Comparison of Two Locations for Recording Intracardiac Conductivity in Anesthetized Dogs

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Abstract

In safety pharmacological and toxicological studies, all new chemical entities should be investigated for the possibility of slow atrioventricular (AV) conduction especially at the infranodal of AV node. The objective of this study was to compare two locations of His bundle electrogram (HBE) recording, the right side (right atrium) and the left side (non-coronary cusp), of the heart in anesthetized dogs. Diltiazem was used to evaluate these two locations. The dogs (n=6) were anesthetized with morphine and α-chloralose. In order to record HBE, two catheters with multiple electrodes were placed in the right atrium via jugular vein and in the non-coronary cusp via femoral artery. Transthoracic electrocardiogram (ECG) was acquired. ECG parameters (HR, PR, QRS, and QT) and HBE parameters (AH and HV intervals) were measured at baseline and after a single bolus dose of 0.5 mg/kg diltiazem (5, 10, and 15 min). PR interval was significantly prolonged after receiving diltiazem when compared to the baseline ($p<0.05$). When HBE was recorded from the catheter placed in the non-coronary cusp, the AH interval was prolonged significantly whereas the HV interval did not change. The AH and HV intervals were prolonged when it was recorded from the catheter placed in the right atrium. The His bundle recording from the non-coronary cusp was preferable since the position was fixed and stable, resulting in the prolongation of the AH interval but not HV interval in response to diltiazem.

Introduction

Cardiac conductivity is an essential property of the heart especially the atrioventricular (AV) conduction system. This property may be affected by balance of autonomic nervous system and many drugs (Smithen and Sowton, 1971). The AV conduction time depends upon the length and velocity of propagation through the pathways (from sinoatrial node to the apical third of the interventricular septum). The AV conduction is expressed in the electrocardiogram (ECG) as the PQ (or PR) interval. Of the normal PQ interval, the percentages of times required for propagation from SAN to atrioventricular node (AVN), through the head of the AVN, and from the AVN to the IVS are 15%, 70%, and 15%, respectively (Hamlin, 2005).

The His bundle electrogram (HBE) contains 3 components as follows: A (occurring during atrial depolarization), H (occurring during activation of either the His bundle or left main bundle), and V (occurring during ventricular depolarization) (Narula et al., 1970). Since HV interval depends upon conduction over those pathways which depend upon voltage-gated sodium channel (I_{Na}) physiology as well as cable properties of the pathways, when HV interval prolongs, the presumption is that the drug affected I_{Na} physiology, which could lead to complete AV block (Smithen and Sowton, 1971). Since AH interval depends upon conduction over internodal pathways (I_{Na} dependent) and conduction over the head of the AV node (more calcium channel [I_{CaL}]-dependent), if AH interval prolongs, but HV interval does not, then it may be presumed that the drug affected I_{CaL} physiology and is less likely to lead to complete AV block or ventricular arrhythmia, at least via reentry (Damato et al., 1969b; Rosen, 1973).

In safety pharmacological and toxicological studies, all new chemical entities should be investigated for the possibility of PQ interval prolongation (i.e. slow AV conduction). If the PQ interval is lengthened, it is also important to know whether it occurs at supranodal or infranodal. Because changes in supranodal conduction are dependent upon autonomic traffic and drugs (e.g. diltiazem, digitalis, beta blockers and class IA antiarrhythmics), this portion of the conduction system is variable and often controllable (Oyama et al., 1978; Motomura and Hashimoto, 1995). However, when conduction over the His-Purkinje system, while also dependent upon drugs (e.g. class IA antiarrhythmics), is retarded, it more often leads to complete AV block. In addition, the cardiac conduction has been shown to relate to paroxysmal atrial fibrillation (Olshansky, 2005; Oliveira et al., 2011).

The conduction of AV node (AVN) may be measured by His bundle electrography (Varghese et. al., 1973, Damato, et. al., 1969a). In general, the technique for recording HBE may be performed by threading a multipolar electrodes catheter through a vein so that the electrodes touch the His bundle in the right atrium and ventricle. However, the measurement of AH or HV intervals from this catheter is very challenging since the catheter location may be affected by heartbeat and respiration making variable AH and HV duration.

