Pifithrin-α protects against doxorubicin-induced apoptosis and acute cardiotoxicity in mice

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DOXORUBICIN (DOX) is a potent, widely used antineoplastic agent in cancer chemotherapy. However, the dose-dependent cardiotoxicity of this drug, which eventually results in refractory cardiac dysfunction, compromises its clinical application (24). Recently, we demonstrated that DOX induces cardiac apoptosis in animals (27). This is in line with previous studies that show that DOX induces acute cardiomyocyte apoptosis (1, 17, 31).

The mechanism of DOX-induced cardiac apoptosis has at least two components. One is the DOX-induced generation of reactive oxygen species (ROS) such as H2O2 and superoxide anion (O2·−); this has been well documented in isolated mitochondria, cultured cardiomyocytes, and intact hearts (11, 12, 23, 39). The generation of these ROS is thought to cause mitochondrial morphological and functional damage (24), which may lead to cardiac myocyte apoptosis by direct initiation of mitochondrial-dependent pathways (2, 8). Recent studies suggest that the mechanism of DOX-induced cardiac apoptosis is dependent on the activation of tumor suppressor p53 (16, 28, 37). In one study, the expression of ventricular p53 and its target gene Bax were upregulated dramatically with a significant increase of myocyte apoptosis following rapid ventricular pacing in dogs (25). In another study, transfection of p53 into ventricular myocytes was shown to induce apoptosis by initiating a mitochondria-dependent apoptotic pathway (34). These studies suggest that p53 may play an important role in cardiac apoptosis and that blockade of p53-dependent apoptosis may facilitate the survival of cardiac cells.

A chemical inhibitor of p53, pifithrin-α (PFT-α), was recently found to inhibit p53-dependent apoptosis (18). For instance, PFT-α protects neurons against p53-dependent apoptosis induced by DNA-damaging agents, amyloid β-peptide, and glutamate (9). Although the mechanism for the antiapoptotic effects of PFT-α is not completely understood, a recent study in endothelial cells showed that PFT-α inhibits both p53 transactivation activity and p53 downstream events (29) including the upregulation of the proapoptotic protein Bax and the activation of caspases.

Although the effects of PFT-α in these studies seem to be promising, no study has been conducted to assess the role of p53 and the effects of PFT-α on DOX-induced cardiotoxicity. In the present study, we hypothesized that PFT-α can block DOX-induced cardiotoxicity by inhibiting p53-mediated cardiac cell apoptosis. To test this hypothesis, phosphorylation of p53 and p53 levels were determined shortly after DOX treatment; in addition, ribonuclease protection assay (RPA) and Western blotting were employed to determine the effects of PFT-α on DOX-induced gene expression of p53 and its target genes 24 h later. In situ oligo ligation (ISOL) analysis was performed to measure cardiac cell apoptosis in mice treated with DOX alone or DOX and PFT-α 5 days later. The effects of PFT-α on DOX-induced cardiotoxicity were measured by assessing cardiac ultrastructural damage, dysfunction, and myocyte injury. Finally, the effects of PFT-α on the antitumor efficacy of DOX were studied using a PC-3 cancer cell line, which is a DOX-sensitive cell line that is widely used to test the chemotherapeutic efficacy of DOX (33).

MATERIALS AND METHODS

Materials and animals. DOX-HCl (2 mg/ml) was purchased from Pharmacia and Upjohn (Kalamazoo, MI). PFT-α was a product of Calbiochem (San Diego, CA). The creatine phosphokinase (CPK) kit (CK-20) and F-12K medium were purchased from Sigma Chemical (St. Louis, MO). PC-3 cells were purchased from American Type...
Culture Collection (Manassas, VA). The ApopTag ISOL kit was obtained from Serological (Norcross, GA). Anti-Bax antibody was a product of Oncogene Research Products (Cambridge, MA). MDM2 and p53 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antibodies against phosphorylated p53 at Ser6, -9, -15, -20, and -392 were purchased from Cell Signaling Technology (Beverly, MA).

