DNA damage is an early event in doxorubicin-induced cardiac myocyte death

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DNA damage is an early event in doxorubicin-induced cardiac myocyte death. Am J Physiol Heart Circ Physiol 291: H1273–H1280, 2006. First published March 24, 2006; doi:10.1152/ajpheart.00738.2005.—Anthracyclines are antitumor agents the main clinical limitation of which is cardiotoxicity (12). The target of anthracyclines is the cardiac myocyte, which is postreplicative and thus unable to regenerate when lethally injured. Most evidence supports oxidative stress as the primary mechanism by which AC induce lethal cardiac myocyte injury, including the demonstration of oxidative stress in the heart (40) and in cardiac myocytes (29) after AC treatment and the protective effect of endogenous (19, 37) and exogenous (30) antioxidants against the detrimental effect of AC. AC treatment leads to myocyte endogenous (19, 37) and exogenous (30) antioxidants against demonstration of oxidative stress in the heart (40) and in cardiac myocytes. Although protein and lipid oxidation have been documented in anthracycline-treated cardiac myocytes, DNA damage has not been directly demonstrated. This study was undertaken to determine whether anthracyclines induce cardiac myocyte DNA damage and whether this damage is linked to a signaling pathway culminating in cell death. H9c2 cardiac myocytes were treated with the anthracycline doxorubicin at clinically relevant concentrations, and DNA damage was assessed using the alkaline comet assay. Doxorubicin induced DNA damage, as shown by a significant increase in the mean tail moment above control, an effect ameliorated by inclusion of a free radical scavenger. Repair of DNA damage was incomplete after doxorubicin treatment in contrast to the complete repair observed in H2O2-treated myocytes after removal of the agent. Immunoblot analysis revealed that p53 activation occurred subsequent in time to DNA damage. By a fluorescent assay, doxorubicin induced loss of mitochondrial membrane potential after p53 activation. Chemical inhibition of p53 prevented doxorubicin-induced cell death and loss of mitochondrial membrane potential without preventing DNA damage, indicating that DNA damage was proximal in the events leading from doxorubicin treatment to cardiac myocyte death. Specific anthracycline-induced DNA lesions included oxidized pyrimidines and 8-hydroxyguanidine. DNA damage therefore appears to play an important early role in anthracycline-induced lethal cardiac myocyte injury through a pathway involving p53 and the mitochondria.

anthracycline; p53; comet assay; deoxyribonucleic acid damage; mitochondrial membrane potential; oxidative stress

ANTHRACYCLINES (AC) are very effective chemotherapy agents the chief limitation of which is the development of cardiotoxicity (12). The target of AC is the cardiac myocyte, which is postreplicative and thus unable to regenerate when lethally injured by a toxic compound or other insult. Most evidence supports oxidative stress as the primary mechanism by which AC induce lethal cardiac myocyte injury, including the demonstration of oxidative stress in the heart (40) and in cardiac myocytes (29) after AC treatment and the protective effect of endogenous (19, 37) and exogenous (30) antioxidants against the detrimental effect of AC. AC treatment leads to myocyte loss, cardiac dysfunction, and eventually clinical symptoms when a sufficient cumulative loss of functioning myocytes has occurred.

Reactive oxygen species, such as generated by AC exposure, induce detrimental modifications to multiple cellular macromolecules, including proteins, lipids, and DNA. Although AC-induced oxidative lesions to cardiac myocyte lipids (26) and proteins (23) have been demonstrated, damage to DNA has been inferred (28) but never directly demonstrated. In contrast, DNA damage is a prominent feature produced by AC and other oxidants in tumor cells (32) and lymphocytes (27). Oxidative DNA base modifications have been demonstrated in hearts and isolated myocytes subjected to oxidant injury, including ischemia-reperfusion (38) and antioxidant depletion (11), indicating that this cell type is susceptible to oxidant-induced DNA damage. In addition to oxidative DNA damage, AC bind avidly to DNA in the nucleus of cancer cells, forming adducts that can interfere with binding of proteins such as transcription factors and RNA polymerase, potentially interfering with the important cellular functions of DNA, including replication and transcription (7).

