Original Article

Protective Effects of Metformin on Doxorubicin-Induced Cardiotoxicity and its Early Detection

Khalida Ajmal[1], Saima Rafique[1], Ayesha Afzal[1], Abeera Sikandar[1] and Uzma Naeem[2]

ABSTRACT

Objective: To evaluate the protective effects of Metformin on Doxorubicin cardiac toxicity and to identify myocardial damage at early phase by quantitative estimation of serum Troponin I (cTnI).

Study Design: Randomized lab base study

Place and Duration of Study: This study was conducted at the Conducted at the Departments of Pharmacology, Army Medical College, Rawalpindi in collaboration with CREAM (Centre for Research in Experimental and Applied Medicine) and completed in four months from April to July 2015.

Materials and Methods: Eighteen healthy male adult rabbits randomly divided into three batches were used. Doxorubicin was administered in a group of rabbits to produce cardiotoxicity; while control group received normal saline. The third experimental group got pretreatment for ten days with Metformin before doxorubicin administration.

Results: Doxorubicin inflicted marked cardiac damage apparent by elevated serum biomarkers (LDH, CK-MB and cTnI) levels and necrosed cardiomyocytes on histological examination. Metformin pretreatment ensued in decreased serum levels of biomarkers and improved the histological grades of heart tissue.

Conclusion: The doxorubicin based chemotherapy can be made efficacious with the concomitant administration of Metformin. The quantitative assessment of serum cTnI for recognition of cardiotoxicity at early stage may direct to substantial financial bearing and improved quality of life in cancer survivors.

Key Words: Doxorubicin (Dox), Metformin, Cardiac Troponin I (cTnI), Lactate Dehydrogenase (LDH), Creatine kinase-MB (CK-MB). Adenosine monophosphate-activated protein kinase (AMPK), Endothelial Nitric Oxide Synthase (-eNOS).

Citation of article: Ajmal K, Rafique S, Afzal A, Sikandar A, Naeem U. Protective Effects of Metformin on Doxorubicin-Induced Cardiotoxicity and its Early Detection Med Forum 2020;31(9):185-189.

INTRODUCTION

Doxorubicin (Dox), one of the anthracyclines, isolated from Streptomyces peuctius, is a potent anticancer agent. It is widely used for chemotherapy of several deadly solid and heamatological cancers since half century however cardiac toxicity documented among 20% patients limits its use in full clinical doses[1, 2].

Its main cytotoxic effects are exerted due to blockade of DNA topoisomerase IIβ & DNA interposing in cancer cells. The semiquinone centered unstable intermediate compounds of Dox generate highly reactive oxidized free radicals that are responsible for cardiotoxicity.

[1.] Department of Pharmacology and Therapeutics, Wah Medical College, Wah Cantt.
[2.] Department of Pharmacology and Therapeutics, Islamic International Medical College, Rawalpindi.

Correspondence: Dr. Khalida Ajmal, Associate Professor of Pharmacology and Therapeutics, WMC, Wah Cantt.
Contact No: 03435654521, 03005347951
Email: mrskhalida57@gmail.com , majmalrana@yahoo.co.uk

Received: May, 2020
Accepted: July, 2020
Printed: September, 2020

The cardiac cells have innate vulnerability to oxidative stress. These free radicals inflict nuclear DNA damage and impair mitochondrial functions.[3,4] These metabolites also lead to myofibrils structural proteins damage causing myocytes necrosis, resulting in release of the cadio-specific cardiac troponins I (cTnI) and cytosolic enzymes like creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH) in blood.[5,6] cTnI is myocardial contractile protein and does not normally circulate in the circulation. It is expressed only in myocardium and is more abundant than CK-MB. cTnT is probably less cardiac specific.

cTnI is considered most specific and highly sensitive biomarker of cardiotoxicity. cTnI estimation reveals the existence of cardiotoxicity at an initial phase, considerably before compromised cardiac function can be diagnosed by any other procedure.[5]

The cardiaotoxicity caused by Dox necessitates lifelong concerns and costly medical management. Cardiac monitoring is largely done by qualitative estimation of cTnI which is often unreliable. As significant number of cancer patients comes from countries with poor socio-economic conditions further imposing financial burden. The quantitative measurement of cTnI, offers a promising alternate in effective monitoring of cancer survivors.[7]

Numerous pharmacological modulations for prevention of cardiotoxicity have been suggested and are currently in practice. Yet, protection imparted by these is often limited and is expensive as well[9, 10, 10].

