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Original Article

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Abstract

The objective of the study was to evaluate the effects of doxorubicin (DOX) at the dose used in clinical practice (12-25 mg/m^2 body surface area) on cardiac electrophysiology and proteinuria. Nine dogs with tumors were treated with 1st dose of DOX while 6 and 3 dogs were left for 2nd and 3rd doses, respectively. Measurements of blood chemistries, blood pressure, electrocardiography, heart rate variability and proteinuria were performed after the 1st, 2nd and 3rd doses of DOX. All data were obtained the day just before the next dose was introduced. Results showed that giving 1 to 3 doses of DOX did not alter serum CPK or AST. However, the BUN increased at the 3rd dose along with significant reduction in plasma albumin (p<0.05). The blood pressures decreased significantly (p<0.05) without heart rate acceleration at the 2nd dose, indicating impaired autonomic response. Electrocardiography demonstrated significant prolonged QTc (p<0.05) at the 2nd dose. Analysis of frequency domain power spectrum of heart rate variability showed increased low frequency (LF) and high frequency (HF) ratio (LF/HF) which suggested the enhancement of sympathetic over parasympathetic. Although proteinuria, which was mainly albumin, was minimal, it showed a dramatic increase as seen after the 1st dose of DOX treatment. These results suggest that routine screening using electrocardiography and proteinuria should be used for monitoring the cardiac and renal toxicities of DOX in dogs during anti-tumor treatment.

Keywords: dogs, doxorubicin, heart rate variability, proteinuria, QTc

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Introduction

Doxorubicin (DOX) is an anthracycline antibiotic and chemotherapeutic agent which is widely used in veterinary medicine for treatment of a variety of cancers such as lymphoma, osteosarcoma, mammary gland carcinoma, hemangiosarcoma, etc. (Allen et al., 2005). Dose was varied between 15-30 mg/kg every 2-3 weeks duration (Argyle et al., 2008). However, the adverse effects of this drug were reported involving abnormality of both electrical and mechanical properties of myocardial tissues (Chatterjee et al., 2009). Development of cardiomyopathy after DOX treatment once developed coincides with poor outcome and fatality (Chatterjee et al., 2009). Thus, understanding the pathophysiology of DOX induced cardiomyopathy and searching the preventive treatments are the main issue. Mauldin and coworker (1992) studied in clinical dogs with cancer receiving doxorubicin and found that 31 of 175 dogs developed electrocardiographic abnormalities including arrhythmias, abnormal R wave, ST segment or QRS duration. Seven dogs had congestive heart failure and died within 90 d. Some of them had arrhythmia and some died within 90 d from congestive heart failure.

Changes in electrocardiogram (ECG) especially in QT duration and QT variability were reported in both human patients (Ritsema van Eck et al., 2009) and dogs (Ducroq et al., 2010). Changes of these parameters were used to predict and prevent the drug-induced Torsades de Points (Thomsen et al., 2006). Systolic and diastolic dysfunctions involve cardiac autonomic nervous system activities. Besides the QT variability, the cardiac autonomic functions can be evaluated by measurement of time domain and frequency domain analysis of heart rate variability using non-invasive technique continuous ECG recording with holter device (Task Force, 1996; Sztajzel, 2004). SDNN, SDANN and SDNN index of time domain were changed corresponding to change in sympathetic, while pNN50 and RMSSD indicated parasympathetic (Calvert, 1998). The LF indicates both sympathetic and parasympathetic, while HF indicates mainly parasympathetic activity (Task Force, 1996; Calvert, 1998; Sztajzel, 2004). Alterations in heart rate variability were demonstrated in dogs with tachycardia induced heart failure (Piccirillo et al., 2010) and in asymptomatic patients receiving anthracycline induced cardiotoxicity (Tjeerdsma et al., 1999). However, the study in clinical dogs receiving DOX therapy has not been elucidated.

Besides the heart, doxorubicin causes renal impairment with increased urinary protein excretion known as nephrotic syndrome. Proteinuria in this model could not be alleviated using carnitine (Boonsanit et al., 2006), angiotensin converting enzyme inhibitor (Hall et al., 1986) or sodium bicarbonate (Galli et al., 2001). Glomerular epithelial cell structure was changes leading to glomerulosclerosis and tubulointerstitial fibrosis (Okuda et al., 1986; Wang et al. 2000; Lee and Harris, 2011). The proteinuria was due to alterations in glomerular charge and size selectivity (Skutelsky et al., 1995; Jeansson et al., 2009). However, there is no report regarding the proteinuric effect and its relation to renal impairment in clinical dogs.

The aims of the present study were to investigate changes in electrophysiology, heart rate variability and renal function in clinical dogs with cancers treated with cumulative doses of doxorubicin.

