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Research Article
Exenatide Attenuation of Cardiac Rhythm Abnormalities and Blood Pressure Changes Induced by Doxorubicin in Rats

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Abstract

Background and Objective: Doxorubicin (DOX) is an anthracycline antibiotic anti-neoplastic drug. The DOX clinical use is limited due to the occurrence of accumulative dose-related cardiotoxicity. The objective of study was to investigate the exenatide effects on cardiac rhythm abnormalities and BP changes induced by DOX. **Methodology:** Male Sprague Dawley rats were used for this study and were distributed into four groups of six animals per group. Groups 1 represented the control, while group 2 were the exenatide group. In group 3, DOX was given alone, while group 4 received a combination of exenatide and DOX. Mode of administration was by intraperitoneally (DOX 3 mg kg^{-1}/every other day and exenatide 10 µg kg^{-1} day^{-1}) for 2 weeks. The DOX induced changes were assessed by recording changes in QT and QRS interval in electrocardiogram (ECG), Heart Rate (HR), Mean Arterial Pressure (MAP), Systolic Arterial Pressure (SAP) and Diastolic Arterial Pressure (DAP). Graph pad prism software was used for statistical analysis, employing Student's t-test, one way ANOVA then Dunnet *Post-hoc* test. **Results:** The DOX augmented QTc, QRS interval, deceased heart rate and increased SAP, DAP and MAP. Treatment with exenatide significantly ($p<0.05$) decreased QTc, QRS interval thus, reversing the changes observed in ECG. Moreover treatment with exenatide ameliorated HR abnormalities induced by DOX in reducing SAP but rather caused an increase in DAP. **Conclusion:** These results suggested that exenatide has the potential of mitigating cardiac rhythm changes induced by the treatment with DOX.

Key words: Exenatide, doxorubicin, electrocardiogram, blood pressure, heart rate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular morbidity and mortality burden necessitate innovative treatments to alleviate the destructive cardiovascular disease effects[1]. Doxorubicin (DOX) is a strong antineoplastic agent regularly used to manage different types of cancers such as leukemias and Hodgkin's lymphoma in addition to bladder, breast and stomach cancers[2]. Nevertheless, DOX clinical usage is limited due to its adverse cardiotoxic properties[2,3]. Gratia *et al.*[4] showed that there are different mechanisms contributing to DOX cardiotoxic effects[4,5]. Documented evidence showed that DOX can induce genotoxic, oxidative stress and inhibits 5' AMP-activated protein kinase (AMPK)[4]. The AMPK inhibition is related to the regulatory cross talk with protein kinase B (Akt) as well as mitogen-activated protein kinase (MAPK) pathways, which is largely elicited by DNA damage. Both AMPK inhibition and mTOR triggering cause negative feedback that exacerbates DOX induced energy deficits[5].

Reports from earlier studies had revealed that Glucagon-like peptide-1 (GLP-1) antidiabetic drugs possess cardiovascular protective effects along with antihyperglycemic activity[6,7]. Ichikawa *et al.*[8] reported in their study that exenatide blunts the inflammatory process triggered by MARK. Evidence also, obtained from literature indicate that exenatide improves cardiac function by ST-segment-elevation in myocardial infarction (STEMI) patients[2,9]. The GLP-1R stimulated by exenatide improves cardiac recovery, mitigates myocardial hypertrophy and reverses cardiac alteration in post-MI animals[10]. Furthermore, H9C2 cardiomyoblasts treated with exenatide led to an intense protection against reactive oxidant stress, an escalation in cell survival rate, decline in reactive oxygen species production and apoptosis mitigation[10-12]. Therefore, the objective of this present study is to investigate the outcome of exenatide on DOX induced cardiac rhythm changes in anaesthetized rats.

