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The droplet size changes of 1% propofol before and after the storage procedure for 6 and 24 hours periods

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ABSTRACT

Propofol (2,6-diisopropylphenol) is a popular anesthetic agent for induction and anesthetic maintenance. Propofol preparation is formulated as a lipid macroemulsion that is thermodynamically unstable and degraded over time, causing the enlargement of droplet size. The degradation is faster when propofol emulsion preparation package has been opened. This droplet enlargement results in decreasing propofol releasing-rate and its concentration. The risk of embolism occurs whenever the mean droplet size (MDS) exceeds Food and Drug Administration (FDA) limit (<0.45) and fat globule population percentage >5 μm (PFAT5) is <0.05%. In the daily practice, some propofol emulsion preparation are often used although they are already opened and saved more than 24 hours. The aim of this study was to evaluate the droplet size changes of propofol emulsion preparation during storage. This was an observational study using cohort prospective design. The droplet size changes of sixteen propofol preparation samples were evaluated before and after storage procedure for 6 and 24 hours in the refrigerator at the temperature of 4ºC. The droplet diameter was determined under a light microscope. Mean droplet size before and after storage were calculated and analyzed by one way analysis of variance (ANOVA), followed by Tukey's post hoc test with 95% confidence interval ($p<0.05$). The droplet diameter of propofol emulsion preparation increased significantly ($p<0.05$) after storage procedure for 6 h (MDS value were: 247 ± 22 nm) and 24 h (278 ± 29 nm) compared to before storage (225 ± 24 nm). The PFAT5 at each interval time was 0%. There was no color and homogeneity changing at each interval time (n=16/100% each time interval). In conclusion, there is a change of propofol emulsion preparation droplet size after storage procedure for 6 to 24 hours at temperature of 4ºC compared to before storage.

ABSTRAK

Propofol (2,6-diisopropylphenol) merupakan obat anestesi yang populer baik untuk induksi maupun pemeliharaan anestesi. Sediaan propofol dibuat dalam bentuk makroemulsi yang secara termodinamik tidak stabil dan terurai selama waktu penyimpanan yang menyebabkan ukuran partikelnya membesar. Peruraian emulsi propofol menjadi lebih cepat jika kemasan propofol telah dibuka. Pembesaran ukuran partikel propofol berakibat penurunan kecepatan pelepasan propofol dan penurunan konsentrasi propofol. Risiko emboli terjadi jika ukuran partikel melebihi batasan yang dipersyaratkan oleh FDA yaitu rata-rata ukuran partikel <0,45 μm dan *fat globule population percentage* >5 μm (PFAT5) <0.05%. Pada praktek sehari-hari sering dijumpai penggunaan emulsi propofol yang diambil dari kemasan yang sudah dibuka dan disimpan lebih dari 24 jam. Tujuan penelitian ini adalah untuk mengetahui perubahan ukuran partikel emulsi propofol sebelum dan setelah prosedur penyimpanan selama 6 dan 24 jam. Penelitian ini adalah penelitian observasional dengan rancangan kohort prospektif. Sebanyak 16 sampel sediaan propofol dievaluasi perubahan ukuran partikelnya sebelum dan setelah penyimpanan selama 6 dan 24 jam dalam almari pendingan pada suhu 4ºC. Diameter partikel diukur di bawah mikroskop cahaya. Rerata ukuran tetesan (MDS) sebelum dan setelah dihitung dan dianalisis

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dengan analisis varian (ANAVA) satu jalan dilanjutkan dengan uji *post hoc* Tukey dengan taraf kepercayaan 95% ($p<0.05$). Diameter partikel menjadi lebih besar secara nyata ($p<0.05$) setelah prosedur penyimpanan selama 6 jam (nilai MDS sebesar 247 ± 22 nm) dan 24 jam (278 ± 29 nm) dibandingkan dengan sebelum penyimpanan (225 ± 24 nm). Nilai PFAT5 tiap interval waktu sama yaitu 0%. Secara makroskopis penampakan fisik, warna dan homogenitas sediaan propofol di setiap interval waktu tidak berubah (masing-masing n = 16/100%). Dapat disimpulkan, terjadi perubahan ukuran partikel sediaan emulsi propofol setelah prosedur penyimpanan selama 6 dan 24 jam pada temperatur 4°C dibandingkan sebelum penyimpanan.