Materials and Methods

The protocol was approved by the Ethics Committee for Using and Care of Animals, Faculty of Veterinary Science, Chulalongkorn University. The study was performed by investigating dogs that came to the Oncology Clinic of Small Animal Teaching Hospital, Chulalongkorn University from August, 2011 untill September, 2012 for treatment of cancer with protocol recruited with doxorubicin. All animals did not have previous history of heart or kidney diseases. All dogs were physically examined by veterinarians and their signalments and medical history were recorded. The dogs were received 1 to 3 administration of standard therapeutic dose of doxorubicin (12-25 mg/m^2 body surface area) intravenously either alone or in combination with other chemotherapeutic agents. Dogs with lymphoma received other chemotherapeutic agents as mention for CHOP protocol (containing Cyclophosphamide, Hydroxy-doxorubicin, Oncovin® or vincristine and Prednisolone). Otherwise, DOX was given as single chemotherapeutic agent. The interval of doxorubicin treatment was between 14 and 63 d depending on the treatment protocol.

The study was investigated on the day the animals came to the Oncology Clinic prior to the 1st, 2nd, 3rd and 4th doses of doxorubicin treatment corresponding to data for baseline, 1st, 2nd and 3rd doses after doxorubicin injection, respectively. Indirect blood pressure was measured when the animal was quiet. Half ml of blood was collected in EDTA tube to measure complete blood count while another 1.5 ml of blood was collected in heparinized tubes to determine blood chemistry profiles. At least 3 ml of urine was collected to perform routine urinalysis and measure urinary protein creatinine ratio. Scalar ECG was recorded for calculating QT variability while a holter device was connected for at least 2 h thereafter for determining short-term heart rate variability.

Analytical procedure: Blood pressure was measured using an oscillometric device (PetiTelemo, Fukuda Denshi, Co, LTD., Japan). A pressure cuff of appropriate width (approximately 40% of the leg's circumference) was placed upon the median artery between the elbow and the carpal pad. Three consecutive readings were performed and averaged.

The complete blood count was analyzed using a hematology analyzer (CELL-DYN 3700, Abbott, USA) while plasma concentrations of creatinine, blood urea nitrogen, albumin and enzyme activities of creatine phosphokinase (CPK) and aspartate aminotransferase (AST) were analyzed by an automate analyzer (I-Lab 650, Icort, England). The protein was analyzed by SDS-page electrophoresis

while the creatinine was measured using the automate analyzer.

Urine SDS page electrophoresis was run on 12.5% resolving gel and 5% stacking gel ran on a mini protein II Tetra Cell (Bio-rad, NY, USA). Standard protein markers with molecular weight from 10-170 kDa (Strep-tag, Thermo-Fisher Scientific, Miami, USA) were used along with bovine serum albumin (66 kDa). The amount of protein loaded was 2 μg per sample. The gel was stained with Coomassie brilliant blue R-250 Staining Solution Kit (Bio-Rad, NY, USA) and scanned with gel scanner (Visioneer OneTouch 7100, CA, USA). The bands were analyzed and calculated using ImageJ software. Total protein and albumin fractions were expressed as urinary albumin and urinary protein creatinine ratios.

The scalar ECG was recorded by an ECG machine (KENZ® ECG 110) in all animals for at least 31 consecutive beats in order to measure standard duration and amplitude of atrial and ventricular vectors. QT interval was corrected for changes in heart rate by conversion to corrected QT (QTc) interval using the formula of Fridericia (Fridericia, 1920). The QT variability was calculated using Thomsen's equation by measuring the QT interval for 30 consecutive beats (Thomsen et al., 2006).

Continuous ECG recording was performed using a holter device (DigitalWalk FM-180, Fukuda Denshi, Co, Ltd., Japan). Data from three consecutive 10 -minute short- term ECG recording were analyzed and averaged for heart rate variability both time and frequency domain using fast Fourier analysis by computer software (SCM 510).

Statistical analysis: Data were presented as mean ± standard error of mean. Data obtained after the 1st, 2nd and 3rd doses of doxorubicin treatments were compared with the baseline before drug administration using Student paired *t*-test or Wilcoxon signed rank test. Significances were considered when P- values were less than 0.05.

Results

The characteristic of dogs in this study is shown in Table 1. Nine dogs were included in this study consisting of 2 castrate males, 4 intact females and 3 spayed females. The mean age was 9.44 ± 0.50 (range 7-11) years of age while the weight was 9.10 ± 2.92 (range 3.4-31.7) kg. The breeds included 4 Shih-Tzus, 3 Poodles, 1 Pomeranian and 1 Golden Retriever. Considering the type of tumors, four were lymphoma, four were mammary gland tumor and one was renal carcinoma. The mammary gland tumors in all dogs were surgically removed 14-49 d prior to doxorubicin treatment. However, no surgical removal of the kidney was performed in the dog with renal carcinoma. The dog with renal carcinoma had normal concentrations of BUN (15 mg/dl) and creatinine (0.8 mg/dl) without proteinuria prior to DOX treatment. The dogs with mammary gland tumors were mostly subjected to chemotherapy with DOX alone while dogs with lymphoma received a combination of chemotherapeutic drugs including vincristine, cyclophosphamide and prednisolone as in CHOP protocol. Most of the dogs received 3 to 4 injections of vincristine prior to DOX treatment. All dogs received antioxidants such as vitamin C, curcumin or CoQ10 throughout the study period, while antibiotics were prescribed periodically.

