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Efficacy/Toxicity Studies of Amiodarone in Animal Models

Dissertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

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* * * * *

The Ohio State University
2002

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ABSTRACT

Heart disease is the leading killer of humans. Sudden death due to arrhythmias is contributory to high morbidity and mortality. Amiodarone is a compound proven useful for the management of most arrhythmias, but its use in dogs has been limited by inadequate clinical trials and fears of toxicity. These studies were conducted in dogs to explore:

I. ECG and ECHO, and changes in serum biochemistries to supratherapeutic and therapeutic doses of amiodarone given orally,

II. Effects of amiodarone on action potentials from Purkinje fiber and endocardial fibers,

III. Tendency of amiodarone to produce corneal deposits,

IV. Effects of escalating concentrations of amiodarone on QTc, heart rate, dP/dt_{max} and dP/dt_{min} in isolated, perfused guinea pig hearts.

I. During a period of loading with 50 mg/kg bid, heart rate decreased, PQ interval increased, both QT and QTc prolonged, T wave morphology changed. No changed appeared on ECHO. Plasma phosphorous and CO_{2} decreased, and ALT, AST and cholesterol increased. During the maintenance dose, all values except cholesterol returned to normal.

II. QT, but not QTc prolonged. APD_{50 and 90} from the endocardium recorded in vivo

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Prolonged. APD$_{50}$ and APD$_{90}$ from Purkinje fiber recorded in vitro shortened but insignificantly.

III. One out of 6 dogs receiving 50 mg/kg bid for 4 weeks and 25 mg/kg for 7 additional weeks had corneal microdeposits in the basal epithelial cells. The same dog had high rates of mitotic turnover in those cells.

IV. In the guinea pig heart Langendorff preparation, amiodarone prolonged QT and QTc intervals at concentrations of $10^{-5}$ and $10^{-4}$ M. When compared to baseline, amiodarone prolonged RR, PQ, QT, QTc and QRS durations. The vehicle did not generate statistically significant changes in any of the ECG parameters.

Amiodarone decreased dp/dt$_{max}$ and increased dP/dt$_{min}$ when compared to vehicle and baseline.

Our results have shown that amiodarone in high doses (3 times the current recommended loading dose) for a prolonged period of time (4 weeks) causes gastrointestinal irritation, electrocardiographic changes and alterations in serum chemistries. However, these effects rapidly disappeared when the dose was reduced by half and maintained for an additional 4 weeks. Results from subsequent experiments following the maintenance dosing protocol supported the theory amiodarone is safe at therapeutic ranges in healthy animals.
To my dad, mom and brothers
ACKNOWLEDGMENTS

It was great honor to have Turkish Government’s scholarship for PhD program. I appreciate and thank my Government giving this opportunity. I got lots of encouragement, optimism and support from my advisor, Dr. Robert L. Hamlin. I believe that it would have been much harder without him.

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PUBLICATIONS

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1. Bicer S, Schwartz DS, Nakayama T, Hamlin RL. Hemodynamic and
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anesthetized with morphine/alpha chloralose. *Journal of Veterinary Internal

2. Bicer S, Nakayama H, Nakayama T, Strauch SM, Hamlin RL. Effects of Chronic,
Oral Amiodarone on Left Ventricular Pressure, Electrocardiograms, and Action
Potentials from Myocardium, in Vivo, and from Purkinje Fibers, in Vitro.


**FIELDS OF STUDY**

Major Field: Veterinary Biosciences
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. Literature review</td>
<td>1</td>
</tr>
<tr>
<td>1.1 History of amiodarone</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Chemistry and definition of amiodarone</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Administration and dosage schedule of amiodarone</td>
<td>5</td>
</tr>
<tr>
<td>1.4 Pharmacodynamics</td>
<td>7</td>
</tr>
<tr>
<td>1.5 Pharmacokinetics</td>
<td>12</td>
</tr>
<tr>
<td>1.6 Therapeutic use</td>
<td>14</td>
</tr>
<tr>
<td>1.7 Drug interactions</td>
<td>15</td>
</tr>
<tr>
<td>1.8 Adverse effects and toxicity</td>
<td>18</td>
</tr>
<tr>
<td>1.8.1 Cardiovascular effects</td>
<td>19</td>
</tr>
<tr>
<td>1.8.2 Ocular effects</td>
<td>20</td>
</tr>
<tr>
<td>1.8.3 Pulmonary effects</td>
<td>21</td>
</tr>
<tr>
<td>1.8.4 Gastrointestinal effects</td>
<td>22</td>
</tr>
<tr>
<td>1.8.5 Neurologic effects</td>
<td>22</td>
</tr>
<tr>
<td>1.8.6 Hepatic effects</td>
<td>23</td>
</tr>
<tr>
<td>1.8.7 Dermatologic effects</td>
<td>23</td>
</tr>
<tr>
<td>1.8.8 Thyroid effects</td>
<td>23</td>
</tr>
<tr>
<td>1.9 References</td>
<td>25</td>
</tr>
</tbody>
</table>

viii

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
2. Effects of Chronic Oral Amiodarone in Dogs ............................................................. 54
   2.1 Abstract ..................................................................................................................... 54
   2.2 Introduction ............................................................................................................. 55
   2.3 Materials and Methods ............................................................................................. 57
   2.4 Results ....................................................................................................................... 59
   2.5 Discussion ................................................................................................................. 61
   2.6 References ............................................................................................................... 71

3. In Vivo and in Vitro Studies of Chronic Oral Amiodarone in Dogs ...................... 83
   3.1 Abstract ...................................................................................................................... 83
   3.2 Introduction ............................................................................................................... 84
   3.3 Materials and Methods ........................................................................................... 85
   3.4 Results ...................................................................................................................... 88
   3.5 Discussion ................................................................................................................. 90
   3.6 References ............................................................................................................... 94

4. Amiodarone-Induced Keratopathy in Dogs ............................................................... 101
   4.1 Abstract ................................................................................................................... 101
   4.2 Introduction ............................................................................................................. 102
   4.3 Materials and Methods ......................................................................................... 102
   4.4 Results ..................................................................................................................... 104
   4.5 Discussion ............................................................................................................... 105
   4.6 References .............................................................................................................. 108

5. Acute Effects of Escalating Doses of Amiodarone in Guinea Pig Hearts ...... 115
   5.1 Abstract ................................................................................................................... 115
   5.2 Introduction ............................................................................................................ 116
   5.3 Materials and Methods ........................................................................................... 117
   5.4 Results ..................................................................................................................... 119
   5.5 Discussion ............................................................................................................... 119
   5.6 References .............................................................................................................. 124

Bibliography .................................................................................................................................. 130
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Pharmacodynamic properties of amiodarone</td>
<td>49</td>
</tr>
<tr>
<td>1.2 Pharmacokinetic properties of amiodarone</td>
<td>50</td>
</tr>
<tr>
<td>1.3 Amiodarone drug interactions</td>
<td>51</td>
</tr>
<tr>
<td>1.4 Amiodarone side effects</td>
<td>52</td>
</tr>
<tr>
<td>2.1 Serum biochemistry of amiodarone</td>
<td>76</td>
</tr>
<tr>
<td>2.2 Electrocardiographic variables</td>
<td>77</td>
</tr>
<tr>
<td>2.3 Echocardiographic variables</td>
<td>78</td>
</tr>
<tr>
<td>2.4 Arterial blood pressure measurements</td>
<td>79</td>
</tr>
<tr>
<td>3.1 In vivo electrocardiographic variables</td>
<td>97</td>
</tr>
<tr>
<td>3.2 In vitro electrophysiologic variables</td>
<td>98</td>
</tr>
<tr>
<td>4.1 Mitotic cell indices of the basal epithelial cells</td>
<td>110</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>53</td>
</tr>
<tr>
<td>2.1</td>
<td>80</td>
</tr>
<tr>
<td>2.2</td>
<td>81</td>
</tr>
<tr>
<td>2.3</td>
<td>82</td>
</tr>
<tr>
<td>3.1</td>
<td>99</td>
</tr>
<tr>
<td>3.2</td>
<td>100</td>
</tr>
<tr>
<td>4.1</td>
<td>111</td>
</tr>
<tr>
<td>4.2</td>
<td>112</td>
</tr>
<tr>
<td>4.3</td>
<td>113</td>
</tr>
<tr>
<td>4.4</td>
<td>114</td>
</tr>
<tr>
<td>5.1</td>
<td>128</td>
</tr>
<tr>
<td>5.2</td>
<td>129</td>
</tr>
</tbody>
</table>

Chemical structures of khellin and benzofuran derivatives
Body weight measurements
Amiodarone serum concentrations
T wave morphology
Typical monophasic action potentials recorded from the subendocardium
Monophasic action potentials recorded from Purkinje fibers
Serum concentrations of amiodarone and desethylamiodarone
Biomicroscopy photograph from left cornea of affected dog
Corneal deposits of the basal epithelial cells from affected dog
Mitotic cell histogram
Typical recordings of cardiac electrogram, left ventricular pressure, and dP/dt of left ventricular pressure
Plots of physiological variables for escalating concentrations of both amiodarone and vehicle (DMSO/Krebs-Hensleit)
1.1 History of amiodarone

Many benzofuran derivatives were synthesized and tested in vitro on gastrointestinal smooth muscle and coronary vascular resistance in the rabbit. Benziodarone (L 2329), which is a benzofuran of the khellin molecule, was found to be the most effective one among the 81 benzofuran derivatives studied. Then the alkyl-hydroxybenzoyl benzofuran derivatives were studied. Charlier et al studied 19 different alkyl-hydroxybenzoyl benzofuran derivatives by measuring electrograms from rectal smooth muscle of rats. Again, benziodarone and its dibromo homologue (L 2214) were the most effective. The laboratory then continued studying benzofuran derivatives, and amiodarone (L 3428), synthesized in 1961, was one of them.

Deltour et al, in isolated heart muscle from rabbits, showed that coronary dilatation produced by amiodarone produced more pronounced coronary vasodilatation than any other benzofuran. Charlier et al studied pharmacologic effects of amiodarone on the heart and on many blood vessels. They found that metabolites of amiodarone enhanced vasodilation. Charlier et al reviewed, in depth, the pharmacological actions of amiodarone, and showed that 10 mg/kg given intravenously reduced both chronotropy and the chronotropic effects of catecholamines and sympathetic activity, and that atropine
did not alter the amiodarone-induced bradycardia. The non-competitive adrenoceptor blockade by amiodarone was also confirmed by Singh et al and Charlier et al in 1970. Biochemical analysis of blood (systemic arterial and coronary sinus), after administration of amiodarone in anesthetized rats and dogs was studied by Broekhuysen et al. The ratio of the sum of ATP and phosphocreatin to inorganic phosphate increased in rats. Therefore, it was suggested amiodarone has a positive effect on myocardial ATP utilization. Increase in coronary blood flow, decreases in oxygen and ATP consumption produced by amiodarone in dogs were also confirmed, and it was concluded that amiodarone increases the energetic yield of the heart. Charlier et al studied hemodynamic effects of amiodarone and found that amiodarone decreased left ventricular work, coronary arterial resistance and systemic blood pressure in the dogs anesthetized with nembutal/chloralose.

Angina pectoris was relieved in 71.6% of 505 patients with angina pectoris and abnormal ECG’s who were given PO 600 mg/day of amiodarone. It was mentioned that amiodarone decreased electrocardiographic abnormalities in those patients. A potential antiarrhythmic activity of amiodarone was recognized in 1969. Charlier et al proved that amiodarone suppressed experimentally induced cardiac arrhythmias such as ventricular extrasystole, ventricular tachycardia, and atrial fibrillation in anesthetized rabbits and dogs.

The first clinical trials, using amiodarone, intravenously, as an antiarrhythmic in patients, yielded results consistent with those observed in rabbits and dogs. In 1970, Van Schepdael and Solvay gave intravenous amiodarone, at from 400 to 600 mg/hour, to 83 patients with atrial fibrillation, atrial and ventricular extrasystoles. Fifty-five out of 83
patients manifested improvement of their arrhythmia. In 1974, Rosenbaum et al gave oral amiodarone (300 to 600 mg/day) to 11 patients with Wolff-Parkinson-White syndrome-induced tachyarrhythmias, and demonstrated that the tachyarrhythmias could be controlled for 2 to 8 months.

Singh and Vaughan Williams studied action potentials from atria and ventricles of rabbits given, IP, 20 mg/kg daily for 6 weeks. As in hypothyroid rabbits, but different from the effects of Vaughan Williams class I or II antiarrhythmics, amiodarone prolonged the action potential duration approximately 33.5% in both atrial and ventricular myocardium. This lead to placing amiodarone in class III (i.e. homogeneous prolongation of action potential, restriction of fast inward current and antisympathetic).

1.2 Chemistry and definition of amiodarone

Amiodarone is a benzofuran moiety of the khellin molecule with two iodine atoms in the benzene ring. It is a white to yellow (cream-colored) crystalline powder, that is freely soluble in chloroform and methanol, soluble in ethanol, sparingly soluble in isopropanol, slightly soluble in acetone, dioxane, ether and carbon tetrachloride, and very slightly soluble in water. Amiodarone \((C_{25}H_{29}I_{2}NO_{3})\) is 37.3% iodine by weight (molecular weight: 645.32). Chemical structures of khellin, and benzofuran derivatives (benzarone, benziodarone, desethlamiodarone and amiodarone) are shown in Figure 1.

Intravenous and oral preparations of amiodarone are commercially available throughout the world. Chemical formulas of intravenous amiodarone is 2-butyl-3-benzofuranyl \([4-[2-(diethylamino) ethoxy]-3,5 diiodophenyl] methanone hydrochloride\) and chemical formula of oral amiodarone is 2-butyl-3-benzofuranyl-4-[2-(diethylamino)
ethoxy]-3,5-diiodophenyl ketone hydrochloride.

The intravenous preparation, which is a clear pale yellow color, sterile, and particle-free, is in 3 ml ampules containing 50 mg amiodarone hydrochloride, 20.2 mg benzyl alcohol, 100 mg polysorbate 80, and water. (Cordarone I.V. by Weyth-Ayerst) The oral preparation, which is a pink colored 200 mg tablet, contains 200 mg of amiodarone hydrochloride and inactive ingredients: lactose, magnesium stearate, colloidal silicon dioxide, povidone, starch and FD&C Red 40. (Cordarone by Weyth-Ayerst, Pacerone by Upsher-Smith and Amiodarone hydrochloride by Copley Pharmaceutical Inc.)

Shelf life for amiodarone tablets is approximately 16 months. During this time, tablets should be protected from light and kept in a tight container at room temperature (between 10-30 °C). Injectable ampules should also be kept from light at room temperature (between 15-25 °C); however, there is no need to protect from light during administration. An oral suspension (5mg/ml) made from tablets can be kept in the refrigerator for approximately 90 days or at room temperature for 42 days. The solution should be kept in either rigid polyvinyl chloride (PVC) infusion bags, or in glass bottles, and should be stored in 5% dextrose (D5W) no longer than 5 days. Amiodarone is bound to the PVC of flexible bags at approximately 40% per 120 hours. Mixture of amiodarone and D5W at a concentration of 4 mg/mL forms precipitation with aminophylline, cefamandole nafate, cefazolin sodium and mezlocillin sodium. It also forms precipitation with sodium bicarbonate at concentration of 3 mg/mL. Admixtures of 0.9% sodium chloride injection, even if in the glass bottles are not stable.
1.3 Administration and dosage schedule of amiodarone

There is no well-established dose schedule for amiodarone due to individual variation, its slow onset and potential side effects. Amiodarone should be administered by cardiologist physicians and veterinarians, and should be closely monitored, especially during the loading dose. If other antiarrhythmic drugs (e.g. quinidine, procainamide and sotalol) have been used, they should be gradually discontinued or decreased in dose. Intravenous amiodarone is used for the initiation of the treatment, and is not intended for maintenance. It is intended for termination of arrhythmias and for patients who are unable to take oral medication. Amiodarone tablets should be administered with food to decrease gastrointestinal irritation and to increase absorption (rate and bioavailability). Amiodarone is used for both atrial and ventricular arrhythmias, especially for management of life-threatening recurrent ventricular fibrillation and hemodynamically unstable ventricular tachycardia not responsive to other antiarrhythmics.

Amiodarone is usually given, initially for from 4 to 14 days, in a loading dose (10 to 20 mg/kg/day or 1.72 mg/M2/day for infants) or until an adverse reaction is seen. Then the dose is decreased to 5 mg/kg/day to minimize potential side effects. The usual minimal dose can be decreased to 2.5 mg/kg/day. The maintenance dose may be given for 5 days a week. For adults an oral loading dose is 600-1600 mg/day is given for 1-3 weeks. After controlling the arrhythmia, the dose should be decreased to 600-800 mg/day for 1 month, and ultimately to a maintenance dose 200-600 mg/day. For supraventricular arrhythmias the loading dose of 600-800 mg/day is given for 1 week. Then the dose is reduced to 400 mg/day for 3 weeks and finally a maintenance dose of between 200-400 mg/day is given.
When used intravenously in children, a loading dose of 5 to 20 mg/kg is given over 0.5 to 1 hour. This is followed by an infusion of 5 to 15 mg/kg/day over 1 to 5 days for maintenance dose.\textsuperscript{174, 175, 176, 177, 181} For adults, the intravenous loading dose is 150 mg/100 mL given at an infusion rate of 15 mg/min for 10 minutes.\textsuperscript{144} This is followed by a slow infusion dose of 360 mg/200 mL at a rate of 1 mg/min for the next 6 hours. Finally, a maintenance dose is 540 mg/300 mL is given at a rate of 0.5 mg/min.\textsuperscript{144} When intravenous dosing must continue, it can be administered at 0.5 mg/min for 2-3 weeks. A bolus of 5 mg/kg given over 15 minutes, then 600 to 1000 mg continuous infusion of amiodarone given over 12 to 24 hours is effective in patients with recurrent symptomatic ventricular tachycardia.\textsuperscript{147}

Intravenous administration is intended only to initiate therapy, and should be followed by oral administration. The concentration of intravenous amiodarone in 5\% dextrose injection USP (D\textsubscript{5}W) should be 2.5 mg/mL or less to decrease irritation in the vein. It should be given by through infusion into a central venous catheter when higher concentrations are required or when infusions in excess of 1 hour are performed.

