

Growth of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* in Propofol, Thiopental and a 1:1 Propofol-Thiopental Mixture

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Abstract: Extrinsic contamination of propofol is thought to be a source of postoperative sepsis. Researchers studied growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* in propofol, thiopental and in a 1:1 propofol-thiopental mixture. All microorganisms were taken from standard stock cultures. Half of the samples were incubated at 20°C and the rest were stored at 4°C for 120 h. Growth of microorganisms in each solution was compared by counting the number of the Colony-Forming Units (CFUs) at 0, 3, 6, 24, 72 and 120 h. Propofol supported the growth of all four microorganisms at both temperatures. In contrast, thiopental and the 1:1 propofol-thiopental mixture exhibited markedly bactericidal properties on *E. coli*, *C. albicans* and *P. aeruginosa* and bacteriostatic effect on *S. aureus*.

Key words: Intravenous anesthetics, propofol, thiopental, mixture, bacteria, growth rate

INTRODUCTION

Propofol is an intravenous anesthetic agent which is used for the induction and maintenance of anesthesia. It is evident that propofol is a suitable growth medium for bacteria and fungi because it contains glycerol, purified egg phosphatide, sodium hydroxide, soy bean oil and water (Tessler *et al.*, 1992; Berry *et al.*, 1993; Sosis and Braverman, 1993; Crowther *et al.*, 1996; Lazar *et al.*, 1998; Sakaragi *et al.*, 1999; Wachowski *et al.*, 1999; Aydin *et al.*, 2002). In a report of the Centre for Disease Control (CDC), Carr *et al.* (1990) reported on 24 cases of infection after the use of propofol.

Addition of thiopental to propofol constitutes a clinically useful induction mixture (Naguib and Sari-Kouzel, 1991). It is reported that the induction of anesthesia with propofol-thiopental mixture provides equally rapid and qualitatively similar recovery to propofol alone (Rashiq *et al.*, 1994). It is also reported in dogs that a 1:1 mixture of propofol and thiopental induced anesthesia of similar quality to propofol or thiopental alone. *In vitro* studies showed that the addition of thiopental to propofol reduces bacterial growth (Sosis and Braverman, 1993). A 1:1 mixture of 1.0% propofol and 2.5% thiopental was reported to be strongly bactericidal

against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Crowther *et al.*, 1996). Chernin *et al.* (1996), reported that 1:1 mixture of propofol and thiopental when stored in polypropylene syringes, propofol 5 mg mL⁻¹ and thiopental 12.5 mg mL⁻¹ was chemically stable for up to 312 h at 4°C and for up to 120 h at 20°C. Although, it is stable for 120 h chemically (Chernin *et al.*, 1996), no investigations on bactericidal effect of propofol-thiopental mixture at 120 h have been previously reported. The purpose of this study was to determine the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* in propofol, thiopental and 1:1 propofol-thiopental mixture for up to 120 h at 4 and 20°C.

MATERIALS AND METHODS

Overnight cultures of *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 35032), *E. coli* (ATCC 25922) and *C. albicans* (ATCC 10231) were diluted to a density of 0.5 McFarland units with 0.9% sterile saline using a crystalspect turbidimeter (Becton Dickinson, USA). Each organism solution was further diluted 1:50 with 0.9% sterile saline. Aliquots of 0.15 mL of each diluted organism were then added to sterile culture vials containing 15 mL

of the following solutions: 1% propofol (The sterile emulsion contains no preservative) (Propofol; Abbott USA); 2.5% thiopental (Pental sodyum; IE Ulagay Turkey); a 1:1 propofol-thiopental mixture and 0.9% sterile saline. The pH values of all anesthetic solutions were measured using a Mettler Toledo MP 220. The pH values of propofol, thiopental and 1:1 propofol-thiopental mixture were 6.5, 10 and 9, respectively.

