Predicting TCA toxicity using *in vivo* and *ex vivo* juvenile safety models.

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Abstract

The tri-cyclic antidepressant (TCA) amitriptyline is used in the treatment of nocturnal enuresis at doses of 2-7 mg/kg in children, despite existing reports of severe cardiac toxicity at doses of 10 mg/kg. Cardiac adverse events are frequent and represent the main cause of TCA mortality, via sinus tachycardia and conduction delays. TCAs inhibit sodium currents and the hERG channel, leading to QRS widening and QT prolongation. The range of cardiac effects reported in children warrants the testing of amitriptyline in conscious juvenile animals and hearts obtained from immature animals. *In vivo*, 1.0 mg/kg/min amitriptyline was infused into telemeterized dogs ranging in age from 2 to 6 month-old, until QRS width doubled from baseline values. Mean arterial pressure (MAP), heart rate (HR), QRS duration, QT interval were measured continuously over a period of 24 hours post-infusion. A 62% increase in HR was measured, with biphasic changes in QT, first decreasing then prolonging as plasma concentrations increased. *Ex vivo*, spontaneously-beating hearts excised from immature dogs (ages 2, 4 and 6 months) were perfused with 1, 5, and 10 µg/mL amitriptyline, with continuous monitoring of HR, QRS, and QT intervals. QRS widening of 20 ms was measured, while 20% QT prolongations were recorded. Administration of 25 mM sodium bicarbonate at the peak of the QRS widening reversed the effect of amitriptyline on the QRS complex. The arrhythmias observed in the conscious dogs and excised hearts, as well as the reversal of the effect of amitriptyline by sodium bicarbonate demonstrate the validity of juvenile animals models in the evaluation of the cardiac safety of drugs intended for paediatric populations.

1. Introduction

Amitriptyline (Elavil®) is a tricyclic antidepressant (TCA) which acts on serotonin-norepinephrine reuptake. It is widely used in adult populations to treat depression, the indication for which it was tested clinically and approved by the world's regulatory agencies. In the targeted population, TCA drugs are well known to produce cardiac adverse effects, ranging from mild sinus tachycardia at low doses to fatal cardiac arrhythmias at higher doses in patients with pre-existing cardiac disease. The excess incidence of sudden TCA overdose deaths emphasizes the existence of an unpredictable conduction-slowing mechanism, which has also been observed at therapeutic doses.

Aside from its potential as a Class 1 anti-arrhythmic, a common side effect of amitriptyline is urinary retention. As a result, amitriptyline is frequently prescribed "off-label" to treat bedwetting in children. Amitriptyline is not FDA-approved for use in children under 12, with good reason; its potency as a conduction depressant and pro-arrhythmic agent has been demonstrated conclusively to be frequency-dependent. i.e. the adverse cardiac effects of amitriptyline increase with heart rate.

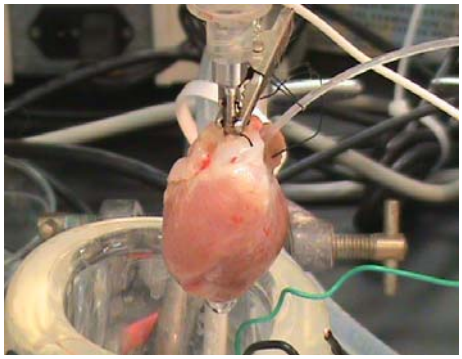
As of this day, the safety of amitriptyline has not been interrogated formally in juvenile safety models, in neither pre-clinical nor clinical studies. From recent interest in juvenile safety models, this investigation focuses on whether amitriptyline acts differently in juvenile and adult dogs, highlighting the need to reconsider the safety margins for juveniles currently based on adult safety data.

***Ex-vivo*: Isolated Heart Preparation (Langendorff's)**

3 to 4 months old dog hearts were excised, mounted in a Langendorff system, and perfused retrogradely at a flow rate of approximately 30 mL/min with oxygenated, warmed (35 ±°2C) Tyrode's solution.