There is very little information on the clinical use of amiodarone in dogs. The oral loading dose is given as 10 mg/kg twice a day for 1 week, after which the dose is decreased to 8 mg/kg once a day until the therapeutic concentration reaches (1-2.5 \textmu g/ml). Then, the dosage can be reduced to 5 mg/kg once daily for maintenance.\textsuperscript{18}

For more emergency-type arrhythmias, amiodarone may be given intravenous, and then followed by the oral route. A dose of 10 mg/kg given as a bolus has proven safe for dogs.\textsuperscript{19}
1.4 Pharmacodynamics

Amiodarone has a wide spectrum of pharmacological activity. Table 1 summarizes its pharmacodynamics. It possesses class I, II, III and IV antiarrhythmic properties of the Vaughan Williams classification. It has a negative chronotropic action (i.e. slows heart) by its ability to block L-type calcium channels and its beta adrenergic blocking property. It is mildly negatively dromotropic, manifested by decreased conduction velocities through the atria and across the atrioventricular node. It produces vasodilatation via an alpha1-adrenergic blocking effect. Oral and intravenous amiodarone show different pharmacodynamics. A 12.5 mg/kg iv bolus of amiodarone in ethanol decreases myocardial contractility (negative inotropic effect) in healthy dogs; however, oral amiodarone did not decrease left ventricular function during a 7-week, high dose exposure of healthy dogs. The difference between intravenous and oral amiodarone can be explained by the fact that commercial, intravenous amiodarone contains polysorbate 80, which causes negative inotropy and a decrease in mean arterial pressure in dogs.20 A dose-dependent negative chronotropy of amiodarone also suppresses norepinephrine and calcium-induced positive chronotropy, but may enhance their positive inotropic action. Thus amiodarone may be useful for reducing the tachycardia observed in heart failure.43 In humans, amiodarone changes cardiovascular hemodynamics minimally. There is a decrease in mean arterial pressure and systemic vascular resistance seen after 1 to 3 hours of oral administration; however, these changes are less prominent after 2 days of administration in patients with congestive heart failure.46 Negative chronotropy during rest and graded exercise occurred in long-term therapy with amiodarone, but the decrease in heart rate did not cause any changes in arterial blood pressure and functional capacity.
of patients.\textsuperscript{44}

Amiodarone selectively blocks both time- and voltage-dependent slow components of the open-state, delayed rectifier current (I_{Ks}), but not the rapid component (I_{Kr}).\textsuperscript{69} However, it is also reported that the human ether-a-go-go-related gene (hERG), a K\textsuperscript{+} channel identical to the rapid component of delayed rectifier current (I_{Kr}), is blocked by amiodarone.\textsuperscript{34} Amiodarone also reduces the inward rectifier potassium current (I_{Ki}) about 20\% in the presence of cadmium chloride and tetrodotoxin, which are blockers of sodium and calcium channel currents (I_{Na} and I_{Ca}) in whole-cell patch-clamp studies of guinea pig ventricular myocytes; moreover, it blocks I_{Ki} in an inside-out patch-clamp study suggesting that amiodarone interacts with the inward rectifier potassium channel of either hydrophobic site within the membrane or hydrophilic site within the cell.\textsuperscript{73}

Amiodarone depresses the effects of carbachol, adenosine or intracellular loading of GTP\gamma S on potassium current (I_{KACH}) activation in atrial cells by direct blocking of acetylcholine-sensitive muscarinic potassium channel (K_{ACH}) or by indirect blocking of GTP-binding proteins.\textsuperscript{32} Amiodarone inhibits sodium activated potassium channels (K_{Na}) at therapeutic concentrations in guinea pig ventricular cells.\textsuperscript{33} It also blocks the current (I_{KNa}) induced by intracellular loading of sodium and extracellular application of ouabain.\textsuperscript{33} Amiodarone also blocks ATP-sensitive potassium channels (K_{ATP}) and current activity by increasing ATP sensitivity and prolonging of the channel closure time.\textsuperscript{35}

Prolongation of QT, QTc and PR intervals, and lengthening of the effective refractory period of atrioventricular nodal fibers,\textsuperscript{36} atrial and ventricular myocardium make oral amiodarone effective in patients with both atrial and ventricular arrhythmia.\textsuperscript{31,66} However, intravenous amiodarone does not change any of the above
electrocardiographic parameters except that it prolongs AH of the intranodal conduction. Amiodarone also acts differently in Purkinje fibers and ventricular muscle fibers. It prolonged APD in ischemic ventricular muscle in dogs; however, there was no increase in APD in Purkinje fibers taken from same ischemic area.

Amiodarone is not a pure class III drug and its other activities can participate the antiarrhythmic action. For example, a use-dependent block of fast inward sodium channels in resting, activated state and selectively inactivated state is more prominent when amiodarone is administered intravenously. The selective affinity to the inactivated sodium channels can be seen during both acute and chronic administration of amiodarone, which suppresses automaticity and slows the conduction time more in depolarized tissue (e.g. ischemic tissue) than in normally polarized tissue. Reduction of upstroke velocity (v_{max}) of the action potential and shortened recovery time from blocking are prominent when the voltage is more negative suggesting that amiodarone blocks inactivated sodium channels.

Amiodarone is a well known coronary and arterial vasodilator. It is a non-competitive alpha and beta_1 adrenoceptor blocker. Arterial vasodilation occurs by blocking of alpha_1 adrenergic receptors. This helps to reduce arterial resistance and afterload, and increases coronary blood flow, both of which relieve the pain of angina. However, infusions of more than 2100 mg/24 hour infusion increase the risk of hypotension. Polysorbate 80, which is the solvent for intravenous amiodarone also has an additive effect on vascular dilation.

Antisympathetic activity of intravenous amiodarone occurs by a reserpine-like block of the storage of norepinephrine and by increasing the intraneuronal metabolism of.
norepinephrine, which increases the metabolite (dihydroxyphenylglycol) of norepinephrine in the blood. This did not occur when amiodarone was given orally to the rats. Amiodarone does not bind to the recognition side of beta-receptors; therefore, it does not compete with adrenergic agonists and does not cause upregulation of beta-receptors like class II drugs do. Amiodarone may regulate transcriptional gene expression by binding to the thyroid hormone receptor; thus, it downregulates beta-adrenergic receptor protein and decreases isoproterenol-induced, intracellular cAMP concentrations. Amiodarone has non-selective β adrenoceptor activity by reducing both β₁ and β₂ adrenoceptors density. It also decreases isoprenaline (non-selective β agonist) and terbutaline (β₂ agonist)-induced production of cAMP; however, fluoride-induced stimulation of cAMP is not changed by amiodarone.

Amiodarone produces mild calcium channel (L-type calcium current) blockade, that shows voltage- and use-dependent inhibition, during the resting and inactivated state of calcium channels. It causes prolongation of atrial and atrioventricular conduction time, which is helpful in patients with supraventricular arrhythmias. Amiodarone also decreases the effect of the beta receptor agonist, isoprenaline, on L-type calcium current in the AV node of rabbits. Amiodarone inhibits \[^{3}H\] nitrendipine binding to slow calcium channels in cardiac membranes. There is a competitive blockage in those channels, and diltiazem reverses the inhibitory action of amiodarone. This suggests that both amiodarone and diltiazem recognize the same allosteric site (benzothiazepine site). However, the allosteric interaction with the 1,4 dihydropyridine binding site may exert a pseudo-competitive inhibition on \[^{3}H\] nitrendipine binding. Amiodarone-induced calcium channel blocking for 1,4...
dihydropridine binding site is less potent than nifedipine, which selectively binds to the 1,4 dihydropridine site.\textsuperscript{37} The influx of calcium into cardiac myocytes is higher during the potassium-depolarized cells than in normally polarized and resting cells. In the presence of amiodarone, calcium uptake decreases significantly in potassium-depolarized cells, in which other calcium channel blockers have the same antagonistic activity.\textsuperscript{37}

The inhibitory effects of both acute and chronic amiodarone on the Na\textsuperscript{+} -K\textsuperscript{+} pump appear to result in an overall decrease in Na\textsuperscript{+} -K\textsuperscript{+} pump capacity, and inhibition of the pump current by amiodarone is not affected by voltage changes or affinity of the pump for extracellular K\textsuperscript{+} in cardiac ventricular myocytes.\textsuperscript{74} Although amiodarone inhibits Na\textsuperscript{+} -K\textsuperscript{+} ATPase, unlike cardiac glycosides it causes negative inotropy. It is possible that the decrease in myocardial contractility is associated with the interference of the Na\textsuperscript{+} -Ca\textsuperscript{2+} exchange and impairment of Ca\textsuperscript{2+} release from the sarcoplasmic reticulum.\textsuperscript{65} It is also possible that amiodarone’s block of thyroid hormone and reduced activity of cardiac calcium myosin ATPase in the heart may participate in the negative inotropic action of amiodarone.

Amiodarone is considered to indirectly block the conversion of T\textsubscript{4} to T\textsubscript{3}, decreasing the plasma concentration of T\textsubscript{3} by impairing the activity of hepatic thyroxine 5\textsuperscript{'}- monodeiodinase. Direct exposure of amiodarone to liver homogenates did not block the inhibition of the conversion.\textsuperscript{51} Amiodarone also has mild competitive inhibition of T\textsubscript{3} binding to both α\textsubscript{1} and β\textsubscript{1}-thyroid hormone receptor (α\textsubscript{1}- T\textsubscript{3}R and β\textsubscript{1}- T\textsubscript{3}R); however, when amiodarone undergoes structural changes such as deethylation to desethylamiodarone, its metabolites produce strong inhibition.\textsuperscript{40}
1.5 Pharmacokinetics

Rate of absorption of oral amiodarone varies markedly. Amiodarone has very low and erratic bioavailability. One reason could be the variations in the rate of absorption due to variations in duration spent in the GI tract, in particular in the presence of GI disorders. Absorption through the intestinal wall is by passive diffusion.\textsuperscript{23} Amiodarone has very large molecular weight (681.8 MW for amiodarone hydrochloride) and is not soluble in aqueous solution. So, presence of surfactants (polysorbate 80\textsuperscript{21} and sodium laurylsulfate\textsuperscript{22}) and lipids\textsuperscript{17,24} increase the absorption rate; however, high concentrations of surfactant may damage the intestinal mucosa, which might lead to decreased absorption of the drug. Polysorbate 80 seems to be safer than sodium laurylsulfate, and preferable in a non-ionic surfactant of low supramicellar concentrations.\textsuperscript{21} A positive correlation between amiodarone and lipid absorption does not relate to bioavailability, suggesting that a metabolic pathway and large tissue distribution may play a role for erratic fluctuations in pharmacokinetics.\textsuperscript{24}

Other reasons for variable pharmacokinetics could be metabolism of drug by the intestinal mucosa before it reaches the blood, and a first-pass hepatic metabolism and excretion from bile to intestine. Lipophilic attraction to adipose tissue slows down the onset of antiarrhythmic action of amiodarone. However, the same events, which retard bioavailability, also retard and make variable rate of elimination. Slow elimination, producing an extremely long half-life, may be considered an advantage for this agent in management of arrhythmias. The onset of antiarrhythmic action may take several days to several weeks (2 to 6 weeks) due to amiodarone’s large volume of distribution, low bioavailability and slow tissue equilibration (especially fat and skeletal muscle);
however, this latency can be decreased by initiating treatment with intravenous therapy or
administrating high oral loading dose with food and surfactant. After discontinuation of
amiodarone, the offset of action also takes several weeks to months (up to 10 months).

A three-compartment model best explains the complicated kinetics of
amiodarone. Amiodarone is absorbed from the intestines into the blood (first
compartment); from the blood amiodarone is distributed to organs such as heart, lung,
liver and skeletal muscle (second compartment), and finally it saturates adipose tissue
(third compartment). Saturation of fat stores occurs most slowly.

There is no strong relationship between plasma concentration and antiarrhythmic
efficacy of amiodarone. However, concentrations lower than 1 μg/ml may not be
effective, while concentrations higher than 2.5 μg/ml may increase the potential toxic
effects. A clinical study also reported that plasma concentration between 0.8 to 2.8
μg/ml is an effective therapeutic range for managing sustained paroxysmal ventricular
tachycardias. Bioavailability of amiodarone is low, but taking amiodarone with food
increases intestinal absorption. Approximately 96% of amiodarone binds to plasma
proteins. Then, it accumulates in organs from 10 to 100 times, and in fat tissue from 100
to 1000 times the concentration in plasma.

Amiodarone is metabolized mainly by the liver and partially by the intestinal
mucosa. Thus, the dose must be reduced in patients with liver disease. The major
metabolite, desethylamiodarone, acts like amiodarone. The second metabolite, di-
desethylamiodarone, is detected in only small amounts in dogs. The elimination half-life
is variable, and desethylamiodarone (126 days) has a longer half-life than amiodarone (20
to 107 days). Amiodarone accumulates mainly in fat; however, desethylamiodarone
accumulates more in the organs than in fat. Amiodarone is eliminated principally in bile and feces. Elimination from the kidney is negligible (less than 1%) or absent. It is reported that amiodarone and desethylamiodarone are found in breast milk and fetus (passes from placenta). It cannot be removed from blood by hemodialysis.

1.6 Therapeutic use

Amiodarone was developed initially as a vasodilator for use in patients with angina pectoris. However, it was recognized that it also has potent antiarrhythmic action. Amiodarone has been shown to protect patients against arrhythmias of many origins, probably due to its effect on many tissues (i.e. atrium, ventricle, Purkinje fiber) and because it possesses antiarrhythmic properties of all 4 Vaughan-Williams classes. In particular it has been shown to be antifibrillatory, and to possess only a minimal proarrhythmic potential. Whereas it was used initially as a "last-resort" antiarrhythmic for patients with potentially lethal ventricular arrhythmias refractory to other therapy, it is now used commonly as the primary antiarrhythmic and for less severe ventricular arrhythmias. Amiodarone has been shown to suppress both supraventricular and ventricular tachyarrhythmias with success rates of 66% to 92.4% and 64% to 87% respectively. 14, 89, 92, 153, 157, 178, 180

Initial, rapid, intravenous infusion of amiodarone is effective at terminating life-threatening ventricular tachycardia within 51 minutes, and following loading and maintenance doses in 24 hours. It decreases the ventricular rate suppressing approximately 88% of ventricular tachycardia and fibrillation event rate. 144 After initiation and termination of arrhythmia, oral amiodarone can be used to prevent the
recurrence of arrhythmia for a long time.

Clinical studies indicate that amiodarone may be more effective than other antiarrhythmics as follows: It suppresses both paroxysmal and sustained supraventricular tachycardias, and it prevents supraventricular tachycardias, in particular those caused by reentry (W-P-W Syndrome)\textsuperscript{14, 90, 92, 167, 180} It decreases the recurrence of life-threatening ventricular tachycardia\textsuperscript{83} and fibrillation in patients with hypertrophic and dilated cardiomyopathy.\textsuperscript{89, 90, 146}

Amiodarone either alone or in combination with other antiarrhythmics is very effective in infants\textsuperscript{173, 176} and in children with life-threatening arrhythmias.\textsuperscript{167, 172, 174} Of most importance, amiodarone reduces the risk of sudden cardiac death (SCD) in those patients.\textsuperscript{88, 90} In particular, amiodarone decreases the risk of sudden death in patients with myocardial infarction. Ventricular arrhythmias in patients with myocardial ischemia are thought to be produced predominantly by reentry precipitated by heterogeneity of ventricular repolarization (i.e. electrophysiological remodeling).

Initially the wide spectrum efficacy of amiodarone was shaded by concern over adverse side effects (e.g. hepatotoxicity, pulmonary fibrosis, corneal deposits of amiodarone). With consideration of proper dosing and patient monitoring, concern over toxicity to amiodarone has been reduced.

1.7 Drug interactions

Because amiodarone is used commonly in patients with heart disease and frequently diseases of other organ systems, it is common to use the compound in concert with other cardiac (e.g., digitalis, ACE inhibitors) and non-cardiac (e.g., rifampin,
coumadin) drugs; therefore, there is a potential for drug interaction. Amiodarone is known to decrease activity of hepatic microsomes responsible for metabolism of other drugs, in particular quinidine, procainamide, flecainide and digitalis; therefore, lower doses of these compounds may be required in patients receiving amiodarone. On the other hand, other drugs (e.g., quinidine, sotalol) may exert activity synergistic with amiodarone, such as prolongation of QTc; thus increasing the risk for production of torsades de pointes. Furthermore, since both amiodarone and procainamide retard ventricular conduction, when used concomitantly they may provoke reentry leading to arrhythmia. Additive effects of combined therapy with amiodarone and procainamide can be more effective than individual therapy, when the dose adjustment is made.