Among the 9 dogs, 6 dogs received the 2nd doxorubicin injections while only 3 dogs received the 3rd doses. The doses of doxorubicin were between 12-25 mg/m^2 body surface area. The first injective dose was 17.9 ± 1.3, while the 2nd and 3rd doses were 17.1 ± 1.3 and 16.8 ± 1.8 mg/m^2 body surface area, respectively. The mean cumulative doses of DOX for the 2nd and 3rd doses were 34.54 ± 2.20 and 50.56 ± 3.65 mg/m^2 body surface area, respectively. The duration between DOX treatments varied depending upon the type of tumors. The duration between treatment in the dogs with mammary gland tumor and renal carcinoma was 21 d, while the dogs with lymphoma had higher duration between 35 to 63 d apart. Most of the dogs received antioxidants such as vitamin C, curcumin or CoQ10 throughout the study.

The packed cell volume, white blood cell count, and blood chemistries are demonstrated in Table 2. No remarkable changes in all parameters were seen except a significant reduction in plasma albumin (p<0.05) after the 3rd dose (Fig 1). The blood urea nitrogen increased almost twice after the 3rd doses (Fig 2).

Changes in blood pressure are shown in figure 3. The baseline of systolic, diastolic and mean pressure in 6 dogs were 130.2 ± 2.5, 90.7 ± 6.6 and 102.5 ± 5.3 mmHg, respectively, However, they fell significantly to 112.7 ± 3.9 (p<0.01), 54.5 ± 4.2 (p<0.05) and 73.5 ± 3.2 (p<0.05), respectively, after they received the 2nd dose of doxorubicin. The reductions of all pressures were still persisted in 3 dogs after the 3rd doses of doxorubicin although they were not significant. The heart rates in beats per minute were unaltered compared with the baseline at the 1st (126 ± 7 vs 128 ± 8), the 2nd (127 ± 3 vs 132 ± 10) and the 3rd (137 ± 9 vs 135 ± 13) doses.

For the scalar ECG measurements, the P duration and height, QRS complex duration and height, PR interval and heart rate were unaltered (Table 3). However, the QTc were lengthened after the 1st, 2nd and 3rd doses of doxorubicin injection (Fig 4). A significance was found after the 2nd drug treatment (p<0.05). The QT variability tended to increase in all doses of doxorubicin treatment.

For the analysis of both time and frequency domains of heart rate variability, no significant differences were found in all parameters after the treatment. However, the LF norm increased by 18%, 17% and 28%, while HF norm decreased by 12%, 11% and 24% in the 1st, 2nd and 3rd doses of doxorubicin treatment, respectively, leading to 38%, 36% and 78% higher LF/HF (Table 4).

Almost all beats were normal beats which was originated from sinus node. Before the doxorubicin treatment, only one dog had single ventricular premature complex (VPC) and another dog had single atrial premature complex (APC) within 30 min. After the 1st dose of doxorubicin ectopic beats

Table 1 Characteristics of dogs in this study

	No	Breed	Sex (M/Mc/F/Fs)	Age (years)	Tumor type	Wt (Kg)	Number of DOX	DOX Interval (Days)	Dose (mg/m² BSA)	Protocol
1	Saengla	Shih-Tzu	Fs	8	Mammary gland tumor	7	1		16.6	
2	Darky	Shih-Tzu	Fs	10	Mixed mammary gland tumor	5	2	21	17.2	
3	Tauwlek	Shih-Tzu	F	10	Renal carcinoma	6.6	3	22	13.7-17.1	
4	Cookie	Shih-Tzu	Mc	9	Lymphoma	7	3	40	13.9-19.4	CHOP
5	Somtum	Poodle	F	7	Mammary gland tumor	3.6	1		15	
6	Lala	Poodle	F	12	Lymphoma	4	2	35	12-16	CHOP
7	Rodtank	Poodle	Fs	12	Multicentric Lymphoma	10.6	2	35	21.7	CHOP
8	Doctor	Pomeranian	Mc	9	Lymphoma	6.6	3	63	17.1-20	CHOP
9	PomPom	Golden Retriever	F	11	Mammary gland tumor	31.7	1		25	

M = male; Mc = castrated male; F = female; Fs = sterile female; wt = weight; CHOP = cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone; mg = milligrams; m^2 = square meters; BSA = body surface area; DOX = doxorubicin

Table 2 Complete blood count and blood chemistries in dogs after different doses of doxorubicin injection

