Journal of Animal and Veterinary Advances 11 (9): 1287-1289, 2012
ISSN: 1680-5593

Early Detection of Cardiac Injuries Related to Transcutaneous Pacing in Dogs Using Cardiac Biomarker Assay

[1, 2]Jong-Sung Lee, [2]Yewon Kim, [2]Suk-Hee Han and [2]Changbaig Hyun
[1]Wooree Animal Hospital, 201-100 Chuncheon, South Korea
[2]Section of Small Animal Internal Medicine,
School of Veterinary Medicine and Institute of Veterinary Medicine,
Kangwon National University, 201-100 Chuncheon, South Korea

Abstract: Evaluation of changes in concentrations of cardiac biomarkers and activities of enzymes in serum as indicators of cardiac injuries may be clinically important because the use of TCP is gaining popularity in the treatment of bradyarrhythmias in small animals. The purpose of the study reported here was to evaluate cardiac biomarker concentrations in serum in dogs with experimentally induced bradyarrhythmias after short duration (1 h) TCP. Marked elevation of serum concentration of cTnI was noticed immediately after TCP (day 0) compared with the baseline value. From day 0 through day 2, serum cTnI concentrations were significantly ($p<0.05$) increased. The expression level of NCX-1 using real-time PCR analysis was not significantly differed in blood samples taken from the different time point. Since, no study has evaluated biomarker levels after transcutaneous cardiac pacing this study may help to understand what kind and what type of cardiac injuries can be occurred.

Key words: Dogs, NCX-1, heart failure, TCP, biomarker, Korea

INTRODUCTION

Cardiac biomarker assays are alternative diagnostic method for detecting heart diseases in dogs. Since, these assays require tiny amount of blood, they are being used for, making a diagnosis of heart disease, screening of asymptomatic heart diseases, predicting prognosis of heart disease, monitoring therapeutic response and cardiotoxicity of some anticancer drugs and rapid differential diagnosis from respiratory diseases in emergency situation (Hyun and Lavulo, 2011). In this sense, many studies have been focused on evaluation of diagnostic and prognostic value of potential cardiac biomarkers. Although, the studies have mainly used circulating biochemical markers because of their practical usefulness, expression level of particular genes related to pathological process of heart failure have been widely studied to overcome limitations from available biochemical markers (Hyun and Lavulo, 2011).

Transcutaneous Cardiac Pacing (TCP) is a Noninvasive Temporary Method for restoring normal heart contractions in humans and animals with abnormally slow heart rates (Zoll *et al*., 1981). Transcutaneous cardiac pacing is relatively easy to perform and only requires minimal training therefore, the procedure is commonly used for the emergency treatment of dogs with a high risk of bradycardiac rhythm disturbances (high-grade heart block, sick sinus syndrome or vasovagal syncope; Zoll *et al*., 1981).

Evaluation of changes in concentrations of cardiac biomarkers and activities of enzymes in serum as indicators of cardiac injuries may be clinically important because the use of TCP is gaining popularity in the treatment of bradyarrhythmias in small animals (Lee *et al*., 2009). The purpose of the study reported here was to evaluate cardiac biomarker concentrations in serum in dogs with experimentally induced bradyarrhythmias after short duration (1 h) TCP.

MATERIALS AND METHODS

Study population: Prior to this study, researchers obtained the approval of the Animal Ethics Committee of Kangwon National University. Ten healthy Beagles that had no evident abnormalities in thoracic cavity conformation were selected for the study. The mean±SD weight of the dogs was 9.24±0.71 kg; there were 6 females and 4 males (sexually intact). Each dog was considered healthy on the

Corresponding Author: Changbaig Hyun, Section of Small Animal Internal Medicine,
School of Veterinary Medicine and Institute of Veterinary Medicine, Kangwon National University,
201-100 Chuncheon, South Korea

basis of results of a CBC and serum biochemical analyses (including assessment of variables indicative of hepatic and cardiac function). Each dog was examined for pre-existing cardiac diseases via 12-lead ECG, thoracic radiography and 2-dimensional (with M-mode) echocardiography. This animal testing programs including animal care, euthanasia and disposal of dead animals was strictly adhered to in accordance to the guidelines of the National Research Council of Korea.