Each organism solution was vortexed before addition to 20 vials (10 vials for 4°C and 10 vials for 20°C). After the organisms were added, each vial was vortexed and 100 µL solution subplated to blood agar plates (Becton Dickinson, USA). Vials were subplated out at 3, 6, 24, 72 and 120 h intervals. The plates were incubated at 35°C for 24 h. Each plated medium was evaluated and Colony Forming Units (CFUs) were counted and recorded by one blinded investigator.

For each microorganism, the CFUs per plate were averaged for each sample period and a comparison was made between time periods by a repeated measures analysis of variance followed by a Scheffe test. Comparison between anesthetics were also made for each time period and each microorganism by analysis of variance (or covariance if required using the baseline value as a cohort) followed by a Scheffe test. Significance was accepted when $p < 0.01$.

RESULTS AND DISCUSSION

In culture vials of *S. aureus*, a significant decrease in CFUs in propofol was noted at 3, 6 and 24 h compared to baseline at 20°C (Fig. 1). No significant growth of *S. aureus* throughout the 24 h period was seen in inoculated propofol emulsion. By contrast, CFUs of *S. aureus* in propofol was significantly increased at 72 and 120 h in 20°C.

CFUs of *S. aureus* in thiopental and 1:1 propofol-thiopental mixture were significantly decreased during 24 h period and no colonies were observed thereafter in 20°C. In culture vials of *S. aureus*, CFUs were unchanged throughout 24 h with all anesthetic solutions in 4°C (Fig. 2). Afterwards, CFUs in all anesthetic solutions significantly decreased.

In culture vials of *P. aeruginosa*, CFUs were significantly increased in propofol at 24 and 120 h in 20°C (Fig. 3). More than 600 CFUs at 24 h and >1000 CFUs at 72 h were counted. No CFUs of *P. aeruginosa* in thiopental and 1:1 propofol-thiopental mixture were observed during all of the study period at 20°C. Although, CFUs of *P. aeruginosa* in propofol were observed until 72 h at 4°C, no CFUs of *P. aeruginosa* were seen in thiopental and 1:1 propofol-thiopental mixture at 4°C (Fig. 4). In culture vials of *E. coli*, significant growth were seen at 24 and 120 h with propofol at 20°C (Fig. 5). CFUs

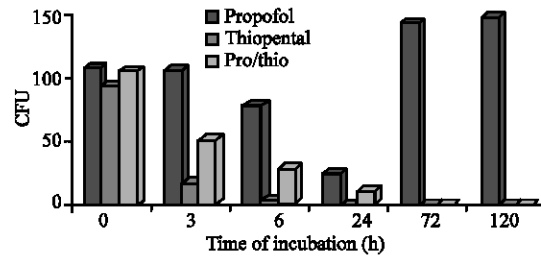


Fig. 1: Number of Colony Forming Units (CFUs) of *Staphylococcus aureus* counted versus time (h) after inoculation in three anesthetic agents in 20°C

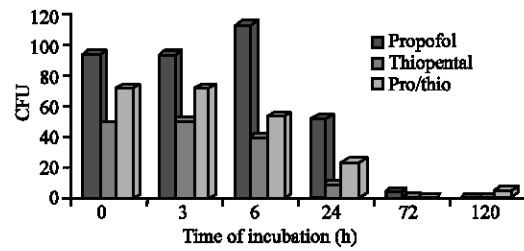


Fig. 2: Number of Colony Forming Units (CFUs) of *Staphylococcus aureus* counted versus time (h) after inoculation in three anesthetic agents in 4°C

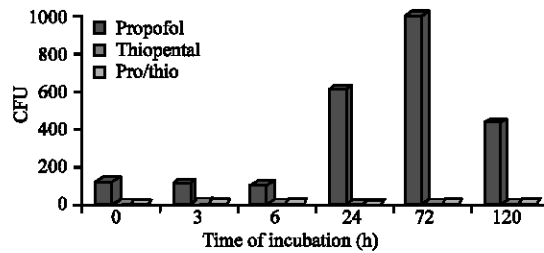


Fig. 3: Number of Colony Forming Units (CFUs) of *Pseudomonas aeruginosa* counted versus time (h) after inoculation in three anesthetic agents in 20°C

gradually decreased until 120 h in propofol emulsion at 4°C (Fig. 6). No colonies were observed in thiopental and 1:1 propofol-thiopental mixture during study periods in both temperatures.