Keywords: propofol 1% – droplet size – physical stability – storage – refrigerator

INTRODUCTION

Propofol (2,6-diisopropylphenol) is a popular anesthetic agent for induction and maintenance, because of its rapid onset, short duration, and minimal side effects. Its use has expanded from solely an anesthetic agent to a sedative–hypnotic agent which is also used for intensive care unit and in outpatient setting.[1] Propofol is a potent lipophilic anesthetic which was initially formulated in Cremophor El for human use. Because of the occurrence of Cremophor El anaphylaxis and improvements in the quality of lipid emulsions, it was ultimately brought to market as 1% propofol formulated in 10% soybean oil emulsion, lecithin (12 mg/mL), and glycerol (22.5 mg/mL) (Diprivan® and Fresofol®).[2,3]

Propofol emulsion and other emulsions for intravenous delivery are manufactured so that the oil droplets average is 0.15–0.3 μm (150–300 nm). These sizes are similar to naturally occurring chylomicrons. In general, emulsions with droplets of 0.1–100 μm are known as macroemulsions, whereas emulsions with droplets of smaller size (< 0.1 μm, or 100 nm) are known as microemulsions.[4] Muller and Harnisch[5] studied the physico-chemical characteristics of 8 emulsion preparations of propofol available in the market produced by several pharmaceutical industry which were still on packages. The result showed that the average diameter of droplet size of the emulsion preparations of propofol varied from 205 to 259 nm. In addition, Ravenelle *et al.*[2] also examined the average droplet size emulsion preparation of propofol Diprivan® 1% which was still on the package with an average diameter that was greater than the one observed by Muller and Harnich (350 ± 50 nm).

The oil in water macroemulsion such as propofol is thermodynamically unstable and will degrade over time. The stability of emulsions can be influenced and accelerated by the presence of external factors such as mechanical stress, temperature, light, pressure, micro-organisms, pH, oxygen, carbon dioxide and the ions of some electrolyte.[6,7] Propofol emulsion degradation occurs through the flocculation, coalition, creaming and cracking processes. Flocculation is caused by the merging of droplets to one another, while coalescence occurs when the surface of droplets has broken and merged into a larger droplet size. If more numerous flocculations happen, there will be creaming and cracking appearing on the emulsion surface.[7]

The droplet size becomes an important parameter in determining the physical stability of propofol emulsion because 1) the emulsion degradation can affect the release of propofol *in vivo*, as a result of surface area reduction due to the enlargement of droplet size; 2) the emulsion degradation can also cause variations in the concentration of propofol in the emulsion due to creaming volume; 3) it is important to note that enlargement of the droplet size may

cause an emboli when given intravenously, if the droplet size is greater than 5-6 μm.[7,8]

According to Driscoll[9] and as required by the Food and Drug Association (FDA), the values of the mean droplet size (MDS) and the percentage of large-diameter fat globules >5 μm (PFAT5) of propofol should be <450 nm (0.45 μm) and <0.05%, respectively. According to Masaki *et al*.[10] the time required for the change of emulsion droplet until it is visible depends on the initial droplet size according to Stokes law, i.e. 3 minutes for the droplet size of 100 ìm, 5 hours for the droplet size 10 μm, and 20 days for the droplet size of 1 μm. There has not been any study yet that examines how long the droplet size of propofol would be if it is more than > 5 μm.

