Published in final edited form as:

Curr Clin Pharmacol. 2014 February ; 9(1): 64–69.

Utilization of Optimal Study Design for Maternal and Fetal Sheep Propofol Pharmacokinetics Study: A Preliminary Study

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Abstract

Multiple blood samples are generally required for measurement of pharmacokinetic (PK) parameters. D-optimal design is a popular and frequently used approach for determination of sampling time points in order to minimize the number of samples, while optimizing the estimation of PK parameters. Optimal design utilizing ADAPT (v5, BSR, University of Southern California, Los Angeles) developed a sparse sampling strategy to determine measurement of propofol in pregnant sheep. Propofol was administered as supplemental anesthetic agent to inhalation anesthesia to mimic anesthesia for open fetal surgery. In our preliminary study, propofol 3 mg/kg was given as a bolus to the ewe, followed by propofol infusion at rate 450 mcg/kg/min for 60 minutes, then decreased to 75 mcg/kg/min for 90 more minutes and then ceased. A three compartment model described the PK parameters with the fetus assumed as the third compartment. Initially, sampling times were chosen from thirteen time points as previously stated in the literature. Using *priori* propofol PK estimates, the final 9 sample time points were proposed in an optimal design with a change in infusion rate occurring between 65 and 75 minutes and sampling proposed at 5, 15, 25, 65, 75, 100, 110, 150, and 180 minutes. D-optimal design optimized the number and timing of samplings, which led to a reduction of cost and man power in the study protocol while preserving the ability to estimate propofol PK parameters in the maternal and fetal sheep model. Initial evaluation of samples collected from three sheep using the optimal design strategy confirmed the performance of the design in obtaining effective PK parameter estimates.

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CONFLICT OF INTEREST

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

Keywords

Optimal design; pharmacokinetics; propofol; sparse sampling

INTRODUCTION

With recent advances in prenatal diagnosis, fetal surgery has become a treatment option for certain life-threatening fetal diseases. However, invasive fetal surgical procedures are some of the most challenging treatments with high risk of fetal morbidity and mortality. General inhalational anesthesia with high minimum alveolar concentrations (MAC) is typically used during open fetal surgery to promote adequate uterine relaxation for optimal surgical exposure of fetus and to minimize the risk of placental separation. The potent vasodilation effect of this high concentration inhalational anesthetic agent (2–3 MAC) may induce maternal hypotension, decrease uteroplacental blood flow, and direct fetal cardiac depression. *In vitro* human studies have shown that propofol [1] decreases isolated gravid uterine muscle contractility in a dose-dependent fashion. This effect has never been demonstrated *in vivo*. However, in our human fetal surgical population, we have observed that Supplemental IntraVenous Anesthesia (SIVA) with propofol infusions provided adequate uterine relaxation for open fetal surgery with decreased, 1 to 1.5 MAC of desflurane.

To investigate how to reduce risk of fetal morbidity and mortality it is important to determine which anesthetics and anesthetic techniques are responsible for adverse maternal and fetal outcomes. This can be done through use of a maternal/fetal sheep model, where it is possible to develop better anesthetic strategies for this population at higher risk of perioperative morbidity and mortality, including tailored dosing of anesthetic agents and use of safer alternative anesthetics. In our human fetal anesthesia practice, we have found that supplemental infusions of propofol along with lower doses of inhalational anesthesia provide better intraoperative fetal hemodynamic parameters [2]. However, it is challenging to get human fetal PK studies performed because of the multiple PK sampling and ethical limitations. We designed our study using fetal-maternal sheep model, a well-established model for fetal instrumentation and drug studies. The application of clinical pharmacology techniques such as optimal design limited sampling strategies and pharmacokinetics can be utilized to develop the study design and elevate the safety and efficacy of the maternal/fetal anesthetics administered. Studies can require multiple blood samples for determination of PK parameters, as well as comparison to the predicted concentrations, especially during modeling development. The cost effectiveness of sampling strategies needs to be justified with the expense of assay analysis, work load, inconvenience, pain and difficulty of blood drawn, especially with difficulty vascular access.