In culture vials of *C. albicans*, CFUs were unchanged until 24 h but showed a significant increase afterwards in propofol emulsion at 20°C ($p < 0.01$) (Fig. 7). A significant gradual decrease of CFUs until 120 h in propofol emulsion were observed at 4°C ($p < 0.01$) (Fig. 8).

No growth of *C. albicans* in thiopental and 1:1 propofol-thiopental mixture were seen during study periods in both temperatures. A 1:1 propofol-thiopental mixture has been clinically used in humans (Naguib and Sari-Kouzel, 1991; Rashiq *et al.*, 1994; Pollard *et al.*, 2002) and dogs (Ko *et al.*, 1999) for induction of anesthesia.

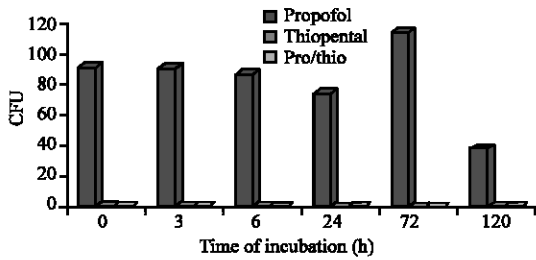


Fig. 4: Number of Colony Forming Units (CFUs) of *Pseudomonas aeruginosa* counted versus time (h) after inoculation in three anesthetic agents in 4°C

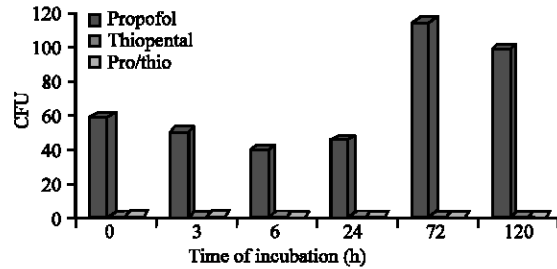


Fig. 7: Number of Colony Forming Units (CFUs) of *Candida albicans* counted versus time (h) after inoculation in three anesthetic agents in 20°C

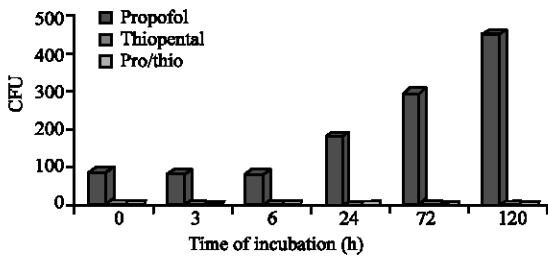


Fig. 5: Number of Colony Forming Units (CFUs) of *Escherichia coli* counted versus time (h) after inoculation in three anesthetic agents in 20°C

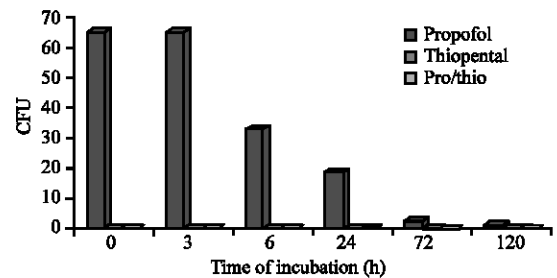


Fig. 8: Number of Colony Forming Units (CFUs) of *Candida albicans* counted versus time (h) after inoculation in three anesthetic agents in 4°C