A canula ending with a fluid-filled balloon connected to a pressure transducer was inserted into the left ventricular through the mitral valve to measure the left ventricular pressure (LVP). Two bipolar silver wire electrodes and one epicardial monophasic action potential electrode were placed on the epicardium for the monitoring of the ECG and MAP respectively.

Continuous recording was initiated following a 20 minute equilibration period. Following baseline recording, the perfusion system was switched to the reservoir containing the first concentration of amitriptyline. Hearts were exposed to each concentration of Amitriptylinefor 10 minutes. Sodium bicarbonate 25 mM was introduced after 7 minutes of exposure to 10 µg/mL amitriptyline.



***In-vivo*: Dog Telemetry**

Animals were naïve and ECG parameters were collected via non surgical telemetry monitoring using modified DSI TA10CTA-D70 transmitters. The transmitters were modified by connecting the implant leads to the ECG leads by soldering the leads together and insulating with heatshrink rubber. The leads were placed directly onto the body surface using a lead II configuration as shown in Figure 1. Amitriptyline was injected i.v. as a 1-minute bolus. The animals were monitored over a period of 24 hours, the first 6 of which are reported here.

Lead placement for dogs.

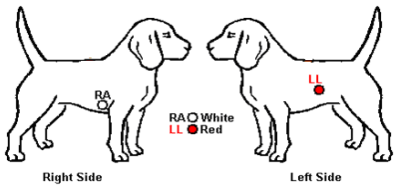
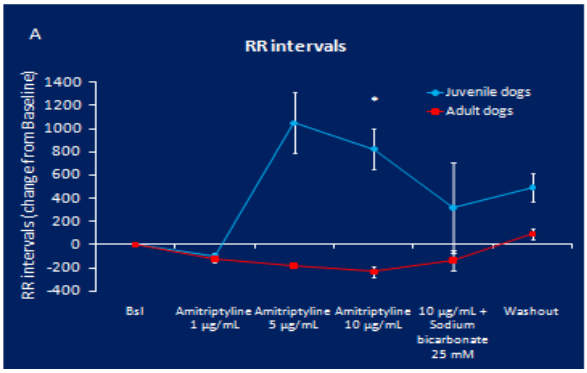
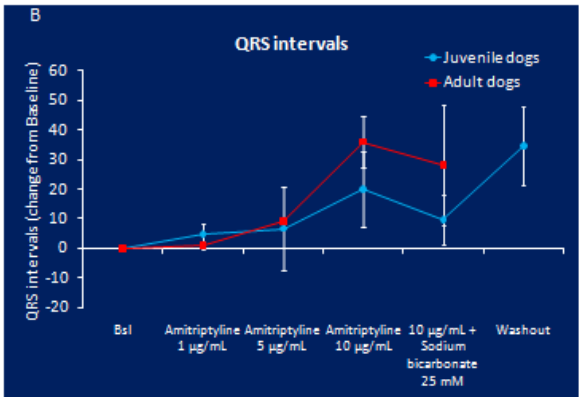


Fig. 1. (A) Effect of Amitriptyline on the RR interval measured from dog isolated hearts.



