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Effect of metformin on ECG, HR and BP of rats administered with cardiotoxic agent doxorubicin

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ABSTRACT

Background: Cardiovascular complications during drug therapy is worrisome and has been known to increase morbidity and mortality. The aim of this study was to investigate the effect of metformin on BP, HR, and ECG, after subacute administration of doxorubicin in rats.

Methods: Laboratory acclimatised animals were divided into 4 experimental groups consisting of group I as the control, given 0.5ml normal saline. Group II, received 3mg/kg doxorubicin on alternate days, while group III was given 300mg/kg metformin. The last group of animals were treated with 300mg/kg metformin, 30 min later, the animals were injected doxorubicin ip. The treatment lasted for 15 days thereafter, ECG, HR and BP were measured with anaesthesia.

Results: Result showed that doxorubicin induced ECG changes in terms of increased PR and QT intervals significantly. Whereas, QRS and RR intervals were at same time significantly decreased. Metformin was observed to significantly attenuate these changes. In addition, metformin restored decreases in HR that was caused by doxorubicin in a significant fashion. However, metformin alone produced a decrease in HR compared with control. The observed reduced SBP and DBP produced by doxorubicin were also alleviated by the administration of metformin. There were increases in BP parameters measured in normotensive rats with metformin alone.

Conclusions: In conclusion, metformin was found to attenuate doxorubicin-induced alteration in ECG pattern and restored the mechanical and physiological functions of the rat heart. Our data further suggests monitoring of CVS changes such as ECG particularly with drugs known to cause myocardial injury.

Keywords: Blood pressure, Doxorubicin, ECG, Heart rate, Metformin, Rats

INTRODUCTION

Cardiovascular complications in the use of drugs are some of adverse effects defying management solution, particularly for those agents whose benefit to risk ratio is on the border line. This worrying phenomenon, has led to the use of multiple regimen to mitigate the likely events of toxicity. Patients who are on certain medications used in the treatment of disease like cancer and psychotics illness do have the added complications of increased risk of cardiovascular complications. This could impair management and increase healthcare cost.¹ Evidence has shown increased mortality from these events like myocardial infarction, hypertension, stroke and

ultimately heart failure. Changes in myocardial malfunctioning will reflect in BP alteration, HR and ECG changes, including ventricular dysfunctions.² Drugs like doxorubicin are known to precipitate cardiac malfunction even in therapeutic doses. Doxorubicin is an effective chemotherapeutic agent used in the treatment of many types of cancers. Reports from studies indicate that it causes cardiac toxicity and this adverse effect limits the use of the drug particularly in some patients' categories.³ Evidence show that the mechanism of doxorubicin toxicity is by reducing adenosine monophosphate activated protein kinase alpha (AMPK α) signalling in the heart.⁴ The inhibition of this signalling pathway causes cellular energy deficits.⁵ Metformin on the other hand is

currently one of the drugs used worldwide to treat diabetes mellitus type 2. It is known to be an insulin sensitizer and has a low potential to produce adverse effects especially in patients with underlying cardiovascular disease.⁶ Several studies have shown that metformin improves vascular functions which reduces cardiovascular events and mortality as a consequence.⁷ The mechanism by which metformin attenuates cardiovascular risks in diabetes is not related to its antidiabetic property but rather due to its ability to activate AMPK.² AMPK is known as a master switch, which regulates glucose and lipid metabolism.⁸ It also modulates salt retention by the kidney.⁸ Studies have shown that angiotensin II inhibits the activities of AMPK in the kidney leading to salt retention and this has huge implications in patients with hypertension.⁹ AMPK activation is also reported to be associated with cardiac hypertrophy regulating contractile responses of the heart.¹⁰ This suggests that decreased AMPK activities will alter its mechanical function and could lead to heart failure. Therefore, reducing the molecular events that leads to cardiovascular complications either by drugs will be beneficial in managing the conditions they precipitate. In addition, the use of AMPK activator like metformin could help to restore cardiac function disrupted by cardio toxic agents like doxorubicin. Research has shown that metformin attenuates the adverse effects induced by doxorubicin on cardiomyocytes.¹¹ However, its effect on doxorubicin altered ECG parameters and changes in normotensive blood pressure as well as heart rate is lacking in literature. Therefore, the aim of this study was to evaluate the beneficial effects of metformin on the ECG, HR and BP of anaesthetised rats administered with doxorubicin.