Propofol has the expired date of 2 years in average after manufactured if it is stored as recommended. The recommended storage temperature ranges between 2° to 25°C. It cannot be stored at temperature over 25°C or freezing. Moreover, it should not be exposed to sunlight or ultraviolet rays directly. Propofol in its manufacture is free from oxygen. Propofol should be administered immediately after the opening of the package (ampoules) for up to 6 hours (Fresofol®; Trivam®) or 12 hours (Diprivan®). The time limit mentioned above is related to the contamination of microorganisms in propofol that has been opened from the package.[11-14]

On daily practice, it is often observed that the propofol preparation has been opened from the package, removed to a 10 cc syringe and then stored in the refrigerator with temperature of 4°C. Then, it is used again in the range of up to 24 hours or even more, ignoring the possibility of contamination, since the growth of bacteria is slow in the refrigerator. Consequently, the possibility of changes in physical stability and in droplet size of the emulsion of propofol preparation after storage can be observed. Hence, it is essential to know whether there has been a change in droplet size in the propofol which is opened up to 6 hours (time limit as recommended by the label) and the one which is opened for 24 hours (the time limit used in the reality of daily practice).

MATERIALS AND METHODS

This was an observational study with a prospective cohort design in order to evaluate the change of droplet size of emulsion preparation of propofol before and after storage procedures for 6 and 24 hours in 4°C. The inclusion criteria for this study was a emulsion preparation of propofol with 20 cc ampoule packaging with the same batch number and expired date and stored in accordance with the recommended label. Sixteen samples of emulsion preparation of propofol (Fresofol®) from the same batch number (Batch No. 16DE0280 ED 31. 05. 11) were used in this study.[15] The exclusion criteria were any macroscopic (visible) emulsion preparation of propofol changes although the batch number and expired date in accordance with the predetermined and emulsion preparation of propofol was allegedly damaged or had a defective packaging. The drop out criteria were the refrigerators no longer had a temperature of 4°C due to power supply failure, broken refrigerator, and so on, not following the procedures of working, and if the value of baseline's MDS was greater than 0.45 μm indicating that emulsion preparation of propofol had been damaged before the study. The MDS of emulsion preparation of propofol was used as the primary outcome whereas the homogeneity and the physical appearance of discoloration were also observed visually and used as the secondary outcome.

The measurement of the droplet diameter of emulsion preparation of propofol was performed by using an light microscope Olympus CX 41 with Image plus DP 12 with a magnification of 50X and 100X. At the 0 hour,

the emulsion preparation of propofol was opened from the package. Shortly, the physical appearance was observed and the droplet diameter and PFAT5 were calculated simultaneously as base line data. Ten cc of emulsion preparation contain of propofol was removed to the 10 cc syringe that had been labeled with sample number, date and hour and then stored in a refrigerator with temperature of 4°C. At the 6th and 24th hours after storage, the physical appearance was re-observed and droplet diameter and PFAT5 were recalculated. Five hundreds droplets size were measured their diameter for each sample at each time interval in order to determine their MDS and PFAT5 values.

All data were presented in the descriptive statistics. The MDS and PFAT5 at 0 hour, 6th and 24th hours after storage in 4°C were analyzed with one-way ANOVA followed by Tukey's test to analyze the MDS and PFAT5 differences between baseline and each time interval of the storage. The p value <0.05 was considered as statistically significant.[15,16]

RESULTS

Homogeneity and physical appearance of discoloration of propofol preparation

The homogeneity and physical apperance dicoloration of emulsion preparation of propofol before (0 hour) and after storage procedures for 6 and 24 hours in 4°C are presented in TABLE 1. The homogeneity and the color of the emulsion preparation of propofol did not change in each time observation.

