Research Article

Doxorubicin-Induced Cardiac Toxicity Is Mediated by Lowering of Peroxisome Proliferator-Activated Receptor δ Expression in Rats

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The present study investigates the changes of peroxisome proliferator-activated receptors δ (PPARδ) expression and troponin phosphorylation in heart of rats which were treated with doxorubicin (DOX). Wistar rats which were treated with DOX according to a previous method. The protein levels of PPARδ and troponin phosphorylation were measured using Western blot. The PPARδ expression in heart was markedly reduced in DOX-treated rats showing a marked decrease in cardiac dP/dT and cardiac output. Also, cardiac troponin phosphorylation was lowered in DOX-treated rats. Meanwhile, combined treatment with the agonist of PPARδ (GW0742) reversed the decrease of cardiac dP/dT and cardiac output in DOX-treated rats. Then, primary cultured cardiomyocytes from neonatal rats were used to measure the changes of calcium concentration in cells. In addition to both decrease of PPARδ expression and troponin phosphorylation in neonatal cardiomyocytes by DOX, a marked decrease of calcium concentration was also observed. Our results suggest the mediation of cardiac PPARδ in DOX-induced cardiotoxicity in rats. Thus, activation of PPARδ may restore the expression of p-TnI and the cardiac performance in DOX-induced cardio toxicity in rats.

1. Introduction

Doxorubicin (DOX) is a widely used chemotherapeutic agent in the treatment of tumors with a major side effect of cardiac toxicity [1, 2], which has not been effectively prevented by cardioprotective drugs [3, 4]. Thus, its clinical use is limited due to a cumulative dose-dependent cardiotoxicity including the electrocardiographic changes, arrhythmias, irreversible degenerative cardiomyopathy, and congestive heart failure [5–8]. The recent report showed that approximately 10% of patients treated with DOX or its derivatives will develop cardiac complications up to 10 years after cessation of chemotherapy [9]. Actually, the mechanism for cardiac toxicity caused by DOX or its metabolites is still not clear. Hypotheses regarding the cardiac toxicity of DOX include perturbation of calcium homeostasis, formation of iron complexes, and generation of radical oxygen species, mitochondrial dysfunction, and damage to cell membranes [10, 11].

PPARs are ligand-activated transcriptional factors that regulate expression of genes involved in lipid metabolism and inflammation [12]. Three subtypes of PPARs, PPARα, PPARγ, and PPARδ, modulate the expressions of many genes and exert various bioactivities [12]. PPARα is relatively abundant in tissues with a high oxidative capacity, such as liver and heart. PPARγ expression is confined to a limited number of tissues, primarily adipose tissue [12, 13]. The ubiquitously expressed PPARδ enhances the lipid catabolism in adipose tissue and muscle [12]. PPARδ-dependent maintenance of inotropic function and metabolic effects is crucial for cardiomyocytes [14–16]. Deletion of cardiac PPARδ, which is
accompanied by decreased contraction, increased left ventricular end-diastolic pressure, and lowered cardiac output, leads to decreased contraction and increased incidence of cardiac failure [14].

Our previous study showed that cardiomyopathy in type-1 like diabetic rats is associated with a marked decrease in cardiac PPARδ expression [17]. It seems possible that cardiac PPARδ expression is involved in the cardiac toxicity of DOX. Thus, in the present study, we used Wistar rats and primary neonatal rat cardiomyocytes to investigate the role of PPARδ in DOX-induced heart failure both in vivo and in vitro.

