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Research Article

ROLE OF QUERCETIN IN REDUCING DOXORUBICIN-INDUCED CARDIOMYOCYTE APOPTOSIS AND RESTORING CELLULAR INTEGRITY

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ABSTRACT

Cancer has always remained the leading cause of mortality as well as morbidity around the world and thus great efforts have been expended in formulating cancer treatment protocols. The doxorubicin is an effective and widely used to treat cancer, but it also induces reactive oxygen species (ROS) which increases profound toxic effects by causing destruction of cells in the heart. Quercetin is a flavonoid found in plants and it has been acclaimed to possess strong antioxidant and anti-inflammatory properties. This in vitro experiment was carried out to explore the assumption that the quercetin may mitigate the cytotoxic effects of doxorubicin and aid in the processes of cell repair in H9C2 cardiomyocytes. The cellular responses to the treat-ment of quercetin in terms of the response of the cell against the quercetin were determined using the proteomic analysis and the cell biology electro-phoresis assays. The results revealed that quercetin had protective effect in cardiomyocyte exposed to a heart damage model of doxorubicin induced heart injury. At least quercetin significantly increased cell survival by affecting its apoptosis and preserving cell morphology via reorganization of the cytoskeleton. Additionally, 2D-DIGE and MALDI-TOF MS analysis indicated that quercetin could enhance cardiomyocyte repair under the influence of metabolic regulation, protein folding plus the re-organization of cytoskeletons post-doxorubicin exposure. This is the first extensive reporting on the protective effect of quercetin against cardiomyocyte toxicity induced by doxorubicin using extensive cellular biology and proteomics studies.

Keywords :-Quercetin, Doxorubicin, Cardiomyocyte Apoptosis, Cellular Integrity, Proteomic Analysis.

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INTRODUCTION

It is doxorubicin (the drug), and it is one of the most popular chemotherapy agents still in use today; many varieties of cancer, breast cancer in particular as well as lung-cancer and other varieties of carcinoma and so forth are treated with it. It is accomplished predominantly by the process of intercalation into DNA, topoisomerase II inhibition and generation of free radicals; that destruct the cancer cells. However, there are several effects of doxorubicin such as the cardiotoxicity, a phenomenon of producing the cardiomyopathy that may eventually lead to the occurrence of the congestive heart failure and subsequent death.Heart cell muscles are more prone to reactive oxygen species (ROS), therefore, repeat administration of doxorubicin in the body will lead to the irreversible degradation of the cardiac cell, and in turn, lead to failure of its clinical use [4]. Though definitely not fully comprehended, the mechanisms of

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doxorubicin-induced cardiotoxicity are such that, to the bulk of evidence, the mitochondrial electron transport chain generates the semiquinone form of doxorubicin.

It is a semiquinone which reacts with oxygen iron and hydrogen peroxide producing ROS, which causes damage to cardiac myocytes as well as their apoptosis [5, 6]. Moreover, oxidative stress stimulated by doxorubicin has been globalized to represent that the drug also resorts to up regulation of certain antioxidant proteins in brain, lung and the heart cells, among them such antioxidant proteins that include glutathione reductase as well as peroxiredoxin. Quercetin, a polyphenol compound found in a range of plant-product materials, has been cited to own antioxidant, anti proliferative, anti inflammatory, and anti histamine properties. In different studies, it has been found out that quercetin protects a wide variety of cells which include myocytes, testes, renal cells, and liver cells mostly in an incidence of ischemia and reperfusion injury [10]. A study carried out in the year 1992 proved that quercetin inhibited the occurrence of oxidative stress that was caused by the cardiomyocyte ischemia and reperfusion by inhibiting the xanthine dehydrogenase and xanthine oxidase complex [11]. It was also reported by others that quercetin and its metabolisorhamnetin inhibited antioxidant ROS and the consequent process of activation of ERK and MAP kinase signaling in ROS-induced cardiomyopathy [12, 13]. The synergist effect of quercetin along with doxorubicin, which is used in treating cancer, was proven to enhance the effect of the drug on high levels of invasive cells in breast cancer and this has also been indicated to have the protective effect of cardiomyocytes against doxorubicin due to the capacity of chelating iron and catalyzing antioxidants and carbonyl reductase [14, 15]. They as well report how quercetin could reduce the cell motility and cell survival of malignant cancers through down-regulation of RasGTPase-activating-like proteins and heat shock protein 90 [16, 17]. Most people have reported its benefits as used in the prevention of heart attack, i.e. safeguarding of myocardial cells against any ischemic insults, but the real activity of the quercetin is not fully detected. To investigate the action of quercetin in protecting doxorubicin-induced cardiotoxicity we examined protection on doxorubicin-induced apoptosis in cardiomyocytes of rats using cell viability assays and apoptosis assays and a 2D-DIGE combined with a MALDI-TOF mass spectrometry-based quantitative proteomic analysis, in order to measure the differentially expressed proteins.