Parameters	Baseline (n=9)	Dox1 (n=9)	Baseline (n=6)	Dox2 (n=6)	Baseline (n=3)	Dox3 (n=3)
PCV (%)	44.89 ± 2.68	44.00 ± 2.63	41.00 ± 2.37	41.00 ± 2.22	40.33 ± 4.67	40.00 ± 2.00
WBC($x10^3$cell/mm^3)	10.87 ± 2.40	10.23 ± 1.20	11.82 ± 3.45	11.42 ± 0.96	14.69 ± 7.09	11.03 ± 0.39
CPK (IU/L)	108.6 ± 25.5	79.78±11.77	122.3 ± 37.9	118.7±31.8	118.3 ± 36.4	93.33±40.54
AST (IU/L)	25.89 ± 7.30	26.11±4.87	29.33 ± 10.97	37.17±13.75	19.67 ± 6.69	22.33±6.44
BUN (mg/dl)	16.88 ± 2.09	21.02± 3.47	16.82 ± 2.09	21.33±3.01	18.00 ± 3.22	30.67±5.49
Creatinine (mg/dl)	0.878 ± 0.094	0.833 ±0.055	0.967 ± 0.126	0.833 ± 0.062	1.067 ± 0.233	1.100 ± 0.153
Albumin (g/dl)	3.478 ± 0.131	3.467± 0.112	3.333 ± 0.123	3.433± 0.126	3.433 ± 0.133	2.967± 0.219*

Data are expressed as mean ± SEM.
Comparison of each injection with baseline was performed using Student paired t-test or Wilcoxon signed rank test. * = $p<0.05$
Abbreviations: PCV = Packed cell volume; WBC = white blood cell count; CPK = creatinine phosphokinase; AST = Aspartate transminase; BUN = blood urea nitrogen; mg = milligrams; dl = deciliters; g = grams; mm^3 = cubic millimeter; Dox = doxorubicin

Table 3 Electrocardigraphic parameters in dogs before and after DOX treatments

Parameters	Baseline (n=9)	Doxo1 (n=9)	Baseline (n=6)	Doxo2 (n=6)	Baseline (n=3)	Doxo3 (n=3)
P duration (sec)	0.039 ± 0.005	0.052 ± 0.008	0.046 ± 0.005	0.048 ± 0.005	0.038 ± 0.004	0.039 ± 0.001
P height (mV)	0.279 ± 0.044	0.288 ± 0.038	0.318 ± 0.359	0.251 ± 0.040*	0.328 ± 0.109	0.349 ± 0.019
QRScomplex (sec)	0.064 ± 0.004	0.068 ± 0.003	0.063 ± 0.006	0.064 ± 0.004	0.064 ± 0.005	0.059 ± 0.007
QRS height (mV)	1.567 ± 0.257	1.599 ± 0.207	1.656 ± 0.296	1.608 ± 0.255	2.065 ± 0.452	1.894 ± 0.380
PR interval (sec)	0.083 ± 0.003	0.089 ± 0.008	0.083 ± 0.003	0.086 ± 0.004	0.084 ± 0.004	0.087 ± 0.002
QT interval (sec)	0.184 ± 0.006	0.191 ± 0.005	0.186 ± 0.008	0.207 ± 0.012*	0.181 ± 0.015	0.192 ± 0.015
QTc (sec)	0.229 ± 0.004	0.235 ± 0.006	0.232 ± 0.006	0.253 ± 0.012*	0.229 ± 0.018	0.240 ± 0.017
QT variability	1.992 ± 0.314	2.474 ± 0.602	2.017 ± 0.428	2.750 ± 0.580	2.513 ± 0.467	2.713 ± 0.258
RR interval (sec)	0.481 ± 0.029	0.463 ± 0.018	0.465 ± 0.035	0.418 ± 0.040	0.453 ± 0.047	0.399 ± 0.007
Heart rate (bpm)	128 ± 8	126 ± 7	132 ± 10	127 ± 2.629	135 ± 13	137 ± 8.9

Data are expressed as mean ± SE.
Abbreviations: QTc = corrected QT values

Table 4 Frequency and time domains parameters of heart rate variability before and after doxorubicin treatment

Parameters	Baseline (n=9)	Dox1 (n=9)	Baseline (n=6)	Dox2 (n=6)	Baseline (n=3)	Dox3 (n=3)
LF (ms^2)	2322 ± 450	1775 ± 242	2398 ± 608	6064 ± 4316	1943 ± 721	580.8 ± 243.9
HF (ms^2)	4650 ± 1129	4072 ± 2105	4777 ± 1494	9969 ± 7610	3179 ± 1308	577.7 ± 360.8
TF (ms^2)	12197 ± 2344	10410 ± 3318	12804 ± 3093	22496 ± 14256	11532 ± 5060	2006 ± 929
LF/HF	0.821 ± 0.202	1.123 ± 0.190	0.670 ± 0.064	0.903 ± 0.151	0.748 ± 0.087	1.323 ± 0.509
VLF (ms^2)	3215 ± 930	2458 ± 518	3442 ± 1397	4982 ± 2114	4446 ± 2865	556.5 ± 233.4
ULF (ms^2)	2011 ± 320	2105 ± 635	2188 ± 235	1481 ± 352	1964 ± 453	291.3 ± 181.7
LF norm. (%)	40.56 ± 3.96	47.58 ± 4.88	38.62 ± 2.25	45.15 ± 3.99	41.45 ± 2.50	52.73 ± 9.09
HF norm. (%)	59.44 ± 3.96	52.42 ± 4.88	61.38 ± 2.25	54.85 ± 3.99	58.55 ± 2.49	47.27 ± 9.09
SDNN (msec)	123.3 ± 21.0	120.6 ± 20.3	136.2 ± 29.1	142.7 ± 46.2	130.3 ± 56.6	48.00 ± 6.66
SDANN (msec)	26.20 ± 7.43	44.55 ± 11.71	32.18 ± 10.41	30.32 ± 11.00	15.88 ± 4.88	9.515 ± 5.293
SDNN index (msec)	101.2 ± 12.5	96.22 ± 14.89	108.1 ± 16.3	119.6 ± 37.8	99.89 ± 27.14	39.56 ± 10.88
pNN50 (%)	34.66 ± 7.89	26.62 ± 7.25	37.46 ± 9.03	32.41 ± 11.43	28.77 ± 12.65	2.912 ± 1.481