2. Materials and Methods

2.1. Materials. Doxorubicin from Sigma-Aldrich (St Louis, MO, USA) and GW0742 from Santa Cruz Biotechnology (Santa Cruz, CA, USA) were used. The fluorescent probe, Fura-2, was obtained from Molecular Probes (Eugene, OR, USA). Antibodies to PPARδ and actin were purchased from Abcam (Cambridge, MA, USA). Antibodies to cardiac TnI and phospho-TnI (Ser 23/24) were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Animal Model. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), as well as the guidelines of the Animal Welfare Act. The male Wistar rats, weighing from 200 to 250 g, were obtained from the Animal Center of National Cheng Kung University Medical College. Heart failure was induced by intraperitoneal injection of 15 mg/kg DOX according to previous report [8]. It is well established that bolus injection of DOX (15 mg/kg) is enough to cause acute cardiomyopathy in rodent [18]. Otherwise, GW0742, a PPARδ-specific activator [19], was dissolved in Dulbecco’s modified Eagle’s medium/dimethyl sulfoxide (DMSO) 6% (Gibco) and injected subcutaneously once a day at 1mg/kg in DOX-treated rats. Another group of DOX-treated rats receiving same treatment with vehicle at same volume was used for comparison. Meanwhile, the age-matched normal rats receiving same treatments were taken as control. Then, under anesthesia with an inhalation of isoflurane (5%), the heart tissue from a 1 to 2 day-old Wistar rat was cut into 1- to 2-mm pieces and predigested with trypsin to remove the red blood cells. The heart tissue was then digested with 0.25% trypsin and 0.05% collagenase. The dissociated cells were placed in uncoated 10 cm dishes and incubated at 37°C in a 5% CO2 incubator for at least 1 h to remove the nonmyocyte cells. This procedure caused most of the fibroblasts to attach to the dishes, while most of the cardiomyocytes remained unattached. The population of cells enriched in cardiomyocytes was then collected and counted. The cells were cultured in DMEM (GIBCO BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 units/mL streptomycin. On the second day after plating, the medium was replaced. Three days after plating, the cells were exposed to hyperglycemic conditions as described in detail later. Animal handling and disposal were performed in accordance with NIH guidelines.

The DOX-treated cardiomyocytes were generated by treating the cells with 10^{-9}−10^{-6} mol/L DOX for 24 h [21]. Then, cells were collected and subjected for Western blotting analysis. The treatment with GW0742 (PPARδ agonist) was performed at 10^{-6} mol/L for 1 hour before the addition of DOX as described previously [15, 22].

2.3. Cell Culture and Treatment. Primary cultures of neonatal rat cardiomyocytes were prepared by a previous method [20] with modification. Briefly, under anesthesia with an inhalation of isoflurane (5%), the heart tissue from a 1 to 2 day-old Wistar rat was cut into 1- to 2-mm pieces and predigested with trypsin to remove the red blood cells. The heart tissue was then digested with 0.25% trypsin and 0.05% collagenase. The dissociated cells were placed in uncoated 10 cm dishes and incubated at 37°C in a 5% CO2 incubator for at least 1 h to remove the nonmyocyte cells. This procedure caused most of the fibroblasts to attach to the dishes, while most of the cardiomyocytes remained unattached. The population of cells enriched in cardiomyocytes was then collected and counted. The cells were cultured in DMEM (GIBCO BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 units/mL streptomycin. On the second day after plating, the medium was replaced. Three days after plating, the cells were exposed to hyperglycemic conditions as described in detail later. Animal handling and disposal were performed in accordance with NIH guidelines.

