EFFECTS OF CIMETIDINE, CHLORAMPHENICOL, AND PHENOBARBITAL ON AND TOLERANCE TO XYLAZINE-KETAMINE INDUCED ANESTHESIA IN DOGS

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PREFACE

Chemicals whether they are medications or environmental agents affect the functioning of all species. The scope of this study involves three therapeutic agents, cimetidine, chloramphenicol, and phenobarbital and their effects on the duration of anesthesia induced by xylazine/ketamine combination in dogs.

I wish to express my deepest gratitude and appreciation to Dr. Subbiah Sangiah, my thesis advisor, teacher, and friend. His sincerity and confidence in me has instilled a quality of professionalism that I will utilize in my endeavors as a physician.

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CHAPTER I

AN INTRODUCTION WITH A REVIEW OF THE LITERATURE

Ketamine and Xylazine are commonly used alone or in combination with sedatives, tranquilizers, and other injectable or inhalational anesthetics in veterinary practice as a chemical restraint of various species including cats, dogs, primates, ruminants, pigs, laboratory and zoo animals, exotic avian birds, reptiles, and fish (Short, 1987a, 1987b; Booth, 1982a, 1982b; Wright, 1982; Mohammad, 1988). In the clinical setting, an animal may be receiving two or more drugs simultaneously, and the interaction of the combined drugs may profoundly affect the intended outcome of one or more drugs. A wide range of chemicals (drugs) and environmental agents are able to alter the activity of drug-metabolizing enzymes by induction or inhibition. The therapeutic and clinical implications of enzyme induction and enzyme inhibition will depend on the relative pharmacologic activity of drugs with narrow therapeutic ranges (anticoagulants, and antiarrhythmic agents) (Conney, 1967; Conney, 1969; Gelehrter, 1976; Katzung, 1987; Park and Breckenridge, 1981).

The term tolerance is used to describe a diminished pharmacologic response to repeated administration of drugs, especially centrally-acting drugs (Kato, 1967; Gilman et al., 1985). Tolerance develops by mechanisms classified as dispositional or pharmacokinetic and functional or pharmacodynamic. Dispositional tolerance is due to induction of hepatic microsomal drug metabolizing enzymes; whereas, functional tolerance is a change in the drug-receptor relationship (Greizerstein, 1978; Gilman, 1985). The development of drug tolerance is an important problem for the evaluation of drug actions and for the determination of a schedule of drug administration (Kato, 1967).

Metabolism (Biotransformation) of Xenobiotics

Humans and domestic animals are constantly exposed to a variety of foreign chemicals called xenobiotics which are substances that are absorbed across the mucous membranes, most commonly those ingested and absorbed from the gastrointestinal tract. Animals may be exposed to these chemicals by contact with environmental pollutants or by receiving drugs for a disease condition (Katzung, 1987). In either case, the animals body must accommodate these chemicals, which may have pharmacologic or toxic activity, by excretion of unchanged chemicals in the urine or metabolism (biotransformation) of the chemicals to another form which then may be eliminated from the body. Most drugs are lipophilic, weak organic acids or bases which are not

eliminated from the body by the kidneys due to filtration and subsequent reabsorption (Gilman, 1985). Biotransformation usually produces metabolites that are more water-soluble than the original substrate, thus the elimination of the parent drug from the body is enhanced (La Du, 1971). The biotransformation reactions of drugs are classified as either phase-I or phase-II reactions. Phase-I reactions consist of oxidation, reduction, or hydrolysis of the parent drug to a more polar metabolite. Phase-II reactions consist of conjugations of the parent drug with an endogenous substance such as: glucuronic acid, sulfuric acid, acetic acid, or an amino acid rendering the parent drug a more polar metabolite (Gilman, 1985). Brodie (1964) states that if the body lacked drug metabolism processes, it would take a hundred years to terminate the actions of pentobarbital which is lipid-soluble and cannot be readily excreted without being metabolized (biotransformed).

Hepatic Drug Metabolism

Although every tissue has some ability to metabolize drugs, the liver is the principal organ of drug metabolism. The liver has two types of drug metabolizing enzyme systems, microsomal and non-microsomal. The term, microsomal, refers to microsomes (vesicles) of smooth and rough endoplasmic reticulum that form after isolation by homogenation and fractionation of the cell. Since these microsomes contain the smooth endoplasmic reticulum which has a rich supply of

oxidative enzymes, the microsomal enzyme system plays a major role in biotransformation of many drugs. The reactions catalyzed by this system are conjugation with glucuronic acid and the majority of oxidative reactions. The oxidation reactions are catalyzed by a group of oxidative enzymes called mixed-function oxidases (MFO) or monooxygenases. Monooxygenases require cytochrome P-450, cytochrome P-450 reductase, NADPH (reducing agent), and molecular oxygen for activity (Gilman, 1985; Katzung, 1987; La Du, 1971). In addition, both microsomal and non-microsomal systems catalyze the reactions concerned with reduction and hydrolysis of drugs.

