Growth curves of *Staphylococcus aureus*, *Candida albicans*, and *Moraxella osloensis* in propofol and other media

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Propofol, 2,6 diisopropylphenol, in an emulsion formulation (Diprivan), has been associated with postsurgical infections caused by Staphylococcus aureus, Moraxella osloensis and Candida albicans. These organisms were individually inoculated into each of the following media: (1) the emulsion preparation of propofol, (2) Intralipid 10%, (3) pure 2,6 diisopropylphenol, and (4) trypticase soy broth (TSB). The organisms were incubated and subcultured hourly for eight hours at room temperature. Propofol supported the growth of all three organisms, but for S. aureus and M. osloensis, the growth rate was slower in propofol than in TSB ($P < 0.05$). There was no difference between the growth rate of any organism in propofol than in Intralipid 10%. The authors conclude that propofol, in the emulsion formulation, supports bacterial growth and, therefore, must be prepared for administration in an aseptic manner. Also, by administering propofol soon after preparation, the risk of introduction of a significant inoculum to the patient will be reduced.

L'utilisation du propofol, ou 2-6 diisopropylphénol, sous forme d'émulsion (Diprivan) a récemment été associée à des infections postopératoires à Staphylococcus aureus, Moraxella osloensis et Candida albicans. Dans la présente étude, ces trois micro-organismes sont placés séparément dans chacun des milieux de cultures suivants : 1) propofol en émulsion, 2) Intralipide 10%, 3) 2-6 diisopropylphénol pur et 4) bouillon trypticase soja (BTS). Ces milieux colonisés sont incubés pendant huit heures à la température ambiante. Des cultures de chacun des milieux sont répétées à chaque heure. Les

résultats des cultures démontrent que le propofol permet la croissance de chacun des trois organismes. Cependant, la croissance du S. aureus et du M. osloensis est plus lente dans le propofol que dans le BTS ($P < 0,05$). La croissance des trois organismes dans le propofol est comparable à celle retrouvée dans l'Intralipide 10%. Le 2-6 diisopropylphénol pur ne permet aucune croissance des organismes. Les auteurs concluent que le propofol sous forme d'émulsion est un milieu de culture potentiel pour différents micro-organismes et que toute manipulation de ce produit pour utilisation clinique doit se faire de façon aseptique. De plus, on recommande d'administrer le propofol peu de temps après l'aspiration dans une seringue afin de diminuer le risque d'infection chez les patients qui doivent recevoir ce médicament.

Propofol, 2,6 diisopropylphenol, a new intravenous anaesthetic virtually insoluble in water, is formulated in an aqueous soya bean oil emulsion similar to Intralipid 10%.^{1,2} Its advantages include smooth, rapid induction of general anaesthesia, a low incidence of nausea or vomiting, and quick recovery.³⁻⁶

Propofol emulsion (subsequently referred to as propofol) has been associated with postsurgical infections, either bacteraemia or wound infections.⁷ The organisms involved were *Staphylococcus aureus*, *Moraxella osloensis* and *Candida albicans*. The article reporting these infections suggested that contamination was extrinsic and stated that when incubated at 33°C in propofol, *S. aureus* rapidly proliferates.⁷

It is not surprising that propofol supports bacterial growth. Intralipid 10% supports the growth of bacteria and yeast, and the Center for Disease Control (CDC) has recommended a 12-hr time limit for infusion of Intralipid 10%.⁸ The effect of adding 2,6 diisopropylphenol to 10% soya bean oil emulsion (Diprivan) on microbial growth is not known.

We therefore investigated and compared the growth rates of *S. aureus*, *M. osloensis* and *C. albicans* in the commercial emulsion formulation of propofol, Intralipid 10%, pure 2,6 diisopropylphenol and trypticase soy broth (TSB).

Key words

ANAESTHETICS, INTRAVENOUS: propofol;
BACTERIA: growth rates.

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Methods

A few colonies of fresh overnight growth from each of *Staphylococcus aureus*, *Moraxella osloensis*, and *Candida albicans* were inoculated into separate test tubes containing TSB. The tubes were then incubated for 2-4 hours at 35°C. This suspension was then diluted with TSB to obtain, visually, a turbidity equivalent to a 0.5 McFarland standard (approximately 1×10^8 colony-forming units $\cdot$ ml⁻¹ (cfu $\cdot$ ml⁻¹).

The suspensions were then diluted 1 in 100 in TSB ($\sim 1 \times 10^6$ cfu $\cdot$ ml⁻¹). They were further diluted 1 in 100 in each of the following: propofol for injection (Diprivan, ICI Pharma), 2,6 diisopropylphenol (Aldrich Chemical Co., Inc), Intralipid 10% (KabiVitrum Canada, Inc.) and TSB (Difco Laboratories).

One in ten serial dilutions in the respective test media of these 1×10^4 cfu $\cdot$ ml⁻¹ suspensions were prepared to obtain the final 1×10^3 , 1×10^2 , and 1×10^1 cfu $\cdot$ ml⁻¹ suspensions to be tested.

