The pharmacokinetics of propofol in greyhounds and mixed breed dogs

by

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EXPLANATION OF THESIS FORMAT

This thesis is presented in the alternate thesis format as defined in the Graduate College Thesis Manual of Iowa State University. There is one paper written as Part I of this thesis which is entitled "The pharmacokinetics of propofol in greyhounds and mixed breed dogs." This paper will be submitted to the American Journal of Veterinary Research. The paper has its own introduction, materials and methods, results, discussion, and reference sections, and its own figures and tables. The placement of the figures and tables in Part I follows the guidelines of the journal. I am the primary author of this publication.

The other sections of this thesis are included to expand upon the information contained in Part I and to present additional data not included in the paper. The literature review contains pertinent information on the pharmacology of propofol in both humans (where the majority of the research and clinical information exists) and companion animals. It provides a basis for the importance of propofol as an alternative anesthetic and also lays the groundwork for the comparisons of greyhounds and mixed breed dogs in their responses to general anesthetics. The importance of pharmacokinetics in the understanding of anesthetics and their most appropriate usage is also discussed. The appendix provides a more detailed description of the methods used in

the analysis of blood samples by high performance liquid chromatography (HPLC) than is presented in the paper. The references listed at the end of the thesis represent those references used in the general introduction, literature review and general summary sections and are separate from the references listed in Part I.

GENERAL INTRODUCTION

The use of intravenous anesthetic agents was introduced in 1934 by two physicians who, working separately, used the barbiturate thiopental to induce general anesthesia in human patients (Dundee 1980). It wasn't until the mid-1950s that the barbiturate anesthetics thiamylal and methohexital were developed. Since that time, numerous studies have described the pharmacokinetics, pharmacodynamics and clinical characteristics of barbiturates in humans and animals. Because of their effectiveness and relative safety, barbiturates became the standard for intravenous anesthesia. However, the search for alternative intravenous anesthetic agents persisted in an attempt to discover the "ideal" anesthetic. In the late 1950s, ketamine, the first of a number of new anesthetics emerged. Several drugs from that group of anesthetics are still available for general use, and include diazepam (1964), etomidate (1973), propofol (1977) and midazolam (1978) (Fragen 1988). Despite the development of these new anesthetics, the barbiturates have remained the standard to which all other intravenous anesthetics are compared.

An ideal intravenous anesthetic agent has been described as an agent which possesses the following qualities: 1) the drug should be water soluble, non-irritating, stable in aqueous solution, and possess a long shelf life, 2) it should

produce rapid (one arm-brain circulation time i.e. less than 60 seconds) and smooth onset of hypnosis without cardiopulmonary depression, 3) it should possess both analgesic and amnestic properties in addition to inducing hypnosis, and 4) it should result in rapid and smooth recovery (return of consciousness) due to redistribution and biotransformation of the drug (Dundee 1980). Propofol is not the ideal anesthetic, but it has many characteristics that make it an excellent alternative intravenous anesthetic agent. Propofol's desirable characteristics include a rapid and smooth onset of hypnosis (sleep) with amnesia, a lack of antanalgesia, a rapid and smooth recovery, and it forms a stable solution with a long shelf life (Reves and Glass 1990). These characteristics have stimulated an intense interest in propofol in both human and veterinary anesthesiology.

One of the problems with thiobarbiturate anesthetics, and especially thiopental and thiamylal, has been the often unpredictable recovery of patients following its administration (Stoelting 1990). The prolonged recovery, or "hangover," that may occur following thiopental anesthesia in humans is especially apparent in situations where rapid, complete recovery from anesthesia is desired (e.g. outpatient medical or surgical procedures). In veterinary patients, the problem of unpredictable recoveries following barbiturate anesthesia is also apparent. This problem is amplified in dogs and cats by the fact that general anesthesia is often

required in situations where human patients may traditionally not be anesthetized. Examples of such situations include dental prophylaxis procedures, specialized radiographic procedures (i.e. skull or vertebral column radiographs, contrast radiography of the urinary tract or colon, etc), or specialized diagnostic procedures such as some ultrasound examinations, biopsy procedures or endoscopic examinations. Thus, in human and veterinary medicine, an anesthetic that is characterized by both a rapid, smooth onset of anesthesia, and also a rapid and predictable recovery would be a particularly useful drug. If propofol has the pharmacodynamic and pharmacokinetic properties in animals that it has in humans, it will be an important new anesthetic in veterinary practice.

In order to fully understand how anesthetic drugs act, it is essential to appreciate the disposition of those drugs in the body. Pharmacokinetics is the quantitative study of the absorption, distribution, metabolism and excretion of injected (or inhaled, swallowed, etc) drugs and their metabolites (Hull 1979). Stated more simply, pharmacokinetics describe the disposition of a drug in the body over the course of time. The practical importance of this information, is to help the clinician: 1) understand dose-effect relationships with respect to onset, intensity, and duration of drug action, 2) recognize dispositional factors as a cause of variability in the responses to a given dose, 3) predict the consequences of different dosage regimens and to design more efficient ones

that are optimally effective with minimal side-effects and toxicity, and 4) design comparative investigations of the potency and efficacy of drugs (Prys-Roberts and Hug 1984). Therefore, determination of the pharmacokinetics of a drug is an especially important step in the development of rational and appropriate drug dosage regimens. In addition, the knowledge of the pharmacokinetics of the drug in the normal animal also provides a basis for comparison of that drug and its interactions with other agents and its effects in diseased, young or old animals.

Propofol was first made available for human use in the United Kingdom in 1986, and is marketed under the trade name Diprivan[1]. The drug was not approved for general use in the United States until 1989. Propofol has been widely studied in humans, both in clinical trials and in pharmacokinetic and pharmacodynamic studies (Adam et al. 1983, Cockshott 1985, Cockshott et al. 1987, White 1988a, Sebel and Lowdon 1989, and Kanto and Gepts 1989). Several clinical trials using propofol in dogs and cats have been conducted in the United Kingdom (Watkins et al. 1987, Morgan and Legge 1989, Brearley et al. 1988, Hall and Chambers 1987); however, only one report of the pharmacokinetics of propofol in animals has been published (Adam et al. 1980). The clinical studies that have been performed have begun to broaden our understanding of the

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[1]Diprivan®, Stuart Pharmaceuticals, Wilmington, DE

effects of propofol in companion animals; however, in order to understand the disposition of propofol in dogs and cats, pharmacokinetic studies in those species must be completed.

The purpose of this research was first to describe the pharmacokinetics of propofol in clinically normal mixed breed dogs. The determination of the pharmacokinetics of propofol in normal dogs not only provides information that will assist veterinarians in the most appropriate dosage regimens, but will also provide a basis for further evaluation of propofol. A second goal of this research was to determine the pharmacokinetics of propofol in greyhound dogs. Greyhounds were included in this research project as a separate group because they respond to intravenous anesthetics, and particularly the thiobarbiturates, much differently than mixed breed or other non-sight hound breed dogs due to their lean body mass and unique drug metabolism (Sams et al. 1985). Because of the unusual pharmacokinetic profile of thiobarbiturates in greyhounds, the use of these drugs in greyhounds is not recommended. Like the thiobarbiturates, propofol is an extremely lipid soluble drug, and thus it would be expected to rapidly redistribute to muscle and fat during the distribution phase, and then be slowly eliminated from the deeper compartments via hepatic metabolism. However, in human pharmacokinetic studies, propofol is more rapidly eliminated via biotransformation and elimination processes than are the thiobarbiturates (Sebel and Lowdon 1989, Kanto

and Gepts 1989). Thus, if the pharmacokinetics of propofol in greyhounds are similar to the kinetics in humans, it may be a safe intravenous anesthetic agent to use in greyhounds. The determination of the pharmacokinetics of propofol in both mixed breed and greyhound dogs will provide important information on the most appropriate uses of propofol as an anesthetic in dogs. Finally, this investigation will lay the groundwork for additional studies of the effects of propofol in diseased, pregnant, pediatric or geriatric patients, which will be necessary if propofol is to be safely used as an alternative intravenous anesthetic agent in general practice.