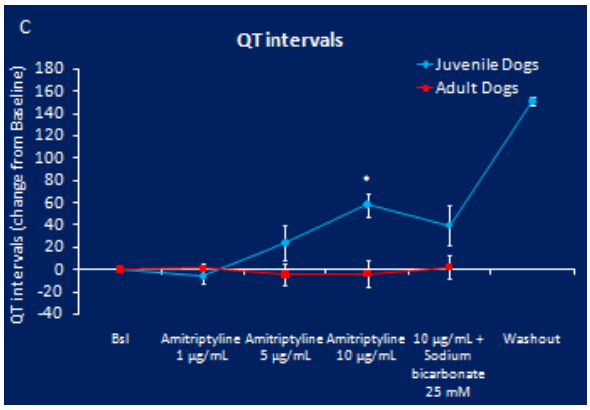
At a low concentration, (1 µg/mL) Amitriptyline caused a statistically significant tachycardia (shortening of the RR interval) while at a high concentration (5 and 10 µg/mL), it caused a statistically significant bradycardia (prolongation of the RR interval). Arrhythmic episodes and fibrillation periods were observed in the presence of 5 µg/mL Amitriptyline (see Table 1). The effect of Amitriptyline on the heart rate was partially reversed by the addition of sodium bicarbonate 25 mM.

(B) Effect of Amitriptyline on QRS intervals measured from dog isolated hearts.



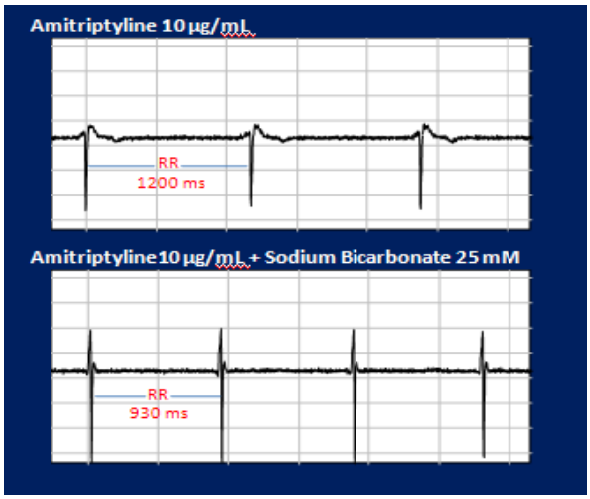
Amitriptyline tended to cause a dose-dependent widening of the QRS interval. The drug also caused a dose-dependent decrease in QRS amplitude (see Table 1). Both effects were partially reversed by 25 mM sodium bicarbonate.

(C) Effect of Amitriptyline on QT intervals measured from dog isolated hearts.



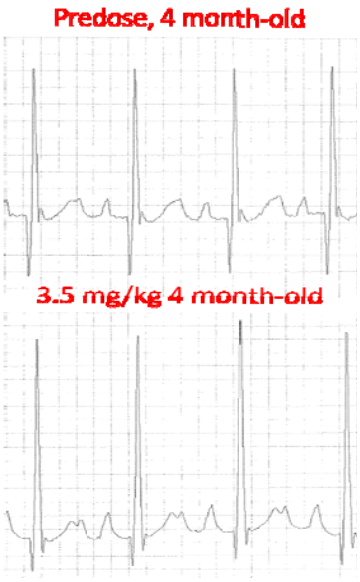
Amitriptyline caused a dose-dependent increase in QT interval. 10 µg/mL significantly prolonged the QT interval by 58 ms. The effect was not reversed by 25 mM sodium bicarbonate.

Fig. 2



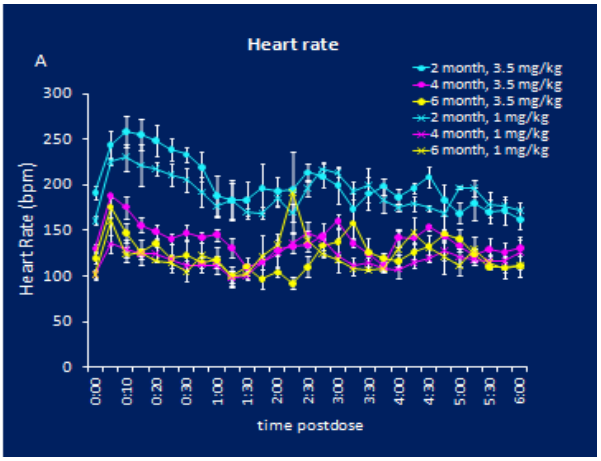
Representative ECG recordings from juvenile dog isolated hearts in baseline condition, in the presence of Amitriptyline at 1, 5 and 10 µg/mL and in the presence of Amitriptyline 10 µg/mL + 25 mM sodium bicarbonate. Although the widening of the QRS complex is difficult to see, the shortening of the QRS (amplitude) is easily observed.