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2.4. Western Blotting Analysis. Protein was extracted from tissue homogenates and cell lysates using ice-cold RIPA buffer supplemented with phosphatase and protease inhibitors (50 mmol/L sodium vanadate, 0.5 mM phenylmethylsulphonyl fluoride, 2 mg/mL aprotinin, and 0.5 mg/mL leupeptin). Protein concentrations were determined with the Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Total proteins (30 μg) were separated by SDS/polyacrylamide gel electrophoresis (10% acrylamide gel) using the Bio-Rad Mini-Protein II system. Protein was transferred to expanded polyvinylidene difluoride membranes (Pierce, Rockford, IL, USA) with a Bio-Rad Trans-Blot system. After transfer, the membranes were washed with PBS and blocked for 1 h at room temperature with 5% (w/v) skimmed milk powder in PBS. The manufacturer’s instructions were followed for the primary antibody reactions. Blots were incubated overnight at 4°C with an immunoglobulin-G polyclonal rabbit anti-mouse antibody (Affinity BioReagents, Inc., Golden, CO, USA) (1:500) in 5% (w/v) skimmed milk powder dissolved in PBS/Tween 20 (0.5% by volume) to bind the PPARδ in the heart specimens. The blot was incubated...
with goat polyclonal antibody (1:1000) to bind the actin serving as internal control. After the removal of primary antibody, the blots were extensively washed with PBS/Tween 20. The blots were then incubated for 2h at room temperature with the appropriate peroxidase-conjugated secondary antibody diluted in 5% (w/v) of skimmed milk powder and dissolved in PBS/Tween 20. The blots were developed by autoradiography using the ECL-western blotting system (Amersham International, Buckinghamshire, UK). The immune blot of PPARδ (49 kDa), actin (43 kDa), cardiac troponin (28 kDa), and phospho-troponin were quantified with a laser densitometer.

2.5. Measurement of Intracellular Calcium Concentration. The changes in intracellular calcium were detected using the fluorescent probe Fura-2 [23]. The neonatal cardiomyocytes were placed in buffered physiological saline solution containing 140 mM NaCl, 5.9 mM KCl, 1.2 mM CaCl₂, 1.4 mM MgCl₂, 11.5 mM glucose, 1.8 mM Na₂HPO₄, and 10 mM Hepes-Tris, to which was added 5 μM fura-2, and incubated for 1h in humidified 5% CO₂ and 95% air at 37°C. The cells were washed and incubated an additional 30 min in PSS. The cells were inserted into a thermostated (37°C) cuvette containing 2 mL of calcium-free PSS. The fluorescence was continuously recorded using a fluorescence spectrofluorometer (Hitachi F-2000, Tokyo, Japan). Values of \([Ca^{2+}]_i\) were calculated from the ratio \(R = F_{340}/F_{380}\) by the formula: \([Ca^{2+}]_i = K_d(B(R – R_{min}))/(R_{max} – R)\), where \(K_d\) is 225 nM, \(F\) is fluorescence, and \(B\) is the ratio of the fluorescence of the free dye to that of the \(Ca^{2+}\)-bound dye measured at 380 nm. Background autofluorescence was measured in unloaded cells and subtracted from all measurements.

2.6. Catheterization for Hemodynamic \(dP/dt\) Measurement. Temporary pacing leads were used for short-term study and were placed in the right atrium and RV apex. A venogram imaged in 2 different angulations (left anterior oblique 30° and anteroposterior) was obtained to determine the anatomy of the coronary sinus venous system. An LV pacing electrode (IX-214; iWorx Systems, Inc., Dover, NH, USA) was placed either in the free wall region via the lateral or posterior vein or in the anterior region via the great cardiac vein. After femoral artery and venous puncture using the Seldinger technique [24], pressure transducer catheters were inserted into the heart to provide the RV, aortic, and LV pressures. Pressure catheters and pacing leads were connected to an external pacing computer (iWorx Systems, Inc., Dover, NH, USA) to execute the pacing protocol and to acquire hemodynamic signals.

2.7. Statistical Analysis. Data are expressed as the mean ± SEM for the number (n) of animals in one group as indicated. Statistical analysis was carried out using repeated measures analysis of variance (ANOVA) and Newman-Keuls post-hoc analysis. Bonferroni’s correction was applied to the data, which were obtained from relatively small groups. A P value of 0.05 or less was considered significant.