Metformin, a bigaunide, is used all over the world in type 2 diabetes mellitus as it lowers basal and postprandial glucose level. It is also prescribed to treat prediabetes, gestational diabetes mellitus and has other off-label uses in polycystic ovarian syndrome, weight loss and cancer[11]. It has been reported to prevent Dox induced cardiotoxicity[12]. Metformin exerts its beneficial effects through various possible mechanisms. It decreases in oxidative stress and free radical generation by prevention of ferritin heavy chain expression in cardiomyocytes. The drug increases the glutathione level in heart tissue and restores mitochondrial bioenergetics[13]. In experimental animal models, metformin has shown the increased tolerance of the myocardium to ischemia-reperfusion injury and decrease in occurrence of heart failure after infarction[16]. Metformin is also said to enhance the efficacy of doxorubicin.[13]

The aim of the study was the definite and early recognition of cardiac damage by estimation of cTnI and its probable amelioration by Metformin, ensuring better results with Dox based chemotherapeutic regimen.

MATERIALS AND METHODS

This experimental study was reviewed and approved by Ethical Committee of "Centre for Research in Experimental and Applied Medicine" (CREAM), and conducted in the Pharmacology Department, Army Medical College, Rawalpindi. Eighteen male healthy adult rabbits, each with average weight of 2.0 kg were divided randomly into groups. Control Group-1: (n=6) was daily administered 2 ml of normal saline solution orally for whole period of study. Experimental Group-2: (n=6) was given injection Doxorubicin 12 mg/kg body weight (BW) into marginal vein of rabbit's ear on tenth day of experiment. Group-3: (n=6) received Metformin 250 mg/kg BW orally for eleven days consecutively and a single dose of Dox 12 mg/kg BW on the tenth day by intravenous route. The blood for biomarker study was drawn from rabbit's ear in the beginning and on the last day of the study. After centrifugation of the blood, plasma was stored in serum storage vials at -20^0 C for analysis of biomarkers.

Doxorubicin HCL was purchased from Park-Davis Pak while Metformin from Merck. cTnI kit was procured from Pharmaceutical Manufacturers' Association while CK-MB and LDH kits from Merck Pak Ltd.

Measurement of cTnI and CK-MB is based on general principle that there is striking similarity between human and rabbit CK-MB sequences and cTnI antibodies. A cut-off value of 0.50 ng/ml cTnI was proposed by kit manufacturer for diagnosing myocardial damage. CK-MB estimation was carried out as per the principles of IFCC. All the rabbits were weighed and slaughtered to take out the heart. Cardiac tissue sections were prepared for histological study under electron microscope. Qualitative and quantitative assessment was done on all myocardial sections. Semiquatitative histological classification and scoring was done in accordance with Billingham technique[14].

Statistical analysis of serum results was computed on SPSS 22 and expressed as means ± standard error of means (SEM) calculated by using One Way ANOVAs. Chi square test was utilized for histopathological analysis. The p value <0.05 was considered significant to measure the difference between two observations.

RESULTS

Observation of individual parameters

Changes in Body Weight: The rabbits in control group-1 gained weight by 12.52±2.69%, however Goup-2 animals (receiving12 mg/kg of doxorubicin) showed fall of 26.88±2.03 %. The Gp-3 animals (receiving both doxorubicin and metformin) displayed less fall in weight of 5.55±7.78 percent as compare to Gp-2.

Serum Cardiac troponin I (cTnI): Cardiac troponin I levels measured in ng/l, were strikingly raised upto 10.00±0.00 among rabbits of Goup-2 while levels in Gp-1 remained normal. The rise in levels of cTnI (2.50±1.00 ng/l) in Gp-3 were much low as compared to Gp-2 although quite raised when matched with levels of group-1 with significant value of p (<0.05) (table-1& fig-1).

