Phospholipase C-δ1 Is a Critical Target for Tumor Necrosis Factor Receptor–Mediated Protection against Adriamycin-Induced Cardiac Injury

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Abstract

The clinical application of adriamycin, an exceptionally good chemotherapeutic agent, is limited by its dose-related cardiomyopathy. Our recent study showed that tumor necrosis factor-α (TNF-α) receptors mediated cytoprotective signaling against adriamycin-induced mitochondrial injury and cardiomyocyte apoptosis. In the present study, we investigated the potential targets of TNF receptor–mediated cytoprotective signaling by global genome microarray analysis using wild-type and TNF receptor–deficient mice. Microarray analysis revealed that adriamycin treatment induced the down-regulation of several mitochondrial functions and energy production–related genes in double TNF receptor–deficient mice, notably, phospholipase C-δ1, a protein involved in fatty acid metabolism and calcium regulation. The role of phospholipase C-δ1 in TNF receptor–mediated cardioprotection against adriamycin-induced injury was evaluated by measuring changes in cardiac function using high-frequency ultrasound biomicroscopy. Selective inhibition of phospholipase C activity in wild-type mice by its inhibitor, U73122, exacerbated adriamycin-induced cardiac dysfunction. Inhibition of phospholipase C-δ1 resulted in the significant decrease of left ventricular ejection fraction and fractional shortening, and the decreased levels were similar to those observed in adriamycin-treated double TNF receptor–deficient mice. The data derived from the global genome analysis identified phospholipase C-δ1 as an important target for TNF receptors and revealed the critical role of TNF receptor signaling in the protection against adriamycin-induced cardiotoxicity. (Cancer Res 2006; 66(8): 4329-38)

Introduction

Adriamycin (doxorubicin) is a broad spectrum anthracycline antibiotic widely used as a chemotherapeutic agent for hematologic and solid tumors. However, the clinical application of adriamycin is limited by its dose-related cardiomyopathy, which may lead to fatal congestive heart failure (1, 2). The most prevailing hypothesis for adriamycin-induced cardiotoxicity is the increase of oxidative stress (2, 3), which may be associated with defects in mitochondrial integrity and function (4–6), alterations in calcium homeostasis (3, 7), and induction of apoptosis (8). Our previous study showed that overexpression of manganese superoxide dismutase, a primary antioxidant enzyme in the mitochondria, protected against adriamycin-induced acute cardiac injury (4), suggesting that mitochondria are major targets of adriamycin-induced cardiac injury.

Tumor necrosis factor-α (TNF-α) is a potent proinflammatory cytokine produced by many cell types, including cardiomyocytes (9). TNF-α has been shown to have both adverse and beneficial effects on cardiac function, such as inducing abnormalities of systolic function and the induction of cytoprotective genes, including manganese superoxide dismutase (10, 11). The main biological actions of TNF-α are initiated by binding to two distinct receptors, TNF receptor I (TNFR1; p55) and TNF receptor II (TNFRII; p75), which mediate different signaling pathways and cellular functions (10).

Previous studies in our laboratory showed that TNF receptors mediated cytoprotective signaling against adriamycin-induced mitochondrial injury and cardiomyocyte apoptosis (12). We also showed that the presence of either TNFR1 or TNFRII was sufficient for protection against adriamycin-induced cardiomyocyte apoptosis. However, the cytoprotective pathways mediated by TNFR1 and TNFRII are unknown. In the present study, we investigated the molecular pathways and potential targets that mediate TNF receptor signaling against adriamycin-induced cardiac injury using global genome microarray analysis of cardiac tissues from TNFR1–deficient mice (p55−/−/p75+/+), TNFRII–deficient mice (p55+/−/p75−/−), and double TNF receptor–deficient mice (DKO), with wild-type mice as controls. Our results showed that adriamycin treatment induced the down-regulation of several mitochondrial functions and energy production–related genes in DKO mice, including phospholipase C-δ1, an enzyme that mediates signaling for regulation of energy metabolism pathways, calcium homeostasis, and intracellular movements (13). Selective inhibition of phospholipase C-δ1 activity in wild-type mice by its inhibitor exacerbated adriamycin-induced cardiac dysfunction similar to that occurring in DKO mice, confirming its role in TNF receptor–mediated cardioprotection against adriamycin toxicity predicted by microarray analysis.