3. Results

3.1. Effects of GW0742 on PPARδ Expression and Cardiac Troponin I Phosphorylation in DOX-Treated Rats. The levels of PPARδ protein expression and cardiac troponin I phosphorylation were significantly reduced in the heart of DOX-treated rats, compared with the control rats (Figure 1). Moreover, the decrease in expression of PPARδ or cardiac Troponin I phosphorylation was markedly reversed by GW0742 in DOX-treated rats (Figure 1).

3.2. Improvement of Cardiac Function in DOX-Treated Rats by GW0742. The values of cardiac output in addition to \(maxP/dt\) and \(mindP/dt\) were significantly (\(P < 0.001\)) reduced in DOX-treated rats as compared with the control rats. Also, the decrease in cardiac output or \(maxP/dt\) and \(mindP/dt\) was markedly reversed in DOX-treated rats after a 3-day treatment with GW0742 (1 mg/kg), as shown in Figures 2(a) and 2(b).

3.3. Effects of Doxorubicin on the Reduction of PPARδ Expression and Cardiac Troponin I Phosphorylation in Neonatal Rat Cardiomyocytes. We used primary neonatal rat cardiomyocytes to investigate the effects of DOX on expression of PPARδ and cardiac Troponin I phosphorylation. After the incubation with \(10^{-6}\) mol/L DOX for 24h, the cells were harvested to compare the expression of PPARδ and the level of cardiac Troponin I phosphorylation in cells. The expression of PPARδ protein and the level of cardiac troponin I phosphorylation in neonatal rat cardiomyocytes were significantly reduced by DOX treatment in a concentration-related manner (Figure 3). Then, the most effective dose (\(10^{-6}\) mol/L) of DOX was used in the following studies.

3.4. Effects of GW0742 on PPARδ Expression and Cardiac Troponin I Phosphorylation in DOX-Treated Neonatal Rat Cardiomyocytes. The decrease in expression of PPARδ protein or cardiac Troponin I phosphorylation was restored by treatment with GW0742 in DOX-treated cells, respectively (Figure 4).

3.5. Effects of GW0742 on the Intracellular Concentration of Calcium in DOX-Treated Neonatal Rat Cardiomyocytes. The fluorescent probe, fura2-AM, was used to detect the intracellular calcium concentration in DOX-treated neonatal rat cardiomyocytes. As compared with control, GW0742 restored the intracellular calcium concentration in DOX-treated cardiomyocytes (Figure 5).

4. Discussion

The present study found that gene expression of PPARδ is reduced in rats with doxorubicin (DOX)-induced cardiac toxicity. Also, activation of PPARδ may improve the cardiac performance damaged by DOX. Thus, we suggest the first report regarding the important role of PPARδ in DOX-induced cardiac toxicity.
According to a previous method [25], we established an animal model showing DOX-induced cardiac toxicity that has been confirmed using the marked decrease in cardiac output and contraction (dP/dt) in addition to the lowering of cardiac Troponin I phosphorylation. Also, gene expression of cardiac PPARδ is markedly reduced in this animal model. Mediation of PPARδ in the lowering of cardiac performance induced by DOX can thus be considered. Actually, we found that a 3-day treatment with GW0742 at the dose sufficient to activate PPARδ reverses the cardiac performance in DOX-treated rats. This is consistent with the view that cardiac PPARδ is mediated in contraction of heart and decrease of PPARδ is related to higher incidence of cardiac failure [14].

Then, we used the primary cultured cardiomyocytes from neonatal rats to investigate the potential mechanism(s). Although cardiac contraction was not determined, Troponin I phosphorylation was reduced in cardiomyocytes by DOX in a concentration-related manner. The reduced
Figure 3: Effects of doxorubicin on PPARδ expression and cardiac troponin I phosphorylation in neonatal rat cardiomyocytes. Cardiomyocytes from neonatal rats were cultured with $10^{-9}$–$10^{-6}$ mol/L doxorubicin for 24 h. These cells were harvested to determine the expression of PPARδ (a) and the level of troponin I phosphorylation (b) using Western blotting analysis. All values are presented as mean ± SEM ($n=6$ per group). *$P<0.05$, **$P<0.01$, and ***$P<0.001$ as compared with control cells.