The test tubes were incubated at room temperature (22.5°C) and 0.1 ml of the different suspensions were subcultured hourly for eight hours on to 5% sheep blood agar plates. At hours seven and eight, because of the increase in turbidity of the *S. aureus* in TSB, 0.001 ml of the suspensions was also plated from the different media using calibrated loops.

These plates were then incubated for 24 hr at 35°C and the number of colonies was counted on each plate.

Data Analysis

The individual organism growth patterns in each media were compared by analyzing the respective regression coefficients derived from a semi-log plot of the growth curves for the different inocula. The ability of the regression equation to account for the variance in the respective growth curve is given by the *r* value for each regression. Statistical comparisons between regression coefficients were done using standard methods.⁹ A *P* value of <0.05 was considered significant.

Results

The pure 2,6 diisopropylphenol was bactericidal. The other three media all supported growth of the three organisms. The growth curves for *S. aureus* are shown in Figure 1. The growth rate of *S. aureus* was significantly faster in TSB versus propofol at an inoculum of 1×10^3 cfu $\cdot$ ml⁻¹ by regression analysis. There were no differences at this inoculum between the growth rates of *S. aureus* in TSB and in Intralipid 10%, or between Intralipid 10% and propofol.

There were also differences between the growth rates of *M. osloensis* in TSB and in propofol and TSB versus Intralipid 10% by regression analysis (at an inoculum of

FIGURE 1 Growth of *Staphylococcus aureus* in different media. Inoculum of 1×10^3 cfu $\cdot$ ml⁻¹. Correlation coefficients (*r*): trypticase soy broth 0.96, Intralipid 10% 0.73, and propofol 0.94. CFU: colony-forming units.

FIGURE 2 Growth of *Moraxella osloensis* in different media. Inoculum of 1×10^3 cfu $\cdot$ ml⁻¹. Correlation coefficients (*r*): trypticase soy broth 0.98, Intralipid 10% 0.91, propofol 0.98. CFU: colony-forming units.

1×10^3 cfu $\cdot$ ml⁻¹). There was no difference between the growth rates of *M. osloensis* in propofol versus Intralipid 10% (Figure 2).

There were no differences among the growth rates of *C. albicans* at any dilution, in any of the media (Figure 3).

There were no significant differences between any of the growth curves in propofol, Intralipid 10%, and TSB for any of the organisms using inocula of 1×10^2 or 1×10^1 cfu $\cdot$ ml⁻¹.

Discussion

We have demonstrated that the emulsion formulation of propofol (2,6 diisopropylphenol in 10% soya bean oil, 1.2% egg phosphatide, and 2.25% glycerol¹) will support the growth of *S. aureus*, *M. osloensis*, and *C. albicans* when this drug is inoculated with these organisms and

FIGURE 3 Growth of *Candida albicans* in different media. Inoculum of 1×10^3 cfu $\cdot$ ml $^{-1}$. Correlation coefficients (r): trypticase soy broth 0.9, propofol 0.97, Intralipid 10% 0.6. CFU: colony-forming units.

incubated at room temperature. We therefore agree with other investigators who recommend that strict aseptic technique must be used when preparing syringes for infusion.^{10,11}

The growth rates for *S. aureus*, *M. osloensis*, and *C. albicans* in propofol were the same as in Intralipid 10% over the eight-hour period of this study. Since the current recommendation is to infuse an intravenous lipid emulsion within 12 hr,⁸ it is appealing to suggest that propofol should have at least an eight hour time limit for infusion. We think it would be an error to make this suggestion because, unlike the situation with Intralipid 10%, there are multiple opportunities for contamination of propofol from aspiration from the vial, until injection into the patient.¹² If the propofol is contaminated, the longer it is allowed to remain in the syringe before use, the larger the inoculum that will be injected into the patient. It is therefore concluded that propofol be prepared for injection in a sterile manner and infused into patients soon after preparation, in order to reduce the possibility of injecting a significant inoculum.

Further, it appears that the size of the inoculum into the propofol affects the growth rate of the organism. With inocula of 1×10^3 cfu $\cdot$ ml $^{-1}$ of *S. aureus* and *M. osloensis*, the growth rates in TSB were faster than in propofol. However, there were no differences of the growth curves in propofol, TSB and Intralipid 10% using lesser inocula of the same organisms. This lack of difference at low levels of bacterial contamination might reflect similar support for growth in propofol, TSB, and Intralipid 10%. Similar support is unlikely, though, because previous authors' experience implies limited growth of bacteria in propofol when the inoculum is low.^{10,11}

In summary, propofol supports the growth of *S. aureus*, *M. osloensis*, and *C. albicans* at room temperature.

Because of the multiple opportunities for contamination of propofol, it is recommended that it be infused shortly after aspiration into a syringe, to minimize any potential bacterial inoculum from being injected into the patient. Further, it is imperative that the propofol be prepared and administered in an aseptic manner to prevent injection of a contaminated solution.

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