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Antibacterial effect of sevoflurane and isoflurane

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ABSTRACT

Introduction. Multidrug resistant bacteria are increasing worldwide and therapeutic options are limited. Some anaesthetics have shown antibacterial activity before. In this study, we have investigated the antibacterial effect of the halogenated anaesthetic agents sevoflurane and isoflurane against a range of resistant pathogens.

Methods. Two experiments were conducted. In the first, bacterial suspensions of both ATCC and resistant strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were exposed to liquid sevoflurane and isoflurane during 15, 30 and 60 minutes. In the second experiment clinical resistant strains of *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *P. aeruginosa*, *Acinetobacter baumannii*, *S. aureus*, and *Enterococcus faecium* were studied. Previously inoculated agar plates were irrigated with the halogenated anaesthetic agents and these were left to evaporate before the plates were incubated. In both experiments colony forming units were counted in resultant plates.

Results. In the first experiment, isoflurane showed faster and higher antimicrobial effect than sevoflurane against all the strains studied. Gram-negative organisms were more susceptible. In the second experiment, *E. faecium* was found to be resistant to both halogenated agents; only isoflurane showed statistically significant activity against the rest of the strains studied.

Conclusions. Both halogenated agents, but particularly isoflurane, showed *in vitro* antibacterial activity against pathogens resistant to conventional antibiotics. Further investigation is required to determine whether or not they also exhibit this property *in vivo*. This might then allow these agents to be considered as rescue treatment against multidrug resistant pathogens, including a topical use in infected wounds.

Key words: Sevoflurane, Isoflurane, Anaesthetics, Inhalation, Anti-Infective Agents.

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Actividad antibacteriana de sevoflurano e isoflurano

RESUMEN

Introducción. Las bacterias multirresistentes están aumentando en todo el mundo y las opciones terapéuticas son limitadas. Algunos anestésicos han mostrado actividad antibacteriana previamente. En este estudio hemos investigado dicha actividad en los anestésicos halogenados sevoflurano e isoflurano frente a un grupo de patógenos resistentes.

Métodos. Se llevaron a cabo dos experimentos. En el primero se enfrentaron suspensiones bacterianas de aislados clínicos resistentes y cepas de referencia (ATCC) de *Staphylococcus aureus*, *Escherichia coli* y *Pseudomonas aeruginosa* a sevoflurano e isoflurano en su forma líquida durante 15, 30 y 60 minutos. Una muestra de la suspensión obtenida se inoculó en agar sólido y se incubó. En el segundo experimento se estudiaron aislados clínicos multirresistentes de *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *P. aeruginosa*, *Acinetobacter baumannii*, *S. aureus* y *Enterococcus faecium*. Placas de agar inoculadas con una cantidad conocida de las cepas se expusieron a los anestésicos líquidos, hasta su evaporación completa, antes de su incubación. En ambos experimentos se determinó el número de unidades formadoras de colonias en las placas obtenidas.

Resultados. En el primer experimento isoflurano demostró una actividad mayor y más rápida que sevoflurano frente a las cepas estudiadas. Los microorganismos gramnegativos

resultaron más sensibles. En el segundo *E. faecium* se mostró resistente a ambos agentes y sólo isoflurano mostró diferencias significativas en su efecto antimicrobiano frente al resto de las cepas.

Conclusiones. Ambos anestésicos halogenados, especialmente isoflurano, mostraron actividad antibacteriana *in vitro* frente a patógenos resistentes a los antibióticos convencionales. Se necesita mayor investigación para determinar si este efecto se confirma *in vivo*. En ese caso se podría considerar a estos agentes como una alternativa frente a bacterias multirresistentes, incluyendo por ejemplo su uso tópico en heridas infectadas.

Palabras clave: Sevoflurano, Isoflurano, Anestésicos, Inhalación, Agentes anti infectivos.

INTRODUCTION

Isolation of multidrug-resistant bacteria is increasing worldwide and there is an urgent need for the development of new antimicrobial drugs. This situation has increased interest in the potential use of alternative antibacterial agents and older, previously discarded drugs. Research on molecules with possible antimicrobial activity has led to a new group of "non-antibiotic drugs" which includes a number of inhalational anesthetics[1-3]. However, previous studies have reported contradictory results on the antimicrobial activity of these molecules. Some of these studies included isoflurane and sevoflurane but, from the data available, they have yet to be tested against resistant strains[4-9].