MATERIALS AND METHODS

Experimental animals: Male Sprague Dawley rats weighing 150–200 g were purchased from the Animal Facility of King Saud Research Center, King Saud University, Saudi Arabia. They were used in the study according to the guidelines, prescribed and approved by the Institutional Review Board, Deanship of scientific research, King Faisal University, Saudi Arabia. Animals were accommodated in polypropylene cages and upheld at 27±2°C under 12 h light/dark sequence. Rats were kept and observed for 1 week in the animal house of College of Medicine to acclimatize. They were fed with standard rat feed and allowed water *ad libitum*. Doxorubicin and exenatide was purchased from commercial retailers.

Study protocol: Animals were distributed into 4 groups, each group containing 6 rats. Group I is the control group in which animal were injected normal saline intraperitoneally (IP). Group II represented the exenatide group in which animal were treated for 2 weeks with exenatide (10 μg kg^{-1} day^{-1}, IP). Group III animals were treated with DOX alone, 3 mg kg^{-1} IP, every other day to reach a total cumulative dose of 15 mg kg^{-1} body weight for 2 weeks. Group IV were then treated with a combination of DOX and exenatide with same doses given in groups 2 and 3 for 2 weeks.

Electrocardiogram (ECG) and Blood Pressure (BP) recording and measurement: Rats anaesthetized with urethane (1.5 g kg^{-1}) were then placed in prone position on the ECG platform of Emka IOX DATA ACQUISITION software, with both fore and hind limbs taped to the leads. Continuous recording of ECG was obtained and later analyzed using ECG Analyze software from Emka Technologies' systems (France). Blood pressure measurements were done using noninvasive computerized tail-cuff system CODA from Kent Scientific (USA).

Statistical analysis: The data is hereby presented as mean ±SEM. Graph[13] pad prism software, version 5.0 was used for statistical analysis, employing Student's t-test, one way ANOVA then Dunnet *Post-hoc* test to ascertain statistical significance ($p<0.05$) among different experimental groups. A $p<0.05$ is taken to indicate statistical significance. The Corrected QT was calculated according to Bazett's formula[13].

RESULTS

Electrocardiographic (ECG) pattern: The results obtained from this study showed significant ($p<0.05$) changes in the ECG patterns of DOX administered rats as compared to control rats. These characteristic changes appeared as altered ECG pattern by DOX administration, displayed significant ($p<0.05$) prolongation of QTc and QRS intervals with 260.7±17.06 and 26.7±1.04 msec, respectively compared to control representing 200±10.3 and 18±0.8 msec, which reflects arrhythmias and conduction abnormalities. However, treatment with exenatide+DOX showed a significant reduction ($p<0.05$) of these ECG alterations induced by DOX, particularly as it relates to QTc and QRS intervals