TABLE 1. Homogeneity and physical appearance of the emulsion of emulsion preparation of propofol before (0 hour) and after storage procedures for 6 and 24 hours in 4 °C

Color	0 hour	6th hour	24th hour
Standard color *	16 (100%)	16 (100%)	16 (100%)
Color changed	0 (0%)	0 (0%)	0 (0%)

* *Standard color: white milk*

Fat Globule Population Percentage >5 μm and MDS of propofol preparation

The PFAT5 of the emulsion preparation of propofol samples before (0 hour) and after storage procedures for 6 and 24 hours in 4°C is presented in TABLE 2. No sample droplet size > 5 μm was observed in each time storage procedures observation indicating that the PFAT5s of the emulsion preparation of propofol were 0 (%).

TABLE 2. The PFAT5 values of emulsion preparation of propofol before (0 hour) and after after storage procedures for 6 and 24 hours in 4 °C

Variable	0 hour	6th hour	24th hour
PFAT5	0 (0%)	0 (0%)	0 (0%)

the emulsion preparation of propofol samples tested was observed before and after storage procedurs. The MDS before storge procedure (0 hour) (225 ±24.2 nm) was significantly lower

($p<0.01$) than after storage procedure for 6 hour (247±22.0 nm) and for 24 hour (278±28.8 nm). Moreover, the MDS after storage procedure for 6 hour was also significantly lower ($p<0.01$) than for 24 hour (TABLE 3).

TABLE 3. Mean droplet size of emulsion preparation of propofol before (0 hour) and after storage procedures for 6 and 24 hours in 4°C

Sample number	Mean droplet size (nm ± SD)		
	0 hour	6th hour	24th hour
1.	212±93.4	250±15.9	337±23.6
2.	214± 12.7	238±14.8	308±22.0
3.	247± 16.4	266±17.3	330±29.0
4.	237 +14.3	249+11.2	275+22.1
5.	207 ±79.0	228±12.7	260±17.2
6.	215 ±89.5	238±12.3	252±15.1
7.	215 ±95.6	237±12.3	263±16.5
8.	231 ±10.5	254±13.7	266±15.7
9.	221 ⊥11.7	241⊥14.1	259⊥15.9
10.	222 +11.0	244+14.8	259+15.7
11.	209 ±89.9	236±13.4	263±17.7
12.	195 ±87.6	216±11.6	254±15.3
13.	218 ±12.5	240±15.6	256±14.8
14.	206 ±83.3	230±11.3	257±16.2
15.	264 ±12.9	281±15.4	290±23.0
16.	290 ±16.2	308±20.7	315±22.6
Total mean	225 ±24.2	247±22.0	278±28.8

*SD = Standard Deviation; ANOVA test; Fo 17.41; F crit 3.204; p<0.01**

The differences of MDS of propofol prepartaion emulsion before (0 hour) and after storage procedures for 6 and 24 hours is presented in TABLE 4. Statistical analysis showed that there was significantly differences of MDS between 0 and 6th hours ($p=0.045$), 0 and 24th hours ($p=0.001$), and 6th and 24th hours ($p=0.040$) observations (TABLE 4).

TABLE 4. The differences of MDS of emulsion preparation of propofol before (0 hour) and after storage procedures for 6 and 24 hours in 4°C

Storage procedure	MDS differences (nm)	p
Between 0 hour and 6th hour	22	0.045
Between 0 hour and 24th hour	53	0.001
Between 6th hour and 24th hour	31	0.040

DISCUSSION

Sixteen samples of emulsion preparation of propofol were included in the study. Before storage procedure or at 0 hour, all physical appearance and homogeneity of samples were the same (n=16; 100%) based on standardized color and homo-geneity of the standards used. The MDS value of samples was 225±24.2 nm before storage procedure, whereas the PFAT5 value was 0%. It indicated that all samples at 0

hours are in accordance with the criteria of FDA (MDS < 450 nm and PFAT5 <0.05%). Morover, the MDS value at 0 hour obtained in this study was similar with the MDS value of others emulsion preparation of propofol i.e. propofol 1% Fresenius® (MDS: 229±2.1 nm) and propofol MCT/LCT emulsion generic (MDS: 212±1.2 nm) as reported by Muller and Harnich.[5] Therefore, the MDS and PFAT5 of samples of this study at 0 hour can be used as control group for samples after 6 and 24 hours storage procedure.