Fig. 3. Representative recordings of ECGs obtained from instrumented juvenile dogs



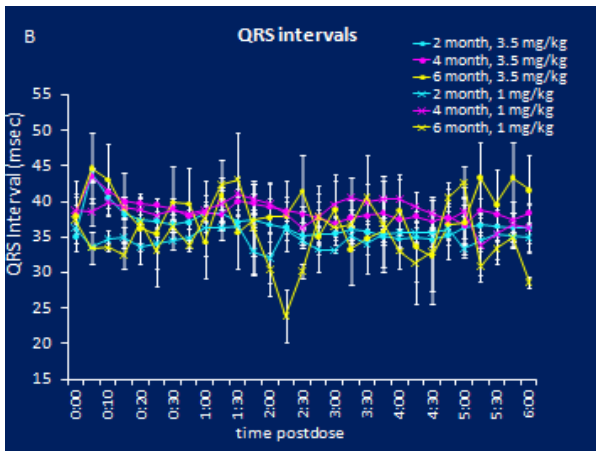
These two 1-second long segments were extracted from recordings obtained from a 4-month-old beagle, instrumented with a JET acquisition system. A: Predose, the dog does not exhibit arrhythmia. B: Following exposure to 3.5 mg/kg amitriptyline, the QRS complex is wider, and suggestion of a right bundle branch block (RBBB) appears. The segments selected do not illustrate the frequent ventricular arrhy thmias recorded in dogs exposed to 3.5 and 7 mg/kg amitriptyline.

Fig. 4. (A) In vivo effect of amitriptyline on the heart rate of instrumented juvenile dogs.



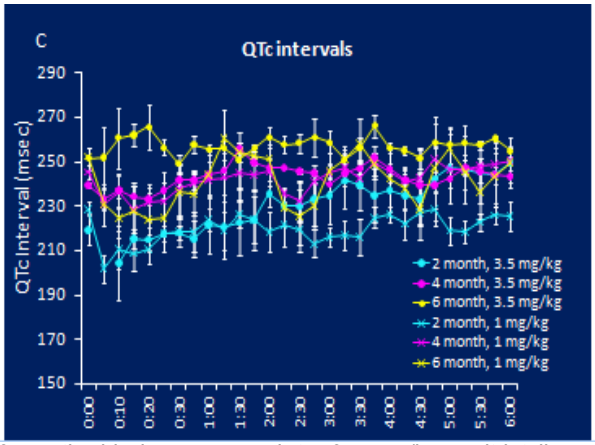
A significant rise in heart rate immediately following the 1-minute infusion of drug is likely associated with the presence of the technicians in the room. The remaining increase in at time 20 minutes is amitriptyline-induced. The effect is dose-related, transient, and greatest in younger dogs (2-months old).

(B) Effect of amitriptyline on the ventricular conduction of instrumented juvenile dogs.



All three age groups exhibited a 10-ms widening of the QRS interval within 5 minutes of the end of 3.5 mg/kg amitriptyline infusion. The effect was dose-dependent, but age-independent, and disappeared rapidly. Other changes further into the experiment are likely associated with staff entering the room.

(C) Effect of amitriptyline on the QTc interval measured from instrumented juvenile dogs.



6-month-old dogs exposed to 3.5 mg/kg amitriptyline exhibited a rapid prolongation of the QTc interval (20 ms), while the 2-month-old dogs presented a QTc interval which prolonged by 30 ms over one hour. Part of the change in QTc duration is likely associated with the QRS widening illustrated in Figure 4B. 1 mg/kg amitriptyline failed to change the QTc interval in dogs from all age groups.