Anesthesia and artificial ventilation: The dogs were weighed prior to the commencement of the study. The dogs were initially anesthetized with an intravenous injection of 8 mg kg^{-1} propofol (Fresofol, Boryung Pharm, Seoul, Korea) titrated to effect. Following endotracheal intubation, the anesthesia was maintained by isoflurane (Forane, Sampoong, Seoul, Korea; 2% concentration) in 100% oxygen as carrier gas. The dogs were mechanically ventilated at a rate of 20-30 times min^{-1} using a volume-cycled respirator (MDS Matrix 3000, Hallowell, USA) in order to maintain eucapnea. End-tidal PCO_2 and SpO_2 were monitored throughout the experiment with a multi-parameter patient monitor (VP-700®, Votem Korea, Chuncheon, Korea).

Induction of bradyarrhythmia: To induce bradya-rrhythmia, diltiazem (Herben, CJ Corp, Seoul, Korea; 20-50 mg/dog) was administered via a cephalic vein in each dog. Bradyarrhythmia (identified by heart rate, <30 beats min^{-1} and ECG detection of sinus node exit block, P-R interval prolongation and atrial standstill) or cardiac arrest was confirmed by use of a continuous digital ECG monitor (PH-1, CU-medical systems, Korea).

Protocol for transcutaneous cardiac pacing: An automated external cardiac pulse generator (Orange-1, B400, Mediana Co., Ltd. Korea) and a transdermal electrode (Mediana Co., Ltd. Korea) were used for TCP (Fig. 1). Five dogs underwent TCP for 1 h (short duration treatment). For each dog, TCP involved application of 5 mA of pacing current/kg of body weight (with 20 msec of pulse duration) via transdermal electrodes (surface area, 40 cm^2) in the left apex-right apex positions (electrode placement over the costochondral junctions of the fourth to seventh ribs on the left and right sides, respectively) at a heart rate of 120 beats min^{-1}; this rate was selected because the mean±SD heart rate among the study dogs was 114.4±12.4 beats min^{-1}. The success of the TCP was confirmed by the presence of spike impulses detected via ECG, the continuous formation of pressure waveforms confirmed viaaortic pressure measurements and left ventricular contraction detected via M-mode

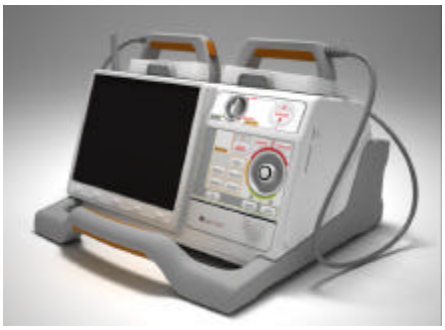

Fig. 1: Transcutaneous cardiac pacemaker (Orange-1, Mediana Co., Ltd. Korea) used in this study

echocardiography. To evaluate detrimental effects associated with short duration TCP, serum concentrations of cardiac biomarkers (cTnI and NCX-1) were measured on the day prior to TCP (baseline; day 1), immediately after TCP (day 0) and at 1, 2, 3, 5 and 7 days after TCP.

Assays for cardiac biomarkers: From each dog, blood samples (2 mL each) were collected via a jugular vein on day 1, on day 0 (immediately after short or long duration TCP) and on days 1, 2, 3, 5 and 7. Samples were immediately placed in a serum separation tube and centrifuged for the separation of serum. Serum cTnI concentration was measured by use of a second-generation cTnl assay (Canine Troponin I ELISA kit Wuhan EIAab Science Co., Ltd. China). Levels of NCX-1 mRNA expression were detected by described in Moon *et al*. (2008).

Statistical analysis: Statistical analysis was performed by use of a two way ANOVA with treatment group as a between animal factor and time as a within animal repeated measures factor to determine whether significant differences existed in cardiac biomarker concentrations over time between groups. When appropriate, a post hoc multiple comparisons was applied between or within groups by use of the Bonferroni correction to ensure an experimental error rate at $\alpha<0.05$. Within and between group comparisons were performed for the baseline values (day 1) and for data at each subsequent time point, respectively. All statistical analyses were performed by use of statistical computer software (SPSS, USA), a value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Marked elevation of serum concentration of cTnI was noticed immediately after TCP (day 0) compared with the baseline value (Fig. 2a). From day 0 through day 2, serum