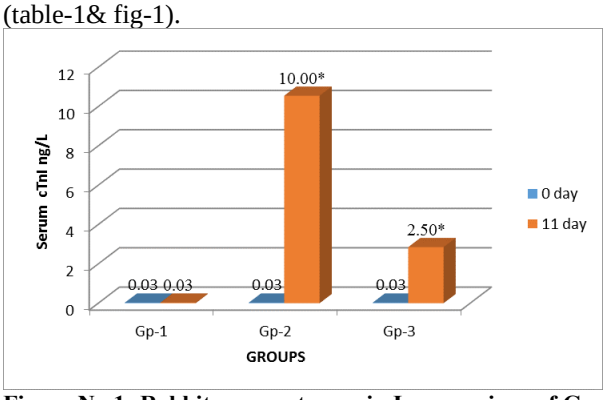

Figure No.1: Rabbits serum troponin I comparison of Gp-1 control, Gp- 2 receiving toxic dose doxorubicin (Dox), Gp-3 treated with metformin and Dox *Significance <0.05

Serum CK-MB: Serum CK-MB (U/L) increased considerably among Goup-2 to 345 ±36 in comparison to 122±4. (Control Gp-1), with statistically significant p value. The Gp-3 rabbits receiving pretreatment with metformin exhibited less rise (180 U/L±10.00) in levels with significant value (p<0.05) when equated with group-2(table-1).

Serum LDH: Serum LDH (U/l) were elevated noticeably up to 1413±112 in Gp-2 in contrast to Gp-1

and with significant p<0.005. In Gp-3, there was no increase (636±54 U/l) in LDH levels seen (table- 1).

Histopathological Analysis: Microscopic study of heart tissue sections among control Gp-1 revealed no damage to the cardiomyocytes. The gross disruption in cardiac cells histological texture was observed in Gp-2 (toxic group) with no obvious normality. The 85.0% heart sections of this group displayed necrosis of grade-3 and of grade- 2 only among 15.0%. The ventricular sections manifested interstitial edema, vacuolization and clumping of nuclear matter. In few slides, there was structural breaks of myofibrils. The mild necrosis of grade-1 was witnessed in 65.6 percent in heart sections of Gp-3 (treated with metformin and doxorubicin) and 16.7% percent of sections exhibited thorough inhibition while the equal number showed modest damage measured as necrosis (table and figure- 2).

Table No.1: The values of estimated Serum biomarkers of rabbits in control Goup-1, doxorubicin Goup-2, Goup-3 receiving both metformin plus doxorubicin

Serum biomarkers	0 day			11th day			P value
	Groups			Groups			
	1	2	3	1	2	3	
Ctni (ng/l) ± sem	0.03 ±0	0.03 ±0	0.03 ±0	0.03 ±0	10.00 ±0	2.50 ±1	0.000*
Ck-mb (u/l) ± sem	120 ±10	125 ±15	121 ±4	122 ±4	345 ±36	180 ±7	0.000*
Ldh (u/l) ± sem	700 ±25	674 ±11	711 ±26	750 ±24	1413 ±112	644 ±50	0.000*

Table No.2: Grades * Groups Cross tabulation

			Groups			Total
			1	2	3	
Grades	Normal	Count	6	0	0	1
		% within Groups	100.0%	100.0%	.0%	5.6%
	Mild	Count	0	0	1	5
		% within Groups	.0%	00.0%	17.2%	27.8%
	Moderate	Count	0	1	4	6
		% within Groups	0.0%	15.0%	65.6%	33.3%
	Severe	Count	0	5	1	6
		% within Groups	0.0%	85.0%	17.2%	33.3%
Total		Count	6	6	6	18
		% within Groups	100.0%	100.0%	100.0%	100.0%

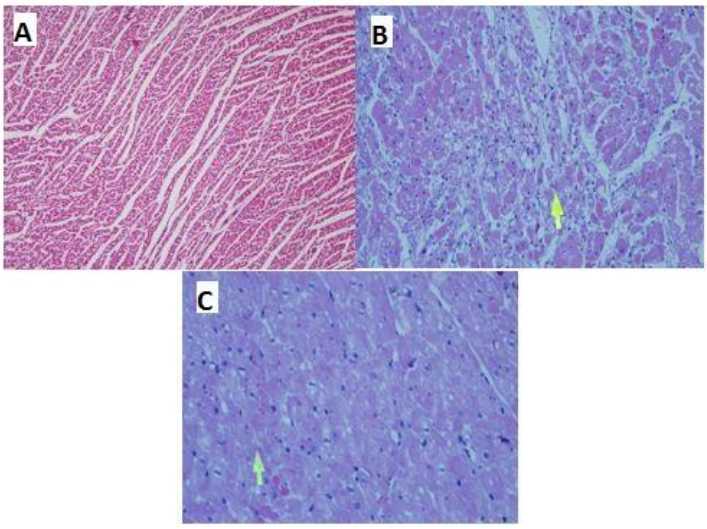

Figure No.2: The (Heamtoxyline & Eosin) stained micrographs (×300) from specimens of rabbit cardiomyocytes: (A) from (Gp-1)) with cardiac cell structure, (B) picture of Gp-2 showing extent of cardiac damage of toxic dose of Dox (C) and micrograph of rabbit heart cell from Gp-3 showing less derangement of myofibrils as compared to Gp-2.