LITERATURE REVIEW

Propofol (2,6 diisopropylphenol) is a substituted phenol derived from a series of alkylphenols which have been found to have anesthetic properties in animals (James and Glen 1980). This drug is chemically unrelated to any of the other currently used intravenous anesthetic agents including barbiturates, imidazoles, benzodiazepines or phencyclidenes (Langley and Heel 1988, Sebel and Lowdon 1989). Propofol was developed in 1973 at Imperial Chemical Industries Pharmaceuticals in Great Britain (Fragen 1988), and was originally known as ICI 35 868, or disoprofol, but now is marketed for human use under the trade name Diprivan®. In addition to its human label, propofol is also marketed in Great Britain under the veterinary label of Rapinovet[2]. Pure propofol is a transparent, pale, straw-colored liquid that has limited water solubility but is highly lipid soluble (Shafer and Stanski 1991). Because of propofol's virtual insolubility in aqueous solution, it was originally formulated as 1% (weight/volume) propofol in a 16% solution of the surfactant Cremophor EL (Glen 1980). The Cremophor formulation of propofol was not marketed for several reasons. One of the biggest problems associated with the use of Cremophor EL was the occurrence of anaphylaxis in humans

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[2]Rapinovet®, Cooper's Animal Health, Hertfordshire, UK

(Clarke et al. 1975, Dye and Watkins 1980, Briggs et al. 1982). In dogs, the Cremophor formulation induced marked increases in plasma histamine levels and the accompanying clinical signs of cutaneous hyperemia, salivation, lacrimation, and defecation were also observed (Glen and Hunter 1984). An additional concern in both humans and dogs, was the high incidence of pain that occurred following an intravenous injection of drugs combined with Cremophor (Glen 1980, Briggs et al. 1982, Glen and Hunter 1984). Thus, investigators began work on a new delivery form for the drug and in 1983, an emulsion formulation consisting of propofol in 10% soybean oil, 2.25% glycerol, and 1.2% purified egg phosphatide was released for clinical trials. The emulsion is identical to Intralipid[3], the commercial product used as a source of fat for total parenteral nutrition. Propofol in this emulsion is packaged in single use, sterile glass ampules to reduce the risk of bacterial contamination. The emulsion formulation was found to be slightly less potent than the original Cremophor EL formulation, but otherwise produced identical anesthetic and hemodynamic responses in the laboratory animals tested (Glen and Hunter 1984). More importantly, the emulsion formulation did not induce histamine release in dogs and was not associated with anaphylaxis in any of the laboratory animals tested. Following numerous clinical

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[3]Intralipid®, Kabivitrum Inc., Clayton, NC

trials in both humans and companion animals, propofol in the 10% Intralipid® emulsion was approved for human use in the United Kingdom in 1986 (Fragen 1988). Clinical trials of propofol in humans were also initiated in the United States in 1984 following the development of the emulsion formulation, but FDA approval was not received until 1989 (Shafer and Stanski 1991).

The pharmacologic characteristics of propofol in the Cremophor formulation were initially described in 1980 by Glen in a variety of laboratory animals, including rats, mice, rabbits, guinea pigs, monkeys, and cats. After propofol was reformulated with the Intralipid® emulsion, the pharmacology of the drug was redescribed by Glen and Hunter (1984) in laboratory animals. The remainder of this thesis will discuss only the emulsion formulation, unless otherwise specified.

Propofol is a sedative, hypnotic anesthetic which produces dose-dependent depression of central nervous system function in a manner similar to the barbiturates and benzodiazepines (White 1988a, Reves and Glass 1990). The onset of anesthesia following intravenous injection of propofol is very rapid (one arm-brain circulation time, i.e. less than 60 seconds) and is not significantly different from that produced by thiopental (White 1988b, Sung et al. 1988, Mackenzie and Grant 1985, Fragen and Shanks 1988). In humans, the speed of injection of propofol has been found to affect its induction characteristics. In a study by Rolly and

coworkers (1985), an increase in the duration of the injection time from 5 to 60 seconds resulted in an increase in induction time from 21 to 50 seconds. However, the speed of induction of anesthesia with propofol did not affect the depth of anesthesia that was attained. In subhypnotic doses, propofol will provide sedation, the depth of which can be controlled by altering the amount of propofol infused (MacKenzie and Grant 1987). Like most non-opioid intravenous anesthetics, propofol does not produce reliable analgesia in subanesthetic doses. However, in contrast to the thiobarbiturates, propofol does not produce an antanalgesic effect (Langley and Heel 1988). Thus, although propofol has no analgesic properties per se, it is superior to thiopental in this respect because it does not increase sensitivity to somatic pain. The sleeping time of propofol in humans is very similar to thiopental; however, in most studies a significant improvement in the recovery characteristics (i.e., less drowsiness with a more rapid return to alertness and clearheadedness, and fewer postoperative sequelae such as headache, nausea/emesis, etc) was observed with propofol (Mackenzie and Grant 1985, Sung et al. 1988, Perry et al. 1988). In addition, the majority of human patients that underwent these trials reported a more satisfactory anesthetic experience than those who had thiopental anesthesia. Since outpatient anesthesia has become increasingly important in human anesthetic practice, the improved recovery characteristics produced by propofol provide

anesthesiologists with an important alternative to other intravenous anesthetic agents. Direct comparisons of the characteristics of thiopental and propofol in dogs or cats have not yet been reported. In clinical trials conducted in dogs and cats, investigators reported that animals recovering from propofol anesthesia awakened in a shorter period of time and were subjectively more bright and alert, than with other anesthetic agents (Watkins et al. 1987, Morgan and Legge 1989, Brearley et al. 1988).

The cardiovascular effects of propofol anesthesia are characterized primarily by a dose-related decrease in mean arterial blood pressure during induction. In normal humans, the decreased blood pressure ranged between 25 and 40 percent (Claeys et al. 1988, Grounds et al. 1985). The decrease in mean arterial blood pressure has been reported to be due to both vasodilation and myocardial depression; however, which of these effects is most important remains controversial (Lippmann et al. 1988, Claeys et al. 1988, Sebel and Lowdon 1989, Reves and Glass 1990, Merin 1990). Some investigators have reported a decreased systemic vascular resistance resulting in a reduced preload as an explanation of the decreased blood pressure (Grounds et al. 1985, Claeys et al. 1988). Other groups report that the decreased mean arterial blood pressure was due to decreased cardiac output, cardiac index, and the stroke volume index as a result of a direct myocardial depressant effect (Lippmann et al. 1988, Stephan et

al. 1986). In a recent study by Lepage and coworkers (1991), propofol was found to decrease preload and cardiac index, but a negative inotropic effect could not be demonstrated and left ventricular performance (ejection fraction) was preserved. In dogs, only one study has been reported to date on the cardiovascular effects of propofol. In that study, Goodchild and Serrao (1989) found that the decrease in mean arterial blood pressure was due to venodilation from the direct effect of propofol on the peripheral vessels, and was not associated with decreases in systemic vascular resistance or cardiac output. Thus, despite the controversy as to the exact cause of the decrease in blood pressure, it is important to recognize that propofol should be used cautiously in patients with cardiac dysfunction. Propofol apparently has no consistent effect on heart rate in either humans or dogs (Watkins et al. 1987, Sebel and Lowdon 1989, Weaver and Raptopoulos 1990, Reves and Glass 1990, Lepage et al. 1991).

Propofol is a respiratory depressant, as are many other intravenous anesthetics. In humans, apnea of greater than 30 seconds duration has been reported in numerous clinical studies (Sebel and Lowdon 1989, White 1988a, Reves and Glass 1990, Shafer and Stanski 1991). However, the incidence of apnea in humans following an intravenous injection of propofol is comparable to that produced by barbiturates (Mackenzie and Grant 1985, White 1988a, Sung et al. 1988, Langley and Heel 1988). In dogs, the mean respiratory rates

and the incidence of apnea were variable following propofol anesthesia, but were not considered to be significantly different than the respiratory effects observed following thiobarbiturate anesthesia (Watkins et al. 1987, Weaver and Raptopoulos 1990).

In humans, numerous studies evaluating the effects of propofol on different body systems have been reported. These include the effects of propofol on intracranial pressure, adrenocortical function, hematologic parameters, and immune system function (Fragen et al. 1987, Sear et al. 1985, Doenicke et al. 1985, Ravussin et al. 1988). In addition, the effects of propofol in combination with other anesthetic agents or drugs has been a major area of research focus. However, the study by Perry and coworkers (1991) on the effects of propofol anesthesia on propranolol pharmacokinetics is the only research in dogs reported to date. Thus, a great deal of information concerning propofol's pharmacologic characteristics in dogs and other companion animals remains to be described.

