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ORIGINAL ARTICLE

Emulsion Effect on the Measurement of Standard Laboratory Blood Parameters

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SUMMARY

Background: **A number of pharmaceutical agents have limited water solubility and are therefore often prepared in a lipid emulsion. Emulsion renders plasma opaque and this could interfere with the accuracy of some standardized laboratory measurements, especially for optical or mechanical based assays. We determined the interference on some laboratory diagnostic values of blood specimens after propofol addition *in vitro* as well as *in vivo* when infused into swine.**

***Methods: In vitro*, laboratory parameters were measured immediately after mixing swine blood diluted with increasing amounts of propofol emulsion in the range of 3 to 23%, v/v. The contact time of a 9% v/v mixture of blood and propofol was also examined over a 3-hours period. Saline-diluted samples served as controls. Cellular volume, hematocrit, hemoglobin, potassium, and coagulation were measured with various instruments. In addition, similar parameters were analyzed from swine blood following a 9 - 10 hours infusion with propofol/fentanyl compared to infusion with ketamine/midazolam.**

***Results: In vitro*, blood cell volume increased immediately upon contact with a mixture exceeding 6% propofol. Above 9% of the mixture, the cellular volume expanded significantly with extracellular K^+ leakage. Hematocrit increased but the hemoglobin was dependent on the instrument type. Coagulation was altered when the emulsion was present. Interestingly, *in vivo* these effects were significant less pronounced in blood collected from experimental swine under total intravenous propofol anesthesia, but they departed from water-based anesthesia in control swine.**

Conclusions: **The *in vitro* studies indicate that results from certain assays of blood samples rich in lipid emulsion may not be accurate due to interference with optical, mechanical or ion selective electrode methodology. Although *in vivo* samples may be less impacted, there is still a risk of deviation from accuracy.**
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KEY WORDS

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Highlights:
- Lipid emulsion may interfere with optical, mechanical or other laboratory assessments.
- Clinical decisions made on laboratory results may require some critical examination.

INTRODUCTION

Many pharmaceutical formulations contain active agents that have limited water solubility and need to be dissolved in a lipid-based solution. If the active agent is rapidly cleared through hepatic metabolism and renal excretion, the lipid emulsion is likely to remain in the blood stream for longer periods of time before being eliminated [1]. Most emulsifying agents are a mixture of soybean oil, glycerol, and egg phosphatide [2], and the resulting opacity of the plasma could interfere with the accuracy of standardized laboratory measurements, especially those with optical or mechanical based assays [3]. One of these anesthetic formulations, propofol, a lipid-based anesthetic, is widely used in both clinical and pre-clinical applications. Our pre-clinical laboratory routinely uses propofol for total intravenous anesthesia (TIVA) regimen in our surgical experiments [4,5]. The anxiolytic active ingredient of propofol, 2,6 di-isopropylphenol, has a fast distribution in the tissues and a short sedation acting time which results in quick clearance from the body and warrants continuous administration to maintain a therapeutic anesthetic plane for long periods [6,12]. This will increase the lipemic index of the blood which in turn may affect the accuracy of results from collected specimens.

The aim of this study was to determine what type of abnormal readings were produced by the emulsion *in vitro* and if these effects were reproducible in the same laboratory assessments from blood containing propofol from swine after a 9-hours long-term sedation.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research.

The propofol emulsifying mixture that was used (Propofol 28, Zoetis, MI, USA) contained soybean oil (100 mg/mL), glycerol (22.5 mg/mL), benzyl alcohol (20 mg/mL), oleic acid (0.6 mg/mL), and egg lecithin (12 mg/mL) with a pH adjusted at 6.0 - 8.5, and was isotonic as per manufacturer. It contained 2,6 di-isopropylphenol, as sedative active component, with a stock concentration of 10 mg/mL (1% w/v). Propofol is sold as a veterinarian product that is similar to the human product Diprivan (Diprivan contains EDTA as a chelator). The bottles were stored at room temperature per manufacturer guidelines. The effect of propofol on hematological parameters was examined *in vitro* on two hematology instruments, the HemaVet (Drew Scientific, Irvine, CA, USA) and the Radiometer ABL 800 (Copenhagen, Denmark) as well as a thromboelastometry device (ROTEM delta, Tem Systems, Inc., Durham, NC, USA), which are currently used in both clinical and pre-clinical laboratories.