ICR mice (body wt, 25–30 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN). PFT-α was injected 30 min before and 3 h after DOX administration at a total dose of 4.4 mg/kg ip in 5% DMSO. Control mice were injected with an equal volume of 5% DMSO. All animal protocols were performed according to the Guidelines of the American Physiological Society and were approved by the University Committee on Animal Care of East Tennessee State University.

Ribonuclease protection assay. Mice were treated with DOX in the presence or absence of PFT-α for 24 h. Total RNA was extracted by TRI Reagent LS (Molecular Research Center; Cincinnati, OH) and was digested by RNase-free DNase I. Gene expression of p53, Bax, and MDM2 was analyzed by RPA with specific templates (Ambion; San Antonio, TX). Synthesis of 32P-labeled cRNA probes was performed using an in vitro transcription kit according to manufacturer’s instructions. Probes were gel purified, hybridized with 5 μg of total RNA at 56°C overnight, and then digested with RNase A and T1. Protected RNA fragments were extracted with phenol-chloroform-isooamyl alcohol (25:24:1 ratio) and separated on a 6% polyacrylamide gel that contained 8 M urea. Gels were dried and subjected to autoradiography. Expression of glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. Protected cRNA fragments were then quantified by densitometry with an Alpha Imaging System (Alpha Innotech; San Leandro, CA).

Western blot analysis. Mice were treated with DOX with or without PFT-α for 0.5–24 h (as indicated in Fig. 2). Mouse hearts were homogenized with a Polytron homogenizer in a buffer that contained 20 mM HEPES (pH 7.5), 150 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT, 1% Nonidet P-40, 0.1% SDS, 2 μg/ml leupeptin, 2 μg/ml pepstatin, and 1 mM PMSF. Heart homogenates were incubated on ice for 30 min and were then centrifuged at 12,000 g for 20 min. The supernatant was transferred, aliquoted, and stored at −80°C. Protein concentrations were determined via BCA protein assay (Pierce Chemical; Rockford, IL). Proteins were separated by 12% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes. Membranes were blocked by gentle agitation in Tris-buffered saline that contained 0.1% Tween 20 and 5% nonfat dry milk for 60 min at room temperature. Blots were subsequently incubated with specific antibodies at 4°C overnight followed by incubation with corresponding secondary antibodies. Blots were washed for 15 min three times and were then detected with ECL Western blotting detection reagent (Amersham Biosciences; Piscataway, NJ).

In vivo cardiac function analysis. In vivo cardiac function was analyzed as previously described (27). Mice were randomly assigned to four groups: control, DOX, DOX + PFT-α, and PFT-α. Mice body weights were measured daily after administration of DOX and/or PFT-α. On day 5, mice were anesthetized with chloral hydrate (360 mg/kg ip), intubated, and ventilated with a rodent ventilator (Columbus Instruments; Columbus, OH). After a left thoracotomy was performed, the pericardium was dissected to expose the heart. A 26-gauge needle connected to a pressure transducer was introduced into the left ventricle after an apical stab to measure the left ventricular (LV) pressure. Two pairs of 1-mm piezoelectric crystals were attached to the apex, aorta root, and anterior and posterior walls of the heart. The intercristal distance, LV pressure, and electrocardiogram measurements were recorded on a beat-to-beat basis. Cardiac function parameters including heart rate (HR), LV end-diastolic pressure (LVEDP), LV developed pressure (LVPD), first derivatives of LV pressure over time (±dP/dt), stroke volume (SV), and cardiac output (CO) were analyzed with a SonoSoft Data Acquisition and Analysis System (SonoMetrics; London, Ontario, Canada).

After in vivo cardiac function was assessed, animals were killed. Blood was collected for the determination of CPK levels. Hearts were removed for ultrastructural and apoptosis studies.