The release of propofol in the emulsion formulation for clinical trials in 1983 was followed by numerous reports of its pharmacokinetic properties in humans (Adam et al. 1983, Schuttler et al. 1985, Kay et al. 1985, Kirkpatrick et al. 1988, Jones et al. 1990, Gin et al. 1990). The pharmacokinetic profile of propofol in humans has been most frequently described by a three compartment open model, which

describes the disposition of propofol as the sum of three exponentials ($C_p = Ae^{-\alpha(t)} + Be^{-\beta(t)} + Ce^{-\gamma(t)}$). The three exponential components of the equation can be broken down into three phases that describe the drug's distribution and elimination. In humans, the first phase ($Ae^{-\alpha(t)}$) consists of the rapid, initial distribution phase that lasts two to four minutes (Kanto and Gepts 1989). The second phase ($Be^{-\beta(t)}$) describes the rapid elimination phase that is characterized by the rapid metabolism and disappearance of propofol from blood. The final or slow elimination phase ($Ce^{-\gamma(t)}$), represents the gradual return of propofol from the peripheral tissue compartments (i.e., fat) and its subsequent biotransformation and elimination. The elimination half-life in humans has been reported to range from three to five hours (Langley and Heel 1988, Kanto and Gepts 1989).

The uniqueness of propofol pharmacokinetics is particularly apparent when they are compared to the kinetics of the thiobarbiturates. The disposition of thiopental in humans was also best described by a three compartment open model; however, the elimination half-life of thiopental was much longer, ranging from 5.1-11.5 hours (Stanski 1984). Several derived pharmacokinetic parameters also illustrate important differences in the two agents. The apparent volume of distribution (Vd) of both propofol and thiopental was large due to the high lipid solubility of both agents. However, the Vd of propofol was 7.6 liters, while the Vd of thiopental was

5.5 liters (Shafer and Stanski 1991). The Vd at steady state [Vd(ss)] for propofol, which is the most accurate term describing the distribution of a drug in the body, ranges from 1.68 to 15.7 liters/kg (Kirkpatrick et al. 1988, Jones et al. 1990). For thiopental, the Vd(ss) was 1.5-3.3 liters/kg (Stanski 1984). The clearance of propofol, which represents the term for drug elimination, has been reported to range from 1.6 to 2.55 liters/min (Schuttler et al. 1985, Cockshott et al. 1987, Gill et al. 1990, Gin et al. 1990). Thiopental clearance ranges between 0.11 to 0.30 liters/min (Stanski 1984). Thus, by examining the differences in the pharmacokinetics of propofol and thiopental in humans, the important differences in the clinical characteristics become apparent. Both drugs are widely distributed, producing both a rapid onset of anesthesia and rapid return of consciousness from the decline in brain drug levels due to redistribution. Even though propofol has a shorter elimination half-life than thiopental, both drugs have relatively long elimination times because of their widespead distribution to muscle and fat. The rapid clearance of propofol due to hepatic and possibly extrahepatic metabolism (Cockshott 1985, Langley and Heel 1988, Kanto and Gepts 1989) is the primary reason that patients recover from propofol anesthesia more rapidly and completely than from thiopental anesthesia.

The pharmacokinetics of propofol in laboratory animals were originally described in 1980 by Adam and coworkers. The

disposition of propofol in the Cremophor EL formulation was described by a two compartment open model for rats, rabbits, pigs and cats (Adam et al. 1980). The concentration versus time graphs from each group of animals was relatively similar, but there were some differences in the derived pharmacokinetic parameters. In cats, the volume of distribution was approximately one-half that of rats and pigs (133% of body weight versus 250% and 275%, respectively), while the elimination half-life was nearly three times as long as that for rats or rabbits (55 min vs. 19 and 16 min, respectively). There were also differences in the concentration at which the animals returned to wakefulness. Rabbits and cats awoke at much higher concentrations of propofol, 7.2 and 4.3 ug/ml respectively, than did rats or pigs (2.8 and 1.1 ug/ml, respectively). With the exception of the rabbit, a blood concentration of 1-4 ug/ml was effective in producing unconsciousness in laboratory animals (Adam et al. 1980). The rapid onset of action was attributed to the lipophilic nature of propofol which allowed the widespread distribution of the drug to well perfused tissues, which includes the brain. The rapid distribution phase of propofol in laboratory animals also accounted for its extremely short duration of action, as the drop in blood concentrations in this phase was associated with the return of consciousness and recovery. The terminal elimination half-life was reported to range from 16-55 minutes, and, in pigs, did not change appreciably after

repeated injections. Several implications were derived on the basis of these findings, including injection of propofol to induce anesthesia must be rapid enough to compensate for the rapid distribution from the brain to the remainder of the body, and recovery will be rapid due to the extremely short elimination half-life and high clearance values (Adam et al. 1980). The duration of sampling times for the determination of propofol pharmacokinetics was only two hours post dosing, thus it is difficult to compare these results to those obtained for human patients. The pharmacokinetics of propofol in other domestic species have not been reported.

The sight hound breeds, which include greyhounds, have often posed problems to the anesthesiologist because of their unique metabolism and anatomical characteristics. One of the most striking examples of the differences between greyhounds and mixed breed dogs is illustrated in their respective pharmacokinetic profiles for thiobarbiturates (Sams et al. 1985). In the study by Sams and coworkers, the disposition of thiopental in mixed breed dogs was characterized by a Vd of 1,359 ml/kg, a clearance of 1.96 ml/min/kg and an elimination half-life of 10.7 hours. However, in greyhounds, the pharmacokinetic values could not be calculated because the thiopental concentration did not decrease exponentially from 45 to 480 minutes (Sams et al. 1985). At 480 minutes, the plasma thiopental concentrations in greyhounds were 9.99 +/- 2.75 ug/ml, which were significantly higher than the plasma

thiopental concentrations in mixed breed dogs at that time (3.42 +/- 1.69 ug/ml). Thus, greyhounds recovered from thiopental anesthesia at significantly higher plasma concentrations of the drug, and also had significantly longer sleeping times than mixed breed dogs. The explanation for the differences in the disposition of thiopental between greyhounds and mixed breed dogs is not known. However, in greyhounds, a reduction in available body fat which thereby reduces the available tissue for redistribution of the drug, or a possible saturation of metabolic clearance mechanisms are postulated as causes for the differences. Because of these drawbacks, the use of thiobarbiturates in greyhounds is not recommended; therefore, other drug regimens have been used for induction of anesthesia in this breed of dog. Propofol is an entirely different chemical compound than the barbiturates. If the pharmacokinetics of propofol in greyhounds are similar to those in mixed breed dogs, it would provide veterinary anesthesiologists with an important new anesthetic for use in the sight hound breeds.

PART I. THE PHARMACOKINETICS OF PROPOFOL IN MIXED BREED AND GREYHOUND DOGS

The pharmacokinetics of propofol in mixed breed and greyhound dogs

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SUMMARY

The pharmacokinetics and recovery characteristics of propofol in greyhounds and mixed breed dogs were compared. In all dogs, the disposition of propofol was adequately described by a two compartment open model, with a rapid distribution phase followed by a slower elimination phase. When greyhounds were compared to mixed breed dogs, significant differences were observed in the whole blood propofol concentration means, recovery characteristics, and the specific parameter estimates for apparent volume of distribution (Vd), volume of distribution at steady state [Vd(ss)], and total body clearance (Cl_b). In addition, greyhounds recovered from anesthesia at higher propofol concentrations than did mixed breed dogs. A secondary peak in the whole blood propofol concentration was noted in 8 of 10 greyhounds and 5 of 8 mixed breed dogs. This peak corresponded to the time of the return of the righting reflex.

INTRODUCTION

Propofol (2,6 diisopropylphenol) is an intravenous hypnotic and sedative anesthetic developed from a series of alkylphenols which have anesthetic properties in animals (James and Glen 1980). The drug was previously known as disoprofol, or by its company name ICI 35 868. Propofol is currently marketed for human use under the trade name of Diprivan[1]. The drug has very limited solubility in aqueous solution, and as a result, was initially formulated in the surfactant Cremophor EL (Glen 1980). However, the Cremophor formulation was never marketed because it was associated with anaphylactic reactions in humans (Briggs et al. 1982). In addition, Cremophor was associated with increased histamine release in dogs and guinea pigs, and a high incidence of pain following intravenous injection (Glen and Hunter 1984). In 1983, propofol was reformulated in a 10% Intralipid[2] emulsion and released for clinical trials in humans. Following numerous clinical, pharmacokinetic, and pharmacodynamic studies in humans, propofol was released for human use in the United States in 1989.

Propofol has been used for intravenous anesthesia in dogs and cats in several clinical trials in the United Kingdom

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[1]Diprivan®, Stuart Pharmaceuticals, Wilmington, DE

[2]Intralipid®, Kabivitrum Inc., Clayton, NC

(Watkins et al. 1987, Hall and Chambers 1987, Brearley et al. 1988, Morgan and Legge 1989, Weaver and Raptopoulos 1990). These studies reported on the induction and recovery characteristics of propofol; its effects on heart rate, blood gases and respiration; and provided dosing guidelines. However, to date, little or no information exists in the literature concerning the pharmacokinetics of propofol in dogs or cats. The purpose of this research is to determine the pharmacokinetic profile of propofol in mixed breed and greyhound dogs. Greyhound dogs were included in this study as a separate group because of their unique responses to anesthetic agents, and to thiobarbiturates in particular (Sams et al. 1985).