Several anesthetic regimens have been used in dogs for safety pharmacological studies, for example, fentanyl/etomidate, isoflurane, sevoflurane, and pentobarbital sodium (Nattel et al., 1990; van Deuren et al., 2009). Among those regimens, a mixture of morphine/α-chloralose is frequently used since it possesses the least impact on cardiovascular function including the baroreceptor reflex.

The objective of this study was to compare two locations of HBE recording, the right side and the left side, of the heart in anesthetized dogs. Duration of AH and HV was assessed after bolus injection of diltiazem.

Materials and Methods

Animals: This study was conducted at QTest Labs, LLC and approved by the Institutional Animal Care and Use Committee of QTest Labs, LLC. The facility is in compliance with USDA regulations. All animal procedures were conducted in accordance with the guideline published in the *Guide for the Care and Use of Laboratory Animals* (NRC, 2011). A total of 6 male beagle dogs, aged 1.5-2.0 years and weighing 12.3-22 kg, were used in this study. Before the beginning of the study, physical examination, complete blood count, blood chemistry profiles (i.e. blood urea nitrogen, creatinine, SGPT, SGOT, AP), and electrocardiogram (ECG) were performed in all dogs.

Experimental procedure: All dogs were anesthetized with morphine sulfate intravenously at 1.5 mg/kg through a venous catheter and a bolus of 100 mg/kg of α-chloralose. Anesthesia was maintained with 43-110 mg/kg/h α-chloralose, intravenously, until completion of the study. The dogs were intubated and ventilated between 9 and 16 breaths/minute with a tidal volume of 12.5 ml/kg (room air) to sustain $PaCO_2$ 35-45 mmHg. The dogs were placed on warm-water circulating pads to sustain normal body temperature. Cut-downs on a femoral artery were performed to permit introduction of a temporary pacing electrode catheter (5F Bard® electrophysiology, Lowell, MA, USA) for collection of His bundle electrogram (left side). This catheter was positioned in the non-coronary cusp (Valsava sinus). Additionally, the animals had cut-downs on a jugular vein performed to permit introduction of a second temporary pacing electrode catheter positioned in the right atrium for collection of His bundle electrogram (right side) simultaneously with the left side (Fig 1).

Bipolar, transthoracic electrocardiogram (ECG) between points rV2 (right, 5th intercostal space at the costochondral junction) and V2 (left, 6th intercostal space at the costochondral junction) were obtained from all dogs at baseline, and 5, 10, 15 min after dosing with diltiazem. ECG data were acquired on the EMKA IOX 2.5.1 system (EMKA Technologies, Falls Church, VA, USA). Signals were sampled at 1 kHz. The band pass of EMKA Amplifiers for HBE recording was set between 100 and 500 Hz. At the end of experiment, all animals were euthanized with 120 mg/kg pentobarbital sodium, intravenously.

Drug administration: Diltiazem (Sigma-Aldrich) was dissolved in sterile water for injection to yield a concentration of 10 mg/ml. Diltiazem was given to the animals as a bolus dose of 0.5 mg/kg.