In Vitro study of emulsion effects

Propofol was mixed *in vitro* with swine blood yielding progressive concentrations of propofol. Swine blood was collected in citrate anticoagulant tubes while the animal was under isoflurane anesthesia at the beginning of the experimental study. Fresh blood was then immediately aliquoted to test the *in vitro* effect of the propofol mixture on hematological parameters. In the first study (concentration-dependent study), blood aliquots were diluted with propofol over a 3%, 6%, 9%, 14%, and 23% (v/v) dilution range, and control samples were diluted over a similar range with 0.9% normal saline (Table 1). Baseline was obtained with the undiluted blood (referred to as 0%) and the reported percentage of propofol represents the volume of propofol (the emulsified product) in the total sample volume (v/v); for example, 100 µL propofol in 1 mL blood is defined as 9%, whereas the active ingredient for 9% propofol will be 0.909 mg of 2,6 di-isopropylphenol per mL blood. In a second study (time-dependent study), a mixture of 9% propofol (or saline for control) was selected to assess the effect of propofol with blood over a 3-hour period of time. This dosing regimen was designed based on the administration dose for our animal study.

Hematology instruments

Three instruments were used in this study to assess clinical hematology parameters. The Radiometer ABL 735 uses a spectrophotometric method to measure hemoglobin (Hb-ABG). The red blood cells (RBCs) in the samples are mechanically lysed by sonication and the free Hb is detected by multiple wavelength scans ranging from 478 to 672 nm. The Hb concentration is computed using a proprietary matrix combining absorption coefficients and wavelength [7]. Ion-selective electrodes using potentiometric principles were used to detect Na^+, K^+, and Cl^- on the Radiometer instrument. The HemaVet is a veterinary hematology blood cell analyzer that uses chemical and physical methods to measure Hb (Hb-HEM), hematocrit (Hct), RBC, platelet (PLT) count, and white blood cell (WBC) count, mean corpuscular volume (MCV), and mean platelet volume (MPV). To determine Hb, the HemaVet uses ammonium salts to lyse the RBCs and the free Hb is transformed into cyanomethemoglobin using a conventional cyanide method [8]. The absorbance is then measured at 540 nm wavelength to calculate Hb concentration. Cell count and size (MCV, MPV) are measured by impedance, measuring directly the particle number and volume. The Hct, is a calculated value as a percentage where Hct = (RBC* MCV)/10. Lastly, a rotational thromboelastrometry (ROTEM) coagulation monitoring device was also used to measure the clot formation time (CFT) as well as the maximum clot firmness (MCF) of the samples. The ROTEM is an established viscoelastic technique to assess hemostasis and clotting kinetics using a mechanical optical detection system.

***In Vivo* observations**

Blood samples were collected from Yorkshire swine (Animal Biotech Industries, Danboro, PA, USA) subjected to an experimental treatment under TIVA and were analyzed separately from the *in vitro* samples. Animals were maintained under anesthesia and sedation with continuous administration of either 1) propofol emulsion (2 - 30 mg/kg/h IV) and fentanyl (5 - 10 μg/kg/h IV) (propofol/fentanyl; PF group; n = 16; animal weight 33.6 ± 3.3 kg) or 2) Ketamine (10 - 30 mg/kg/h) and Midazolam (0.4 - 1.5 mg/kg/h) (ketamine/midazolam; KM group; n = 10; animal weight 32.6 ± 4.0 kg) to maintain a steady anesthetic plane. All animals received saline at 3.5 mL/kg/h as a carrier for TIVA. Swine were ventilated using synchronized intermittent mechanical ventilation mode (Apollo®, Draeger Medical Inc. Telford, PA, USA) with FiO_2 of 0.4 and end-tidal carbon dioxide ($ETCO_2$) between 35 - 40 mmHg. Animals received 3 mL/kg/h IV 0.9% saline as a maintenance fluid. The experimental swine protocol required the animals to be maintained under anesthesia for as long as 10 hours. In the PF group, the total volume of fluid infused (PF and saline) was 1,651 ± 200 mL. With an estimated blood volume in swine of 70 mL/Kg, and an average administration of 20 mg/kg/h (2 mL/kg/h) propofol emulsion intravenous infusion, the infusion yielded to ~15% and 25% of propofol in blood over 5 hours and 10 hours, respectively. In the KM group treated for the same period of time, the animals received ketamine ranging from 6.2 to 6.8 mL/h. The total fluid volume infused in KM group (KM and saline) was 1,168 ± 103 mL.