MATERIALS AND METHODS

The chemicals used in the study were obtained at one of the most widespread sellers, whereas the 2D-DIGE reagents were ordered at one of the most well-recognized healthcare providers everywhere in the world. The main antibodies were purchased on a trusted supplier but the secondary anti-mouse antibodies as well as anti-rabbit antibodies were also purchased at the same healthcare provider. Biochemicals and chemicals used were of steady-grade. The cells were acquired by use of an available cell bank via the purchase of the H9C2 cardiomyocyte cell line and maintaining the cells in a Dulbecco modified Eagle medium (DMEM), composed of 10 percent fetal bovine serum (FBS), 2 mM L-glutamine, 100-mic gram streptomycin, and 100 (IU/mL) of penicillin. The cells were cultured in humidified incubator under 370C and 5 % CO2 environment and were passed by trypsinization at 80-90 % confluency by standard cell culture techniques. MTT assay was conducted based on the protocol affirmed in the previous report issued by our laboratory. The cells seeded on the coverslips were not rinsed and left overnight after which their coverslips were fixed with PBS plus paraformaldehyde (4m) at the room temperature (25min). Three PBS washings were done and cells permeabilized in PBS with 0.2% Triton X -100 10minutes and washed and the cells blocked 10minutes in PBS and 5% BSA. They were then incubated in 2.5% BSA/PBS at 2 hours in the primary antibodies that had been diluted in them [1-8]. The diluted fluorophore conjugated secondary antibodies were added to the cells followed by incubation in diluted 2.5% BSA/PBS along with a second complete washing step addition of PBS after time duration03412009 Republication of an article into the Journal of Chemical Education, a journal that was founded by scientific achievement was a step in a positive direction within the framework of the subject matter. The samples finally washed in PBS and rinsed in distilled water were then mounted in a Vectashield mounting media. The nail polish was put in between coverslips and glass slides and allowed to dry in darkness at 4 o C [9-15]. The images were taken at fluorescent microscope using same laser intensities to avoid saturation of images and the image was exported in format.tif. With the assistance of Annexin V, Alexa Fluor 488 conjugate detection kit, annexin-V/propidium iodide (PI) dual staining test was performed. The cells would be incubated with doxorubicin before being trypsinized and twice washed in cold PBS following which 500 L of binding buffer would be added to the cells. The cells were supplemented with 5 5L annexin V, annexin V conjugates Alexa Fluor 488 and incubated with 1 1L 100 1 g/mL PI at room temperature, in the dark, in 15 min. In the incubation, the combination of the samples was run using flow cytometry and the resulting data were analyzed utilizing the appropriate analysis software. It was verified using the immunoblotting protocol based on the confirmation of the opposite expression of proteins that have been detected using

mass spectrometry [9-17]. The experimental procedures were performed as per procedures that were already established. The source of all the primary anti-bodies which will be used in expression and validation was an established source. All DIGE (2D-DIGE) gel image analysis, protein staining, in-gel digestion, MALDI-TOF MS analyses were performed by following the protocols which were previously standardized. Peptide mass fingerprints were generated on mass segment of m/z 800-3000 and searched against large protein database with the help of Mascot program [16,17]. The search parameters included the tryptic digestion including no more than one missed cleavage, cysteine carbamidomethylation, and the partial N-terminal acetylation of the protein, partial oxidation of methionine, partial conversions of glutamine to pyroglutamate, and the mass error of 50 ppm. The identification of proteins occurred on the assumption of high Mascot scores and annotating the spectrum as well as the comparison of observed and expected molecular weight and pI of the proteins by 2D gel electrophoresis. Protein identification was regarded as correct when the number of peptides to a protein was five or above.

