Development of an Experimental Method for Long-Term Electrocardiographic Recordings in a Heart Failure Rabbit Model

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Abstract

A method has been developed where the electrocardiogram (ECG) is monitored continuously using implantable monitors in a doxorubicin rabbit model of heart failure. The primary goal is to devise strategies to detect and analyze changes in the ECG as the disease develops. Doxorubicin was injected weekly in four rabbits at two different doses, 2mg/kg and 4mg/kg per week. Two control rabbits received saline injections. The ECG was continuously tracked and recorded (1000 samples per sec) for three control and up to ten treatment weeks. Data was produced 180 MB per ECG channel per day per rabbit, totaling around 200 GB for the study. Eleven classic heart rate variability (HRV) parameters were derived for every usable 5-minute segment for the entire period of the recordings. A new visualization technique was designed. The results graphically show changes in the circadian rhythm in treatment group with a general loss in circadian rhythm and decrease in HRV.

1. Introduction

One to two million adults are affected in the United States by heart failure with 200,000 deaths annually. In addition, 30-50% of these deaths are sudden and have attributed to ventricular tachyarrhythmias, bradycardia, and electro-mechanical dissociation [1,2]. Electro-cardiology plays a major role in prediction and prevention of heart failure and sudden cardiac death. Although much research has been performed to attempt to predict heart failure and sudden cardiac death, no long-term studies have tracked the electrocardiographic progression from normal to disease state. Longitudinal tracking of ECG changes could have impact on the understanding of the disease and the changes that occur over time as the disease develops.

Today's technological advances in high performance computers, miniature radio-transmitters, and affordability of massive data storage resources allow physiological monitoring efforts to extend to months or even years to track physiological changes in the long term. Despite these advances, little work of this type has been performed in the biomedical area to discover insight into physiologic mechanisms and disease. In addition, few tools have been developed and utilized to analyze and visualize massive amounts of times-series data. The difficulties encountered when collecting long-term, detailed physiological data sets is that the data sets are very large and complex and have the additional component of time. Methods for data handling and processing, detecting relevant and significant changes in physiological signals, analyzing and visualizing large data sets need to be established. The goal of this research is to develop strategies to detect and analyze changes in continuous electrophysiological measurements, collected in vivo, over extended periods of time (i.e., weeks to months).

A new experimental setup uses a doxorubicin rabbit model of heart failure which develops progressively over ten weeks. Animals are outfitted with a long-term implanted electrocardiogram (ECG) system. ECGs are recorded continuously through radiofrequency-telemetry for three weeks prior to treatment, over the course of the treatment, and for ten weeks post-treatment or death, whichever comes first. A significant to this work is the sheer mass of data. The experiment generated 180 megabytes per ECG channel per day per rabbit, totalling up to 200 gigabytes for the study reported in this paper. Eleven classical heart rate variability parameters were calculated before and during the development of heart disease, and a new visualization tool was devised to aid in data interpretation.

2. Methods

The experiment divided animals into two groups. Three female white New Zealand rabbits were used in each group where one is control and two are treatment animals. Doxorubicin was injected to treatment animals at 2mg/kg and 4mg/kg per week in groups one and two, respectively [3]. Control animals received saline injections. Animals were outfitted with implantable telemetry systems which recorded the electrocardiogram (Data Sciences Intl., St. Paul, MN.). The monitoring

system was designed to record signals for thirteen weeks, three control and ten treatment weeks, at sampling rate of 1000 Hz.

Implantable biopotential transmitters were placed subcutaneously in a pocket located on the scapulae (in the back) and wired ECG leads were tunnelled beneath the skin to specific positions. Four lead configurations were used. 1.) Lead II: ECG leads are placed at right upper chest and upper part of left thigh. 2.) MX: This is a modified lead position between manubrium sternum and xiphoid process. This configuration was better than Lead II during motion [4]. 3.) Orthogonal lead: This lead is located at sternum and center of the back. 4.) Intracardiac lead: A bipolar electrode lead is placed with fluoroscopy in the right side of the ventricle through the jugular vein. The intracardiac lead has benefits over the subcutaneous leads because the movement does not introduce interference to the sensing ECG. Details of lead configurations used in the experimental animals are shown in Table 1.