Statistical analysis

Data was analyzed using the statistical package IBM SPSS Statistics 21.0 (IBM Corporation, Armonk, NY, USA). All laboratory measures were assessed for normal distribution. Continuous variables were assessed with ANOVA. Dichotomous variables were analyzed with the Wilcoxon signed rank test. Statistical significance was a priori set at $p \leq 0.05$. The propofol samples were grouped based on their percentage mixture (0%, 3%, 6%, 9%, 14%, or 23%) in either propofol or saline and a one-way ANOVA was conducted to determine if there were group percentage differences between the propofol and the saline control samples. One-way ANOVA was similarly conducted to assess the effects of time exposure on propofol administration. The different time points were modeled as groups, and comparisons among physiological measures were made between saline and propofol samples. A Wilcoxon signed rank test was used for paired values analysis for statistical comparison. Anticipated results over the dilution range were calculated parameters based on the undiluted blood parameters and dilution factors.

RESULTS

***In Vitro* study of propofol effects**

Concentration-dependent study: effects of increasing propofol concentration in blood

In order to establish a baseline control, we compared the measured parameters from saline-diluted controls with the calculated values based on dilution (anticipated values; dotted lines in figures) we found no significant difference for all parameters tested, providing confidence that the saline dilution accurately followed the dilution pattern and can, therefore, be used as controls. The cellular volumes, mean corpuscular volume (MCV), and mean platelet volume (MPV) remained the same for the dilution range, whereas Hct, Hb-HEM and Hb-ABG, potassium, cell counts and maximum clot firmness (MCF) decreased, and the clot formation time increased with the dilution. As the concentration of propofol increased within the blood samples, both MCV and MPV also constantly increased compared to the saline diluted samples; this trend became significant at a 6% propofol concentration (Figure 1A, B) ($p < 0.05$). Hb-HEM also departed significantly from saline; however, Hb-ABG did not and followed a trend similar to the saline dilution (Figure 1C, D). Departure from the anticipated dilution for the hematocrit became significant above 10% (Figure 1E). Potassium (K^+) levels were increased above anticipated values while Na^+ levels were decreased ($p < 0.05$) (Figure 1F, G). Cell count such as RBC, WBC, and platelets were not affected by propofol and were comparable to the saline dilution, although platelet count was more variable (Figure 1H, I, J). When samples were diluted in saline, the coagulation profile illustrated by the clot forming time (CFT) tended to be below the anticipated dilution profile, whereas the propofol diluted samples showed a significant increase at 14% ($p < 0.05$). The maximum clot formation (MCF) indicated a similar but reverse pattern compared to the CFT (Figure 1K, L). No hemolysis was noticed in these samples for all dilutions.

Time-dependent study: Exposure of a mixture of 9% propofol in blood over time

The effects of propofol on each of the parameters were evident immediately after the addition of propofol to the blood (time point; T0) and followed the same amplitude of change as in the dilution study. Most of these effects remained constant over the 2-hour period without much change in the measured parameters (Figure 2). For the parameters such as cell counts (RBC, WBC, and platelets) there was no time effect (data not shown). MCV for the saline control samples followed the anticipated values for the 3-hour time course, but the propofol samples had MCV values that remained above the saline controls similar to that obtained in the dose response ($p < 0.05$) (Figure 2A, B). A similar pattern was seen with the MPV for the first 2 hours but after 2 hours the MPV was increased for saline controls and decreased for propofol samples. The Hct values followed the

Table 1. Example of propofol mixture with swine blood.

Volume used (µL)	Example of dilution of blood and propofol					
Blood	1,000	1,000	1,000	1,000	1,000	1,000
Propofol 28	0	30	60	100	160	300
Percent of propofol (v/v)	0%	3%	6%	9%	14%	23%

Table 2. *In vivo* TIVA experiments.

A

Propofol/fentanyl TIVA n = 16		Baseline	After TIVA anesthesia	p-value
Hemavet measurements	WBC (10^6/mL)	17.5 ± 4.5	15.2 ± 4.5	0.07
	Hb (g/dL)	9.2 ± 0.8	8.5 ± 1.1 *	0.01*
	HCT (%)	33.0 ± 4.8	30.6 ± 3.4 *	0.03*
	MCV (fL)	55.0 ± 3.8	53.3 ± 3.6	0.14
	MPV (fL)	9.2 ± 0.9	8.8 ± 0.8	0.18
ABG measurements	Hb (g/dL)	10.0 ± 1.7	8.9 ± 1.8 *	0.01*
	Na^+ (mM)	138 ± 3	137 ± 3	0.1
	K^+ (mM)	4.0 ± 0.2	4.1 ± 0.2	0.48
Rotem measurements	CFT (sec)	165 ± 32	115 ± 48 *	0.05*
	MCF (%)	62.6 ± 8.6	68.8 ± 6.9 *	0.01*