MATERIALS AND METHODS

Animals and Experimental Design

This study was approved by the animal care and use committee of the university. A total of eighteen dogs were included in this research. The dogs were divided into two groups consisting of ten greyhounds (GH) and eight, non-greyhound, mixed breed dogs (MD). The dogs were all adults of various ages and sexes (GH = 6 males, 4 females; MD = 5 males, 3 females). The greyhounds weighed between 24.0 and 40.5 kg (mean = 32.7 kg), while the mixed breed dogs weighed from 5.0 to 28.6 kg (mean = 13.3 kg). Dogs were housed in standard kennels and fed a diet of commercial dry dog food with free choice water. The dogs were vaccinated and dewormed two weeks prior to the study. All dogs were evaluated by a pre-study physical examination and hematologic profile prior to their inclusion in the study. Sample collection was performed over a three week experimental period, with the exception of two of the mixed breed dogs. Food, but not water was withheld during the 12 hours preceding the experiment. On the day of each experiment, a cephalic vein was percutaneously catheterized, using a 20 gauge needle and over the needle catheter[3]. The cephalic catheter was used to facilitate the intravenous injection of propofol. Additionally, one of the

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[3]Wingless Quik Cath®, Baxter Healthcare, Deerfield, IL

jugular vein regions was clipped and surgically scrubbed. The jugular vein was percutaneously catheterized with a 14 or 16 gauge needle and silicone elastomer catheter[4], and then the catheter was secured with skin ligatures and wrapped to protect the catheter site. Following the initial preparations, a 4 ml control blood sample was withdrawn from the jugular catheter, and all subsequent blood samples were withdrawn from the jugular catheter. Patency of each catheter was maintained by flushing with 0.9% saline or heparinized saline as needed.

Propofol (Diprivan®) was obtained from a commercial source. One vial of propofol (20 ml, 10 mg/ml) was used per dog to prevent contamination and possible sepsis, due to propofol's lipid emulsion carrier. The dose of propofol (5 mg/kg) was administered, intravenously, to each dog over a one minute time period. This dosage was based upon personal experience and previous clinical trials in which the induction doses of propofol in unpremedicated dogs was reported (Watkins et al. 1987, Weaver and Raptopoulos 1990). Additional propofol was made available if a larger dose of propofol was needed to induce anesthesia to the level of intubation. Immediately following the propofol injection, the dog was intubated and allowed to breathe 100% oxygen. The depth of anesthesia for each dog was assessed by the degree of

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[4]Centrasil®, Baxter Healthcare Co., Deerfield, IL

relaxation, toe pinch withdrawl, and palpebral reflex. The duration of anesthesia was recorded according to the time of extubation, time to return of the righting reflex (return to sternal position), and the time to standing unassisted.

Because propofol is intimately associated with the formed elements of blood, whole blood is considered best for pharmacokinetic analysis (Plummer 1987). For determination of whole blood propofol concentrations, four milliliter blood samples were collected into a 7 ml glass vacutainer[5] tube containing potassium oxalate. The sampling times began before drug administration (time zero), and then were continued at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, 720, and 1440 minutes post-injection. A waste sample was first collected from the jugular catheter prior to withdrawing the timed sample for analysis. The catheter was flushed with 4 ml of 0.9% saline following sample collection for replacement of blood volume. Blood samples were immediately placed on ice in a covered container until they could be refrigerated (4 degrees Celsius) while awaiting analysis. When stored in whole blood at 4 degrees C, the concentration of propofol has been found to be stable for 12 to 18 weeks (Plummer 1987, Shafer et al. 1988).

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[5]Vacutainer®, Becton Dickinson, Rutherford, NJ

Determination of Blood Propofol Concentrations

The propofol concentration in oxalated whole blood samples was determined by a modification of the method described by Plummer (1987). The reader is referred to the thesis appendix for complete details of the analytical method used in this study. One of the modifications to the procedure included filtering the samples prior to analysis, which was performed after reconstitution of the dried extract with 250 ul of mobile phase. The samples were filtered through a 0.45 micron pore, nitrocellulose membrane filter[6] via centrifugation at 1000 rpm for five minutes. A 100 ul sample of the filtered extract was then analyzed by high performance liquid chromatography (HPLC). Propofol was detected by ultraviolet light at a wavelength of 276 nm. The limit of detection for this method was approximately 10 ng ml^{-1}. Propofol standards were prepared from pure propofol[7] stock solutions and propofol free whole dog blood to produce standards containing 0.025, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 ug/ml of propofol per milliliter of blood. The standards were extracted in the same manner as were unknown samples. The standards were divided into two groups to form a standard curve for each absorbance units full scale (AUFS) setting used for ultraviolet detection. At the highly sensitive 0.01 AUFS

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[6]Bioanalytical Systems Inc., West Lafayette, IN

[7]Propofol, Stuart Pharmaceuticals, Wilmington, DE

setting, the standards 0.025, 0.05, and 0.1 ug/ml were used to construct the curve. The remaining standards, 0.5, 1.0, 5.0, and 10.0 ug/ml, were used to construct the curve for the 0.05 AUFS setting. Standard curves were constructed by linear regression analysis[8], obtained by plotting the peak height of the propofol standard against the concentration in the standard sample. Standard curves were linear, and passed through the origin. The concentration of the unknown samples was determined by plotting the peak heights onto the standard curve. Blood samples from each dog were extracted and analyzed within 18 hours of removal from storage at 4°C. The maximum length of sample storage until analysis was 12 weeks; however, the majority of samples were analyzed within 3 weeks of collection. The concentrations of propofol in the unknown samples were corrected for within-batch variation by averaging the peaks of thymol standards. Whole blood propofol concentrations for each dog were then adjusted according to the differences in the internal standards for that day.

Pharmacokinetic Calculations

Pharmacokinetic analysis of the propofol concentration vs. time data from each group of dogs was performed using the least squares nonlinear regression analysis program,

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[8]Pharmacologic Calculations®, Tallirida and Murray, NY

PCNONLIN[9], with weighting of the data proportional to $(1/C_p)$, where C_p is the whole blood propofol concentration. Initial parameter estimates were obtained by using a standard stripping technique[10]. Pharmacokinetic values were calculated from the coefficients and exponents of the biexponential equation best describing the data for each dog. The choice of pharmacokinetic model used was based upon criteria by Endrenyi (1981), and the best fit according to the Akaike criterion (Sakamoto et al. 1986). For a two compartment open model, the biexponential equation is defined:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

The parameter estimates obtained from the pharmacokinetic analysis included: A (distribution phase intercept), B (elimination phase intercept), α (distribution phase rate constant), and ß (elimination phase rate constant). The pharmacokinetic parameter equations listed below were obtained from Gibaldi and Perrier (1982).

The area under the curve (AUC) is determined by the formula:

$$AUC=A/\alpha + B/\beta$$

The volume of distribution (Vd) was determined by the formula:

$$Vd(area)=Dose/(AUC)(\beta)$$

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[9]PCNONLIN®, Statistical Consultants Inc., Lexington, KY

[10]EStrip®, Statistical Consultants Inc., Lexington, KY

The volume of distribution at steady state (Vd[ss]) was described by the formula:

$$Vd(ss)=Dose(AUMC)/AUC^2$$

The term AUMC describes the area under the (first) moment curve, and was determined by the formula:

$$AUMC=A/\alpha^2 + B/\beta^2$$

The formula for the calculation of total body clearance is:

$$Cl_b=Dose/AUC$$

The mean residence time (MRT) was calculated according to the formula:

$$MRT=AUMC/AUC$$

The mean propofol blood concentrations and recovery times data from the greyhounds and mixed breed dogs were analyzed for differences between the groups by the Student's t test. Statistical differences between the groups for derived pharmacokinetic parameters were analyzed by the Kruskal-Wallis test (using Chi-square approximation), since these parameters cannot be considered to be normally distributed (Powers 1990). The level of significance was $P \leq 0.05$.