ISOL analysis. Staining of DNA strand breaks was performed on 5-μm sections of hearts from mice treated with DOX in the presence or absence of PFT-α for 5 days using the ApopTag ISOL kit according to the manufacturer’s instructions with modifications as described previously (5). Endogenous biotin was blocked with an avidin-biotin blocking kit (BioGenex Laboratories; San Ramon, CA). Trexigen Apoptotic Cell System (TACS) blue peroxidase substrate (CardioTACS, Trevigen; Gaithersburg, MD) was used to label positive nuclei, and nuclear fast red was used to counterstain normal nuclei. The percentage of ISOL-positive cardiac cells was determined by counting 10 random fields/section.

Ultrastructural heart examination. Hearts from mice treated with DOX in the presence or absence of PFT-α for 5 days were fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature for 4 h and were processed as described previously (27). Samples were observed under a Philips Tecnai-10 electron microscope. At least three tissue blocks from each treatment group were randomly picked for the ultrastructural study.

Measurement of serum CPK activities. CPK activities in mouse serum 5 days after treatment were assayed using a CPK test kit (Sigma Chemical) as previously described (27). One unit of CPK was defined as 1.0 μmol of product formed per minute at 37°C.

Fig. 1. Inhibitory effects of pitirhin-α (PFT-α) on the expression of p53 target genes in doxorubicin (DOX)-treated mouse hearts. Mice were treated with DOX and/or PFT-α and were killed 24 h later. Total heart RNA was subjected to ribonuclease protection assay analysis. A: PFT-α inhibited the expression of Bax and MDM2 but not p53. Lane 1, control (Con); lane 2, DOX; lane 3, DOX + PFT-α; and lane 4, PFT-α. B: intensity of protected bands was quantified by densitometric analysis. *P < 0.05, DOX vs. control, DOX + PFT-α, and PFT-α; n = 3.
RESULTS

PFT-α inhibits DOX-induced expression of p53 target genes. Our first set of experiments was designed to measure the mRNA levels of apoptotic genes in mouse hearts following DOX challenge in the presence or absence of PFT-α. As shown in Fig. 1, mRNA levels of Bax and MDM2 were increased two- to threefold in mouse hearts 24 h after DOX treatment. This increase was blocked by PFT-α. Neither DOX nor PFT-α had any effects on p53 gene expression in mouse hearts (Fig. 1).

PFT-α inhibits protein expression of p53 target genes. As discussed above, DOX did not induce the upregulation of p53 gene expression. Because phosphorylation is known to play an important role in p53 transactivation activity (13, 20, 21) and because p53 is a direct transcriptional activator of Bax during apoptosis (30), the mechanism for DOX-induced upregulation of Bax and MDM2 was hypothesized to involve p53 phosphorylation. The protein levels of p53 and its phosphorylated forms at different serine residues (Ser6, -9, -15, -20, and -392) were measured by Western blot analysis. As shown in Fig. 2A, levels of p53 phosphorylated at Ser15 increased 1 h after DOX treatment and peaked 2 h after DOX treatment. Phosphorylation of p53 at other serine residues was not significantly upregulated (data not shown). The protein level of p53 was elevated 4 h after DOX treatment (Fig. 2A). Cotreatment with PFT-α did not affect the level of phospho-p53 at Ser15 (Fig. 2B).

The effects of PFT-α on DOX-induced protein expression of the p53 target genes Bax and MDM2 in mouse hearts were analyzed. Although PFT-α did not attenuate DOX-induced elevation of p53 levels, it dramatically inhibited DOX-stimulated protein expression of Bax and MDM2 in mouse hearts (Fig. 2, C and D). These data are consistent with our RPA study, which showed that PFT-α inhibited DOX-upregulated gene expression of Bax and MDM2 (see Fig. 1).