Table 1. ECG lead configurations used in rabbits and numbers of weeks after the first injection that ECG was recorded

Rabbit ID	# of weeks after 1st injection	ECG lead configurations			
		II	MX	Ortho-gonal	Intra-cardiac
R710V (C,G1)*	9	X			
R733W (T,G1)	9		X		
R735W (T,G1)	8		X		X
R929X (C,G2)	5		X	X	X
R926X (T,G2)	2		X	X	X
R928X (T,G2)	3		X	X	X

* C = Control animal, T = Treatment animal, G1 = Group 1 (2mg/kg dose), G2 = Group 2 (4mg/kg dose)

In group 1, the ECG was continuously recorded for the entire period of experiment, while in group two the ECG was recorded for only three days before injection day due to battery limitations with 3-channel devices. Table 1 shows the number of weeks that the ECG was recorded in each animal. R735, R926X, and R928X died spontaneously because of heart failure. R733W was euthanized after the 10th injection due to excessive pain and discomfort from heart failure. The control animals had no complications.

The monitored ECG is transmitted over modulated radiofrequency to a receiver which is connected to a local computer. The signal is sampled at 1000 Hz and uniformly digitized with resolution of 16 bits per sample. The ECG amplitude ranges from -10 mV to 10 mV. The data was generated at a rate of approximately 180 megabytes per channel every day. At the end of day, the data was automatically transferred from the local hard

drive to a data server over the network. Total amount of data transfer a day is approximately 750 and 1,700 megabytes for experiment group 1 and 2, respectively.

For the analysis, eleven heart rate variability parameters were computed. The computation was accomplished in several steps. The raw ECG was cleaned (removal of saturation and when receiver was out of range), and RR intervals were derived by using a beat detection program [5]. Then, an artifact rejection routine was applied to remove noise in the RR interval series [6]. For every useable 5-minute segment of RR intervals, the following time-domain and frequency-domain HRV parameters were calculated; mean (MEAN), median (MEDIAN), standard deviation (SDNN), coefficient of variance (CV), interquartile range (IQR), standard deviation of ΔRR (SDSD), interquartile range of ΔRR (DDIQR), root mean square of ΔRR (RMSSD), low frequency (0.0625 – 0.1875 Hz) power (LF), high frequency (0.4373 – 0.5625 Hz) power (HF), and ratio LF/HF (LHF) [7,8]. For a 24-hour period (starting from midnight), each HRV parameter has a vector of 288 elements.

3. Results

The calculated HRV parameters are time series which corresponds to progression from normal heart condition to heart failure. Research has shown that the development of disease is related to the changes of 24-hour pattern (circadian rhythm) of HRV [9,10]. A new visualization program was designed to aid in data interpretation and display changes of the HRV circadian pattern. There are three plot modes, image plot, contour plot, and 3-D plot. Figures 1, 2, and 3 show the image plots. The gray level of each palette is assigned according to the intensity indicator bar below the graph. The software has the capability of color plots also. The horizontal axis is time of day starting from midnight, whereas day is the vertical axis. The data is selected to display from first day (Day 1) of second control week (2 weeks before the first injection). The first injection is Day 15. Portions of missing data are displayed as white horizontal stripes. Figure 4 displays a contour plot, and a 3D plot is illustrated in Figure 5.