DNA damage in proliferative cells activates a pathway that arrests cell division to allow either DNA repair or the induction of cell death by apoptosis. p53 is an effector protein in this pathway that plays a critical role in the induction of cell cycle arrest and apoptosis (39). Once activated, p53 translocates to the nucleus where it induces expression of genes that prevent cell division (i.e., p21) and cause apoptosis (e.g., Bax; see Ref. 6). In more differentiated skeletal muscle cells, DNA lesions induced by AC exposure also induce apoptosis in a p53-dependent fashion (17). An alternate response by cells subjected to DNA damage is lesion repair rather than apoptosis. DNA repair utilizes enzymes that excise oxidized bases before DNA replication (3), remove oxidized bases from the nucleotide pool (8), or remove oxidized bases from DNA after replication (33). Because cardiac myocytes are postreplicative, repair of DNA lesions is important to avoid deterioration of cardiac function that would eventually attend myocyte loss. Indeed, nucleotide excision repair, particularly of the transcribed strand, is very active in myocytes after DNA injury induced by irradiation (35). This study was undertaken to define whether AC induce DNA lesions in cardiac myocytes, whether myocytes are able to repair DNA lesions, and to define the pathway transducing the DNA damage into lethal cell injury.

MATERIALS AND METHODS

Cell culture and treatment. The H9c2 cardiac cell line was derived from embryonic rat heart by selective serial passage (15). Cells were plated on 35- or 100-mm dishes in DMEM with 10% FCS and used

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when 70–90% confluent. The AC doxorubicin (DOX) was added to
complete medium and incubated at 37°C for various intervals at a
concentration (0.5 μg/ml) similar to plasma concentrations encoun-
tered in clinical use (10). In some experiments, the free radical
scavenger amifostine was included during DOX treatment at a
concentration of 14 mM (4). Alternatively, a chemical inhibitor of p53,
pifithrin (16), was added at a concentration of 30 μM during DOX
treatment. Pifithrin appears to act downstream of accumulation of p53,
reducing apoptosis by inhibiting production of pro-apoptotic proteins
(21). Amifostine and pifithrin were added to cultures 60 min before
addition of DOX. Cells were alternatively treated with H2O2 at a
concentration of 10 μM in PBS on ice for 5 min. At the end of the
 treatment interval, cells were washed two times with PBS before
harvesting. In repair experiments, treated cells were allowed to re-
cover in fresh complete medium at 37°C for varying intervals before
harvest.

**Single-cell electrophoresis assay.** Oxidative stress, radiation, and
AC cause DNA damage that can be detected and quantified using the
alkaline single-cell electrophoresis assay (comet assay; see Refs. 5, 11,
and 31). During electrophoresis, undamaged DNA is largely confined
to the nucleus, whereas damaged DNA migrates apart from the
nucleus in the shape of a comet. The length and fluorescent intensity
of the comet are proportional to the number of DNA strand breaks.

The extent of DNA damage can be quantified by the use of this
sensitive technique, with the most frequently reported measure being
the tail moment, a product of tail length and percent tail DNA (34).
We performed the assay essentially as described by Singh et al. (31).

**Evaluation of DNA damage.** After electrophoresis, DNA was
stained with 1% propidium iodide, and slides were coverslipped and
analyzed by fluorescent microscopy using an excitation filter of
515–560 nm and a barrier filter of 590 nM. An image analysis system
(Kinetic Imaging, Bromborough, Wirral) was used to measure indexes of
DNA damage. Digitized images were obtained of 50 randomly
selected cells per slide, and the tail moment was determined from two
slides per condition. The median tail moment was determined for each
slide, and the mean of at least eight of these values per condition (i.e.,
at least 2 slides/condition from at least 4 separate days) was used as
the index of DNA damage. The tail moment was expressed as the
degree of increase in elevation above a negative control slide from the
same day to account for daily variability in cell health. Addition of
repair enzymes to the comet assay allows identification of specific
lesions in DNA induced by DOX (5). DOX-treated lysed cells were
washed three times in buffer supplied with each enzyme, drained,
covered with 25 μl of either enzyme buffer or buffer with 0.01 unit
enzyme per microliter, sealed with a cover slip, and incubated for 30
min at 37°C. Cells were subjected to electrophoresis and scoring of
DNA damage as described above.