Materials and Methods

Generation of TNF receptor–deficient mice. p55 (TNFR1)-deficient (p55−/−/p75+/+) and p75 (TNFRII)-deficient (p55+/−/p75−/−) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and homozygote double TNFR1/TNFRII-deficient mice (DKO) were obtained by an appropriate cross of TNFR1- and TNFRII-deficient mice. Receptor-deficient
mice were maintained on a C57BL/6 background. Wild-type littermates, maintained on the same C57BL/6 background, were used as controls. Male mice (10-14 weeks old) were used for experiments. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Microarray analysis. To detect the changes of early response genes involved in TNF receptor–mediated cardioprotection against adriamycin toxicity, we treated animals with a single injection of 20 mg/kg adriamycin, an equivalent dose of a single i.v. infusion given to patients with small cell lung cancer (14). RNA was isolated from cardiac tissue 3 hours after treatment of mice with saline or adriamycin using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). The RNA was further purified with an RNeasy Mini Kit (Qiagen, Valencia, CA). The same amount of total RNA from each of two hearts was pooled for each sample. Three samples per treatment group were subjected to microarray analysis. The integrity of the RNA was verified prior to reverse transcription by Agilent bioanalyzer (Agilent Technologies, Palo Alto, CA). Labeled cRNA was generated by in vitro transcription of CDNA with incorporation of labeled ribonucleotides. The cRNA was then fragmented and hybridized to Affymetrix murine genome MOE 430A arrays following the protocol described in the GeneChip expression analysis technical manual (Affymetrix, Santa Clara, CA). The expression level of each mRNA was estimated by calculating the hybridization signal intensity using GCOS software (Affymetrix). Sixty-four Affymetrix quality control probesets, and 6,835 probesets that were absent across all chips, were removed before the statistical analysis. The expression signals of each of the remaining 15,791 probesets were analyzed by three-way ANOVA. The three factors in three-way ANOVA are drug, p55, and p75. A post hoc analysis was done by orthogonal decomposition of the overall significant probesets into a three-way interaction significant group, two-way interaction significant group, and main effects significant group. The two-way interaction significant group was further decomposed into two-way interaction significant groups (drug × p55, drug × p75, and p55 × p75) and the main effects significant group was further decomposed into three main effect significant groups (drug, p55, and p75). Of the 15,791 probesets, 1,834 were significant statistically overall at \( P \leq 0.05 \). These 1,834 probesets were further categorized into three-way interaction significant groups (183 drug × p55 × p75 significant probesets), two-way interaction significant groups (217 drug × p55 significant probesets, 193 drug × p75 significant probesets, and 224 p55 × p75 significant probesets), and main effect significant groups (914 drug effect significant probesets, 202 p55 effect significant probesets, and 323 p75 effect significant probesets). Statistical analysis for differential mRNA expression was done using SAS Software (SAS Institute, Inc., Cary, NC). Biological analysis was done using EASE Software (BHI, Bethesda, MD) and Genespring Software (Silicon Genetics, Redwood City, CA).