Figure 1 is an image plot of mean heart rate of 5-minute segments. The 24 hour pattern includes an increase in heart rate at approximate 6 am and 6 pm. They are related to time of switching lights on and off. During the first few days after the first saline injection (Days 15 to 18), heart rate increases, and then returns back to normal. Mean heart rate of R733W is displayed in Figure 2. The circadian pattern is established in control weeks and for approximately two weeks after the 1st injection followed by a decrease in variation of the pattern and a very small variation in the last two weeks. Similar phenomenon also occurs in other treatment rabbits. In
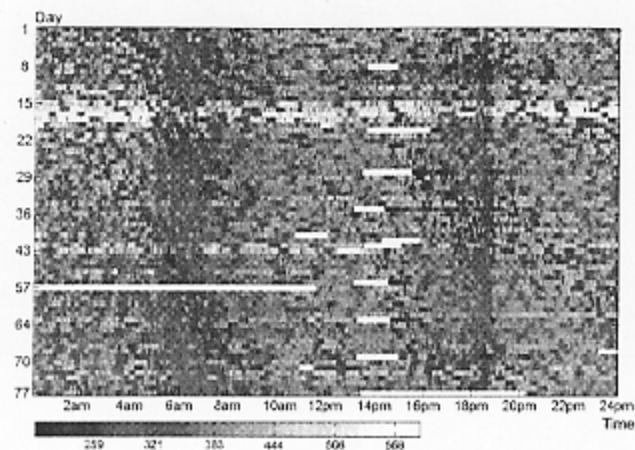
Figure 1. Image plot of MEAN of R710V: Gray level of each palette is assigned according to intensity indicator bar below the graph. Horizontal axis is time of day. Day is vertical axis. The plot starts at first day of second control week which is Day 1. There is a jump from Day 50 to 56 due to missing data. The white horizontal stripes indicate missing data.
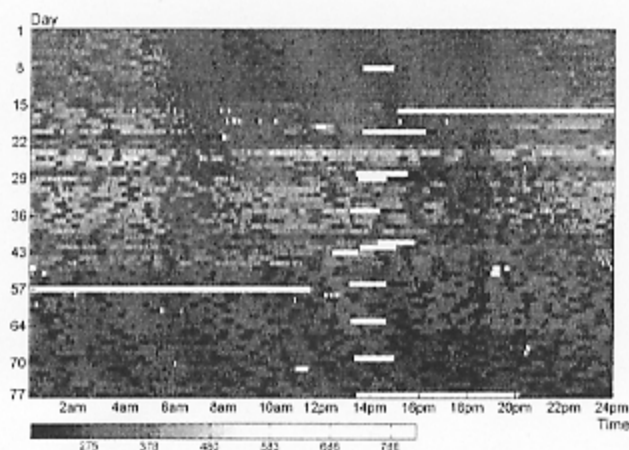