In clinical pharmacokinetic (PK) studies, multiple blood samples at different time points are collected from each individual subject, then further analyzed by nonlinear mixed effects regression models with multiple responses. During the past ten years, various methods and models to estimate pharmacokinetic parameters, as well as to determine the optimal sampling time sequence have been explored and developed. The impacts of optimal design

on the precision of the estimated parameters in Pharmacokinetic/Pharmacodynamic (PK/PD) studies have been published [3–5]. D-optimal design is the most popular design metric used in PK/PD optimization [6]. The mathematic theory for determining the optimal sampling times is mostly based on the Fisher Information Matrix (FIM). The optimality criterion used in generating the D-optimal design, or D-optimality criterion, is maximization of the determinant of the FIM, and also minimizing the determinant of variance-covariance matrix [7].

The proposed study requires both maternal and fetal blood samples for propofol concentrations to be collected. Minimizing sampling times with accurate prediction of PK parameters has tremendous potential to reduce analyses cost and time. An optimal design was proposed for optimizing the cost effectiveness of this on-going study. The primary objective was to develop an optimal design with a sparse sampling strategy for propofol in pregnant sheep and then to evaluate the effectiveness and accuracy of the optimal design from three maternal/fetal sheep pairs investigated in the study.

METHODS

The anesthetic techniques used in this on-going study involved the use of propofol. For the purpose of the optimal design, the focus was on development of an optimal limited sampling strategy for propofol to assess the pharmacokinetics. Phoenix® WinNonlin version 6.2 (Pharsight, Mountain View, CA) was used to undertake a non-compartmental analysis (NCA) to calculate area under the blood concentration-time curve (AUC), maximum concentration (C_{max}) and half-life.

Anesthesia Techniques

The anesthesia technique proposed for this study includes induction with propofol 3 mg/kg and endotracheal intubation with succinylcholine 1.5 mg/kg [8]. Maintenance of anesthesia is achieved with propofol 450 µg/kg/min for 60 minutes. After 60 minutes, desflurane is commenced and maintained at 1.5 MAC (end-total desflurane 10.2%), and subsequently the propofol infusion rate will be decreased to 75 µg/kg/min maintained for 90 more minutes. Remifentanyl was infused for intraoperative pain control.

Initial Suggested Sampling Times

The initial suggested sampling times for this study, which were adapted from study by Andaluz *et al.*, required that 13 plus baseline plasma samples would be taken from the ewes and the fetus, totaling 28 samples. This sampling schedule below was based on a previous reported PK study in pregnant sheep [9].

Start Propofol Infusion

Baseline (0), 5, 10, 30 and 60 min

After change in infusion rate

5, 10, 30, 60, 90 min after the rate change (65, 70, 90, 120, 150 min)

End of Infusion

5, 10, 15, 30 min after stopping infusion (155, 160, 165, 180 min)

Optimal Design Methodology

Analysis and model simulation are important tools for study design optimization, a number of software tools exist to aid in development of optimal design strategies. There are a few open source software packages available that can be used to develop optimal designs for population PK studies. The following use D-optimal criteria: ADAPT [10], PFIM [11], PopDes [12], PopED [13], and POPT and its associated standalone graphical user interface WinPOPT [14]. The software packages above all use the same derivation of the Fisher information matrix to evaluate sampling designs; they vary only in how extensive the packages are [15]. In this study, both ADAPT [10] and WinPOPT were considered for development of the optimal design strategy. However, one of the limitations of WinPOPT is that it considers doses to be given at a set frequency and does not allow for irregular dosing as required in this study.

The initial optimal design software used to develop a sparse sampling strategy used ADAPT [10] (v5, BSR, University of Southern California, Los Angeles), to evaluate and to determine the optimal sampling strategy. The application of D-optimality to design an optimal design requires a priori knowledge in PK parameters. Review of the literature provided several PK models for propofol in sheep. Table 1 provides estimates obtained from a two compartment model undertaken in sheep. For the given PK model determined from the literature [16–18], PK parameter estimates, dose and optimal sampling times, the effects of subject variation and sample size on precision of PK parameter estimates were investigated. Following obtainment of a priori data from the literature as outlined above, the D-Optimality optimal design model in ADAPT was used to assess varying sample designs. A population analysis using population parameter estimation with maximum likelihood EM (Expectation-Maximization algorithm) was also used.