Real-time PCR. RNA was isolated from cardiac tissue 3 hours after mice were treated with saline or adriamycin using Trizol reagent (Invitrogen Life Technologies). The RNA was further purified with an RNeasy Mini Kit (Qiagen). Total RNA (1 \( \mu \)g) was used for reverse transcription reaction by Advantage RT for PCR kit (BD Biosciences, Palo Alto, CA). Real-time PCR was done with a LightCycler System (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer's instructions. The primers were designed from published mouse sequences (15–21): Plcd1 sense-GCCGAAGGTCAATAAGATAAGAA, and antisense-AAGTGTTCTTCAAGAGGGAGTCGA (222 bp); Nat sense-GCTAATGCTCATTCTGGAGGATAT, and antisense-GTATCGACCCTTTAGCCACAAGAG (174 bp); UCP3 sense-GACTCTCATCAAGATTCTGGGAAA, and antisense-TAAAACATTGTTCTCGAGCCTTAC (234 bp); Ezh2 sense-ACCTGATCCACATGCAACAACT, and antisense-AGGGCAACAGATGATTCCATTATAG (190 bp); Ube1x sense-TCTGGTTCAAATAAGTACCCCAAT; and antisense-GAATCCATTGTTCAATCGAG (204 bp); Acadm sense-GAGATTTGCGACACAAATTCAGA, and antisense-AAACTCGTCTTTGATGAAGGTACTGCACAAATTCAGA, and antisense-GCAGCTAGTAGTGATCATGGAGAA (204 bp); Tuba2 sense-CTCTGTGGATAACGGAAAATGAAGGTACTGCACAAATTCAGA, and antisense-AAACTCGTCTTTG(T)GATGAAGGTACTGCACAAATTCAGA (204 bp); Ezh2 sense-ACTGATCCACATGCAACAACT, and antisense-AGGGCAACAGATGATTCCATTATAG (190 bp); and antisense-TAAAACATTGTTCTCGAGCCTTAC (234 bp). Western blotting. For protein analysis, total heart tissue homogenates were separated by SDS-PAGE. The following antibodies were used: anti-phospholipase C-\( \delta \), and anti-tubulin-\( \alpha \) (Santa Cruz Biotechnology, Santa Cruz, CA).

Echocardiography. Wild-type mice were treated with olive oil or 2.5 mg/kg U73122 (Biomol International L.P., Plymouth Meeting, PA), alone or 30 minutes prior to 20 mg/kg adriamycin treatment. DKO mice were treated with olive oil alone or 30 minutes prior to 20 mg/kg adriamycin treatment. The mice were dosed once daily with olive oil or U73122 for 3 days. Mice were anesthetized with isoflurane (2%) for ultrasound biomicroscopic evaluation with a high-frequency 30 MHz probe (Vivo 660, VisualSonics, Toronto, Ontah, Canada). Short axis M mode images were recorded at the papillary muscle level for cardiac function analysis.

Statistical analysis. Data were analyzed by ANOVA with a post hoc Student-Newman-Keuls multiple comparison test, or two-sample Student's \( t \) test. A difference of \( P < 0.05 \) was considered significant.

Results

Adriamycin treatment induced down-regulation of mitochondrial function and energy production–related genes in DKO mice. To identify the potential target genes involved in TNF-\( \alpha \) receptor–mediated protection against adriamycin-induced cardiac injury, RNA harvested from cardiac tissues of animals treated \( in \) \( vivo \) for 3 hours with saline or adriamycin was analyzed using Affymetrix GeneChip Arrays. The data were analyzed by three-way ANOVA. The three-way interaction significant group contained genes in which their different regulations of adriamycin-induced changes of mRNA levels between the presence and the absence of p55 would be modified by the presence/absence of p75, or genes in which their different regulations of adriamycin-induced changes of mRNA levels between the presence and the absence of p75 would be modified by the presence/absence of p55. The drug × p55 interaction significant group contained genes whose mRNA levels would be different between the changes induced by adriamycin treatment in the presence and the absence of p55. The drug × p75 interaction significant group contained genes whose mRNA levels would be different between the changes induced by adriamycin treatment in the presence and the absence of p75. Genes in the three-way interaction significant group, drug × p55 interaction significant group, and drug × p75 interaction significant group were further analyzed by Gene Ontology categories and biological functions to investigate the roles of TNF receptors on adriamycin-induced cardiac injury, and to identify the potential candidate genes important for TNF receptor–mediated cardiac protection.