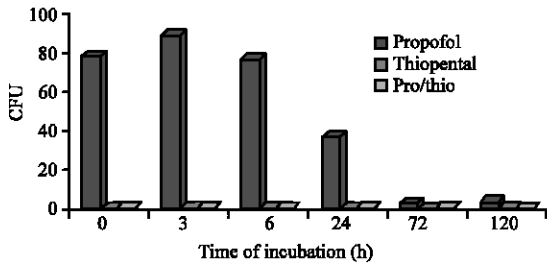


Fig. 6: Number of Colony Forming Units (CFUs) of *Escherichia coli* counted versus time (h) after inoculation in three anesthetic agents in 4°C

Since the combination of propofol with thiopental has synergistic activity, this mixture reduces the amount of propofol required, thus lowering total drug cost (Naguib and Sari-Kouzel, 1991; Rashiq *et al.*, 1994).

It is reported that a single bolus dose of a 1:1 propofol-thiopental mixture induces a rapid and smooth plane of anesthesia in dogs that helps easier endotracheal intubation (Ko *et al.*, 1999).

The quality of induction with mixture was similar to that with propofol alone, indicating that 1:1 mixture propofol-thiopental was suitable for anesthetic induction in dogs (Ko *et al.*, 1999) and humans (Naguib and Sari-Kouzel, 1991; Rashiq *et al.*, 1994; Pollard *et al.*, 2002). Its' recovery characteristics are

similar to propofol alone and has bactericidal effects on various organisms. It was demonstrated that the 1:1 mixture propofol-thiopental was stable for up to 312 h at 4°C and for up to 120 h at 23°C (Lazar *et al.*, 1998).

This present research is the first study to compare the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* in propofol, thiopental and 1:1 propofol-thiopental mixture at 4 and 20°C for up to 120 h period. The results revealed that propofol was the only anesthetic agent tested in this investigation that clearly supported the growth of four different micro-organisms at both temperatures. These are parallel with the reports of rapid growth of *Staphylococcus aureus* within 24 h in propofol (Tessler *et al.*, 1992; Berry *et al.*, 1993; Carr *et al.*, 1990; Sosis and Braverman, 1993). The findings of significant growth of *Pseudomonas aeruginosa* in propofol between 24-120 h after inoculation has not been previously reported in any controlled laboratory study but is parallel with the manufacturer's recommendation that propofol has to be used within 6 h of its handling. The bactericidal effect of thiopental is thought to be secondary to its high pH (Clinton *et al.*, 1992). Most pathogenic bacteria grow in a narrow pH range of 6-8 and strong alkalis media exert marked bactericidal effects (Wolin and Miller, 1985). Interestingly, the 1:1 propofol-thiopental mixture had similar effects as thiopental, the only difference was

longer time was needed to eradicate the *Staphylococcus aureus* growth. Propofol-thiopental mixture had a pH of 9.0 which may explain why its bactericidal properties were similar to those of thiopental alone.

In summary, the study shows that thiopental and 1:1 propofol-thiopental mixture unlike propofol suppressed the growth of micro-organisms in both temperatures with the exception of *Staphylococcus aureus* which remained at static levels during 6 h. If already prepared mixture are not used in the first 6 h, it can be used until 120 h afterwards instead of propofol alone. This study has shown that despite the presence of nutrients (Propofol®) in a 1:1 mixture of propofol-thiopental, this mixture does not support the growth of the microorganisms tested and is bactericidal towards *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli* during 120 h. Because of 1:1 mixture of propofol and thiopental was proved to be chemically stable up to 120 h both at 4 and at 20°C and the findings have shown that this mixture does not support the growth of the microorganisms in the same time periods, this mixture can be safely used and stored at both temperature for at least 120 h.

CONCLUSION

In this study, thiopental and propofol-thiopental mixture, unlike propofol suppressed the growth of certain microorganisms for at least 120 h.

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