The aim of this study was to investigate the possible *in vitro* antibacterial effect of sevoflurane and isoflurane on reference and multidrug-resistant strains of species commonly found in hospital settings.

MATERIALS AND METHODS

Liquid-liquid experiment. In this experiment, we tested microorganisms usually associated with ventilator associated pneumonia (VAP) (table 1). American Type Culture Collection strains of *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were studied as reference strains. For each species, one multidrug-resistant strain isolated from clinical samples was included. Minimal inhibitory concentrations (MIC) were determined by broth microdilution (Microscan Walkaway) and antimicrobial susceptibility profiles were set following Clinical and Laboratory Standards Institute (CLSI) breakpoints.

One tube with 5 mL of nutrient broth (bioMérieux) was inoculated with each strain. The tubes were then incubated overnight at 37ºC. Half a millilitre of these cultures was added to 4.5 mL of fresh nutrient broth and incubated at 37ºC for 90 minutes. Further dilutions of each suspension were performed in sterile physiological saline to give approximately 10^3 CFU/mL.

One-millilitre samples of this suspension were inoculated into sterile evacuated tubes (BD Vacutainer®) containing either 1 mL of sterile saline solution (controls) or 1 mL of the anaesthetic. Pure sevoflurane and isoflurane (Abbott) are supplied with neither additives nor preservatives. The tubes were then incubated at 37ºC with continuous shaking. After 15, 30 and 60 minutes of exposure, a 100 µL sample was spread on a sterile blood agar plate (bioMérieux). The plates were incubated for 24 hours at 37ºC (5-10% CO_2) and then colonies were counted. Each experiment was repeated three times.

Solid-liquid experiment. In a second experiment, we tried to mimic the conditions of topical use on infected ulcers by pouring liquid sevoflurane and isoflurane over bacteria spread in solid medium. Only multidrug-resistant strains from the laboratory collection were included (table 1).

Bacterial suspensions adjusted to a density of 0.5 McFarland (1.5 x 10^8 CFU/mL) from cultures of 18-24 hours were prepared in sterile physiological saline. Serial 10-fold dilutions were performed to give approximately 10^5 CFU/mL. For each strain, 5 blood agar plates were inoculated, each with 5 µL of the resulting suspension. One was incubated 24 hours at 37ºC (5-10% CO_2) with no other manipulation and used as a growth control. Two plates were treated with sevoflurane and two with

Table 1 **Strains included in each experiment.**

Liquid-Liquid Experiment
Reference Strains:
S. aureus (ATCC* 29213)
E. coli (ATCC 27853)
P. aeruginosa (ATCC 25922)
Clinical Isolates (antimicrobial resistance[a]):
S. aureus (Met, Ery, Ami, Cip)
E. coli (ESBL, Cip)
P. aeruginosa (Gent, Tob, Carbap)
Solid-Liquid Experiment
Clinical Isolates (antimicrobial resistance[a]):
E. coli (ESBL, Amino, FQ, TMP/SMX)
K. pneumoniae (ESBL, Amino, FQ, TMP/SMX)
E. cloacae (Cepha, Amino, FQ)
P. aeruginosa (Carbap, Amino, FQ)
A. baumannii (Carbap, Amino, FQ)
S. aureus (Met, Ery, Amino, FQ)
E. faecium (Van, Str, Ery, FQ)

***ATCC; American Type Culture Collection.**

[a]**Met: methicillin; Ery: erythromycin; Ami: amikacin; Cip: ciprofloxacin; Gent: gentamycin; Tob: tobramycin; TMP/SMX: trimethoprim-sulfamethoxazole; Van: vancomycin; Str: streptomycin (high-level resistance); Carbap: carbapenems; Amino: aminoglycosides; FQ: fluoroquinolones; Cepha: cephalosporins; ESBL: extended-spectrum beta-lactamase.**

Table 2 **Growth of strains after exposure to anesthetics. Liquid-liquid experiment.**