Data are expressed as mean ± SE.

Abbreviations: LF= low frequency; HF = high frequency; TF = total frequency; VLF = very low frequency; ULF= ultra low frequency; LF norm = low frequency in normalized units; HF norm = high frequency in normalized units; SDNN = standard deviation of the normal-to-normal interval; SDANN = standard deviation of the averages of NN intervals in all 5 min segments of the entire recording; pNN50 = NN50 count divided by the total number of all NN intervals

were found with higher frequency. Three dogs had VPC of 7, 8 and 2 beats. The dog with 2 VPCs also had 3 APCs. However, the ectopic beats seemed to be less after the 2nd and the 3rd dose. After the 2nd dose, a single VPC and single APC were found in 1 dog and 2 dogs, respectively. After the 3rd dose, one dog had a single APC while another one had 3 VPCs and 1 APC. The incidences of VPC and APC was less than 0.2% compared with normal beats in all dogs after the doxorubicin injections.

The urine specific gravity was unchanged while urinary albumin and urinary total protein creatinine ratios increased dramatically. However, they were not significantly different and fell within normal limits (Fig 5).

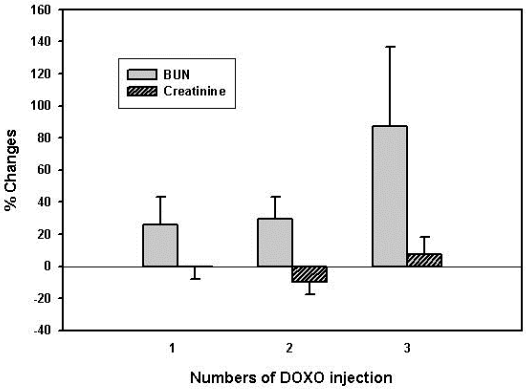

Figure 2 Percentage changes in blood urea nitrogen and plasma creatinine concentrations after DOX injections.

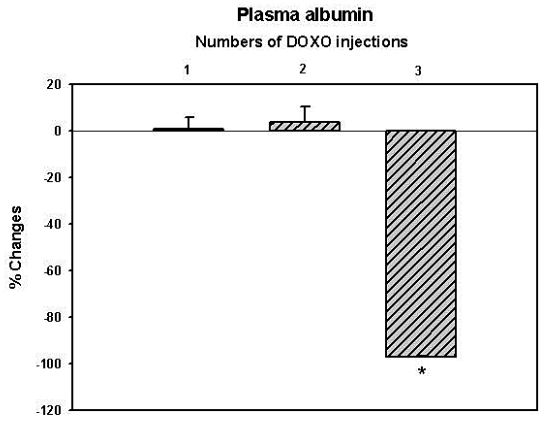

Figure 1 Percentage changes in plasma albumin concentration after DOX injections. Comparison of each injection with baseline was performed using Student paired *t*-test or Wilcoxon signed rank test. * = $p<0.05$.

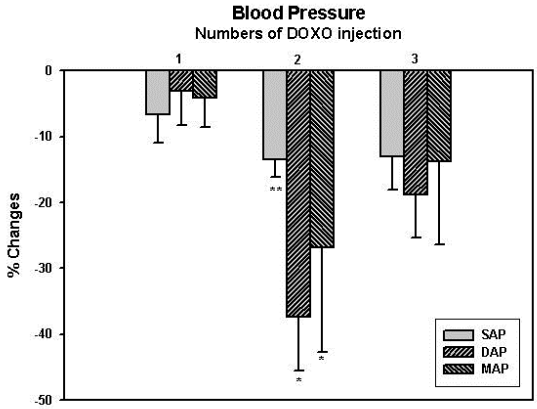

Figure 3 Percentage changes in blood pressure after DOX injections. Comparison of each injection with baseline was performed using Student pair *t*-test or Wilcoxon signed rank test. **, P= 0.01; *, = $p<0.05$.