PFT-α attenuates DOX-induced myocardial ultrastructural alterations. Administration of DOX led to myocardial ultrastructural damage such as cytoplasmic vacuolization, mitochondrial swelling, and the presence of dense bodies in cardiac

![Fig. 2. Effects of PFT-α on the expression of apoptosis-related proteins. A: mice were killed at indicated times after DOX injection, and mouse heart proteins were blotted with specific antibodies against phospho-p53 at Ser15, p53, and actin. B: mice were killed 2 h after DOX treatment with or without PFT-α administration. Levels of p53 and phosphorylated p53 at Ser15 were detected by Western blot analysis. C: mice were killed 24 h after DOX and/or PFT-α treatment, and heart proteins were analyzed by Western blotting with specific antibodies against Bax, MDM2, p53, and actin. Lane 1, control; lane 2, DOX; lane 3, DOX + PFT-α; and lane 4, PFT-α. D: densitometric analysis of Bax, MDM2, and p53 expression in mouse hearts after treatment. All values were standardized against actin. *P < 0.05, DOX vs. control and PFT-α; **P < 0.05, DOX vs. DOX + PFT-α; n = 5.

as the reduction of 1 μmol NAD⁺ to NADH per minute in a Bis-Tris buffer (pH 6.9) at 25°C.

Cell density assay. PC-3 cells were cultured in F12-K medium supplemented with 10% fetal bovine serum. Approximately 3,000 PC-3 cells/well were plated into 96-well dishes and allowed to attach overnight.

PFT-α at various doses was added 3 h before the administration of 80 nM DOX. The number of surviving cells was measured 4 days after DOX application with a sulforhodamine B colorimetric assay (27).

Statistics. All values are expressed as means ± SE. Statistical differences between each group were determined by one-way ANOVA followed by Tukey’s multiple-comparison test if there was a significant difference between groups. Statistical results were considered significantly different at P < 0.05.

RESULTS

PFT-α attenuates DOX-induced myocardial ultrastructural alterations. Administration of DOX led to myocardial ultrastructural damage such as cytoplasmic vacuolization, mitochondrial swelling, and the presence of dense bodies in cardiac

![Fig. 3. Effects of PFT-α on DOX-induced apoptosis in mouse hearts. Mice were treated with DOX and/or PFT-α and killed 5 days later. Heart sections were stained with an in situ oligo ligation kit. *P < 0.05, DOX vs. control, DOX + PFT-α, and PFT-α; n = 6.](http://ajpheart.physiology.org/)

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mitochondria (Fig. 4B). Treatment of DOX along with PFT-α dramatically suppressed the damage, particularly the degree of cytoplasmic vacuolization, mitochondrial swelling, and cristae disappearance. No myofibril loss was observed despite the existence of occasional myofibril disorganization (Fig. 4C). Control (Fig. 4A) or PFT-α treated (Fig. 4D) hearts showed normal ultrastructure.

**PFT-α protects against DOX-induced cardiac dysfunction.** In vivo cardiac function was analyzed in mice treated with DOX and/or PFT-α. Although DOX treatment did not result in cardiac hypertrophy and dilatation during a 5-day period (27), it greatly suppressed cardiac function under anesthetized conditions (Table 1). DOX treatment resulted in a significant elevation of LVEDP and suppression of LVDP, which indicates a decline of LV contractility. Owing to this suppression of LV contractility, reductions of SV and CO were also observed in DOX-treated mice. In contrast, mice administered both DOX and PFT-α showed greatly improved cardiac function.

**PFT-α inhibits DOX-induced serum CPK release.** Because DOX causes disruption of the cardiac myocyte membrane, the release of intracellular CPK into serum was used to evaluate the extent of myocyte injury (10). Mouse serum CPK values were elevated 5 days after DOX treatment. PFT-α significantly reduced DOX-induced release of serum CPK (Fig. 5).

