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Cardiovascular tolerance and safety of intravenous lidocaine in the broiler chicken (Gallus gallus domesticus)

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CARDIOVASCULAR TOLERANCE AND SAFETY OF INTRAVENOUS LIDOCAINE IN THE BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*)

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ABSTRACT

Lidocaine, an amide local anesthetic agent, is commonly used in mammals, including humans. There is a general assumption that birds are more sensitive to lidocaine than mammals. Relatively low doses of lidocaine have been suggested to cause toxic effects in birds. While this information appears to be anecdotal, it has been perpetuated in the literature. The overall objective of this thesis research was to determine the tolerance and safety of intravenous lidocaine in broiler chickens. To assess the cardiovascular effects of lidocaine, relative changes on heart rate and mean blood pressure were calculated. Clinically significant cardiovascular effects were defined as relative decrease of heart rate and/or mean blood pressure equal to or greater than 30%. On the first study, doses below the reported toxic dose were assessed. The effects of 2.5, 3.0, 3.5 mg/kg intravenous lidocaine were compared with a control (saline) group. Each dose was used in 2 randomly selected animals. No significant cardiovascular effects were detected; therefore, higher doses were investigated. On a 2nd study, using an up-and-down study design, a total of 11 subjects were evaluated. The up-and-down method is a sequential design with binary response variables within a certain population which allows the determination of an effective dose to 50% of the population (ED_{50}). The ED_{50} was defined as the dose that would cause clinically insignificant cardiovascular depression to 50% of the population. Using two statistical methods, the ED_{50} of cardiovascular function was 6.3 mg/kg and 6.22 mg/kg (95% confidence interval, 5.3 – 7.13 mg/kg). The safety of this dose was then tested in a new group of broiler chickens. The dose of 6 mg/kg was administered to 6 animals. No clinically significant cardiovascular effects were detected in any animal. In conclusion, the 3 studies performed for this thesis indicates that the reported toxic dose of lidocaine appears to be erroneous.

Furthermore, this thesis determined the highest tolerable dose and its safety in a specific group of broiler chickens. Further studies assessing analgesia and anesthetic effects of lidocaine are necessary, both in chickens and other avian species.

CHAPTER ONE
INTRODUCTION

Birds have always drawn the attention of man and have been maintained in captivity for thousands of years. The use of birds by man, in particular birds of prey for the purpose of hunting, has been described for centuries, possibly tracing back to 10,000 BP in the Near East.(Epstein, 1943; Jaques and Dobney, 2002; Prummel, 1997) Although the use of birds by humans can be considered an ancient tradition, the discipline of avian medicine has advanced significantly in the last 40 years. The lack of scientific research that focuses on avian medical issues has led to a substantial amount of anecdotal information being reported and perpetuated in the literature. The use of allometric scaling for the conversion of data from, but not limited to, human medicine and small animal medicine has been reported for birds.(Frazier et al., 1995; Kabat et al., 2008; Pokras et al., 1993) However, lidocaine is said to be a poor candidate for pharmaceutical allometric scaling, although, no clear explanation is stated.(Hunter, 2010) The extrapolation of medical data from other species is useful and its contribution to avian healthcare is invaluable, however this nonspecific information may lead to overdose or underdose of therapeutic agents in birds. (Cunningham et al., 2010; Hunter et al., 2008) Comparison between predicated and observed data has proved to be grossly erroneous in some species for specific drugs.(Hunter et al., 2008)

Avian medicine includes the treatment of any living bird. Currently, at least 9,993 species of live birds have been reported.(Jetz et al., 2012) In the opinion of the author, it is unlikely that all avian species will equally respond to or require the same dose of a specific drug. An example of the inconsistencies of doses of the same drug between different avian species is tramadol. While Hispaniolan Amazon parrots (*Amazona ventralis*) require doses of 30 mg/kg orally, 3 to 4

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times a day to reach and maintain human therapeutic levels, peafowl (*Pavo cristatus*) require 7.5 mg/kg orally, once to twice a day.(Black et al., 2010; Souza et al., 2013) Among birds of prey, bald eagles (*Haliaeetus leucocephalus*) and American kestrels (*Falco sparverius*) require 5 mg/kg orally twice a day, however, red-railed hawks (*Buteo jamaicensis*) require 15 mg/kg orally, twice a day.(Guzman et al., 2014; Souza et al., 2009; Souza et al., 2011) Although interesting, such variability of doses exists among the avian species listed above. Moreover it is important to acknowledge that each of the identified avian groups have not only different anatomy but also different biology, feeding habits, and gastrointestinal tract anatomy and physiology which may lead to dissimilar bioavailabilities of the therapeutic agent.

Current thoughts do not consider lidocaine as an adequate therapeutical agent in avian species because of concerns associated with toxicity.(Figueiredo et al., 2008) It has been perpetuated in the literature that birds are more sensitive to local anesthetic agents than mammals. (Hall et al., 2001; Machin, 2005; West et al., 2007) Lidocaine has been reported to cause toxicity in birds, even at relatively low doses.(Carpenter, 2005; Carpenter and Marion, 2013) It is said that seizure, cardiac arrest, and mortality is likely to occur when lidocaine is administered in small bird species.(Fedde, 1978; Murray, 1967) The recommended therapeutic dose range of lidocaine is published as 1 to 3 mg/kg, although the route of administration is not clearly defined.(Carpenter, 2005) Reports in the literature suggest that a dose of 4 mg/kg or higher (unknown route) can be toxic to birds.(Carpenter, 2005; Huckabee, 2000; Ludders and Matthews, 2007; Machin, 2005; Paul-Murphy and Ludders, 2001; West et al., 2007) While the origin of such information is unclear, it appears to come from a publication that assessed the toxicological effect of subcutaneous lidocaine administration in budgerigars (*Melopsittacus undulatus*).(Grono, 1961) Although the dose of lidocaine and the body weight of the study

subjects are not clearly stated, the dose can be extrapolated. Using the body weight of 29g (Sibley, 2000), the doses were calculated and are summarize in table 1. Two animals died within 2 minutes after administration of approximately 345 mg/kg of lidocaine, 1 animal died within 2 minutes after administration of 172 mg/kg of lidocaine, and 1 animal died within 20 minutes after administration of 172 mg/kg of lidocaine. One animal became ataxic but recovered after the administration of 86 mg/kg of lidocaine. Based on the calculated doses and in the assumption that the toxic dose of 4 mg/kg has originated in this publication, the toxic dose is grossly wrong. The doses of 2.7–3.3 mg/kg intra-articular administration in chickens have also been suggested to cause toxic effects.(Carpenter and Marion, 2013) However, the citation appears to be erroneous as the original publication describes the effect of intra-articular bupivacaine but not lidocaine.(Hocking et al., 1997) Lidocaine may not be commonly considered an adequate therapeutic agent due to the presumptive risk of toxicity.(Figueiredo et al., 2008) Contrary to those recommendations, other studies have described the use of lidocaine in avian species. Pharmacokinetics of intravenous lidocaine at 2.5 mg/kg has been assessed in chickens (n=6). (Da Cunha et al., 2012) Intravenous lidocaine in chickens had a shorter half-life than humans, pigs, dogs, cats, and rabbits.(Da Cunha et al., 2012) Lidocaine has been used for the purpose of nerve plexus block in chickens (n=6), mallards (n=2), and Hispaniolan Amazon parrots (n=18) at dose rates of 20 mg/kg lidocaine with 10 µg/ml epinephrine, 15 mg/kg lidocaine with 3.8 µg/ml epinephrine, and 2 mg/kg lidocaine, respectively.(Brenner et al., 2010; da Cunha et al., 2013; Figueiredo et al., 2008) No mortality or morbidity related to the regional block was reported (Brenner et al., 2010; Figueiredo et al., 2008), however, one duck died as a result of endotracheal tube obstruction by tracheal secretions.(Brenner et al., 2010) Moreover, lidocaine has also been used for non-clinical research. (Dial, 1992; Takahashi et al., 1984)

Table 1. Dose calculation based on the information provided in a study assessing the effects of subcutaneous lidocaine in budgerigars (*Melopsittacus undulatus*).(Grono, 1961) Dose was calculated based on the body weight of 29 g.(Sibley, 2000)

Parakeet	Volume (ml)	Concentration (mg/ml)	Dose (mg/kg)	Outcome
1	0.5	20	345	Death within 2 minutes
2	0.5	20	345	Death within 2 minutes
3	0.25	20	172	Death within 2 minutes
4	0.5	10	172	Death within 20 minutes
5	0.25	10	86	Death within 20 minutes

Based on the available literature, there is a lack of scientific information regarding the use of lidocaine in avian species and the available information appears to be contradictive. The purpose of this thesis is to scientifically assess the use of lidocaine under controlled conditions. The author hypothesized that the previously referenced toxic lidocaine doses are erroneous and that higher dosages of this drug can be safely used in a specific strain of broiler chickens. The up-and-down methodology provided evidence that the currently published high dose of lidocaine, when administered intravenously, would not cause clinically significant cardiovascular effects in 50% of the study population. Further scientific evaluation described in this thesis was performed in order to confirm the safety of the determined dose.

CHAPTER TWO
LITERATURE REVIEW

2.1 Historical review

2.1.1 Local anesthetics

Local anesthetics have a common chemical structure which consists of a lipophilic aromatic ring, a link and a hydrophilic amine group, commonly tertiary amines.(Columb and MacLennan, 2007; Ostercamp and Brunsvold, 2006) Local anesthetics can be divided into 2 groups according to the link; amides (-NH-CO-) or esters (-O-CO-).(Columb and MacLennan, 2007) The class amide local anesthetics includes lidocaine, mepivacaine, bupivacaine, levobupivacaine, ropivacaine, etidocaine, prilocaine, and articaine.(McLure and Rubin, 2005) The ester group includes cocaine, procaine, chloroprocaine and amethocaine.(Columb and MacLennan, 2007)

2.1.2 Ester group: From cocaine to procaine

Prior to the development of the amide class of local anesthetics, cocaine was commonly used for this purpose. Sigmund Freud (1856-1939) and Carl Koller (1857-1944) were two of the first researchers to investigate the medical use of cocaine.(Galbis-Reig, 2002) Joseph Brettauner (1835-1905) and Carl Koller demonstrated the numbing effect of cocaine when applied to the eye of a dog in 1884.(Drasner, 2014; Grzybowski, 2008; Markel, 2011; McLure and Rubin, 2005) In front of an audience, Brettauner was able to touch the eye of an awake dog with surgical instruments without any kind of response after applying drops of cocaine to the corneas of the animal.(Markel, 2011) However, due to the addictive and toxic characteristics of cocaine as well as an inability to sterilize the drug, there was a need to develop safer drugs.(Drasner,

2010; Drasner, 2014; Scholz, 2002) In order to replace cocaine, Ritsert (1890) created benzocaine and Einhorn (1904) created procaine.(Boren et al., 2007) Although procaine quickly supplanted cocaine, in part because of a higher therapeutical index, procaine was still associated with toxic and abnormal reactions.(Drasner, 2010; Drasner, 2014; Scholz, 2002) Among mortality cases linked to the use of procaine, some were later determined to be due to the use of cocaine, most likely because of miscommunication since procaine and cocaine are similar in name and spelling.(Drasner, 2010) The use of procaine and other amino-esters for spinal anesthesia was popular for many decades.(Maltby et al., 2000) An important event led to criticism of the use and methodology of such drugs for spinal anesthesia. In 1947, Albert Woolley and Cecil Roe, two healthy middle-age men became paraplegic after each received spinal anesthesia for minor procedure at the Chesterfield Royal Hospital.(Maltby et al., 2000) Both anesthestic procedures were performed by the same anesthesiologist, in the same day, using the same drug (hypobaric 1:1500 cinchocaine).(Maltby et al., 2000; Morgan, 1995) This case had significant media and legal impact which led to questions relating to the use of spinal anesthesia and subsequent induction of paralysis.(Drasner, 2014; Hutter, 1990; Morgan, 1995) Although the anesthesiologist was considered not guilty and the drug contamination was believed to have occurred through a crack in the ampule, questions remained.(Cope, 1954; Drasner, 2014; Forrester, 1968) The "invisible crack" theory has been questioned over the years, however, no clear explanation has ever been provided.(Drasner, 2014; Hutter, 1990; Maltby et al., 2000; Morgan, 1995)