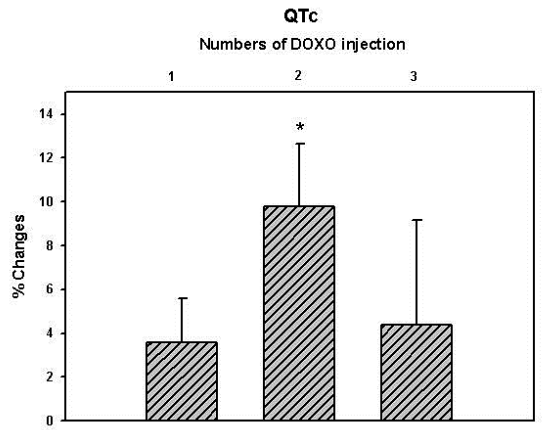

Figure 4 Percentage changes in QTc after DOX injections. Comparison of each injection with baseline was performed using Student paired *t*-test or Wilcoxon signed rank test. * = $p<0.05$.

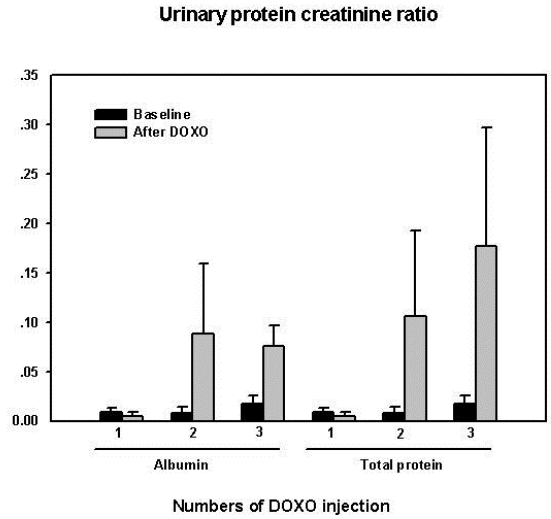

Figure 5 Changes in urinary albumin and urinary protein creatinine ratio after DOX injections

Discussion

Most of the dogs in this study were old age females. The tumor types that required DOX treatments were mammary gland tumor, lymphoma and renal carcinoma. The results corresponded to the study by Merlo et al. (2008) in 6,743 dogs with tumor which showed that in females, tumor types that have the highest incidence rate were mammary gland cancer and non-Hodgkin's lymphoma while in male, they were non-Hodgkin lymphoma and skin cancer. The incidence rate increased with age.

No remarkable changes in complete blood count and biochemical profiles were seen. The serum GOT and CPK were unchanged, which indicated that these enzymes had low sensitivity and the cardiac damage was minimal. The CPK which is released greater within 4 h after subendocardial infarction was useful in human patients (Norris et al., 2014). Plasma albumin concentrations decreased significantly after the 3rd dose of doxorubicin injection, which may be due to decreased albumin synthesis by the liver. DOX is excreted mainly by the liver, while a dose reduction is required in patients with hepatic insufficiency (Superfin et al., 2007). However, the increased urinary loss of albumin may not be a case since proteinuria although increased, fell within a normal range.

Doxorubicin causes cardiotoxicity in both humans and animals. Tachycardia, paroxysmal nonsustained supraventricular tachycardia, premature atrial and ventricular beats, decreased QRS amplitude, non-specific ST-T changes and left axis deviation may be evidences for acute effects (Chatterjee et al., 2009). The chronic toxicity will be evident within 30 d of administration of its last dose. In human the cardiac changes may occur later, 6-10 years after the treatment. The incidence could also be related to the dose of administration, which is considered around 500-600 mg/m^2 in human (Lefrak et al., 1973). These doses after drug treatment are way too high compared with the dose used in this study. Moreover, the study was carried out while the protocol of the treatment was not yet terminated.

A previous study in 175 dogs with various malignancies and treated with DOX showed that 31 dogs had electrocardiographic abnormalities including arrhythmia and non-specific alterations in R wave, ST segment or QRS duration (Mauldin et al., 1992). Seven dogs had congestive heart failure and died within 90 d. The toxicity was dose dependent as shown by intracoronary infusion of DOX 5-15 mg weekly for 4 weeks in dogs (Astra et al., 2003). All dogs had lower cardiac output after receiving the drug. Groups that received 10 and 15 mg had mortality rate of 33% and 67%, respectively. Thus, the electrocardiographic and echocardiographic measurements have been used as screening tools prior to DOX administration and were adopted in institution guidelines since the incidence rate in dogs was 8% (8/101) in one study (Ratterree et al., 2012). Echocardiography seems to be superior to electrocardiography for evaluating the DOX-induced cardiomyopathy (Singal and Iliskovic, 1998). One study in dogs to which DOX at the dose of 1.5 mg/kg iv 6 times at intervals of 3 weeks was administered showed reduction in fractional shortening with mitral regurgitation (Hanai et al., 1996). The ST-segment and T-wave were depressed and QRS complex was widened in severe dogs. No measurement of QT was performed in this study.