Figure 4: Effects of GW0742 on PPARδ expression and cardiac troponin I phosphorylation in doxorubicin-treated neonatal rat cardiomyocytes. Changes of PPARδ expression (a) and cardiac troponin I phosphorylation (b) in DOX-treated cells by GW0742. Cells were treated with $10^{-6}$ mol/L GW0742 for 1 hour before the incubation with $10^{-6}$ mol/L DOX. After 24 h, they were harvested for measuring PPARδ protein expression (a) and cardiac troponin I phosphorylation (b) by Western blot. All values are expressed as mean ± SEM ($n=6$ per group). *$P<0.05$, **$P<0.01$, and ***$P<0.001$ as compared with DOX-treated cells.

phosphorylation of Troponin I has been identified in the failing hearts of human studies [26]. Thus, the reduced Troponin I phosphorylation may indicate the contractile defects as shown in hearts induced by DOX. Moreover, PPARδ expression is also parallel reduced by DOX in cardiomyocytes. DOX is one of the widely used agents for the treatment of cancer with a limitation in clinical utility due to the irreversible cardiac toxicity [2, 27, 28]. In the current work, we demonstrated that DOX impaired cardiac function with a decrease in cardiac PPARδ expression both in vivo and in vitro.
The hearts of DOX-treated rats. Also, treatment with GW0742 at
Troponin I phosphorylation was also reversed by GW0742 in
view has been identified in the present study. Furthermore,
result in the lowering of Troponin I phosphorylation and this
expression as mean ± SEM for measuring intracellular calcium concentration. All values are
for 1 hour before incubation with
activation of PPAR
[26, 36–39]. Recent studies also demonstrated that direct
acceleration of relaxation and an increase in cardiac output
[40–43]. A decrease in cardiac intracellular calcium may
could be reversed after the treatment of calcium chelators
[40–43]. A decrease in cardiac intracellular calcium may
result in the lowering of Troponin I phosphorylation and this
view has been identified in the present study. Furthermore,
Troponin I phosphorylation was also reversed by GW0742 in
the hearts of DOX-treated rats. Also, treat with GW0742 at
effective dose as described previously [44, 45] was observed
to improve cardiac performance in DOX-induced cardiotoxicity in rats. Taken together, it is suitable to speculate that the
cardiotoxic effect of GW0742 on DOX-induced cardiotoxicity seems related to an increase of Ca\(^{2+}\) concentration.

5. Conclusions
Cardiac PPARδ plays an important role in DOX-induced cardiotoxicity in rats. Thus, activation of PPARδ may restore
the expression of p-
TnI and the cardiac performance in DOX-induced cardiotoxicity in rats.

Conflict of Interests
The authors have no conflict of interests about this study.