2.1.3 Amide group: From procaine to lidocaine

The first amide local anesthetic to be produced was niraquine, however, it was quickly abandoned because of local irritation to the surrounding tissue where it was administered.(Ruetsch et al., 2001) Other anesthetic compounds, including lidocaine were developed following the adverse reactions noted with the use of niraquine. The origin of lidocaine comes from a compound called gramine (Figure 1.).(Vale et al., 2005; Wildsmith, 2011) Gramine was initially isolated from a chlorophyll defective mutant of barley (*Hordeum vulgare*) by a Nobel prize awardee, Hans van Euler, and later from great reed (*Arundo donax*) by a group of Russian researchers.(Dahlbom and Hollman, 1991; Holmdahl, 1998) An isomer, isogramine, was isolated by Holger Erdman who noticed the numbing effect of isogramine but not gramine, when tasted.(Dahlbom and Hollman, 1991; Holmdahl, 1998) In the early 1940's, Löfgren and Erdtman synthesized several compounds originating from isogramine.(Wildsmith, 2011) Among the different compounds, one had an additional second methyl group in a ortho position on the ring structure.(Wildsmith, 2011) Using this compound, Löfgren and Lundqvist in 1943 were able to produced LL30 (Löfgren & Lundqvist, compound number 30), later known as lidocaine.(McLure and Rubin, 2005) Lidocaine, also known as lignocaine or xylocaine, was initially used as a replacement for procaine and other amino-ester local anesthetics. Once the significance and potential medical use of this compound was reported, Astra Pharmaceutical took over the commercialization and further development of lidocaine.(Holmdahl, 1998) For the next half a century, lidocaine was considered the gold standard of local anesthetics and since that time it has been widely used although newer drugs have been developed.(Drasner, 2014; Holmdahl, 1998)
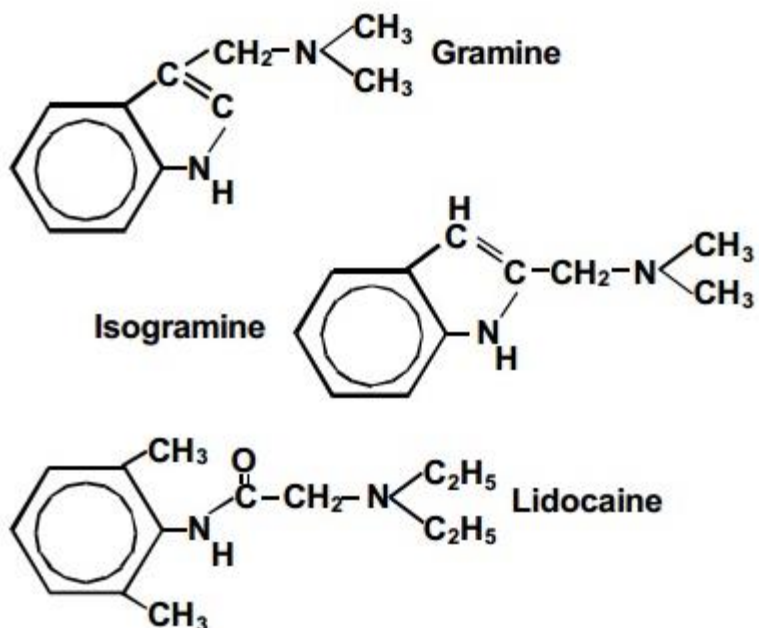
Figure 1. Chemical formula for gramide, isogramine and lidocaine. (Wildsmith, 2011)

2.2 Pharmacology

2.2.1 Local anesthetic pharmacology

The chemical structure of lidocaine consists of an aromatic group, 2,6-xylidine which is coupled to diethyglycine through an amide bond. (Collinsworth et al., 1974) The physiochemical properties of local anesthetic correlate to the lipid solubility, protein binding, and acid dissociation constant (pK_a) of the different drugs (Table 2).(Cousins and Bridenbaugh, 2009) The aromatic ring improves lipid solubility; increased lipid solubility implies increased potency.(Becker and Reed, 2006) The amine terminal may be in a lipid soluble tertiary form (3 bonds) or as a quaternary form (4 bonds) which is positively charged and water-soluble.(Becker and Reed, 2006) The amine terminal acts as an "on-off switch" allowing the local anesthetic to exist either on a lipid-soluble or water-soluble conformation.(Becker and Reed, 2006) As with other local anesthetic agents, lidocaine produces its effect by affecting nerve

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conduction.(Mofenson et al., 1983) Most local anesthetics impede the permeability of the neuron cell membrane to sodium.(Ragsdale et al., 1994; Subramaniam and Tennant, 2005) The sodium channels contain a larger α-subunit and one or two smaller β-subunits.(Neal and Rathmell, 2007) The α-subunit is the site for ion conduction and local anesthetic binding site.(Neal and Rathmell, 2007) Local anesthetic drugs are assumed to inhibit sodium channels by occupying the binding sites at the α-subunit.(Chernoff, 1990) However, more recent information has suggested that this pathway may be more complex.(Columb and Ramsaran, 2010) Local anesthetics also interact with potassium and G-protein-regulated channels.(Scholz, 2002)

Ion channels are transmembrane proteins which can control the passive transport of ions, usually categorized as K^+, Na^+, Ca^{2+} or Cl^-.(Zhorov and Tikhonov, 2004) The generation and propagation of the afferent (sensory) and efferent (motor, sympathetic) information requires the flow of specific ions through the sodium channels.(Eappen and Datta, 1998) The action potentials are transient membrane polarizations which occur due to increase of sodium and delayed increase of potassium.(Strichartz and Ritchie, 1987) In normal conditions, the sodium channel opens briefly during the action potential allowing the extracellular sodium to enter the cell and depolarize the membrane, after which the sodium channel is inactivated.(Neal and Rathmell, 2007) The sodium channels exist in three native conformations; resting, open, and inactivated.(Neal and Rathmell, 2007) Local anesthetic agents have a higher affinity towards open or inactivated sodium channels.(Neal and Rathmell, 2007) Local anesthetics reversibly bind and block certain membrane channels which prevent electrical impulses from being generated or propagated.(Eappen and Datta, 1998)

Table 2. Psycochemical properties of selected local anesthetics in Humans. Adapted from Lagan 2004.(Lagan and McLure, 2004)

Agent	Molecular weight	pK$_a$	Speed of onset	Partition co-efficient	Potency	Protein binding (%)	Duration	Toxicity	Maximum plain dose (mg/kg)	Maximum dose with vasoconstrictors (mg/kg)
Amide agents										
Lidocaine	234	7.7	Fast	43	Intermediate	64	Intermediate	Low	5	7
Mepivacaine	246	7.9	Slow	21	Intermediate	77	Intermediate	Low	5	7
Bupivacaine	288	8.1	Slow	346	High	95	Long	High	2	3
Levobupivacaine	288	8.1	Slow	346	High	96	Long	Intermediate	2.5	3
Ropivacaine	274	8.1	Slow	115	Intermediate	94	Intermediate	Intermediate	2.5	4
Prilocaine	220	7.9	Fast	25	Intermediate	55	Intermediate	Low	5	8
Articaine	321	7.8	Fast		Intermediate	95	Intermediate	Low	7	
Ester agents										
Cocaine	303	8.7	Slow		High	98	Long	Very high	1.5	
Procaine	236	8.9	Slow	1.7	Low	6	Short	Low	8	10
Tetracaine	264	8.2	Slow	221	Intermediate	76	Intermediate	Intermediate	1.5	2.5

2.2.2 Lidocaine pharmacology

The main metabolic pathway of lidocaine is hepatic oxidative dealkylation into monoethylglycinexylidide (MEGX) followed by hydrolysis of this metabolite to 2,6-xylidide.(Reichel et al., 1998; Stoelting and Hillier, 2006) Studies on liver homogenates have indicated that the microsomal enzyme system is primarily responsible for the hepatic metabolism of lidocaine.(Collinsworth et al., 1974) Monoethylglycinexylidide has a prolonged elimination half-life and accounts for approximately 80% of the drug's activity, while xylidide only provides approximately 10% of the lidocaine activity to protect against cardiac dysrhythmias.(Stoelting and Hillier, 2006) In humans, the main clearance mechanism of lidocaine is through the liver which results in a half-life elimination of 1.4 to 8 hours.(Stoelting and Hillier, 2006) Further conversion of 2,6-xylidine to 4-hydroxy-2,6-xylidine is apparent with lidocaine, as more than

70% of the oral dose of this drug is detected in the urine over a 24 hour period.(Collinsworth et al., 1974) Other degradative pathways have been reported to produce small amounts of metabolites (e.g. 3-hydroxylidocaine, 3-hydroxymonoethylglycinexylidide, glycinexylidide).(Collinsworth et al., 1974)

The therapeutic plasma concentration of lidocaine in humans has been reported to be 1 to 5 µg/ml.(Stoelting and Hillier, 2006) In humans, lidocaine is stated to have an age-dependent kinetic effect.(Rademaker and de Vries, 2008) Moreover, lidocaine was shown to have similar interactions with the sodium channels of the heart, nerve, and skeletal muscle.(Bean et al., 1983) As previously stated lidocaine, similar to other local anesthetic drugs, is associated with a decrease in neuronal excitability by blocking voltage-dependent sodium channels. The pharmacologic effect of lidocaine on the nervous system enables this drug to have anti-seizure, and antiarrhythmic properties.(De Giorgio et al., 1992; Walker and Slovis, 1997) The potency of local anesthetic drugs is directly correlated to the lipid solubility of the agent in question.(Becker and Reed, 2006, 2012) Since bupivacaine has increased lipid solubility and is therefore more potent than lidocaine, the commercially available formulations are 0.5% versus 2%, respectively.(Becker and Reed, 2006) Interestingly, class I antiarrhythmic drugs, which block the calcium channels in a manner similar to local anesthetic agents, also have local anesthetic effects.(Tzeng et al., 2007)

As lidocaine is metabolized by the liver, hepatic disease, or decreased hepatic blood flow (i.e. not uncommon during anesthetic procedures), may decrease lidocaine metabolism.(Stoelting and Hillier, 2006) In human patients administered oral lidocaine, prior to being anesthetized for laparoscopic procedures, study results suggested a delayed rise of plasma concentration secondary to slow absorption and decreased elimination of lidocaine. Consequently an impaired

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metabolism of lidocaine in the human surgery subjects was suggested.(Adjepon-Yamoah et al., 1973) Although not statistically significant, intravenous lidocaine clearance rate was higher in patients receiving chronic antiepileptic therapy, which suggested a secondary effect associated with stimulation of hepatic first-pass metabolism by antiepileptic drugs.(Perucca and Richens, 1979) Other drugs have been shown to interact with lidocaine (e.g. atropine (Adjepon-Yamoah et al., 1974), erythromycin (Olkkola et al., 2005), cimetidine (Feely et al., 1982; Knapp et al., 1983) propranolol (Branch et al., 1973)). Maternal clearance of lidocaine is also prolonged by pregnancy induced hypertension.(Ramanathan et al., 1986) Moreover during pregnancy placental transfer occurs leading to higher fetal concentrations of lidocaine than that of the mother at the time of delivery.(de Carvalho Cavalli et al., 2004)

Due to its pharmacological characteristics, lidocaine is used for a wide range of medical purposes. The most basic description of the mechanism of action of local anesthetics has been reported as blocking the inward movement of sodium at the sodium ionophore during depolarization which prevents the propagation of the axonal action potential.(Columb and MacLennan, 2007) As with other local anesthetic agents, an important use of lidocaine is for regional or topical anesthesia. Furthermore, lidocaine can be used to treat arrhythmias (Collinsworth et al., 1974), convulsions (Ragsdale et al., 1996), pain (Rowbotham et al., 1991), anti-inflammatory (Stoelting and Hillier, 2006), and cough (Adcock et al., 2003). Intravenous anesthesia has also been used as a part of a balanced anesthetic protocol in order to decrease the minimum alveolar concentration of volatile anesthetics.(Doherty and Frazier, 1998; Hendrickx et al., 2008; Valverde et al., 2004) The uses of lidocaine for different medical presentations are related to the drug's effects on the sodium channels.

The velocity of effect of sensorial anesthesia using local anesthetics is dependent on the pK_a which represents the amount of local anesthetic that exists in the active nonionized form at the pH of the surrounding tissue.(Stoelting and Hillier, 2006) The clinical onset of lidocaine activity is approximately 3 minutes, while bupivacaine, levobupivacaine, or ropivacaine require approximately 15 minutes. The shorter onset of action of lidocaine is a result of the compound having a greater molecular fraction that exist in the lipid-soluble nonionized form.(Stoelting and Hillier, 2006)

The pharmacokinetics of lidocaine has been described in humans, rabbits, cats, horses, pigs, sheep, and dogs.(Bennett et al., 1982; Boyes et al., 1971; Feary et al., 2005; Finholt et al., 1986; Morishima et al., 1979; Orszulak-Michalak et al., 2002; Satas et al., 1997; Thomasy et al., 2005; Wilcke et al., 1983) To the author's knowledge, no such study has been performed in reptiles. Among avian species, pharmacokinetics of lidocaine has only been investigated in the chicken (n=6).(Da Cunha et al., 2012) After the administration of 2.5 mg/kg intravenous lidocaine, the plasma levels of lidocaine, MEGX and glycinexylidine (GX) were determined.(Da Cunha et al., 2012) The elimination half-life of lidocaine was 25.54 (±9.39) minutes, which was faster than humans, dogs, cats, and rabbits; however, it was similar to the results of sheep.(Da Cunha et al., 2012) The pharmacokinetic profile of GX in chickens was not described as the maximum concentration could not be determined within sampling period.(Da Cunha et al., 2012) The mechanism of metabolism and elimination of lidocaine in the chicken and mammals appear to be similar as there is a rapid initial increase of MEGX followed by rapid decrease in concentration post intravenous administration of lidocaine as well as a steady increase of GX overtime.(Da Cunha et al., 2012)

2.3 Lidocaine uses

2.3.1 Lidocaine as an anticonvulsive

Status epilepticus (SE) is characterized by prolonged seizure activity.(Lowenstein, 1998) Status epilepticus is commonly defined as seizure activity that last for 5 or more minutes of continuous clinical and/or electrographic seizure activity or recurrent seizure activity without recovery.(Brophy et al., 2012) The definition of SE is not precise although this condition has been long been recorded in human history.(Lowenstein, 1999) In children, the greatest seizure risk occurs during the neonatal period (1.8-3.5/1,000 live births in the United States).(Silverstein and Jensen, 2007) Several characteristics are used to distinguish between seizures that occur during the neonatal period and those observed in older children; neonatal seizures are usually behaviorally subtle and the electroencephalogram reflects a multifocal process while in older children, coordinated seizure activity is more common.(Silverstein and Jensen, 2007) Among adults, generalized convulsive status epilepticus and non-convulsive status epilepticus are important neurological conditions potentially associated with significant mortality and morbidity rates, with the annual incidence reported in Europe being 3.6 to 6.6 per 100,000 and 2.6 to 7.8 per 100,000, respectively.(Meierkord et al., 2006; Meierkord et al., 2010) The initiation of status epilepticus is a consequence of an inability of normal mechanisms to terminate seizures, decrease inhibition, and regulate persistent excessive excitation.(Meierkord et al., 2006) The main causes of SE are, low blood concentration of antiepileptic medication with chronic epilepsy (34%), remote symptomatic causes (24%), cerebrovascular accidents (22%), anoxia or hypoxia (~10%), metabolic causes (~10%), and alcohol and drug withdrawal (~10%).(Chen and Wasterlain, 2006) Several other underlying etiologies have been reported in humans, which are divided in acute processes (e.g. sepsis, central nervous system infection, head trauma, hypertensive

encephalopathy, autoimmune encephalitis) or chronic processes (e.g. chronic ethanol abuse, CNS tumors, remote CNS pathology).(Brophy et al., 2012) Both intravenous lidocaine and mepivacaine have suppressed grand mal seizures due to initial depression of hyperexcitable cortical neurons.(Stoelting and Hillier, 2006)