Theoretically, as an emulsion preparation, propofol will degrade over time, which can be observed as droplet size change.[7] This study focused on propofol emulsion preparation droplet size changes after 6 and 24 hours storage procedures in 4ºC refrigerator. The result of this study showed that the MDS of emulsion preparation of propofol samples has changed significantly ($p<0.05$) after 6 and 24 hours storage procedure in ºC (TABLE 3). This result indicated that the emulsion preparation of propofol which was already opened from its packaging had a tendency to rapidly degrade.

The stability of emulsions can be influenced and accelerated by the presence of external factors such as mechanical stress, temperature, light, pressure, microorganisms, pH, oxygen, carbon dioxide and the ions of some electrolyte.[6,7] The storage procedure in a refrigerator with a temperature of 4ºC, as recommended in package insert i.e. in range of temperature of 2-25ºC, was performed in order to minimize the influence of external factors. However, if the emulsion preparation of propofol has been already opened from the ampoules and transferred in a syringe, the storage procedure in the refrigerator can not prevent the influence of external factors especially oxygen to propofol in the syringe. The propofol undergoes oxidative degradation in the presence of oxygen. As reported by Ryoo[14] lipid peroxidation can also lead to destabilization of the emulsion preparation of propofol through degradation of soybean oil droplets contained in the preparation, although its process is relatively slow. For this reason, an emulsion preparation of propofol manufactured by pharmacetical industry should be prepared in condition of free of oxygen that is conducted by evacuating all the oxygen in the ampoules and replacing it with hydrogen.[6,14]

After the storage procedure for 6 and 24 hours, change in the homogeneity and the color of the emulsion preparation of propofol was not observed macroscopically or visually (TABLE 1). Baker *et al.*[7] reported that the visual change of an emulsion preparation of propofol will be observed if the droplet size of the emulsion preparation exceeds 50 μm.

However, microscopic obsevation showed that the droples size of emulsion preparation of propofol before (0 hour) was different with after storage procedures for 6 and 24 hours in 4ºC. The largest droplet at 0 hour and after storage procedure for 6 and 24 hours were 1.5, 2 and 3 ìm, respectively. Moreover, the MDS of emulsion preparation of propofol increased significantly ($p < 0.05$) and gradually after storage procedure for 6 and 24 hours compared to before storage procedure (0 hour) (TABEL 3 and 4). It was indicated that droplet size of the emulsion preparation of propofol that was already opened from the packaging has significantly changed within 6 hour storage procedure in 4ºC. Although the droplet size of emulsion preparation of propofol after 6 hour storage changed, none of the droplet size exceeded 5 μm. Therefore, the PFAT5 value at three time observation were respectively 0% (n=16; 100%) (TABLE 1).

The MDS and PFAT5 values are important risk factors associated with embolism incidence.[7,8] This study showed that the change of droplet size of emulsion preparation of

propofol up to 24 hours was still below the value required by the FDA (MDS-24 <450 nm and PFAT5 <0.05%). The degradation of the emulsion can affect the release of propofol *in vivo*, as a result of reduced surface area due to the enlargement of droplet size. Moreover, it can also cause variations in the concentration of propofol in volume emulsion.[8]

Masaki *et al*.[10] studied the physicochemical stability of combinations of propofol-lidocaine mixtures frequently used in clinical practice. In this study, various dose of lodocaine i.e. 5, 10, 20, or 40 mg were added to propofol preparation. The result indicated that the addition of 20 and 40 mg of lidocaine to propofol preparation resulted in macroscopically colorless layers at 3 and 24 h after preparation, whereas, the mixture with 5 or 10 mg of lidocaine was macroscopically stable. Moreover, the addition of 40 mg lidocaine decreased propofol concentrations significantly, whereas, the mixture with 5, 10 or 20 mg of lidocaine was unchanged compared with baseline concentrations. Scanning electron microscopy showed that droplets with diameters > 5 μm first appeared after the addition of 40 mg of lidocaine to propofol preparation, and the emulsion droplets were enlarged in a time- and dose-dependent fashion. Depending on the dose of lidocaine and the duration between its preparation and administration, this combination might pose the risk of pulmonary embolism. Further study is needed to evaluate the implication of the combinations of propofol-lidocaine frequently used in clinical practice.