For this study, a three compartment model was used for the design to describe the pharmacokinetics of propofol in pregnant sheep, with the third compartment simulating distribution to the fetus. As propofol PKs have both been described in the literature using a three compartment model approach [16–18], choosing this structural model should provide a reasonable basis for the optimal design. Following proposal of the optimal design strategy and sampling time, the experiment was performed initially on three maternal/sheep pairs to assess the validity of the design.

SAMPLE COLLECTION

Blood samples were drawn from an arterial or venous line in the ewe (2 mL) and from the fetus (1 mL). The blood sample was transferred into tubes containing 10 units of heparin per mL of blood. After collection of the blood sample, the tube was gently inverted to make sure that the heparin was mixed with the blood. Samples were then immediately centrifuged at 2000 g for 15 minutes at 4 °C. Plasma samples were then transferred into pre-labeled screw top polypropylene tubes. Samples were stored immediately after plasma separation at –70°C

or below in a GLP-compliant freezer until shipping. Maternal and fetal propofol concentrations (mcg/mL), LLOQ = 0.05 mcg/mL were measured with high sensitivity LC-mass spectrometry assays (by Dr. Uwe Christians' Clinical Research & Development, Department of Anesthesiology, University of Colorado, Denver).

RESULTS

Optimal Sampling Strategies

Using ADAPT [10], an optimal sampling strategy was developed in order to maximize the PK information obtained in pregnant ewes while minimizing the number of blood samples required and the volume of blood collected for the study.

The proposed optimal study design was based on including 10 pregnant ewes with mean weight of 58.3 ± 11.5 kg [9]. *A priori* propofol PK estimates were available from a variety of sources, including a two compartment maternal-fetal sheep model, a three compartment human adult model and a three compartment pediatric model. As the proposed PK model was a three compartment model, the optimal design required *a priori* PK estimates for all PK parameters. The sheep model was only a two compartment model and therefore estimates were required for the missing parameters for the proposed model. The design involved testing of the *a priori* PK estimates from the adult and pediatric models in the three compartments optimal design strategy. The PK parameters utilized were: V_1 , K10, K12, K21, K13 and K31.

Baseline Design

The initial design generated the baseline design using 10 ewes and the initial design for testing 13 sampling times at 5, 10, 30, 60, 65, 70, 90, 120, 150, 155, 160, 165 and 180 min. The design was tested with and without lower and upper constraints on sampling times (0, 200 and 5, 180). Variability for the PK parameter estimates was simulated between 20–30%. The proposed study involves changes in the infusion rate of the administered drugs and the use of a propofol and remifentanyl. To simplify the ability to generate an optimal design for this study, propofol and remifentanyl were evaluated separately. This study focuses on the optimal design and development of sampling times for propofol. Initially, the design used propofol at 450 µg/kg/min and subsequently at 75 µg/kg/min. The design simulated administration of a propofol infusion at time 0 and a change in infusion rate at 65 min. In the propofol baseline design, the following PK parameters were utilized: V_1 8.91 (L), K10 0.86, K12 0.39, K21 0.21, K13 0.1 and K31 0.005 min^{-1} . These were a combination of estimates from the sheep model and pediatric models.

The baseline design performed adequately, which was defined based on percent Standard Error of the mean (SE%) less than 50% for the fixed effects parameters, designs with SE% over 50% for the fixed effects parameters were considered poor designs. In subsequent tested designs, the PK parameters were adjusted to obtain information related to uncertainty in the parameter estimates and the plausibility of the proposed design strategy. Variability for the PK parameter estimates was simulated to be 20%. In further evaluation of the optimal design strategies, sampling times were optimized to obtain 9 sample times, using the

following initial times: 5, 10, 30, 60, 70, 90, 120, 150, and 180 min. The final PK parameters utilized were: V_1 8.91 (L), K_{10} 0.86, K_{12} 0.31, K_{21} 0.15, K_{13} 0.24 and K_{31} 0.2 min⁻¹.