B

Ketamine/midazolam TIVA n = 10		Baseline	After TIVA anesthesia	p-value
Hemavet measurements	WBC (10^6/mL)	16.5 ± 4.7	16.1 ± 4.8	0.61
	Hb (g/dL)	8.0 ± 0.7	8.0 ± 1.0	0.88
	HCT (%)	27.3 ± 3.7	27.3 ± 4.5	0.97
	MCV (fL)	52.4 ± 5.0	51.9± 5.2	0.57
	MPV (fL)	9.9 ± 2.0	9.9 ± 2.0	0.48
ABG measurements	Hb (g/dL)	8.9 ± 1.5	8.8 ± 1.3	0.62
	Na^+ (mM)	138 ± 2.5	137 ± 2.8	0.54
	K^+ (mM)	4.3 ± 0.4	4.4 ± 0.4	0.24
Rotem measurements	CFT (sec)	142 ± 48	139 ± 85	0.86
	MCF (%)	68.5± 7.6	69.7 ± 7.7	0.40

Variation in measured parameters from blood samples collected in an anesthetized swine after a 10-hour infusion of A: propofol/fentanyl emulsion and B: ketamine/midazolam. Changes are presented as a percent from the initial sampling before the infusion (100%). Means and Standard deviation. *p < 0.05.

anticipated values for both samples over the 3 hours. Hb-HEM, Hb values read with the Hemavet, indicated an increase compared to saline samples ($p < 0.05$), whereas the Hb-ABG, hemoglobin read with the ABL, was comparable to saline and anticipated control values (Figure 2C, D). Sodium in the presence of propofol was lower than saline (137 ± 1 vs. 145 ± 1, respectively, at 3 hours; $p < 0.05$), and although not significant, potassium was higher than in saline, increasing slowly over the 3 hours (3.4 ± 0.3 vs. 2.9 ± 0.4, respectively at 3 hours) (Figure 2E, F). CFT and MCF were highly variable over the course of the 3-hour observation and there was no significant difference between the propofol and saline samples (data not shown). There were no remarkable changes within the other parameters over time. Plasma from the *in vitro* blood samples was inspected after centrifugation at 2,000 g for 20 minutes after the 3-hour experimental time and none of the samples showed signs of hemolysis.