METHODS

Doxorubicin and metformin, all sigma Aldrich products were used in present study.

Equipments used were ECG Tunnel non-invasive ECG and respiration Data Acquisition and Analysis Systems emka Technologies for ECG measurement. CODA™; Non-Invasive Blood Pressure System 2013 Kent Scientific Corporation Software Version 4.1

Dosing

Twenty four male specie of rats Sprague Dawley weighing between 150-200gm were used obtained from King Saud University Animal house. The animals were housed under standard laboratory conditions and maintained on 12-hour light and dark cycle. They were allowed free access to food and water, also procedure for animal experiment prescribed by the Deanship for Scientific Research Ethical Committee was followed. Animals were however allowed to acclimatize in laboratory conditions for 2 weeks before the commencement of the experiment. They were then randomly assigned into 4 groups consisting of 6 rats in

each group. Group 1 represented the control group that was given normal saline orally. The second group received metformin (500mg/kg orally), whereas group 3 was given doxorubicin (3 mg/kg ip). The animals in group 4 represented the treatment group, with metformin given orally and 30mins later, doxorubicin was administered intraperitoneally. The experimental dosing was done according to the method of Ashour et al.¹²

ECG

Animals were anaesthetised with 1.5g/kg urethane given ip and then placed in a supine position with spontaneous breathing for the measurement ECG. The measurement was performed with the use of Emka IOX Data Acquisition software. Thereafter, Emka ecg Analyser software was used to analyse tracings recorded during data acquisition. ECG measurements were recorded in two phases of 30 mins each consisting of 20 cycles each. Parameters such as RR, PR, QRS and QT intervals were determined using the ECG Analyse software.

BP and HR

Systolic, diastolic and mean arterial blood pressures and heart rate were measured using Kent non-invasive blood pressure instrument (CODA). Animals were anaesthetised with 1.5g/kg urethane and then placed in a restrainer. Their tails were connected to a cuff and volume pressure recorder, which in turn was connected to the CODA software with computer monitoring and acquisition of data. Blood pressure and heart rate were monitored for 1 hr in 15 cycles.

Statistical analysis

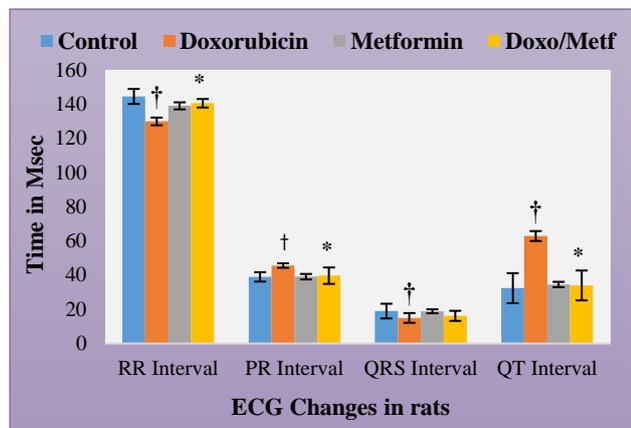
Data acquired was represented as mean±SEM for ECG and HR data. Whereas, BP data was presented as mean±SD. Analysis was done with Graph Pad Prism Software. Comparison was done for group means using T- test and one-way ANOVA followed by post-test.

RESULTS

Figure 1 describes results obtained from ECG changes in rats of various groups studied, while Figure 2 displays the tracings from different groups. From the results obtained, doxorubicin was observed to have reduced the RR-intervals significantly ($p<0.001$), when compared with the control. The decrease was however restored by co-administration of metformin which was also statistically significant.