**Trypan blue exclusion.** Trypan blue is a vital dye excluded by
viable cells with intact cell membranes; nonviable cells fail to exclude
the dye. After treatment with DOX, floating and attached cells were
collected by gentle trypsinization followed by neutralization with
serum-containing medium. Subsequently, a small volume of 0.4%
trypsin was added to cells collected from each dish, and the number of
cells excluding or staining with the dye was immediately counted on a Nikon microscope as described previously (19). To confirm equal loading, an antibody to
β-actin (no. A5316; Sigma, St. Louis, MO) was used at a concentration of 1:20,000 dilution to probe immunoblots. Densitometry of p53 immunoreactive
bands was used to quantify the amount of protein present, which was
expressed as the degree of increase in elevation of the quantity present in
the untreated control condition.

**Assessment of mitochondrial membrane potential.** Redistribution
of proapoptotic proteins from the mitochondria to the cytoplasm with
subsequent apoptosis occurs after loss of mitochondrial membrane
potential (ΔΨ) and opening of the mitochondrial permeability transi-
tion pore. ΔΨ was assessed using the fluorescent indicator 5,5′,6′,
-tetrachloro-1,1′,3′,3′-tetrachlorobenzimidazolocarbocyanine iodide (JC-1;
Molecular Probes, Eugene, OR). Cells exposed to various conditions
were incubated with 1 μg/ml JC-1 for 30 min at 37°C and were
visualized on a fluorescent microscope using a ×20 objective. A shift
from red to green fluorescence indicates a loss of ΔΨ, with appear-
ance of green monomeric JC-1 in the cytosol. Because cells in all
conditions express a degree of green fluorescence, the percentage of
cells expressing red fluorescence was used as an index of ΔΨ,
alogous to the change in red fluorescent intensity previously de-
scribed by Akao et al. (2). Multiple merged images of the fluorescein
and rhodamine channels were acquired from each condition. Cells
were counted as demonstrating red fluorescence if red or orange
staining was visible on a merged image. At least 200 cells were scored
per condition per day as green (indicating loss of ΔΨ), red, or orange
(indicating intact ΔΨ). Data are expressed as the percentage of cells
with intact ΔΨ. In additional experiments (data not shown), we found
similar results when the JC-1 reagent was used in conjunction with
flow cytometry rather than microscopy.

**Statistical considerations.** SPSS software for Windows 98 version
10.0.2 was used for statistical comparisons, as described previously (18).
Observations are presented as means ± SE, and one-way ANOVA
was used to compare means, followed by the post hoc Sidak or
Bonferroni test (a P < 0.05 is considered significant).

**RESULTS**

**H2O2 induces myocyte DNA injury.** To document that nu-
clear DNA damage occurred in cardiac myocytes treated with a
known oxidant, we applied the alkaline comet assay to measure DNA damage in H9c2 cells treated with H2O2. Rep-
resentative micrographs of a control cell and a cell treated with
10 μM H2O2 for 5 min on ice (to prevent DNA repair) are
shown in Fig. 1. In control cells, the undamaged DNA migrates
in an electrophoretic field within the nucleus (Fig. 1A), whereas
some DNA from cells treated with H2O2 migrates away from
the nucleus, forming a tail, or comet (Fig. 1B). Software
analysis allows semiautomated collection of indexes of DNA
damage from multiple cells, which can be analyzed statisti-
cally. As shown in Fig. 2, DNA damage occurs rapidly in H9c2
cells treated with 10 μM H2O2, with a 5-min exposure pro-
ducing a mean tail moment significantly above untreated control
cells [100 ± 3.1 (SE); P < 0.00001]. If cells were returned to
the incubator at 37°C with fresh medium after the 5-min
Treatment with H2O2, DNA damage was repaired rapidly, as
shown in Fig. 2. The mean tail moment after all repair intervals
was significantly less than that without repair (P < 0.0001) and
after a 90- or 120-min repair interval was not significantly
different from untreated control cells (P = 0.145 and 1.0,
respectively). These experiments indicate that oxidative stress rapidly induces cardiac myocyte DNA damage and that H9c2 myocytes have significant DNA repair capacity from a purely oxidative stress. We found that more extensive DNA damage occurred with higher H2O2 concentrations (data not shown).

**DOX induces myocyte DNA injury.** In the next series of experiments, H9c2 cells were treated with the anthracycline DOX for varying intervals before performing the alkaline comet assay. DOX was used at a concentration of 0.5 μg/ml, a concentration achieved after bolus use in human pharmacokinetic studies (10). Although DNA damage was detectable after treatment intervals of 2 h, the mean tail moment did not become statistically different from the untreated control cells until 4 h at 23 ± 4.3 (Fig. 3; \( P < 0.0001 \)). Inclusion of the free radical scavenger amifostine in the culture medium during DOX treatment decreased the mean tail moment to 7.7 ± 1.8 (\( P < 0.0001 \) vs. DOX alone), suggesting that the injury induced by DOX was at least partly oxidative.