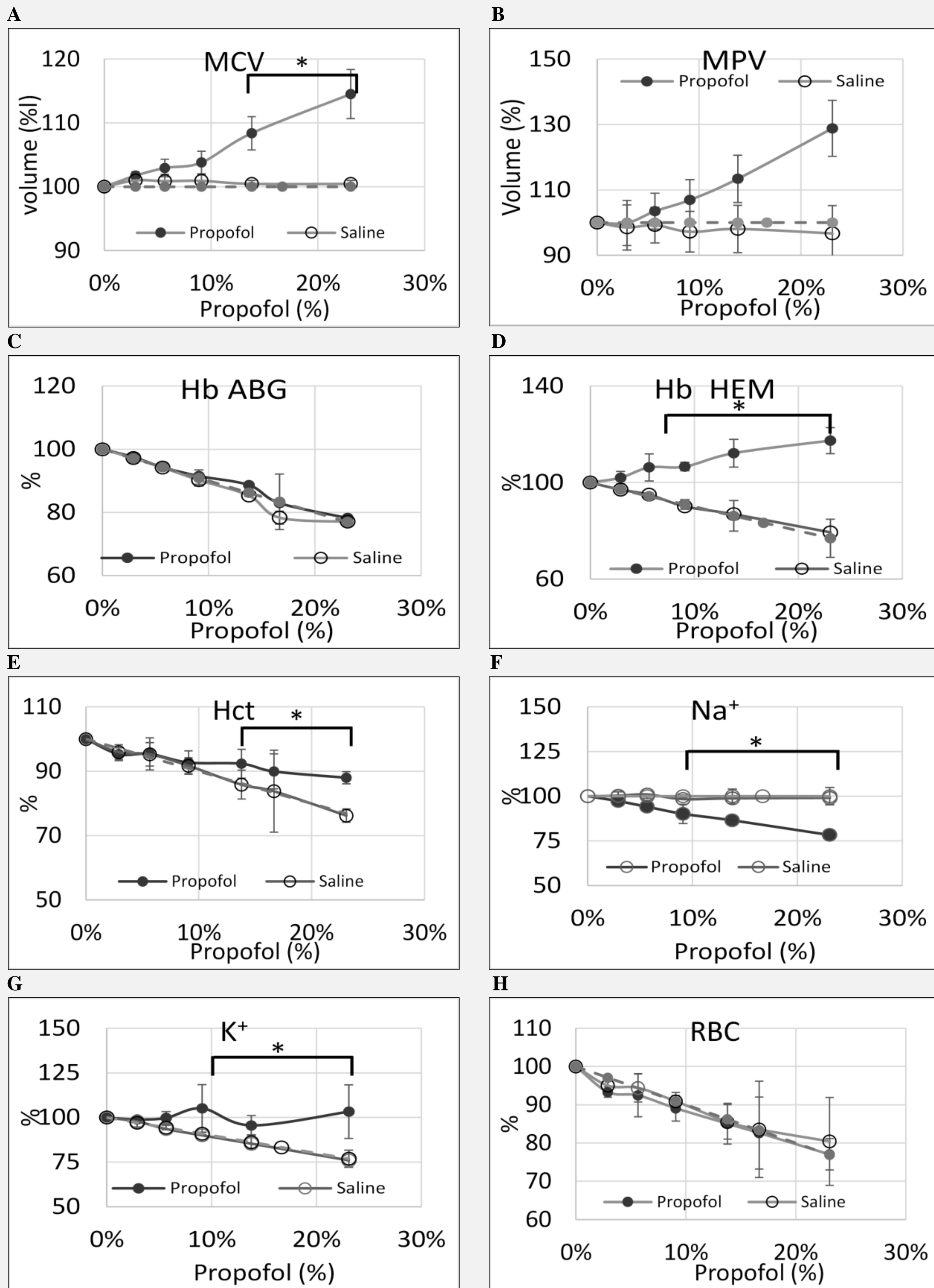

Figure 1.