In animal models, the tendency of SE is to become self-perpetuating as seizures rapidly become self-sustaining and continue long after the withdrawal of the epileptogenic stimulus, although this does not seem to occur in humans.(Chen and Wasterlain, 2006) With the previous premise that SE may be self-perpetuating there is a need for early seizure control, therefore optimum treatment may be achieved by early administration of intravenous anticonvulsants.(Chen and Wasterlain, 2006) Several medications (e.g. benzodiazepines and antiarrhythmics) have been studied to induce early seizure control.(Shorvon, 1994) In humans, only a few controlled, double-blinded, clinical studies have assessed treatments for SE. Lorazepam and diazepam were compared and no difference was detected.(Leppik et al., 1983) Other studies comparing lorazepam and diazepam reported that both drugs were useful for the treatment of SE, but lorazepam is recommended as a first-line therapy.(Alldredge et al., 2001; Cock and Schapira, 2002; Gathwala et al., 2012) Lorazepam was also considered superior to phenytoin but no significant difference was reported in comparison with phenobarbital and diazepam in combination with phenytoin.(Treiman et al., 1998) Research investigations have reported a time-dependent loss of benzodiazepine potency suggesting this class of drugs should not be used alone or to control seizures lasting longer than 30 minutes.(Kapur and Macdonald, 1997; Mazarati et al., 1998) There is a need for the assessment of other therapies for SE using ketamine(Borris et al., 2000) or levetiracetam alone or in combination with diazepam(Mazarati et al., 2004), or lidocaine(Hamano et al., 2006).

Some authors suggest that lidocaine does not provide advantage over conventional medications for treatment of SE and have more severe side effects.(Manno, 2003) Nevertheless, lidocaine is considered a second line drug that can be used in cases of refractory SE.(Chen and Wasterlain, 2006; Fallah, 2009; Fallah and Gofrani, 2007; Rossetti and Lowenstein, 2011; Sugiyama et al., 2004) The usefulness of lidocaine is its short half-life and relative lack of respiratory or cerebral depressant effects.(Shorvon, 1994) Intravenous lidocaine for the treatment of SE has been assessed in human infants. Lidocaine was efficient to control seizure activity in 19 of 53 convulsive episodes in children with a mean age of 3 years and 7 months (SD 3y 5mo).(Hamano et al., 2006) Favorable properties of lidocaine include prompt response, less alterations of consciousness, and fewer adverse effects, however, less than 50% of the convulsive episodes responded to therapy.(Hamano et al., 2006) In other studies, lidocaine was considered useful or very useful in approximately 50% of the assessed SE cases.(Hattori et al., 2008; Sugiyama et al., 2004; Yildiz et al., 2008) In a scientific study investigating the use of lidocaine, continuous infusion of the drug was recommended over bolus administration.(Hattori et al., 2008) After intravenous administration, distribution of lidocaine to vascular organs was fast and the drug able to cross the blood-brain barrier.(Shorvon, 1994) Lidocaine has also originally reported to have neuroprotective characteristics (Mitchell, 2002; Mitchell et al., 1999), however later studies have indicated that the drug has none of these properties (Mitchell et al., 2009). The initial erroneous conclusions were reported to be a consequence of a type 1 error.(Mitchell et al., 2009)

2.3.2 Lidocaine as an antiarrhythmic

The antiarrhythmic effects of local anesthetic agents including procaine and related compounds have been described.(Burstein, 1946; Joseph et al., 1951; Kayden et al., 1958; Mark et al., 1951) However, these drugs have significant cardiovascular effects (e.g. hypotension), possibly due to impaired myocardial contractibility, lower cardiac output, and systemic arterial pressure.(Aserman, 1953; Harrison et al., 1963; Mason and Pelmore, 1953) Lidocaine when administered at clinically therapeutic doses is an effective antiarrhythmic and preferable to procaine amide.(Harrison et al., 1963) Historically lidocaine has been mainly used for the suppression of ventricular dysrhythmias, in particular for suppressing reentry cardiac dysrhythmias (e.g. premature ventricular contractions, ventricular tachycardia).(Stoelting and Hillier, 2006) Cardiac effects of lidocaine have been reported to be; tonic block, faster recovery kinetics, shortens the action potential, shortens effective refractory period, and decreases normal automaticity.(Gintant and Hoffman, 1987) Furthermore, lidocaine has a fast clinical effect as well as a prompt cessation of effects once intravenous administration is discontinued.(Stoelting and Hillier, 2006) Lidocaine has become a primary antiarrhythmic drug and its introduction has resulted in a 30% decrease in the fatality rate of humans that present with acute myocardial infarction.(Lown, 1981) However, the prophylactic use of lidocaine to treat the early stages of acute myocardial infarction is not recommended as it may increase the occurrence of fatal bradysdysrhythmias.(Stoelting and Hillier, 2006) Recently, other medications, including amiodarone has been reported to be superior to lidocaine for the treatment of shock-resistant out-of-hospital ventricular fibrillation.(Dorian et al., 2002) The use of intravenous lidocaine has also been shown to increase the defibrillation threshold.(Stoelting and Hillier, 2006)

The antiarrhythmic effect of lidocaine is due to delayed rate of spontaneous phase 4 depolarization either by prevention or diminishing the gradual decrease in potassium ion

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permeability, and changes to the action potential of the cardiac myocytes secondary to sodium permeability.(Neal and Rathmell, 2007; Stoelting and Hillier, 2006) The effect of lidocaine in voltage clamped rabbit Purkinje fibers was a block effect on the cardiac sodium channels.(Bean et al., 1983) The blocking effect may be antagonized by external sodium ions with blocking/unblocking rates being voltage-dependent.(Zamponi et al., 1993) At therapeutic doses, lidocaine has no significant side effect on QRS or QTc intervals, however, at high doses it can decrease conduction in the atrioventricular node and His-Purkinje system.(Stoelting and Hillier, 2006)

The antiarrhythmic effects of lidocaine are an effect of the drug on the rested, activated, and inactivated sodium channels of the cardiac tissue.(Hondeghem and Katzung, 1977) Changes on the myocardial electrical field cause rapid structural transformations of the cardiac sodium channels.(Balser, 2001) Cardiac sodium channels are key molecular substrates in both inherited and acquired disorders of cardiac excitability, therefore, the blockade of the sodium channels by local anesthetics can be used for the treatment of specific disease conditions.(Balser, 2001) However, significant mortality secondary to the use of class I antiarrhythmic drugs for ventricular ectopy suppression after myocardial infarction has been associated with arrhythmias and acute recurrent myocardial infarction.(Echt et al., 1991)

Cardiac toxicity secondary to local anesthetics has also been reported. The rank order, from highest to lowest cardiotoxic potency of local anesthetic drugs is tetracaine, etidocaine, R(+) bupivacaine, racemic bupivacaine, levobupivacaine, ropivacaine, mepivacaine, lidocaine, and prilocaine.(Heavner, 2002) Although lidocaine can have cardiotoxic effects, it is also used for the purpose of local anesthetic neuro- and cardiotoxicity treatments. After an accidental intravascular administration of bupivacaine via epidural catheter, the patient was treated with

lidocaine, propofol, and 20% lipid emulsion which resulted in a rapid recovery from the cardiotoxic event.(Zimmer et al., 2007) However, in canine patients, the use of lidocaine to treat bupivacaine induced cardiovascular toxicity was not effective.(Kasten and Martin, 1985) Conversely, lipid emulsion appears to be useful in treating bupivacaine induced cardiovascular toxicity. Although double blinded, placebo controlled studies are lacking, the use of lipid emulsion to treat local anesthetic induced cardiac arrest appears to be beneficial.(Brull, 2008; McCutchen and Gerancher, 2008)

2.3.3 Lidocaine as an analgesic drug

As previously mentioned the different therapeutic effects of lidocaine result from its effect on the sodium channels. The analgesic effect of lidocaine is also related to the blockade of sodium channels. Furthermore, although not clearly described, systemic lidocaine administration may suppress spontaneous ectopic discharges of injured nerves without blocking normal nerve conduction.(Mao and Chen, 2000) In cases of diabetic neuropathic pain, the analgesic effect may be centered at the spinal level.(Bach et al., 1990) Reports indicate that lidocaine will provide local analgesia when the animal is treated in one of the following methods: local administration, transdermal patches, or intravenous administration.(Boas et al., 1982; Gammaitoni et al., 2003; Kolesnikov et al., 2000) Intravenous lidocaine and procaine produce significant analgesic effects but the use of these drugs in this manner is limited by the margin of safety between intravenous analgesia and systemic toxicity. However low-dose continuous rate infusion of both lidocaine and procaine decreases post-operative pain and reduces the need of opioids.(Stoelting and Hillier, 2006)

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2.3.3.1 Lidocaine as an intravenous analgesic agent

In humans, the use of intravenous lidocaine for the treating painful disease conditions has been well described and its first use was first reported in 1961.(Bartlett and Hutaserani, 1961) Since the first reported use of lidocaine, several studies have investigated the use of intravenous lidocaine in humans, specifically to determine its effectiveness in treating neuropathic pain.(Kastrup et al., 1987; Mao and Chen, 2000) Lidocaine has been compared to morphine for the treatment of post-amputation pain.(Wu et al., 2002) Although there are reports that lidocaine is more efficient than morphine for the treatment of stump pain, it was not effective in reducing phantom pain.(Wu et al., 2002) Nevertheless, lidocaine appears to be similar to morphine in reducing the intensity of neuropathic pain.(Rowbotham et al., 1991) Lidocaine is also effective in reducing pain associated with rocuronium injection.(Cheong and Wong, 2000) Moreover, perioperative low dose lidocaine infusion decreased postoperative pain and postoperative analgesic consumption.(Baral et al., 2010) Other studies indicate that perioperative and/or postoperative intravenous lidocaine was beneficial in diminishing post-operative pain, bowel function restoration, and length of hospitalization.(Baral et al., 2010; Groudine et al., 1998; Marret et al., 2008; Tikuisis et al., 2013) The use of intravenous lidocaine for analgesic purposes is not without controversy. Some publications have reported no significant analgesic effects associated with lidocaine use; furthermore, it is hypothesize that in order to reach adequate plasma levels, toxicity may occur.(Baranowski et al., 1999; Hempenstall et al., 2005; Martin et al., 2008)

The analgesic effects of intravenous lidocaine have also assessed in animals, primarily laboratory animals used as human models. In Sprague-Dawley rats, intravenous infusion of lidocaine prevented or reversed development of neuropathic pain.(Smith et al., 2002) The use of

intravenous lidocaine appears to be useful controlling some aspects of experimental allodynia in rats.(Sinnott et al., 1999) Clinical analgesic effects of intravenous lidocaine have also been assessed in dogs. A pilot study reported that lidocaine produced similar effects to that of morphine in anesthetized dogs undergoing intraocular surgery.(Smith et al., 2004) However, in another study, high rates of lidocaine did not show any antinociceptive effects when compared to saline in conscious dogs.(MacDougall et al., 2009) Nevertheless in the same study the canine subjects had a mild to moderate sedative effect and signs of toxicity associated with lidocaine administration.(MacDougall et al., 2009)

2.3.3.2 Lidocaine as part of a balanced analgesia protocol

Pain is considered to be the most important post-operative adverse side-effect that contributes to patient distress, prolonged hospitalization, and increased postoperative re-hospitalization.(Jin and Chung, 2001) Opioid and anti-inflammatory drugs alone may not result in successful post-operative pain management.(Jin and Chung, 2001) Therefore, a multimodal analgesic protocol is the currently recommended technique to properly manage post-operative pain. Multimodal analgesia is based on the combination of several analgesic agents that produce synergistic effects thereby minimizing the potential adverse side-effects of the drugs if administered alone.(Jin and Chung, 2001) A combination of local anesthetic agents and general anesthesia improves the management of a patient's pain after surgery.(Kaufman et al., 2005) Multimodal anesthesia using local anesthetic agents and gabapentin led to reduced acute and chronic pain after breast cancer surgery in humans.(Fassoulaki et al., 2005) When a multimodal analgesic (morphine and local anesthetic) protocol was implemented in human patients on which a total knee arthroplasty procedure was performed, an improved analgesic effect, with minimal

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adverse side effects, was reported.(Vendittoli et al., 2006) Significant attention has been focused by researchers on prevention of pain associated with the injection of propofol. Among 6,264 patients, 70% reported pain or discomfort when propofol was administered.(Picard and Tramer, 2000) Lidocaine mixed with propofol has been reported to prevent propofol injection induced pain, however, it is suggested to be due to pH changes in the "cocktail" rather than local anesthetic effects.(Eriksson et al., 1997) Combination of lidocaine and dexamethasone has also been reported to be efficient in controlling pain associated with propofol injection.(Kwak et al., 2008) Lidocaine alone or in combination with remifentanil abolished moderate to severe pain associated with propofol injection.(Aouad et al., 2007) However, ketamine followed by vein occlusion has been reported to be more efficient than lidocaine in reducing pain associated with propofol injection.(Saadawy et al., 2007)

The use of lidocaine as part of a multimodal or balanced analgesic protocol has not been established in avian medicine. In mammals lidocaine is considered safe, lacks adverse side-effects when compared with other drugs, and its analgesic effect appears to be more consistent than that of ketamine.(Corletto, 2007) The use of lidocaine as part of a balanced analgesia has been assessed in dogs.(Almeida et al., 2010) Also, lidocaine confers hemodynamic stability and prevents ischemic/reperfusion injury in experimental animal models.(Corletto, 2007)

2.3.4 Lidocaine as an anesthetic agent

2.3.4.1 Lidocaine as part of a local and regional anesthetic protocol

In the clinical perspective, the terminology local anesthetic implies a substance that blocks sensory and motor innervation of a peripheral area or region of the body.(Garfield and Gugino, 1987) As previously stated, Sigmund Freud and Carl Koller were two of the first

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researchers to investigate the medical use of cocaine.(Galbis-Reig, 2002) The first attempt to use cocaine as a local anesthetic in a canine patient was performed by Koller and Brettauer in 1884.(Grzybowski, 2008; Markel, 2011) In front of an audience, Brettauer was able to touch the eye of an awake dog with surgical instruments without any kind of response after applying drops of cocaine to the corneas of the animal.(Markel, 2011) Since this first demonstration of the medical use of local anesthetics, many technological developments have been made.