Proposed Designs to Optimize Sampling Times

In subsequent tested designs, the sampling times and number of required samples were adjusted to determine the minimum number of samples that would need to be obtained to provide an effective and efficient PK sampling profile. Designs investigated obtaining between 7–9 sample time points. Table 2 provides the final proposed sparse sampling times using 9 time points which was the design used to obtain the samples for the initial analysis to assess the PK of propofol (Fig. 1).

INITIAL ANALYSIS

Prism 5 (version 5.04, GraphPad software, San Diego, CA) was used for the statistical analysis. We used to assess the PK of propofol for the maternal/sheep pairs administered propofol infusion dose of 450 mcg/kg/min for 60 min then 75 mcg/kg/min for 60 min. Sheep used in the study had a body weight range of 65 to 78 kg, mean 72.7 kg. Gestation of the fetus when propofol concentrations were obtained ranged from 112 to 118 days, mean 115.3 days.

Propofol concentration data were obtained from three sheep in the study to evaluate the study design and the use of the sampling times proposed in the optimal design (Table 3). The mean $\pm$ SD propofol concentration in the ewes was 4.37 $\pm$ 3.05 mcg/mL and 0.61 $\pm$ 0.37 mcg/mL in the fetuses. Using Phoenix® WinNonlin (version 6.2) a non-compartmental analysis calculated area under the blood concentration-time curve (AUC) for the ewes had a mean $\pm$ SD of 768.3 $\pm$ 146.1 min*mcg/mL and for the fetuses 110.7 $\pm$ 21.89 min*mcg/mL. In the samples obtained from the ewes, C_{max} mean $\pm$ SD was 9.33 $\pm$ 0.98 mcg/mL and for fetuses 1.2 $\pm$ 0.32 mcg/mL. The mean $\pm$ SD half-life in the ewes was 0.64 $\pm$ 0.03 h and in the fetus 1.23 $\pm$ 0.26 h.

Optimal design under predicted peak plasma concentration was most likely due to overly conservative values used for variance in the model parameters. However, PK curve obtained from the ewes and fetuses has a similar shape to the simulated in the optimal design (Fig. 2). The sample times proposed in the optimal design and used to obtain the propofol concentrations in the sheep appear to have provided reasonable times to obtain parameter estimates.

DISCUSSION

In preliminary study of propofol pharmacokinetics in fetal-maternal sheep model, we demonstrated that the use of D-optimal design for selection of sampling time points provided accurate estimation of PK parameters and reduced the number of required blood samples. The choice of design was based on clinical practicality and ease to implement under clinical conditions. For clinical convenience the proposed sampling times have been rounded to the nearest 5–10 mins (Table 2, Fig. 1). Sampling at these times as proposed by the model will yield a design that is clinically attainable and provides reasonable estimates

for the PK parameters of interest. Sampling times have been constricted to between 5–180 minutes in the final proposed design strategy. It is suggested from a practical point of view that the smallest allowable time between consecutive sampling times should be fixed at 10 minutes.

It has been outlined that the precision of the estimated parameters in PK/PD studies is correlated with sampling design [6]. This relationship has led to the development of design metrics, which can provide the optimal design or determine the most precise parameter estimates.[6]. Optimal design methods have gained more application in the past 10 years with improvement in technology and have been utilized in designing many population PK studies [3–5, 19, 20].

The minimum number of samples in the design that allowed convergence to be achieved was with 7 sample time points. However, due to the complexity of the dosing strategy of the study, which includes changes in infusions rates, dose and intervals, 9 sample time points were chosen. ADAPT was used to evaluate the design as it can be used to perform a single subject analysis. The design requires more samples than parameters, to provide good estimates for the fixed effects in the design. Bayesian methods can be very useful to incorporate *a priori* knowledge when the PK of the population under investigation is unique or only limited data are available. Optimal sampling strategies can be constructed using either the D-or C-optimality criterion. It has been outlined that the precision of the estimated parameters in PK/PD studies is correlated with sampling design [6]. The D-optimality criterion selects sampling designs minimizing the determinant of the variance-covariance matrix[6]. An optimization algorithm searches for the D-optimal design as defined by the constraints of the study design[21].