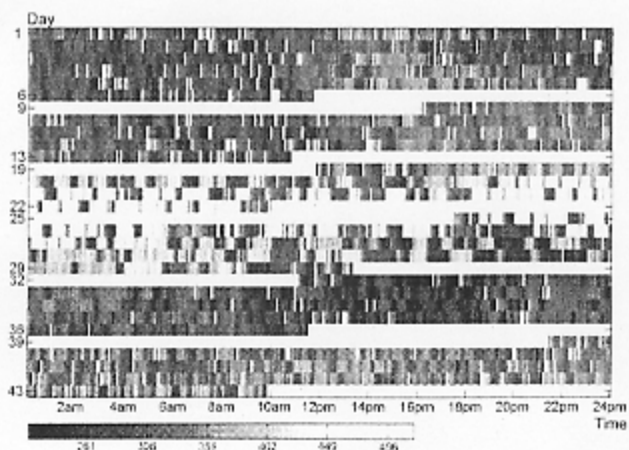
Figure 2. Image plot of MEAN of R733W

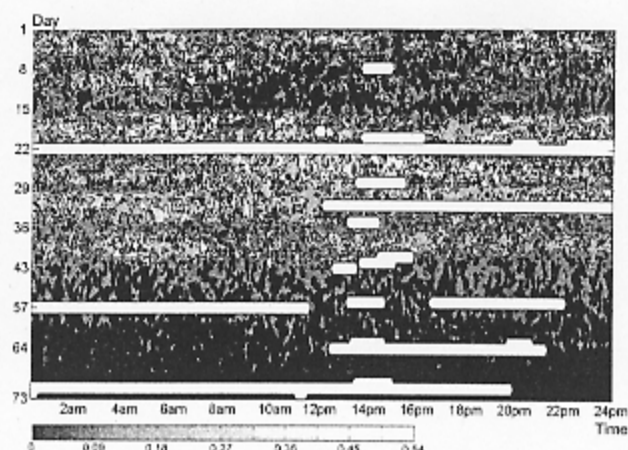Figure 3. Image plot of MEAN of R929X

Figure 4. Contour plot of CV of R735W: The plot shows noticeable changes occurring at 1st week and 6th week of injection. Value of CV is very low during last week before the rabbit died.
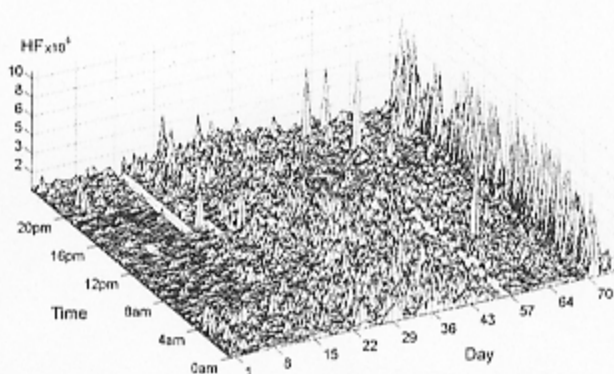

Figure 5. 3D plot of HF of R733W: Value of HF dramatically increases after 8th week of injection.
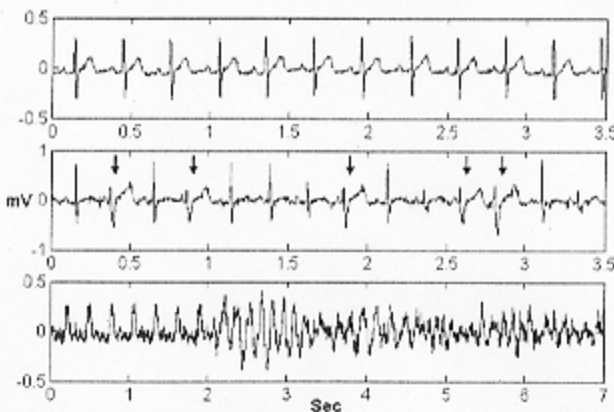

Figure 6. MX-lead ECG of R926X: Top is ECG before the 1st injection. Middle displays premature beats, indicated by arrows in the 2nd week of injection. Bottom shows ventricular fibrillation occurring 21minutes before the rabbit died. Note time scale differences.

335

addition, dramatic changes occur in the last week of the treatment rabbit as illustrated in Figures 2, 4, and 5. No statistical analysis was performed due to the limited dataset. It is unknown whether these trends will continue in larger studies. In Figure 3, no circadian pattern is established in R929X, a control rabbit from group 2, compared to control weeks of the other rabbits.

The ECG was scanned by use of an ECG template matching algorithm to find abnormal beats [11]. ECG morphology changes, premature beats, and bradycardia were found in all treatment rabbits. Ventricular fibrillation occurred in R926X after two weeks of injections shown in Figure 6. A 14-second pause occurred approximately 2 minutes prior to the VF passage. While spontaneous VF is interesting to note, it is unknown whether VF will be seen in future studies. Furthermore, with the 4mg/kg per week level, animals rapidly declined which gave limited information regarding progression of the disease.

4.   Conclusion

In this paper, a technique was described for a long-term recording of electrophysiological measurements collected in vivo in a rabbit model of heart failure. Doxorubicin, a cardiotoxicity substance, was administered to two groups of rabbits weekly on amount of 2mg/kg and 4mg/kg over ten weeks. The electrocardiogram was monitored for the entire experimental three-week control and ten-week treatment period. The challenge is the sheer mass of data, which is generated 180 megabytes per ECG channel per day per animal. A new visualization tool was devised and used to interpret derived heart rate variability parameters. The results show a decreasing variation in circadian pattern of heart rate and HRV in treatment rabbits. In addition, ECG morphology changes, premature beats, and bradycardia were found in all treatment rabbits, and one treatment rabbit had ventricular fibrillation after the second week of injection (4mg/kg per week). While conclusions cannot be drawn due to the limited dataset, feasibility for studies of this type has been demonstrated.

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