The microarray data showed that the majority of statistically significant genes were involved in the metabolism processes and regulation of cell growth and cell death (Fig. 1A). A number of genes were also involved in the regulation of signal transduction, transcription and translation, transport processes, and maintenance of cytoskeleton and cell adhesion. Hierarchical clustering of genes in the metabolism category (Fig. 1B) indicated a cluster of genes with reduced expression (transition to blue), and another cluster of genes with increased expression (transition to red) after adriamycin treatment. The transcription profiles in different groups also showed the effects of TNF-\( \alpha \) receptors on the expression of each gene in response to adriamycin treatment. The identification of potential target genes involved in the regulation of TNF receptor–mediated cardioprotection (Table 1) was determined by comparing the differences among genotypes. Genes that play a role in energy production/metabolism processes and that contribute to the regulation of calcium homeostasis and mitochondrial function were among those most significantly affected by TNF receptors and adriamycin treatment.
Several mitochondrial proteins are involved in energy production processes and regulation of mitochondrial function that showed significantly reduced expression in DKO mice after adriamycin treatment. They are, phospholipase C-\(\gamma_1\), nicotinamide nucleotide dehydrogenase, thymidine kinase 2, and acetyl-CoA dehydrogenase-medium chain. To verify the microarray data, we compared the changes in mRNA levels by real-time reverse transcription-PCR for all three-way interaction significant genes and genes encoded for mitochondrial proteins (Fig. 2). Generally, the changes in microarray analysis and real-time PCR analysis showed similar patterns.

Figure 3 summarizes the genes and their functions that potentially play an important role in TNF receptor–mediated protection against adriamycin-induced cardiac injury. The function of genes regulated by both TNFRI (p55) and TNFRII (p75) (middle column, Fig. 3) seems to be essential to TNF receptor–mediated cardioprotection, including regulation of energy production and calcium homeostasis (phospholipase C-\(\gamma_1\)), maintenance of electron transport chain function and energy production (phospholipase C-\(\gamma_1\) and nicotinamide nucleotide transhydrogenase), and regulation of protein ubiquitinylation (ubiquitin-activating enzyme E1, ChrX). Genes regulated by TNFRI (p55) are likely involved in TNFRI-mediated cardioprotection (left column, Fig. 3). Genes regulated by TNFRII (p75) are likely involved in TNFRII-mediated cardioprotection (right column, Fig. 3).

Adriamycin suppressed phospholipase C-\(\delta_1\) expression in TNF receptor–deficient mice. Microarray analysis indicated that the maintenance of energy production/metabolism processes and regulation of calcium homeostasis and mitochondrial function may be important pathways for TNF receptor–mediated cardioprotection. Phospholipase C-\(\delta_1\) and nicotinamide nucleotide
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NOTE: Selected genes that potentially regulate TNF receptor-mediated cardioprotein against ADR toxicity representative of functional groups. Fold changes and statistical P values with a range reflect three probesets for the same gene. See supplementary tables for complete list of genes in three-way significant, Drug × p55 interaction significant, and Drug × p75 interaction significant groups.
transhydrogenase (Nnt) were genes in the three-way interaction significant group that showed significant reduction in DKO mice, as detected by both microarray and real-time PCR analyses (Fig. 3).

Because Nnt functions to maintain electron transport chain activity, it is not surprising that the protection of this gene is important for adriamycin-induced cardiac injury.

The role of phospholipase C-\(\gamma\) activity in TNF receptor–mediated protection against adriamycin cardiotoxicity is unclear. To determine whether the effects of TNF receptors and adriamycin treatment on phospholipase C-\(\gamma\) expression in cardiac tissues would lead to a corresponding change in protein levels, phospholipase C-\(\delta\) mRNA levels and protein levels were analyzed by real-time PCR (Fig. 4A) and Western blotting (Fig. 4B). The data showed that phospholipase C-\(\delta\) mRNA levels were significantly decreased in p55\(^{-/-}\) p75\(^{++/+}\) mice, p55\(^{-/-}\) p75\(^{+++/-}\) mice, and DKO mice after adriamycin treatment.

The protein levels of phospholipase C-\(\delta\) were also significantly decreased in TNF receptor–deficient mice, but there were no changes in the wild-type mice after adriamycin treatment. The phospholipase C-\(\delta\) protein levels in adriamycin-treated DKO mice were also significantly lower than adriamycin-treated p55\(^{-/-}\) p75\(^{++/-}\) mice or p55\(^{++/+}\) p75\(^{+++/-}\) mice, suggesting that both