RESULTS

Greyhounds

In greyhound dogs after single intravenous injections of propofol (mean dose = 5.28 mg/kg), whole blood propofol concentrations were described by a biexponential equation. In three of the ten greyhounds, the data could also be described by a triexponential equation (three compartment open model). However, because all of the greyhound data were adequately described by the two compartment open model, it was used in the parameter calculations. Whole blood propofol concentrations were 3.29 +/- 1.18 ug/ml at 2 minutes post injection and 0.017 +/- 0.019 ug/ml at 480 minutes (Table 1). Disposition of propofol was characterized by a rapid disposition phase (alpha t1/2 = 10.97 minutes) and by a slower elimination phase (beta t1/2 = 175.68 minutes, Figure 1, Table 2). The Vd(ss) was 6,289 +/- 4,886 ml/kg and the total body clearance (Cl_b) of propofol was 54 +/- 12.7 ml/kg/min. The mean residence time was 144 +/- 159 minutes (Table 2).

Greyhound dogs required significantly longer times to achieve sternal and standing positions than did mixed breed dogs (Table 3, Figure 2). Whole blood propofol concentrations were higher in greyhound dogs when they achieved sternal and standing positions than in mixed breed dogs. The greyhound dogs generally had smooth inductions and recoveries, with no apnea of greater than 30 seconds duration or emesis noted.

One greyhound did exhibit seizure-like activity (mild tonic-clonic activity) during the first 10 minutes of anesthesia, but was normal upon recovery. A second greyhound, who was in estrus and hyperactive, required a much larger induction dose (7.5 mg/kg) to achieve intubation and a moderate level of anesthesia.

Mixed Breed Dogs

In mixed breed dogs following a single intravenous injection of propofol (mean dose = 5.44 mg/kg), whole blood propofol concentrations were best described by a biexponential equation. In two of the eight mixed breed dogs, the data could also be described by a three compartment open model. However, since all mixed breed dogs were adequately described by the biexponential equation, the two compartment open model was used in all parameter calculations. The disposition of propofol in mixed breed dogs was characterized by a rapid distribution phase (alpha t1/2 = 7.67 minutes) followed by a slower elimination phase (beta t1/2 = 122 minutes, Figure 1, Table 2). Peak whole blood propofol concentrations, which occurred at two minutes post-injection, were 2.301 +/- 0.72 ug/ml, and the concentrations decreased to 0.012 +/- 0.008 ug/ml at 480 minutes (Table 1). The concentration of propofol in whole blood could not be reliably detected at 720 or 1440 minutes in either greyhounds or mixed breed dogs with this method. The total body clearance of propofol was 114.8 +/- 46

ml/kg/min, and the Vd(ss) was 9,748 +/- 1,937 ml/kg. The mean residence time of propofol in the mixed breed dogs was 94.7 +/- 37.2 minutes. Significant differences between greyhounds and mixed breed dogs were detected for apparent Vd, Vd(ss), and Cl_b values (Table 2).

Propofol anesthesia in mixed breed dogs was uncomplicated, with no apnea, nausea or emesis noted. One dog exhibited mild excitement during the initial phase of induction, and paddling and nystagmus were observed during recovery in three of eight mixed breed dogs. The times recorded for the return to the sternal and standing positions were significantly ($P>0.004$ and $P>0.001$, respectively) shorter than those of the greyhound dogs (Table 3, Figure 2).

In five of the eight mixed breed dogs and eight of the ten greyhounds, secondary peaks were observed in the plots of the concentration versus time data. These peaks corresponded to the times when dogs were awake and able to return to a sternal position. The phenomenon of the secondary peak as recorded in one of the greyhounds is illustrated in Figure 3.

DISCUSSION

Thiobarbiturates have long been the "standard" for intravenous anesthetic induction agents. However, the drawbacks associated with use of the barbiturates, including unpredictable or prolonged sleeping times, have prompted the ongoing search for alternative intravenous induction agents. Propofol is a highly lipophilic drug derived from the series of alkylphenols which are a unique class of anesthetic agents (James and Glen 1980). Because of propofol's high lipid solubility, it readily crosses the blood/brain barrier and other blood/tissue membranes. In a study by Schuttler and coworkers in 1985, the mean blood-brain equilibrium half-life of propofol in humans was found to be 2.9 minutes. This short equilibrium time, which is due to the lipid solubility of propofol, correlates with the rapid onset of anesthesia following intravenous injection of propofol. Propofol's lipid solubility enables it to readily cross cell membranes, not only during the initial distribution phase, but also during its redistribution from the well perfused (vessel-rich) tissues, which includes the brain, to the less well perfused tissues of muscle and fat. The termination of propofol's effect has been attributed both to the redistribution of propofol to muscle and fat, and to biotransformation by the liver (Kanto and Gepts 1989). The primary difference between propofol and the thiobarbiturates resides in propofol's rapid

clearance due to hepatic (and possibly extrahepatic) metabolism. The rapid clearance of propofol from the body results in a faster and more reliable recovery from anesthesia. Propofol has many characteristics that make it an ideal anesthetic induction agent including rapid induction of hypnosis, a short duration of anesthesia due to its rapid redistribution and elimination, and a rapid predictable recovery.

The pharmacokinetics of propofol in humans after rapid intravenous injection have been extensively investigated. The pharmacokinetic profile of propofol has been most frequently described by a three compartment open model, which characterizes its disposition as the sum of three exponentials (Cockshott 1985, White 1988, Sebel and Lowdon 1989, Kanto and Gepts 1989). In humans, the important differences in the pharmacokinetic parameters of propofol and thiobarbiturates are illustrated in the Vd(ss) and Cl_b. Thiopental has a Vd(ss) ranging from 1.5-3.3 liters/kg (Stanski 1984), while the Vd(ss) of propofol is 1.7 to 15.7 liters/kg (Kirkpatrick et al. 1988, Jones et al. 1990). The Cl_b of thiopental ranges from 0.11 to 0.30 l/min (Stanski 1984), while the Cl_b values of propofol have been reported to range from 1.6 to 2.55 l/min (Gill et al. 1990, Gin et al. 1990, Cockshott et al. 1987, Schuttler et al. 1985). In humans, this high total body clearance exceeds liver blood flow. Therefore, the mechanisms of elimination of propofol must include some extrahepatic or

extrarenal metabolism or elimination routes (Kanto and Gepts 1989).

In this study, the disposition of propofol in the majority of greyhound and mixed breed dogs was best described by a biexponential equation. Both greyhounds and mixed breed dogs had rapid distribution phases which were characterized by a rapid drop in the whole blood propofol concentration due to the redistribution of the drug to body tissues. The decrease in propofol concentration in each group was associated with the return of consciousness and righting reflexes. In the mixed breed dogs the distribution half life was 7.67 +/- 6.78 minutes, while in greyhounds the distribution phase half-life was 10.97 +/- 6.94 minutes. These times corresponded to blood concentrations of approximately 1.05 ug/ml and 1.6 ug/ml for mixed breed and greyhound dogs, respectively (Figure 3, Table 2). Thus, the initial redistribution of propofol accounts for most, but not all, of the time required for the rapid return of consciousness following anesthesia. However, in order to confirm this hypothesis, tissue concentrations of propofol would have to be measured and correlated to the distribution and elimination phases of the pharmacokinetic profile. It is important to note that greyhounds recovered from propofol anesthesia at significantly higher concentrations than mixed breed dogs (Figure 2).

Several terms are used to describe the volume of distribution of a drug. The Vd(area), or apparent volume of

distribution, will often over-estimate the true Vd of a drug that is described by a multi-compartment model. The volume of distribution at steady state [Vd(ss)] is mathematically independent of the rate of drug elimination, and thus is the most accurate and useful pharmacokinetic parameter for defining the volume of distribution (Riviere 1988). The Vd(ss) of the greyhound dogs was significantly ($P \leq 0.036$) different from the Vd(ss) of the mixed breed dogs (Table 2). In mixed breed dogs, the estimation of Vd(ss) was almost three times the value in the greyhound dogs, indicating that the mixed breed dogs had a much greater distribution of propofol. This difference was most likely due to the fact that mixed breed dogs, in general, have a greater percentage of body fat than do greyhounds (Sams et al. 1985).