To determine whether DNA damage induced by DOX could be repaired, cells were treated with 0.5 μg/ml DOX for 4 h and allowed to repair DNA damage in fresh medium at 37°C for varying intervals before the comet assay was performed. As shown in Fig. 4, DOX treatment for 4 h induced significant DNA damage, with a mean tail moment of 21 ± 1.7 (\( P < 0.0001 \) vs. untreated control). DNA damage progressed in the first 4 h of repair, with the mean tail moment increasing to 29 ± 1.7 (\( P < 0.0001 \) vs. negative control, \( P < 0.006 \) vs. 4 h treatment without repair). The mean tail moment remained significantly elevated after repair intervals as long as 24 h at 18 ± 1.2 (\( P < 0.0001 \) vs. untreated control). Some treated cells began to detach during repair intervals longer than 24 h, making it difficult to assess DNA injury after longer repair intervals. The sum of these results indicate that DOX induces DNA damage in cardiac myocytes that is less extensive and
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After DOX treatment compared with H2O2 treatment suggests treated with 0.5 μg/ml DOX for 4 h and processed for the comet assay immediately or allowed various repair intervals in fresh medium at 37°C before the assay. Mean of tail moments as the degree of increase of negative control is shown as index of DNA damage. *Significantly different from negative control at P < 0.0001.

Fig. 4. DOX-induced DNA damage is incompletely repaired in H9c2 cells. Quantitative presentation of comet assay data from 4 independent experiments performed as described in MATERIALS AND METHODS. Cells were untreated or treated with 0.5 μg/ml DOX for 4 h and processed for the comet assay immediately or allowed various repair intervals in fresh medium at 37°C before the assay. Mean of tail moments as the degree of increase of negative control is shown as index of DNA damage. *Significantly different from negative control at P < 0.0001.

Fig. 5. Specific DOX-induced DNA lesions recognized by DNA repair enzymes. The alkaline comet assay detects single- and double-stranded DNA breaks as well as alkaline-labile sites (34). When the assay is coupled with enzymes that repair specific lesions in DNA, it is possible to define the DNA lesions induced in DOX-treated cells more specifically. An increase in the tail moment is noted in enzyme-treated cells compared with buffer-treated cells if the specific DNA lesion recognized by the enzyme is present in the cell population. In these studies, we first used endonuclease (Endo) III to detect oxidized pyrimidines. As shown in Fig. 5, DOX-treated cells subsequently treated with Endo III had a mean tail moment of 42 ± 5.5, significantly above untreated control cells (P < 0.001) and DOX-treated cells subsequently treated with Endo III buffer alone (19.8 ± 2.1; P = 0.018). This result indicates that DOX induces oxidative damage to pyrimidines in cardiac myocytes, as it does in lymphocytes (5). Similarly, the base excision repair enzyme formamidopyrimidine-DNA glycosylase (Fpg) recognizes and nicks 8-oxoguanine, the most common DNA lesion in cells subjected to oxidative stress (9, 38). As shown in Fig. 5, DOX-treated cells subsequently treated with Fpg had a mean tail moment of 56 ± 7.0, significantly above untreated control cells (P < 0.0001) and DOX-treated cells subsequently treated with Fpg buffer alone (22.8 ± 2.2, P < 0.0001). This result indicates that DOX induces 8-oxoguanine in cardiac myocytes.