Lidocaine is one of the most widely used drugs to produce local anesthesia in humans.(Vahatalo et al., 1993) Local anesthesia is the corner stone of anesthesia in several medical specialties (e.g. dentistry). Some authors suggest that it is impossible to provide proper dental care without the use of local anesthetics.(Becker and Reed, 2006) Reports indicate that approximately 20% of dental patients undergoing endodontic procedures experience moderate to severe pain, while 1 to 3% describe an sudden increase of severe pain.(Hargreaves and Keiser, 2002) Local infiltration of local anesthetic agents implies the extravascular administration of the drug in the area to be anesthetized.(Stoelting and Hillier, 2006) In human dentistry, lidocaine is considered the gold standard for local anesthetic drugs.(Kanaa et al., 2006) Several other therapeutic agents have been compared to lidocaine. When evaluating 2% lidocaine (1:100,000 epinephrine) to 4% prilocaine and 3% mepivacaine, no significant difference was reported between the drugs when the inferior alveolar nerve was blocked for 50 minutes.(McLean et al., 1993) However, articaine was believed to be superior for mandibular buccal infiltration anesthesia.(Kanaa et al., 2006; Robertson et al., 2007) Articaine appears to enhance the effectiveness of lidocaine when anesthetizing the inferior alveolar nerve.(Kanaa et al., 2009) It appears that articaine, when administered in larger volumes may cause local discomfort.(Corbett et al., 2008)

Addition of epinephrine to the local anesthetic agent at 1:200,000 epinephrine can double the duration time of the local anesthesia effect.(Stoelting and Hillier, 2006) Furthermore, the addition of vasoconstrictors to local anesthetic therapeutic agents is beneficial in terms of depth of anesthesia, blood loss, and reduction of systemic toxicity associated with the drug use..(Brown and Rhodus, 2005) Other authors also suggest decrease in the peak plasma concentration of the local anesthetic agent, increase quality of anesthesia, and reduction of the minimum concentration of the anesthetic agent required for the nerve block.(Sisk, 1992) The addition of epinephrine to local anesthetic drugs may lead to increase presynaptic β_2 receptors on sympathetic nerve endings and the adrenomedulla which also causes a release of endogenous epinephrine.(Takahashi et al., 2005) The combination of epinephrine and local anesthetic agents has been said to have possible side effects when administered to areas supplied by end-arteries (e.g. fingers, ears and, nose).(Stoelting and Hillier, 2006) The use of such drug combinations for digital blocks has long been considered dangerous as it has been associated with digital necrosis.(Krunic et al., 2004) However, literature reviews have failed to provide evidence to substantiate the association of epinephrine/local anesthetic drug use to digital necrosis.(Krunic et al., 2004) Nevertheless, the use of local anesthetic agents in dentistry procedures is said to have a low adverse side effect risk with complications reported to be 5.7% (risk factor group) and 3.5% (nonrisk factor group).(Daublander et al., 1997)

Regional anesthesia is a therapeutic protocol that provides either central or peripheral anesthetic effect to a large part of the body. Administration of local anesthetics to the epidural space (central) or plexus (peripheral) are examples of regional anesthesia. Epidural administration is considered one of the most difficult procedures to be learned by human anesthesia residents.(Konrad et al., 1998) During the early onset of regional anesthesia, the

impulse transmission is not completely blocked; local anesthetics decrease the frequency and the amplitude of the action potential.(Grabinsky, 2005) When the local anesthetic drug is deposited in the vicinity of the nerve, the drug diffuses from the outer surface or mantle to the center (core) due to the concentration gradient, therefore, nerve fibers of the mantle are anesthetized first.(Stoelting and Hillier, 2006) The mantle fibers are more abundant in more proximal nerves, therefore anesthetic onset occurs first on the more proximal tissue.(Stoelting and Hillier, 2006)

Peripheral nerve block, as means of regional anesthesia, are widely used in human medicine.(Casati et al., 2007) Peripheral nerve blocks has few cardiovascular or pulmonary side effects, however, some potential complications can occur including systemic side effects of local anesthetics, phrenic nerve block, and blockade of nerves which compromise respiration.(Kettner et al., 2011) Nerve stimulation has become the gold standard for appropriate delivery of local anesthetic agents to achieve a nerve blockade.(Casati et al., 2007) Recently, the use of ultrasound imaging has increased allowing clinicians to visualize the nerve when administering the drug to perform regional anesthesia procedures.(Casati et al., 2007) It is suggested that the use of imaging guidance improves nerve block success and reduces the likelihood of possible adverse side effects.(Eichenberger et al., 2009; Latzke et al., 2010; Marhofer and Chan, 2007; Marhofer et al., 2010; O'Donnell and Iohom, 2009) The use of ultrasound imaging for brachial plexus blockade using lidocaine has been studied in Hispaniolan Amazon parrots.(da Cunha et al., 2013) Although the ultrasound guided technique led to a faster onset of the nerve block in parrots, neither technique produced an effective block.(da Cunha et al., 2013) In humans, multiple injections using ultrasound imaging provided similar success with nerve stimulation guidance.(Casati et al., 2007) Currently, an increased use of ultrasound guided administration of

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local anesthetic agents for the purpose of nerve blockade may not be possible due to financial constraints associated with this procedure.(De Andres and Sala-Blanch, 2002)

The use of lidocaine for the purpose of regional block has been studied in a small number of avian species. Brachial plexus blockade in mallards (n=8) using lidocaine at 15 mg/kg with 3.8 μg/ml epinephrine and bupivacaine at 2 mg/kg and 8 mg/kg, has been reported.(Brenner et al., 2010) In that study, 2 novel brachial plexus nerve block techniques (dorsal and axillary) were assessed by electrophysiologic methods.(Brenner et al., 2010) Results were highly variable and no technique significantly decreased cord dorsum potentials or resulted in consistent wing droop.(Brenner et al., 2010) In chickens (n=6), brachial plexus blockade by nerve detector guidance has been investigated using lidocaine (20mg/kg with 10μg/ml epinephrine) or bupivacaine (5 mg/kg with 10μg/ml epinephrine).(Figueiredo et al., 2008) Overall, the success rate of the nerve block described above was 66.6%. (Figueiredo et al., 2008) Lidocaine caused faster loss of motor and sensory function than bupivacaine; however, it was shorter acting.(Figueiredo et al., 2008)

The administration of local anesthetic agents into the epidural space, provide anesthesia by 2 mechanisms. Local anesthetics diffuse across the dura and act on the nerve roots and spinal cord and also diffuse into the paravertebral area through the intervertebral foramina which produces several paravertebral blocks.(Stoelting and Hillier, 2006) The description of epidural space access has been well described in small and large animals.(Bettschart-Wolfensberger and Larenza, 2007; Jones, 2001; Lee et al., 2001; Lee et al., 2006; Robinson and Natalini, 2002) and epidural anesthesia has been reported.(Bozkurt et al., 1995; Lichtenberger and Ko, 2007; Otero et al., 2012) This technique is not commonly used in birds because of anatomical considerations. Although epidural anesthesia access is not well described, myelography has been reported.(Harr

et al., 1997; Krautwald-Junghanns et al., 2008; Naeini et al., 2006) While in mammals the caudal lumbar and occipital-C1 regions are commonly used for myelographic contrast administration, in birds the synsacrum is composed of fused lumbar and sacral vertebrae and pelvis, therefore, thoracolumbar access is needed rather than lumbar puncture.(Harr et al., 1997) Epidural administration of local anesthetics for regional anesthesia in avian species is difficult to perform.

2.3.4.2 Lidocaine as part of a balanced anesthesia

In veterinary medicine, the greatest attention to intravenous administration of lidocaine has been for the purpose of a multimodal or balanced anesthesia. The concept of balanced anesthesia is based on the assumption that the mixture of small amounts of several neuronal depressants provides the sum of the advantages but not the disadvantages of the individual pharmaceuticals. The objective of multimodal or balanced anesthesia is calming the patient, minimizing pain, and reducing potential adverse side effects. The major advantage of this approach is the reduction of the pharmaceutical dose used, therefore decreasing the occurrence of side-effects. This concept was first introduced in human anesthesia by George W. Crile (1910), the so-called anociassociation.(Tonner, 2005) However, veterinary anesthesia still relies heavily in inhalant drugs alone (Ilkiw, 1999) although efforts have been made to introduce balanced anesthesia/perioperative analgesia. The use of perioperative analgesia in Canada increased significantly between 1994 and 2001, with 62% of the questioned veterinarians reporting to use at least 2 classes of analgesic prior to surgery.(Hewson et al., 2006) In New Zealand, a relatively high percentage of veterinarians report to use peri-operative analgesia, including pre-emptive and multi-modal analgesia.(Williams et al., 2005) However, a similar study from South Africa, shown that approximately 86.3% of cats and 80.7% of dogs did not received peri-operative

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analgesia.(Joubert, 2001) In the United Kingdom, the use of peri-operative analgesia was commonly considered and it was administered more often by female veterinarians and recent graduates.(Capner et al., 1999)

In veterinary anesthesia, a relatively large number of pharmaceuticals have been assessed in terms of decreasing the minimum alveolar concentration (MAC) of volatile drugs. The effect of drugs like morphine, ketamine, butorphanol, fentanyl, alfentanil, buprenorphine, diazepam and dexmedetomidine on the inhalant drug MAC of dogs, cats, and horses has been studied.(Hellyer et al., 2001; Ilkiw et al., 1997; Ilkiw et al., 2002; Ko et al., 2000; Muir and Sams, 1992; Muir et al., 2003; Pascoe et al., 2006; Solano et al., 2006) The use of intravenous lidocaine has also been assessed. Lidocaine produced dose dependent halothane MAC decrease in ponies.(Doherty and Frazier, 1998) Similar findings were reported on isoflurane MAC in dogs and rabbits.(Schnellbacher et al., 2013; Valverde et al., 2004) In another study, lidocaine alone or in combination with morphine and ketamine was showed to cause a decrease on isoflurane MAC in dogs.(Muir et al., 2003) In comparison with morphine, lidocaine produced a lower effect on the isoflurane MAC, however, it appeared to be slightly superior to ketamine.(Muir et al., 2003) The use of lidocaine alone or in combination allowed clinically important reduction on isoflurane MAC in goats.(Doherty et al., 2007) In the case of sevoflurane, lidocaine alone or in combination with ketamine also led to decrease MAC in dogs.(Matsubara et al., 2009; Wilson et al., 2008) In cats, the use of lidocaine in combination with general anesthesia is not recommended as it has been reported to be associated with greater cardiovascular depression than isoflurane alone.(Pypendop and Ilkiw, 2005)

2.3.5 Lidocaine as an anti-inflammatory

Inflammation has been described as a stereotypic response of vascularized tissue to an injury and as a protective response which serves to destroy, dilute or wall off both the injuring agent and the injured tissue.(Cassuto et al., 2006) Inflammation is necessary for structural and functional repair of injured tissue, which is complemented by granulocytes, macrophages, and other cell mediators to provide proper wound healing.(Durieux and Hollmann, 2004) In some situations, the inflammatory response may be over reactive and harmful so anti-inflammatory therapy is necessary.(Cassuto et al., 2006) Local anesthetics modulate the inflammatory response after surgical trauma, by inhibition of the nervous conductivity at the site of the trauma.(Beloeil and Mazoit, 2009) As local anesthetics attenuate the nervous system sensitization and consequently the inflammatory phenomena, exert intrinsic anti-inflammatory properties by modulating the local and systemic liberation of inflammatory mediators, it has been suggested that these therapeutic agents can be used for anti-inflammatory purposes.(Beloeil and Mazoit, 2009) Local anesthetics appear to inhibit the release of several inflammatory mediators and also direct effect on polymorphonuclear granulocytes (PMNs) and macrophages.(Sallam and El-Kafrawy, 2011) Lidocaine and procaine are suggested to decrease phospholipase A_2 at low doses.(Sallam and El-Kafrawy, 2011) For example, intravenous lidocaine administered pre- and early post-induced lung injury in rabbits decreased PMNs accumulation in the lung and inhibited superoxide anion production by PMNs; leading to decreased edema.(Nishina et al., 1998) It is said that local anesthetics administered through the intravenous or epidural route could be considered for modulation of postoperative inflammatory response.(Hollmann and Durieux, 2000) In a recent literature review, lidocaine was reported to have potential as an anti-inflammatory agent although there is limited scientific evidence to back up this claim.(Caracas et al., 2009)