For electrocardiographic measurement, DOX caused significant increase in QT interval and QTc in the 2nd dose. The lengthening of QTc was also found in the 3rd dose but it was not significant due to the limited number of dogs. The mechanism of QT prolongation was proved to be due to selective I_{Ks} blockade as shown in guinea pig heart (Ducroq et al., 2010). Prolonged QTc was previously demonstrated in dogs injected with 1.5 mg/kg DOX once (Xin et al., 2011). The ECG abnormalities were detected along with increased CPK and AST. However, the enzymes reached the peak at 24 h and dropped to baseline within 48-72 h. Thus, the enzyme level in this study was unaltered due to the measurement taken late (21-63 d apart). One study in dogs given DOX 30 mg/m^2 iv once a week for 3 times showed increased JTc and

QTc intervals. The changes corresponded to the ultra-structural changes of cardiac tissue (Danesi et al., 1989). Another study in patients with non-Hodgkin's lymphoma who received DOX to cumulative dose of 400-500 mg/m^2 showed prolonged QTc, increased QT dispersion and late potential occurring independently of the impaired left ventricular function (Nousianen et al., 1999). In patient receiving DOX, short-term QT variability increased when measured within 1 day after dosing in 4 course injections (Ritsema van Eck et al., 2009). Therefore, changes in QTc and QT variability may occur before echocardiographic abnormalities and could be used to predict arrhythmia rather than the myocardial damage since DOX affected left ventricular ejection fraction when the dose reached 90 mg/m^2 (Alves de Souza and Camacho, 2006). In the present study, the QT variability was increased, however, it was not significant. The QTc was more sensitive but the arrhythmia could not be detected by the holter. These may be due to the low dose of DOX and the arrhythmogenic effects might have appeared shortly after the DOX treatment, therefore, unrecorded.

Changes in cardiac function involve the cardiac autonomic control. By measuring the power spectrum of heart rate, the balance between sympathetic and parasympathetic could be evaluated. The present study showed increased LF with decreased HF resulting in higher LF/HF. The data suggested enhanced sympathetic over parasympathetic tone. The results were similar to a study in a symptomatic patient treated with anthracycline and recorded for 29 months after the end of chemotherapy, the diastolic variables were abnormal in 50% of the patients. The heart rate variability (HRV) was abnormal in 85% with decreases in both time and frequency domains especially the parasympathetic indices (Tjeerdsma et al., 1999). Another study in lymphoma patient receiving cumulative dose of DOX of 200, 400 and 500 mg/m^2 showed the reduction in left ventricular ejection fraction. Increased sympathetic tone was detected at the dose up to 400 mg/m^2 by increased normalized low frequency, LF/HF, plasma norepinephrine (NE) concentration with decreased normalized high frequency (Nousiainnen et al., 2001). However, these changes disappeared after cumulative dose of 500 mg/m^2. Thus, sympathetic stimulation may occur at the beginning of ventricular failure.

Although sympathetic system seems to be dominant, the blood pressure was reduced while the heart rate did not accelerate. A study of HRV in rabbit showed that DOX caused progressive increase in resting LF power. However, the paradoxical fall in LF power with exercise found (Moguilevski et al., 1995). The results suggest impaired baroreflex sensitivity or reduced norepinephrine responsiveness. A study in rats given adriamycin showed a reduction in blood pressure compared to baseline but the baroreflex response to phenylephine and nitroprusside did not change (Rabelo et al., 2012). Other mechanisms include decreased baroreflex sensitivity in children and adolescents (Zavodna et al., 2005), increased plasma NE concentration but decreased NE contents in both ventricles and decreased maximal number of binding sites (Bmax) of myocardial beta-receptor (Yoshikawa et al., 1994), decreased beta-adrenergic receptors in cardiac tissues (Kenk et al., 2010), impaired vascular contraction via reduced alpha-adrenoceptors in the aorta (Ahmadiasl et al., 2002), impaired noradrenaline release from sympathetic nerve endings via presynaptic beta-adrenoceptors (Uno et al., 1993), blunted vasomotor reactivity to both alpha and beta adrenergic receptor (Filippelli et al., 1994) were suggested.

In the present study, the dogs received therapeutic dose of doxorubicin and the azotemia was unnoticed except after the 3rd dose in which the blood urea nitrogen concentration was elevated. Impaired renal function with protein loosing nephropathy was suspected. Increased urinary protein loss measured by electrophoresis was found. Although the protein loss in the urine was minimal, the patterns of increase in albumin and total protein were observed after the 2nd dose of doxorubicin injection. Many researchers demonstrated the effect of DOX on urinary protein loss with varying dose and time of administration. In rats, proteinuria was detected as early as 7 and 15 d after 5.0 and 3.5 mg/kg of DOX treatment, respectively (Galli et al., 2001), suggesting the dose dependent effects. Giving 7.5 mg/kg adriamycin in rats showed severe proteinuria in 10 d with a gradual decrease in glomerular sialic acid content (Skutelsky et al., 1995). Adriamycin given at 25 mg/kg could increase urinary albumin to creatinine ratio from 0.1 to 0.67 at day 6 but failed to increase at the dose of 10 mg/kg (Jeansson et al., 2009). In BALB/c mice, the proteinuria was present as early as 5 d after treatment with 10-11 mg/kg DOX, while the reabsorption droplet in tubular cell and intraluminal casts appeared at 2-3 weeks and after 2 weeks, respectively (Wang et al., 2000). The mechanism of proteinuria includes a significant loss of charge density and size selectivity of the glomerular barrier. The thickness of endothelial surface layer (glycocalyx) was 20% of that normal, which may be due to downregulating proteoglycan synthesis when study using glomerular endothelium of isolated kidney. Focal segmental glomerulosclerosis was evident and the final stage composed of tubulointerstitial fibrosis and global glomerulosclerosis (Okuda et al., 1986; Wang et al., 2000, Lee and Harris, 2011). The present study in dogs demonstrated that the undetectable loss of protein excretion might exist in clinical practice when therapeutic dose was introduced. In the present study, the proteins, mainly albumin, appeared in the urine and persisted for the whole course of treatment. The increased BUN concentration was found at the 3rd dose of DOX treatment