DOX activates p53 in H9c2 cells. The transcription factor p53 has a short half-life and is present at a low level in normal cells. Exposure to DNA damaging agents leads to stabilization and accumulation of p53, translocation to the nucleus, and expression of its target genes (6). As shown in Fig. 6A, p53 is not detectable by immunoblot analysis in untreated cells but becomes detectable within 4–8 h of treatment with DOX, and demonstrates a time-dependent accumulation over the first 16 h before returning toward control levels by 48 h. As shown in Fig. 6B, densitometry of p53 immunoblots confirmed significant induction by DOX treatment within 8 h (P < 0.006). Equal loading was confirmed by probing representative immunoblots for β-actin. Activation of p53 correlates with accumulation of the protein and with expression of proapoptotic proteins, including Bax and Bad (17). We were unable to consistently document induction of p53-responsive proapoptotic proteins after treatment with DOX, possibly reflecting inadequate sensitivity of immunoblotting for detection of these proteins in cardiac cells. To confirm the importance of p53 in DOX-induced cell death, H9c2 cells were treated with DOX in the presence or absence of the p53 inhibitor pifithrin, and cell viability was assessed using trypan blue. As shown in Fig. 6C, untreated H9c2 control cells have a rate of spontaneous cell death of 11 ± 0.4% after 48 h in culture. Treatment of cells with DOX significantly increased the percentage of trypan blue-positive cells at 48 h to 19 ± 0.3% (P < 0.0001 vs. untreated control). Inclusion of 30 μM pifithrin during DOX treatment reduced the cell death rate to the untreated control rate of 11 ± 1%. These experiments indicate that p53 activation occurs after DOX treatment and suggest that it plays an important role in mediating DOX-induced H9c2 cell death. To rule out the possibility that pifithrin protects against cell death by preventing DOX-induced DNA damage, a comet assay was performed on H9c2 cells treated for 4 h with DOX in the presence or absence of pifithrin. As shown in Fig. 7, DOX significantly increased the mean tail moment above untreated control to 18 ± 2.5 (P < 0.001), and the inclusion of pifithrin had no significant effect on this DOX-induced DNA damage, indicating that DNA damage induced by DOX is proximal to p53 activation and eventual cell death.

DOX induces ΔΨ loss in cardiac myocytes. Oxidative stress induces p53 accumulation in cardiac myocytes, with eventual apoptosis mediated by release of proapoptotic proteins from the mitochondria, including cytochrome c (36). Activation of p53 is followed by loss of ΔΨ and downstream caspase...
activation in HeLa cells independent of Bax translocation or Bid activity (20). H9c2 cells were treated with DOX in the presence or absence of pifithrin. After cells were loaded with JC-1, the percentage of cells expressing red fluorescent aggregates (indicating intact ΔΨ) was obtained from each condition. Merged images of the fluorescein and rhodamine channels of a representative culture from each condition are shown in Fig. 8. Nearly all cells are well spread and exhibit red or orange fluorescence in an untreated culture, as shown in Fig. 8A. A n image from a culture treated with DOX for 4 h shown in Fig. 8B, which does not look significantly different from the untreated control, indicating that ΔΨ is intact. In contrast, a culture treated with DOX for 16 h is shown in Fig. 8C, demonstrating multiple rounded cells, a majority of which fluoresce green exclusively, indicating loss of ΔΨ. A culture treated with DOX for 16 h in the presence of pifithrin appears similar to untreated control, with spread cells exhibiting red or orange fluorescence, as shown in Fig. 8D. The percentage of cells with intact ΔΨ is displayed in Fig. 9. In untreated control cells, 90 ± 1.1% demonstrated intact ΔΨ, with the percentage similar for cells treated with DOX for 4 h. The percentage of cells with intact ΔΨ decreased significantly after 16 h treatment with DOX to 58 ± 4.0% (P < 0.0001 vs. untreated control or 4 h DOX treatment), and this decrease was prevented by inclusion of pifithrin in the medium, indicating that loss of ΔΨ after DOX treatment is mediated by p53.

DISCUSSION

In summary, our results demonstrate for the first time that the anthracycline DOX induces specific DNA lesions in cardiac myocytes. These lesions occur at clinically relevant concentrations of DOX within several hours of exposure and are not completely repairable. This pattern of DNA damage/repair is distinctly different from that seen in purely oxidative lesions, where DNA damage occurs more rapidly and is rapidly and completely repaired. The data also show that DOX causes activation of p53 after DNA lesion development, with subsequent loss of ΔΨ and loss of cell viability. Furthermore, inhibiting p53 eliminated DOX-induced cell death and loss of ΔΨ without reducing the extent of DNA lesions. Taken together, our results demonstrate that DNA damage is an early event in DOX-induced lethal cardiac myocyte injury and that cell death is mediated through p53 and the mitochondria.

