Journal of Pharmacological Reviews and Reports

Extended Abstract
Vol. 2, Iss. 2
2019

Pharma Sci- Comparative Study of the Protective Effect of Metformin and Sitagliptin against Doxorubicin-Induced Cardiotoxicity in Rats- Mahmoud M Kamel- Cairo University

Mohamed EL Shabrawy Abdo1 , Afaf Sayed Osman1 , Omayma Anwar Khorshid1 , Lubna Omar El-Farouk2 and Mahmoud M Kamel3 *

1 Department of Medical Pharmacology, Cairo University, Egypt

2 Department of Pathology, Cairo University, Egypt

3 Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt

Introduction

Doxorubicin (DOX) is one of the most potent anticancer agents, but its use is associated by development of cardiotoxicity that may lead to cardiomyopathy and congestive heart failure. Heart is sensitive to Reactive Oxidative Species (ROS) induced damage because of its highly oxidative metabolism and fewer antioxidant defences compared to other organs. Doxorubicin induces mitochondrial damage which causes continued production of free radicals and release of cytochrome c which induces apoptosis. Doxorubicin-induced cardiotoxicity is usually dose dependent . If the doxorubicin-induced cardiac complications prevented or at least reduced, higher doses could be utilized, thereby increasing cancer cure rates. The prevalence of glucose intolerance is increased in patients with malignancy especially those treated by DOX. Insulin resistance is correlated with increased risk for cancer. In addition, the rate of tumor recurrence, metastatic spread and fatal outcome is higher in cancer patients with hyperglycemia or type II diabetes. Metformin is an oral biguanide antihyperglycemic drug which can enhance insulin sensitivity. Metformin has been shown to have antioxidant properties and can decrease lipid peroxidation in various tissues. Noteworthy, Metformin significantly improved left ventricular function and survival in vivo murine model of heart failure, improves outcomes in patients with advanced systolic heart failure and may reduce the cardiovascular complications in diabetic patients. Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor which improves hyperglycemia by inhibiting the inactivation of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide. The GLP-1 receptor is expressed in islet cells, kidney, lung, brain, the gastrointestinal tract and in the heart. The DPP-4 inhibitors caused decrease in the expression of myocardial fibrosis-related proteins and markers of cardiomyocyte stress in a rat model of uremic cardiomyopathy.

This work aimed to compare the potential protective effect of the antihyperglycemic drugs; metformin and sitagliptin against doxorubicin-induced cardiotoxicity in male Wistar rats.

Materials and Methods Materials:

Doxorubicin (Sigma Chemicals Company)

Adriblastina ampoules (10 mg/5 ml) given by Intraperitoneal (i.p.) injection to rats. Metformin (Mina Pharm Company), Sitaglipitin (MERCK SHARP and DOHME Company) and Isoprenaline (Pharco Chemicals Company): all drugs are obtained as powder and dissolved in distilled water immediately before use. Reagent and chemicals: reagents used for measurement of Creatinine Kinase-M

band Isoenzyme (CK-MB), Lactate Dehydrogenase (LDH) and blood glucose (Sigma Chemicals Company), kits for Measurement of Tissue Malondialdhyde (MDA) (Bio-diagnostic Company) and chemicals for modified Krebs-Henseleit buffer (Sigma Chemicals Company).

Animals

Experiments were performed on adult male Wistar rats 200-250 g. Animals were housed for at least 2 days before experiments under a 12 h light/dark cycle. Food and water were provided ad libitum. The study protocol was approved by the Institutional Reviewer

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

Board of Faculty of Medicine, Cairo University and the animal experiments were carried out in accordance with the ethical guidelines of animal welfare.

Tissue sampling for lipid peroxidation:

Six rats from each group were sacrificed by decapitation under ether anesthesia and hearts were rapidly isolated. Isolated hearts were placed into Petri dishes containing ice-cold isolation medium consisting of 125 mM KCl, 15 mM Tris, pH 7·4. The heart great vessels and valves were trimmed away and the ventricles and atria were cut open and rinsed free of blood. 20% homogenates were prepared with a Potter-Elvehjem homogenizer set at a standard velocity (500 r.p.m.) for determination of tissue Malondialdhyde (MDA) content. Malondialdhyde reacts with thiobarbituric acid producing Thiobarbituric Acid Reactive Substance (TBARS), a pink chromogen, which is measured spectrophotometrically at 532 nm.

Measurement of cardiac contractility:

Six rats from each group were sacrificed by decapitation under ether anesthesia and hearts were rapidly isolated. The amplitude of ventricular contractions was recorded in response to variable concentrations of isoprenaline (3, 6 and 12 µg) using Power Lab Data Acquisition and Analysis Systems for Monitoring and recording signals from the isolated rat heart connected to the Langendorff's coronary perfusion set. Modified Krebs-Henseleit buffer solution at temperature 37°C was used. The heart was continuously bubbled with a mixture of 95% O2 and 5% CO2 taking care that no air bubbles entered to the aorta. Coronary perfusion is maintained at a constant pressure of 90 mmHg in a nonrecirculating way.