**Effects of PFT-α on the antitumor activities of DOX.** DOX killed ~50% of PC-3 cells at a concentration of 80 nM as determined previously by a sulforhodamine-stained cell density test (27). Treatment of cells with PFT-α at a concentration <10 μM did not have any effect on the growth of PC-3 cells or inhibition of PC-3 cells by DOX. In fact, PFT-α showed toxicity at 50 μM and enhanced the cytotoxicity of DOX (Fig. 6).

**DISCUSSION**

The present study demonstrates a novel protection of PFT-α against DOX-induced cardiac cell apoptosis and cardiotoxicity. This protection is primarily due to the inhibition of apoptosis via the inhibition of p53 downstream activity, which includes Bax upregulation.
Table 1. Effects of PFT-α on DOX-induced cardiac dysfunction

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>DOX</th>
<th>DOX + PFT-α</th>
<th>PFT-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>435±11</td>
<td>373±28</td>
<td>383±25</td>
<td>446±15</td>
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<tr>
<td>LVDP, mmHg</td>
<td>90±6</td>
<td>63±3*</td>
<td>88±3</td>
<td>92.5±1</td>
</tr>
<tr>
<td>HR × LVDP, mmHg-beats-min⁻¹</td>
<td>39,179±1,978</td>
<td>23,180±1,309*</td>
<td>33,618±2,556</td>
<td>41,251±1,634</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>3.85±0.31</td>
<td>10.7±1.2*</td>
<td>4.25±0.45</td>
<td>4.11±0.43</td>
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<tr>
<td>+dP/dt, mmHg/s</td>
<td>4,362±477</td>
<td>1,890±104*</td>
<td>3,624±310</td>
<td>4,188±188</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>3,247±437</td>
<td>1,484±27*</td>
<td>2,778±266</td>
<td>3,400±180</td>
</tr>
<tr>
<td>SV, µl</td>
<td>10.0±1.0</td>
<td>4.84±0.78*</td>
<td>9.34±0.60</td>
<td>9.86±0.91</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>4.32±0.43</td>
<td>1.76±0.43*</td>
<td>3.89±0.25</td>
<td>4.05±0.53</td>
</tr>
</tbody>
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Values are means ± SE; n = 6–10 mice. Mice were injected with doxorubicin (DOX) and/or pifithrin-α (PFT-α). Whereas 6 of 9 DOX-treated mice survived, all DOX-treated animals that received PFT-α survived for 5 days. In vivo cardiac function was measured 5 days after DOX injection by a SonoMetrics data acquisition and analysis system. Con, control; HR, heart rate; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; ±dP/dt, first derivatives of left ventricular pressure; SV, stroke volume; CO, cardiac output. *P < 0.05, DOX vs. Con, DOX + PFT-α, and PFT-α.

PFT-α blocks DOX-induced p53-dependent gene expression. This study strongly suggests that DOX-induced apoptosis involves upregulation of Bax. As shown in Fig. 1, the mRNA levels of Bax were increased two- to threefold in mouse hearts after DOX treatment, an increase that was blocked by PFT-α. However, DOX did not affect p53 gene expression; therefore, DOX-induced increases in Bax mRNA levels were not mediated by increased p53 gene expression. Rather, it appears that the elevation of Bax mRNA levels depends on DOX-induced phosphorylation of p53 at Ser15 (see Fig. 2A). Our results suggest that phosphorylation of p53, which is known to play an important role in p53 transactivation activity (13, 20, 21), increases the upregulation of Bax gene expression.

Our study shows that PFT-α does not affect p53 gene expression but does block the p53-dependent expression of Bax and MDM2. These data suggest that PFT-α blocks transactivation activities of p53. In previous studies, PFT-α was shown to suppress the translocation of p53 into nuclei and reduce the stability of nuclear p53, but it did not affect the protein expression or sequence-specific DNA binding activity of p53 (18). We have shown that DOX enhanced the expression of the p53 downstream genes Bax and MDM2 in mouse hearts. Bax is known to promote apoptosis, and it is involved in cardiac myocyte apoptosis of right ventricles from rats after pulmonary artery banding (16) and coronary occlusion (26). The upregulation of Bax observed in this study suggests that cytochrome c may be released from mitochondria to initiate the activation of caspase-9, which cleaves procaspase-3 into active caspase-3. Indeed, DOX has been shown to induce cytochrome c release in cultured cardiac myocytes (6, 40).