Strain		Drug	Exposure time		
			15 min	30 min	60 min
S. aureus	ATCC	Sevo	$8x10^2 \pm 0.5x10^2$	$4.8x10^2 \pm 2.2x10^{2*}$	$2.6x10^2 \pm 0.9x10^{2*}$
		Control	$8.1x10^2 \pm 0.8x10^2$	$7.8x10^2 \pm 1.9x10^2$	$8.3x10^2 \pm 1.8x10^2$
		Iso	UDL*	UDL*	UDL*
		Control	$2x10^3 \pm 9x10^2$	$1.2x10^3 \pm 5x10^2$	$8.9x10^2 \pm 3x10^2$
	Clinical	Sevo	$8.6x10^2 \pm 0.8x10^2$	$5.6x10^2 \pm 1.3x10^{2*}$	$3.8x10^2 \pm 1.2x10^{2*}$
		Control	$9.9x10^2 \pm 1.2x10^2$	$9.4x10^2 \pm 1x10^2$	$9x10^2 \pm 0.9x10^2$
		Iso	$0.1x10^2 \pm 0.2x10^{2*}$	$0.1x10^2 \pm 0.2x10^{2*}$	UDL*
		Control	$2.6x10^3 \pm 1.4x10^2$	$2x10^3 \pm 4.2x10^2$	$1.4x10^3 \pm 7.1x10^2$
P. aeruginosa	ATCC	Sevo	$3.6x10^2 \pm 4.9x10^2$	$0.3x10^2 \pm 0.5x10^{2*}$	$0.2x10^2 \pm 0.3x10^{2*}$
		Control	$1.7x10^3 \pm 7.4x10^2$	$1.6x10^3 \pm 6.9x10^2$	$1.6x10^3 \pm 6.3x10^2$
		Iso	UDL*	UDL*	UDL*
		Control	$1.1x10^3 \pm 3x10^2$	$9.9x10^2 \pm 1.3x10^2$	$6.7x10^2 \pm 1.4x10^2$
	Clinical	Sevo	$2.5x10^2 \pm 1.1x10^{2*}$	$1.4x10^2 \pm 0.4x10^{2*}$	$0.3x10^2 \pm 0.3x10^{2*}$
		Control	$5.7x10^2 \pm 0.5x10^2$	$6.3x10^2 \pm 0.5x10^2$	$6.8x10^2 \pm 0.4x10^2$
		Iso	UDL*	UDL*	UDL*
		Control	$2x10^3 \pm 5.8x10^2$	$1.4x10^3 \pm 2x10^2$	$6.6x10^2 \pm 1.5x10^2$
E. coli	ATCC	Sevo	$2x10^2 \pm 1.7x10^{2*}$	$1.6x10^2 \pm 1.4x10^{2*}$	$1.3x10^2 \pm 0.3x10^{2*}$
		Control	$2x10^3 \pm 0.9x10^2$	$1.9x10^3 \pm 1.5x10^2$	$1.9x10^3 \pm 1.1x10^2$
		Iso	UDL*	UDL*	UDL*
		Control	$2x10^3 \pm 3.4x10^2$	$2x10^3 \pm 6.5x10^2$	$2x10^3 \pm 2x10^2$
	Clinical	Sevo	$2.9x10^2 \pm 0.2x10^{2*}$	$2.8x10^2 \pm 0.8x10^{2*}$	$2.2x10^2 \pm 2x10^{2*}$
		Control	$1.9x10^3 \pm 1.5x10^2$	$1.9x10^3 \pm 2.2x10^2$	$1.9x10^3 \pm 0.7x10^2$
		Iso	UDL*	UDL*	UDL*
		Control	$2.1x10^3 \pm 5.3x10^2$	$2x10^3 \pm 6.3x10^2$	$2.1x103 \pm 1.1x10^3$

Sevo: sevoflurane; Iso: isoflurane; UDL: under detection limit. Values are CFU/mL (mean ± SD). *Significantly different from growth control ($P < 0.05$).

isoflurane as follows: in a laboratory safety cabinet with plates placed face up, 2.5 mL of the anaesthetic was poured over each plate, one being immediately covered with a lid while the other was left uncovered. Exposure in each case lasted until the anaesthetic had completely evaporated at room temperature. Evaporation times were recorded in each experiment. Plates were then incubated for 24 hours at 37ºC (5-10% CO_2) and colonies were then counted. Experiments were repeated seven times.