The elimination half-life represents the time it takes the body to remove 50% of the drug from the blood and tissues (Riviere 1988). This term is useful because it generally estimates the duration of drug effects in the body. The elimination half-life of propofol in greyhounds versus mixed breed dogs was not statistically different. In greyhounds, the terminal elimination half-life (ß) was 175.68 +/- 179.58 minutes while in mixed breed dogs the half-life was 122 +/- 55.62 minutes. Despite the usefulness of elimination half-life, the total body clearance (Cl_b) represents the most important pharmacokinetic parameter used to define drug elimination. Clearance estimates the drug elimination from

the body by all mechanisms, including hepatic, renal, and from other organs such as lungs, saliva, etc. In this study, significant differences were found between the clearances for the greyhounds and mixed breed dogs (Table 2). Greyhounds were able to eliminate propofol at approximately 50% the rate of mixed breed dogs. The definitive mechanisms for this difference were not addressed by this investigation. However, one likely explanation may reside in the differences in body fat composition of greyhounds versus mixed breed dogs. In greyhounds, the greater proportion of lean muscle mass and relative lack of fat suggests that hepatic biotransformation mechanisms may play a greater role in the early stages of propofol metabolism. In humans, hepatic biotransformation mechanisms include the metabolism of propofol into glucuronide and sulfate conjugates (Kanto and Gepts 1989). Greyhounds may not have the same capacity for biotransformation of propofol via their glucuronide or sulfate conjugation pathways as mixed breed dogs. In mixed breed dogs, the redistribution of propofol (presumably to fat) probably accounts for a small proportion of the overall propofol volume, but still results in a smaller amount of propofol being presented to the liver for biotransformation. Therefore, the difference in the Cl_b for greyhounds versus mixed breed dogs could be due to: 1) the differences in body fat content (less redistribution of propofol to fat in greyhounds) or 2) differences in hepatic biotransformation or elimination mechanisms of propofol.

In addition to the model derived parameters, mean residence times (MRT) were calculated for each group of dogs. The MRT represents the statistical moment analog to the elimination half-life, but is calculated from primary parameter estimates and is independent of model derived estimates (Gibaldi and Perrier 1982). In effect, the MRT represents the time required for 63.3% of the administered dose of propofol to be eliminated. The MRT, as with the elimination half-life, was different for greyhounds and mixed breed dogs, but these differences were not statistically significant.

The clinical recovery characteristics of propofol are also significantly different between the greyhounds and mixed breed dogs (Table 3, Figure 2). While the times to extubation were essentially the same for both groups, the times to return to a sternal or standing position were quite different. The return of a sternal position corresponded in the individual dogs to the time when the propofol concentration was less than 1.0 ug/ml in mixed breed dogs, and slightly greater than 1.0 ug/ml in greyhounds. These data correspond to results in human studies where the return of consciousness occurred when the propofol concentration was 1.0 ug/ml or less (Adam et al. 1982, Adam et al. 1983, Cockshott et al. 1987). The differences observed in recovery characteristics in the two groups is reflected by several differences in their pharmacokinetic parameters, but especially in the Cl_b.

A unique observation concerning the pharmacokinetic profile of propofol occurs upon evaluation of the concentration versus time data. A secondary peak was observed in the whole blood propofol concentration graphs in a majority of the dogs (Figure 3). The second peak corresponded to the time of return of consciousness and the righting reflex. The presence of a secondary peak has also been reported in several propofol studies in humans (Kay et al. 1985, Cockshott et al. 1987, White 1988). The reasons for this peak are not completely understood, but have been attributed to: 1) the development of a new equilibrium between propofol in the sampled limb and blood, or 2) to changes in cardiac output and regional blood flow during that period of anesthesia that lead to a release of drugs from tissue depots (Kanto and Gepts 1989). In this study, the blood samples were taken from the intrathoracic jugular vein, thus the former explanation seems unlikely. However, the peaks were observed in both greyhounds and mixed breed dogs at times of arousal and when skeletal muscle movements were occurring in an attempt to right themselves, which could be associated with changes in cardiac output and blood flow. Propofol is known to be a direct venodilator (Goodchild and Serrao 1989). Thus, with recovery from propofol anesthesia, as the dynamics of the peripheral circulation return to the preanesthetic state, an influx of propofol from peripheral tissues could be occurring. Further work is required to elucidate the answer to this question.

In summary, the disposition of propofol in mixed breed and greyhound dogs is similar. In particular, both groups can be adequately described by a biexponential equation with a rapid distribution phase and slower elimination phase. But, there are important differences. The greyhounds have a smaller Vd(ss), slower Cl_b, and tend to sleep longer than the mixed breed dogs. However, the drug can still be safely used in greyhounds as an induction agent, as its recovery characteristics are significantly more predictable and of shorter duration than those of the thiobarbiturates. Overall, the pharmacokinetics of propofol in mixed breed dogs and greyhounds support the results of clinical trials and suggest that propofol is an extremely useful and viable alternative induction agent to the thiobarbiturates. The pharmacokinetics of propofol also suggest that, because of its rapid distribution and clearance values, it will be a useful drug for intravenous infusion. However, clinical trials using propofol in total intravenous anesthesia in veterinary patients are just beginning (Hall and Chambers 1987).

TABLES

TABLE 1. Whole blood propofol concentrations

The whole blood propofol concentration means (+/- standard deviations) for greyhounds (GH) and mixed breed dogs (MD) are given. Statistically significant differences ($P \leq 0.05$) between the groups are highlighted with the asterisk (*). Means are expressed in ug/ml. The ND represents propofol concentrations not detected.

Time (min)	GH MEANS n=10	STD DEV	MD MEANS n=8	STD DEV	P value
2	3.290	1.178	2.301	0.716	0.0541
4	2.670	0.911	1.724	0.894	0.0422*
6	2.307	0.847	1.145	0.548	0.0041*
8	2.109	0.637	1.026	0.475	0.001*
10	1.788	0.493	0.753	0.484	0.0004*
15	1.494	0.482	0.676	0.338	0.0009*
20	1.280	0.278	0.553	0.476	0.0009*
30	0.825	0.288	0.351	0.285	0.003*
45	0.412	0.234	0.185	0.143	0.029*
60	0.308	0.082	0.161	0.059	0.006*
90	0.181	0.076	0.102	0.036	0.016*
120	0.134	0.049	0.077	0.024	0.009*
150	0.096	0.045	0.057	0.032	0.0563
180	0.096	0.042	0.047	0.023	0.0096*
240	0.073	0.056	0.042	0.026	0.1711
300	0.044	0.036	0.028	0.018	0.2713
360	0.032	0.024	0.021	0.013	0.2642
480	0.017	0.019	0.012	0.008	0.4741
720	ND	---	ND	---	----

TABLE 2. Pharmacokinetic parameters

The means (+/- standard deviations) of the parameter estimates (A, B, α, ß) and the pharmacokinetic values derived from these estimates are given. The † represents those data compared by the Student's t test. The asterisk (*) represents a level of significance of $P \leq 0.05$. Co=blood propofol concentration at time zero, α t1/2=alpha half-life, ß t1/2=beta half-life, K12=rate constant from compartment 1 to 2, K21=rate constant from compartment 2 to 1, Kel=elimination rate constant, Vc=volume of the central compartment.

PARAMETER ESTIMATES	UNITS	GH MEANS n=10	STD DEV	MD MEANS n=8	STD DEV	t TEST† OR KRUSKAL-WALL
Co	ug/ml	4.2643	2.07	3.1332	1.26	0.2863
A	ug/ml	3.7573	1.65	2.9533	1.21	0.2667†
ALPHA	min-1	0.1360	0.16	0.1963	0.18	0.4676†
B	ug/ml	0.5069	0.60	0.1799	0.07	0.1494†
BETA	min-1	0.0088	.007	0.0070	.004	0.5410†
α t1/2	min	10.977	6.94	7.6705	6.78	0.2135
ß t1/2	min	175.68	179	122.04	55.6	0.859
K12	min-1	0.0703	0.10	0.1131	0.12	0.183
K21	min-1	0.0304	0.05	0.0184	0.01	0.789
Kel	min-1	0.0442	0.02	0.0718	0.05	0.155
Vc	L/kg	1.4668	0.74	1.8166	0.67	0.286
Vd	L/kg	11.158	7.68	17.998	6.60	0.033*
Vd(ss)	L/kg	6.289	4.89	9.7479	1.94	0.036*
Cl_b	L/kg/m	0.054	0.01	0.1148	0.05	0.0004*
MRT	min	144.0	159	94.68	37.2	0.594
DOSE	mg/kg	5.288	0.23	5.44	0.39	-----

TABLE 3. Recovery characteristics data

Means (+/- standard deviations) for three recovery characteristics following a single intravenous injection of propofol in greyhound (GH) and mixed breed dogs (MD). The asterisk (*) denotes those characteristics which had statistically significant ($P \leq 0.05$) differences between the groups.