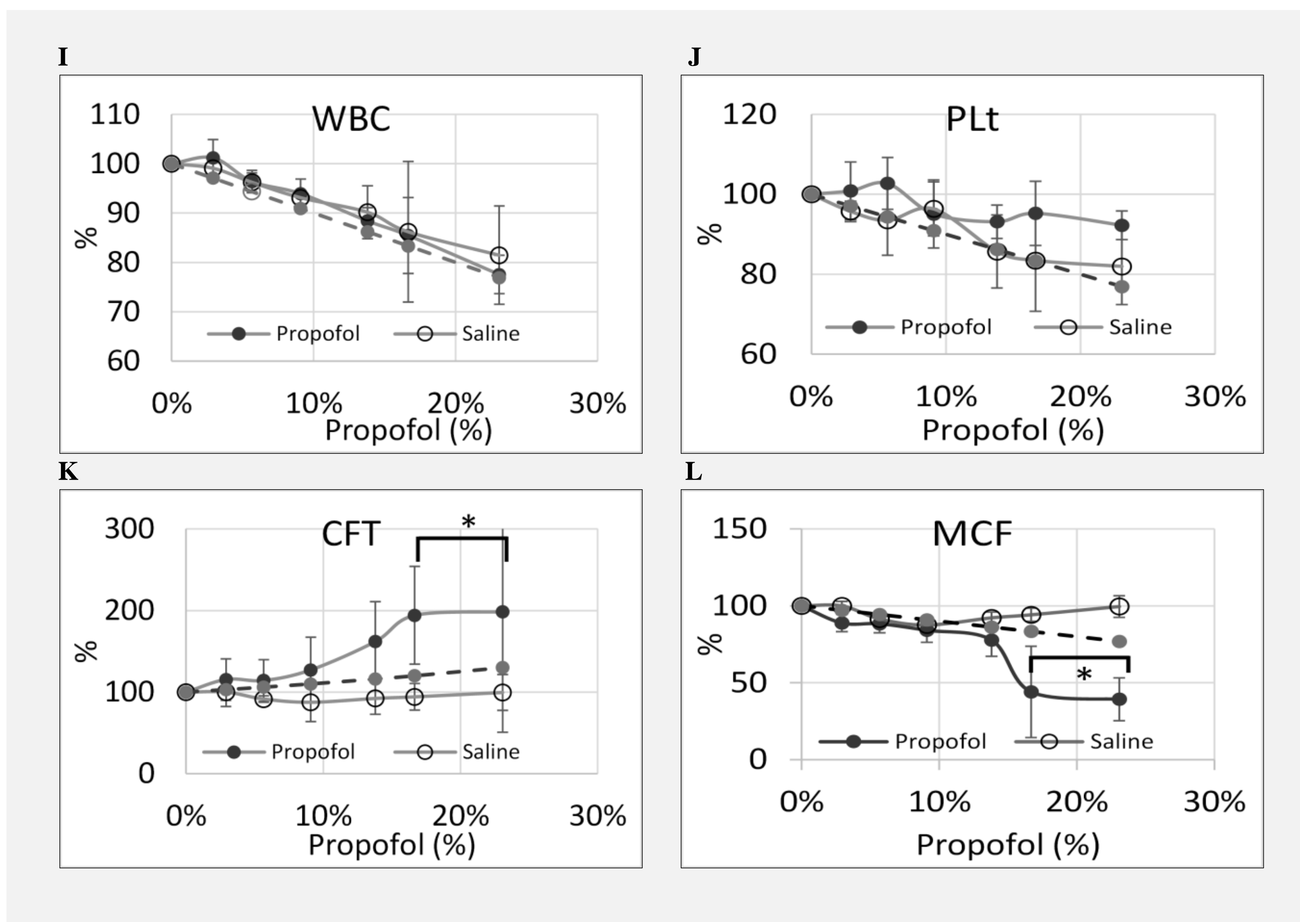

Figure 1 (continued).

Change in % of baseline (0%) in measured parameters *in vitro* in the presence of up to 23% (v/v) propofol. A) MCV: mean corpuscular volume, B) MPV: mean platelet volume, C) Hb-ABG: hemoglobin read with ABL, D) Hb-HEM: hemoglobin read with Hemavet, E) Hct: hematocrit, F) sodium and G) potassium concentration, H) RBC: red blood cells, I) WBC: white blood cells, J) PLt: platelets, K) CFT: clot forming time, L) MCF: maximum clot firmness; Propofol –•–; Saline –o– ; anticipated - - -. Mean and standard deviation. Data are presented a percent of baseline. * p < 0.05 between propofol and saline.

Expresses the span of the difference -

In vivo observations

Swine blood samples collected at the end of the propofol/fentanyl (PF) anesthesia were very turbid compared to clear samples following ketamine/midazolam (KM) anesthesia upon gross visual inspection; neither group showed evidence of hemolysis. Compared to animals in the KM group those in the PF group received more fluid volume (1,437 ± 121 mL vs. 928 ± 99 mL, respectively; $p < 0.01$) due to the higher infusion rate of propofol (2-fold more than ketamine). Cell count, sodium, and potassium levels remained normal and unchanged in both groups. In the PF group some measured parameters after anesthesia showed changes compared to initial sampling before TIVA (Table 2). Hemoglobin and hematocrit were lower than baseline on both Hemavet and ABG ($p < 0.05$) in the PF group but similar in the KM group. Although without significant difference, in the PF group cellular volumes (i.e., MCV, MPV) trended toward a decrease instead of increasing; they remained unchanged in the KM group. The coagulation parameter CFT was lower, whereas MCF was higher after infusion compared to baseline in the PF group ($p < 0.05$); they remained unchanged in the KM group.

DISCUSSION

The purpose of this study was to determine how emulsion might influence the accuracy of laboratory hematology, electrolyte, and coagulation measures when lipid-based sedatives such as propofol are used. The effects of propofol were first studied in an *in vitro* dilution experiment, followed by a time exposure experiment on whole blood. Effects were not noticeable for lower propofol doses, but became statistically more evident with propofol dilutions beginning at 6%. An ad-