Figure 2. Verification of representative microarray results for three-way significant and energy production and metabolism-related genes by real-time PCR. Fold changes (ADR/Saline) of mRNA levels for selected genes were determined by microarray analysis (\(n = 3\)) and real-time PCR (\(n = 6\)). Columns, mean; bars, ±SD, evaluated using two-sample Student’s \(t\) test or one-way ANOVA with a post hoc Student-Newman-Keuls multiple comparison test. *, \(P < 0.05\) compared with wild-type mice; **, \(P < 0.05\) compared with wild-type, p55\(^{-/-}\) p75\(^{+++/-}\), and p55\(^{++/+}\) p75\(^{+++/-}\) mice; #, \(P < 0.05\) compared with wild-type and p55\(^{-/-}\) p75\(^{+++/-}\) mice; @, \(P < 0.05\) compared with p55\(^{-/-}\) p75\(^{+++/-}\) and DKO mice.
TNFR-I and TNFR-II alone partially regulate phospholipase C-δ1 expression in cardiac tissues.

**Inhibition of phospholipase C activity or lack of TNF receptors exacerbated adriamycin-induced cardiac dysfunction.** To directly test the role of phospholipase C in TNF receptor-mediated cardioprotection against adriamycin-induced injury, we evaluated cardiac function changes with or without the selective phospholipase C inhibitor (U73122) after adriamycin treatment, using high-frequency ultrasound biomicroscopy. There was no difference in the basal cardiac function between wild-type mice and DKO mice in all the variables measured (data not shown). Inhibition of phospholipase C activity alone by its inhibitor did not affect cardiac function (Fig. 5). Three days of adriamycin treatment resulted in cardiac dysfunction in both wild-type mice and DKO mice as indicated by the decreases of heart rate, left ventricular ejection fraction, fractional shortening, stroke volume, and cardiac output. However, the heart rate, ejection fraction, and fractional shortening in adriamycin-treated DKO mice were significantly lower than adriamycin-treated wild-type mice, confirming that TNF receptors had cardioprotective effects against adriamycin. Inhibition of phospholipase C activity reversed the cardioprotective effect of TNF receptors, as shown by the significantly decreased ejection fraction and fractional shortening in U73122 plus adriamycin-treated wild-type mice compared with wild-type mice treated with adriamycin alone. These results suggested that the inhibition of phospholipase C activity or the lack of TNF receptors exacerbated adriamycin-induced cardiac dysfunction by decreasing cardiac contractility.

**Discussion**

The most prevailing hypothesis for adriamycin-induced cardiac injury is the increase of oxidative stress. Our previous study showed that overexpression of manganese superoxide dismutase, a primary antioxidant enzyme in the mitochondria, protected against adriamycin-induced acute cardiac injury (4), suggesting that mitochondria are major targets of adriamycin-induced cardiac injury. Clinically, there is ∼20% incidence of adriamycin-induced heart failure in patients receiving a cumulative dose >500 mg/m² resulting from the inability of the cardiac pump to meet the energy requirements of the body (1, 22). One mechanism that contributes to heart failure is an abnormality in the metabolic pathway,
including decreased energy production, energy transfer, and energy utilization. The heart has high-energy demands for maintenance of cellular processes, and mitochondria constitute a major component of cardiomyocytes. Mitochondria serve as the power plant in the heart providing ATP by oxidative metabolism, and the heart is almost entirely dependent on ATP generated by mitochondria for its function and contractility. Because cardiomyocytes are the cells with the highest mitochondria density, having ~40% total intracellular volume (23), mitochondrial injury could contribute to cardiac failure. It has also been shown that impaired regulation of mitochondrial function and mitochondrial biogenesis was associated with the increases of cardiovascular risk factors (24). We have recently shown that TNF-α receptors mediated cytoprotective effects against adriamycin-induced mitochondrial injury and cardiomyocyte apoptosis (12). However, the molecular pathways mediating TNF receptor–associated protection were unclear. In the present study, we determined the potential targets of TNF receptor–mediated cytoprotective signaling by microarray analysis after adriamycin treatment for 3 hours, the earliest time point that nuclear factor κB and activator protein-1 DNA binding activities increased significantly in the cardiac tissues observed in our unpublished study using mice of a different strain. These changes reflect an early response to adriamycin treatment in cardiac tissues. It is possible that gene expression might be different as a function of time posttreatment. Thus, it is possible that changes in expression of other genes may occur at later time points not investigated in this study. The results from the present study revealed that TNF receptor signaling pathways prevented adriamycin-induced down-regulation of several mitochondrial functions and energy production–related genes, including genes directly regulating energy production, such as nicotinamide nucleotide transhydrogenase (Nnt), and genes regulating both calcium homeostasis and mitochondrial function, such as phospholipase C-δ1 (Plcd1).