2.3.6. Lidocaine side effects

Although used for a multitude of purposes, lidocaine as well as other local anesthetics, can have toxic effects. Some authors suggest that because of its common use in over 1,000,000 daily regional anesthestic procedures, it is not surprising that adverse reactions occur.(Yagiela, 1985) Side effects include allergic reactions, systemic toxicity, ventilatory response to hypoxia and hepatotoxicity.(Stoelting and Hillier, 2006)

Allergic reactions to local anesthetics are rare, estimated to correspond to less than 1% of all adverse reactions.(Stoelting and Hillier, 2006) Among 197 cases of adverse drug reactions to local anesthetic agents, only 3 reacted after subcutaneous challenge with the causative drug (amide type local anesthetic).(Gall et al., 1996) Of those 3 cases, one had delayed-type response to mepicaine and two had immediate-reactions non-IgE mediated to articaine and lidocaine.(Gall et al., 1996) In another study, among 236 patients referred to an allergy clinic for local anesthetic hypersensitivity, skin prick and interdermal challenge was negative in all patients.(Berkun et al., 2003) It has been suggested that the methods to test hypersensitivity to local anesthetics be revisited.(Berkun et al., 2003) Incremental challenge tests have been suggested as part of the revision process.(Nettis et al., 2001) Considering the low number of confirmed allergic reactions, it is possible that the allergic reactions may be related to other ingredients within the drug.(Stoelting and Hillier, 2006) Among 208 patients with adverse reactions to local anesthetic drug administration, 39 were considered responses to additives but this consideration was never verified.(Fisher and Bowey, 1997) Several compounds are reported to have caused allergic reactions; methylparaben, sodium metabisulfite, and bisulfite.(Dooms-Goossens et al., 1989; Schwartz and Sher, 1985; Stoelting and Hillier, 2006) Although not confirmed, it is hypothesized

that latex contaminated, local anesthetic allergic reaction, can also occur.(Shojaei and Haas, 2002) This assumption is based on information that latex can be released from the container either by penetration through or direct contact with natural latex stoppers.(Shojaei and Haas, 2002)

Systemic toxicity occurs when there is an excessive plasma concentration of the drug. (Stoelting and Hillier, 2006) There is a direct correlation between local anesthetic plasma concentration and symptoms of toxicity (Table 3).(Eappen and Datta, 1998) The results of a comparative study on intravenous toxicity of local anesthetics (lidocaine, procaine, chloroprocaine, and tetracaine) indicated that lidocaine was least tolerated by the subjects while chloroprocaine was the most tolerated.(Foldes et al., 1960) It was suggested that the low toxicity of chloroprocaine was due to rapid hydrolysis by plasma cholinesterase.(Foldes et al., 1960) The most common cause of systemic toxicity is due to accidental intravascular administration of local anesthetics (e.g. peripheral nerve block, because of excessive absorption from the injection site).(Stoelting and Hillier, 2006) Clinical signs of neurotoxicity associated with systemic toxicity occur as the local anesthetic crosses the blood-brain barrier leading to restless, vertigo, tinnitus and difficulty focusing followed by slurred speech and skeletal muscle twitching.(Stoelting and Hillier, 2006) The cardiovascular tissue is more resistant to the systemic toxicity than the central nervous system.(Stoelting and Hillier, 2006) However, cardiotoxicity is a common adverse effect of local anesthetics. If a sufficient number of sodium channels is blocked due to excessive plasma concentration of local anesthetics, conduction and automaticity becomes depressed.(Stoelting and Hillier, 2006) Although cardiotoxicity has been described since the 1930's, more attention was devoted since 1979 when Albright reported cardiac arrest after regional anesthesia with bupivacaine and etidocaine.(Groban and Butterworth, 2007) Long

acting local anesthetics like bupivacaine are disproportionately more cardiotoxic than their shorter acting counterparts (ropivacaine and levobupivacaine).(Mather and Chang, 2001) The rank order from lowest to highest cardiotoxic potency of local anesthetics has been reported to be prilocaine, lidocaine, mepivacaine, levobupivacaine, racemic bupivacaine, R(+) bupivacaine, etidocaine and tetracaine.(Heavner, 2002) Although the shorter acting more modern local anesthetics are safer, toxicity can still occur.(Mather and Chang, 2001) While plasma concentrations of lidocaine below 5 µg/kg only lead to decrease rate of spontaneous phase 4 depolarization, plasma concentrations of 5 to 10 µg/kg may produce profound hypotension and direct myocardial depression.(Stoelting and Hillier, 2006) Furthermore, lidocaine can also cause slow cardiac impulse conduction, prolongation of the P-R interval and QRS complex.(Stoelting and Hillier, 2006)

Table 3. Dose dependent effects of lidocaine.(Stoelting and Hillier, 2006)

Plasma lidocaine concentration (µg/kg)	Effect
1-5	• Analgesia
5-10	• Circumoral numbness • Tinnitus • Skeletal muscle twitching • Myocardial depression
10-15	• Seizures • Unconsciousness
15-25	• Apnea • Coma
>25	• Cardiovascular depression

2.3.6.1. Lidocaine side effects in birds

Lidocaine is not commonly considered an adequate therapeutic agent in avian species because of concerns associated with toxicity.(Figueiredo et al., 2008) It has been perpetuated in the literature that birds are more sensitive to lidocaine than mammals, and that lidocaine dose should not exceed 4 mg/kg because toxic effects may occur.(Carpenter, 2005; Carpenter and

Marion, 2013; Hall et al., 2001; Machin, 2005; West et al., 2007) Higher doses than 4 mg/kg are suggested to possibly lead to seizures and cardiac arrest.(Machin, 2005) However, this information appears to be anecdotal. Also, no clear information on route of administration and species variability are available in the literature. As stated previously, lidocaine has been used in several studies and no reports of mortality, morbidity or toxic effects have been published.(Brenner et al., 2010; Da Cunha et al., 2012; da Cunha et al., 2013; Figueiredo et al., 2008)

Bupivacaine has been used in avian species but the available information is limited. Bupivacaine is reported to relieve pain in chickens (n=72) with experimentally induced arthritis.(Hocking et al., 1997) It was suggested that the dose of 2.7–3.3 mg/kg intraarticular bupivacaine cause toxic effects in 10/12 animals; recumbency, abnormal posture, and distress.(Hocking et al., 1997) All animals were covered completely within 1 hour.(Hocking et al., 1997) The overall results of the study suggested that the minimal dose of 3 mg in 0.3 ml given intraarticularly was efficient in order to provide analgesia in chickens with experimentally induced arthritis.(Hocking et al., 1997) Differential mortality of spectacled eiders (*Somateria fischeri*) and king eiders (*Somateria spectabilis*) subsequent to field anesthesia with propofol (mean total dose, 26.2–45.6 mg/kg IV), bupivacaine (2–10 mg/kg SC surgical site), and ketoprofen (2–5 mg/kg IM) has been reported.(Mulcahy et al., 2003) Within 4 days following the anesthetic event, 4/10 male spectacled eiders, 5/6 male king eiders and 1/5 female king eiders died.(Mulcahy et al., 2003) The suggested cause of death was the perioperative use of non-steroidal anti-inflammatory drug although the combination of propofol, bupivacaine and ketoprofen was recommended to be avoided in field situations.(Mulcahy et al., 2003) The use of propofol (8-15 mg/kg IV) in combination with bupivacaine and lidocaine (s.c. and i.m.

infiltration of 1.5–2.0 mg/kg of a 2:1 mixture [total concentration of 1 mg/ml]) for field coelomic satellite transmitter implantation is reported to be safe in bar-tailed godwit (*Limosa lapponica*) and the bristle-thighed curlew (*Numenius tahitiensis*).(Mulcahy et al., 2011)

CHAPTER 3
STATISTICAL ANALYSIS

3.1 Statistical analysis and optimal dose determination

The determination of the optimal dose is a difficult procedure that most clinical researchers need to deal with. The definition of optimal dose varies with the drug and the therapeutical objective. Nevertheless, this utopic pursuit of clinical research has greatly improved since the use of statistics. The use of statistical analysis allows researchers to extrapolate from a limited population to a general population.

One of the critical steps in drug development is proper understanding and characterization of its drug response relationship; erroneous determination may lead to inappropriate recommendations which may be shown to be drastic.(Dette et al., 2013) Commonly, dose determination or MAC studies in veterinary medicine use a bracketing design. Although less common, the up-and-down method has also been used. In toxicological studies, both these methods are commonly used.

3.1.1. The bracketing system

In the past, acute lethal dose 50 (LD_{50}) determinations in toxicological studies used to depend on a bracketing system. Using this design, several dose levels (3 to 5 different doses, ideally including the LD13 and the LD87) were experimented in several groups (usually 5 animals of each sex per group).(Rispin et al., 2002) A dose increase should lead to decrease survival and two or more doses should show partial responses; in this case responses being mortality.(Rispin et al., 2002) However, this assays may have to be repeated as the confidence intervals may not fulfill the regulatory requirements.(Kelly, 2001) Minimum alveolar anesthetic

concentration studies in humans are commonly performed in a quantal (categorical; all-or-none) study design, which implies exposing each individual to an anesthetic concentration for a defined period of time, applying a noxious stimulus (commonly a skin incision) and recording the response.(Sonner, 2002) Data is fitted into a logistic or sigmoid Emax equation which allows determination of the probability of non-movement in half of the population, the 50% effective dose (ED_{50}) or MAC for the population but not to the individual.(Sonner, 2002) In the bracketing study design, which is commonly used in animals, the animal is exposed to the anesthetic and the movement or lack of is noted. Depending on the initial response, the dose is increased until the animal does not respond or decreased until a response is detected.(Sonner, 2002) This design allows determination of MAC per individual as the minimum dose to preventing movement and the largest concentration permitting movement.(Sonner, 2002) The population MAC is the average individual MAC.(Sonner, 2002) In the case of toxicology studies, the determination of LD_{50} is usually required. Using older methods, a large number of animals is needed, therefore, alternative studies designs have been proposed as the Acute-Toxic-Class-Method, the Fixed-Dose-Procedure and the Up-and-Down method.(Rusche, 2003) The up-and-down has been reported to reduce the number of animals needed without affecting the reliability.(Lichtman, 1998)

3.1.2 The Up-and-Down study design

The so-called up-and-down method was initially described by Dixon and Mood (1948) to test the sensitivity of explosives.(Dixon and Mood, 1948) Later, Dixon (1965) described the up-and-down method for statistical analysis of small samples.(Dixon, 1965) Since then, this method has been used and modified for different purposes from toxicological studies (Sunderam et al.,

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2004) to human and veterinary MAC studies (Mercado et al., 2008; Niu et al., 2013). The use of the up-and-down method for MAC determination has been performed in birds, mainly in endangered species as the thick-billed parrot (*Rhynchopsitta pachyrhyncha*).(Mercado et al., 2008; Phair et al., 2012)

The up-and-down method is a sequential design with binary response variables within a certain population which allows the determination of an effective dose to 50% of the population.(Pace and Stylianou, 2007) The target dose is the mean dose measured during these crossover events.(Mercado et al., 2008) With this method each animal is used only once. This is extremely important because if one animal is used more than once, this animal will have too much influence on the final dose determination, therefore, inducing bias to the data. A predetermined dose is chosen. This dose can be randomly selected or based on prior experience; however, ideally it should be close to the ED_{50} or LD_{50}. Strict quantifiable binary response variables should be established a priori. For MAC studies, a response or lack of response to stimuli is commonly used. Crossover events occur when two sequential animals have contradictory results (one positive response followed by a negative response or vice versa).