The non-microsomal enzyme system refers to enzymes that catalyze reactions that are and not catalyzed by the microsomal enzyme system. The reactions catalyzed by non-microsomal enzymes consist of conjugations (except for glucuronide formation), reduction, oxidation, and hydrolysis of drugs (Gilman, 1985).

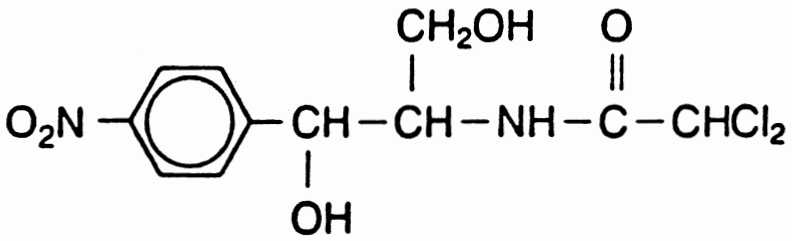
Inhibition of Hepatic Drug Metabolism

Since many patients receive multiple drug therapies, the action of one drug to inhibit or induce the metabolism of another is of common occurrence. This fact is vitally important when dealing with the inhibited metabolism of drugs with immense toxic potential (anticoagulants, theophylline, and phenytoin). The list of agents that inhibit drug metabolism varies, but the list contains drugs such as:

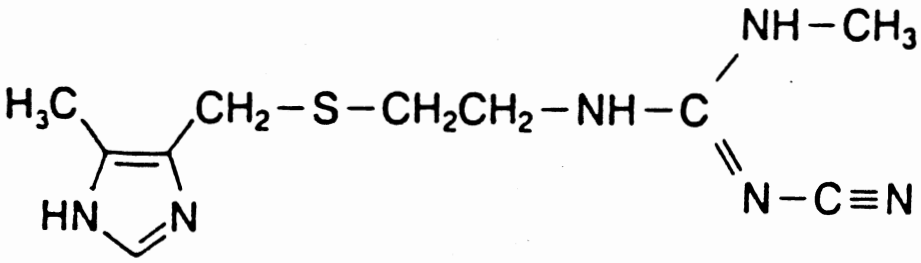
interferons, ketoconazole, cimetidine, and chloramphenicol. It has been shown that interferons (IFN) and inducers of IFN inhibit hepatic microsomal drug metabolism (Mannering and Deloria, 1986). Ghezzi et al. (1985) has proposed the inhibitory mechanism of IFN to be an increase in xanthine oxidase activity. Ketoconazole, a broad spectrum antifungal agent, is proposed to bind to the heme moiety of the hepatic cytochrome P-450 thereby diminishing substrate interaction with the enzyme (Mosca et al., 1985).

Pharmacology of Cimetidine

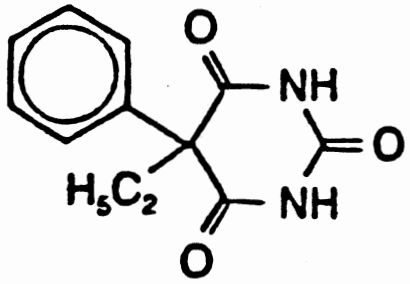
Cimetidine, a 4,5-substituted imidazole (Figure 1) with histaminergic type 2 antagonist activity is commonly used for the treatment of peptic ulcer disease (Konturek, 1983), duodenal ulcer (Parsons, 1985; Melville, 1985), gastric ulcer (Melville, 1985; Graham et al., 1985), Zollinger-Ellison Syndrome, and other hypersecretory states (McCarthy, 1978). In addition, new studies provide information on the potential use of cimetidine in areas of immunology and toxicology. Cimetidine has an immunomodulator effect on T-lymphocytes by diminishing T-suppressor activity and an antineoplastic effect on some tumors when used alone or in combination with interferons (Mavligit, 1987). It has also been viewed as an additive or alternative antidote for acetaminophen overdose by inhibiting the cytochrome P-450 system of drug metabolism (Speeg, 1987). Cimetidine has been known to alter the pharmacokinetics of about 25 different drugs (Powell and



Chloramphenicol



Cimetidine



Phenobarbital

Figure 1. Chemical Structure of Cimetidine, Chloramphenicol, and Phenobartital

Down, 1984) by increasing intragastric pH, inhibition of hepatic cytochrome P-450 microsomal enzymes, and alteration of renal tubular secretion (Sax 1987). Recent studies indicate that cimetidine inhibits the metabolism of various drugs by reversible binding of hepatic cytochrome P-450 (Knodell et al. 1982; Speeg et al. 1982). Cimetidine has been recently introduced in the therapeutic management of gastrointestinal disorders in veterinary medicine.