PARAMETER	UNITS	GH MEANS	SD	MD MEANS	SD	t TEST
EXTUBATE	min	6.40	4.2	6.75	1.7	0.829
STERNAL	min	16.70	2.9	10.88	4.4	0.004*
STAND	min	21.70	3.3	14.63	3.6	0.001*

FIGURES

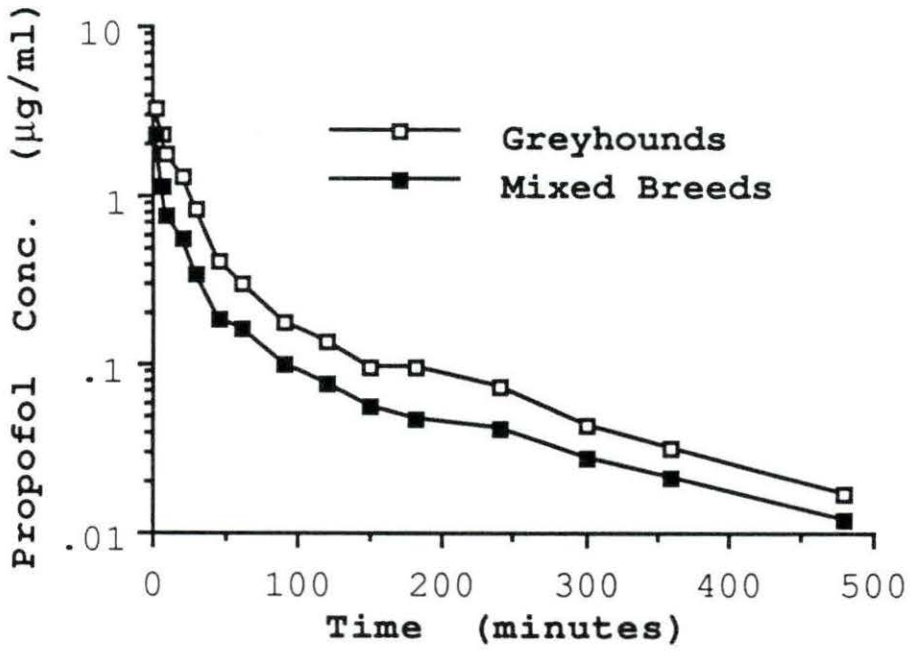

FIGURE 1. Whole blood propofol concentration vs. time data

Whole blood propofol concentrations in greyhounds and mixed breed dogs after a single intravenous dose of propofol. The means are reported over 480 minutes (see Table 1 for specific values).

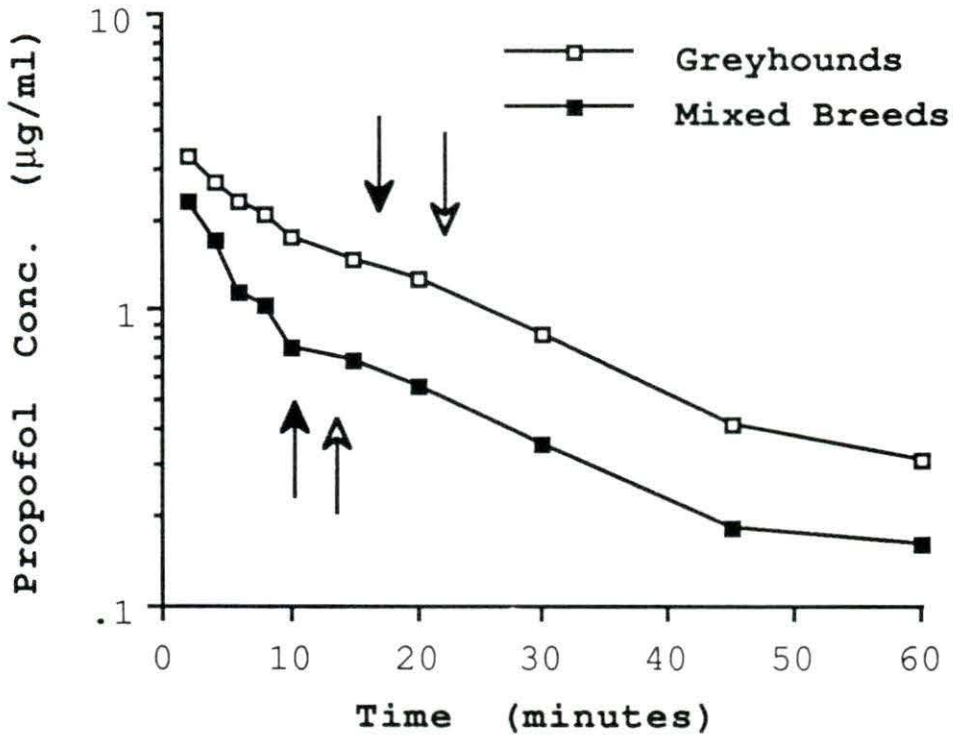

FIGURE 2. Whole blood propofol concentrations vs. time data with associated recovery times

Whole blood propofol concentrations in greyhounds and mixed breed dogs for the initial 60 minutes following a single intravenous injection of propofol. Solid arrows indicate the times on the respective curves to the return of the righting reflex, while open arrows indicate the time to standing unassisted in each group. Statistically significant differences were observed between the greyhounds and mixed breed dogs for both parameters.

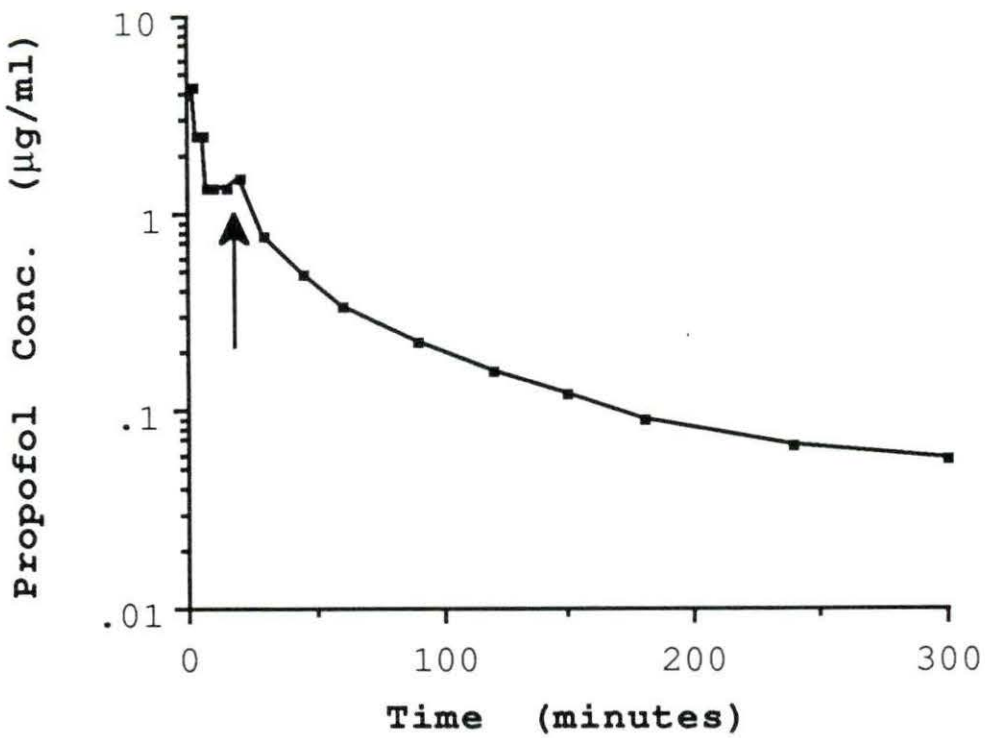

FIGURE 3. Secondary peak in blood propofol concentrations

Whole blood propofol concentrations in one greyhound following a single intravenous injection of propofol. This figure illustrates the occurrence of the secondary peak in blood propofol concentrations. The arrow indicates the time (17 minutes) of return of the righting reflex, which corresponded to the onset of the secondary peak.

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GENERAL SUMMARY AND CONCLUSION

The results of this research project confirm that propofol is a unique but useful intravenous anesthetic agent in normal mixed breed and greyhound dogs. The pharmacokinetic profile of propofol in greyhounds is similar to that of mixed breed dogs, but there are important differences. In particular, greyhounds had significant differences in the pharmacokinetic variables of Vd(ss) and Cl_b. Despite the smaller Vd(ss) and slower Cl_b of propofol in greyhounds, the recovery characteristics were still more predictable and shorter in duration than those observed with thiobarbiturates. However, because of the differences in the clearance values of propofol in greyhounds, it is possible that propofol biotransformation and elimination mechanisms could be easily and rapidly saturated in those dogs. Thus, further evaluation of the hepatic biotransformation mechanisms and additional pharmacokinetic studies utilizing propofol in a constant rate infusion or with repeated injections in greyhounds is indicated.