This study suggested that propofol preparation should be used according to the instruction in the packed insert that recommends using propofol immediately and as soon as possible after opened from the package. The instruction in the packed insert suggests that propofol preparation should be used not up to 6-12 hours after opened or stored. This study showed that after 6 hours after storage procedure at ºC, enlargement of droplet size of the emulsion preparation of propofol has been observed although the droplet size was still below the value required by the FDA.

CONCLUSION

The droplet size of emulsion preparation of propofol enlarges significantly after the storage procedures for 6 and 24 hours in refrigerator with temperature of 4ºC. However, the enlargement of the droplet size is still below the value required by the FDA. Moreover, macroscopical homogeneity and physical apperance of the propofol preparation is stable after the storage.

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REFERENCES

1. Morgan EG, Mikhail MG, Murray MJ. Clinical anesthesiology. 4th ed. New York: Lange Medical Book, McGraw-Hill, 2005.
2. Ravenelle F, Gori S, Garrec DL, Lessard D, Luo L, Palusova D, *et al.* Novel lipid and preservative-free propofol formulation: properties and pharmacodynamics. Pharm Res 2008; 25(2): 313-9.
3. Li X, Zhang Y, Fan Y, Zhou Y, Wang Z, *et al.* Preparation and evaluation of novel mixed micelles as nanocarriers for intravenous delivery of propofol. Nanoscale Res Lett 2011; 6:275-84.
4. Driscoll DF, Dunbar JG, Marmarou A. Fat-globule size in a propofol emulsion containing sodium metabisulfite. AJHP 2004; 61:1276-80.
5. Muller RH, Harnisch S. Physicochemical characterization of propofol-loaded emulsions and interaction with plasma proteins. Berlin:

Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University, 1997.

6. Han J, Davis SS, Washington C. Physical properties and stability of two emulsion formulations of propofol. Int J Pharm 2001; 215:207–20.
7. Baker MT, Naguib M. Propofol: the challenges of formulation. Anesthesiology 2005; 103(4): 860-76.
8. Tamilvanan S. Oil-in-water lipid emulsions: implications for parenteral and ocular delivering systems. Prog Lipid Res 2004; 43(6): 489-533.
9. Driscoll DF. Lipid injectable emulsions. Nutr Clin Pract 2006; 21:381–6.
10. Masaki Y, Tanaka M, Nishikawa T. Physico-chemical compatibility of propofol-lidocaine mixture. Anesth Analg 2003; 97(6):1646-51.
11. Anonim. Propofol (Trivam®), package insert. Korea: Hana Pharm Co., Ltd, 2009.
12. Anonim. Propofol (Diprivan®), package insert. United Kingdom: Astra Zeneca, Macclesfield, 2010.
13. Anonim. Propofol (Fresofol®), package insert. Australia: Fresenius-Kabi, 2011.
14. Ryoo HK, Park CW, Chi SC, Park ES. 2005. Development of propofol-loaded microemulsion systems for parenteral delivery. Arch Pharm Res 2005; 28(12):1400-4.
15. Hopkins WG. 2003. A new view of statistics 2003. [cited 2011 january 5th] Available from URL:www.sportsci.org/resource/stats.
16. Sastroasmoro S, Ismail S. Dasar-dasar metodologi penelitian klinis, ed 2. Jakarta: Sagung Seto, 2006.