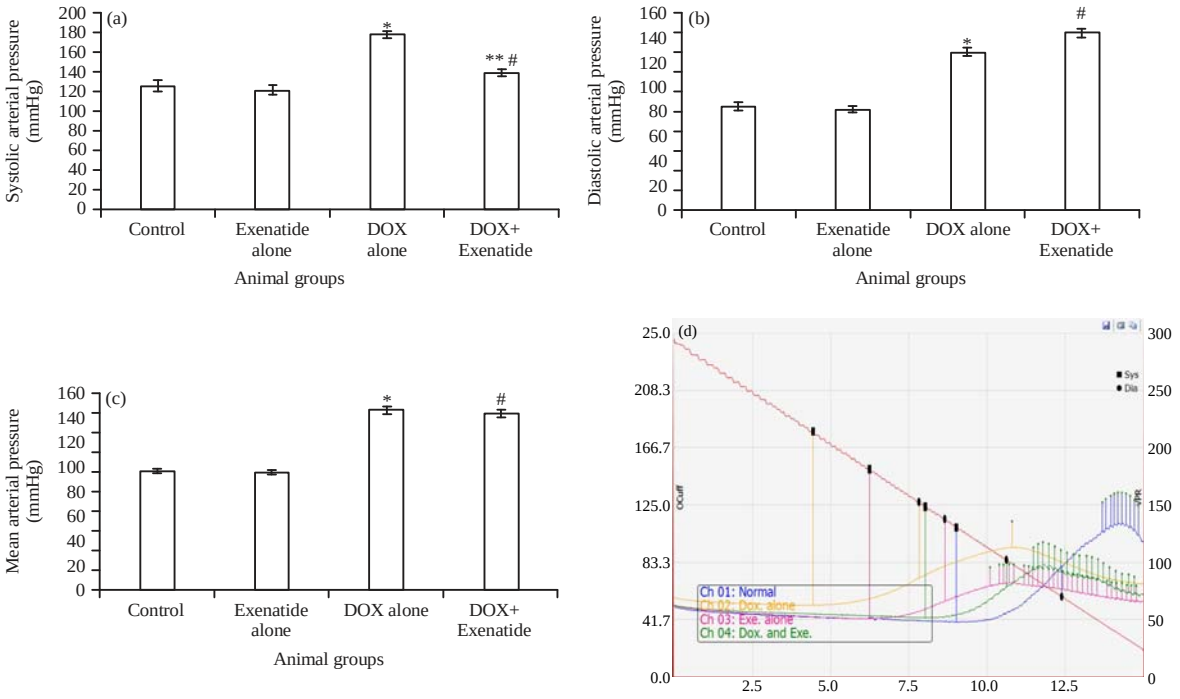

Fig. 1(a-d): Effect of exenatide and DOX on DOX induced changes in blood pressure, Effect of treatment with a combination of exenatide (10 μg kg^{-1} day^{-1}, IP) and DOX (3 mg kg^{-1}/every other day, IP) for 2 weeks on DOX induced changes in (a) Systolic arterial pressure, (b) Diastolic arterial pressure, (c) Mean arterial pressure in rats and (d) Computerized tracings obtained during BP recordings

Data are the Mean±SEM, *Significant difference compared to control (p<0.05), **Difference compared to DOX group (p<0.05), #Significant difference compared to exenatide group (p<0.05)

Table 1: Effect of exenatide and DOX on DOX induced changes in ECG and heart rate

Treatments	Heart rate (beat min^{-1})	QRS interval (msec)	QTc interval (msec)
Normal control	386.5±21.3	18.0±0.8	200.00±10.3
Exenatide alone	350.5±7.32	16.0±0.6	210.50±14.7
DOX alone	260.4±14.43*	26.7±1.04*	260.70±17.06*
DOX+exenatide	361.0±15.37**#	19.0±1.0**#	233.56±22.4**#

DOX: Doxorubicin, Data are the Mean±SEM.*Significant difference compared to control (p<0.05), **Difference compared to DOX group (p<0.05), #Significant (p<0.05) difference compared to exenatide group

(233.56±22.4 and 19±1.0 msec, respectively) (Table 1). This indicates a form of cardioprotective effect elicited by exenatide. In addition, DOX treated group showed significant (p<0.05) bradycardia (260±14 beat min^{-1}) compared to control rats with 386.5±21.3 beat min^{-1}. Experimental tracings showed normal cardiac activity in the control and exenatide groups, with heart rates of 386±21.3 and 350±7.32 beat min^{-1}, respectively. The DOX induced heart rate changes were ameliorated with the treatment of exenatide as indicated in group IV animals which were given a combination of DOX and exenatide. The result produced an increase in heart rate from 260.4±14.43 to 361±15.37 beat min^{-1}.

Blood pressure: This study showed significant (p<0.05) increase of Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP) and Mean Arterial Pressure (MAP) in DOX treated rats as compared to control group. Figure 1d shows computerized tracings obtained during BP recordings. The DOX induced blood pressure changes is as shown in Fig. 1a. Similarly, treatment with exenatide alone produced BP changes comparable to the control measurements. With co-administration of exenatide and DOX, result showed a reduction only in SAP. However, there were increases in both DAP and MAP as indicated in Fig. 1b and c. These observed increased levels in DAP and MAP by exenatide combination with DOX is significant (p<0.05) when compared with exenatide and control groups.