The pharmacokinetic profile of propofol in mixed breed dogs reveals that their disposition of propofol is very similar to that in humans. One major difference between human studies and the research reported here was that in most humans propofol disposition was best described by a three compartment open model. While some of the dogs in this study were best

described by a triexponential equation, most were best fit to the two compartment open model. The precise reason for these differences is unknown, but may reflect the unique disposition of propofol in dogs, differences in the weighting of pharmacokinetic data for model fitting or differences in model-fitting programs. The pharmacokinetic profile in mixed breed dogs is characterized by an initial rapid distribution phase which corresponds to the rapid onset and short duration of propofol's effects, and a longer elimination phase which corresponds to the slow elimination of propofol from the deeper, less well perfused compartments. The onset and duration of propofol anesthesia in mixed breed dogs is very similar to the thiobarbiturates; however, the Cl_b exceeds that of other reports of the thiobarbiturates. The rapid clearance of propofol is observed clinically in the rapid and complete recovery of these dogs following propofol anesthesia.

In conclusion, propofol is a viable alternative to the thiobarbiturates for intravenous induction of anesthesia in greyhounds and mixed breed dogs. The dose of propofol in unpremedicated dogs ranges between 5.0-6.0 mg/kg, and should be given to effect over 30-60 seconds. Further studies concerning the pharmacokinetics of propofol in sick, geriatric or young animals, as well as studies examining the interactions of propofol with other drugs are warranted to assure that propofol is acceptable for general use.

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APPENDIX

The method used in this research for high performance liquid chromatography (HPLC) analysis of propofol concentrations in whole blood was originally described by Plummer (1987). This section will describe in depth the materials and methods used in determination of propofol concentrations in greyhounds and mixed breed dogs.

HPLC Materials and Methods

Mobile phase-The mobile phase consisted of 600 ml Acetonitrile, 400 ml triple distilled water, and 1 ml Trifluoroacetic acid. The mobile phase was filtered under vacuum through a 0.45 micron Millipore filter[1] and then degassed by submitting it to an ultrasonic device for 15 minutes.

HPLC Equipment-The solvent delivery system consisted of a Waters[2] model 6000A Chromatography Pump with a Rheodyne[3] Model 7125 syringe-loading injector. A Hamilton[4] 100 ul syringe was used to introduce samples to the system. The mobile phase flow rate was maintained at 1.3 ml/min, which

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[1]Millipore® filter, Waters Associates, Milford, MS

[2]Waters Associates Chromatography Pump, Milford, MS

[3]Rheodyne® injector, Rheodyne Inc., Cotati, CA

[4]Hamilton® syringe, Hamilton Co., Reno, NV

produced a pump pressure of approximately 1500 psi. The analytical column was a Hypersil[5] 3 C18 column (length 100 mm, internal diameter 4.6 mm) packed with silica, that had a particle size of 3 microns. A 10 micron Waters® stainless steel precolumn filter and a 0.45 micron Hypersil® precolumn were used to protect the analytical column. A Lambda-Max Model 480 LC Spectrophotometer[6] was used for detection of the propofol and thymol (internal standard) compounds as they came off the column. A wavelength of 276 nm was used for detection at a time constant of 2 seconds, and at an absorbance units full scale setting (AUFS) of 0.05 to 0.01. An OmniScribe[7] strip chart recorder was used to record the data. The retention time for thymol (internal standard), was approximately 3 minutes. Propofol had a retention time of 5.5 minutes. Detection of thymol and propofol was not compromised by other substances in the extract.

Whole Blood Extraction of Propofol-All samples from one experimental dog were analyzed within eighteen hours of removal from refrigeration, to reduce the day to day variation between dogs. Into acid washed 10 ml glass tubes, one milliliter of whole blood was added to 1 ml of phosphate

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[5]Hypersil®, Phenomenex, Torrance, CA

[6]Lambda Max® Model 480, Waters Associates, Milford, MS

[7]Omniscribe®, Houston Instruments Co., Austin, TX

buffer (0.1 M sodium dihydrogen orthophosphate), 20 ul of internal standard (thymol prepared in methanol), and 5 ml of cyclohexane (HPLC grade). The concentration of internal standard added was dependant on the sensitivity setting. At 0.01 AUFS, 20 ul of 10 ug/ml thymol was used, while at 0.05 AUFS, 20 ul of 100 ug/ml thymol was added to the standards. The samples were mixed for 15 minutes on an inversion mixer, then centrifuged for 5 minutes at 1100 g. Following centrifugation, 4.5 ml of the cyclohexane layer was transferred to 10 ml glass tubes containing 50 ul tetramethyl ammonium hydroxide (TMAH). The TMAH was added to help preserve propofol concentrations during the drying and analysis period due to propofol's tendency to oxidize. The samples were dried under a stream of nitrogen (approximately 60 minutes at room temperature), and then were reconstituted in 250 ul of mobile phase. The reconstituted samples were stable in the refrigerator for up to 18 hours (Plummer 1987). Prior to submitting the samples for analysis by HPLC, they were filtered through a 0.45 micron nitrocellulose membrane filter[8] via centrifugation at 1000 RPM for 5 minutes. All extracted samples were analyzed during the same time period, and within 18 hours of reconstitution. A typical chromatogram obtained during the analysis is illustrated in Figure 4.

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[8]Bioanalytical Systems Inc., West Lafayette, IN

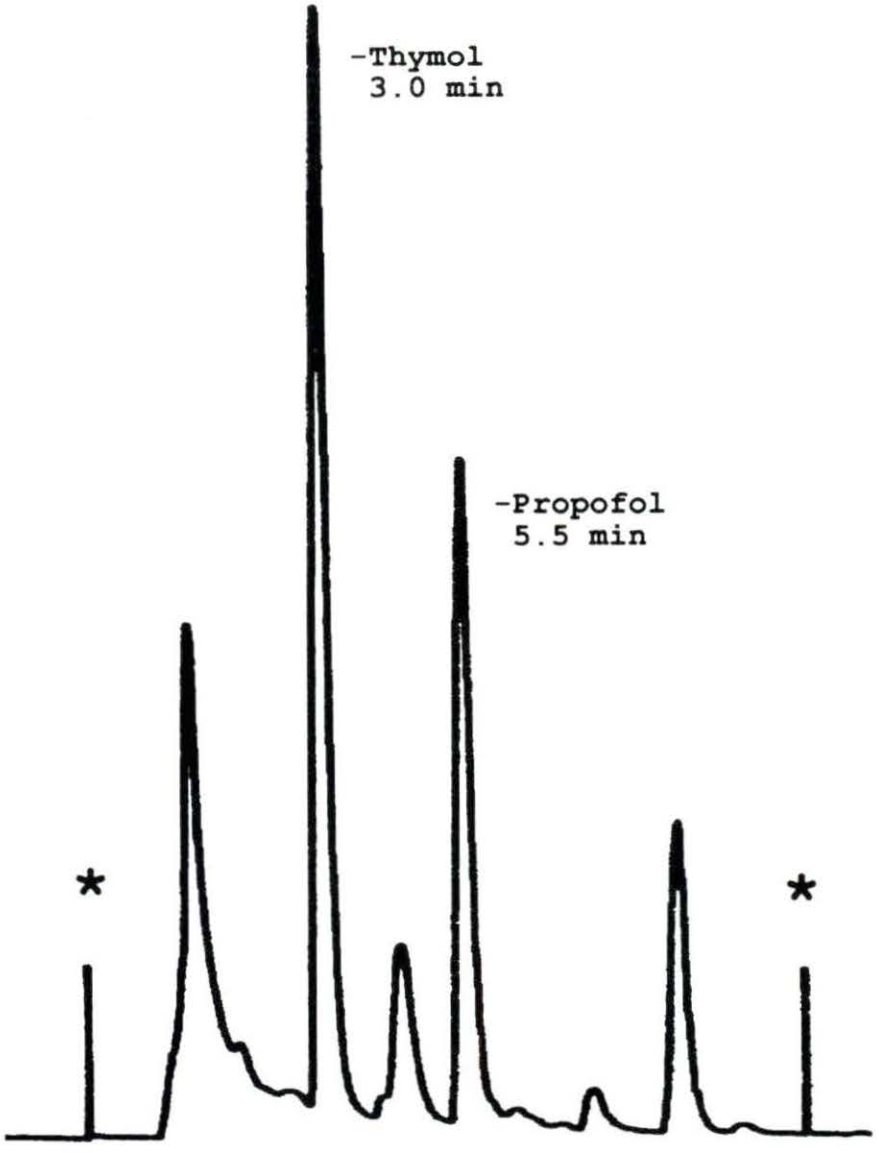

FIGURE 4. Typical chromatogram

Typical chromatogram obtained during HPLC analysis of whole blood from a mixed breed dog following a single intravenous injection of propofol. The peak at 3.0 minutes represents the internal standard (thymol) used in this study. The retention time of propofol was approximately 5.5 minutes for both greyhounds and mixed breed dogs. The asterisks denote the separation of sample chromatograms, with injections made at approximately 10 minute intervals.