Pharmacology of Chloramphenicol (ChPC)

Chloramphenicol (ChPC, Figure 1), is a broad spectrum antibiotic which was isolated from Streptomyces venezeulae in 1947 and produced synthetically in 1949 for commercial use. ChPC is bacteriostatic especially for gram-negative bacteria and rickettsiae; also, some gram-positive bacteria are inhibited (Gilman et al., 1985; Katzung, 1987). ChPC is indicated or is a possible choice in the treatment of (1) typhoid fever and other Salmonella infections, (2) bacterial meningitis of unknown origin especially a-lactamase producing organisms, (3) anaerobic and mixed infections of the CNS and bowel, and (4) rickettsial diseases in patients sensitized to tetracyclines or compromised in some other way. ChPC was used indiscriminately between 1948 and 1951, until the toxicities associated with its use were discovered (Hird and Knifton, 1986; Gilman et al., 1985; Katzung, 1987). ChPC can produce an irreversible dose-independent blood dyscrasia (aplastic anemia being the

most common) or a reversible dose-dependent erythroid suppression of the bone marrow. ChPC when given in high doses to neonates or especially premature babies can produce a fatal toxic condition called the "gray syndrome", however 60% of cases survive (Gilman et al., 1985). ChPC inhibits protein synthesis in bacteria and to a lesser extent in mammalian bone marrow cells, but it also affects other enzymes (ATPase, ferrochelatase, and cytochrome oxidases) (Smith and Weber, 1983). It is the above interaction with protein synthesis that probably produces the toxicity associated with chloramphenicol, and the interaction with the cytochrome oxidases that affects the metabolism of other xenobiotics of clinical importance such as: tolbutamide, diphenylhydantoin, and dicoumarol (Smith and Weber, 1983; Christensen and Skovsted, 1969).

Induction of Hepatic Drug Metabolism

The hepatic drug-metabolizing enzymes, especially the mixed-function oxygenases located in the microsomal fraction of the liver, can be induced by a wide range of biologically and chemically unrelated compounds (Park and Breckenridge, 1981; Gelehrter, 1976) including barbiturates, antihistamines, oral hypoglycemic and uricosuric agents or chemicals encountered in the environment (DDT, chlordane, and 3,4-benzpyrene) (Conney, 1969). The process of enzyme induction is a dose-dependent relationship that involves an increase in the number of molecules of a specific enzyme in

response to a enzyme-inducing agent (Breckenridge et al., 1973; Gelehrter, 1976). The induction characteristic of these compounds are classified as either those with induction similar to that produced by phenobarbital or those with induction similar to that produced by polycyclic hydrocarbons (Gilman et al., 1985).

Pharmacology of Phenobarbital

Phenobarbital, a derivative of barbituric acid (Figure 1), was synthesized in 1912 and became the first effective organic drug therapy for management of epilepsy. Phenobarbital is effective in managing partial seizures and tonic-clonic seizures in humans (Gilman et al., 1985) and animals (Booth, 1982a). The exact mechanism of action is unknown, but phenobarbital potentiates GABA (Gammaamino butyric acid) mediated pre- and postsynaptic inhibitions, thereby inhibiting the spread of impulses from the neural source (foci) (Katzung, 1987).

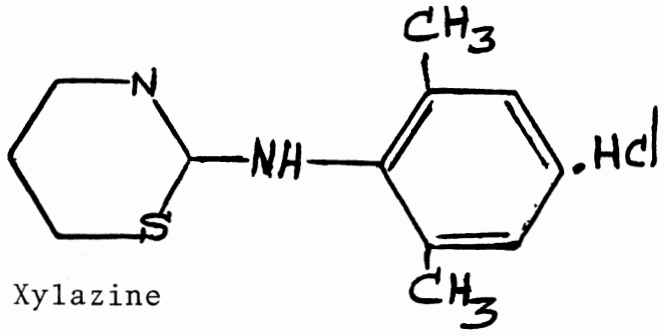
Phenobarbital has been the most widely studied enzyme-inducer in both man and animals (Park and Breckenridge, 1981). The induction by phenobarbital produces an increase in the metabolism of a large number of substrates by increasing the quantity of cytochrome P-450, cytochrome P-450 reductase, and other hepatic enzymes involved. It is also associated with proliferation of the endoplasmic reticulum, increases in liver weight, blood flow, and bile flow which is a characteristic not possessed by all inducers (Gilman et al., 1985).