On the other hand, MDM2 appears to have a very different function. Sequence analysis of the MDM2 promoter reveals the existence of a p53 DNA-binding consensus sequence, and p53 is known to augment MDM2 expression (42). Previous studies indicate that MDM2 is an important negative regulator of p53 that controls the degradation rate of p53 (14, 15). MDM2 binds to the NH₂-terminal domain of p53 and acts as a ubiquitin ligase that leads to p53 proteolysis by the 26S proteosome (15). As such, there is a negative-feedback loop involved in the regulation of p53; p53 augments MDM2 expression, which in turn augments the degradation of p53. In this study, MDM2 was upregulated in the hearts of DOX-treated mice. Presumably, this would decrease p53 levels. However, p53 levels were elevated following the phosphorylation of p53 (Ser15) in the hearts of DOX-treated mice (see Fig. 2). These seemingly contradictory findings might be due to the fact that phosphorylation of p53 at Ser15 hinders its interaction with MDM2 (36) and thus prevents MDM2 from augmenting p53 degradation. Additional studies on this feedback loop may be helpful in finding approaches to block DOX-induced cardiac cell apoptosis.

DOX-induced cardiotoxicity is mediated by DOX-induced cardiac apoptosis. Several lines of evidence indicate that DOX-induced cardiotoxicity (e.g., cardiac dysfunction) is mediated by cardiac cell apoptosis. First, apoptosis has been
shown to contribute to the development of cardiac failure in paced dogs (25); as such, inhibition of cardiac cell apoptosis would presumably protect cardiac function. Second, inhibition of cardiac cell apoptosis by the p38 inhibitor SB-203580 greatly improved the recovery of cardiac function in ischemia-reperfusion injury (44). Third, overexpression of caspase-3 suppresses cardiac function recovery and exacerbates ischemia injury by enhancing cardiac cell apoptosis, which indicates that protection against cardiac injury could be achieved by inhibiting cardiac myocyte apoptosis (7). Finally, activation of poly(ADP-ribose)polymerase, a substrate of caspases, promotes cardiac damage and development of heart failure in mice after DOX injection (32).

The present study adds additional support for the link between DOX-induced cardiotoxicity and DOX-induced apoptosis. As shown in Fig. 3, DOX significantly increased the percentage of apoptotic cells and also caused ultrastructural damage, cardiac dysfunction, and cardiac myocyte injury (see Figs. 4 and 5 and Table 1). The mechanism of DOX-induced cardiotoxicity is most likely related to DOX-mediated upregulation of Bax. DOX is able to induce apoptosis via the Fas pathway that is independent of Bax (31), and activation of Fas has been shown to be mediated by a p53-dependent pathway in primary endothelial cells (29). Given that PFT-α may block p53-dependent pathways, it is unclear whether Fas mediates the DOX-induced apoptosis observed in our study. This possibility needs to be further examined.

PFT-α attenuates DOX-induced cardiac dysfunction and myocardial damage. DOX treatment is known to result in acute cardiac dysfunction in mice (32, 41). In this study, severe depression of LV function developed 5 days after DOX treatment (see Table 1). This depression manifested itself as reduction of LVDP and elevation of LVEDP, thereby decreasing SV. Administration of PFT-α along with DOX dramatically improved mouse cardiac function. Mice injected with DOX and PFT-α showed relatively normal LVDP and LVEDP values. This finding suggests that PFT-α can protect against DOX-induced cardiac dysfunction. It is feasible that the observed reduction of LVDP could be the result of decreased arterial pressure in response to anesthetics. Such a decrease in arterial pressure would compromise the coronary circulation and could therefore have induced the observed alterations in SV, CO, and LVDP × HR in this study.