Modified Krebs–Henseleit buffer of the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.52, MgSO4 1.64, NaHCO3 24.88, KH2PO4 1.18, glucose 5.55, sodium pyruvate 2.0. Histopathological examination: Isolated hearts from six rats of each group were fixed in 10% formalin, embedded in paraffin wax, sectioned (3-5 µm), and stained with Hematoxylin and Eosin. The sections were examined under light microscope and then photomicrographs were taken for assessment of histopathological changes. Statistical analysis Data were coded and entered using the statistical package SPSS version 22. Data were summarized using mean ± standard deviation. Comparisons between groups were done using Analysis of Variance (ANOVA) with multiple comparisons post hoc Tukey test. P-values less than 0.05 were considered as statistically significant.

Results

Groups treated with either metformin (group II) or sitagliptin (group III) didn't show any significant change regarding any of the tested parameters compared with control untreated group (group I). There was no mortality among rats of group I, II or III.

Histopathological examination

The heart from Groups I, II and III showed normal histologic appearance, that is showing normal arrangement of cardiac muscle fibres, regularity of cell and nuclear membrane, normal nuclear pattern of cells, absent vacuolation of cells, normal blood vessels pattern, no inflammatory infiltration around blood vessels, as well as normal appearances of endocardium and pericardium

Histopathological examination of isolated rats` hearts in doxorubicin induced cardiotoxicity group showed marked interstitial edema, inflammatory cellular infiltration around blood vessels, perinuclear vacuolation, highly oesenophilic cytoplasm, interfibrillar hemorrhage, disarrangement and degeneration of the myocardium.

Co-administration of metformin with doxorubicin produced improvement in the histopathological findings which appear as reversion to normal cellular pattern, little cellular infiltration and absent interfibrillar hemorrhage. Co-administration of sitagliptin with doxorubicin produced improvement in the histopathological findings which appear as mild interstitial edema, disarrangement of muscle fibres, cellular infiltration and absent interfibrillar hemorrhage (**Figure 4**).

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

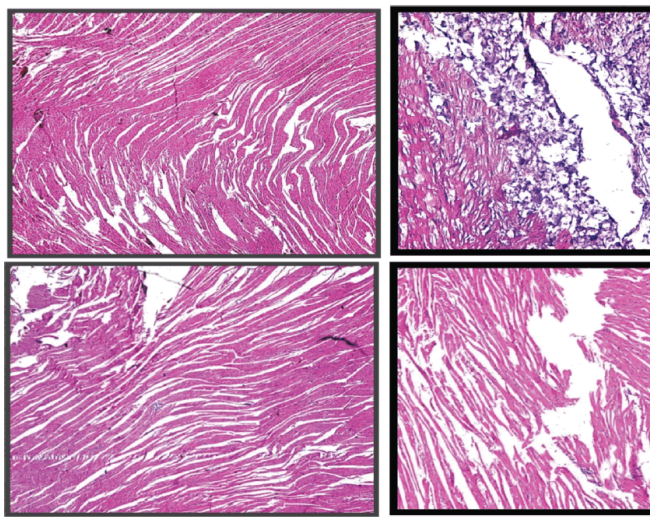

Figure 4: Histopathology of cardiac muscle in doxorubicin-induced cardiotoxicity groups (H&E, 100X).

Figure 4: Histopathology of cardiac muscle in doxorubicin-induced cardiotoxicity groups (H&E, 100X).

(MET: Metformin, STG: Sitagliptin and DOX: Doxorubicin), A: Group I: Control untreated rats, B: Group Π (DOX-induced cardiotoxicity group) shows small cluster of myocardial fibers with small and large cytoplasmic vacuoles (indicated by left arrow) and inflammatory infiltration (indicated by bent-up arrow), C: Group Π (MET+DOX group) shows normal myocardium, reversion to normal pattern, no vacuolated fibers and no hemorrhage, D: Group IV (STG +DOX group) shows disarrangement of fibers, no vacuolated fibers and no hemorrhage.

Discussion

The aim of the present work was to compare the ability of metformin and sitagliptin to protect the heart against the cardiotoxicity induced by cumulative dose of doxorubicin in male rats which may help in cardio protection of the patients treated with doxorubicin.

In the present study, a cumulative dose of doxorubicin induced significant deterioration in body weight, blood pressure and heart rate. Significant elevation of ST segment and prolongation of QT interval, significant elevation in the serum level of CK-MB, LDH, blood glucose and cardiac MDA, histopathological signs of cardiotoxicity and reduced myocardial contractility in response to isoprenaline compared to control untreated group.