DISCUSSION

The study was aimed to evaluate possible prevention of dox-induced cardiotoxicity with Metformin and its early, reliable detection by quantitative estimation of cTnI that might help to institute any interventional strategy.

In our study, group of animals exposed to toxic dose of doxorubicin showed considerably deranged serum biomarkers and grade-3 necrosis of heart tissue. There was increase of 58.32% in LDH, 147.39% increase in CK-MB and serum cTnI values with increase of 30110.53% all with significant difference in comparison with control group rabbits. Histologically, heart sections of Goup-2 expressed necrosis of grade-3 while picture from Gp-1 animals was completely normal. Similar changes both in morphology and biomarkers following the use of doxorubicin in rabbits were observed by many other researchers. Cardinale and colleagues in clinical study on cancer patients and Sawyer and his colleagues in both their in -vitro and in -vivo preclinical experiments recognized that doxorubicin in varying concentrations produced a substantial cardiac cells death and apoptosis within 1-2 days of its administration. It was depicted in our histological slides too and our results are fairly homogenous with these studies[,16].

Doxorubicin remains a preferred therapeutic agent for various solid and heamatological malignancies. However, a noteworthy number (20%) of patients develop cardiotoxicity causing lifelong co-morbidities and this clinical impact is growing with increasing cancer survivors.[3,4] In this experiment, the reason for inculcating measurement of LDH and CK-MB was their chronological worth and main emphasis remained on highly sensitive and specific biomarker cTnI. As recognized by The Food and Drug Administration and European Medicines Agency, it is considered the benchmark serum biomarker of cardiac damage in all species of mammals to detect the early anthracyclin-induced cardiotoxicity. cTnI is exclusively a myocardial biomarker, no evidence of cTnI presence in injured or healthy skeletal muscle and in other tissue has been found. Jaffe and others, in clinical study established that measuring cTnI levels perceives cardiotoxicity very early, positively before cardiac dysfunction can be discovered by any other diagnostic modalities. cTnI has also been integrated into the National Cancer Institute (NCI) for classifying anticancer therapy induced cardiotoxicity [17].

The subsequent part of experiment work was conducted to appraise the protection conferred by metformin. The Goup-3 rabbits pretreated with 250 mg/kg metformin for ten consecutive days prior to administration of doxorubicin revealed the value of cTnI depicting significant difference with 75.84% less upsurge, 42.93% less elevation in CK-MB and LDH with 57.42% less rise in Goup-3 in relation to Goup-2. Hisopathological alterations were also analogous with significant difference ($p<0.000$). The morphological grading shifted from necrosis of grade 3 to 1 whereas one-quarter of the tissue slidee exhibited fairly normal myofibrillar arrangement. The weight loss (4.57±2.48%) among the animals of this group was of lesser degree.

The cardioprotective actions of metformin are mediated by an AMPK-eNOS signaling pathway. Activation of adenosine monophosphate-activated protein kinase, increased formation of adenosine, and the prevention of the mitochondrial injury all contribute to cardioprotection by metformin.[20] Metformin therapy attenuates post infarction cardiac remodeling.[19] Moreover metformin has been explored to reduce radiation-induced cardiac toxicity risk in women with early-stage breast cancer being treated with doxorubicin containing regimen.[20].

CONCLUSION

The promising results from this study supported that the quantitative evaluation of cTnI may be crucial for early detection of Doxorubicin inflicted cardiotoxicity. Subjects with negative troponin may be barred from costly and long term programs for cardiac monitoring. Similarly, pretreatment with metformin is beneficial as it has attenuated the doxorubicin induced cardiotoxicity.