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References
[1] S. Kumar, R. Marfatia, S. Tannenbaum, C. Yang, and E. Avelar,
“Doxorubicin-induced cardiomyopathy 17 years after chemotherapy,”
*Texas Heart Institute Journal*, vol. 39, no. 3, pp. 424–427, 2012.
[2] G. Takemura and H. Fujiwara, “Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management,”
*Progress in Cardiovascular Diseases*, vol. 49, no. 5, pp. 330–352,
2007.
[3] M. I. Gharib and A. K. Burnett, “Chemotherapy-induced cardiomyopathy: current practice and prospects of prophylaxis,”
*European Journal of Heart Failure*, vol. 4, no. 3, pp. 235–242,
2002.
[4] J. Wojtacki, E. Lewicka-Nowak, and K. Lesniewski-Kmak,
“Anthracycline-induced cardiotoxicity: clinical course, risk factors,
pathogenesis, detection and prevention—review of the literature,”
*Medical Science Monitor*, vol. 6, no. 2, pp. 411–420, 2000.
[5] P. W. Fisher, F. Saloum, A. Das, H. Hyder, and R. C. Kukreja,
“Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocyte apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardiotoxicity,”
*Circulation*, vol. 111, no. 13, pp. 1601–1610, 2005.
[6] G. Fajardo, M. Zhao, J. Powers, and D. Bernstein, “Differential
cardiotoxic cardioprotective effects of \(\beta\)-adrenergic receptor
subtypes in myocytes and fibroblasts in doxorubicin cardiomyopathy,”
*Journal of Molecular and Cellular Cardiology*, vol. 40, no. 3, pp. 375–383,
2006.
[7] F. Villani, E. Monti, and F. Piccinini, “Relationship between
doxorubicin-induced ECG changes and myocardial alterations
in rats,” *Tumori*, vol. 72, no. 3, pp. 323–329, 1986.
[8] H. Alimoradi, A. Barzegar-Fallah, G. Hassanzadeh et al., “The
cardioprotective effects of an antiemetic drug, tropisetron,
on cardiomyopathy related to doxorubicin,” *Cardiovascular Toxicology*, vol. 12, no. 4, pp. 318–325, 2012.
[9] Y. Octavia, C. G. Tocchetti, K. L. Gabrielson, S. Janssens, H. J. Crijs, and A. L. Moens, “Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies,” *Journal of Molecular and Cellular Cardiology*, vol. 52, no. 6, pp. 1213–1225, 2012.

[10] A. Mordente, E. Meucci, G. E. Martorana, B. Giardina, and G. Minotti, “Human heart cytosolic redoxs and anthrycline cardiotoic toxicity,” *IUBMB Life*, vol. 52, no. 1-2, pp. 83–88, 2001.

[11] L. E. Olson, D. Bedja, S. J. Alvey, A. J. Cardounel, K. L. Gabrielson, and R. H. Reeves, “Protection from doxorubicin-induced cardiac toxicity in mice with a null allele of carnobyl reductase 1,” *Cancer Research*, vol. 63, no. 20, pp. 6602–6606, 2003.

[12] Q. Yang and Y. Li, “Roles of PPARs in regulating myocardial energy and lipid homeostasis,” *Journal of Molecular Medicine*, vol. 85, no. 7, pp. 697–706, 2007.

[13] I. Issemann and S. Green, “Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators,” *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[14] L. Cheng, G. Ding, Q. Qin et al., “Cardiomyocyte-restricted peroxisome proliferator-activated receptor-δ deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy,” *Nature Medicine*, vol. 10, no. 11, pp. 1245–1250, 2004.

[15] L. Cheng, G. Ding, Q. Qin et al., “Peroxisome proliferator-activated receptor δ activates fatty acid oxidation in cultured neonatal and adult cardiomyocytes,” *Biochemical and Biophysical Research Communications*, vol. 313, no. 2, pp. 277–286, 2004.

[16] G. D. Barish, V. A. Narkar, and R. M. Evans, “PPARδ: a dagger in the heart of the metabolic syndrome,” *The Journal of Clinical Investigation*, vol. 116, no. 3, pp. 590–597, 2006.

[17] B. C. Yu, K. C. Chang, H. Y. Ou, K. C. Cheng, and J. T. Cheng, “Decrease of peroxisome proliferator-activated receptor delta expression in cardiomyopathy of streptozotocin-induced diabetic rats,” *Cardiovascular Research*, vol. 80, no. 1, pp. 78–87, 2008.

[18] M. Rahimi Balaei, M. Momeny, R. Babaekliloshimi, S. Ejtemaei Mehr, S. M. Tavangar, and A. R. Dehpour, “The modulatory effect of lithium on doxorubicin-induced cardiotoxicity in rat,” *European Journal of Pharmacology*, vol. 641, no. 2-3, pp. 193–198, 2010.