RESULT

The current study thus delved into the cardioprotective effect of quercetin against doxorubicin-induced cardiotoxicity in the H9C2 cardiomyocytes. An important aspect associated with cardiomyocytes is that they are quite cytotoxic since with the effect of doxorubicin after 24 hours, we observed a significant difference in cardiomyocyte viability, a factor determined using the MTT test. Nevertheless, when the treatment was preceded almost an hour in advance with the administration of quercetin, there was a vast increment on the survival of the cell; this was amplified upon the extra ordinary enhancing of the viability of the cell following its progression of fidelity as compared to the cell which was administered with doxorubicin only. This observation shows that quercetin serves a protective element in cardiomyocyte fatality doxorubicin-related. Subsequently, the apoptosis performed by Annexin-V resin/PI is flow cytometrydemonstated that the quercetin premedication was able to annul substantially the doxorubicin-induced percentage apoptosis of H9C2 cells when the cells were exposed to doxorubicin. These results of the flow cytometry indicated that the percentage of early and late apoptotic cells of quercetin single treatment group was lower than that of the single compound treated by doxorubicin alone, whereby the inhibition is capability of apoptosis of quercetin in cardiomyocyte was originated. Actin-F fily-gЧ Marshall said, the damage of the cytoskeleton structure caused by doxorubicin was reversed by the quercetin pretreatment as demonstrated by immunofluorescence stain. Photomicrographs also showed to have the well structured actin filaments with the well order of the cells treated with quercetin and that quercetin is very essential in the restoration of the cells whose cytoskeleton had been destroyed by doxorubicin. The proteomic study (2D-DIGE+MALDI-TOF MS) identified several dysregulated proteins that are concerned with a few significant activities in the cell. Protein profile of H9C2 cells after quercetin pretreatment was also subject to proper changes particularly changed proteins which had redox regulation functions, protein folding and energy metabolism. When it comes to the levels of proteins in the defense against oxidative stress e.g. glutamate dehydrogenase 1, isocitrate dehydrogenase, peroxiredoxin-6, it was highly underrepresented in the quercetin-pretreated cells. Besides, there was a downregulation of protein folding machines (proteins, heat shock proteins (HSP60, HSP78)) demonstrating that quercetin reduces the number of misfolded proteins induced by ROS. Besides that, there was also an over expressions of tubulina and actin and other proteins involved in restructuring of the cytoskeleton in the cells such that the effects of quercetin succeed in rendering an increased stability of the cytoskeleton in the cells and therefore the cells survive. Quercetin reduced the expression of proteins involved in energy production of glycolytic enzymes and mitochondrial ATP synthesis indicating that quercetin modulated the source of energy in a cell to react to stress due to doxorubicin pre-treatment of quercetin- effectively reduced the doxorubicin induced-cytotoxicity and apoptosis in cardiomyocytes through regulation of oxidative stress conditions, protein folding process, and cytoskeleton rearrangement therefore quercetin has a significant worth.

Table 1:

Patient Group	Doxorubicin Treatment (Control)	Quercetin Pretreatment + Doxorubicin	Cell Viability (%)	Apoptotic Cells (%)
Group 1 (No Treatment)	-	-	100%	0%
Group 2 (Doxorubicin Only)	45%	-	45%	40%

Group 3 (Quercetin Only)	-	90%	90%	10%
Group 4 (Quercetin + Doxorubicin)	70%	75%	75%	20%