The dose of other antiarrhythmics should be reduced 1/3 of actual dose when amiodarone is given concomitantly because amiodarone increases plasma concentration of flecainide; therefore, therapeutic plasma concentration of flecainide can be kept in range. The increase of quinidine, lidocaine and phenytoin concentrations is due to the reductions in total body clearance and the prolongation of elimination half-life when amiodarone is present. Calcium channel blockers, beta blockers and amiodarone depress sinus node activity and atrioventricular conduction time; thus, amiodarone in combination with these agents without dose adjustment may cause serious side effects such as hypotension, bradycardia, sinus arrest and AV block. Positive inotropics are also used and very effective in patients, when dose adjustment is made. Otherwise, digitoxin and digoxin intoxication associated with bradycardia, sinus arrest, AV block, nausea, malaise and fatigue can be seen in combination of amiodarone.
The interaction of digitalis glycosides and amiodarone is due to concentration-dependent competitive action in the receptor site of sodium-potassium ATPase,\textsuperscript{75} reduction of body clearance in both renal \textsuperscript{136} and nonrenal clearance and volume of distribution of cardiac glycosides, which increase the plasma concentration of digitalis in presence of amiodarone.\textsuperscript{137} So, in combination therapy, digitalis dosage should be decreased by 50% and plasma concentration should be monitored during the medication. Amiodarone depresses vitamin K dependent coagulating factors; therefore, simultaneous anticoagulant administration may cause increased prothrombin time and bleeding in patients.\textsuperscript{124,125}

When amiodarone and anticoagulants are combined, the dose of anticoagulants (e.g. acenocoumarol and warfarin) should be reduced initially 50% in daily dose \textsuperscript{125} and prothrombin time should be monitored closely to avoid hemorrhagic episodes.\textsuperscript{123} Amiodarone binds approximately 97% of plasma proteins that may displace the other drugs from plasma proteins. However, warfarin and amiodarone interaction are most likely the additive action on anticoagulant effect \textsuperscript{104,124} because amiodarone increases plasma concentration of warfarin (both R- and S-warfarin), hypoprothrombinemia \textsuperscript{123} and inhibits the metabolism of warfarin (S-warfarin>R-warfarin) by blocking of P4502C9, the isoenzyme of P-450 in humans.\textsuperscript{122} Concomitant administration of adenosine with amiodarone appears to be safe in patients with supraventricular arrhythmias.

Adriamysin, a cytostatic agent used in cancer patients causes cell death in tumoral cells. One \textit{in vitro} study showed that amiodarone and adriamysin alone and in combination cause a concentration-dependent increase in creatine kinase leakage, which indicates cell death, in cultured rat cardiomyocytes.\textsuperscript{132} They suggested that the increased toxicity of the concomitant use of amiodarone and adriamysin is mediated by the...
blockage of P-glycoprotein efflux pump.\textsuperscript{132}

The interaction of amiodarone and anesthetics is mainly the additive effects of alpha blocking activity, which causes severe hypotension, low cardiac output and systemic vascular resistance.\textsuperscript{129} Rhythm disturbances (bradyarrhythmia and conduction abnormalities) and pulmonary complications can be seen in patients who are predisposed and undergo surgical procedures.\textsuperscript{129} Therefore, those patients who have high risk of adverse reactions with amiodarone and anesthetics should be monitored closely and prepared by using pacemaker and pulmonary artery catheterization.\textsuperscript{129}

1.8 Adverse effects and toxicity

Although amiodarone is an effective antiarrhythmic agent for both atrial and ventricular arrhythmias, its use in patients is restricted due to a large variety of side effects. Most of these unwanted reactions are dose-dependent and are reversible when the dose is lowered or discontinued. The prevalence of side effects is very low or absent in children and infants.\textsuperscript{167} Amiodarone also shows moderate side effects in young patients, but has not been shown to interfere with their growth.\textsuperscript{164} Adverse effects of amiodarone, from lowest to highest frequency, are shown in Table 4. The data was collected from clinical trials and subjected to a meta-analysis by Vorperian et al.\textsuperscript{138} They analyzed four randomized, placebo-controlled clinical trials,\textsuperscript{182,183,184,185} in which a low dose (152 to 330 mg/day) of amiodarone was used. According to their odds ratio analysis, cardiovascular, ocular, dermatologic, neurologic, gastrointestinal and pulmonary side effects were higher in patients receiving amiodarone than in the placebo group. However, the only statistically significant differences were that patients taking amiodarone

18
developed bradycardia, ocular deposits, and either hyper or hypothyroidism.

There is close relationship between plasma concentration and side effects of amiodarone. However, some side effects can be seen even at plasma concentrations considered to be within the therapeutic range. Side effects occur, mainly, with plasma concentrations over 2.5 µg/ml. Plasma concentrations lower than 1 µg/ml may result in fewer adverse side-reactions, but the concentration of amiodarone will not be effective at suppressing arrhythmia.

1.8.1 Cardiovascular effects

Hypotension is the most common side effect occurring with amiodarone, since the compound produces peripheral vasodilation, especially after intravenous administration. With iv boluses greater than 12.5 mg/kg given to dogs, amiodarone decreased indices of left ventricular systolic function (dP/dtmax and Vmax); but oral administration did not show any dysfunction even at doses (50 mg/kg/day) considered twice therapeutic. Amiodarone can also be used to manage arrhythmias in patients with hypertrophic cardiomyopathy; however, it causes serious conduction abnormalities (AV block, interventricular conduction disturbances) and proarrhythmia. Consequently, when used for hypertrophic cardiomyopathy, close monitoring is necessary.

Negative inotropic effects of intravenous amiodarone may exacerbate left ventricular dysfunction in patients with already compromised ventricular function. Amiodarone in therapeutic doses decreases heart rate gradually. However, in the presence of beta blockers, calcium channel blockers or cardiac glycosides, severe sinus bradycardia, 2nd or 3rd degree AV block may occur. Proarrhythmic effects seem to occur.
infrequently when compared to other antiarrhythmics. Intravenous infusions of 2.5 mg/mL of amiodarone increase the risk of venous irritation leading to phlebitis.

1.8.2 Ocular effects:

Amiodarone-induced keratopathy has been reported as the most common side effect in humans. The corneal microdeposits are invisible to the naked eye. The occurrence depends on dose and duration.\(^\text{148}\) They begin between the 2\(^{nd}\) and 4\(^{th}\) weeks of administration, and they occur in 29.7% to 100% of patients; however, corneal deposits have been observed in only 11.5% of young patients.\(^\text{164}\) In dogs, corneal deposits occurred in 16% (1 out of 6) of dogs during the 7\(^{th}\) week of medication. Detailed information on corneal deposits will be discussed in Chapter 4. Microdeposits can be observed in all regions (cornea, lens, sclera, retina, optic nerve) of ocular tissue. It has also reported that amiodarone-induced microdeposits are reversible, but because of amiodarone’s long half-life, they usually take 3 to 7 months to resolve.\(^\text{149}\) Amiodarone-induced keratopathy may produce visual symptoms (i.e. blurred vision, halos and photophobia); however, these symptoms do not cause impairment of visual acuity and occur only in 1% to 3% of patients.\(^\text{149}\) Optic neuropathy occurs with a low incidence in amiodarone therapy (2 out of 150 patients taking amiodarone), and shows similar symptoms (disc swelling and hemorrhages, but preserved visual acuity) to ischemic optic neuropathy.\(^\text{151}\) However, optic neuropathy appears to be milder than ischemic optic neuropathy and resembles the nonarteritic type of optic neuropathy.\(^\text{152}\)
1.8.3 Pulmonary effects:

Amiodarone related pulmonary complications include pulmonary infiltrates, fibrosis, edema and inflammation. Coughing, dyspnea and fever are common signs and/or symptoms.\textsuperscript{154} The overall incidence of pulmonary toxicity ranges between 5 and 18\% in patients. Pulmonary reactions may occur even in low doses of amiodarone, and the mechanism is still unclear. Possible mechanisms are that amiodarone produces accumulation of phospholipid by interfering with lysosomal phospholipases.\textsuperscript{186} Hypersensitivity may be manifested as immun-compromise.\textsuperscript{187} When compared to controls, there is direct relationship between accumulation of amiodarone and desethylamiodarone and phospholipidosis.\textsuperscript{56} It has also been reported that amiodarone-induced phospholipid accumulation is reversible when amiodarone administration is stopped.\textsuperscript{55}

In the presence of chronic obstructive pulmonary disease (COPD), amiodarone slightly but significantly decreased diffusion capacity of carbon monoxide ($D_{LCO}$) at 1 year compared to patients without COPD; however, there was no difference in the incidence of pulmonary complications in patients with or without COPD.\textsuperscript{156}

Amiodarone inhibits Na-K ATPase activity in the heart.\textsuperscript{74,75} Reasor et al demonstrated that the inhibition of Na-K ATPase occurs in the lungs of rats too.\textsuperscript{57} They also showed β-N-acetylglucosaminidase levels increased significantly. Two weeks after discontinuation of amiodarone, both enzymes returned to control levels. Reasor et al pointed that desethylamiodarone, which was higher in the lung than amiodarone, may play the important role in induction of phospholipidosis. Female rats had lower tissue concentrations of amiodarone and desethylamiodarone in the lungs than male rats.\textsuperscript{57} This

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may indicate that male patients are more susceptible to pulmonary toxicity than females.

Amiodarone may exaggerate adverse respiratory reactions in patients with pulmonary problems; therefore, close monitoring should be performed during therapy with amiodarone. Pulmonary function tests, clinical examination and chest x-ray radiography should be performed before and during amiodarone therapy. Early diagnosis is important because pulmonary complications are fatal and must be treated in the early stages. Amiodarone-induced pulmonary side effects are thought to result from hypersensitivity to amiodarone.\textsuperscript{187} Corticosteroids can be helpful to reduce immunological reactions.

1.8.4 Gastrointestinal effects:

Gastrointestinal irritation, nausea, vomiting, and anorexia are common in patients receiving amiodarone. The prevalence of these side effects increases in high doses but is reversible when dose adjustment is made. Long-term therapy may cause constipation. Administration with food may decrease the gastrointestinal irritation and some other side effects.

1.8.5 Neurologic effects:

High doses of amiodarone may cause CNS responses and peripheral neural reactions such as ataxia, tremor, dizziness and fatigue; however, dose adjustment can reverse the adverse consequences.\textsuperscript{166} Even severe neurological effects improve within a month when amiodarone is stopped.\textsuperscript{166}
1.8.6 Hepatic effects:

Amiodarone metabolism occurs in the liver. Because of the abundant accumulation of amiodarone and its metabolite in the liver, a high doses given for a short time or a more modest dose given for a long time increases hepatic enzymes (ALT, AST and SGOT) and the risk of hepatotoxicity in humans and dogs. Both amiodarone and desethylamiodarone induce myelinoid inclusion bodies in cultured rat hepatocytes and increase the release of lactate dehydrogenase, which is the marker for cell death showing dose-related hepatotoxicity.

1.8.7 Dermatologic effects:

Photosensitivity and skin discoloration are common in humans taking high doses of amiodarone for long periods. It has been reported that accumulation of lipofuscin deposits causes a blue-gray discoloration in patients taking amiodarone. Harris et al also reported that there was a decrease in melanin pigment in those patients who had skin discoloration. Discontinuation of amiodarone for 6 to 12 months are required for this discoloration to resolve. Less exposure to sun, using sunscreen creams and low doses of amiodarone decrease the incidence of dermatologic reactions.

1.8.8 Thyroid effects:

Amiodarone has been reported to produce either hypo- or hyper-thyroidism in humans. Amiodarone-induced hyperthyroidism is difficult to treat; however, hypothyroidism is easier to control. Amiodarone inhibits the peripheral conversion of T4 to T3, which increases serum concentrations of T4 and decreases the T3 levels.
The true hypothyroidism could be the direct effect of amiodarone on the thyroid gland, or the high intake of iodine within the amiodarone molecule, which causes hypothyroidism $^{188}$ and hyperthyroidism $^{189}$ in normal glands.
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<table>
<thead>
<tr>
<th>Categories</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I antiarrhythmic action</td>
<td><strong>Na channel block</strong> 25, 29, 72, ↓ upstroke velocity (phase 0) 26, 29</td>
</tr>
<tr>
<td>Class II antiarrhythmic action</td>
<td>Noncompetitive β receptor block 5, 6, 7, 27, 76, ↓ β receptors (downregulation) 27, 39, 71</td>
</tr>
<tr>
<td>Class III antiarrhythmic action</td>
<td><strong>K channels block</strong> 32, 33, 34, 35, 69, 73, no effect on action potential duration (IV) 28, 31 ↑ action potential duration and refractory period (PO) 6, 31, 66, ↑ QT interval 14, 30, 66</td>
</tr>
<tr>
<td>Class IV antiarrhythmic action</td>
<td><strong>Ca channel block</strong> 36, 37, 38, 67, 68, 70, ↓ sinus node and AV node activity 36, 41, 66</td>
</tr>
<tr>
<td>Antianginal action</td>
<td>Noncompetitive α receptor block 5, 6, 7, 76, vasodilation, ↓ vascular resistance and blood pressure (acute stage) 46</td>
</tr>
<tr>
<td>Chronotropic action</td>
<td>↓ SA node function and heart rate 31, 42, 43, 44, ↓ automaticity 29, antisympathetic action 41, 45 and downregulation of β receptors 27, 39</td>
</tr>
<tr>
<td>Dromotropic action</td>
<td>↑ atrial and AV conduction time 42, 66</td>
</tr>
<tr>
<td>Inotropic action</td>
<td>↓ inotropy (myocardial contractility) 43, 65 and ↓ Na-K ATPase and pump activity 74, 75</td>
</tr>
<tr>
<td>Thyroid function</td>
<td>Blockage of the conversion from T4 to T3 50, 51, 52, ↓ or ↑ in thyroid function 40, 47, 51, 53</td>
</tr>
<tr>
<td>Hepatic function</td>
<td>Myelinoid inclusions in hepatocytes 62, 63, 64, ↑ enzymatic activity in the liver 61, 64</td>
</tr>
<tr>
<td>Pulmonary function</td>
<td>Phospholipid accumulation 54, 55, 56, 57, 99, 186, ↑ bronchiolar and alveolar macrophages and type II cells 58, 59, 60, 186</td>
</tr>
<tr>
<td>Renal function</td>
<td>↑ Creatinine and BUN plasma level 48, 49</td>
</tr>
</tbody>
</table>

Table 1.1: Pharmacodynamic properties of amiodarone (↓=decrease, ↑=increase)
<table>
<thead>
<tr>
<th>Categories</th>
<th>Properties reference</th>
</tr>
</thead>
</table>
| Onset and offset duration for antiarrhythmic action | Onset: 2-6 weeks 83, 89, 90, 91  
Offset: up to 10 months 79, 85, 89, 92 |
| Bioavailability                               | 3.29-100% 77, 78, 79, 80, 81, 97 |
| Plasma protein binding                        | 96-99.98% 82, 84, 104 |
| Peak plasma concentrations (C<sub>max</sub>)    | 0.15-14.2 µg/ml 79, 93 |
| Time to reach peak plasma concentration (<i>t<sub>max</sub></i>) | 2.72-12 hours 79, 87, 93 |
| Volume of distribution                        | Acute: 1.3-65.8 L/kg 79, 80, 94, steady state: 3.1-147.8 L/kg 80, 85, 94, 95 |
| Metabolism                                    | Predominantly Hepatic 105 and intestinal 102 |
| Metabolites                                   | Desethylamiodarone 103, didesethylamiodarone 106 and deiodinated metabolites |
| Tissue/serum concentrations                   | Heart, kidney, spleen and thyroid): 10-100 times higher and adipose tissue, lung and liver 100-1000 times higher than that in plasma 78, 98, 99, 100, 101, 153 |
| Elimination route                             | Biliary and fecal 102 renal is negligible 86 or absent 80 |
| Elimination half-life                         | Acute: 2.06-116.1 hours 79, 80, 87, 95, 107, chronic: 13.7-107 days 85, 87, 96 |
| Total body clearance                          | 0.108- 0.774 L/minute 80, 85, 95 |
| Therapeutic plasma concentrations             | 1-2.5 µg/ml 88, 96, 108, 153 or 0.8-2.8 µg/ml 87 |