The results of the present study are in agreement with Xu who showed weight loss, who demonstrated significant reduction in systolic blood pressure who detected ST segment elevation and QT interval prolongation in doxorubicin treated rats. Demonstrated increase cardiac MDA and Shao detected cardiac histopathological changes in rats treated with doxorubicin.

Arunachalam et al. showed that doxorubicin can alter glucose metabolism through inhibition of insulin secretion or increase the insulin resistance. Elevation of cardiac enzymes in rats following cumulative dose of doxorubicin was detected by Ref.'s.

Different cellular mechanisms may explain doxorubicin-induced cardiac toxicity as increase intracellular iron accumulation causing increased oxidative stress, preventing the repair of damaged DNA strands, changes in vascular endothelium-derived vasoactive mediators (endothelin-1 and cardiac nitric oxide) and alteration of cardiac-specific gene expression including structural, metabolic and enzyme activities.

Co-administration of either metformin with doxorubicin (group V) or sitagliptin with doxorubicin (group VI) in the present study resulted in significant improvement of the body weight, blood pressure and heart rate, significant attenuation of doxorubicin-induced ST segment elevation and QT interval prolongation, significant reduction in the elevated serum level of CK-MB, LDH, blood glucose and the elevated cardiac MDA level, significant improvement of cardiac contractility in response to isoprenaline and significant improvement of histopathological signs of cardiotoxicity compared to doxorubicin treated group (group IV).

The present study prove that metformin produced more significant improvement than that produced by sitagliptin regarding systolic blood pressure, ST segment elevation, serum level of CK-MB, LDH, cardiac MDA level and histopathological finding and isolated heart contractility in response to isoprenaline.

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

The findings of the present study are in agreement with Aleisa et al. who reported that pre-treatment with metformin could improve doxorubicin-induced increase in MDA in mice and Kelle et al. who detected the protective effect of metformin could reduce cardiac MDA and the serum level of CK-MB and LDH in doxorubicin treated rats.

Metformin can ameliorate doxorubicin-induced oxidative stress through; increase cardiac superoxide dismutase (SOD) activity, increase in the glutathione level in heart tissue, enhancement of endothelium-dependent relaxation factor protection, modulation of ferritin heavy chain (FHC), that is responsible for sequestration of any excess intracellular iron and prevent mitochondrial damage induced by doxorubicin.

Metformin through its activation of 5′-Adenosine Monophosphateactivated Protein Kinase (AMPK) reduces the generation of ROS and increases endothelial nitric oxide synthase phosphorylation which prevents the opening of the mitochondrial permeability pores. Also, AMPK can up regulate the activity of peroxisome proliferatoractivated receptor gamma coactivator-1α (PGC-1α), a tissue-specific transcriptional co-activator which plays a key role in regulating energy metabolism and mitochondrial bioenergetics responses.

Metformin increases the circulating levels of adiponectin and increase the expression of adiponectin receptors (adipoR1 and adipoR2) in skeletal muscle and adipose tissue. Adiponectin is an adipokine synthesized in the adipose tissue, exerts vasodilator, antiapoptotic, anti-inflammatory and anti-oxidative activities in both cardiac and vascular cells and attenuate the apoptosis induced by doxorubicin.

Prevention of the activation of caspase-3 may attribute in the metformin induced cardio protection. The caspase-3 protein is a member of protease (caspase) family that plays a central role in the execution phase of cell apoptosis.

Cardio protective effect of sitagliptin may be due to reduction in inflammatory markers by down-regulation of COX-2 expression and iNOS expression, decreasing free radicals and nitro oxidative stress parameters (MDA and NOx) by increase SOD activity.

Stromal cell-derived factor 1α (SDF1α) is degraded by DPP4, so, Sitagliptin by inhibition of DPP4 can increase the stromal cell-derived factor 1α (SDF1α) concentration that promote vascular repair and neoangiogenesis. Also, DPP4 inhibition exerts ant atherosclerotic effects and reduces inflammation via inhibition of toll-like receptor 4- mediated up regulation of IL1β, IL6, and other proinflammatory cytokines.

Furthermore, sitagliptin may decrease the expression of fibrosis markers as transforming growth factor b (TGF-b1), tissue inhibitor of matrix metalloproteinases (TIMP-1) and collagen (Col1a1 & Col3a1) and vascular calcification marker, osteopontin that is known to be associated with vascular calcification and cardiovascular morbidity in humans.

The observed beneficial effects of metformin and sitagliptin could generate increasing interest in their potential use as adjunctive therapy to patients under treatment with doxorubicin. Clinical trials are needed to confirm the protective effect of metformin and sitagliptin on cardiotoxicity induced by doxorubicin. Also, safety profile for the concomitant use of either metformin or sitagliptin with doxorubicin for longer periods and in patients with malignant tumours must be studied.