Pharmacology of Xylazine

Xylazine (Figure 2), an analogue of clonidine, is a non-narcotic sedative and analgesic as well as a muscle relaxant (Feldberg and Symonds, 1980; Lumb and Jones, 1984). Sedative and analgesic activities are related to central nervous system depression mediated by stimulation of Alpha-2 adrenergic receptors (Hsu, 1981). Muscle relaxation is related to inhibition of intraneuronal transmission of impulses in the central nervous system (Booth, 1982b). There is a lack of information in the literature on the biotransformation of xylazine in domestic animals. This problem is related to the lack of a good analytical method for measuring the metabolites of xylazine (Garcia-Villar et al., 1981). However, studies using radio-labeled xylazine have shown that only a small percentage of unchanged xylazine is excreted in the urine, thus suggesting that xylazine is highly metabolized (Putter and Sagner, 1973; Garcia-Villar et al., 1981).

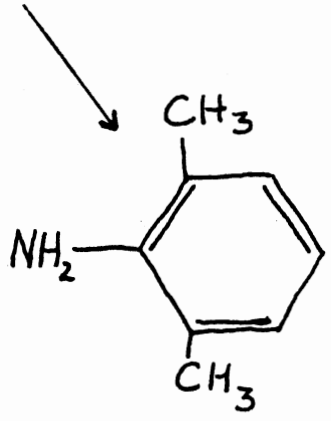
Pharmacology of Ketamine

Ketamine, a cyclohexanone derivative (Figure 3), is related to phencyclidine (PCP) and tiletamine (Stephenson et al., 1978). It is classified as a short-acting dissociative (cataleptic) anesthetic producing hypnosis and analgesia, but when used alone produces poor muscle relaxation due to its hypertonic effects on skeletal muscle (Booth, 1982b; Lumb and



Xylazine

[2(2,6 dimethyl amino) - 4H-5,6
dilydro 1,3 thiazide hydrochloride]



1,2 dimethyl amino benzene

Figure 2. Chemical Structure and Metabolic Pathways of Xylazine

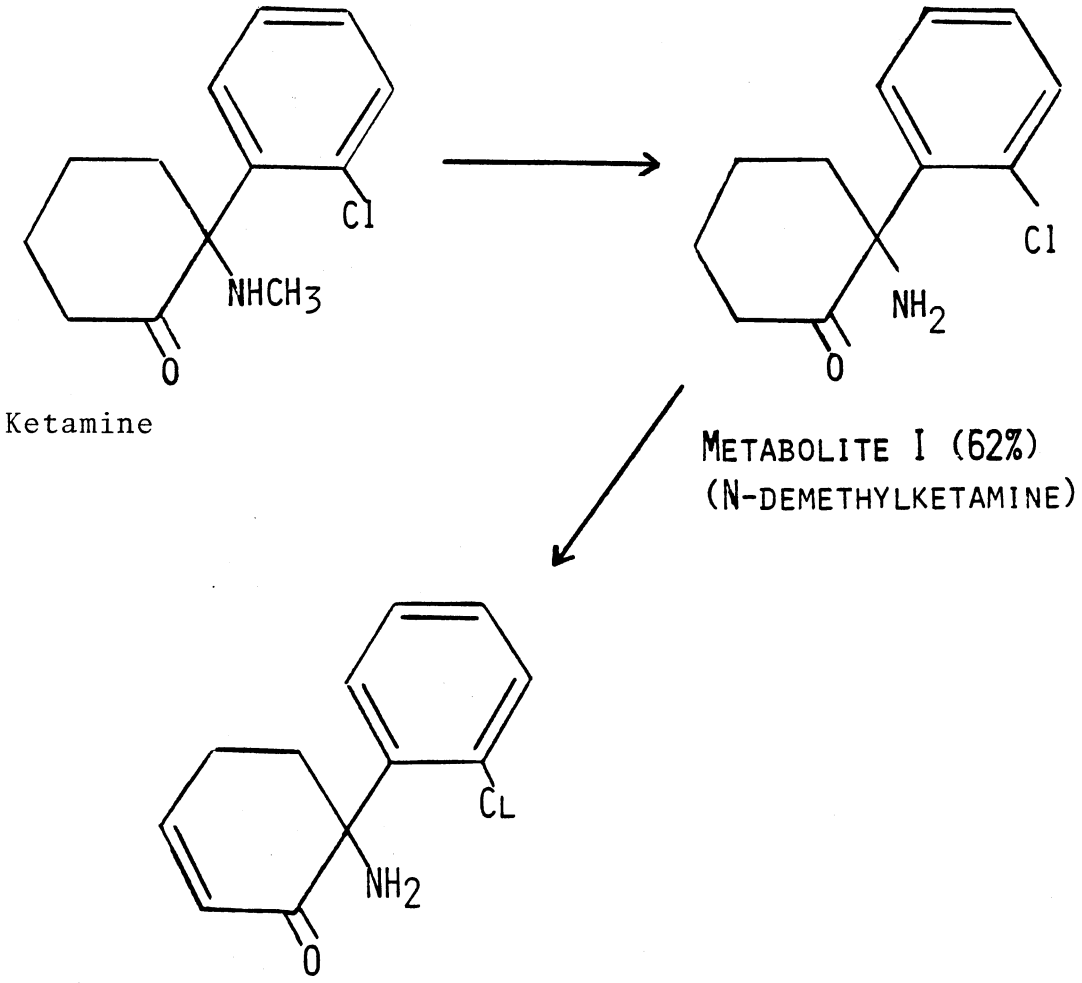


Figure 3. Chemical Structure and Metabolic Pathways of Ketamine

Jones, 1984). The clinical unresponsiveness observed during its use is not due to CNS depression, but is due to functional disorganization of the CNS (Stephenson et al., 1978).

Ketamine is rapidly metabolized in the liver of most animal species to as many as four metabolites (Figure 2). These metabolites are formed and eliminated rapidly by the kidney through expulsion into the urine (Kaka and Hayton et al., 1980; Lumb and Jones, 1984).

Tolerance

Tolerance develops by mechanisms classified as pharmacokinetic (dispositional) or pharmacodynamic (functional). Dispositional tolerance is due to induction of hepatic microsomal drug metabolizing enzymes, whereas functional tolerance is a change in the drug-receptor relationship (Greizerstein, 1978; Gilman et al., 1985). Acute tolerance describes a tolerance that has developed with a single dose or at most a few doses in a time period of minutes to a few days. It has been shown that clonidine (Ishii and Kato, 1984; Ishii et al., 1982; Finberg and Koplin, 1987), pentobarbital (Greizerstein, 1979), morphine (Kato, 1967), alcohol (Lee and Becker, 1987), anticholinergics, chlorpromazine, imipramine (Gilman et al., 1985), and other sedative-hypnotic drugs develop tolerance when administered chronically.

With regard to receptors, "desensitization" has become the best accepted term for receptor tolerance due to agonist stimulation. The mechanism involved in the desensitization of beta-adrenoceptors is due to an uncoupling of the receptor with adenylate cyclase. This produces a reduction in cytoplasmic cAMP during subsequent beta-agonist stimulation (Jones et al., 1986). The mechanism for the development of desensitization in alpha-adrenoceptors has not been elucidated. Cheung (1986) showed that alpha-2-adrenoceptors became desensitized when subjected to cumulative doses of adrenoceptor agonist, but alpha-1-adrenoceptors did not show the same desensitization. In another study subjecting platelets to a short term infusion of alpha-2-agonists (Jones et al., 1986), the affinity of the platelet receptors for epinephrine decreased. Whether the desensitization of alpha-2-adrenoceptors is related to the same mechanism of desensitization in beta-adrenoceptors remains to be seen.

Conclusion and Objectives of the Proposed Study

1. In a clinical setting for minor surgical procedures, the animal frequently receives a combination of ketamine/xylazine to induce anesthesia; however, the animal may be currently receiving medication for a preexisting illness such as: phenobarbital for management of epilepsy or chloramphenicol (ChPC) for infectious diseases produced by ChPC susceptible pathogens, and cimetidine for treating

gastritis, gastric ulcer, and reflux esophagitis. It appears that ketamine and xylazine are metabolized by the liver; therefore, the concomitant use of drugs that alter the function of hepatic P-450 drug metabolizing enzymes could change the duration of anesthesia. The objective of this study is to investigate the effects of cimetidine and chloramphenicol (inhibitors of hepatic P-450 drug metabolizing enzymes) and phenobarbital (an inducer of hepatic P-450 drug metabolizing enzymes) on the duration of clinical anesthesia induced by ketamine/xylazine combination in dogs.

2. It was noted that the sedative effects of xylazine in dogs decreased when given the same dose a week later thus indicating that tolerance had developed for xylazine from the single dose injection initially (Moreau et al., 1983). It was also observed in our laboratory during studies in which the use of xylazine was employed that tolerance had developed from single doses given a week prior. Several studies in which mice and rats were subjected to acute and chronic doses of clonidine developed tolerance not only to clonidine but to other presynaptic inhibitory agents (Yamazaki and Kaneto, 1985; Ishii and Kato, 1984; Ishii et al., 1981). Xylazine, an analogue of clonidine, is also a selective alpha-2-agonist (Vizi, 1986; Docherty and McGrath, 1980) which inhibits the release of norepinephrine from nerve terminals as does clonidine (Langer, 1981). Clonidine is an alpha-2 adrenergic agonist that has displayed tolerance when used chronically.

But, it has been observed that Clonidine can develop acute tolerance to a single dose injection (Mastrianni and Ingenito 1985; Yamazaki and Kaneto 1985; Cheung 1986).

The objective of this study is to look for the effects of acute tolerance when administering single doses of xylazine a week apart.

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CHAPTER II

EFFECTS OF CHLORAMPHENICOL, CIMETIDINE, AND PHENOBARBITAL ON AND TOLERANCE TO XYLAZINE-KETAMINE ANESTHESIA IN DOGS

INTRODUCTION

The combination of xylazine and ketamine is a commonly used anesthetic agent in veterinary medicine. Xylazine is an alpha$_2$-adrenergic agonist with sedative, analgesic and muscle relaxant properties (1) and ketamine is a dissociative anesthetic (2).

Many chemicals and environmental agents can alter the activity of drug metabolizing enzymes by induction or inhibition. Commonly used drugs such as cimetidine, chloramphenicol and ketoconazole have been shown to alter the activity of other drugs by inhibition of hepatic drug metabolizing enzymes (3-6). Phenobarbital, a commonly used anticonvulsant, has been the most widely studied hepatic enzyme inducer in both man and animals (7).

The development of drug tolerance is an important problem for the evaluation of drug actions and for the determination of a schedule of drug administration (8). It

has been shown that chronic administration of clonidine (9-11), pentobarbital (12), morphine (8), alcohol (13), anticholinergics, chlorpromazine, imipramine (14) and other sedative-hypnotic drugs leads to development of tolerance.

Previous studies have shown tolerance to some pharmacological effects of clonidine (9-11, 15-17). Tolerance to sedative effect of xylazine, also an $alpha_2$ adrenergic agonist, has been noted in dogs (18).

The objectives of this study were (1) to investigate the effects of chloramphenicol, cimetidine and phenobarbital on the duration of clinical anesthesia induced by xylazine-ketamine combination and (2) to determine if there is any tolerance to sedative effects of xylazine in dogs.

MATERIALS AND METHODS

Animals: Thirty healthy male mongrel dogs weighing between 15 to 20 kg were purchased and used in this study. Each animal was allowed to adjust to the environmentally controlled conditions in a single restraining cage for a minimum of five days before the experiments were initiated. The animals were fasted for approximately 15 hours prior to the administration of any agents.

Drugs: Chloramphenicol (Chloromycetin® sodium succinate, Park-Davis, Morris Plains, NJ), phenobarbital (Elsin-Sinn Inc., Cherry Hill, NJ), cimetidine (Tagamet®, SK&F, Carolina,

PR), xylazine (Rompun®, Mobay, Shawnee, KS) and ketamine (Ketaset®, Bristol-Myers, Syracuse, NY) were purchased commercially.

Pretreatment Experiments: The animals were randomly divided into groups of 5 each. Group I served as control and received xylazine (1.1 mg/kg, i.v.) followed 10 min. later by ketamine (10 mg/kg, i.v.). The other groups were pretreated as follows: Group II, chloramphenicol sodium succinate (33 mg/kg, i.v., 15 min.); group III, cimetidine hydrochloride (5 mg/kg, i.v., 24 hours) and group IV, phenobarbital sodium (15 mg/kg, i.v., 96 hours). After pretreatment period, each animal received xylazine (1.1 mg/kg, i.v.) followed 10 min. later by ketamine (10 mg/kg, i.v.).

Tolerance Experiments: Animals in group V and VI were used in tolerance experiments. Group V received xylazine (1.1 mg/kg, i.v.) followed 10 minutes later by ketamine (10 mg/kg, i.v.). Group VI received xylazine (1.1 mg/kg, i.v.) alone. The treatment in group V and VI was repeated at 3 days intervals for 9 days.

Determination of the Duration of Anesthesia and Recumbency: The dogs were placed in lateral recumbency at the onset of anesthesia. The following behavioral parameters were measured and recorded: duration of absence of pedal reflex, duration for return of consciousness (eg. movement of

eyelids, ears, tail, or any body part), and duration for return of ambulation (ability to walk one step without aid of leash).

Statistical Analysis: Mean ± S.E.M. time in minutes for each group was calculated and the significance of treatments was determined by analysis of variance (19). A 95% probability level of was considered significant.

RESULTS

Effects of Cimetidine, Chloramphenicol, and Phenobarbital on Xylazine-Ketamine Anesthesia: Administration of xylazine (1.1 mg/kg, i.v.) followed by ketamine (10 mg/kg, i.v.) produced anesthesia with the duration or absence of pedal reflex, duration for return of consciousness, and duration for return of ambulation to be 33.2 ± 4.9, 33.4 ± 3.7 and 86.4 ± 11.7 minutes, respectively (Table 1). Administration of chloramphenicol (33 mg/kg, i.v.) and cimetidine (5 mg/kg, i.v.) prior to xylazine (1.1 mg/kg, i.v.) and ketamine (10 mg/kg, i.v.) combination did not produce significant changes in any of the above parameters (Table 1). When phenobarbital sodium (15 mg/kg, i.v.) was administered 96 hours prior to xylazine (1.1 mg/kg, i.v.) and ketamine (10 mg/kg, i.v.), a significant ($p < 0.05$) reduction in the duration of absence of the pedal reflex (33.2 ± 4.9 vs. 18.6 ± 1.6 min.), duration of loss of consciousness (33.4 ± 3.7 vs. 22.8 ± 2.4

min.), and no significant change in the time for return of ambulation (86.4 ± 11.7 vs. 62 ± 4.5 min.) was observed (Table 1).

Tolerance to Xylazine-Ketamine and Xylazine: Administration of xylazine (1.1 mg/kg, i.v.) followed by ketamine (10 mg/kg, i.v.) produced no significant reduction in the duration of absence of pedal reflex (33.2 ± 4.9 vs. 21.8 ± 1.7 vs. 22.2 ± 4.9 min.), duration of loss of consciousness (33.4 ± 3.7 vs. 28.8 ± 2.6 vs. 35.2 ± 3.5 min.), and duration for return of ambulation (86.4 ± 11.7 vs. 79 ± 9.7 vs. 67.6 ± 6.5 min.) of trials one, two, and three, respectively (Table 2). Administration of xylazine (1.1 mg/kg, i.v.) alone produced no significant reduction in the duration of lateral recumbency (28.6 ± 6.2 vs. 22.7 ± 6.5 vs. 14.6 ± 2.0 min.) over three consecutive trials (Table 3).

Table 1

Effects of chloramphenicol (33 mg/kg, i.v., 15 min.), cimetidine (5 mg/kg, i.v., 24 hrs) and phenobarbital (15 mg/kg, i.v., 96 hrs) on xylazine (1.1mg/kg, i.v.) and ketamine (10 mg/kg, i.v.) anesthesia in dogs.

Treatment	Duration of Absence of Pedal Reflex (min)	Duration of Anesthesia (min)	Ambulation Time (min)
Control	33.2 ± 4.9	33.4 ± 3.7	86.4 ± 11.7
ChPC	36.4 ± 8.5	46.6 ± 7.3	138.6 ± 20.0
CIM	29.6 ± 6.2	44.4 ± 6.1	74.0 ± 5.4
PHB	18.6 ± 1.6*	22.8 ± 2.4*	62.0 ± 4.5*

* Significantly different from control group ($p < 0.05$).

Table 2

Effects of repeated administration of xylazine (1.1 mg/kg, i.v.) and ketamine (10 mg/kg, i.v.) on the duration of anesthesia in dogs

Treatment	Duration of Absence of Pedal Reflex (min)	Duration of Anesthesia (min)	Ambulation Time (min)
1	33.2 ± 4.9	33.4 ± 3.7	86.4 ± 11.7
2	21.8 ± 1.7	28.8 ± 2.6	79.0 ± 9.7
3	22.2 ± 4.9	35.2 ± 3.5	67.6 ± 6.5

Table 3

Effects of repeated administration of xylazine (1.1 mg/kg, i.v.) on the duration of recumbency in dogs

Trial	Duration of Recumbency (min)
1	28.6 ± 6.2
2	22.7 ± 6.5
3	14.6 ± 2.0

DISCUSSION

The data presented in this study indicate that pretreatment with chloramphenicol (ChPC) and cimetidine (CIM) did not produce a significant reduction in the duration of xylazine-ketamine anesthesia. This is in contrast with the recent report that both chloramphenicol and cimetidine prolonged the duration of xylazine-ketamine anesthesia in rats (20). Information concerning the metabolic fate of xylazine in various species is limited. Xylazine is rapidly metabolized yielding about twenty metabolites in rats (21). It has been reported that xylazine is rapidly distributed and eliminated in horses, cattle, sheep, and dogs; thus, suggesting the rapid elimination may be due to intense metabolism instead of renal excretion, as evidenced by absence of the parent compound in the urine of sheep (22). These studies suggest that xylazine is metabolized by hepatic cytochrome P-450 drug metabolizing enzymes similar to ketamine. The major metabolic disposition of ketamine is N-demethylation by cytochrome P-450 enzymes (23, 24). The failure of chloramphenicol and cimetidine to prolong xylazine-ketamine anesthesia in dogs could be due to various factors. The dose of ChPC, shown to produce a significant prolongation of pentobarbital anesthesia in various laboratory animals varies from 1-300 mg/kg in dogs, cats, monkeys, rats, and mice (25-29). Within this group, a dose-dependent and dose-independent prolongation of

pentobarbital anesthesia was noted in mice and dogs, respectively (29, 27). The dosage (33 mg/kg) in this study was within the therapeutic range commonly used in veterinary medicine. Although there is a trend in prolongation of duration of anesthesia, it appears that in dogs, the inhibition of cytochrome P-450 is dose-dependent. In this study, a higher dose of ChPC could have affected the duration of anesthesia significantly. The hepatic cytochrome P-450 enzymes are composed of specific isozymes. ChPC has been shown to only inhibit a few but not all of the isozymes of the P-450 complex (3, 25, 30-32). Since only specific cytochrome P-450 isozymes are shown to be inhibited by ChPC, it is also possible that isozymes involved in xylazine and/or ketamine metabolism may not be affected by ChPC.

It appears that dose, route, frequency and method of administration could influence the degree of inhibition of hepatic cytochrome P-450 enzymes by cimetidine (33). Cimetidine could inhibit specific isozyme of cytochrome P-450 which are not involved in metabolism of xylazine and/or ketamine.

In addition, species variations may play a role. In contrast to the present study, both CIM and ChPC have been shown to significantly prolong xylazine-ketamine induced anesthesia in rats (20).

A significant reduction in xylazine-ketamine anesthesia with a therapeutic dose of phenobarbital (PHB) observed in dogs is consistent with previous studies (7, 14, 34, 35).

This suggests that PHB inducible cytochrome P-450 isozymes are involved in the metabolism of xylazine and/or ketamine in dogs in contrast to a recent study in rat (20).

The data from this study show acute tolerance, although not significant, to repeated administration of xylazine-ketamine combination and xylazine alone. The trend in development of acute tolerance to xylazine-ketamine was not as pronounced as that with xylazine alone. This suggests that xylazine might play the major role in the development of tolerance. It has been shown that repeated administration of clonidine leads to tolerance (9, 10, 15-17, 36). Tolerance to xylazine, like clonidine, may develop possibly due to desensitized central alpha-2 adrenoceptors (15). It is also possible that the development of acute tolerance to repeated administration of ketamine is mediated by self-induction of hepatic cytochrome P-450 drug metabolizing enzymes (37).

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CHAPTER III

SUMMARY AND CONCLUSIONS

The effects of chloramphenicol, cimetidine, and phenobarbital on the xylazine-ketamine induced anesthesia and the development of tolerance to xylazine-ketamine combination and xylazine alone were studied in dogs. The duration of absence of pedal reflex, duration of return of consciousness, and duration for return of ambulation were determined. Pretreatment with chloramphenicol (33 mg/kg, i.v., 15 min) and cimetidine (5 mg/kg, i.v., 24 hrs) did not influence any of the above parameters significantly. Phenobarbital pretreatment (15 mg/kg, i.v., 96 hrs) significantly reduced the duration of anesthesia. Although not significant, there is a trend toward the development of tolerance to repeated administration of xylazine (1.1 mg/kg, i.v.) and ketamine (10 mg/kg, i.v.) combination and xylazine (1.1 mg/kg, i.v.) alone once daily at 3 days intervals for 9 days. These results indicate that 1) hepatic cytochrome P-450 drug metabolizing enzymes, inhibited by chloramphenicol and cimetidine, might not be involved in the metabolism of xylazine and/or ketamine, 2) phenobarbital inducible hepatic cytochrome P-450 enzymes might play a role in metabolic

disposition of xylazine and/or ketamine, and 3) repeated administration of xylazine alone or in combination with ketamine might lead to the development of tolerance in dogs.

VITA 2

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Master of Science

Thesis: EFFECTS OF CIMETIDINE, CHLORAMPHENICOL, AND PHENOBARBITAL ON AND TOLERANCE TO XYLAZINE-KETAMINE INDUCED ANESTHESIA IN DOGS

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