Results are expressed as mean CFU/mL ± SD (standard deviation). Detection limits are 10 UFC/mL in the first experiment and 200 UFC/mL in the second one. Statistical analysis was performed by analysis of variance (SPSS 15.0). Comparisons between group means were made using Scheffe or Games-Howell procedures according to Levene's test on equality of variances. $P < 0.05$ was regarded as significant.

RESULTS

In the liquid-liquid experiment, both halogenated anaesthetic agents showed antibacterial activity against bacterial suspensions. Isoflurane sterilized almost all bacterial suspensions, even after the shortest exposure time. The antibacterial effect of sevoflurane was higher against gram-negative bacilli and needed at least 30 minutes to significantly reduce *S. aureus* CFU/mL with respect growth control. No significant differences were detected between reference and multidrug-resistant strains (table 2).

Table 3 **Growth of strains after exposure to anesthetics. Solid-liquid experiment.**

Strain	Drug				
	Control	Sevo uncovered	Sevo covered	Iso uncovered	Iso covered
E. coli	$1.5x10^4 \pm 5.6x10^3$	$1.8x10^4 \pm 8.4x10^3$	$1.8x10^4 \pm 6.1x10^3$	$1.5x10^3 \pm 1.4x10^{3*}$	$0.6x10^2 \pm 1.6x10^{2*}$
K. pneumoniae	$3.4x10^4 \pm 1.1x10^4$	$3.1x10^4 \pm 1.1x10^4$	$3.1x10^4 \pm 6.9x10^3$	$6.7x10^3 \pm 3.4x10^{3*}$	UDL*
E. cloacae	$6.3x10^4 \pm 1.3x10^4$	$6.1x10^4 \pm 8.3x10^3$	$6.8x10^4 \pm 1.2x10^4$	$2.9x10^4 \pm 9.5x10^{3*}$	UDL*
P. aeruginosa	$2.8x10^4 \pm 1.2x10^4$	$3.5x10^4 \pm 2x10^4$	$3x10^4 \pm 1.3x10^4$	$1.2x10^4 \pm 6.1x10^{3*}$	$0.2x10^2 \pm 0.8x10^{2*}$
A. baumannii	$4.5x10^4 \pm 1.9x10^4$	$5.7x10^4 \pm 2.8x10^4$	$4.9x10^4 \pm 2.1x10^4$	$3.8x10^3 \pm 2.2x10^{3*}$	$0.2x10^2 \pm 0.8x10^{2*}$
S. aureus	$3.4x10^4 \pm 7x10^3$	$3.6x10^4 \pm 8.3x10^3$	$3.5x10^4 \pm 7.8x10^3$	$2.4x10^4 \pm 5.2x10^{3*}$	$0.8x10^3 \pm 1.2x10^{3*}$
E. faecium	$5x10^4 \pm 1.2x10^4$	$5x10^4 \pm 1.4x10^4$	$5x10^4 \pm 1.2x10^4$	$4.8x10^4 \pm 1.1x10^4$	$5x10^4 \pm 1.3x10^4$

Sevo: sevoflurane; Iso: isoflurane; UDL: under detection limit. Values are CFU/mL (mean ± SD). * Significantly different from control ($p < 0.05$). Mean evaporation times: isoflurane 7.5 min for uncovered and 103.5 min for covered plates, sevoflurane 12.8 min and 142.8 min.

Different results were observed for both drugs in the solid-liquid experiment (table 3). Neither of them showed effect against *E. faecium* regardless of the experimental conditions. Isoflurane however had excellent antibacterial activity on the rest of the strains studied, yielding counts below the detection limit in most of them. Sevoflurane did not reduce the count of viable microorganisms compared to growth control even when the experiment was performed in covered plates.

DISCUSSION

Our main finding is that liquid halogenated anaesthetics studied (sevoflurane only against bacterial suspensions) show antimicrobial effect *in vitro*. Previously reported results on antibacterial activity of volatile anaesthetics have been contradictory. The different methods used by investigators may explain the variety of results obtained. In general, a greater effect was detected when bacteria in liquid media or spread on the surface of cellulose membranes were exposed to anaesthetics in either liquid or vapour form[5-8,10-13]. Bacteria grown in solid media were more resistant to gas exposure[4,9,11,14,15]. Some authors, however, found no activity[4,5,7,9]. Our results are consistent with those observed in other liquid-liquid experiments[6,12]. Johnson and Eger also reported isoflurane to be faster than other drugs tested, but they did not include sevoflurane. In their study, *S. aureus* was also more resistant than *E. coli* and *P. aeruginosa*[6]. However, the liquid-liquid experiment is of doubtful clinical application and we lack a model of mechanical ventilation.