In order to clarify the up-and-down method, the following hypothetical example is given in which X is the initial dose and Y is the variation of the dose. (Figure 2 and 3) The first animal is given a dose X. If the response of the first animal was considered negative, the second animal receives the dose of X+Y. If the response of the first animal is positive, the second animal will receive X-Y. The sequence and dose calculation is based on the response of the previous animal. Dose will receive increments or reductions in a sequential faction.
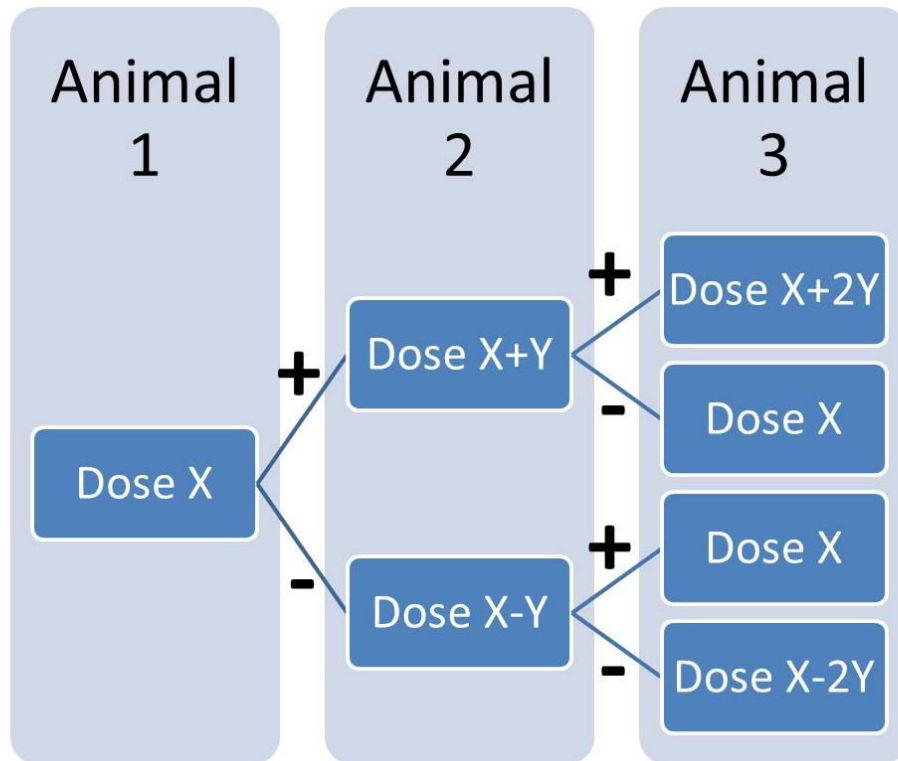
Figure 2. Flow chart demonstrating the sequence of dose selection using the up-and-down method in a hypothetical case.
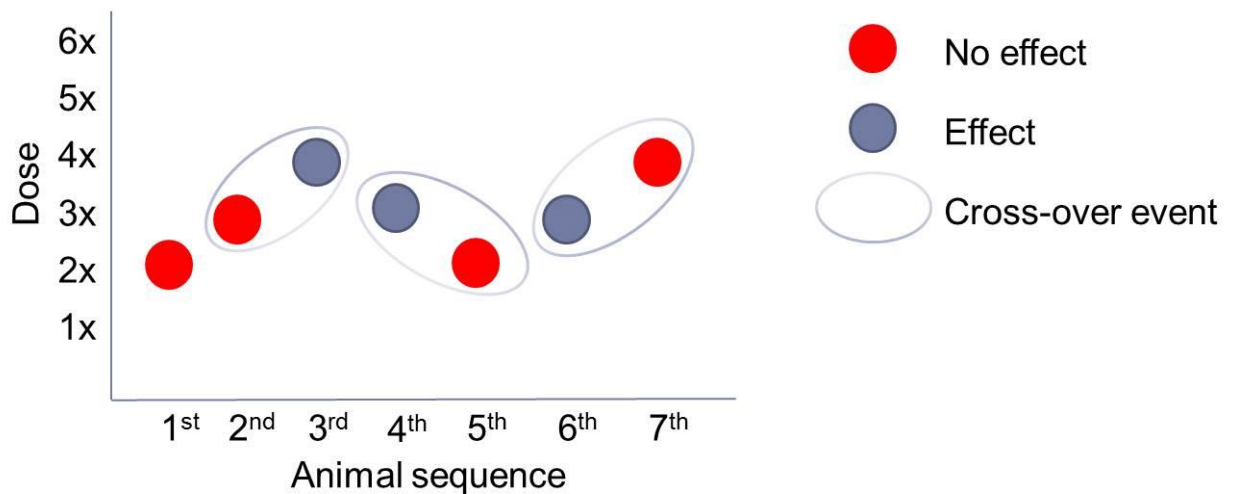
Figure 3. Graphical distribution of a hypothetical study using the up-and-down design. Animals are used only once in a sequential manner. Dose is increased or decreased by equal dose spacing depending on response of the previous animal. Binary responses (effect [solid blue circle] or no effect [solid red circle]) are previously defined. Cross over events are contradictive responses between two sequential animals.

The up-and-down method has been compared with the fixed dose procedure and conventional LD_{50} tests.(Bruce, 1987; Lipnick et al., 1995; Yam et al., 1991) In one study, the up-and-down method was consistent with the conventional LD_{50} in 23 of 25 cases while the fixed dose procedure was consistent in 16 of 20.(Lipnick et al., 1995) In another study, although similar results were gathered with both the up-and-down method and the fixed dose procedure, the up-and-down required a lower number of study subjects.(Yam et al., 1991) In one study, about 40 to 50 animals were needed using the traditional LD_{50} method while with the up-and-down method only 6 to 9 animals were needed.(Bruce, 1987) Although to the author's knowledge no comparison between the conventional methods and the up-and-down has been reported in anesthesia, one could consider this method as an adequate method for efficient dose determination. Some limitations to the up-and-down have been reported. As the determination of dose is based on the response of the previous animal, it is necessary to allow time for the effect of the test. This is a concern in some toxicological studies in which the mortality may only occur 7 days after drug experimentation, therefore, the up-and-down method is not recommended for cases in which death is expected within more than 2 days post dosing.(Bruce, 1985) The use of the up-and-down method, as previously stated, allows the determination of the ED_{50}, however, it does not provide information on $ED_{95/99}$ as it does not give any insight into the upper tail of the tolerance distribution.(Pace and Stylianou, 2007; Rispin et al., 2002)

CHAPTER 4
ASSESSMENT OF CARDIOVASCULAR EFFECT OF PREDEFINED DOSES OF INTRAVENOUS LIDOCAINE IN BROILER CHICKENS (*GALLUS GALLUS DOMESTICUS*) – PILOT STUDY

4.1 Objective and hypothesis

The objective of this pilot study was to determine the cardiovascular effects of predefined intravenous doses of diluted preservative free lidocaine in broiler chickens. The dose selection was based on the published lidocaine doses of 1 to 3 mg/kg (route of administration not reported) in birds, while 4 mg/kg (route of administration not reported) is suggested to be toxic.(Carpenter, 2005) Therefore, three treatment doses of 2.5, 3.0 and 3.5 mg/kg of intravenous lidocaine were selected for this study. Intravenous 0.9% saline would serve as the control. The hypothesis was that intravenous lidocaine at 2.5, 3.0 and 3.5 mg/kg would not cause clinically significant cardiovascular side-effects when compared to the saline group.

4.2 Study design

The LSU Institutional Animal Care and Use Committee (IACUC) approved this study. Eight female broiler chickens (Ross 708, Aviagen, Huntsville, AL, USA), 6 to 8 weeks of age, acquired from the LSU poultry unit, were used for this study. Chickens weighed an average of 2.5 kg (range 2.1 – 3.7 kg). To ensure the health of the birds, a physical exam, complete blood cell count (CBC) and plasma biochemistry panel were performed on all birds upon arrival at the housing facility. The CBC's were performed by the LSU Clinical Pathology Laboratory. The plasma biochemistries were analyzed using the Abaxis Avian/Reptile biochemistry profile (VetScan, Abaxis, California, USA). All animals were considered healthy based on the results of the physical exam, CBC, and biochemistries. Chickens were individually identified with a

numbered leg band and allowed to acclimatize for 1 week prior to the experimental period. The animals were housed as a group and cared for in accordance to protocols set forth by the LSU Division of Laboratory Animal Medicine. The birds were offered *ad lib* commercial maintenance chicken pelleted diet (Layena SunFresh, Purina Mills, St. Louis, MO) and unfiltered tap water in plastic containers. Fluorescent lighting was maintained on an automatic timer with a 12 hour photoperiod.

Animals and order of treatments were randomized using statistical software (R, R Foundation for Statistical Computing, Vienna, Austria) to limit order effect. Treatments consisted of: treatment I (0.9% saline control of equal volume to treatment IV), treatment II (2.5 mg/kg lidocaine), treatment III (3 mg/kg lidocaine) and treatment IV (3.5 mg/kg lidocaine). Two animals were assigned to each treatment group. Each animal was used only once. Preservative free lidocaine hydrochloride 2% (Lidocaine Hydrochloride Injection, International Medication Systems, California, USA) was diluted with 0.9% saline (0.9% Sodium Chloride Injection USP, Hospira, Illinois, USA) to reach a concentration of 10 mg/ml.

Clinically significant cardiovascular effects were defined as a relative decrease in the mean blood pressure (MAP) and/or heart rate (HR) of 30% or greater from the baseline value. Conversely, clinically insignificant effects were defined as a relative decrease in the MAP and HR of less than 30%.

4.3 Experimental design

Each chicken was physically restrained for anesthetic induction via face mask with isoflurane (Isoflo; Abbott Laboratories, Illinois, USA) delivered at 5% in 100% oxygen at a flow rate of 2 L/min using a Bain breathing system. Once anesthetic induction was achieved, the birds

were intubated with a non-cuffed Murphy's endotracheal tube (3.0, 3.5 or 4.0 mm internal diameter as needed) and placed in dorsal recumbency. Isoflurane concentration was decreased until the end-tidal concentration of isoflurane was between 1.4 and 1.7% as measured by a gas analyzer (Datascope Passport 2, MAQUET, New Jersey, USA). Core temperature was monitored with an esophageal temperature probe (Datascope Passport 2, MAQUET, New Jersey, USA) placed at the level of the heart. Surface electrocardiogram pads (LL Electrode Series, Lead-Lok, Idaho, USA) were placed bilaterally on the cranial pectoral muscles and unilaterally on the left proximal tarsometatarsus connected to a data acquisition system (Power Lab Acquisition System, Power Lab, Colorado, USA). A 22-gauge 1 inch long venous catheter (Abbocath-T, Hospira, Illinois, USA) was placed in either the right or left ulnar vein and a 22-gauge 1 inch long arterial catheter (Abbocath-T, Hospira, Illinois, USA) was placed in the left or right deep radial artery or in the right cranial tibial artery. Selection of venous and arterial access placement was based on subjective interpretation of access quality. Direct arterial blood pressures were measured using a disposable pressure transducer system (Menscap, SP844 physiological sensor, North Coralina, USA) connected to a data acquisition system (Power Lab Data Acquisition System, Colorado, USA). Spontaneous breathing was allowed as long as end-tidal carbon dioxide concentration (EtCO$_2$) remained between 35 and 45 mmHg. If higher values of EtCO$_2$ were detected, positive-pressure ventilation was administered by a mechanical ventilator (SAV2500 Ventilator, Smiths Medical PM, Wisconsin, USA) at a rate of 5 breaths/min with a peak inspiratory pressure of 15 cm H$_2$O until EtCO$_2$ levels returned to the 35 and 45 mmHg range. Constant rate infusion of lactated ringer's solution (Lactated Ringer's Injection USP, Hospira, Illinois, USA) was delivered at 4 ml/kg/h via venous catheter using a fluid pump (Vet/IV 2.2, Heska Corporation, Colorado, USA). A hot-water blanket (T/Pump, Gaymar Industries Inc., New York, USA) set at

90°F (32°C) was covered with a towel and placed underneath the animal. The animals' temperature was maintained between 102 and 105°F (39 and 40.5°C). If temperature was below target, additional thermoregulatory support was provided with a forced-air warmer (Bair Hugger, Arizant Inc., Minnesota, USA).

Once all instrumentation was placed, and target values reached, data collection using a data acquisition system (Power Lab Acquisition System, Power Lab, Colorado, USA) was initiated. Baseline values were recorded for 30 seconds prior to treatment administration. After baseline was acquired, administration of lidocaine at the target dose was initiated. Total volume was administered over a 2 min period using a syringe drive pump (Medfusion 2010i Syringe Pump, Medex Inc., Georgia, USA). Data was collected for 30 minutes post drug administration.

4.4 Data analysis

Mean HR and MAP was calculated using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA) for 30s (baseline) prior to lidocaine administration. The lowest HR and MAP values were determined from the data collected post lidocaine administration using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA). Relative changes between baseline and post lidocaine values were calculated. Calculation was performed with the following formulas:

$$Relative\ decrease\ of\ HR = \left(1 - \frac{Lowest\ HR\ post\ lidocaine}{Mean\ HR\ prior\ lidocaine}\right) \times 100$$

$$Relative\ decrease\ of\ MAP = \left(1 - \frac{Lowest\ MAP\ post\ lidocaine}{Mean\ MAP\ prior\ lidocaine}\right) \times 100$$

Data was analyzed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) software. Values were calculated to the first decimal unit. Effect was considered when relative changes of MAP and/or HR were equal to or greater than 30%. Conversely, no effect implied relative changes of MAP and HR were less than 30%.

4.5 Results

During the course of the experiment, none of the animals showed clinically significant cardiovascular depression. Values of HR and MAP pre- and post-lidocaine administration as well as relative changes are reported on table 4. Variable responses to the administration of preservative free lidocaine were detected. The highest doses of intravenous lidocaine did not appear to consistently lead to the most significant decreases of HR or MAP. As an example, one of the control animals (saline) had a decrease in HR of 28%, which was similar to the effect cause by the dose of 3.5 mg/kg and 2.5 mg/kg in one animal, each (animal #6 and #2, respectively). The animal #2 which received the dose of 2.5 mg/kg had similar relative decrease of MAP to animal #6, which received the dose 3.5 mg/kg.

Table 4. Heart rate and mean blood pressure values prior and post intravenous lidocaine (at 2.5, 3.0, 3.5 mg/kg) or control, and relative changes.

Animal	HR baseline (bpm)	HR post lidocaine (bpm)	HR relative changes (%)	MAP baseline (mmHg)	MAP post lidocaine (mmHg)	MAP relative changes (%)	Response	Dose (mg/kg)
1	236.2	190.1	19.5	96.8	75.5	22.0	No effect	3.5
4	266.1	237.4	10.8	106.2	81.9	22.9	No effect	3.0
6	313.3	226.3	27.8	113.7	80.1	29.6	No effect	3.5
2	240.5	174.6	27.4	83.4	58.8	29.5	No effect	2.5
5	225.4	162.2	28.0	96.7	89.1	7.9	No effect	Control

Table 4 continued

Animal	HR baseline (bpm)	HR post lidocaine (bpm)	HR relative changes (%)	MAP baseline (mmHg)	MAP post lidocaine (mmHg)	MAP relative changes (%)	Response	Dose (mg/kg)
8	266.1	211.5	20.5	106.9	78.8	26.3	No effect	3.0
3	238.9	168.7	29.4	95.9	83.9	12.5	No effect	2.5
7	178.9	168.4	5.9	77.2	67.7	12.3	No effect	Control

4.6 Conclusions

Based on these results, it was concluded that doses of 2.5, 3.0 and 3.5 mg/kg were not sufficient to cause clinically significant cardiovascular effects in broiler chickens. This supports the previous recommended doses of 1 to 3 mg/kg in birds. It appeared that higher doses had to be used in order to determine the tolerable limit.