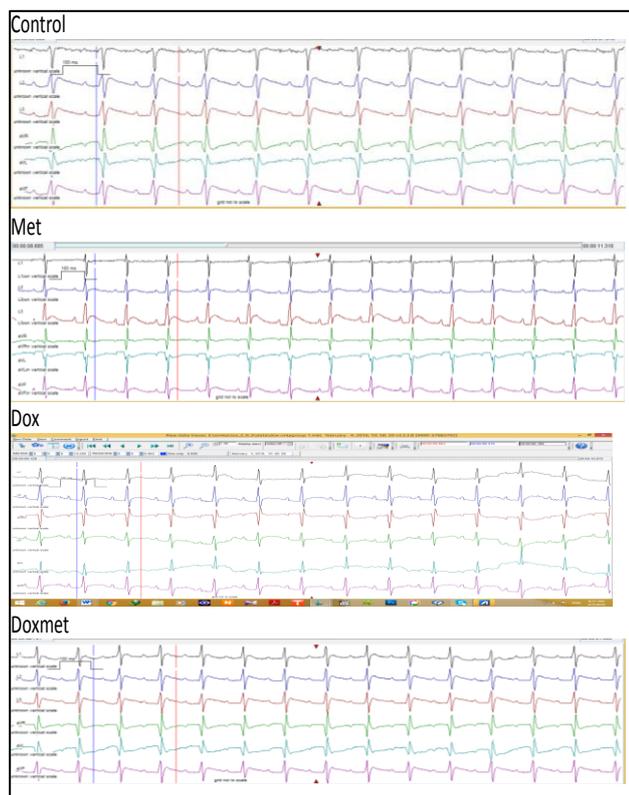
On the PR-intervals, a significant ($p<0.001$) increase by doxorubicin was observed whose effects were attenuated in the presence of metformin significantly too. The effect of doxorubicin on QRS was a significant decrease which was statistically significant ($p<0.001$). However, the attenuation by co-administration with metformin reduced it but not significantly. Results on QT intervals induced-

effects by doxorubicin showed a significant increase ($p < 0.001$), which again reduced by the metformin considerably in a significant fashion ($p < 0.001$). Metformin was able to significantly attenuate the effects of doxorubicin in all the parameters assessed.



†=indicates significant changes with doxorubicin administration compared with the control ($p < 0.001$). *=represents significant differences between the effects produced by Doxorubicin and that of Doxorubicin/metformin combination.

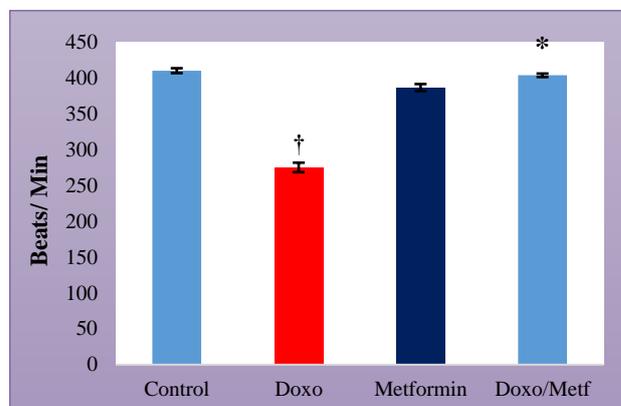
Figure 1a: ECG changes of rats administered with doxorubicin, metformin and doxorubicin/metformin.



Control=normal saline, Met=metformin 500mg/kg, Dox=doxorubicin 3mg/kg, Doxmet= doxorubicin and metformin group.

Figure 2: ECG tracings showing changes in rats treated with different drugs used in the study.

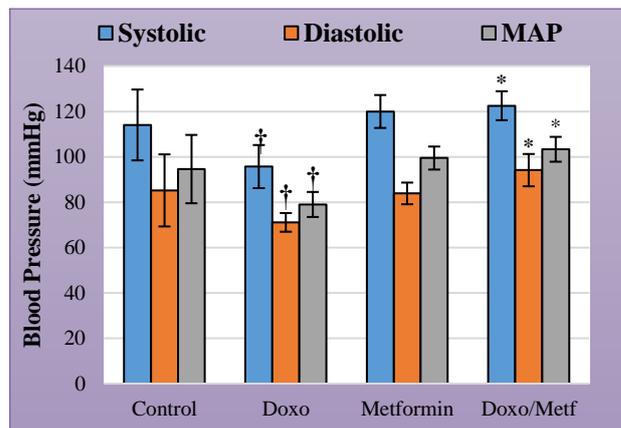
The effects of doxorubicin on heart rate (HR) showed a significant decrease ($p < 0.001$) as shown on Figure 3. As can be seen, administration of metformin restored the HR in rats and therefore significantly ($p < 0.001$) reduced the bradycardia caused by doxorubicin. In addition, metformin on its own produced a decrease in the heart rate which was also statistically significant.



†=indicates significant changes with doxorubicin administration compared with the control ($p < 0.001$), *=represents significant differences between the effects produced by Doxorubicin and that of Doxorubicin/metformin combination.

