



Extended Aseptic Handling and Anaesthetic Efficacy of Propofol Preserved under Different Conditions

Njoku, N.U.^{1*}; Babalola, S.A.²; Unamba-Oparah, C.¹; Anaba, C.E.¹; Ukwani, C.P.¹

¹Department of Veterinary Surgery and Radiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria ²Department of Veterinary Medicine, Surgery and Radiology, Faculty of Veterinary Medicine, University of Jos, Nigeria. *Corresponding author: Email njoku.uchenjoku@gmail.com; Mobile+234 8063397918

SUMMARY

Twenty-seven vials of propofol and 20 Albino rats were used in this study to determine the effect of refrigeration and aseptic handling on contamination and anaesthesia of propofol. The vials were assigned to three groups of nine (9) vials per group in 3 replicates. They were opened aseptically and the contents transferred to sterile bottles. VR group were stored in a refrigerator at 40C, VS in an infrared hood chamber, and VT stored on a bench. Propofol was aseptically aspirated from each vial immediately after opening and at 8, 10, 12, 24, 48 and 168 hours for culture on nutrient and McConkey agar. Qualitative and quantitative analysis of the isolates were carried out. Twenty adult Albino Wistar rats were assigned to 4 groups of 5 rats per group. One group (VF) of rats was anaesthetized with fresh propofol, while the remaining 3 groups were anaesthetized with the preserved propofol from each drug group, stored at the longest duration at which contamination was not evident at culture. Durations of anaesthesia, corneal reflex loss, and abdominal twitch and tail sensation loss, were measured. Data was analyzed using ANOVA and Duncan Multiple Range Test using SPSS version 22. Values of $p < 0.05$ were considered significant. Contamination occurred in the VS, VR and VT groups at 168, 48 and 12 hours respectively. This contravenes the opinion that opened propofol vials become contaminated after 8 hours. *Bacillus subtilis*, *Streptococcus pneumonia*, *Lactobacillus* spp, *Klebsiella pneumonia* and *Staphylococcus epidermidis* were isolated from the various groups. The indices of anaesthesia did not differ significantly among the groups. Therefore, following strict adherence to aseptic techniques, propofol may be safely used for up to 24 hours if stored either in a refrigerator or in a sterile environment, without any significant alterations in its anaesthetic characteristics.

Key words: Propofol. Anaesthesia. Aseptic. Preservation. Sterile

INTRODUCTION

Propofol (2, 6 Diisopropylphenol) is a popular drug for intravenous induction and maintenance of anaesthesia in humans and several animal species. Propofol is not water soluble, and is prepared as a milky white emulsion containing 10mg propofol, 100mg soybean oil, 12mg egg lecithin and 22.5mg glycerol per ml (Hota *et al.*, 2020). It contains no preservative, thus the emulsion supports bacterial growth and endotoxin production (Crowther *et al.* 1996). It is preferred as an anaesthetic agent because of its smooth induction and recovery (Miner and Burton, 2007; Njoku, 2015). Propofol is prone to contamination, either during manufacture (intrinsic) or more frequently, after vial opening (extrinsic) (Arduino *et al.*, 1991). It is recommended that once exposed to the atmosphere, the content in the vial must be used within 8 hours or thereafter discarded (Lee 2015). Propofol has been associated with many outbreaks of post surgical wound infection and septicaemia resulting in death of humans (Bennett *et al.*, 1995, Kuehnert *et al.*, 1997, Henry *et al.*, 2001), dogs and cats (Heldmann *et al.*, 1999). In spite of the post-surgical infections associated with the use of propofol, it is still one of the most widely preferred narcotics by anaesthetists because of its advantageous properties such as short duration, rapid systemic clearance, and smooth induction and recovery (Schraag *et al.*, 2018). Its high cost, relative to duration of action discourages anaesthetists from using it in patients of low body weight in which a vial may not be exhausted, or in long duration procedures lasting beyond the recommended 8 hours in which propofol may be included in the maintenance agents. This concern necessitated this research aimed at determining to what extent refrigeration and aseptic handling affected propofol sterility and also to determine the anaesthetic efficacy of aseptically handled

and preserved propofol used after the recommended 8 hours.