Table 1. Clinical observations of the effects of Amitriptyline on the ECG of juvenile dog isolated hearts.

	Bsl	Amitryp. 1 µg/mL	Amitryp 5 µg/mL	Amitryp. 10 µg/mL	Amitryp. 10 µg/mL+ $NaHCO_2$ 25 mM
Arrhythmia	-	-	+++	++	+
Fibrillation	-	-	+	+	+
Second degree AV block	-	-	+++	++	+
Bundle Branch Block	-	+	++	+++	+

Bsl: Baseline Amitrip. = Amitryptiline

DISCUSSION

Amitriptyline is known to cause tachycardia at low doses, an effect which reverses at higher exposure levels. In this study, we tested a wide range of concentrations, *in vivo* using instrumented juvenile dogs (ages 2,4, 6 m-o), and *ex vivo* using isolated hearts excised from dogs (ages 2 to 5.5 m-o). The concentrations selected for the *in vivo* experiments were kept

low (1, 3.5, and 7 mg/kg), while the excised, spontaneously-beating hearts were exposed to amitriptyline levels of 1, 5 and 10 µg/mL. The juvenile data was compared to peer-reviewed data obtained in adult dogs

Heart Rate

A slight sinus-node-driven increase in heart rate was observed in 2-month-old dogs. The effect was greater than in older dogs, but significantly less than the tripling of the heart rate associated with 1 mg/kg in adult dogs. 5 and 10 µg/mL - approximately equivalent to doses of 0.5 and 1 mg/kg- amitriptyline perfused into excised hearts caused important sinus-node bradycardia, which could be reversed by 25 mM sodium bicarbonate. Amitriptyline and $NaHCO_3$ could be completely washed out over 10 minutes of perfusion, suggesting a rapid rate of dissociation from the sodium channels.

QRS interval / ventricular conduction

Surprisingly, amitriptyline exposure induced little change in QRS in juvenile conscious dogs, and the absence of effect was observed throughout the range of ages for the dogs. A 10-ms widening of the QRS was observed consistently after 5 minutes of infusion of 3.5 mg/kg amitriptyline; the absence of equivalent increase when 1 mg/kg was added suggests that the change in QRS is amitriptyline-related. Ex vivo, 1, 5, 10 µg/mL amitriptyline caused a concentration-dependent widening of the QRS which could be half-reversed by 25 mM $NaHCO_3$.

QT and QTc intervals

A slight age-dependent increase in QT and QTc intervals appears when 2-month-old conscious dogs are exposed to 3.5 mg/kg. The rapid heart rate of younger dogs likely enhances the effect of amitriptyline. *Ex vivo*, a 60-ms prolongation of the QT interval (30 ms change in QTc) was observed across hearts, irrespective of age. Most of the QT/QTc prolongation was associated with the change in **QRS.**

The inhibition of sodium channels by amitriptyline likely represents the main mechanism through which ventricular conduction is slowed, translating into QRS widening on the ECG. The block of sodium currents is rate-dependent, and enhanced by depolarisation. Amitriptyline unbinds from the channels during diastole, such that slower heart rates allow the drug to unbind from the channels, limiting its pro-arrhythmic effects. In contrast, a faster heart rate, as recorded from the younger dogs and hearts in this study, re-enforces the inhibition of the sodium current. AV-block, bundle branch block, ventricular tachyarrhythmia, and fibrillation ensue. Finally, it is suggested that NaHCO3 alkalinizes amitriptyline, decreasing its affinity for sodium channels and reversing the drug's adverse effects.

The results of this study highlight the greater toxicity of amitriptyline -a widely-prescribed drug- in juvenile animals. They argue in favour of more thorough safety testing for drugs intended for a paediatric population, using appropriately-validated juvenile animal models which display key characteristics found in the intended target population**.**

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