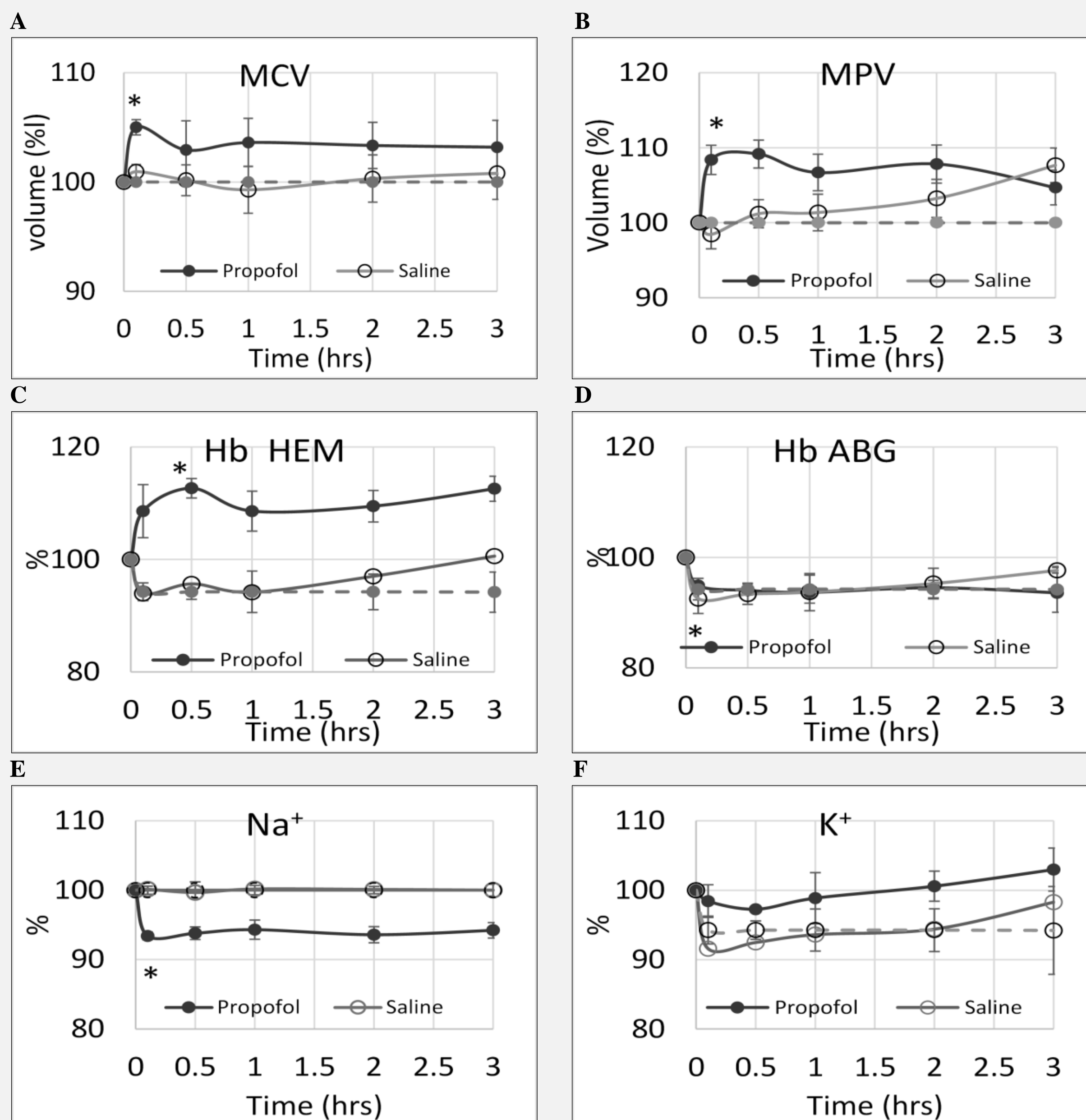

Figure 2.

Change in % of baseline (T0) in measured parameters *in vitro* over time up to 3 hours in the presence of 9% (v/v) propofol: A) MCV: mean corpuscular volume, B) MPV: mean platelet volume, C) Hb-HEM: hemoglobin read with Hemavet, D) Hb-ABG: Hemoglobin read with ABL, E) sodium and F) potassium concentration; Propofol –●–; Saline –o–; anticipated - - -. Mean and standard deviation. Data are presented a percent of baseline. * $p < 0.05$ between baseline and immediately after addition of propofol.

ministration rate *in vivo* of 2 mL/kg/h for a 2-hour continuous infusion of propofol was calculated to correspond approximately to the 6% dose when the effects became visible. In the concentration-dependent study, we observed effects of propofol on some common clinical laboratory parameters such as MCV, MPV, Hb-HEM, Hct, K^+, and CFT that proportionally increased with the percentage of propofol, and MCF values that showed an inverse relationship to propofol concentrations. The Hb-ABG, RBCs, and WBCs values were not significantly affected as they were similar to those of saline-treated samples. Platelet values started to increase with increasing amounts of propofol, but this trend was not statistically significant. Optical interfer-

ence was noted with HemaVet readings (hematocrit, hemoglobin increased upon addition of propofol) but not with ABG readings, which may have been due to the different wavelengths used for optical detection by the two clinical diagnostic instruments (see methods). The photometric effect was likely due to the emulsion in which the active agent is dissolved, which created a turbid environment interfering with readings at visible wavelengths. Further, independent of optical effects, sodium (Na^+), and potassium (K^+) changes were evident. However, the presence of propofol, even in a minute amount, did impair the measurement of electrolytes by ion-selective electrodes (we confirmed that Na^+, K^+, Cl^- levels for any dilution of propofol in water could not be obtained using the ion electrode technique or dry chemistry; data not shown). Therefore, Na^+ and K^+ from the propofol emulsion was not measurable; the decrease of Na^+ in Figure 1F can be explained by the dilution of blood Na^+. The Na^+/K^+ ATPase seems to have been altered over time as reflected in the increased of K^+ (Figure 2F) likely originating from blood cells. Related to this observation, MCV and MPV were also increased with increasing concentration of propofol; as this is measured by impedance which did not affect cell count, cellular swelling is an indication of ion imbalance. In a previous study, using a perfluorocarbon emulsion, Oxycyte, similar observations were made [9]; a cellular volume increase was observed to reach hemolysis with 40% Oxycyte. In the current study, hemolysis was not present at the concentration used.