Data analysis: ECG and HBE were analyzed using the EMKA ECG auto software. ECG parameters (PR, QRS, RR (HR) and QT) as well as AH and HV intervals of HBE were calculated from one-minute recording of each time-point. The QT interval was corrected for changes in heart rate by conversion to the corrected QT (QTc) interval using the formula of Fridericia (1920). Means and standard error of mean, for the baseline and 5, 10, 15 min after receiving diltiazem, were calculated for all parameters. Values were compared utilizing repeated measures one-way ANOVA using baseline as a control and followed by Dunnet's test. Differences were considered significant for *P* values less than 0.05.
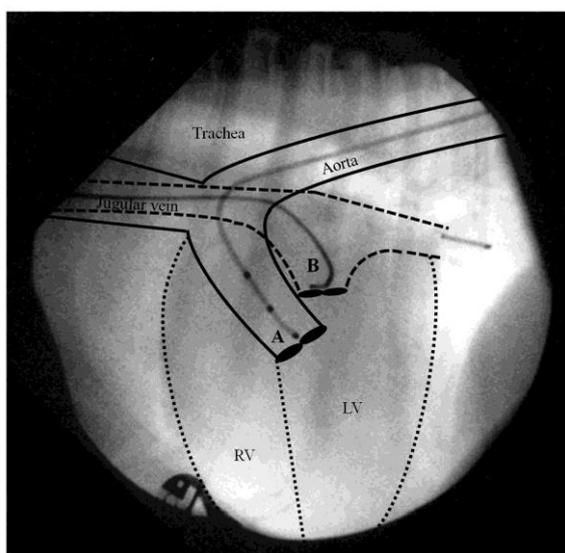

Figure 1 Fluoroscopic picture from anesthetized dogs instrumented with 5Fr pacing electrode catheters to record His bundle electrogram. A = pacing catheter positioned in the non-coronary cusp wedged in the Valsava sinus, B = pacing catheter positioned in right atrium and ventricle, RV = right ventricle, LV = left ventricle.

Results

During instrumentation and stabilization period, the animals were under the stage of surgical anesthesia and remained in this stage until the end of experiment. Hence, a mixture of morphine and α-chloralose provided a reliable anesthetic preparation. The heart rate at baseline was 71 ± 5.6 bpm. There was no change in heart rate after dosing with 0.5 mg/kg diltiazem. The PR interval was increased significantly at 5 min after diltiazem administration (30.7%, *p*<0.05) (Fig 2). The PR interval continued to increase at 10 and 15 min after dosing. At baseline, the QRS duration, QT, and QTc intervals were 65 ± 2.4 ms, 254 ± 9.7ms, and 270 ± 11.6 ms, respectively. After dosing, there were no changes for QRS, QT, or QTc intervals when compared to the baseline. When HBE was recorded from the non-coronary cusp, the AH interval was increased significantly at 5 min (30.1%, p<0.05) and continued to

increase until 15 min after injection. On the other hand, when HBE was recorded from the right atrium, the AH interval was peaked at 5 min after dosing (12.5%, *hour*0.05) and started to fall after 5 min (Fig 3). In response to diltiazem, the HV interval recorded from the non-coronary cusp was unaltered. Conversely, the HV interval recorded from the right atrium was decreased dramatically after diltiazem was given for 5 min. Then, the HV interval was increased significantly at 10 and 15 min after dosing when compared to the baseline (*p*<0.05, Fig 4).
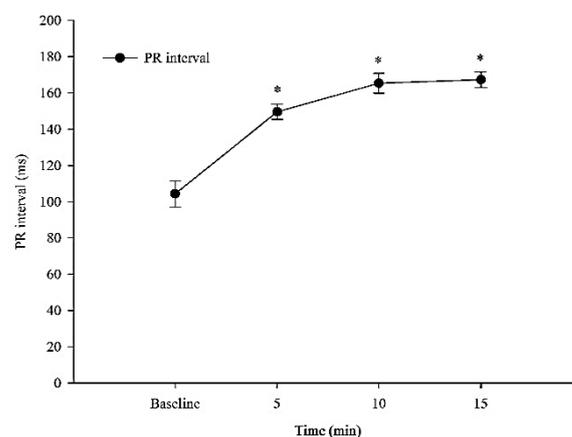