Nicotinamide nucleotide transhydrogenase (Nnt) is a proton pump located in the mitochondrial inner membrane that catalyzes the reduction of NADP+ by NADH to NADPH and NAD+. It regulates the proton gradient through the NAD- and NADP-dependent isocitrate dehydrogenase couple in the citric acid cycle. Our results showed that 3 hours after adriamycin treatment, the mRNA levels of Nnt in DKO mice were significantly decreased compared with wild-type, p55−/−p75+/+ and p55+/+p75−/− mice, suggesting the direct effect of adriamycin on decreasing energy production capacity and the roles of TNFRI and TNFRII in maintaining normal mitochondrial function.

Phospholipase C is an enzyme crucial for the phosphoinositol pathway and calcium homeostasis regulation (13). Phospholipase C hydrolyzes phosphatidylinositol-4,5-bisphosphate to diacylglycerol and inositol-1,4,5-tris-phosphate. Diacylglycerol is involved in the biosynthesis of arachidonic acid and eicosanoid and the regulation of protein kinase C activity. Inositol-1,4,5-tris-phosphate also mediates calcium homeostasis regulation and intracellular calcium movements. Calcium is a key regulator of mitochondrial function.
and regulates ATP synthesis at several organelle levels (27). Dysregulation of mitochondrial calcium homeostasis has been shown to play a key role in normal tissue pathologies, including cardiac ischemia reperfusion injury and neuronal excitotoxicity. Under physiologic conditions, calcium is beneficial for mitochondrial function and energy production; however, in the presence of pathologic stimulus, mitochondrial calcium overload leads to increased generation of reactive oxygen species, triggering permeability transition pore openings, decreased energy production, cytochrome c release, and the induction of apoptosis.

Phospholipase C-61 is the most abundant phospholipase C isozyme in normal cardiac tissues (28). It is a nuclear factor κB target gene (29), and contains consensus binding sites of activator protein-1 in the promoter sequence (30). Hwang et al. (28) have shown that, in ischemic heart and hypoxic neonatal cardiomyocytes, phospholipase C-61 was selectively degraded and overexpression of phospholipase C-61 rescued intracellular calcium overload. Tappia et al. (31) also showed a defect in the phosphatidic acid-phospholipase C signaling pathway in diabetic rat cardiomyocytes, which may significantly contribute to heart dysfunction during

Figure 5. Inhibition of phospholipase C activity or lack of TNF receptor–exacerbated ADR-induced cardiac dysfunction. Cardiac function was evaluated by high-frequency ultrasound biomicroscopy 3 days after ADR treatment (n ≥ 5). Columns, mean; bars, ±SE, evaluated using two-way ANOVA with a post hoc Student-Newman-Keuls multiple comparison test. *, P < 0.05 compared with WT-Oil and WT-U73122 groups; #, P < 0.05 compared with DKO-Oil group; @, P < 0.05 compared with WT-ADR group.
diabetes and many different types of cardiac pathologies. Our microarray data have shown that phospholipase C-d1 could be regulated by both TNFRI and TNFRII and its levels decreased significantly in DKO mice 3 hours after adriamycin treatment, suggesting the role of phospholipase C-d1 in TNF receptor-mediated cardioprotection. This result has been verified by real-time PCR and Western analysis, showing that adriamycin suppressed phospholipase C-d1 expression in TNF receptor-deficient mice. The expression levels in DKO mice were also lower than those in p55+/−/p75−/− and p55+/p75−/− mice. Our data showing that phospholipase C-d1 protein levels decreased by ~40% in DKO mice after adriamycin treatment are consistent with those reported by Asemu et al. (32), which showed a 40% decrease of phospholipase C-d1 protein levels, and a 35% decrease of phospholipase C-d1 activities in ischemic hearts. Dent et al. (33) also showed similar results in volume-overloaded cardiac tissues.