[19] M. L. Sznaidman, C. D. Hauffner, P. R. Maloney et al., “Novel selective small molecule agonists for peroxisome proliferator-activated receptor δ (PPARδ)—synthesis and biological activity,” *Bioorganic and Medicinal Chemistry Letters*, vol. 13, no. 9, pp. 1517–1521, 2003.

[20] W. B. Kannel, M. Hjortland, and W. P. Castelli, “Role of diabetes in congestive heart failure: the Framingham study,” *American Journal of Cardiology*, vol. 34, no. 1, pp. 29–34, 1974.

[21] T. Tokudome, T. Horio, M. Fukunaga et al., “Ventricular nonmyocytes inhibit doxorubicin-induced myocyte apoptosis: involvement of endogenous endothelin-1 as a paracrine factor,” *Endocrinology*, vol. 145, no. 5, pp. 2458–2466, 2004.

[22] D. Kitz Krämer, L. Al-Khalili, S. Perrini et al., “Direct activation of glucose transport in primary human myotubes after activation of peroxisome proliferator—activated receptor δ,” *Diabetes*, vol. 54, no. 4, pp. 1157–1163, 2005.

[23] I. G. Poornima, P. Parikh, and R. P. Shannon, “Diabetic cardiomyopathy: the search for a unifying hypothesis,” *Circulation Research*, vol. 98, no. 5, pp. 596–605, 2006.

[24] S. I. Seldinger, “Catheter replacement of the needle in percutaneous arteriography: a new technique,” *Acta Radiologica*, vol. 49, no. 434, pp. 47–52, 2008.

[25] C. F. Brilhante Wolle, L. De Aguilar Zollmann, A. Etgess, G. S. Vitalis, C. E. Leite, and M. M. Campos, “Effects of the antioxidant agent tempol on periapical lesions in rats with doxorubicin-induced cardiomyopathy,” *Journal of Endodontics*, vol. 38, no. 2, pp. 191–195, 2012.

[26] A. E. Messer, A. M. Jacques, and S. B. Marston, “Troponin phosphorylation and regulatory function in human heart muscle: phosphorylation of Ser23/24 on troponin I could account for the contractile defect in end-stage heart failure,” *Journal of Molecular and Cellular Cardiology*, vol. 42, no. 1, pp. 247–259, 2007.

[27] S. E. Lipshultz, M. A. Grenier, S. D. Colan et al., “Doxorubicin-induced cardiomyopathy,” *The New England Journal of Medicine*, vol. 340, no. 8, pp. 653–655, 1999.

[28] A. Berdichevski, G. Meiry, F. Milman et al., “TVPl022 protects neonatal rat ventricular myocytes against doxorubicin-induced functional derangements,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 332, no. 2, pp. 413–420, 2010.

[29] G. Minotti, P. Menna, E. Salvatorelli, G. Cairo, and L. Gianni, “Anthryclines: molecular advances and pharmacologie developments in antitumor activity and cardiotoxicity,” *Pharmacological Reviews*, vol. 56, no. 2, pp. 185–229, 2004.

[30] M. F. Xu, P. L. Tang, Z. M. Qian, and M. Ashraf, “Effects of doxorubicin on the myocardium are mediated by oxygen free radicals,” *Life Sciences*, vol. 68, no. 8, pp. 889–901, 2001.

[31] J. H. Doroshow, “Effect of anthyacyline antibiotics on oxygen radical formation in rat heart,” *Cancer Research*, vol. 43, no. 2, pp. 460–472, 1983.

[32] N. R. Bachur, S. L. Gordon, and M. V. Gee, “A general mechanism for microsomal activation of quinone anticancer agents to free radicals,” *Cancer Research*, vol. 38, no. 6, pp. 1745–1750, 1978.

[33] J. H. Doroshow, “Doxorubicin-induced cardiac toxicity,” *The New England Journal of Medicine*, vol. 324, no. 12, pp. 843–845, 1991.