Acknowledgements: The experiment was partly funded by National University of Sciences and Technology (NUST), Islamabad, Pakistan

Author's Contribution:

Concept & Design of Study:	Khalida Ajmal
Drafting:	Abeera Sikandar
Data Analysis:	Uzma Naeem
Revisiting Critically:	Ayesha Afzal, Rashada Farooqi
Final Approval of version:	Saima Rafique

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Schulz D, Nachtigall J, Geisen U, Kalthoff H, Imhoff JF, Fiedler HP, Süssmuth RD. Aranciamycin anhydride, a new anthracycline-type antibiotic isolated from Streptomyces. J Antibiot 2010;63(7):397-9.
2. Thorn CF, Oshiro C, Marsh S, Hernandez-BoussarT, McLeod H, Klein TE, et al. Doxorubicin pathways: pharmacodynamics and adverse effects Pharmacogenet Genomics 2010; 21(7):440-6.
3. Matt S, Hofmann TG. The DNA damage-induced cell death response: a roadmap to kill cancer cells. Cellular Molecular Life Sci 2016;73(15):2829–50.

4. Cappetta D, Angelis AD, Sapio L, Prezioso L, Illiano M, Quaini F, et al. Oxidative Stress and Cellular Response to Doxorubicin: A Common Factor in the Complex Milieu of Anthracycline Cardiotoxicity. Oxid Med Cell Longev 2017:1521020.
5. Desai VG, C Kwekel J, Vijay V, Moland CL, Herman EH, Lee T, et al. Early biomarkers of doxorubicin-induced heart injury in a mouse model. Toxicol Appl Pharmacol 2014;281:221–9
6. Singh D, Thakur A, Tang WH. Utilizing cardiac biomarkers to detect and prevent chemotherapy-induced cardiomyopathy. Current Heart Failure Reports 2015;12(3):255-262.
7. Tan LL, Lyon AR. Role of Biomarkers in Prediction of Cardiotoxicity during Cancer Treatment. Curr Treat Options Cardiovasc Med 2018;20(7):55.
8. Henriksen PA. Anthracycline cardiotoxicity: An update on mechanisms, monitoring and prevention. Heart 2018;104:971–7.
9. Ajmal K, Ajmal M, Afzal A, Saima, Naeem U, Sikandar A. Comparison of protective effects of carvedilol and a-tocopherol on doxorubicin-induced cardiotoxocity. Int J Basic Clin Pharmacol 2019; 8:1944-50.
10. Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. J Am Coll Cardiol 2014;64:938–45.
11. El Messaoudi S, Rongen GA, de Boer RA, Riksen NP. The cardioprotective effects of metformin. Curr Opin Lipidol 2011;22:445–53.
12. Asensio-Lopez MC, Sanchez-Mas J, Pascual-Figal DA, Abenza S, Perez-Martinez MT, Valdes M, et al. Involvement of ferritin heavy chain in the preventive effect of metformin against doxorubicin-induced cardiotoxicity. Free Radic Biol Med 2013;157:188–200.
13. Ashour AE, Sayed-Ahmed MM, Abd-Allah AR, Korashy HM, Maayah ZH, Alkhalidi H, et al. Metformin rescues the myocardium from doxorubicin-induced energy starvation and mitochondrial damage in rats. Oxid Med Cell Longev 2012:434195.
14. Billingham ME, Mason JW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat Rep 1978;62(6):865-72.
15. Cardinale D, Sandri MT. Role of biomarkers in chemotherapy-induced cardiotoxicity. Prog Cardiovasc Dis 2010; 53(2):121-9.
16. Sawyer DB, Zuppinger C, Miller TA, Eppenberger HM, Suter TM. Modulation of anthracycline-induced myofibrillar disarray in rat ventricular myocytes by neuregulin-1ß and anti-erbB2: potential mechanism for trastuzumab-induced cardiotoxicity. Circulation 2010;105:1551-4.
17. Howlader N, Noone AM, Krapcho M, Miller Brest A, Yu M, et al. SEER Cancer Statistics Review, 1975-2016. National Cancer Institute, Bethesda, MD.
18. Kobashigawa LC, Xu YC, Padbury JF, Tseng YT, and Yano N. Metformin protects cardiomyocyte from doxorubicin induced cytotoxicity through an AMP-activated protein kinase dependent signaling pathway: an in vitro study. PLoS One 2014; 9:e104888.
19. Mustafa A, Kazım Ü, Mehmet F.S, Abdullah Ö, DeryaKt, Zeynep, et al Cardioprotective effect of metformin against doxorubicin cardiotoxicity in rats. Anatol J Cardiol 2016;16(4): 234–241.
20. Jung-Min Yu, Mao-Chih Hsieh, Lei Qin, Jiaqiang Zhang, Szu-Yuan Wu Metformin reduces radiation-induced cardiac toxicity risk in patients having breast cancer. Am J Cancer Res 2019;9(5):1017–1026.