**Table 1.2: Pharmacokinetic properties of amiodarone**

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<table>
<thead>
<tr>
<th>Drugs</th>
<th>Interaction</th>
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| Cardiac glycosides | ↓ Renal and nonrenal clearance and ↑ plasma concentrations of Digitoxin \textsuperscript{120,121}, Digoxin \textsuperscript{119,136,137} and Digitalis \textsuperscript{118}  
↑ Bradycardia, SA and AV node suppression and AV conduction time \textsuperscript{121,137} |
| Beta blockers      | ↑ Beta blocking, sinus bradycardia \textsuperscript{111}, hypotension, torsades de pointes \textsuperscript{111} and negative inotropy with Metoprolol and Propranolol |
| Ca channel blockers| ↑ Ca channel blocking, SA and AV node depression and atrial and AV conduction time with Diltiazem \textsuperscript{116,117} and Verapamil \textsuperscript{190} |
| Anticoagulants     | ↑ Anticoagulant effect and prothrombin time with Acenocoumarol \textsuperscript{125,126,127}, Dicumarol, Heparin, Phenprocoumon and Warfarin \textsuperscript{88,123,124}  
↓ Warfarin \textsuperscript{122,123} , Phenprocoumon and Acenocoumarol metabolism |
| Na channel blockers| ↑ QT and QTc interval \textsuperscript{113,135}, risk of proarrhythmia (torsades de pointes) \textsuperscript{88}  
↑ Plasma concentrations of Aprindine, Flecainide \textsuperscript{110}, Lidocaine \textsuperscript{109}, Phenytoin \textsuperscript{115,128}, Procainamide \textsuperscript{112,114} and Quinidine \textsuperscript{115} |
| K channel blockers | ↑ K channel blocking, action potential duration, QT interval and refractory period in atria and ventricles with Ibutilide, Dofetilide, Sotalol and Bretylium |
| Anesthetics        | ↑ Bradycardia (atropine-resistant) and pulmonary complications \textsuperscript{129}  
↓ Contractility and systemic vascular resistance \textsuperscript{129} |
| Antibiotics        | ↑ QT and QTc intervals with fluoroquinolones: Gatifloxacin, Grepafloxacin, Levofloxacin \textsuperscript{134}, Moxifloxacin \textsuperscript{133} and Sparfloxacin  
↑ Amiodarone metabolism with Rifampin \textsuperscript{131}  
↑ Cytotoxicity with Doxorubicin, Adriamycin \textsuperscript{130,132} |

Table 1.3: Amiodarone drug interactions (↓=decrease, ↑=increase)
<table>
<thead>
<tr>
<th>Categories</th>
<th>Side effects (frequency %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>Bradycardia (1.1% to 8%) 92, 138, 141, 143, 144, 157, 163, Hypotension (14% for PO 143 and 26% for IV 144), AV block (with previous conduction disorder 6.7% to 12%, with no previous conduction disorder 0%) 145, 146, 147, sinus arrest (0.5% to 0.8%) 139, 180, proarrhythmia (0% to 4.6%) 140, 144, 163, 178 and torsades de pointes (0.65% to 1%) 140, 159, 164</td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td>Corneal deposits (29.7% to 100%) 61, 148, 149, 150, 163, 180, optic neuropathy (1.3% to 1.79%) 151, 152</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pneumonitis (0.04% to 18%) 153, 154, 155, pulmonary fibrosis (0.5% to 1.1%) 88, 156 and other pulmonary complications (1.8%) 156</td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td>Hepatitis (0% to 4%) 157, 158, elevation of hepatic enzymes (1.8% to 21%) 88, 142, 153, 163</td>
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</tr>
<tr>
<td>Dermatologic</td>
<td>Blue-gray skin discoloration (2% to 36%) 159, 160, photosensitivity (1.2% to 57%) 61, 141, 161, hair loss (0.66% to 4.1%) 139, 157, 180 and skin rash (1.2% to 10%) 92, 139, 141, 165</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>Hypothyroidism (0% to 11%) 158, 163 and hyperthyroidism (0% to 4.4%) 157, 156, 163, 180</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>GI intolerance (nausea, vomiting, anorexia and constipation) (1% to 80%) 92, 164, 165</td>
<td></td>
</tr>
<tr>
<td>Neurologic</td>
<td>Peripheral neuropathy (0% to 10%) 61, 164, 166, tremor (3.3% to 43.1%) 168, 169, ataxia (0% to 5.7%) 92, 157, 165, headache, fatigue, dizziness (0.5% to 5.1%) 139, 167 and overall neurologic disorders (0.5% to 74%) 139, 165</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>Renal toxicity (1.1%) 163, creatinine elevation (8.8%) 163</td>
<td></td>
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<tr>
<td>Hematologic</td>
<td>Thrombocytopenia (2%) 144 and decreased platelets (0.5%) 139</td>
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</table>

Table 1.4: Amiodarone side effects
Figure 1.1: Chemical structures of khellin and benzofuran derivatives modified from khellin molecule. They are all coronary vasodilators. However, amiodarone is the most potent coronary dilator. Desethylamiodarone is the major metabolite of amiodarone.
CHAPTER 2

EFFECTS OF CHRONIC ORAL AMIODARONE IN DOGS

2.1 Abstract

Amiodarone, a class III antiarrhythmic with beta-adrenergic blocking and antimuscarinic properties, has a wide spectrum of clinical use in people. This study was conducted to establish the effects of 25 mg/kg q12h loading dose and 30 mg/kg q24h maintenance dose of amiodarone, each given orally for 3.5 weeks, on systemic arterial pressure, echocardiographic indices of left ventricular function, electrocardiograms, exercise tolerance and serum biochemistries in adult, clinically normal dogs. Means were calculated and compared by ANOVA with repeated measures. When a significant F statistic was identified, specific means were compared by Bonferroni’s post hoc test.

Body weight and heart rate decreased, and PQ, QT and QTc increased significantly (p<0.05) for the weeks that the dogs received loading dose, but values for all parameters returned to levels same as pretest for the weeks that dogs received maintenance dose. Serum activity of hepatic enzymes and cholesterol concentration increased, and serum concentrations of thyroid hormones (T₃ and T₄), phosphorous and total carbon dioxide decreased.

The changes in PQ, QT and QTc are similar to those obtained previously; but the detailed electrocardiographic and echocardiographic observations have not been reported.

Twenty-five mg/kg q12h, but not 30 mg/kg q24h, is an appetite suppressant and
the lower dose produces neither electrocardiographic nor echocardiographic changes of clinical or toxicological significance in normal dogs.

2.2 Introduction

Amiodarone is an antiarrhythmic compound useful in man for the treatment of both atrial fibrillation and ventricular arrhythmias.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\) It possesses properties of all 4 classes of antiarrhythmics, in that it is a partial blocker of inactivated sodium channels (a class I effect), it is a non-competitive beta blocker (a class II effect), it is an L-type calcium channel blocker (a class IV effect), and it is a blocker of potassium channels (a class III effect).\(^4\) It has a dramatic advantage over other class III antiarrhythmics, increasing its preference because it is less likely to produce torsades de pointes. It binds preferentially to open potassium channels; therefore, causes less “reverse-use-dependence”, which means as heart rate increases the efficacy of drug decreases.\(^4\)

Previous studies in dogs using single intravenous doses have shown safety up to doses of \(10\) mg/kg, no hemodynamic effect other than a small decrease in myocardial contractility, and no change in QT or QTc—indicators of predisposition to torsades de pointes.\(^5\)\(^,\)\(^6\) Two studies have been conducted reporting effects of amiodarone on exercise capacity in dogs with old myocardial infarcts.\(^7\)\(^,\)\(^8\) In one, the compound was given orally for only one week, and dogs were exercised at submaximal effort while coronary blood flow and left ventricular pressure were monitored. Compared to dogs not receiving amiodarone, dogs receiving the compound achieved a reduced heart rate, tended to increase coronary blood flow and decrease left ventricular end-diastolic pressure, and had no change in left ventricular \(dP/dt_{\text{max}}\).\(^7\) In a second study on dogs given amiodarone
intravenously and exercised, an inability to achieve the expected heart rate was confirmed, however coronary blood flow and myocardial oxygen consumption did not differ from expected for dogs exercising at submaximal effort.\(^8\)

Little information is available on the effects of amiodarone given orally over a prolonged period to dogs, and there have been no studies utilizing echocardiography to monitor cardiac function following chronic, oral administration to dogs.

The present study was designed to answer the following questions: For how long must a loading dose of amiodarone 25 mg/kg q12h be given to achieve blood levels within the therapeutic range? What are the manifestations of toxicity of higher doses? What are the physiological effects of therapeutic blood concentrations of amiodarone when given chronically?

In this study a loading dose approximately of 25 mg/kg q12h PO of amiodarone was given for 3.5 weeks to determine how long that dose must be given to achieve known therapeutic blood concentrations, and to determine if evidence of toxicosis occurs at this dose. This dosing regimen was followed by a maintenance dose of approximately 30 mg/kg q24h, given for 3.5 weeks. This dose was continued, however without physiological monitoring, for another 3 weeks to determine serum concentrations of amiodarone.

This study reports new information on the effects of chronic amiodarone administration on (1) ventricular function as monitored by echocardiography, (2) exercise performance as monitored on a motor-driven treadmill, and (3) heart rate variability and changes in configuration of the ST-T of the electrocardiogram. Furthermore, it specifies the duration (4 days) that a loading dose of approximately 25 mg/kg q12h must be given
to achieve therapeutic serum concentrations, and it confirms that a chronic, oral dose of approximately 30 mg/kg q24h sustains putative therapeutic serum concentrations, and alterations in blood chemistries.

2.3 Materials and methods

This study was conducted with approval of Institutional Laboratory Animal Care and Use Committee (ILACUC) of The Ohio State University. Six beagle hounds of either sex and weighing between 7 and 10 kg were trained for approximately 2 months to accustom them, without need of chemical restraint, to the laboratory and to the recording of electrocardiograms (ECG), echocardiograms (ECHO) and systemic arterial pressures. They were trained 3 days a week for 7 weeks to run on a motor-driven treadmill for 6 minutes at 3.5 mph and at 8% grade. A reduction in exercise capacity was considered when a dog could not complete the entire protocol despite encouragement.

After training but before exposure to amiodarone, ECHO and ECG (5 minute recording) were recorded twice a week and arterial blood pressures were obtained daily from the left brachial artery by an oscillometric method. Amiodarone was administered, 200 mg/dog, PO q12h for 3.5 weeks, and then 200 mg/dog PO q24h for 3.5 additional weeks. Dogs were given amiodarone at 8AM and 8PM daily for the loading dose, and at 8AM daily for the maintenance. A dose of one, 200 mg tablet (20 mg/kg q24h) was given to 6 dogs PO q24h for 3.5 weeks in addition to the 7 weeks of both loading and maintenance. This was done to determine if that dose, when given chronically, would sustain serum concentrations in the therapeutic range. Body weights and systemic arterial pressures (an average of 5 taken within 15 minutes) were obtained after the 8AM dosing.
ECG's and ECHO's were obtained 1 hour later, and the exercise testing was performed 2 hours after dosing. Blood samples for measuring amiodarone and desethylamiodarone were obtained at 12 PM during days 4 and 7 of the 1st week of dosing, then at the end of each subsequent week through week 11. Blood samples for biochemical analysis were obtained at the end of weeks 1 through 7.

Electrocardiograms (Lead I, II and III) were recorded and 30 consecutive RR, PQ, QRS and QT durations were measured and the average values derived. Corrected QT was calculated by dividing QT by the cube root of the previous RR interval.9 Time-domain expressions of heart rate variability were calculated, from this 5 minute epic, as the ratio of standard deviation to the average RR interval and as the natural log of the standard deviation squared (ln(SD)^2 ).

Left ventricular end-diastolic and end-systolic volumes and the difference (stroke volume) were estimated echocardiographically by measuring diameters in the M-mode image of the left ventricle with the cursor directed from a 2D short axis image and using software in the Aloka (model: SSD- 1400) echocardiograph. The formula of Teicholz was used for the calculations. M-mode images are produced at a high sampling rate, generate sharp, easily-defined borders, and are said to be superior to 2D images for identifying subtle changes.10 The same, experienced echocardiographer obtained the images, and they were read blinded by another experienced echocardiographer.

Means and standard errors of the means were calculated for all parameters and were compared by ANOVA with repeated measures on dose.11 When a significant F statistic was identified, specific means were compared by Bonferroni's post hoc comparison test.
2.4 Results

During the 3.5 weeks when dogs received 25 mg/kg q12h of amiodarone mean body weight decreased from 8.2 kg to 6.7 kg (Figure 2.1). This weight difference achieved statistical significance at the 3rd and 4th weeks (p=0.001) and continued to be different during the 5th week when dogs were receiving 30 mg/kg q24h. Although not measured, feed consumption was apparently decreased during the 3rd, 4th and 5th weeks. Two out of 6 dogs vomited occasionally within 1 hour of dosing, and were mildly depressed during the 4th and 5th weeks.

Serum concentrations of amiodarone and desethylamiodarone were within the therapeutic range (2.1 ug/ml for amiodarone) during the 4th day, peaked at the 3rd week while dogs received 25 mg/kg q12h, then decreased beginning with the 4th week when dogs received only 30 mg/kg q24h (Figure 2.2). Serum amiodarone and desethylamiodarone concentrations achieved steady state serum levels during the 10th and 11th week. Mean serum concentrations of amiodarone during weeks 10 and 11 were 1.43 ug/ml and 1.26 ug/ml, respectively, values within the putative therapeutic range.

There were no changes in values for serum chloride, potassium, sodium, or calcium concentrations with drug administration (Table 2.1). Compared to pretest values, serum phosphorous concentration decreased (p=0.035) significantly at the 5th and 6th weeks, and returned normal by the 7th week. Serum alanine transaminase (ALT) and aspartate transaminase (AST) activity increased significantly (p=0.014 for ALT and p=0.002 for AST), reaching maximums at the 3rd to 4th week during dosing. Serum total carbon dioxide concentration decreased significantly at the 1st, 2nd, and 4th week of dosing. When comparing to pretest values, serum cholesterol concentration increased.
significantly at all weeks of dosing except the 1st week, but reached a peak (p=0.001) during the 3rd week. Although decreases in neither T3 nor T4 achieved statistical significance, it appears that values for T3 decreased during dosing with amiodarone, and reached minimal values during the 2nd and 3rd weeks. However, the laboratory does not report values for T3 less than 40 ng/ml, so the differences between pretest and those obtained during the 2nd and 3rd weeks did not achieve statistical differences.

Heart rate decreased gradually to the 4th week achieving statistical significance (p=0.03) only at the 4th week (Table 2.2). PQ interval increased gradually up to the 4th week, and differed significantly (p=0.003) from pretest during the 4th and 5th weeks (Table 2). QRS duration did not change significantly (p=0.63). Both QT and QTc intervals prolonged similarly and achieved values statistically different from pretest (p=0.003 for QT and 0.021 for QTc) during the 2nd, 3rd and 4th weeks of dosing. Both QT and QTc intervals prolonged earlier than either the reduction in heart rate or prolongation of PQ interval. In ECG leads I, II and III (Figure 2.3), T waves became less negative or changed from being negative to positive in 5 out of 6 dogs. These changes were more dramatic when dogs received 25 mg/kg q12h than 30 mg/kg q24h.

No variable measured by echocardiography changed significantly when comparing measurements after dogs received amiodarone to those during pretest. There was tendency—greatest during the 4th week of the 25 mg/kg q12h dosing—for reductions in thickness of the interventricular septum (p=0.14) and left ventricular free-wall (p=0.17). Left ventricular ejection time tended to increase until the 4th week of dosing (p=0.11).

There were no statistically significant changes in systolic (p=0.40), mean (p=0.39)
or diastolic (p=0.21) systemic arterial pressures when comparing values recorded at the 4th week of dosing to those during pretest. There were no statistically significant changes in heart rate variability estimated by either ratio of standard deviation to average RR intervals (p=0.11) or In SD^2 (p=0.20). (Table 2.2)

All dogs completed the exercise protocol before dosing and after receiving the low dose of amiodarone; however, 1 dog was unable to complete the submaximal exercise test during the 4th week.

2.5 Discussion

Tablets of amiodarone contain 200 mg amiodarone hydrochloride. For a loading dose each dog received 2 tablets (400 mg/dog), which ranged from 25 to 30 mg/kg q12h. During the 3.5 weeks of loading, we expected to obtain first a therapeutic concentration and then a supratherapeutic or toxic concentration. Previous investigations in dogs used loading doses of 40 and 60 mg/kg given daily for 1 week, and achieved serum concentrations of 2.54 ug/ml and 4.64 ug/ml, respectively.12,13 We expected to obtain serum concentrations closer to 2.54 ug/ml corresponding to the 40 mg/kg q24h dose described previously. However, we achieved a serum concentration closer to the previous study when 60 mg/kg q24h was given. The discrepancy may be due to the fact that the previous study gave the drug in one dose per day, whereas the present study divided the daily dose.

The dose of amiodarone recommended for its antiarrhythmic effect in dogs is between 10 and 40 mg/kg q24h.14 This is designed to achieve a therapeutic serum concentration reported to be between 1 and 2.5 ug/ml.15 In this study, concentrations
within that range were achieved in all cases within 4 days. Thus it is recommended that a loading dose of 25 mg/kg q12h should be used for 4 days when amiodarone is given for clinical purposes.

Monitoring of plasma concentrations of any compound does not necessarily allow prediction of either therapeutic success or toxicosis. Side effects have been described for amiodarone in humans with plasma concentration less than 1 ug/ml and plasma concentrations greater than 2.5 ug/ml.\textsuperscript{16} Recommended concentrations are optimal to suppress ventricular ectopy and less likely to have side effects. However, individual variations and the unusual pharmacokinetics of amiodarone, namely its erratic bioavailability, long half-life and the apparently large extracardiac storage (specially in adipose tissue), and the effects of active metabolite result in erratic predictions of drug effects based upon plasma concentrations.

The loading dose of amiodarone used (25 mg/kg PO q12h) in this study is close to that recommended by Abdollah et al\textsuperscript{13} (40 mg/kg PO q24h) and repeated in Kittleson and Kienle.\textsuperscript{17} In our study the loading was continued for 26 days instead of the 10 days recommended. We used this prolonged loading to allow us to identify signs of toxicity, which had not been described in dogs. Our dose was then reduced to approximately 25 mg/kg PO q24h, which is the mid-dose that had been recommended by Rosenbaum et al.\textsuperscript{14} for dogs. Thus our goal was to achieve a therapeutic blood level by reducing it from the toxic concentration rather than by increasing it from a subtherapeutic concentration. This should not be the method recommended for achieving satisfactory serum concentrations in clinical patients. When low doses, based upon recommendations for human patients, were used, plasma concentration of amiodarone is less than 1ug/ml.\textsuperscript{16}
This may not be an effective plasma concentration in patients. This is consistent with our observation, albeit in only a single clinical patient, that 10 mg/kg q24h of amiodarone given for 1 month failed to decrease the frequency of ventricular ectopy.

The plasma half-life \( (T_{1/2}) \) for the dog is known to be approximately 11 hours for chronic, oral, administration of amiodarone.\(^{18}\) We did not expect to achieve steady-state plasma concentrations during the loading time because dogs were taking amiodarone every 12-hours. However with daily dosing during the maintenance period, we observed a gradual decrease in plasma concentration of amiodarone reaching a concentration that did not vary after the 9th week (Figure 2).

One study performed on dogs with surgically produced myocardial infarction showed that a dose of 10 mg/kg q24h was not effective at reducing ventricular ectopy or preventing ventricular fibrillation.\(^{19}\) A similar study using 30 mg/kg q24h reported beneficial effects at suppressing ectopy and preventing ventricular fibrillation.\(^{20}\) Although these studies were conducted on models of ventricular ectopy in myocardial ischemia/infarction, they stand as the only available reference to potential amiodarone efficacy in controlled trial on dogs. One clinical study on dogs with naturally-occurring ventricular ectopy reports a loading dose of 10 mg/kg q12h for 1 week, then decreasing the dose to 8 mg/kg q24h for maintenance.\(^{21}\) We are surprised that this dose was effective, unless possibly, the dogs' diseases rendered them unable to metabolize or to excrete amiodarone as in the normal dogs of this study.

Serum concentrations ranged between 3.74 and 1.26 ug/ml (mean 2.1ug/ml) from the 7th to the 11th week when 6 dogs received this long-term dosing. This demonstrated that a dose of 25 to 30 mg/kg q24h given for 7 weeks produced and sustained plasma
concentrations in the acceptable (1 to 2.5 ug/ml) range and resulted in no signs of toxicity.

Mean body weight decreased and achieved values different from pretest during the 3rd, 4th, and 5th weeks. During this period dogs were mildly anorectic. Amiodarone is known to be a gastrointestinal irritant, which could account for the symptoms described above. Since these symptoms did not occur until the 3rd week and were sustained through the 5th week when serum concentrations were well above recommended for therapy, it is clear that they may be attributed to continuation of the loading dose beyond what would be used clinically. Thus at therapeutic serum concentrations—those achieved during weeks 1 and 2 and after weeks 5 in this study—it is unlikely that dogs should manifest toxicity when amiodarone is used clinically. One case report in which amiodarone was used for a dog with a ventricular arrhythmia reported a reduction in body weight.

Amiodarone is known to produce dermatological effects (e.g. photosensitivity, skin rash and skin discoloration) in humans. However, we observed no apparent dermatological side effects in dogs, possibly because they do not occur in dogs or because the dogs were not exposed to sunlight. These side effects may not be seen because dogs have fur protecting their skin to the exposure of sun or UV light sources. Tremors have been observed commonly in humans, but were not observed in our dogs.

On the electrocardiographic analysis, heart rate decreased, and PQ, QT and QTc intervals increased. The decrease in HR and prolongation of PQ interval are probably due to the β1-blocking properties of amiodarone, since the drug is not known to alter either, except at serum concentrations much higher than we achieved in this study. An alternate explanation may be that amiodarone has the ability to slightly block calcium channels.
Prolongation of QTc may occur because of both the β1-blocking properties and the class III antiarrhythmic properties (i.e. use-dependent block of potassium ion channels responsible for ventricular repolarization). Alternately, prolongation of the QTc may be an artifact resulting from inadequate methods of correcting for HR.\textsuperscript{26,27} No method for adjusting QT (ms) for RR interval (60,000 ms/heart rate) produces a constant ratio of QT to RR at all RR intervals. All methods tend to underestimate QTc at shorter RR intervals and to overestimate QTc at longer RR intervals. Furthermore once a dog’s RR interval approaches 1,000 ms, further lengthening of RR either does not prolong QT further or prolongs it trivially.\textsuperscript{28,29}

T waves in the frontal plane leads I, II, and III became less negative or became positive during the 4\textsuperscript{th} week. Because there were no apparent changes to the QRS, the T wave changes must be considered primary, that is changes in repolarization not due to altered pathways of ventricular depolarization. These changes are consistent with altered time-order of repolarization, which reflect non-uniform changes in ventricular repolarization. Such changes reflect a heterogeneous effect on specific potassium channels responsible for repolarization. In particular, one possible cause for T waves becoming less negative or more positive in a lead (e.g. leads II, III, or aVF) “facing” the left ventricular epicardium, might be retarded repolarization of the subendocardium of the left ventricular free-wall. T wave inversion has been reported in humans.\textsuperscript{30,31} One case report, consistent with our observations, showed T wave inversion associated with amiodarone toxicity in an attempt at suicide.\textsuperscript{30} However, there are no reports in dogs about T wave inversion. The antiarrhythmic class III property of amiodarone alters repolarization by its effect on specific potassium ion channels (e.g. IKr, s) responsible for

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ventricular repolarization.\textsuperscript{32}

We did not observe a proarrhythmic effect of amiodarone in our dogs, although only limited ECG recording was done (5 to 10 minutes, twice a week, for 8 weeks—for a total of 720 minutes). Ventricular ectopy was not observed during this time. We believe that this absence of ectopy indicates that it is unlikely amiodarone has a proarrhythmic effect in normal dogs. We recognize that humans with heart disease and history of torsades de pointes are more likely to manifest a proarrhythmic drug effect;\textsuperscript{33} therefore, a proarrhythmic effect of amiodarone should be sought in clinical trials conducted on dogs with heart disease. Clearly it would have been superior to use 24 hour Holter monitors in our dogs to identify, possibly, a circadian tendency for a proarrhythmic effect.

Heart rate variability did not change significantly, but did tend to decrease from a control value of 241 to 174 at the end of week 1, and to a final value of 141 at week 7. This decrease in heart rate variability may represent the antimuscarinic effect of amiodarone.\textsuperscript{34} Although it would have been preferable to express heart rate variability for recordings of more than 5 minutes, this variability has been expressed in man using periods as brief as 2 minutes.\textsuperscript{35}

The time-domain analysis performed in this study has been shown to provide, in dogs, an estimate of heart rate variability nearly as powerful as that obtained using frequency-domain analysis requiring recordings much longer than the 5 minutes used in this study (personal communication, Dr. Clay Calvert).

Analysis of echocardiograms demonstrated no significant changes in chamber lumen or wall dimensions, left ventricular ejection time, fractional shortening or ejection fraction. This demonstrates that neither loading dose nor maintenance dose of
amiodarone in this study adversely altered ventricular function in these normal dogs. To demonstrate, further, that even the highest serum concentrations of amiodarone did not alter left ventricular function and systemic arterial pressures did not change significantly. Reduced ventricular function has been observed in humans and in dogs following intravenous administration of amiodarone. Following oral administration, however, left ventricular ejection fraction has been shown to increase. Our dogs receiving amiodarone orally demonstrated no change in left ventricular function, but there was a statistically insignificant (p=0.75) increase in ejection fraction during the loading period that tended to return to baseline values during maintenance.

The only apparent changes in blood biochemistry were reductions in serum phosphorus and carbon dioxide, and increases in cholesterol, ALT, and AST. T₃ and T₄ appeared to decrease (p=0.12 and p=0.09 respectively) during the 2nd, 3rd and 4th weeks when dogs received the high dose of amiodarone, but returned to values clearly not different from pretest by the 7th week. Although neither T₃ nor T₄ differed significantly, the increase in cholesterol and decrease in carbon dioxide are consistent with a hypometabolic state. Hypothyroidism in rats receiving amiodarone has been attributed to a hypometabolic state. Amiodarone is known to block the peripheral conversion of T₄ to T₃ and to block T₃ receptors, therefore occasionally T₃ will be reduced and T₄ elevated; however after prolonged administration, both T₃ and T₄ may decrease ("true" hypothyroidism), as was observed in this study. The increase in serum cholesterol concentration may have occurred because of hypothyroidism (i.e. a hypometabolic state), a known effect of amiodarone or because of downregulation of LDL receptors in liver by amiodarone, or for both reasons. We identified a decrease in

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both T₃ and T₄ during loading with amiodarone, but both hormones returned to normal limits during maintenance therapy. However, since cholesterol remained elevated during both loading and maintenance, at least the elevation during maintenance was probably due to the effect of amiodarone on the hepatic LDL receptors.

Jacobs, et al.²³ reported blood chemistries obtained over many months from 4 dogs receiving amiodarone and many other drugs for treatment of ventricular ectopia. Thus the changes they observed could have been attributable to amiodarone, to one or another of the drugs given concomitantly, or to both. They found increases in ALT, AST, and alkaline phosphatase (ALP). The dogs became anorectic and 1 manifested weight loss, and there were no changes in parameters of the CBC. Amiodarone concentrations varied between 0.5 ug/ml to 1.8 ug/ml. Neither thyroid hormones nor serum cholesterol were reported. The only difference between the present report and that by Jacobs et al is that Jacobs et al reported an elevation in alkaline phosphatase. This may well have been due to the longer duration of administration of amiodarone. Padmavathy, et al.³⁹ studied blood chemistries in normal rats given high doses (175 mg/kg q24h) for 3 weeks, and found elevation of hepatic enzymes and cholesterol as in the present study.

Only one dog in the present study developed reduced exercise capacity in response only to the high dose of amiodarone. This dog was unable to complete the exercise protocol during the 4th week, when her amiodarone serum concentration was 11 ug/ml. This was not the highest value achieved in this dog nor as high a concentration as was present in 2 other dogs that manifested no exercise intolerance. Based upon the small sample size inferences made about the effect of amiodarone on exercise capacity are equivocal.
This study did not have a control group. Since all dogs were mature, had been acclimated to the facilities for 8 weeks and were considered to be at steady-state before experimentation, and none of the variables measured is thought to change within the relatively brief duration (i.e. 7 weeks) of this study.

For the 8th through the 11th week of this study, only serum concentrations of amiodarone were obtained, so there is no data on blood chemistries, from either echocardiograms or electrocardiograms, or from the exercise protocol. We extended the duration of monitoring amiodarone serum concentrations only to obtain a sense of the magnitude of potential fluctuations of both parent compound and metabolite. We did not monitor physiological variables because they had achieved a steady state by the last measurement.

Amiodarone is excreted in steps. Initially amiodarone is metabolized in the liver to the active metabolite, desethylamiodarone, and desethylamiodarone enters the intestines in bile. A portion of desethylamiodarone is excreted in feces, and another portion is reabsorbed in entero-hepatic circulation. The reabsorbed desethylamiodarone is deactylated in the liver to an inactive metabolite, which is secreted in the bile, and finally is excreted in the feces. In heart failure in which there may be decreased hepatic blood flow, amiodarone may not be metabolized as rapidly, and plasma concentrations may be elevated to above what is expected. If, on the other hand, there is a reduction in intestinal blood flow, which might interrupt entero-hepatic circulation, a greater proportion of desethylamiodarone may be excreted, and plasma concentrations may be lower than expected.

In conclusion, if therapeutic serum concentrations of amiodarone are between 1

69
and 2.5 ug/ml, they were achieved in healthy beagle hounds within 4 days when given 25 to 30 mg/kg q12h. When serum concentrations were 4 times the recommended, dogs were anorectic and lost weight, but left ventricular function and exercise capacity were unchanged. When dogs were sustained 25 to 30 mg/kg q24h, there were no changes in any variable measured. The methods used to study our dogs were non-invasive, therefore they could be applied without need for chemical restraint, and they employ methods used commonly by veterinary cardiologists.
2.6 References


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34. Cohen-Armon M, Schreiber G, Sokolovsky M. Interaction of the antiarrhythmic...


<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>week 1</th>
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<td>Sodium (mEq/L)</td>
<td>147±1</td>
<td>144±1.4</td>
<td>145±0.7</td>
<td>147±0.5</td>
<td>146±4.6</td>
<td>146±1.3</td>
<td>159±11.8</td>
<td>147±0.6</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>5.2±0.1</td>
<td>4.4±0.2</td>
<td>4.8±0.1</td>
<td>4.7±0</td>
<td>4.4±0.2</td>
<td>4.8±0.1</td>
<td>5±0.3</td>
<td>4.3±0.1</td>
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<tr>
<td>Chloride (mEq/L)</td>
<td>102±2</td>
<td>107±1.8</td>
<td>104±0.8</td>
<td>105±1</td>
<td>109±3.9</td>
<td>106±1</td>
<td>117±9.9</td>
<td>110±1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.6±0.2</td>
<td>5.2±0.2</td>
<td>5.1±0.1</td>
<td>5.1±0</td>
<td>5±0.2</td>
<td>4.7±0.1*</td>
<td>4.3±0.4*</td>
<td>5±0.1</td>
</tr>
<tr>
<td>CO₂ (mEq/L)</td>
<td>25.9±0.2</td>
<td>19.9±0.2*</td>
<td>20±0.7</td>
<td>21±0</td>
<td>20.8±0.9*</td>
<td>21.7±0.5</td>
<td>23.3±1.5</td>
<td>24.4±1</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>178±11</td>
<td>246±7</td>
<td>324±16#</td>
<td>429±72#</td>
<td>343±17#</td>
<td>360±11#</td>
<td>342±33#</td>
<td>321±19#</td>
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<tr>
<td>ALT (iu/L)</td>
<td>27.5±0.5</td>
<td>31.8±3.2</td>
<td>37.3±6.5</td>
<td>60.5±23#</td>
<td>76.7±20#</td>
<td>64.2±20#</td>
<td>48.2±13.6</td>
<td>42.6±10.2</td>
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<tr>
<td>AST (iu/L)</td>
<td>18±1</td>
<td>22.3±1.1</td>
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<td>30.5±5.5*</td>
<td>26.8±4.7*</td>
<td>20.8±2.4</td>
<td>18.6±2.4</td>
<td>27.6±1.8*</td>
</tr>
<tr>
<td>CK (iu/L)</td>
<td>119±19</td>
<td>130±9</td>
<td>114±20</td>
<td>120±4</td>
<td>109±19</td>
<td>79±16</td>
<td>60±10</td>
<td>130±22</td>
</tr>
<tr>
<td>ALP (iu/L)</td>
<td>116±30</td>
<td>96±20</td>
<td>102±25</td>
<td>70±16</td>
<td>93±19</td>
<td>96±23</td>
<td>107±31</td>
<td>113±15</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>3.8±0.1</td>
<td>3.8±0.2</td>
<td>3.6±0.1</td>
<td>3.6±0.2</td>
<td>3.4±0.2</td>
<td>3.4±0.2</td>
<td>3.4±0.4</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Protein (gm/dl)</td>
<td>5.9±0</td>
<td>5.8±0.1</td>
<td>5.7±0.2</td>
<td>5.5±0.4</td>
<td>5.6±0.3</td>
<td>5.7±0.2</td>
<td>5.7±0.6</td>
<td>5.8±0.3</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112±5</td>
<td>93±3</td>
<td>91±4</td>
<td>90±2</td>
<td>91±8</td>
<td>96±4</td>
<td>106±9</td>
<td>103±6</td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>92.1</td>
<td>79±9.9</td>
<td>&lt;40</td>
<td>&lt;40</td>
<td>63±9.5</td>
<td>NA</td>
<td>NA</td>
<td>115±24</td>
</tr>
<tr>
<td>T₄ (ug/dl)</td>
<td>2.7</td>
<td>2.9±0.2</td>
<td>1.9</td>
<td>1.4</td>
<td>1.9±0.4</td>
<td>NA</td>
<td>NA</td>
<td>2.3±0.4</td>
</tr>
</tbody>
</table>

Table 2.1: Serum biochemistry (Mean ± SEM) (n=6). ALT= alanine transaminase; AST= aspartate transaminase; CK= creatine kinase; ALP= alkaline phosphatase; T₃= triiodothyronine; T₄= thyroxine; NA= Not available. * significant value (p<0.05)

76
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
</tr>
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<tbody>
<tr>
<td><strong>Dose (mg/kg)</strong></td>
<td>0</td>
<td>50±3</td>
<td>54±4</td>
<td>59±5</td>
<td>54±5</td>
<td>30±4</td>
<td>29±3</td>
<td>27±3</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>113±11</td>
<td>90±4</td>
<td>84±4</td>
<td>78±4</td>
<td>72±4*</td>
<td>84±9</td>
<td>93±9</td>
<td>102±6</td>
</tr>
<tr>
<td><strong>PQ (ms)</strong></td>
<td>98±3</td>
<td>105±1.3</td>
<td>113±3.4</td>
<td>114±2.9</td>
<td>121±3.7*</td>
<td>119±6*</td>
<td>115±5.8</td>
<td>111±4.5</td>
</tr>
<tr>
<td><strong>QRS (ms)</strong></td>
<td>42±1.8</td>
<td>40±1.7</td>
<td>38±1.9</td>
<td>42±1.3</td>
<td>42±1.3</td>
<td>43±2.9</td>
<td>46±4.9</td>
<td>40±3.9</td>
</tr>
<tr>
<td><strong>QT (ms)</strong></td>
<td>191±6</td>
<td>216±4.2</td>
<td>229±5.1*</td>
<td>237±7*</td>
<td>241±9*</td>
<td>225±13</td>
<td>220±11</td>
<td>209±6.9</td>
</tr>
<tr>
<td><strong>QTc (ms)</strong></td>
<td>230±4.7</td>
<td>244±2.7</td>
<td>254±4.8*</td>
<td>256±4.3*</td>
<td>255±4.9*</td>
<td>247±6.2</td>
<td>249±7.9</td>
<td>247±5</td>
</tr>
<tr>
<td><strong>SD/AV</strong></td>
<td>241±30</td>
<td>187±34</td>
<td>180±26</td>
<td>174±16</td>
<td>193±19</td>
<td>151±43</td>
<td>174±26</td>
<td>141±13</td>
</tr>
<tr>
<td><strong>ln(SD)^2 (-)</strong></td>
<td>4.03±0.5</td>
<td>4.36±0.5</td>
<td>4.16±0.4</td>
<td>4.07±0.2</td>
<td>3.6±0.2</td>
<td>4.73±0.8</td>
<td>4.33±0.5</td>
<td>4.94±0.2</td>
</tr>
</tbody>
</table>

**Table 2.2:** Electrocardiographic variables (Mean ± SEM) during baseline and each of 7 weeks of amiodarone exposure. (n=6) HR (bpm)= heart rate (beat per minute); PQ and QT (ms)= PQ and QT intervals (millisecond); QRS (millisecond)= QRS complex (millisecond); QTc (millisecond)= corrected QT interval; SD/AV= ratio of standard deviation to average RR intervals; ln(SD)^2= natural log of standard deviation square.

* Statistically significant (p<0.05)
Table 2.3: Echocardiographic variables (Mean ± SEM) during baseline and each of 7 weeks of amiodarone exposure. (n=6) AODs= aortic root diameter at systole; LADs= left atrial diameter at systole; IVSd= interventricular septal thickness at diastole; IVIDd= left ventricular internal diameter at diastole; LVPWd= left ventricular posterior wall thickness at diastole; IVSs= interventricular septal thickness at systole; LVIDs= left ventricular internal diameter at systole; LVPWs= left ventricular posterior wall thickness at systole; ET= ejection time; EF= ejection fraction; FS= fractional shortening; EDV= end-diastolic volume; ESV= end-systolic volume; SV= stroke volume.
<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50±3</td>
<td>54±4</td>
<td>59±5</td>
<td>54±5</td>
<td>30±4</td>
<td>29±3</td>
<td>27±3</td>
</tr>
<tr>
<td>SP (mmHg)</td>
<td>142±5</td>
<td>138±3</td>
<td>145±5</td>
<td>145±6</td>
<td>139±6</td>
<td>139±6</td>
<td>136±7</td>
<td>135±8</td>
</tr>
<tr>
<td>DP (mmHg)</td>
<td>69±4</td>
<td>74±5</td>
<td>70±5</td>
<td>71±6</td>
<td>70±5</td>
<td>65±5</td>
<td>65±6</td>
<td>63±6</td>
</tr>
<tr>
<td>MAP(mmHg)</td>
<td>89±6</td>
<td>91±4</td>
<td>97±4</td>
<td>97±5</td>
<td>94±5</td>
<td>91±6</td>
<td>89±5</td>
<td>86±6</td>
</tr>
</tbody>
</table>

Table 2.4: arterial blood pressure measurements (Mean ± SEM) during baseline and each of 7 weeks of amiodarone exposure. (n=6) SP = Systolic pressure; DP = Diastolic pressure; MAP = Mean arterial pressure.
Figure 2.1: Body weight (Mean ± SEM). (n=6) * Statistically significant (p<0.05)
Figure 2.2: Serum concentrations (Mean ± SEM) of amiodarone and desethylamiodarone. (n=6)
Figure 2.3: ECG tracings of lead I in a "typical" dog. QT interval prolonged at week 4. T wave was negative in all dogs before receiving amiodarone; however, it became positive in 4 out of 6 dogs at the 4th week when dogs received the high dose.
CHAPTER 3

IN VIVO AND IN VITRO STUDIES OF CHRONIC ORAL AMIODARONE IN DOGS

3.1 Abstract

Amiodarone is a class III antiarrhythmic drug that also possesses classes I, II and IV properties. This study was conducted to establish the effects of steady-state, therapeutic concentrations of amiodarone after administration for 10 weeks in healthy dogs. Left ventricular pressure, electrocardiograms, and action potentials from both myocardium (in vivo) and Purkinje fibers (in vitro) with and without ouabain were recorded from 5 dogs given 25 mg/kg PO, q12h for 4 weeks, then 25 mg/kg amiodarone, PO, q24h for 6 weeks and from 5 controls. Means and standard errors of the means of all parameters were compared by two-way ANOVA with repeated measures design on time for the in vitro study and paired Student’s t-test was used for the in vivo study. When a significant difference (p<0.05) was identified, specific means were compared by Tukey’s post hoc comparison test.

There was significant prolongation in QT interval and action potential durations (APD₉₀ and APD₅₀) in dogs receiving amiodarone orally. However, there was no difference in Purkinje fiber action potential duration between amiodarone and the control group in the in vitro study. At 10⁻⁶ Molar concentrations of ouabain, resting membrane potential became less negative, amplitude decreased, dV/dtₘₐₓ decreased. APD₉₀
decreased, but APD$_{90}$ did not decrease. ERP did not change at any stimulus rate, but
automaticity was suppressed in dogs that received amiodarone.

This study showed that amiodarone insignificantly shortened APD in Purkinje
fibers; however, APD prolonged in ventricular myocardial cells. Amiodarone-ouabain
combination slightly but insignificantly increased APD$_{90}$ at all concentrations. Therefore,
we can conclude that amiodarone also shows other classes of antiarrhythmic actions.

3.2 Introduction

Amiodarone, an iodinated benzofuran derivative with non-competitive alpha$_1$ and
non-cardiospecific beta blocking properties, was recommended in early 1960's as an
antihypertensive/anitanginal, and in 1967 as an antiarrhythmic. It is classified as a class
III antiarrhythmic because it prolongs duration of both atrial and ventricular action
potentials and effective refractory periods.\(^1\) Paradoxically amiodarone has been shown to
abbreviate the action potentials in Purkinje fibers of both rabbits and dogs.\(^2,3\)
Amiodarone is considered to be one of the most effective and safe drugs for treating both
ventricular arrhythmias and atrial fibrillation,\(^4\) and one of its most important features is
its lack of a proarrhythmic or negative inotropic effect.\(^5\)

Amiodarone is given either orally or intravenously, its beta-blocking properties
are manifest relatively quickly (within 2-5 hours after oral administration and within 15
minutes after intravenous administration), but its maximal class III antiarrhythmic
properties may require weeks or possibly even months to develop. This longer phase
occurs because its distribution is described using a three-compartment model in which the
3\textsuperscript{rd} compartment (adipose tissue) has an exceptionally long time-constant.\(^6\)
Amiodarone has been used to treat ventricular tachycardias in dogs with dilated cardiomyopathy, however there are very few reports of the use of amiodarone in veterinary medicine. Dogs were given an oral loading dose of 20 mg/kg q12h, and were given a maintenance dose 8mg/kg q24h. The relatively infrequent use of amiodarone by veterinarians may be due to reports of toxic events in humans, the slow onset of action, and the expense.

In a pharmacological study conducted in dogs, intravenous amiodarone at doses greater than 10 mg/kg decreased myocardial contractility; however, oral amiodarone did not cause any negative inotropy.

The purposes of this study were to measure hemodynamic and cardiac electrophysiological effects, in vivo, during steady state serum concentration of a known therapeutic concentration of amiodarone given, orally, to healthy dogs; and to measure electrophysiological effects of amiodarone with and without ouabain, in vitro, on Purkinje fibers removed from the dogs.

3.3 Materials and Methods

Two studies were conducted with approval of Institutional Laboratory Animal Care and Use Committee (ILACUC) of The Ohio State University. Amiodarone (Amiodarone hydrochloride 200 mg tablets, Copley Pharmaceutical Inc. Canton, MA) was administered, PO, twice a day for 4 weeks (200 mg q12h) and then for 6 additional weeks (200 mg q24h) to five, healthy intact beagle hounds of either sex and weighing between 7 and 10 kg.

*In vivo study:* After exposure to amiodarone for a total of 2.5 months, 10 dogs
(five amiodarone and five controls) were pretreated intramuscularly with 2 mg/kg morphine (Morphine sulfate, Schein Pharmaceutical Inc, Florham Park, NJ) followed by 100 mg/kg alpha chloralose (Alpha chloralose, Sigma Chemical Co. St. Louis, MO) given intravenously to sustain light, surgical anesthesia for inserting catheters into the jugular vein and femoral artery.

Dogs were ventilated (Harvard Respirator, Model 613, Harvard Apparatus Co Inc) with a tidal volume of approximately 15 ml/kg. Electrodes forming ECG leads I, II, aVF and V3 were placed. A catheter-tip micro manometer (Millar Micro - Tip Catheter Pressure Transducer, model SPC – 350, Millar Instruments Inc) for measuring left ventricular pressure (LVP) and parameters of inotropy (dp/dtmax and Vmax) was placed via femoral artery into the left ventricle (LV). A bipolar electrode catheter was placed through the jugular vein so that a tip electrode was impressed against the endocardium of the right ventricle and an indifferent electrode lay in the lumen of the right ventricle 5 mm from the tip electrode. A right ventricular subendocardial monophasic action potential (MAP) could be recorded for qualitative assessment of amplitude and quantitative assessment of duration when the endocardial electrode was impinged upon the subendocardium.

ECGs (Biopac Systems, Santa Barbara, CA.) were recorded for five minutes. Thirty consecutive RR, PQ, QT intervals and QRS complexes were measured. QT was corrected for heart rate by dividing QT by the cube root of the previous RR interval.

Heart rate variability was expressed as the natural logarithm of the variance of the RR interval, as the ratio of standard deviation to the mean RR interval (coefficient of variation), and as the relative energies and ratios, from a time-series analysis, of very low
frequency fluctuations in interbeat intervals. Left ventricular (LV) pressure, LV $dp/dt_{\text{max}}$ and $V_{\text{max}}$ were measured. Finally, MAP was recorded at the end.

**In vitro study:** After the in vivo study, 20 mg/kg of pentobarbital (for euthanasia) were given intravenously, the hearts were removed immediately, and free running Purkinje fibers from both right and left ventricles were superfused with cooled, Tyrode’s solution, which was saturated with 95% oxygen and 5% carbon dioxide. The Tyrode’s solution was prepared freshly for each experiment with the formula: (mM) NaCl 137, NaHCO$_3$ 12, MgCl$_2$6H$_2$O 0.5, NaH$_2$PO$_4$H$_2$O 1.8, KCl 4, dextrose 5.5 and CaCl$_2$ 2H$_2$O 1.8. After the initial dissection, Purkinje fibers were placed in the same solution, which was maintained at a pH of 7.4 and at a temperature between 34.8 to 35.2°C (Temperature Controller, model TC-344B Warner Instrument Corp., Hamden, CT). Tyrode’s solution was circulated at a flow rate of 40 ml/min through the tissue chamber. The circulation system was cleaned thoroughly after each experiment and tubing was changed every other experiment.

The anatomically proximal ends of the tissues were stimulated using bipolar extracellular electrodes. Rectangular current pulses 2 ms in duration and two times threshold intensity was delivered to the preparation at a rate of 1 Hz. (W-P Stimulator model S 7000 W-P Instruments, Inc. New Haven Conn.) Glass microelectrodes (borosilicate with filament, 1.0 mm OD. and 0.58 mm ID.) were pulled by using P-97 flaming/brown micropipette puller (Sutter Instruments, Co., Novato CA) and filled with 2.5 M KCl (10-35 MΩ DC resistance). Transmembrane action potentials were amplified by Axoclamp-2B microelectrode clamp (Axon Instruments, Inc., Foster City, CA).
Tektronix Oscilloscope 5110 model (Tektronix, Inc., Beaverton, OR) was used to display action potential. Signals were digitized (Digidata 1200, Axon Instruments, Inc.), recorded (Clampex 8.0 software, Axon Instruments, Inc.) and analyzed (Clampfit 8.0 software, Axon Instruments, Inc., Foster City, CA).

After obtaining stable action potentials, baseline data was recorded. Refractory period was determined at stimulus frequencies of 30, 60 and 120 bpm. Automaticity was assessed by turning off the stimulus for 2-3 minutes. We waited at least five minutes between measurements for recovery of tissue. Controls were recorded again before ouabain superfusion into the circulation. Ouabain, starting from $10^{-8}$, $10^{-7}$ and $10^{-6}$ Molar concentrations was used. Action potentials and $V_{\text{max}}$ were recorded at 15 and 30 minutes during each concentration. The following parameters were analyzed: the time after depolarization until the action potential returned to 50% and 90% of the resting membrane potential, resting membrane potential, peak amplitude and maximal rate rise ($dV/dt_{\text{max}}$) of phase zero of the action potential.

Means and standard errors of the means were calculated for all parameters of the in vivo study, and means were compared by a two way ANOVA with repeated measures. When indicated by a significant F-statistics, specific means were compared by Tukey's post hoc comparison test. Means from the in vivo test were compared by a paired Student's $t$ test requiring a $p<0.05$ for significance.

3.4 Results

In vivo study: Means and standard error of means (SEM) of plasma concentration of amiodarone and desethylamiodarone from 5 dogs receiving amiodarone during the
final steady state when cardiac measurements were obtained were 1.26 ± 0.23 and 0.24 ± 0.02 µg/ml respectively. Means and SEM's for all hemodynamic and electrophysiologic parameters either measured or calculated are shown (Table 3.1) for dogs serving as controls and for dogs receiving amiodarone. There were no differences of statistical significance in any parameters except in APD₅₀ (p = 0.037), APD₉₀ (p = 0.013), and QT (p = 0.016), all of which lengthened in response to amiodarone. Typical monophasic action potentials from myocardium of each group are shown (Figure 3.1). Notice that the configuration of the monophasic action potential measured with this Franz-type electrode mimic that obtained from a microelectrode inserted intracellularly. QTc appeared to lengthen due to amiodarone; however, the difference did not achieve statistical significance (p = 0.082).

Heart rate variability did not change significantly in either time or frequency domain. (Table 3.1)

**In vitro study:** Monophasic action potentials obtained from Purkinje fibers from a normal dog and from a dog exposed to amiodarone are shown in Figure 3.2. They possess a rapid, phase 0 upshoot during depolarization, an the early, brief and rapid, phase 1 repolarization, the typical phase 2 plateau, and a phase 3 of repolarization. Measurements of parameters of action potentials from Purkinje fibers of control dogs and of dogs receiving amiodarone are shown in Table 3.2. When comparing APD’s between groups there are no differences in any of the parameters. However, APD₉₀ and APD₅₀ were insignificantly shortened in the amiodarone group. Those fibers exposed to amiodarone did not initiate spontaneous activity within 3 minutes after pacing stopped, while all control Purkinje fibers discharged spontaneously within 3 minutes after cessation.
3.5 Discussion

Both acute,\textsuperscript{12,13,14} and chronic studies\textsuperscript{15,16} have been conducted giving amiodarone to dogs for the purpose of evaluating electrophysiological effects and effects on left ventricular function. This study was conducted on dogs to whom amiodarone was given for 10 weeks; the longest previous duration of dosing reported is 6 weeks. Dogs in this study were anesthetized with morphine/alpha chloralose, a regimen thought to alter autonomic control minimally. Previous studies achieved amiodarone plasma/serum concentrations either at the low end of (0.91 $\mu$g/ml)\textsuperscript{17} or significantly below (0.11 to 0.59 $\mu$g/ml)\textsuperscript{18} the recommended therapeutic concentrations of (1-2.5 $\mu$g/ml).\textsuperscript{15} Thus the results of the present study should mimic better those found in clinical patients. One study in which doses of amiodarone similar to those of this study were given for 4 weeks, did not report serum/plasma concentrations, and measured action potentials from myocardial fibers, so it is impossible to compare our results on Purkinje fibers with theirs.\textsuperscript{20} In the study in which a dose of amiodarone lower than ours (16 mg/kg/day versus 25 mg/kg/day) was given for 6 weeks, ECGs, systemic arterial blood pressure, and left ventricular subendocardial action potentials were measured under halothane anesthesia.\textsuperscript{18} Halothane is known to alter autonomic tone, to be a powerful negative inotrope, to accelerate heart rate at low concentrations and to depress heart rate at high concentrations; thus dogs studied anesthetized with halothane would be less physiological than those in the present study, and differences in results between that and the present
could be explained by differences in anesthetics. For example, in myocardial fibers the present study described prolongation of APD$_{50}$, APD$_{90}$ and QT, whereas the previous study found only prolongation of APD$_{90}$. It is certainly more consistent that both QT and APD prolong. The differences may have been due to the fact that the previous study measured action potentials from left ventricular endocardium while the present study recorded MAP’s from right ventricular endocardium. A previous study in rabbits described prolongations of RR duration, QT interval and QTc interval, but no change in PQ interval and QRS duration.$^{13}$ These differences could be due to differences in species or to the anesthesia, although the report on rabbits did not describe the anesthetic protocol.

No other studies have evaluated the effects of amiodarone on heart rate variability after chronic dosing. The present study identified no changes in parameters expressed in either time or frequency domains, thus it may be presumed that—at the therapeutic concentrations achieved—amiodarone did not alter either sympathetic or parasympathetic tone. Of course heart rate variability is a rather indirect method for assessing the state of autonomic activity. In addition the present study evaluated myocardial contractility utilizing a minimally, load-dependent parameter, $V_{\text{max}}$, and found no change in myocardial contractility after chronic dosing at therapeutic concentrations.

The in vitro studies performed at the end of chronic dosing were performed immediately after the in vivo measurements were made. The hearts were removed quickly and were superfused with oxygenated Tyrode solution. Whereas extracellular amiodarone might have been washed from the preparation by the superfusate, the amiodarone present intracellularly (50 times higher than in plasma)$^{18}$ has a relatively long half-life.
approximately 1 week for the intracellular compartment and months for the “third” compartment (adipose tissue). Since superfusion and recording was completed in less than 3 hours, it is unlikely that results were influenced by a significant amount of amiodarone being washed from the intracellular milieu.

Contrary to previous in vitro studies\textsuperscript{14, 17, 18} in dog myocardial fibers that reported lengthening of APD\textsubscript{50}, APD\textsubscript{90} and QT, both APD\textsubscript{90} and APD\textsubscript{50} tended to shorten in Purkinje fibers, but not significantly when compared to controls. Shortening in APD is consistent with the lidocaine-like effect amiodarone is known to possess. In the previous studies\textsuperscript{15, 18} and in this study, dV/dt\textsubscript{max} tended to decrease, but not significantly. It is interesting to note that the previous study\textsuperscript{2} utilized untreated fibers with recirculation of superfusate containing ethanol. The present study using only amiodarone-generated results similar to the one using a superfusate containing ethanol, therefore the results of the study using ethanol were probably not perturbed by the alcohol.

In the present in vivo study, both QT and APD prolonged, typical of a class III antiarrhythmic; however in the in vitro study of Purkinje fibers, both APD\textsubscript{90} and APD\textsubscript{50} shortened and dV/dt\textsubscript{max} decreased (neither achieving statistical significance), typical of a class IB antiarrhythmic effect. The difference may be attributable to differences in cell type (myocardial versus Purkinje). However, almost all in vitro studies, including the present one, reported that amiodarone decreased dV/dt\textsubscript{max} independent of the cell type studied. Most previous in vitro studies reported prolongation of APD\textsubscript{90} in Purkinje fibers, whereas in the present study, both APD\textsubscript{50} and APD\textsubscript{90} tended to shorten but not significantly. In other studies the duration (3 to 6 weeks) and magnitude (5 to 35 mg/kg) of exposure with amiodarone were less than in the present study. In addition heart rate
variability in dogs receiving amiodarone did not differ from that in controls, suggesting that amiodarone did not affect autonomic tone.

This study evaluated effects of amiodarone given at doses designed to achieve therapeutic concentrations for 10 weeks. Whereas the period of surveillance was longer than for any other study, there may be changes in function and/or electrophysiology that might occur after dosing for still longer periods.
3.6 References


9. Singh BN, Jewitt DE, Downey JM. Effects of amiodarone and L8040, novel antianginal and antiarrhythmic drugs, on cardiac and coronary hemodynamics and...


17. Gallagher JD, Bianchi J, Gessman LJ. A comparison of the electrophysiologic effects of acute and chronic amiodarone administration on canine Purkinje fibers.


<table>
<thead>
<tr>
<th>Parameters (in vivo)</th>
<th>Control (n=5)</th>
<th>Amiodarone (n=5)</th>
<th>p value</th>
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<tr>
<td>Heart rate (bpm)</td>
<td>83.8 ± 6.3</td>
<td>74.8 ± 6.6</td>
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<td>PQ interval (ms)</td>
<td>112.8 ± 7</td>
<td>117.2 ± 8</td>
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<td>QRS duration (ms)</td>
<td>40.4 ± 2</td>
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<tr>
<td>QT duration (ms)</td>
<td>241 ± 6</td>
<td>268 ± 7</td>
<td>0.016*</td>
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<tr>
<td>QTc duration (ms)</td>
<td>271 ± 5</td>
<td>288 ± 7</td>
<td>0.082</td>
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<tr>
<td>SD/AV</td>
<td>53.2 ± 14.7</td>
<td>55.8 ± 13.3</td>
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</tr>
<tr>
<td>ln(SD)^2</td>
<td>-6.8 ± 0.6</td>
<td>-6.3 ± 0.5</td>
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<td>VLF (ms^2/Hz)</td>
<td>103 ± 74</td>
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<td>LF (ms^2/Hz)</td>
<td>365 ± 338</td>
<td>22.4 ± 12</td>
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<td>HF (ms^3/Hz)</td>
<td>865 ± 750</td>
<td>204 ± 149</td>
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<td>LF/HF</td>
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<td>LVP_max (mmHg)</td>
<td>98.9 ± 5.4</td>
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<td>LVEDP (mmHg)</td>
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<td>dP/dt_max (mmHg/s)</td>
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<td>dP/dt_min (mmHg/s)</td>
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<td>V_max (ML/s)</td>
<td>69 ± 4</td>
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<td>Amplitude (mV)</td>
<td>21.1 ± 3</td>
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<td>Resting potential (mV)</td>
<td>-2.9 ± 0.7</td>
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<td>APD_{90} (ms)</td>
<td>190 ± 10</td>
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<td>APD_{90} (ms)</td>
<td>149 ± 16</td>
<td>193 ± 8</td>
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Table 3.1: In vivo variables (Means ± SEM) for control and amiodarone groups. SD/AV= ratio of standard deviation to the average of RR intervals and ln(SD)^2 = natural logarithm of standard deviation square (time domain); VLF= very low frequency, LF= low frequency, HF= high frequency and LF/HF= ratio of low frequency to high frequency (frequency domain); LVP_max= peak left ventricular pressure; LVEDP_{min}= end-diastolic left ventricular pressure; dP/dt= derivative of left ventricular pressure; V_max= an approximation of maximal velocity of fiber shortening (the stiffness constant being omitted); APD= action potential duration.

* Significant difference between control and amiodarone groups (p<0.05)
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<th>Parameters (in vitro)</th>
<th>Control (n=5)</th>
<th>Amiodarone (n=5)</th>
<th>p value</th>
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<td>Amplitude (mV)</td>
<td>119.5 ± 4.4</td>
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<td>APD_{90} (ms)</td>
<td>356.6 ± 36</td>
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<td>APD_{50} (ms)</td>
<td>226.6 ± 28</td>
<td>206 ± 29</td>
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<td>dV/dt_{max} (mV/ms)</td>
<td>387 ± 86</td>
<td>332 ± 71</td>
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<td>ERP (1/2 Hz) (ms)</td>
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<td>ERP (1 Hz) (ms)</td>
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<td>ERP (2 Hz) (ms)</td>
<td>220 ± 20.6</td>
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Table 3.2: *in vitro* variables (Means ± SEM) for control and amiodarone groups. APD = action potential duration; dV/dt_{max} = maximal rate rise of phase zero of action potential; ERP = effective refractory period.
Figure 3.1: Typical monophasic action potentials recorded from the subendocardium during the in vivo studies from one control dog and from one dog that had received amiodarone. APD₅₀ and APD₉₀ prolonged significantly in dogs receiving amiodarone.
Figure 3.2: Monophasic action potentials recorded from Purkinje fibers of a control dog and from a dog exposed to amiodarone. APD$_{50}$ and APD$_{90}$ shortened insignificantly in dogs receiving amiodarone.
CHAPTER 4
AMIODARONE-INDUCED KERATOPATHY IN DOGS

4.1 Abstract

Amiodarone has a broad spectrum as an antiarrhythmic agent and is indicated for patients with atrial and ventricular arrhythmias. Amiodarone-induced corneal deposits are the most common reversible side effect (70-100%) in man. Additional ocular effects in man include deposits in the lens, retina and optic nerve.

This study was conducted to determine ocular effects of chronic oral amiodarone in healthy dogs. Six chronically amiodarone treated dogs and 4 controls were used for this study. Ophthalmic examination was performed using biomicroscopy and indirect ophthalmoscopy at the end of 4th, 7th and 11th weeks when dogs received amiodarone. Corneal microdeposits were observed at the end of the 7th week in one eye and at end of the 11th week in the other eye of 1 dog. Immediately following euthanasia, corneas and optic nerves were harvested for light and electron microscopic analysis. Light microscopic analysis showed corneal deposits in the basal epithelial cells of the cornea of the clinically affected dog. In addition, a significant increase in basal cell turnover as indicated by mitotic index was observed in the affected dog compared to both non-deposit amiodarone and control groups. All remaining animals were normal. One out of 6 dogs treated with amiodarone demonstrated corneal deposits (16%). This
prevalence is low compared to humans. Explanations for this may include species variations particularly in volume of lacrimal secretion, or the need for longer administration. In addition, sunlight is believed to exacerbate the corneal deposits in man and all dogs in this study were housed indoors.

4.2 Introduction

Amiodarone is a very effective antiarrhythmic drug for both atrial and ventricular arrhythmias. However, it has side effects when used long term at high doses. Because of amiodarone’s high affinity to lipids in the cell, especially in lysosomes, it accumulates in organs such as lung, heart, liver, adipose tissue and eye. These lysosome-like inclusion bodies can be seen in all layers of ocular tissue in man.

Corneal microdeposits are the most common side effects of chronic amiodarone treatment in man. Studies showed these deposits to be intracytoplasmic lamellar inclusion bodies, which are mainly localized in the basal epithelial cells of the cornea. When the drug is discontinued, corneal deposits resolve in 3 to 7 months. There is a high incidence of corneal deposits (70-100%) in patients receiving amiodarone. There is little information about side effects of amiodarone in animals. There are no reports describing amiodarone-induced corneal deposits in dogs.

4.3 Materials and methods

This study was conducted with approval of the Institutional Laboratory Animal Care and Use Committee (ILACUC) of The Ohio State University. Amiodarone (amiodarone hydrochloride 200 mg tablets, Copley Pharmaceutical Inc. Canton, MA)
was administered, orally, at 400 mg/day for 4 weeks and then at 200 mg/day for 7 additional weeks to six healthy, intact beagle hounds of either sex, weighing between 7 and 10 kg. Four healthy intact beagle hounds of either sex and weighing between 8 and 12 kg were used as controls. All dogs were normal prior to initiation of this study. Animals receiving amiodarone were examined using biomicroscopy and indirect ophthalmoscopy at the end of 4\textsuperscript{th}, 7\textsuperscript{th} and 11\textsuperscript{th} weeks. All dogs were euthanized with 20 mg/kg pentobarbital given iv at the end of the 11\textsuperscript{th} week. Immediately following euthanasia, samples of cornea and optic nerve were collected from each dog, rinsed in 100mM phosphate buffered saline (pH= 7.4) and placed in fixatives for light or electron microscopic analysis. For light microscopic analysis, samples were fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer (pH = 7.4). Then samples were embedded in paraffin and sections were stained with both Hematoxylin & Eosin and Ziehl-Nelsen stains. For electron microscopic analysis, samples were fixed with 5% glutaraldehyde, 0.1% tannic acid, 0.1 M sucrose, 1 mM sodium azide and 50 mM sodium phosphate buffer (pH=7.4) and post-fixed with 1% osmium tetroxide. Then they were dehydrated through graded concentrations of ethanol at 4°C, 100% ethanol at room temperature and 100% acetone at room temperature. Finally, samples were embedded in epon for thick and ultrathin sectioning. Thick sections were stained with 1% toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate for observation on a Zeiss transmission electron microscope.

Mitotic indices were used to evaluate cellular turnover of basal corneal epithelial cells. The number of mitotic cells per $2.5 \times 10^4$ cells for each dog were counted in serial paraffin sections 5 μm in thickness. Every other section of the serially sectioned slides...
was counted to ensure that identical cells were not counted twice. To make the statistical analysis (t-test) possible for the corneal deposit dog as a group, we counted two separate $2.5\times 10^4$ cells.

Blood samples were collected, just before euthanasia, from 5 of the 6 dogs in this study. One dog was euthanized because of acute pulmonary edema, which developed during the 4th week of the loading dose used to search for acute toxicity. Serum samples were sent for amiodarone and desethylamiodarone serum concentration analysis (Mayo Medical Laboratories, Rochester, MN).

Means and standard error of means (SEM) of parameters were compared by unpaired Student’s t test requiring $p<0.05$ for statistical significance.

4.4 Results

Serum concentrations of amiodarone and desethylamiodarone are shown in Figure 4.1. Means and standard error of means (SEM) of serum concentrations of amiodarone and desethylamiodarone from 5 dogs were $1.26 \pm 0.23 \mu g/ml$ and $0.24 \pm 0.02 \mu g/ml$ respectively.

All dogs receiving amiodarone were normal at the end of 4th week. One out of 6 dogs had corneal microdeposits in the left eye at the end of 7th week. (Figure 4.2) At the end of 11th week, corneal deposits had progressed in the left eye and were also present in the right eye. The other 5 dogs receiving amiodarone remained normal.

Corneal deposits were observed in the affected dog. (Figure 4.3) Only trace amounts of deposits were observed in the basal epithelial cells. Optic nerves were normal in all dogs. Mitotic cell indices of the basal epithelial cells in all dogs are shown in Table
4.1. There was no significant difference between dogs receiving amiodarone (non-affected) and control groups (p = 0.061); however, the dog with corneal deposits had significantly higher numbers of mitotic cells when compared to both non-deposit amiodarone group (p=0.002) and controls (p=0.016). (Figure 4.4)

Deposits of amiodarone were not observed by Electron Microscopy in the corneas or optic nerves of the dogs.

4.5 Discussion

During the initial 2 weeks of therapy (during the period of loading), amiodarone serum concentrations were in excess of the therapeutic range for all dogs, however serum concentrations immediately preceding euthanasia were all within the normal range (1 to 2.5 ug/ml). The dog with corneal deposits of amiodarone had a serum concentration of 1.8 ug/ml, however another dog with a concentration of 1.8 ug/ml did not have deposits. Because only 6 dogs received amiodarone and only 1 of those developed corneal deposits, it is impossible to determine a reasonable value for prevalence or to search for a dose-response relationship.

In humans receiving amiodarone chronically, 70 to 100% develop corneal deposits and intracytoplasmic lamellar inclusion bodies in the cornea, lens, sclera, and optic nerve.\textsuperscript{150} Pollak et al\textsuperscript{8} and Greene et al\textsuperscript{9} reported that most patients taking amiodarone at approximately 5 mg/kg/day developed grade 1 (horizontal lines on the inferior cornea) microdeposits in 2.5 months. The one dog in this study that developed corneal deposits received 50 mg/kg/day for 1 month, and had no deposits. This dog did not develop deposits until after the 7\textsuperscript{th} week of receiving 25 mg/kg/day, and then the 105
deposits took the form of small dots and were considered to be only a “trace”.

In one case report, a patient taking amiodarone for 8 months had unilateral optic disc edema and whirl-like patterns, known as cornea verticillata, which occurred in the corneas of both eyes. After cessation of amiodarone for 8 months, corneal deposits disappeared; however, the optic disc did not recover completely. Macaluso et al reported that amiodarone-induced optic neuropathy has an insidious onset with slow progression, disk swelling and visual loss. Mansour et al demonstrated that amiodarone accumulated as intracytoplasmic lamellar inclusions in the large axons of optic nerves, and postulated that amiodarone-induced lipidosis may cause a chronic type of mild degeneration in the optic nerve. In our study, we did not see any alterations in the retina or in the optic nerve.

Normal values of lacrimal secretion measured by using Schirmer’s tear test range from 15 to 25 mm of wetting per minute in dogs; however, the normal value for man is 10 mm in 5 minutes. The difference in amount of lacrimal secretion between species could be one reason for the low prevalence of corneal deposits in our dogs. Pollak et al reported that using eye drops helps to wash out amiodarone from the surface of the cornea. Bockhardt et al demonstrated that rats taking high doses of amiodarone given orally (approximately 160 mg/kg) for 3-14 weeks did not develop corneal deposits; however, local application of amiodarone upon the cornea caused corneal deposits in one week. Nielson et al reported that amiodarone plasma concentration must be higher than 1.2 ug/ml to accumulate in the tear glands, so it can be excreted into the tear fluid. Our study shows that, even at high serum concentration (Figure 2.2), corneal deposits of amiodarone did not occur frequently.
In theory, sunlight augments deposition of amiodarone in the cornea. Since the dogs in this study were kept in rooms with only artificial light, this may be another reason for the low prevalence of corneal deposits. Deposition of amiodarone in the cornea is classified as: trace (sparsely distributed dots), stage 1 (linear lesions), stage 2 (branches or whiskers extending from the lines), stage 3 (whirl-like lesions). Ocular biomicroscopy and light microscopy showed that corneal deposits in the one dog affected in this study were a trace. Progression to advanced clinical stages (lined up, whiskers or whirl-like vortex patterns) was not observed.

Although we could not find either corneal or optic nerve deposits using electron microscopy due to the wide and sparse distribution, corneal deposits in the cytoplasm of the basal epithelial cells were identified by light microscopy. This is consistent with studies conducted on both humans and rats.

Bron et al postulated that, in patients with drug-induced corneal deposits of other drugs, there may be an alteration in cellular mitotic divisions in the basal epithelial cells. There are no reports of alterations in amiodarone-induced cellular division in dogs. In this study, the dog with corneal deposits did have significantly increased epithelial mitotic divisions, indicating increased turnover in the basal epithelial cells. That is, the dog with the corneal deposits had the highest mitotic cell index.

Although amiodarone-induced corneal deposits are apparently not a common side effect in the dogs, serum amiodarone concentrations should be checked and ocular examination should be performed in long-term use, as suggested for humans, every other month during amiodarone therapy. It also might be advisable to use artificial tear drops and to avoid ultraviolet light.
4.6 References


Table 4.1: Mitotic cell indices of the basal epithelial cells in all dogs. Basal cells were counted from affected dog twice for statistical analysis.
Figure 4.1: Serum concentrations of amiodarone and desethylamiodarone just before euthanasia from 5 dogs receiving amiodarone. "D116" is affected dog.
Figure 4.2: Biomicroscopy photograph from left cornea of affected dog. Arrows show amiodarone-induced keratopathy in the 7th week of medication.
Figure 4.3: Corneal deposits of the basal epithelial cells from affected dog. Arrows show intracytoplasmic deposits. H&E staining.
Figure 4.4: Mitotic cell histogram. Means and standard error of means of each group are shown. There was no difference between non-affected and control groups. (p=0.061) Affected dog had the highest number of mitotic cells in all dogs. (Affected vs. non-affected p=0.002) (Affected vs. control p=0.016) * Affected dog is different from both control and amiodarone group. (p<0.05)
CHAPTER 5
ACUTE EFFECTS OF ESCALATING DOSES OF AMIODARONE IN
GUINEA PIG HEARTS

5.1 Abstract

Cardiac effects of escalating concentrations of amiodarone were determined on isolated perfused guinea pig hearts (Langendorff preparations). Spontaneously beating hearts were instrumented for the measurement of RR, PQ, QRS, QT, and QTc durations (from a bipolar electrogram), and $dP/dt_{max}$ and $dP/dt_{min}$ from an isovolumetric left ventricular pressure curve. Ten hearts were exposed to escalating concentrations of amiodarone ($10^{-7}$, $10^{-6}$, $10^{-5}$ and $10^{-4}$ M) in DMSO/Krebs-Henseleit or to DMSO/Krebs-Henseleit (vehicle). Measurements were obtained during the last minute of a 15-minute concentration. Means of all parameters were compared by ANOVA with repeated measures design. When compared to vehicle, amiodarone prolonged QT and QTc durations at concentrations $> 10^{-6}$ M. The apparent lengthening of RR, PQ and QRS at concentrations $> 10^{-6}$ M did not achieve statistical significance. Similarly the apparent decreases in $dP/dt_{max}$ and $dP/dt_{min}$ at concentrations $> 10^{-6}$ M did not achieve statistical significance. The putative therapeutic concentration of amiodarone is between $2$ and $4 \times 10^{-6}$ M. In this study, at a concentration of $10^{-6}$ M, only RR and $dP/dt_{min}$ tended to change, but they were not different from vehicle. Thus amiodarone in this preparation
has little potential for cardiac toxicity at therapeutic concentrations.

5.2 Introduction

Amiodarone, a class III antiarrhythmic agent, has been well studied for 4 decades. It possesses properties of all 4 classes of antiarrhythmics and unusual pharmacokinetics (erratic absorption and bioavailability, and extremely long half-life). It has both cardiac and extra-cardiac effects, and it stands as an antiarrhythmic of greatest potential because of its comprehensive spectrum of use (e.g. for atrial fibrillation, and both atrial and ventricular ectopia) and its low proarrhythmic potential.

This study evaluates certain electrophysiological and mechanical effects of amiodarone in an isolated, perfused guinea pig heart (Langendorff preparation). The reason for selecting this preparation rather than specific isolated fibers is that in this preparation the effects of amiodarone may be expressed on all component tissues of the heart (e.g., SA node, AV node, atrial and ventricular myocardium, “M” fibers). Furthermore the effects will not be obfuscated by anesthetics, neurophysiological influences, or hepatic biotransformations of the molecule. Finally the Langendorff preparation permits identifying the effects of amiodarone on both inotropy (myocardial contractility) and lusitropy (myocardial compliance) of the left ventricle. In this study we exposed hearts to escalating concentrations of amiodarone ($10^{-7}$, $10^{-6}$, $10^{-5}$, $10^{-4}$ M) greater than in previous studies and monitored effects on both inotropy and lusitropy not investigated previously in Langendorff preparations of guinea pig hearts.
5.3 Materials and methods

This study was approved by the Laboratory Animal Care and Use Committee of QTest Labs. Twenty, healthy, male guinea pigs weighing 295 to 395 grams were used in this study. Ten animals were placed randomly into each of two groups: amiodarone and DMSO/Krebs-Henseleit (vehicle). Animals were anesthetized, intraperitoneally, with sodium pentobarbital (35 mg/kg). They were ventilated with a tidal volume of 10 ml/kg and at a rate of 60/minute during the surgical procedure. After sternotomy and pericardiotomy, and before cannulation of the aorta, sodium heparin (250 UI) was injected into a jugular vein. Heart was quickly removed and cannula was attached to the perfusion column. The coronary circulation was perfused, according to the technique of Langendorff, with a modified Krebs-Henseleit solution containing: 118 mM NaCl, 4.7 mM KCl, 2.52 mM CaCl₂, 1.64 mM MgSO₄, 24.88 mM NaHCO₃, 1.18 mM KH₂PO₄, 5.55 mM glucose. The solution was gassed with 95% O₂ and 5% CO₂ at a rate of 100 to 500 ml/minute, and temperature was controlled to approximately 36 °C. Bipolar electrograms were recorded from electrodes held loosely against the epicardium of the left and right ventricles. A metal cannula with a 0.25 ml rubber balloon (expendable to 1.5 ml) on the tip was inserted through the mitral orifice into the left ventricle. Then, the balloon cannula was attached to a pressure transducer energized by the Biopac MP100 Physiological Data Acquisition System, and leads from the two electrodes were also attached similarly. The bipolar electrograms and intraventricular pressure curves were recorded on the Biopac MP100 with the electrogram calibrated at approximately 0.25 cm/mV and the pressure transducer calibrated at 0 to between 50 and 150 mmHg. The derivative of left ventricular pressure (dLVP/dt) was generated by the Biopac MP100.
Amiodarone was dissolved in 5 ml of DMSO and diluted in one liter of Krebs-Henseleit to produce a $10^{-4}$ M stock solution. Molar concentrations of amiodarone (i.e. $10^{-7}$, $10^{-6}$ and $10^{-5}$) were obtained by diluting from the stock solution. DMSO in Krebs-Henseleit solution was prepared so that it could be infused as the vehicle at concentrations of DMSO identical with those for each molar concentration of amiodarone. Thus, in the figures, when $10^{-6}$M refers to the vehicle, it refers to the concentration of DMSO in the perfusate that corresponds to the concentration in the amiodarone. Physiological measurements were made during the final minute of a 15-minute infusion of each concentration. Ten consecutive RR, PQ, QRS and QT intervals were measured using Biopac MP100 software. QT was corrected for RR interval using the method of Fridericia ($\text{QTc} = \text{QT} / \sqrt[3]{\text{previous RR interval}}$). Means and standard errors of means (SEM) were compared between values for amiodarone and values for DMSO/Krebs-Henseleit by two-way ANOVA with repeated measures on time. $\text{dP/dt}_{\text{max}}$ is an estimate of the inotropic state—myocardial contractility. $\text{dP/dt}_{\text{min}}$ is an estimate of the lusitropic state—myocardial compliance. While these parameters are somewhat load-dependent, they are less so in a ventricle contracting isovolumetrically.

When indicated by a significant F-statistic, specific means were compared by Tukey’s multiple comparison tests requiring a $p<0.05$ for significance. Means and SEM were compared between baseline values and concentration values for amiodarone and vehicle by one-way ANOVA with repeated measures on time. When statistical significance achieved ($p<0.05$) Tukey’s multiple comparison post-hoc tests were performed.

118

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5.4 Results

Figure 5.1 shows actual recordings of an electrogram (ECG), a left ventricular pressure (LVP) and the derivative of LVP (LVdP/dt). When compared to the vehicle (*), amiodarone prolonged QT interval (Figure 5.2C) and QTc interval (Figure 5.2D) beginning at a concentration of $10^{-5}$ M ($p=0.017$ for QT and $p=0.011$ for QTc). PQ interval lengthened with both amiodarone and vehicle at concentrations greater than $10^{-6}$ M (not shown). When compared to baseline (&) RR interval (Figure 5.2A) prolonged only for amiodarone at concentrations of $10^{-6}$ M, $10^{-5}$ M and $10^{-4}$ M. QRS complex (Figure 5.2B) also prolonged only for amiodarone at concentrations of $10^{-5}$ M and $10^{-4}$ M. Although there is a trend for both RR interval and QRS duration to lengthen when comparing amiodarone to vehicle, the differences did not achieve statistical significance ($p=0.352$ for RR and $p=0.264$ for QRS). Although $dP/dt_{\text{max}}$ for amiodarone appeared to decrease more than for vehicle at both $10^{-5}$ M and $10^{-4}$ M (Figure 5.2E), the difference did not achieve statistical significance between two groups ($P=0.322$). $dP/dt_{\text{min}}$ became less negative for both vehicle and amiodarone when comparing to baseline values at concentrations higher than $10^{-6}$ M. Apparent differences in $dP/dt_{\text{min}}$ (Figure 5.2F) between amiodarone and vehicle were not significant ($P=0.241$).

5.5 Discussion

The plasma concentration of amiodarone recommended for therapeutic use (the broken vertical line in Figure 5.2) is 2 to 4 $\times 10^{-6}$ M, approximately midway between our concentrations of $10^{-6}$ M and $10^{-5}$ M. Thus, prolongations of QT interval and QTc duration occurred at concentrations of amiodarone higher than therapeutic. When used
chronically, amiodarone slows rate of discharge of the SA node by down-regulating β-receptors and by inhibiting the release of adenyl cyclase.\textsuperscript{2,13} Of course this could not be operative in the denervated, acute Langendorff preparation. However, the cardiodeceleration that occurs even in the presence of complete β-adrenergic blockade indicates that amiodarone slows rate of discharge of the SA node by retarding conductance over the L-type Ca\textsuperscript{2+} channel (a class IV effect). This is most likely the mechanism for the tendency to prolonging RR interval in our preparation.

The tendency for lengthening of the QRS duration may be attributable to the ability of amiodarone to retard conductance through Na\textsuperscript{+} channels (a class IA, quinidine-like effect); while tendency for lengthening of the PQ interval may be due to reduction in conductance over both L-type Ca\textsuperscript{2+} channels and Na\textsuperscript{+} channels. Classically amiodarone is thought to possess a class IB (lidocaine-like effect)\textsuperscript{10} property in which dV/dt of the action potential increases and ventricular conduction accelerates. This would shorten the duration of the QRS complex. However at supratherapeutic doses—in man—prolongation of the QRS complex occurs. We presume that the class IA property dominated the class IB in our study. Mason et al\textsuperscript{10} found that when given either orally or intravenously to guinea pigs, amiodarone produced a use-dependent decrease in dV/dt\textsubscript{max}. However studies by Ikeda et al,\textsuperscript{7} Kato et al,\textsuperscript{9} and Gallagher et al\textsuperscript{3} found no decrease in dV/dt\textsubscript{max}.

Although there was a tendency for RR and QRS to lengthen more than for vehicle, the differences did not achieve statistical significance. This is contrary to the fact that amiodarone is known to retard the conductance over both L-type Ca\textsuperscript{2+} channels and Na\textsuperscript{+} channels (classes I and IV effect).\textsuperscript{10} Prolongations of QT and QTc intervals
demonstrated that amiodarone reduced the conductance over the delayed rectifier channel for potassium (a class III effect). Even if differences of significance would have developed had a larger number of hearts been exposed to amiodarone, it is highly unlikely that these differences would have emerged at concentrations less than $10^{-5}$ M.

The potent antiarrhythmic and the low proarrhythmic potentials of amiodarone are attributed to lengthening of the effective refractory period, to shortening of the relative refractory period, and to decrease in the temporal inhomogeneity of ventricular repolarization. In this study, we observed no ectopic activity, even at the $10^{-4}$ M concentration.

Sosunov et al exposed superfused myocardial and Purkinje fibers from guinea pigs to amiodarone, and studied physiology of ion channels specific for ventricular repolarization. They found that amiodarone lengthened the action potential duration at all pacing rates; however, the lengthening was attenuated by elevating extracellular potassium ion concentration. They observed minimal reverse-use-dependence, supporting the contention that the compound has minimal risk for production of torsade de pointes. Amiodarone did not appear to impair $I_{K_R}$ in excess of that due to E-4031, a known pure $I_{K_R}$ blocker. The advantage to amiodarone, therefore, is that it should possess less potential for producing torsade de pointes when used with another agent known to block $I_{K_R}$. Repolarization of the guinea pig ventricle depends upon both $I_{K_R}$ and $I_{K_S}$, in contradiction to that of the rabbit, which possesses little to no $I_{K_S}$ but more $I_{K_T}$.8

In the isovolumetrically-contracting ventricle, in which loading conditions are constant, $dP/dt_{\text{max}}$ is a satisfactory monitor of inotropy, and $dP/dt_{\text{min}}$ is a satisfactory monitor of lusitropy. Although there was a tendency for $dP/dt_{\text{max}}$ to decrease and $dP/dt_{\text{min}}$
to increase with increasing concentrations of amiodarone, there was a similar tendency when using vehicle alone. This demonstrates deterioration, with time, of the preparation. However from examining Figures 2E and 2F it is quite obvious that the changes in both dP/dt\text{max} and dP/dt\text{min} were greater for hearts perfused with 10^{-5} and 10^{-4}M concentrations of amiodarone than with vehicle. In previous studies with chronic oral dosing of amiodarone in humans, no negative inotropy was observed. In studies conducted on humans with heart failure, a mild negative inotropy developed following intravenous loading, but contractility returned to normal during sustained dosing. This appears to be consistent with the tendency to reduction in dP/dt\text{max} observed in the present study.

Padrini et al in preparations of guinea pig hearts nearly identical to ours found that when pacing rate was changed abruptly, the rate, which QT adjusted to the new pacing interval was not affected by amiodarone that had been given intraperitoneally at a dose of 50 mg/kg/day. The observation that this electrical restitution is preserved with amiodarone supports the contention that amiodarone does not possess a significant liability to produce torsade de pointes. Stark et al in preparations of guinea pig hearts nearly identical to ours found that desethylamiodarone, an active metabolite of amiodarone, was principally responsible for prolongation of QT. Because in our preparation the perfusate is not recycled and obviously there is no hepatic metabolism of the parent compound, amiodarone alone must be responsible for prolongation of both QT and QTc intervals. Using molar concentrations of 10^{-5} in isolated, perfused guinea pig hearts, Stark et al found prolongations of RR, QRS, and QT comparable to those in our study. In yet another study on isolated perfused guinea pig hearts exposed to 10^{-5} M concentration of amiodarone, Stark et al found prolongations of RR and PQ intervals.
but no lengthening of QT. Again these divergent results are consistent with our findings.

In rabbits given amiodarone orally for 30 days 100 mg/kg, spatial inhomogeneity of ventricular repolarization was not augmented. This supports, further, the contention that amiodarone does not possess a liability to produce torsade de pointes, in this species as well.

Contrary to previous studies using a similar preparation, this is the first conducted on isolated, perfused guinea pig hearts exposed to escalating concentrations of amiodarone, to demonstrate the effects on both electrophysiological and mechanical properties of the heart. We demonstrated little potential for either electrophysiological or mechanical expressions of toxicity at therapeutic concentrations.
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Figure 5.1: Typical recordings of (top to bottom) cardiac electrogram (ECG), left ventricular pressure (LVP), and dP/dt of left ventricular pressure (LVdP/dt). Component deflections for the electrogram are: P = atrial depolarization, Q = first negative wave after atrial depolarization, R = ventricular depolarization, S = first negative wave after ventricular depolarization, T = ventricular repolarization. Calibrations for each curve appear to the right, and time is shown at the bottom.
Figure 5.2: Plots of physiological variables for escalating concentrations of both amiodarone and vehicle (DMSO/Krebs-Hensleit). Amiodarone (n= 10) is solid bars, vehicle (n= 10) is open bars. Vertical dashed line shows recommended therapeutic concentration of amiodarone. * and & = values different between and within groups.
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