Figure 2 Effects of a bolus diltiazem followed by an observation period on PR interval in anesthetized male beagle dogs. Values are presented as mean ± SEM (n=6). *indicates *p*<0.05 when compared with baseline.
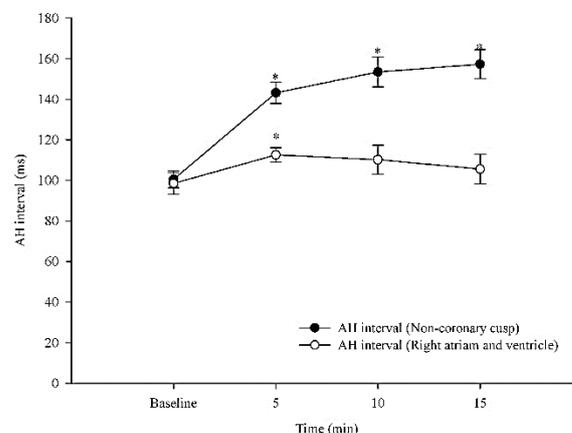

Figure 3 Effects of a bolus dose of diltiazem followed by an observation period on AH interval in anesthetized male beagle dogs. Values are presented as mean ± SEM (n=6). *indicates *p*<0.05 when compared with baseline of the same group.

Discussion

The anesthetic regimen used in this study was morphine and α-chloralose. In our experiment, this regimen produced a stable, physiologically immobilized and unconscious state. The result of this anesthetic preparation was consistent with previous studies in dogs. Holzgrefe and colleagues (1987) revealed that α-chloralose was a good anesthetic agent for cardiovascular studies because it preserved myocardial function and minimally depressed

baroreceptor function. Da Cunha and colleagues (2008) demonstrated that morphine-chloralose, when given repeatedly for 3 episodes, was safe and might be used for studies to evaluate torsadogenicity of test articles. However, there is disagreement in the literature about the use of α-chloralose as a sole anesthetic agent for major or painful minor surgical procedures (Rath et al., 1995).
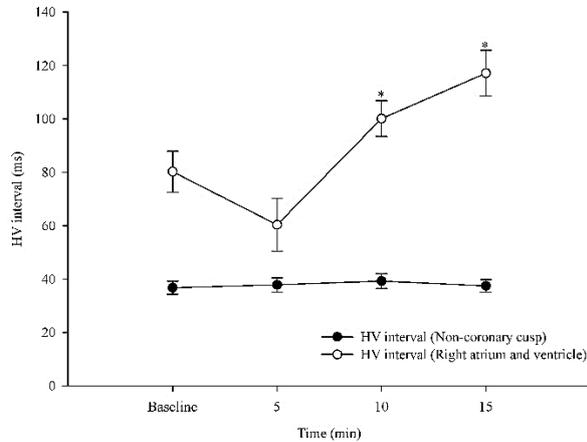

Figure 4   Effects of a bolus dose of diltiazem followed by an observation period on HV interval in anesthetized male beagle dogs. Values are presented as mean ± SEM (n=6). *indicates $p<0.05$ when compared with baseline of the same group.

It is important to obtain a His bundle electrogram that is stable, i.e. measured from the same location on the His bundle (Damato et. al., 1969a). When the His bundle is interrogated from the right side of the heart, the catheter and its electrodes move with each heartbeat and breath (Fig 5). For this reason, the left portion of the His bundle located in the septum

in contact with the non-coronary cusp was interrogated. With this method the tip of the catheter is wedged in the Valsalva sinus, and neither it nor the electrodes it contains can move. Therefore, the His bundle electrogram is always recorded from the same region of the conduction pathway.

The AH interval in anesthetized dogs recorded at either from the left or right side of the heart varies between 55 ms and 135 ms, whereas the HV interval varies between 28 ms and 52 ms (Hart et al., 1988; Traunecker, 1988; de La Coussaye et al., 1992; Sen et al., 2002). In our study, the AH and HV intervals recorded from either the right or left side of the heart were longer than previous studies. This could be due to either the difference in anesthetic used or the method of interrogation. It has been known that anesthetic drugs alter autonomic activity results in variation of heart rate. The inverse relationship between heart rate and AV conduction system has been reported previously (Carruthers et al., 1987). Therefore, comparison of these intervals among studies with different anesthetic regimens should be interpreted with caution.

