0.05$ was considered significant.

The growth of *P. aeruginosa*, *A. lwoffii*, and *S. aureus* is shown in Table 1.

Comparisons with control values showed that although the growth of *P. aeruginosa* was promoted after the exposure to nitrous oxide alone (2.8×10^3 CFU ml^{-1}; $P < 0.05$), it was not altered after the exposure to sevoflurane, or to sevoflurane with nitrous oxide at T2 (3 h). The growth of *A. lwoffii* was enhanced after exposure to nitrous oxide and to sevoflurane with nitrous oxide (8.7×10^3 and 8.0×10^3 CFU ml^{-1}; $P < 0.05$), respectively, at T2 (3 h). There were no significant changes in *S. aureus* growth.

Comparisons with initial values showed that the growth of *P. aeruginosa* and *A. lwoffii* increased at T_1 (1 h) and T_2 (3 h) in all exposured groups and controls compared to the initial values ($P < 0.05$). There was no change in *S. aureus* (Table 1). The effects of time, group, and group by time on the control and growth of *P. aeruginosa* and *A. lwoffii* were significant. For *S. aureus*, the effects of time, group, and group by time were nonsignificant.

The main finding of this laboratory investigation was that exposure to sevoflurane and/or nitrous oxide altered the growth of *P. aeruginosa* and *A. lwoffi* under experi-

mental conditions similar to those in clinical practice. We found different responses of the different bacteria to the gas exposure. In all groups, the same oxygen concentration was provided, as it is known that oxygen concentration has a major impact on bacterial growth [6]. The contact between the gases and the bacteria was performed in liquid at 37°C, which corresponds to clinical conditions and gives optimal conditions for bacterial growth. Soda lime was not used in the circuit because of its controversial bactericidal effects [5,7]. Furthermore, it is known that soda lime is a carbon dioxide absorbant and the use of soda lime is not necessary in clinical pediatric anesthesia practice, unless carbon dioxide is present in the anesthetic circuit. There was no carbon dioxide in this experiment, due to the absence of patients. *P. aeruginosa*, *A. lwoffii* and *S. aureus* were chosen as the experimental species because they were common agents isolated in nosocomial bacteremia associated with anesthesia [4]. The liquid nutrient broth was selected due to its high protein content, which closely approximates bronchial or alveolar fluid content. Under these conditions, we observed the effects of sevoflurane and/or nitrous oxide on bacterial growth at 3 h of exposure and at clinically relevant concentrations. In our study, the observed relationship between the exposure time and the change of bacterial growth at 1 h did not differ among the groups. All significant changes in bacterial growth occurred at 3 h. The growth rates between the three control values for the same bacteria were different. Although it is known that the growth requirements vary for specific bacteria, the possible mechanisms of the effects of anesthetic agents on bacterial growth are, as yet, unknown.

Under the simulated clinical conditions, we were not able to find any significant differences in the growth of *S. aureus* with and without anesthetic gases. Also, Asehnoune et al. [2] have reported that isoflurane did not affect the growth of *S. aureus*. The observation period of 3 h that we used may seem short for *S. aureus*, but, 3 h is sufficient according to anesthetic practice.

We concluded that nitrous oxide alone and sevoflurane with nitrous oxide increased the growth of *P. aeruginosa* and *A. lwoffii* in vitro, but whether this effect is clinically important or not remains to be determined. It appears that the effect of anesthetic agents on bacterial growth may differ according to the type of anesthetic used, the duration of the anesthesia, and the type of pathogenic microorganism.

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