As far as we know, there have been no previous experiments with liquid-form halogenated anaesthetics against bacteria in solid medium, but that seems logical since they are administered in vapour form in clinical practice.

Very little is known about antibacterial mechanisms of anaesthetic agents. It has been proposed that halogenated compounds have solvent properties on the cell envelopes[16]. The similar results obtained in the first experiment with reference and resistant strains suggest that the mechanism of activity might differ from those of conventional antibiotics. Thus, halogenated anaesthetics could be an attractive option to overcome the problem of multidrug resistance in some situations.

Volatile anaesthetics have traditionally been used as sedating drugs in surgery. Now, new devices (e.g. AnaConDa®) allow prolonged sedation using sevoflurane or isoflurane with a common intensive care ventilator[17]. It is known that VAP is associated with colonization of and the subsequent formation of a slime layer in, the endotracheal tube[18]. Our experimental conditions are far from those in anaesthetic practice. However it is possible that continuous exposure of the endotracheal tube to these drugs could be useful for delaying or preventing both processes and in turn, might delay VAP development. On the other hand, this antimicrobial effect could result in negative cultures of respiratory samples from patients actually suffering from VAP, an undesirable result which has been previously suggested by other authors[4]. None of these theoretical scenarios have been investigated in clinical practice.

More recently, we have focused on another clinical application which, from an infectious point of view, seems more interesting and realistic. We decided to investigate the possible topical antibacterial effect of halogenated anaesthetics in liquid form. In fact, the second part of the experiment was designed to mimic the irrigation of these drugs over infected wounds. The use of this type of molecules in infected wounds and ulcers is not new. Halogenated drugs are derived from ether. Early in the 20th century some authors found ether to be an excellent antimicrobial drug, either in infected wounds and other clinical situations in humans[19,20] or in animal experimental models of infections[14].

Clinical experience with topical use of liquid sevoflurane has shown also an analgesic/anaesthetic effect which allows patients to better tolerate debridement in infected wounds[21] and helps in controlling refractory pain caused by venous ulcers[22,23], even when infected[24,25].

These properties, combined with good antibacterial activ-

ity against multidrug-resistant strains, especially gram-negative, make these drugs attractive not only from an analgesic point of view, but also in terms of infection. Until now, we have only communicated clinical experience with topical irrigations of sevoflurane. The first one was an infected postsurgical wound[26], in which a multidrug-resistant strain of *P. aeruginosa* was eradicated, but not *S. aureus,* from the wound bed and allow it to close. These results are consistent with the different susceptibility observed in gram-positive and gram-negative bacteria against isoflurane but not sevoflurane *in vitro*. Some clinical important aspects like repeated applications of the drug and surgical debridement of the wound which are not taken into account *in vitro* could explain the good results observed *in vivo*. The second experience involved a multi-recurrent frontal epidural infection resistant to surgical debridement. The last recurrence was caused by a sensitive strain of *E. coli* and the infection was cured by injecting sevoflurane trough de fistulous trajectory[27]. Of importance, this case was treated in a cost-saving outpatient basis. Besides, topical sevoflurane was well tolerated by the patients until now reported when employed on wounds both as analgesic or antimicrobial topical agent[21-27], the only adverse effect being a slight itching on the surrounding skin of the wound. Successful *in vivo* microbiological results are expected with isoflurane in the light of our *in vitro* results. However, it is well know that isoflurane has pungent effects on respiratory airways, whereas sevoflurane is much better tolerated[28]. Moreover, isoflurane has shown direct toxic effects on peritoneum and subjacent tissues, whereas sevoflurane did not[29]. Thus, although effective *in vitro*, risk-benefit balance should be evaluated to clarify if isoflurane would be an effective option to treat wounds.

In conclusion, our results suggest that sevoflurane and isoflurane have antibacterial activity *in vitro* against reference and multidrug-resistant strains. The clinical impact of our findings remains unclear but topical use in infected wounds or ulcers could be a potential new clinical indication.

FUNDING

None to declare.

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