Figure 3: Effects of doxorubicin, metformin and doxorubicin/metformin administration on the heart rate of experimental rats.

BP effects were also determined in this study. Doxorubicin, was found to decrease systolic, diastolic and mean arterial blood pressure. These effects were significant ($p < 0.001$) compared to the control.



†=indicates significant changes with doxorubicin administration compared with the control ($p < 0.001$), *=represents significant differences between the effects produced by Doxorubicin and that of Doxorubicin/metformin combination.

Upon the administration of metformin, all the parameters were restored beyond normal and significantly to ($p < 0.001$). It was however observed that metformin alone increased both the systolic and diastolic blood pressure as was shown in Figure 4. However, the mean arterial blood pressure remained unchanged.

DISCUSSION

Doxorubicin is a widely used chemotherapeutic agent employed for different cancer types. Its application in oncology protocol is growing daily, however this use is being limited by its associated myocardial injury.¹³ Early detection of its adverse effect on the heart will help in better prognosis and management. Research has shown that metformin, another drug widely used in the management of type 2 diabetes mellitus, can reduce the adverse effects of doxorubicin, on the heart.^{12,14} Various mechanisms has been reported to be involved in doxorubicin induced cardiotoxicity.¹⁵⁻¹⁷ However, its inhibitory effects on AMPK has been well documented.^{4,11,16}

Evidence has shown that AMPK is activated through adenine nucleotide dependent mechanism.¹⁸

Metformin also has been reported to reduce the risk to myocardial injury via the activation of AMPK, thereby decreasing reactive oxygen species generation and hence reducing the associated oxidative stress.^{3,11} This action is reported to promote mitochondrial function through enhancement of Ca^{++} .^{19,20} Cardiac abnormality manifesting in conduction alterations has been associated with mutations in AMPK regulating gene thus implicating this signalling pathway.²¹ From our study, we found that metformin restored doxorubicin reduced RR interval. This phenomenon of reduced RR interval is reflective of slowed heart beat and hence bradycardia. In the same fashion, QRS complex was decreased depicting slow depolarization. Also, prolonged QT intervals was observed by doxorubicin which again was attenuated by the administration of metformin. Prolongation QT interval is viewed as an indication of ventricular functional abnormality due to oxidative stress and a consequent membrane damage by doxorubicin.²² It can be also due to inhibition of Ca^{2+} release.^{23,24} The reduction of RR interval, QRS complex and prolongation of QT intervals by doxorubicin was consistent with the results already reported in literature.^{22,25} Monitoring changes in ECG has a high propensity to positively predict underlying myocardial disease. Therefore, it can be a useful tool for cardiac function assessment during doxorubicin use.

Observations from HR assessment revealed that doxorubicin reduced them in our experimental rats. This result complimented the findings earlier observed by Jensen showing lack of contractile responses in rats administered with doxorubicin. He attributed this phenomenon to Ca^{++} deficiency.²⁶ Our findings were also in total agreement with the report of Ozdoğan et al.²⁷ It shows that doxorubicin also affects the mechanical functions as well as the ventricular depolarisation and repolarisation of heart during its action potential. The administration of metformin restored the HR close to normal levels. This effect was not reflected when metformin was administered alone. Reports from other

workers showed that metformin alone could cause a transient rise in the HR contrary to our observations.²⁸ We also found that metformin on its own, increased both SBP and DBP in rats after 15 days administration. Hence, the lowered SBP and DBP by doxorubicin was restored with the administration of metformin together. Our findings was collaborated by the work of Tomczyk et al.²⁸ But was contrary with the results reported by Luque-Ramírez et al, Huang et al showed in their study that regulation of AMPK signalling pathways affected the blood pressure of rats.^{29,30} This view was supported by Sun and Zhou an effect which they described was prevented by metformin administration.³¹ Therefore, metformin could be said to be able to restore both normal action potential and mechanical functions as seen in our study. In addition, past results had shown its amelioration of doxorubicin induced myocardial injury.^{12,14,32,33}

CONCLUSION

From this study, metformin was able to attenuate the conduction abnormalities and mechanical dysfunction induced by doxorubicin administration. Also, our data further suggests monitoring of cardiac function parameters such as ECG, HR, and BP in the patients been treated with agents known to produce myocardial injury.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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