MATERIALS AND METHODS

The research was approved by the Research Ethics Committee of the Department of Veterinary Surgery and Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria, with number REC-15-0012. Twenty seven (27) vials of propofol (POFOL[®] INJECTION I.V. Dongkook Pharmaceutical, Korea) were assigned to three groups of nine (9) vials per group. There were 3 replicates in each group. The vials were opened aseptically and the contents transferred to air-tight sterile bottles near flame, using sterile syringes and needles. Group 1 vials (VR) were stored in a refrigerator at 4^oC, group 2 (VS) in a sterile infrared hood chamber, and group 3 (VT) on a trolley in the surgical theatre. Immediately after opening the vials, 0.2 mls of propofol was aspirated from each of the vials in the respective groups and cultured on nutrient agar for 24 hours. After 8 hours of initial sample collection, 1 ml of propofol from each vial in the VS, VR and VT groups was aspirated near flame, using sterile syringes, and immediately transported to the laboratory for bacterial culture on nutrient and McConkey agar. The procedure was repeated at 10, 12, 24, 48 and 168 hours after the initial sample collection. One milliliter of the drug from each group of vials was transferred into 10 mls of sterile water and serially diluted. The agar were inoculated with the diluted sample by pouring 0.1 ml of the sample to the agar and spreading it with a glass spreaders (the spread plate technique).

The colony forming units were examined based on physical appearance such as size, color, edge appearance, elevation, surface appearance, density, consistency, smell and the effect of the growth on the media. Colony forming units were counted using colony counters and contaminating

organisms were identified through microscopic properties (color, size, shape and arrangement), and biochemical tests, including catalase, coagulase, indole, oxidase, urease, voges-proskauer, nitrate reduction and citrate utilization tests. Motility was also determined.

Twenty (20) eight-week old Albino Wistar rats, weighing 218.6 ± 10.28 g, were acclimatized for two weeks and randomly assigned to four groups of five rats per group. The rats were anaesthetized with the preserved propofol from each group, stored at the longest duration at which contamination was not evident at culture, at the dose of 100mg/kg through the intraperitoneal route., Rats in Group 1 (VF) were anaesthetized with freshly opened propofol, Group 2 rats were anaesthetized with propofol stored on the surgical table (VT) for 8 hours. Rats in Group 3 were anaesthetized with propofol stored for 24 hours in an infrared inoculating hood chamber (VS), while rats in group 4 were anaesthetized with propofol refrigerated for 24 hours (VR). Anaesthesia was induced by intraperitoneal injection of propofol. The rats were weighed on an electronic balance, and the volume of drug to be administered was determined using the weight of the animal, dose and

concentration of the drug. The proposed site of injection was clipped and cleaned with chlorhexidine. Following induction of anaesthesia, the indices of anaesthesia were evaluated as follows: duration of anaesthesia measured as the time (minutes) interval from loss of righting reflex to restoration of righting reflex, duration of corneal reflex loss measured as the time (minutes) interval from loss of palpebral and corneal reflexes to restoration of palpebral and corneal reflexes, duration of abdominal twitch and tail sensation loss measured as time interval (minutes) from loss of pain sensation in the abdomen and tail till when they were regained. Data obtained from the response variables were expressed as mean \pm SEM and subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1986) and Duncan New Multiple Range Test (Duncan 1955) using SPSS version 22. Values of $p < 0.05$ were considered significant.

RESULTS

Results of bacterial contamination (Table I) showed that there was contamination in the VS group after 48 hours, while the VT and VR groups showed evidence of contamination at 12 and 48 hours respectively. Bacteria isolated from the various groups are listed in Table II.