DOX is known to cause ultrastructural changes of myocytes including cytoplasmic vacuolization, mitochondrial damage, and myofibrillar loss (3, 22, 35). Because DOX triggers membrane peroxidation and disruption, release of CPK from myocytes is a marker of myocardial damage, which can be quantified by measuring the CPK levels in the blood or culture medium (10, 27). In this study, ultrastructural changes were observed 5 days after DOX treatment at a high dose of 22.5 mg/kg (see Fig. 4B). Cotreatment of mice with PFT-α significantly attenuated all of these myocardial ultrastructural changes (see Fig. 4C) as well as the release of CPK (see Fig. 5). It is well known that CPK could be released from the brain, kidney, and skeletal muscle in pathological conditions. However, significant ultrastructural changes in the kidney and brain were not detected in this study (data not shown). In the absence of kidney or brain injury, CPK is a relatively specific marker for cardiac injury.

The reversal of DOX-induced ultrastructural changes and the attenuation of CPK release by PFT-α indicate that PFT-α can dramatically reduce DOX-induced myocardial damage. The exact mechanisms of the protective effects of PFT-α are not clear but most likely involve the inhibitory effects of PFT-α on apoptosis (see Fig. 3). A recent study showed that PFT-α is able to block p53-independent signaling pathways in mouse thymocytes such as the activation of heat shock transcription factor and glucocorticoid receptors (19). As such, the mechanism of the inhibitory effects of PFT-α on apoptosis may be partially attributed to the blockade of a p53-independent pathway. Additional studies are needed to explore this possibility.

Effects of PFT-α on the antitumor potency of DOX. PC-3 is a DOX-sensitive prostate carcinoma cell line that is widely used to investigate the cytotoxic effects of various anticancer drugs including DOX and other anthracycline derivatives (4, 33, 38). DOX inhibits PC-3 cell growth and proliferation via a p53-independent apoptotic pathway; indeed, these cells lack expression of functional p53 (43). More than 50% of human malignancies are associated with missense mutation or deletions of p53, as it is one of the most commonly mutated genes found in human tumors. To simulate the majority of cancer patients in a clinical setting, we employed a p53-mutated PC-3 cancer cell line to test whether PFT-α compromises the antitumor effects of DOX. As shown in Fig. 6, PFT-α did not attenuate the antitumor activities of DOX; in fact, it actually enhanced the antitumor activity at higher concentrations. Such an enhancing activity would be an especially valuable advantage in a combination of PFT-α and DOX treatment in cancer chemotherapy. Our results demonstrate that PFT-α does not compromise the anti-tumor activity of DOX in p53-mutated cancer cells. It remains to be established whether PFT-α would compromise the antitumor effects of DOX in cancer patients with wild-type p53.

In conclusion, our findings suggest a role of p53 in DOX-induced cardiac apoptosis and indicate that PFT-α could be a novel cardioprotective agent that may be used clinically to help offset the negative side effects of DOX. This is important for clinical treatment of patients undergoing chemotherapy and especially for cancer patients at high risk for heart disease. We acknowledge that the current animal model, which used a high dose of DOX, is an imperfect tool for modeling the chronic cardiotoxicity of DOX. The rationale for using an acute, high dose of DOX stemmed from the fact that many DOX-induced signal transduction cascades are time dependent; as such, activity of these cascades may be easily missed in a chronic model. In the future, it will be important to evaluate the clinical relevance of this study in a chronic model. Although this study suggests that PFT-α inhibits DOX-induced cardiotoxicity via blockade of a p53-dependent pathway, it will be important to determine whether inhibition of a p53-independent pathway also plays a role. Additional studies are needed to establish the efficacy and safety of a combination PFT-α and DOX treatment regimen in clinical settings.

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