To obtain a recording as accurate as possible, the beginning of the V wave is taken as the beginning of the high frequency component to the total V deflection. The AH interval is taken from the onset of the A wave to the beginning of the H wave. This recording methodology produces an AH slightly longer, and an HV slightly shorter, than when recording an electrogram from above the tricuspid valve (Fogoros, 2006). However, the dominant portion of AH is still due to slow propagation across the head of the AV node (reflecting autonomic efferent activity or an I_{CaL} effect), whereas the HV reflects rapid propagation from an infranodal position to the apical-third of the interventricular septum (reflecting His-Purkinje propagation and a predominant I_{Na} effect).
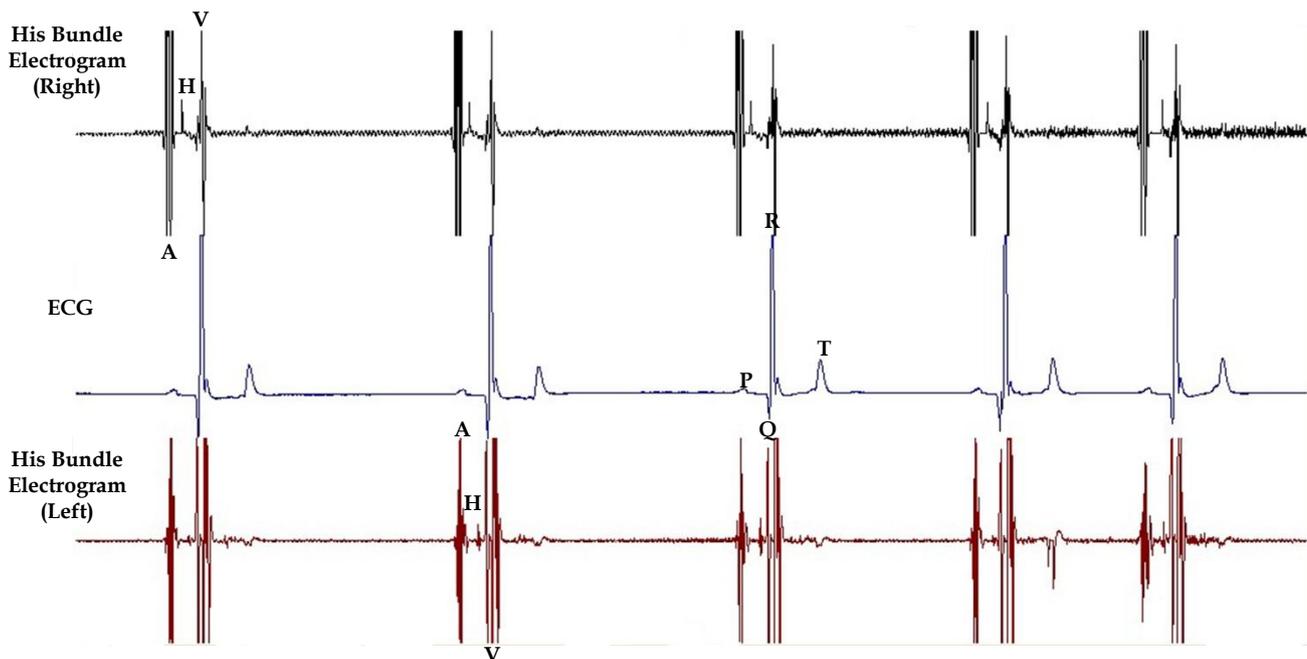

Figure 5   His bundle electrograms. Top trace is a His bundle electrogram recorded from the right side of the heart at the level of the His bundle (black line), middle trace is transthoracic electrograms (blue line), and bottom trace is a His bundle electrogram recorded from the left side of the heart at the level of the non-coronary cusp (brown line).