p53 activation in cardiomyocytes by AC. p53 is activated in the hearts of DOX-treated animals. Inhibition of p53 reduced morphological, biochemical, and functional consequences of DOX treatment (21). The function of p53 is best characterized in proliferative cells, where it maintains genomic stability in response to DNA damage, thereby preventing malignant transformation. Highly differentiated cells such as myocytes are also subjected to DNA damage, where p53 activation may maintain the integrity of the transcribed genome to avoid loss of cell function (17). Presumably, death of differentiated cells occurs when DNA lesions exceed the capacity for repair. The
earliest proteins activated in proliferating cells in the DNA damage pathway are ataxia telangiectasia-mutated and ataxia telangiectasia Rad3-related kinase. Subsequent events in this pathway include activation of transducers, including BrcA, Chk-1 and Chk-2, and p53 (39). Apoptosis is one of the responses effected by these transducers. The DNA damage pathway has been demonstrated subsequent to DNA damage in skeletal muscle cells (17), but to our knowledge it has not been demonstrated in cardiac myocytes. Our data demonstrating myocyte DNA damage and p53 activation suggest that the DNA damage pathway is active in this cell type.

Anthracycline-induced DNA injury. Oxidative stress occurs rapidly after DOX treatment of cardiac myocytes (19), and oxidative stress is a known activator of p53 (36), possibly because of oxidative DNA damage. We show in this report that DNA damage is an early event after DOX treatment in cardiac myocytes that is reduced by inclusion of a free radical scavenger. This extends mechanistic observations in cancer cells showing that DOX causes site-specific DNA damage and subsequent generation of H_2O_2 mediated by NAD(P)H oxidase activation (25). However, it appears that DNA lesions in cardiac myocytes may not be exclusively oxidative, since we observed that purely oxidative DNA lesions induced by H_2O_2 were rapidly and completely repaired when the oxidant was removed, whereas the less extensive DOX-induced lesions were not. The alkaline comet assay detects multiple DNA lesions, including single- and double-stranded breaks and alkaline-labile sites. It is possible that the type of DNA lesion(s) induced by H_2O_2 is different and more readily repaired than lesions induced by DOX, a possibility that can be explored by performing the comet assay under neutral conditions, which only detects double-stranded DNA breaks (11). In a previous report, we demonstrated that overexpression of the α4-isoformal of the antioxidant glutathione transferase in cardiac myocytes eliminated DOX-induced oxidative stress and reduced, but did
not eliminate, DOX-induced total and apoptotic cell death (19). Our previous data therefore are consistent with the current report that oxidative stress may not be the exclusive mediator of cardiac myocyte death after DOX treatment. However, DOX has been shown to influence DNA independent of generating oxidative lesions. DOX rapidly localizes to the nucleus in tumor cells, related to its high affinity for DNA. The drug intercalates rapidly in the DNA strand at specific sequences. Specific DNA-binding proteins, including transcription factors, may thereby be denied access to their usual binding sites by these adducts, potentially inhibiting transcription of specific genes (7).

Specific DNA lesions induced by AC include oxidized pyrimidines and 8-hydroxyguanine. These lesions occur in malignant cells after AC treatment (24) but have not been previously demonstrated in cardiac myocytes. AC treatment of lymphocytes induces DNA damage more rapidly, and repair is more rapid and complete (5) than we observed in cardiac myocytes, suggesting that the DNA damage response is cell type specific.

Therapeutic implications. Although oxidative stress is generally accepted as the mechanism by which AC cause toxicity to the heart, antioxidant therapy has not significantly reduced the magnitude of the clinical problem. For example, the iron chelator dexrazoxane reduces oxidative stress by reducing iron availability for the Fenton reaction, a generator of toxic oxygen-centered free radicals. This agent in clinical trials has reduced, but not eliminated, the DOX-induced decline in cardiac function (22). Moreover, antioxidants are not cardiac specific but rather reduce oxidative stress nonspecifically. This potentially reduces the desired effect of AC, since oxidative stress plays a role in the tumor-killing effect of these agents (14). Knowing that DNA injury occurs in myocytes treated with DOX and that elements of the DNA damage pathway are subsequently activated identifies additional steps that can be inhibited to reduce AC-induced cardiac myocyte death. Inhibition of p53 specifically in the heart, for example, may be a treatment to pursue, and this approach has been validated in animal studies (21). The observation that ΔΨ is lost after p53 activation in cardiac myocytes provides an additional target, since cyclosporine, diazoxide, and pinacidil prevent ΔΨ loss in response to cardiac myocyte oxidative stress (1). Agents that specifically act on cardiac myocyte mitochondria to preserve ΔΨ offer the potential to block the detrimental effects of AC while preserving antitumor activity, and such agents are currently under investigation (13).

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