With addition of propofol in both the concentration-dependent and the time-dependent studies, we observed an *in vitro* increase of CFT (indicating a decrease of the coagulation) as measured by viscoelastic principle, and the increase in the sample's viscosity could contribute to an elongated clotting time. Interestingly, it was reported that 2,6 di-isopropylphenol, the active ingredient, has an inhibitory effect on platelet aggregation [10]. Although we have not measured the aggregation parameters in this study, weaker platelets could have contributed to the reduction of the maximum clot firmness (MCF) and the clot forming time (CFT) by providing less anchorage on the fibrin strand leading to a weaker clot.

Noteworthy, we have observed that these effects occurred immediately or within the first 30 minutes upon exposure of propofol with blood, and thereafter, the parameters remained unchanged at the level reported with the 9% dose response experiment for the total 3-hour study. Thus, the propofol effects occurred on contact with blood and this is supportive of an immediate osmotic effect altering cellular water exchange and of photometric interference impaired by the lipidic emulsion.

In vivo there were no noticeable physiological side effects of the propofol infusion on the animals. The blood samples collected on anesthetized animals after the procedure were visually turbid when propofol was infused, and laboratory results from PF and KM TIVA groups indicated that only propofol influenced the coagulation assay and Hb and Hct that were lower than baseline in the PF compared to KM TIVA groups. This may be partially attributed to a hemodilution effect as this change was shown with both instruments and the propofol/fentanyl volume was greater than ketamine/midazolam; however, it may also correspond to propofol interference. Contrary to what was observed *in vitro*, cellular volumes (MCV, MPV) showed no significant changes from *in vivo* samples. *In vivo,* vascular water exchange allows cellular osmosis equilibrium, whereas *in vitro*, blood cells are confined to the finite tube environment which limits cellular adjustments leading to abnormal electrolytes [1,6]. From a clinical perspective, we observed misleading laboratory results in the presence of a lipid-based anesthetic emulsion. The diagnostic values of the blood samples cannot truly be measured and should be considered as inaccurate depending on the device and the type of parameters measured.

The active agent, 2,6 di-isopropylphenol, in propofol has a rapid metabolic clearance which follows compartment distribution; initially the concentration in tissue equilibrates with that of plasma and it is then cleared through hepatic metabolism and renal excretion. Therefore, it is expected that solely the emulsion is likely to remain systemically *in vivo* for longer periods of time before being totally cleared which may also depend on the emulsion lipid composition [1]. In addition, due to lower lipase activity in swine and different fatty tissue metabolism rates compared to humans, it is possible that lipid-based emulsions may remain longer in a swine model. Interestingly, lipids could act as scavengers (lipid sink) or as activators of Akt kinases, which will protect tissue and organs [11]; this may influence the pharmacokinetics of the anesthetic agent and the restoration of the hemodynamic function [12].

There were some limitations to this study. The *in vitro* results differed from *in vivo* observations. To investigate a possible initial propofol osmotic effect, it may have been advantageous to immediately collect the blood samples after the initial bolus of propofol was administered to the animal and then followed *in vitro* over time. This should be replicated in future studies with significantly larger sample sizes. Another source for laboratory unpredictability could also be the formulation of the emulsion and variability in their osmolality [9,13]. Finally, lipid metabolism in swine might differ from that in humans, and therefore, changing the long-term accumulation of lipids in the human plasma might have a different effect than in porcine model, particularly regarding coagulation parameters.

CONCLUSION

The results indicate that *in vitro* high levels of propofol induce mainly photometric interference with ion selective electrodes, photometric and viscoelastic laboratory assays. Cellular osmotic imbalance is visible *in vitro* but

is corrected *in vivo*. The lipid emulsion that has a longer half-life than 2,6 di-isopropylphenol, the active agent in propofol, is responsible for the optical interference. These *in vitro* results prompt the question of how results from blood samples collected from subjects administered with propofol/fentanyl could possibly be affected if they were left unattended before being tested. The analysis of blood collected after propofol infusion does not show obvious departure from normalcy, but when compared to ketamine/midazolam infusion viscoelastic measuring coagulation parameters indicated a slight tendency for hypocoagulation due to the emulsion. Future long-term experimentation may consider ketamine, alphaxalone or other water-based anesthesia.

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Declaration of Interest:
The authors declare that there are no conflicts of interest.

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