Due to the nature of PK and PD modeling, often models and parameter estimates are not well defined due to the influence of unknown physiological mechanisms and large unexplained variability in the population estimates. In this situation, construction of a design using D-optimality will have difficulty providing useful designs [6]. D-optimality does not account for the accuracy of a parameter estimate.

To assess the performance of the design and sampling times and to determine if there would be a need to modify the design should be evaluated early in the study. Although the proposed sampling design is expected to result in adequate PK estimates, it is suggested that an interim PK analysis following the availability of concentration data from a minimum of three sheep should be performed. Individual Bayesian PK estimates should be derived using the data and concentration-time profiles used to evaluate the study design performance. Assessment of the PK parameters as predicted by the concentration-time profiles will help to determine safety and whether dose or sampling time adjustment is necessary. Computer simulation can be used to explore the accuracy of the parameter estimation and be compared to those estimates produced by the estimation procedure [6].

CONCLUSION

The optimal study design and proposed sampling times were initially evaluated from propofol PK obtained from three ewes and fetuses. Many prediction strategies have been developed to get the most accurate PK parameter estimates when obtaining a limited number of samples. These strategies aim to maximize cost effectiveness by collecting samples at the optimum time to get the most precise and accurate estimation of PK parameters. This study demonstrated that the use of D-optimal design to optimize the sampling times in a study evaluating administration of a drug with changing infusion rates would lead to reduce cost and easier study implementation. Application of optimal design methods has great potential in both research and clinical practice to optimize the precision and time of sample collection and to implement studies under clinical conditions.

Acknowledgments

The authors would like to acknowledge financial support from the following NIH grants 5T32AR007594-15(CMTS); 1K24HD050387-05 (AAV) and FAER grant RFG-02/15/2010(PN).

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Fig. (1).
The final sparse sampling time to obtain blood samples for propofol pharmacokinetics study. D-Optimal design using lower (5 min) and upper (180 min) constraints propofol model optimized the design to nine sample time points. Each square represents a sampling time point. The Y-axis is propofol concentration and the X-axis is time.

Fig. (2).
Results of propofol concentrations from three ewes and three fetuses using the optimal study design time points (grey dashed line) as outlined in Table 2 and Fig. (1).

Table 1

A priori Sheep Propofol PK Estimates from a Two Compartment Model in Sheep (Based on 450 mg/45 min Administered Dose) [17]

PK Parameter	Estimates (Mean $\pm$ SD)
Volume of distribution (V_1) (L)	8.9 ± 3.35
Elimination rate constant (K_{el}) (min^{-1})	0.86 ± 0.32
Rate of distribution between central and second compartment (K_{12}) (min^{-1})	0.39 ± 0.33
Rate of distribution between second and central compartment K_{21} (min^{-1})	0.2 ± 0.12
Rate of distribution between central and brain compartment (K_{eo}) (min^{-1})	0.11 ± 0.006

Table 2

Final Proposed Sampling Times for Propofol Pharmacokinetics in Pregnant Sheep, using 9 Sample Time Points

Sample Time	Initial value (min)	Final Sampling Time (min)
Pre-infusion		
1	5	5
2	10	15
3	30	25
4	60	65*
5	70	75**
6	90	100
7	120	110
8	150	150
9	180	180

Note: Change in infusion rate would need to occur between these two sampling times (*, **)

Table 3

Evaluation of Mean Data Obtained from the Maternal Fetal Sheep Model Compared to Simulated Optimal Design Predicted Values

Sample Time	Means from Ewes (n=3) Fetuses (n=3)	SD	SEM	Optimal Design Predicted Values
5	2.95	2.88	1.17	2.9
15	2.79	3.23	1.32	3.5
25	4.67	4.10	1.67	3.8
65*	5.17	4.42	1.80	2.9
75**	2.25	1.78	0.72	2.1
100	1.56	1.36	0.55	1.5
110	1.40	1.15	0.47	1.0
150	1.19	0.96	0.39	0.98
180	0.35	0.18	0.07	0.97