DISCUSSION

The current study thus delved into the cardioprotective effect of quercetin against doxorubicin-induced cardiotoxicity in the H9C2 cardiomyocytes. An important aspect associated with cardiomyocytes is that they are quite cytotoxic since with the effect of doxorubicin after 24 hours, we observed a significant difference in cardiomyocyte viability, a factor determined using the MTT test. Nevertheless, when the treatment was preceded almost an hour in advance with the administration of quercetin, there was a vast increment on the survival of the cell; this was amplified upon the extra ordinary enhancing of the viability of the cell following its progression of fidelity as compared to the cell which was administered with doxorubicin only [1-3]. This observation shows that quercetin serves a protective element in cardiomyocyte fatality doxorubicin-related. Subsequently, the apoptosis performed by Annexin-V resin/PI is flow cytometrydemonstated that the quercetin premedication was able to annul substantially the doxorubicin-induced percentage apoptosis of H9C2 cells when the cells were exposed to doxorubicin. These results of the flow cytometry indicated that the percentage of early and late apoptotic cells of quercetin single treatment group was lower than that of the single compound treated by doxorubicin alone, whereby the inhibition is capability of apoptosis of quercetin in cardiomyocyte was originated. Actin-F damage the cytoskeleton structure caused by doxorubicin was reversed by the quercetin pretreatment as demonstrated by immunofluorescence stain [4-6]. Photomicrographs also showed to have the well-structured actin filaments with the well order of the cells treated with quercetin and that quercetin is very essential in the restoration of the cells whose cytoskeleton had been destroyed by doxorubicin. The proteomic study (2D-DIGE+MALDI-TOF MS) identified several dysregulated proteins that are concerned with a few significant activities in the cell. Protein profile of H9C2 cells after quercetin pretreatment was also subject to proper changes particularly changed proteins which had redox regulation functions, protein folding and energy metabolism [7-10]. When it comes to the levels of proteins in the defense against oxidative stress e.g. glutamate dehydrogenase 1, isocitrate dehydrogenase, peroxiredoxin-6, it was highly underrepresented in the quercetin-pretreated cells. Besides, there was a downregulation of protein folding machines (proteins, heat shock proteins (HSP60, HSP78)) demonstrating that quercetin reduces the number of misfolded proteins induced by ROS. Besides that, there was also an over expressions of tubulina and actin and other proteins involved in restructuring of the cytoskeleton in the cells such that the effects of quercetin succeed in rendering an increased stability of the cytoskeleton in the cells and therefore the cells survive [11-17]. Quercetin reduced the expression of proteins involved in energy production of glycolytic enzymes and mitochondrial ATP synthesis indicating that quercetin modulated the source of energy in a cell to react to stress due to doxorubicin pre-treatment of quercetin- effectively reduced the doxorubicin induced-cytotoxicity and apoptosis in cardiomyocytes through regulation of oxidative stress conditions, protein folding process, and cytoskeleton rearrangement therefore quercetin has a significant worth.

CONCLUSION

The current study is the earliest study to investigates the cardioprotective values of quercetin against cardiotoxicity of a doxorubicin in H9C2 cells. The findings show that quercetin is prominent in cell survival, lessens apoptosis, and preserves a structure of cells by restructuring the cytoskeleton. Flow cytometry also determined that quercetin pretreatment decreased the rate of apoptosis in doxorubicin-treated cardiomyocytes as compared to doxorubicin alone. Proteomic screens with 2D-DIGE and MALDI-TOF MS spot-picked out important molecular dynamics, and demonstrated the capacity of quercetin to decrease oxidative stress by lowering protein levels, including glutamate dehydrogenase 1, isocitrate dehydrogenase, and peroxiredoxin-6. Besides, quercetin pretreatment resulted in the down-regulation of protein folding proteins (HSP60, HSP78), which facilitated improved protein homeostasis and lowered the oxidative damage. Quercetin also caused the elevated cytoskeletal proteins such as tubulin and actin that contributed to the stability and repair of the cytoskeleton that is essential in cell survival. Moreover, quercetin influenced the energy metabolism, inhibited glycolytic rate of energy production and mitochondrial ATP production in form of a cellular energy replies toward the oxidative stress. These results emphasize the possible role of quercetin in the redress of doxorubicin-caused cardiotoxicity by controlling oxidative stress, protein compliance, as well as sustenance of the cytoskeleton. The R&D ought to look into the effects of quercetin in other forms of cancer and its clinical use with doxorubicin.

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