Although the suggested toxic dose of 4 mg/kg was not tested, the 3.5 mg/kg dose did not result in clinically significant cardiovascular depression. It appeared unlikely that an increase of 0.5 mg/kg would lead to a severe cardiovascular depression. The cardiovascular changes caused by the dose of 3.5 mg/kg on animal #6 (HR decrease 27.8%, MAP decrease 29.6%) were similar to the dose of 2.5 mg/kg on animal #2 (HR decrease 27.4%, MAP decrease 29.5%). Animal #1 which received a dose of 3.5 mg/kg had lower cardiovascular depression than an animal receiving the 2.5 mg/kg dose (animal #2). It is unclear why the lower doses occasionally led to similar cardiovascular effect as the higher doses.

Although the initial suspicion was that the toxic and recommended doses of lidocaine were anecdotal, this pilot study was needed to assess the accuracy of this information. Furthermore, it would be unethical to test the toxic or higher doses without first confirming the

accuracy of the published information. The purpose of this pilot study was to confirm the published information.

Based on the information gathered during this study, it would be necessary to test higher doses to determine the highest tolerable dose in terms of cardiovascular effects.

CHAPTER 5
CARDIOVASCULAR TOLERANCE TO INTRAVENOUS LIDOCAINE IN THE BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*) – EFFECTIVE DOSE 50 DETERMINATION PHASE

5.1 Objective and hypothesis

The goal of this study was to determine the cardiovascular tolerance to intravenous lidocaine administration in broiler chickens. The objective was to determine the effective dose (ED_{50}) using up-and-down methodology (Dixon, 1965). The ED_{50} was defined as the dose that would cause clinically significant cardiovascular effects in 50% of the population. Based on the previously reported pilot study in which no clinically significant cardiovascular depression was detected at the predefined doses of 2.5, 3.0 and 3.5 mg/kg, higher doses than previously test were necessary. The initial dose was selected to be 7 mg/kg. The dose selection was determined by applying a factor of 2 to the previously higher dose tested. The rationale to such dose selection was based on the fact that the highest doses tested previously were insufficient to cause clinically significant cardiovascular depression. Furthermore, the lowest doses tested led to similar cardiovascular changes as the highest dose. The hypothesis was that the ED_{50} of intravenous lidocaine would be lower than 7 mg/kg for a specific population of broiler chickens.

5.2 Study design

The study was approved by the LSU IACUC. Eleven female broiler chickens (Ross 708, Aviagen, Huntsville, AL, USA) 8 to 10 weeks of age, acquired from the LSU poultry unit, were used for this study. Chickens mean weight was 3.6 kg (range 2.6 – 4.3 kg). Animals were assessed and housed as previously described in chapter 3.

Order of animals was randomized using freeware statistical software (R, R Foundation for Statistical Computing, Vienna, Austria). Each animal was used only once. The dose of 7 mg/kg was used as the baseline dose for the first animal. Increments and deductions of the dose were elected to be 1 mg/kg (one unit). Effect was defined as a relative decrease of MAP and/or HR equal to or greater than 30%. Conversely, no effect implied relative decrease of MAP and HR of less than 30%. Relative changes were calculated using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Clinically significant and insignificant relative changes on MAP and HR were noted.

5.3 Experimental design

Anesthesia, monitoring, and determination of baseline values were performed as described in chapter 3. The first animal received a dose of 7 mg/kg of preservative free lidocaine hydrochloride 2% (Lidocaine Hydrochloride Injection, International Medication Systems, California, USA) diluted with 0.9% saline (0.9% Sodium Chloride Injection USP, Hospira, Illinois, USA) to a concentration of 10 mg/ml. Total volume was administered over 2 minutes via a syringe drive pump (Medfusion 2010i Syringe Pump, Medex Inc., Georgia, USA). Based on the response in the first animal, the second randomly selected animal would receive either an increment or deduction of one unit (1 mg/kg) from the 7 mg/kg dose if no effect or effect response, respectively, was observed. The third animal would receive either an increment or deduction of 1 mg/kg from the dose of the second animal if a no effect or effect response, respectively, were observed. This pattern would be continued for all animals in study. Crossover events were allowed and noted. Crossover events were classified as contradictory effects between sequential animals. A minimum of 4 cross overs were required. Each animal was used

only once. The HR and MAP was monitored and recorded for 30 minutes after the administration of lidocaine as described on Chapter 3. After the end of the treatment, all equipment was removed and anesthesia was discontinued. Animals were allowed to recover under supervision.

5.4 Data analysis

Mean HR and MAP was calculated using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA) for 30s (baseline) prior to lidocaine administration. The lowest HR and MAP values were determined from the data collected post lidocaine administration using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA). Relative changes between baseline and post lidocaine values were calculated. Calculation was performed with the following formulas:

$$Relative\ decrease\ of\ HR = \left(1 - \frac{Lowest\ HR\ post\ lidocaine}{Mean\ HR\ prior\ lidocaine}\right) \times 100$$

$$Relative\ decrease\ of\ MAP = \left(1 - \frac{Lowest\ MAP\ post\ lidocaine}{Mean\ MAP\ prior\ lidocaine}\right) \times 100$$

Data was analyzed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) software. Values were calculated to the first decimal unit. Effect was considered when relative decrease of MAP and/or HR was equal to or greater than 30%. Conversely, no effect implied relative decrease of MAP and HR were less than 30%.

The ED_{50} of intravenous lidocaine was calculated using the Dixon's up-and-down analysis (Dixon, 1965) and quantal analysis. For the up-and-down analysis, the ED_{50} was defined as the mean of lidocaine doses measured during crossover events. The 95% confidence intervals were calculated by use of binomial probability. Quantal analysis was used to calculate the

probability of significant cardiovascular effects as a function of intravenous dose, where ED_{50} was the dose at which that probability was 50%. Data underwent logistic and nonlinear regression by use of computer software. The ED_{50} and fiducial intervals were estimated from the probit curve. For all statistical analyses, a value of $p < 0.05$ was considered significant.

5.5 Results

Animals were considered healthy based on physical examination, CBCs and plasma biochemical. Values of HR and MAP pre- and post-lidocaine administration as well as relative changes are reported on table 5. Six of 11 animals had effect (decrease of MAP and/or HR $\geq$ 30%). The remaining 5 animals had no effect (decrease of MAP and HR < 30%). Five cross over events occurred as shown on Figure 4. Using the Dixon's up-and-down analysis (Dixon, 1965), the ED_{50} of the population was 6.3 mg/kg. Using logistic regression, we obtained a calculated dose of 6.22 mg/kg with a 95% Wald confidence interval of 5.3 – 7.13 mg/kg.

Table 5. Heart rate and mean blood pressure values prior and post intravenous lidocaine, and relative changes.

Animal order	HR baseline (bpm)	HR post lidocaine (bpm)	HR relative changes (%)	MAP baseline (mmHg)	MAP post lidocaine (mmHg)	MAP relative changes (%)	Response	Dose (mg/kg)
1st	309.6	216.9	29.9	101.6	91.5	9.9	No effect	7
2nd	331.9	204.6	38.4	116.1	58.3	48.2	Effect	8
3rd	188.9	146.5	22.4	117.8	73.0	38.0	Effect	7
4th	166.3	145.1	12.8	94.9	61.0	35.7	Effect	6
5th	160.6	188.9	15.0	120.0	95.7	20.3	No effect	5
6th	274.2	207.4	24.4	117.8	86.6	26.5	No effect	6
7th	181.1	144.5	20.2	100.9	62.7	37.9	Effect	7
8th	242.1	187.1	22.7	112.3	75.5	32.8	Effect	6

Table 5 continued

Animal order	HR baseline (bpm)	HR post lidocaine (bpm)	HR relative changes (%)	MAP baseline (mmHg)	MAP post lidocaine (mmHg)	MAP relative changes (%)	Response	Dose (mg/kg)
9th	258.3	247.5	4.2	121.4	113.6	6.4	No effect	5
10th	229.9	179.6	21.9	101.5	92.3	9.1	No effect	6
11th	242.2	178.7	26.2	124.7	78.9	36.7	Effect	7
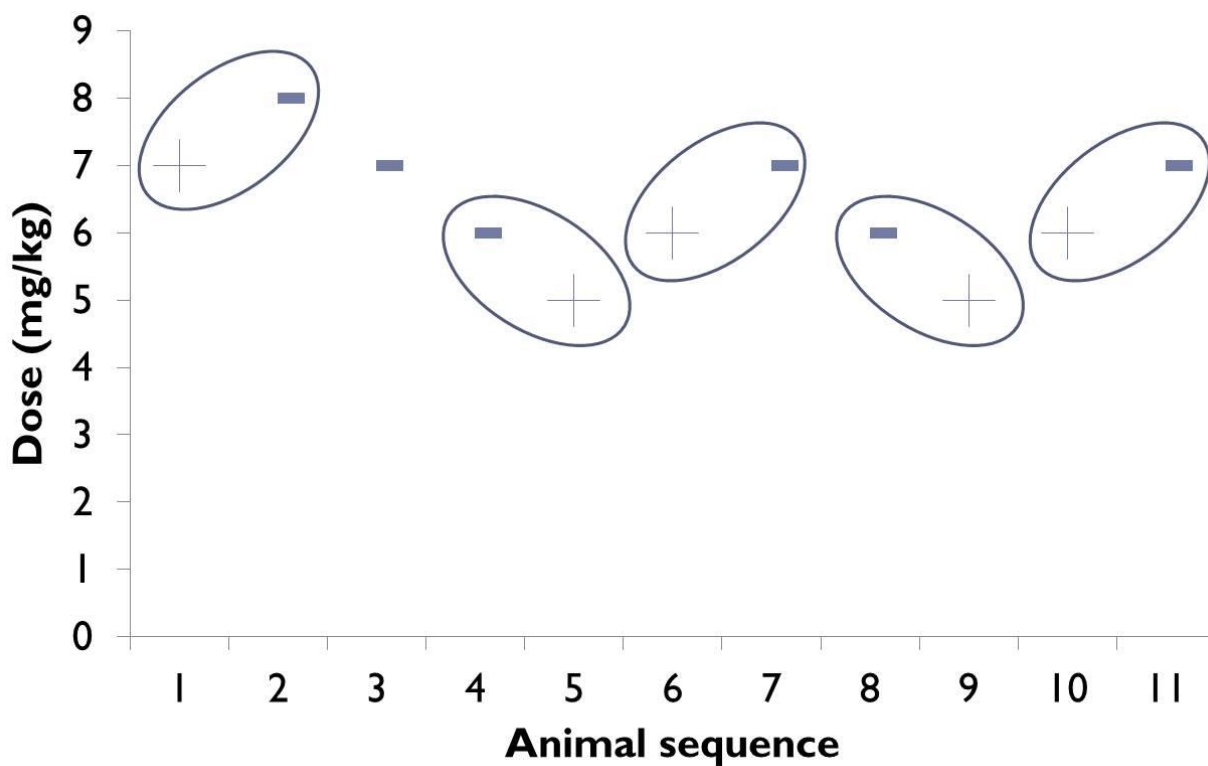
Figure 4. Sequence of animals used during the tolerability study. No effect (+) and effect (-) responses are shown. Cross-over events (circles) are highlighted.

5.6 Conclusions

Mortality or morbidity events were not detected. The highest dose used throughout the experimental period was 8 mg/kg. The relative decreases at that dose were 38.4% (HR) and 48.2% (MAP). At the dose of 7 mg/kg (2nd highest dose), one animal had effect while three

animals had no effect. In 3 of 4 animals, only the MAP was clinically significantly affected (38%, 37.9%, 36.7%). None of the 4 animals had a clinically significant decrease of HR. No abnormality was detected during recovery in any of the animals. Although the objective of this study was not to determine the toxic dose, it appears that higher doses than 7 and 8 mg/kg may be necessary to determine the toxic dose.

The dose calculation based on the Dixon's up-and down statistical analysis allowed the determination of the ED_{50}. For the up-and-down technique, the ED_{50} was defined as the mean of lidocaine doses measured during crossover events. Using the up-and-down method, it was possible to detect the maximum dose without clinically significant cardiovascular side-effects for 50% of the population as 6.3 mg/kg.

The quantal analysis was used to calculate the probability of clinically significant cardiovascular depression (HR and/or MAP decrease equal to or greater than 30%) as a function of intravenous lidocaine dose. Using the quantal analysis the dose of 6.22 mg/kg with intervals of 5.3 – 7.13 mg/kg was calculated.

With this study, it was possible to determine the highest tolerable dose of intravenous lidocaine in 50% of a broiler chicken population. Using two different statistical methods, the dose determination resulted in similar results (6.3 vs. 6.22 mg/kg). The quantal analysis also allowed determination of the confidence interval of 5.3 – 7.13 mg/kg with 95% Wald confidence.

The doses determined in this study were higher than the 4 mg/kg toxic dose previously reported.(Carpenter, 2005) However, this information was representative of a particular population of broiler chickens. Another study would be required to further characterize the use of

intravenous lidocaine in a different group of broiler chickens and assess the safety of the determined dose.

CHAPTER 6
CARDIOVASCULAR SAFETY OF INTRAVENOUS LIDOCAINE IN THE BROILER CHICKEN (*GALLUS GALLUS DOMESTICUS*) – EFFECTIVE DOSE 50 CONFIRMATION PHASE

6.1 Objective and hypothesis

The objective of this study was to determine the safety of the previously determined doses of 6.22 and 6.3 mg/kg. Based on the previous study, these doses should cause clinically insignificant cardiovascular depression in 50% of the population. The goal of this study stage was to determine a safe dose that could be used clinically in broiler chickens. Therefore, it was elected to reduce the dose to 6 mg/kg. The hypothesis of this study was that the dose of 6 mg/kg of intravenous lidocaine would not cause significant cardiovascular effects on the study population of broiler chickens.