[34] A. Aviles, N. Arevila, J. C. Diaz Maqueo, T. Gomez, R. Garcia, and M. J. Nambo, “Late cardiac toxicity of doxorubicin, epirubicin, and mitoxantrone therapy for Hodgkin’s disease in adults,” *Leukemia and Lymphoma*, vol. 11, no. 3-4, pp. 275–279, 1991.

[35] A. Avilés, N. Neri, M. J. Nambo, J. Huerta-Guzman, A. Talavera, and S. Cleto, “Late cardiac toxicity secondary to treatment in Hodgkin’s disease. A study comparing doxorubicin, epirubicin and mitoxantrone in combined therapy,” *Leukemia and Lymphoma*, vol. 46, no. 7, pp. 1023–1028, 2005.

[36] C. A. Tate, M. F. Hyek, and G. E. Taffet, “The role of calcium in the energetics of contracting skeletal muscle,” *Sports Medicine*, vol. 12, no. 3, pp. 208–217, 1991.

[37] X. Liu, N. Takeda, and N. S. Dhallan, “Troponin I phosphorylation in heart homogenate from diabetic rat,” *Biochimica et Biophysica Acta*, vol. 1316, no. 2, pp. 78–84, 1996.

[38] L. Li, J. Desantiago, G. Chu, E. G. Kranias, and D. M. Bers, “Phosphorylation of phospholamban and troponin I in β-adrenergic-induced acceleration of cardiac relaxation,” *American Journal of Physiology*, vol. 278, no. 3, pp. H769–H779, 2000.

[39] Y. Pi, K. R. Kemnitz, D. Zhang, E. G. Kranias, and J. W. Walker, “Phosphorylation of troponin I controls cardiac twitch dynamics: evidence from phosphorylation site mutants expressed on a troponin I-null background in mice,” *Circulation Research*, vol. 90, no. 6, pp. 649–656, 2002.

[40] M.-T. Chou, S.-H. Lo, K.-C. Cheng, Y.-X. Li, L.-J. Chen, and J.-T. Cheng, “Activation of β-adrenoceptors by dobutamine
may induce a higher expression of peroxisome proliferator-activated receptors δ (PPARδ) in neonatal rat cardiomyocytes,” *The Scientific World Journal*, vol. 2012, Article ID 248320, 8 pages, 2012.

[41] Z.-C. Chen, B.-C. Yu, L.-J. Chen, and J.-T. Cheng, “Increase of peroxisome proliferator-activated receptor delta (PPARδ) by digoxin to improve lipid metabolism in the heart of diabetic rats,” *Hormone and Metabolic Research*, 2012.

[42] S. C. Fan, B. C. Yu, Z. C. Chen, L. J. Chen, H. H. Chung, and J. T. Cheng, “The decreased expression of peroxisome proliferator-activated receptors (PPAR) is reversed by digoxin in the heart of diabetic rats,” *Hormone and Metabolic Research*, vol. 42, no. 9, pp. 637–642, 2010.

[43] Z.-C. Chen, L.-J. Chen, and J.-T. Cheng, “Activation of peroxisome proliferator-activated receptor δ (PPARδ) by agonist named gw0742 increases cardiac contractility but not heart beating in animal,” *British journal of pharmacology*. In press.

[44] I. Paterniti, E. Mazzon, L. Riccardi et al., “Peroxisome proliferator-activated receptor β/δ agonist GW0742 ameliorates cerulein- and taurocholate-induced acute pancreatitis in mice,” *Surgery*, vol. 152, no. 1, pp. 90–106, 2012.

[45] B. Faiola, J. G. Falls, R. A. Peterson et al., “PPAR alpha, more than PPAR delta, mediates the hepatic and skeletal muscle alterations induced by the PPAR agonist GW0742,” *Toxicological Sciences*, vol. 105, no. 2, pp. 384–394, 2008.