In conclusion, doxorubicin given to the dogs with tumors at clinical therapeutic dose treatment caused prolonged ventricular repolarization as shown by the lengthened QTc. The drug also caused blood pressure reduction without heart rate acceleration. The enhanced cardiac sympathetic activity after DOX treatment was proposed as shown by the increased LF/HF power spectrum. Slight proteinuria was presented as cumulative dose dependent concurrent with increased BUN levels. Routine monitoring for

cardiac arrhythmia and proteinuria in dogs with multiple doses of DOX following tumor treatment protocol should be performed.

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บทคัดย่อ

การเปลี่ยนแปลงสรีรวิทยาทางไฟฟ้า ความแปรปรวนของอัตราการเต้นของหัวใจ และการขับทิ้งโปรตีนทางปัสสาวะในสุนัขป่วยที่ได้รับการรักษาด้วยยาดอกโซรูบิซิน

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การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของการใช้ยาดอกโซรูบิซินขนาดที่ใช้ในคลินิก (12-25 มิลลิกรัมต่อตรม. พื้นที่ผิวกาย) ต่อการเปลี่ยนแปลงทางไฟฟ้าของหัวใจและการขับทิ้งโปรตีนทางปัสสาวะ สุนัขจำนวน 9 ตัวได้รับการฉีดดอกโซรูบิซิน แต่มีเพียง 6 ตัว และ 3 ตัวที่ได้รับดอกโซรูบิซินเข็ม 2 และ 3 ตามลำดับ ได้ทำการตรวจเลือด วัดคลื่นไฟฟ้าหัวใจ ความแปรปรวนของอัตราการเต้นของหัวใจ และปริมาณการขับทิ้งโปรตีนในปัสสาวะภายหลังการฉีดยาทั้ง 3 เข็ม โดยเก็บข้อมูลก่อนการฉีดยาเข็มถัดไป การศึกษาพบว่าภายหลังได้รับยาทั้ง 3 เข็ม ไม่พบการเปลี่ยนแปลงของเอนไซม์ครีอะทีนฟอสโฟไคเนส (CPK) และเอนไซม์แอสปาเตต ทรานสไมเนส (AST) พบการเพิ่มขึ้นของพลาสมายูเรียไนโตรเจนในเข็มที่ 3 พร้อมการลดลงของอัลบูมินในพลาสมาอย่างมีนัยสำคัญทางสถิติ 0.05) ค่าความดันโลหิตลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) โดยไม่พบการเพิ่มขึ้นของอัตราการเต้นของหัวใจในเข็มที่ 2 ซึ่งบ่งชี้ว่าอาจมีความผิดปกติของการตอบสนองของระบบประสาทอัตโนวัติ การตรวจคลื่นไฟฟ้าหัวใจพบการเพิ่มขึ้นของระยะคิวทีซี (QTc) ($p<0.05$) ในเข็มที่ 2 เมื่อศึกษาความแปรปรวนของอัตราการเต้นของหัวใจโดยวิเคราะห์จากคลื่นความถี่พบการเพิ่มขึ้นของอัตราส่วนความถี่ต่ำ (LF) ต่อความถี่สูง (HF) (LF/HF) ซึ่งแสดงถึงการกระตุ้นระบบประสาทซิมพาเทติกมากกว่าพาราซิมพาเทติก นอกจากนี้ตรวจพบโปรตีน ซึ่งส่วนใหญ่คืออัลบูมิน เพิ่มขึ้นในปัสสาวะตั้งแต่ในเข็มแรกแม้จะมีปริมาณน้อย ผลการศึกษานี้สนับสนุนการวัดคลื่นไฟฟ้าหัวใจและการตรวจโปรตีนในปัสสาวะ เพื่อเฝ้าระวังความเป็นพิษของยาดอกโซรูบิซินต่อหัวใจและไตในขณะมีการใช้รักษาโรคเนื้องอกในสุนัข

คำสำคัญ: สุนัข ดอกโซรูบิซิน ความแปรปรวนของอัตราการเต้นของหัวใจ โปรตีนในปัสสาวะ คิวทีซี

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