In the present study, the HBE recorded by two different locations provided inconsistent HV interval in response to diltiazem. The reliable data were obtained from the left side of the heart. Diltiazem, a voltage-gated, L-type calcium channel blocker, has been demonstrated in sodium pentobarbital anesthetized dogs to increase AH interval in a dose dependent manner without changing the HV interval (Nagao et al., 1981). In addition, a study in humans suggested that diltiazem depressed the intracardiac conduction of the AVN by inducing AH prolongation without an alteration in HV and RR intervals (Oyama et al., 1978). Thus, the AH and HV intervals recorded from the left side of the heart in our study was in agreement with other investigators recording HBE from humans. In conclusion, HBE recorded from the catheter placed in the non-coronary cusp is favorable since the duration of HBE is reliable from a catheter position that is fixed and wedged in the Valsalva sinus. As a consequence, the prolongation of the AH interval in response to diltiazem was observed.

Acknowledgements

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บทคัดย่อ

การประเมินค่าการนำไฟฟ้าภายในหัวใจจากการบันทึกคลื่นไฟฟ้าฮิสบันเดิล

ในสุนัขที่ได้รับการวางยาสลบ

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          ในการศึกษาความปลอดภัยและความเป็นพิษของยา ยาที่ผลิตใหม่ควรได้รับการประเมินการลดลงของการนำไฟฟ้าที่เอตริโอเวนตริคูลาร์โนด (atrioventricular node) โดยเฉพาะการนำไฟฟ้าภายในเอตริโอเวนตริคูลาร์โนด การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบตำแหน่งของการบันทึกคลื่นไฟฟ้าที่ฮิสบันเดิล (His bundle) หรือเอชบีอี (HBE) ระหว่างการบันทึกจาก electrode catheter ที่ตำแหน่งหัวใจห้องบนขวา และที่ตำแหน่ง non-coronary cusp และใช้ยาดิลไทอะเซม (diltiazem) ช่วยในการประเมิน สุนัขทั้งหมด 6 ตัวได้รับการวางยาสลบโดยใช้มอร์ฟีน (morphine) และ α-chloralose ทำการบันทึกเอชบีอีโดยใช้ multiple electrodes catheter สอดเข้าทางหลอดเลือดดำจูกูลาไปที่หัวใจห้องบนขวา และใช้ multiple electrodes catheter อีกอันสอนผ่านหลอดเลือดแดงฟีโมรอล (femoral) ที่ขาไปที่บริเวณ non-coronary cusp ทำการบันทึกคลื่นไฟฟ้าหัวใจหรืออีซีจีบนผิวหนังบริเวณนอก พารามิเตอร์ของอีซีจี (ECG) ประกอบด้วยอัตราการเต้นของหัวใจ ระยะพีอาร์ (PR interval) ระยะคิวอาร์เอส (QRS complex) และระยะคิวที (QT interval) และพารามิเตอร์ของเอชบีอีประกอบด้วยระยะเอเอช (AH interval) และระยะเอชวี (HV interval) ทำการบันทึกก่อนให้ยาและหลังให้ยาดิลไทอะเซมแบบ single bolus ขนาด 0.5 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม บันทึกผลของยาที่ 5 15 และ 30 นาที พบว่าระยะพีอาร์ยืดยาวออกอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) หลังจากได้รับยาดิลไทอะเซมเมื่อเทียบกับก่อนให้ยา การบันทึกเอชบีอีที่ตำแหน่ง non-coronary cusp พบว่าระยะเอเอชยืดยาวออกอย่างมีนัยสำคัญทางสถิติ ในขณะที่ระยะเอชวีไม่มีการเปลี่ยนแปลง เมื่อบันทึกเอชบีอีจากตำแหน่งหัวใจห้องบนขวาพบว่าระยะเอเอชและเอชวียืดยาวออก การบันทึกคลื่นไฟฟ้าฮิ สบันเดิลจากบริเวณ non-coronary cusp ให้ผลการทดลองที่ดีกว่าเนื่องจาก electrode catheter จะอยู่ในตำแหน่งที่คงที่ ทำให้สามารถบันทึกได้ว่าระยะเอเอชยืดยาวออกแต่ระยะเอชวีไม่เปลี่ยนแปลงจากการได้รับยาดิลไทอะเซม

คำสำคัญ: วางยาสลบ หัวใจ การนำไฟฟ้า ยาดิลไทอะเซม สุนัข เฮิสบัลเดิล

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