6.2 Study design

The study was approved by LSU IACUC. Six female broiler chickens (Ross 708, Aviagen, Huntsville, AL, USA), 8 to 10 weeks of age, acquired from the LSU poultry unit, were used for this study. The chickens mean weight was 3.2 kg (range 2.8 − 3.6 kg). Animals were assessed and housed as previously described in chapter 3.

Order of animals was randomized using freeware statistical software (R, R Foundation for Statistical Computing, Vienna, Austria). Each animal was used only once. Effect was defined as a relative decrease of MAP and/or HR equal to or greater than 30%. Conversely, no effect implied relative decrease of MAP and HR of less than 30%. Results of relative decrease were calculated using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Clinically significant and insignificant relative changes on MAP and HR were noted.

6.3 Experimental design

Anesthesia, monitoring, and determination of baseline values were performed as described in chapter 3. Each animal received a dose of 6 mg/kg of preservative free lidocaine hydrochloride 2% (Lidocaine Hydrochloride Injection, International Medication Systems, California, USA) diluted with 0.9% saline (0.9% Sodium Chloride Injection USP, Hospira, Illinois, USA) to a concentration of 10 mg/ml. Total volume was administered over 2 minutes via a syringe drive pump (Medfusion 2010i Syringe Pump, Medex Inc., Georgia, USA). The HR and MAP was monitored and recorded for 30 minutes after the administration of lidocaine as described on Chapter 3.

6.4 Data analysis

Mean HR and MAP was calculated using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA) for 30s (baseline) prior to lidocaine administration. The lowest HR and MAP values were determined from the data collected post lidocaine administration using computer software (Power Lab Acquisition System, Power Lab, Colorado, USA). Relative changes between baseline and post lidocaine values were calculated. Calculation was performed with the following formulas:

$$Relative\ decrease\ of\ HR = \left(1 - \frac{Lowest\ HR\ post\ lidocaine}{Mean\ HR\ prior\ lidocaine}\right) \times 100$$

$$Relative\ decrease\ of\ MAP = \left(1 - \frac{Lowest\ MAP\ post\ lidocaine}{Mean\ MAP\ prior\ lidocaine}\right) \times 100$$

Data was analyzed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) software. Values were calculated to the first decimal unit. Effect was considered when relative

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decrease of MAP and/or HR was equal to or greater than 30%. Conversely, no effect implied a relative decrease of MAP and HR of less than 30%.

6.5 Results

All animals were determined to be healthy based on physical exam, CBC and plasma biochemistry. All 6 animals received an intravenous lidocaine dose of 6 mg/kg over a period of 2 minutes. No mortality or morbidity was detected during the course of the study. No clinically significant cardiovascular effects were detected in any of the animals. Results are reported on table 6.

Table 6. Heart rate and mean blood pressure values prior and post intravenous lidocaine, and relative changes.

Animal order	HR baseline (bpm)	HR post lidocaine (bpm)	HR relative changes (%)	MAP baseline (mmHg)	MAP post lidocaine (mmHg)	MAP relative changes (%)	Response	Dose (mg/kg)
1st	239.7	222.9	7.0	79.8	79.6	0.3	Positive	6
2nd	308.5	288.0	6.7	83.6	75.4	9.8	Positive	6
3rd	324.4	307.5	5.3	112.4	113.9	-1.3	Positive	6
4th	261.6	242.3	7.4	90.2	82.6	8.4	Positive	6
5th	260.9	223.2	14.5	91.6	99.6	-8.7	Positive	6
6th	315.6	279.8	11.3	71.4	59.8	16.2	Positive	6

6.6 Conclusions

This study phase was intended to test the safety of intravenous lidocaine of 6 mg/kg in the broiler chicken. The dose of 6 mg/kg was based on the previous ED_{50} determined on chapter 4. This dose is 2 mg/kg higher than the previously suggested toxic dose. (Carpenter, 2005) If the published information was accurate, it would be expected that the dose of 6 mg/kg would lead to

significant morbidity and even possibly mortality. However, throughout the entire study, no significant cardiovascular effects were noticed. Furthermore, no mortality or morbidity was observed.

Interestingly, the relative changes detected in the course of this study phase, were lower than the ones detected during the ED_{50} study. These different findings may be related to the slightly lower dose (6 mg/kg versus 6.22 and 6.3 mg/kg) used on the third phase.

As previously mentioned, the rationale of reducing the dose to 6 mg/kg was in order to test a dose that could be used in a clinical setting. As the entire population did not show clinically significant cardiovascular effects, it appears that this dose could be safe under clinical setting for broiler chickens.

This study showed that the dose of 6 mg/kg of intravenous preservative free lidocaine did not lead to clinically significant cardiovascular effects. Further studies are needed to assess the safety of 6 mg/kg lidocaine dose in a larger population as well as in other species.

CHAPTER 7
CONCLUSIONS

Based on the currently published information, it is said that lidocaine can cause toxicity at relatively low doses (4 mg/kg).(Carpenter, 2005) Based on this assumption, lidocaine may not be commonly used in avian species. However, this information appears to be anecdotal and lack scientific validation. The use of non-scientifically validated information is a common problem in avian medicine. Due to the large number of species and limited scientific research, there is a need to adapt information from other species and use personal experience. In the case of the suggested therapeutic (1-3 mg/kg) and toxic (4 mg/kg) doses of lidocaine for birds, the origin of such information is not clear.(Carpenter, 2005; Carpenter and Marion, 2013; Huckabee, 2000; Ludders and Matthews, 2007; Machin, 2005; Paul-Murphy and Ludders, 2001; West et al., 2007) Furthermore, no information on the route of administration or species is provided in those references. As previously mentioned, several studies have used much higher doses for the purpose of regional nerve block without reporting mortality or morbidity related to the use of lidocaine.(Brenner et al., 2010; Figueiredo et al., 2008) The route of administration is a factor that could have a significant impact on the occurrence of adverse side effects. Also, certain species may be more sensitive to lidocaine than others. Due to this contradictive data, there was a need to assess the veracity of such information.

The objective of this thesis research was to assess the cardiovascular effects of intravenous lidocaine in a specific strain of broiler chickens. To do so, a 3 stage study was planned. On an initial stage, validity of the published information was tested. On a pilot study, doses below the published toxic level were tested. Based on a lack of clinically significant cardiovascular toxicity during that study, testing higher doses was performed. The 2nd study,

using the Dixon's up-and-down method, allowed the determination of the highest tolerable dose of intravenous lidocaine that would not cause significant cardiovascular depression in chickens. On the 3rd study, the previously gathered information was tested in a different group of broiler chickens.

The 3 studies allowed the determination of the highest tolerable dose in terms of cardiovascular effects and further assessed the safety of such dose. The results gathered during these studies indicate that the previously reported toxic dose were erroneous, at least for this study population. These studies were performed in a specific strain of chickens. Future studies assessing the use of lidocaine in other species are needed, nevertheless, the results show that lidocaine may be a useful therapeutical agent and that it may be worth to investigate its effects further in avian species. However, the studies had limitations that are important to consider.

The lidocaine used was preservative free lidocaine. In humans, allergic reactions to local anesthetics are rare. (Stoelting and Hillier, 2006) Considering the low number of confirmed allergic reactions, it can be considered that the allergic reactions could be related to other compounds of the drug rather than the pharmaceutical agent *per se*. (Stoelting and Hillier, 2006) Several compounds commonly used as preservative have been reported to cause allergic reactions; methylparaben, sodium metabisulfite, and bisulfite.(Dooms-Goossens et al., 1989; Schwartz and Sher, 1985; Stoelting and Hillier, 2006) Because of the assumption that other compounds could be related to lidocaine toxicity, it was elected to use a lidocaine formulation that did not contain preservatives. It is unclear if the findings of this study could have been different if another lidocaine formulation was used, but it is an important consideration in terms of clinical use.

All animals used during this study had similar genetic background. The broiler chickens used during the study were Ross 708 (Aviagen, Huntsville, AL, USA). These animals are highly selected and the genetic background is very similar. This provides less genetic variability, therefore less bias to the study but may limit the usefulness of the information to other chicken breeds and other avian species. Congenital cardiovascular disease predisposition of broiler chickens has been well described.(Julian, 1998; Julian et al., 1987; Moghadam et al., 2005; Rauw et al., 1998) In a recent study assessing the gross pathological finding on dead-on-arrival broiler chickens (Ross 308 and Ross 708) to a Danish abattoir, atrial dilation (222/295), myocardial congestion (54/295), right ventricular dilation (32/295), and biventricular dilation (7/295) were reported.(Lund et al., 2013) The reason to use such animals was that if no clinically significant cardiovascular effects would be noted in a strain of chickens genetically predisposed to cardiovascular disease, then it would be less likely to occur in species without this genetic predisposition. Nevertheless, this hypothesis needs to be scientifically validated.

During the 3 studies, all anesthetic monitoring and instrumentation placement was performed in the same manner. Attention was given to standardize certain parameters like $EtCO_2$, temperature, and end-tidal concentration of isoflurane. This was achieved by defining target values for these parameters. The reason behind such standard was to reduce bias to the research. Furthermore, if these parameters were not similar among the animals, significant differences among the metabolic rates of the individuals could occur associated with the anesthesia. The isoflurane MAC of chickens with controlled ventilation is reported to be $1.25 \pm 0.13\%$ (n=9 female cross-bred) and $1.24 \pm 0.05\%$.(Concannon et al., 1995; Naganobu and Hagio, 2000) In the current study, the measurement of the end-tidal isoflurane concentration was

performed using a gas analyzer (Datascope Passport 2, MAQUET, New Jersey, USA). The selected target range was 1.4 and 1.7%.

The effect of the intravenous lidocaine on the cardiovascular parameters (MAP and HR) was used to quantify the cardiovascular depression. Prior to the lidocaine infusion, the average value of MAP and HR were calculated. These data was acquired for 30s prior to the drug infusion. Following the lidocaine administration, the lowest values of HR and MAP were determined. The comparison between the average value of cardiovascular parameters prior to the drug administration and the lowest values obtain post-lidocaine administration, allowed the determination of a relative decrease (or increase) of those cardiovascular parameters. This also allowed the definition of binary responses (effect or no effect). A binary response definition is essential for the use of the up-and-down method.

The definition of clinically significant cardiovascular depression was considered to be a threshold of 30% decrease for HR and MAP. Effect was considered when relative decrease of MAP and/or HR was equal to or greater than 30%. No effect implied relative decrease of MAP and HR of less than 30%. To the author knowledge, there is no clear definition of hypotension for chickens or avian species in general; therefore, the definition of hypotension for the purpose of this thesis was based on published data on normal MAP/HR and the effects of anesthesia on those parameters. Mean blood pressure measured from the abdominal aorta of conscious white leghorn chickens (n=45, 30 to 35 weeks old) has been reported to be 137.6 ± 2 mmHg. (Nishimura et al., 1981) However, the MAP of anesthetize chickens at 1x MAC (1.25% isoflurane) has been reported to be 88 ± 10 (n=9).(Naganobu and Hagio, 2000) In the same study, the MAP at 2x MAC (2.5% isoflurane) was 63 ± 9 mmHg (n=9).(Naganobu and Hagio, 2000) Once the isoflurane was reduce to 1x MAC, the MAP returned to the original

values.(Naganobu and Hagio, 2000) The decrease of MAP pressure between 1x MAC and 2x MAC was approximately 30%, which was considered statistically significantly (p<0.01).(Naganobu and Hagio, 2000) In another study, the administration of reserpine, a indole alkaloid antipsychotic and antihypertensive agent, led to statistically significant decrease of MAP.(Nishimura et al., 1981) The HR prior to the administration of reserpine was 272 bpm while the HR post administration was 186 bpm, which corresponded to approximately 32% decrease.(Nishimura et al., 1981) Based on this data, it was elected to use 30% as the clinically significant threshold. Although the author considers that a decrease of 30% HR and MAP may not be clinically significant in healthy individuals, this may not be the case in unhealthy animals.

Overall, this study has shown that lidocaine can be used safely in broiler chickens. Furthermore, even at the highest doses used during the study (7 and 8 mg/kg), no mortality or morbidity was detected. Nevertheless, 4 of 5 animals that received those doses had clinically significant cardiovascular effects. Although the objective of this study was not to determine the toxic dose, it appears that the toxic/lethal intravenous dose may be higher than 7 or 8 mg/kg.

In conclusion, this study provides information on the use of intravenous lidocaine in chickens. As mentioned above, lidocaine can be used as an analgesic and anesthetic. This is not commonly reported in avian species, possibly due to concerns with toxicity. The information provided by this study shows evidence that further attention to research on the use of intravenous lidocaine in avian species should be performed. Studies assessing the use of constant rate infusion, MAC reduction, and analgesic effect of lidocaine need to be performed to investigate lidocaine's efficacy for these purposes. The multitude of lidocaine uses makes it a very appealing and interesting drug. The versatile use of lidocaine is, as described previously, due to its effects on the sodium channels. Analgesic effects are a consequence of this channel interaction as well.

This appears to be extremely interesting in avian analgesia. Avian analgesia still relies greatly on the use of non-steroidal anti-inflammatories and opioids. Opioids depend on the interaction with opioid receptors, which are of unknown distribution in most avian species. Using a drug, such as lidocaine, that does not rely on opioid receptors may be promising. Further studies are needed to assess such hypothesis. The author expects that this study can provide useful information for the use of lidocaine in avian species.

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VITA

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