DISCUSSION

This study was undertaken in other to ascertain the effect of exenatide on DOX induced cardiac rhythm abnormalities and changes. The results in this study has shown ECG patterns alterations in DOX group compared to control group including significant ($p<0.05$) prolongation of QTc and QRS intervals. Glucagon-like peptide-1 (GLP-1) receptor agonists were reported to be beneficial in cardiovascular disease risks such as blood pressure lowering[14]. Preclinical studies in both animal and human, support cardioprotective role of exenatide in variety of the cardiac syndrome[14]. These observations appeared to be similar to the findings of Wu *et al.*[15], as they reported prominent ECG changes induced by DOX. The mechanism by which DOX induced abnormalities in cardiac rhythm was not however elucidated in the present study. Although, evidence from previous studies supported the knowledge that DOX administration is usually accompanied by endogenous antioxidants defense reduction and generation of oxygen free radicals leading to increased oxidative stress[4,5]. These are reported to be followed by numerous myocardium subcellular alterations instigating cardiac injury[11,12].

Electrocardiograph abnormalities are the key criteria largely used to define myocardial injury diagnosis. Studies have shown that exenatide does not significantly alter QT interval baseline on its own as was also observed in this study[16,17]. However, exenatide diminished increased QT interval induced by DOX and in addition, significantly reduced ($p<0.05$) the QRS interval as well. The QT interval is reflected to be a cardiac repolarization measure. An increased QT interval could indicate torsades de pointes arrhythmias and might lead to abrupt cardiac cell death[17].

Documented evidence suggested that exenatide exerts cardioprotective effects in myocardial infarcted animal models[18]. Other researchers have also reported that exenatide has substantial antioxidant properties against oxidative stress-induced damage[19].

Therefore, exenatide cardioprotective effects might be due to its scavenging activity of oxidative stress products, its ability to increase the antioxidant defense enzymes concentrations and inhibiting cardiomyocyte apoptosis[19]. Reports indicated that exenatide anti-apoptotic activities were accompanied with the PI3K/Akt activation[4,19]. It could be due to the blunting of MAPK and activation of the AMPK signaling pathways as adduced by Ichikawa *et al.*[8]. Another outcome observed in this study is a decreased HR by Extanide alone and a reversal of DOX induced increased HR. Nevertheless, other studies reports on the effect of exenatide on HR indicated a significant ($p<0.05$) increase in HR from baseline. This observed increase in HR by exenatide according to their report was attributed to a compensatory mechanism due to decreased SAP[20]. On the effect of exenatide on BP, there was no significant lowering of the BP when it was administered alone. It is inconsistent with other reports, which have shown that exenatide had BP lowering effects[21,22]. These effects were seen with both SAP and DAP in human clinical trials[20-22]. However, exenatide did reduce the SAP significantly ($p<0.05$) which was increased in the wake of DOX administration but DAP was increase beyond that of DOX alone on the contrary. This observed phenomenon could not be explained in this present study.

CONCLUSION AND FUTURE RECOMMENDATION

The findings of the study suggested that exenatide could protect against DOX induced abnormal cardiac rhythm changes in rats. This was evidenced by the observation that exenatide corrected ECG changes induced by DOX and maintained the blood pressure by stabilizing the SAP. This was also demonstrated by the improvement in the heart rate. GLP-1 receptor agonists may represent a unique approach to protect patients taking DOX. However, more study needed to be done to investigate the mechanisms underlying exenatide phenomenon on altered cardiac rhythm.

SIGNIFICANCE STATEMENTS

Exenatide possess cardioprotective efficacy as found in this study for the first time that it mitigate the cardiac rhythm changes induced via DOX treatment.

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