TABLE I: Colony forming units of samples preserved under different conditions

Group	0hr	8hr	10hr	12hr	24hr	48hr	168hr
VR	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	4.00 \pm 4.00 ^a	72.00 \pm 24.25
VS	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	64.33 \pm 8.09
VT	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	11.33 \pm 11.33 ^b	23.00 \pm 9.29 ^b	32.00 \pm 10.82 ^b	132.33 \pm 74.88

(Different superscripts indicate a significant ($p < 0.05$) difference among the groups)

TABLE II: Bacteria isolated from the cultures from the various groups of samples after contamination

Group	Bacteria isolated
VR	<i>Bacillus subtilis</i> , <i>Streptococcus pneumonia</i>
VS	<i>Lactobacillus spp</i> , <i>Bacillus subtilis</i>
VT	<i>Bacillus subtilis</i> , <i>Klebsiella pneumonia</i> , <i>Staphylococcus epidermidis</i>

The result for anaesthetic efficacy did not show any significant difference in any of the anaesthetic indices measured (Table III).

TABLE III: Anaesthetic indices of the rats in the three (3) groups

Groups	Duration of Anaesthesia (min)	Induction Time (min)	Duration of PR (min)	Duration of CR (min)	Duration of ABD (min)	Duration of Tail (min)
VF	100.75±21.49	3.75±0.25	70.50±27.67	53.00±48.00	40.00±6.46	50.00±38.82
VT	100.00±30.86	4.50±0.50	81.50±27.86	32.50±7.50	57.50±17.90	59.50±15.63
VS	67.33±4.37	5.00±0.00	46.50±13.50	15.00±10.29	25.00±2.89	21.67±4.40
VR	141.00±26.34	3.35±0.50	72.50±28.10	51.67±34.92	34.50±8.91	53.73±11.79

No significant difference was observed among the means

DISCUSSION

Propofol preserved in the refrigerator and inoculating chamber remained sterile until 24 and 48 hours respectively. This finding contravenes the globally accepted recommendation that the vial of propofol becomes contaminated after 8 hours of opening, and therefore should be discarded 8 hours of initial opening (Lee 2015). However, after 24 hours of initial opening, some bacteria were isolated from the samples stored under different conditions. Bacterial contamination of propofol vials and needles has been reported (Zorilla-Vaca et al., 2016). There has also been a reported correlation between the use of propofol on one hand and bloodstream infection (BSI) and surgical site infectious (SSI) disease risk on the other, which has been seen to cause severe sepsis, systemic inflammatory response syndrome (SIRS) and even death in healthy animals and humans (Muller et al., 2010, Zorilla-Vaca et al., 2016, Franci et al., 2014) following propofol administration after surgery. The presence of *Bacillus subtilis*, *Streptococcus pneumonia*, *Lactobacilli*, *Klebsiella pneumonia*, *Streptococcus epidermidis* and *Escherichia coli* in contaminated propofol vials have been documented in various research to be a contaminant of propofol (Wachowski et al 1999; Harvey and Ganzgerg, 2003; Zorrilla-Vaca et al., 2016). The difference in these bacteria found at the different storage condition could be as a result of

the presence of those bacteria in the environment where the storage facility was located. The source of the contaminating bacteria is unclear, as samples were aseptically collected and handled. This finding indicates that in spite of strict adherence to aseptic procedures, contamination of the vial cover, lid, needle or syringe used for multiple aspirations was possible. Bacteria might therefore have been introduced to the vials in the course of the study. Strict adherence to sanitary practices in the surgical theatre such as disinfection of personnel hands and lids of vials, reducing the number of withdrawals made from the vial, avoiding injection of environmental air into the vial during drug aspiration, controlling duration of use and storage conditions of drugs may have prolonged the time of contamination in all groups. One may therefore assert that the content of the vial could remain sterile for a longer duration if the vial is aseptically stored and not aspirated frequently.

There was no significant difference in the anaesthetic indices between the different groups of preserved propofol and the freshly opened propofol vial. This implies that the mode of preservation did not affect the pharmacokinetics and pharmacodynamics of the drug.

CONCLUSION

This study shows that propofol, when opened, not used up immediately, and preserved aseptically can remain sterile for up to 24 hours, contrary to prevailing knowledge. Furthermore, propofol from sterile vials opened for more than 8 hours did not have any significant effect on the anaesthetic efficacy compared to the control. It is therefore concluded that following strict adherence to aseptic techniques, propofol may be safely used for up to 24 hours if stored either in a refrigerator or inoculating chamber after initial opening, without any significant alterations in its anaesthetic characteristics.

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