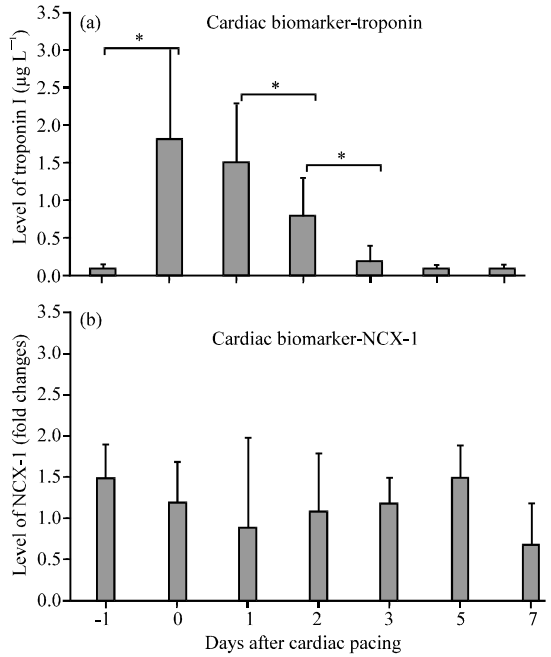

Fig. 2: a) Changes in the level of troponin I and b) NCX-1[$] after the short duration (1 h) and long-duration (3 h) cardiac pacing: -1 = 1 day before cardiac pacing, 0 = immediately after cardiac pacing, 1= 1 day after cardiac pacing; [$]Fold change of each group was calculated by $2^{-\Delta\Delta Ct}$ Method; *A difference was considered significant at a value of $p<0.05$

cTnI concentrations were significantly ($p<0.05$) increased. The expression level of NCX-1 using real-time PCR analysis was not significantly differed in blood samples taken from the different time point (Fig. 2b).

Accurate staging and monitoring methods for HF are crucial for making a correct diagnosis and for setting up tailor-made therapeutic strategy of HF in small animals. Although, the advance in diagnostic imaging technologies makes feasible the correct diagnosis of HF, these technologies have still many limitations for general veterinary practitioners since, they require training in cardiology and expensive equipments. This study was conducted to determine whether early detection of cardiac injuries is possible by means of two cardiac biomarker assays reflecting different cardiac pathologies (troponin I for myocardiacl damage and NCX-1 for myocardial contractivility).

Cardiac troponin I is a cardiospecific biomarker and changes in circulating concentration of this protein reflect various cardiac injuries. Assessment of serum cTnI concentration is widely used for detection of acute myocardial infarction in humans (Hyun and Lavulo, 2011). Serum cTnI concentration was increased in dogs with TCP but the elevation lasted only for 48 h after injury. The findings of the present study clearly suggest that Troponin I test can detect early stage of cardiac injury and the severity of myocardial damage may be correlated with the duration of TCP. Increased expression patterns of NCX-1 in blood cells have been well documented in human with HF and dogs with CMVI (Moon *et al*., 2008; Nam *et al*., 2010).

CONCLUSION

In this study, researchers found that the expression levels of NCX-1 were not significantly different from the different time point, unlike other biomarker such as troponin, probably because myocardial contractibility may not be affected from the short tem of cardiac pacing. Since, no study has evaluated biomarker levels after transcutaneous cardiac pacing this study may help to understand what kind and what type of cardiac injuries can be occurred. Further study should be directed to unveil different type of cardiac pathology using different type of cardiac biomarkers reflecting different aspect of cardiac pathology.

ACKNOWLEDGEMENT

This study was supported by research grant from Kangwon National University.

REFERENCES

Hyun, C. and L. Lavulo, 2011. Calcium related genes in dogs as potential cardiac biomarkers for the detection of chronic mitral valve disease. Recent Pat Biomark, 1: 68-80.

Lee, S., S.I. Pak and C. Hyun, 2009. Evaluation of changes in cardiac biomarker concentrations and enzyme activities in serum after short- and long-duration transcutaneous cardiac pacing in dogs. Am. J. Vet. Res., 70: 599-603.

Moon, H.S., E. Choi and C. Hyun, 2008. The cardiac sodium-calcium exchanger gene (*NCX-1*) is a potential canine cardiac biomarker of chronic mitral valvular insufficiency. J. Vet. Int. Med., 22: 1360-1365.

Nam, S.J., S.H. Han, H.W. Kim and C. Hyun, 2010. The cardiac biomarker sodium-calcium exchanger (*NCX-1*) can differentiate between heart failure and renal failure: A comparative study of *NCX-1* expression in dogs with chronic mitral valvular insufficiency and azotemia. J. Vet. Int. Med., 24: 1383-1387.

Zoll, P.M., R.H. Zoll and A.H. Belgard, 1981. External noninvasive electric stimulation of the heart. Crit. Care Med., 9: 393-394.