The role of phospholipase C signaling in TNF receptor–mediated protection against adriamycin-induced cardiac injury was supported by a finding that the selective phospholipase C inhibitor, U73122 (34, 35), exacerbated adriamycin-induced cardiac dysfunction similar to that observed in DKO mice after adriamycin treatment for 3 days. The 3-day time point was chosen because our previous study observed the highest serum creatine kinase and lactate dehydrogenase activities after a single injection of adriamycin (36). Our findings, which indicated that there was no difference in the basal cardiac function between wild-type and DKO mice determined by all functional variables evaluated, suggested that under normal physiologic conditions, TNF receptor deficiency does not result in cardiac dysfunction. However, lack of TNF receptors significantly exacerbate adriamycin-induced cardiac dysfunction by decreases of heart rate, ejection fraction, fractional shortening, stroke volume, cardiac output, and reduction of phospholipase C activity. These results suggested that phospholipase C activity was important in TNF receptor–mediated cardioprotection, and the effects were sustained for at least 3 days, which were confirmed by the effect of U73122 on adriamycin-induced decrease of cardiac contractility. Although there is no clear evidence demonstrating the linear relationship between phospholipase C-d1 activities and cardiac function in the literature, Asemu et al. (32) have shown that there was ~40% decrease of phospholipase C-d1 activities and phospholipase C-d1 protein levels (the same decreased levels we observed in adriamycin-treated DKO mice) in the ischemic heart, which corresponded to the significant decreases of cardiac function. Tappa et al. (37) also showed that a 30% decrease of phospholipase C activity corresponded with significant decreases in the cardiac function in the diabetic cardiomyopathic rat model. Thus, a 40% decrease of phospholipase C-d1 protein levels may contribute significantly to the cardiac dysfunction observed in our adriamycin-treated DKO mice. However, it should be noted that the relationship between loss of phospholipase C-d1 and cardiac dysfunction may not be linear and loss of phospholipase C-d1 may have to exceed a minimum level before cardiac function is affected. Our results suggested the importance of phospholipase C activity in TNF receptor–mediated cardioprotection. This may, in part, be due to phospholipase C signaling that helped to maintain normal mitochondrial function and the ATP production required for normal cardiac contractility and function. However, it has also been shown that U73122 could affect muscarinic receptor expression (38), receptor-mediated PI turnover (39), and neutrophil adhesion (40). Thus, we could not completely rule out the possibility of the involvement of other signaling pathways.

Our results are the first to show that TNF receptor signaling prevented adriamycin-induced down-regulation of mitochondrial function and energy production–related genes. Our data also suggested that cytoprotective signaling via TNFRI and TNFRII against adriamycin-induced cardiac dysfunction was mediated, at least in part, by the phospholipase C signaling pathway. These results showed a link between TNF receptor–mediated cardioprotection and adriamycin-induced mitochondrial injury. Thus, enhancing phospholipase C-d1 activity may be a good strategy to prevent adriamycin-induced cardiotoxicity and other mitochondrial dysfunctions related to cardiovascular diseases. Although the phospholipase C-γ signaling pathway has been shown to be a key molecular switch for tumor cell progression (41, 42), the effect of phospholipase C-d1 on tumor cells is unclear. The mechanism by which adriamycin kills tumor cells is thought to be different from the mechanism which regulates the activity of adriamycin in cardiac tissue. Whereas adriamycin kills tumor cells mainly by intercalating into the DNA double helix and interfering with DNA replication and transcription, the production of free radicals is considered a predominant mechanism for adriamycin-induced cardiac injury. Thus, enhancing phospholipase C-d1 activities might be expected to have little effect on the efficacy of adriamycin chemotherapy. However, the effects of enhancing phospholipase C-d1 activities on tumor response to adriamycin need to be investigated. The identification of other mitochondria and energy production–related genes in TNF receptor–deficient mice following adriamycin treatment, as predicted by microarray analysis, could also serve as a basis for future investigation of the mechanisms of adriamycin-induced cardiac dysfunction.

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