Footnotes

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologie developments in antitumor activity and cardio toxicity. Pharmacol Rev 56: 185-229.

2. Herman EH, Zhang J, Chadwick DP, Ferrans V (2000) Comparison of the protective effects of amifostine and dexrazoxane against the toxicity of doxorubicin in spontaneously hypertensive rats. Cancer Chemother Pharmacol 45: 329-334.

3. Danz ED, Skramsted J, Henry N, Bennett JA, Keller RS (2009) Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway. Free Radic Biol Med 46: 1589-1597.

4. Horenstein MS, Vander Heide RS, L'Ecuyer TJ (2000) Molecular basis of anthracycline-induced cardiotoxicity and its prevention. Mol Genet Metab 71: 436-444.

5. Ioannidis JP, Hesketh PJ, Lau J (2000) Contribution of dexamethasone to control of chemotherapy-induced nausea and vomiting: a meta-analysis of randomized evidence. J Clin Oncol 18: 3409-3422.

6. Suba Z, Ujpál M (2006) Correlations of insulin resistance and neoplasms. Magy Onkol 50: 121-135.

7. El Messaoudi S, Rongen GA, De-Boer RA, Riksen NP (2011) The cardioprotective effects of metformin. Curr Opin Lipidol 22: 445-453.

8. Bhamra GS, Hausenloy DJ, Davidson SM (2008) Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. Basic Res Cardiol 103: 274-284.

9. Gundewar S, Calvert JW, Jha S (2009) Activation of AMP-activated protein kinase by metformin improves left ventricular function and survival in heart failure. Circ Res 104: 403-411.

10. Shah DD, Fonarow GC, Horwich TB (2010) Metformin therapy and outcomes in patients with advanced systolic heart failure and diabetes. J Card Fail 16: 200-206.

11. Kooy A, De Jager J, Lehert P, Bets D, Wulffelé MG, et al. (2009) Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. Arch Intern Med 169: 616-625.

12. Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, et al. (2004) Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation 109: 962-965.

13. Chaykovska L, von Websky K, Rahnenfu¨hrer J, Alter M, Heiden S. et al. (2011) Effects of DPP-4 inhibitors on the heart in a rat model of uremic cardiomyopathy . PLoS ONE 6: e27861.

14. Reagan-Shaw S, Nihal M, Ahmed N (2008) Dose translation from animal to human studies revisited. FASEB J 22: 3659-3661.

15. Irvine RJ, White J, Chan R (1997) The influence of restraint on blood pressure in the rat. J Pharmacol Toxicol Methods 38: 157-162.

16. Buelke-Sam J, Holson JF, Bazare JJ, Young JF (1978) Comparative stability of physiological parameters during sustained anesthesia in rats. Lab Anim Sci 28: 157-162.

17. Kobashigawa LC, Xu YC, Padbury JF, Tseng YT, Yano N (2014) Metformin protects cardiomyocyte from doxorubicin induced cytotoxicity through an AMP-activated protein kinase dependent signaling pathway: an *in vitro* study. PloS ONE 9: 104888.

18. De Nigris F, Rienzo M, Schiano C, Fiorito C, Casamassimi A (2008) Prominent cardioprotective effects of third generation beta blocker nebivolol against anthracycline-induced cardiotoxicity using the model of isolated perfused rat heart. Eur J Cancer 44: 334-340.

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

19. Kozlovski VI, Lomnicka M, Chlopicki S (2006) Nebivolol and carvedilol induce NO-dependent coronary vasodilatation that is unlikely to be mediated by extracellular ATP in the isolated guinea pig heart. Pharmacol Rep 58: 103-110.

20. Chan YH (2003) Biostatistics 102: quantitative data-parametric & non-parametric tests. Singapore Med J 44: 391-396.

21. Xu Y, Liu Z, Sun J, Pan Q, Sun F, et al. (2011) Schisandrin B prevents doxorubicin-induced chronic cardiotoxicity and enhances its anticancer activity *in vivo*. PloS ONE 6: 28335.

22. Tatlidede E, Şehirli Ö, Velioğlu-Öğünç A, Çetinel Ş, Yeğen BÇ, et al. (2009) Resveratrol treatment protects against doxorubicin-induced cardiotoxicity by alleviating oxidative damage. Free Radic Res 43: 195-205.

23. Khatib NA, Medi S, Patel J (2011) Evaluation of concomitant treatment of simvastatin and zingiber officinale in doxorubicin induce cardiotoxicity in wistar rats. IJRAP 2: 660-664.

24. Zhou S, Palmeira CM, Wallace KB (2001) Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicol Lett 121: 151-157.

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences