Amlodipine Inhibits Doxorubicin-Induced Apoptosis in Neonatal Rat Cardiac Myocytes

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The anthracycline derivative doxorubicin is widely used to treat various types of cancer; however, its clinical use is limited by dose-dependent cardiotoxicity, leading to severe congestive heart failure, often termed “doxorubicin cardiomyopathy.” Several hypotheses have been proposed to account for doxorubicin cardiotoxicity, including free radical formation (1,2), impaired adrenergic regulation (3), release of vasoactive amines (4), altered calcium handling (5), mitochondrial impairment (6), suppression of muscle-specific genes (7), and ceramide generation (8). The most thoroughly investigated hypothesis for doxorubicin-induced cardiotoxicity is based on the formation of reactive oxygen species, such as superoxide and hydrogen peroxide, by reactions catalyzed by the quinone moiety of doxorubicin (6). Moreover, recent studies have demonstrated that, in addition to necrosis, doxorubicin can induce myocyte apoptosis through the Fas-mediated pathway (9), ceramide generation (8), or reactive oxygen species (10,11). These findings suggest that myocyte apoptosis contributes to the development of myocardial loss and severe contractile dysfunction during the pathogenesis of doxorubicin cardiomyopathy.

Several antioxidants, including probucol (12) and dextrazoxane (13), have been suggested to prevent anthracycline-induced cardiotoxicity. Antioxidant therapy may therefore be useful in the management of doxorubicin cardiomyopathy. To date, however, treatment with antioxidants has not been shown to be clinically effective. Furthermore, the molecular mechanisms by which doxorubicin-activated oxidative stress induces myocyte injury and antioxidants prevent doxorubicin-induced myocyte death remain poorly understood. As recent studies have demonstrated that reactive oxygen species may also induce myocyte apoptosis (14,15), we hypothesized that some antioxidants can effectively inhibit doxorubicin-induced myocyte apoptosis by scavenging reactive oxygen species.

Amlodipine is a newer member of the dihydropyridine subclass of calcium channel antagonists. It has strong membrane-lipid antioxidant activity that is entirely inde-
dependent of calcium channel modulation. A lack of adverse effects on morbidity and mortality in patients with severe congestive heart failure (as demonstrated in the Prospective Randomized Amlodipine Survival Evaluation [PRAISE] trials) indicates that amlodipine has an unusual pharmacologic profile, providing advantages over other calcium antagonists (16). In pilot studies, we have confirmed that amlodipine strongly inhibits doxorubicin-induced apoptosis of cultured neonatal rat cardiac myocytes. In the present study, we examined the mechanisms by which doxorubicin induces and amlodipine inhibits myocyte apoptosis. We have clearly demonstrated that amlodipine attenuates doxorubicin-induced oxidative stress and significantly inhibits myocyte death by inhibiting the mitochondrial apoptotic pathway.

METHODS

Culture of neonatal rat cardiac myocytes. Primary neonatal rat cardiac myocytes were prepared as described previously, with some modifications (17,18). We routinely obtained contractile, myocyte-enriched cultures with >95% myocytes. Before the experiments, the cells were grown for 36 to 48 h in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 0.5% fetal bovine serum.

Experimental protocols. Cell cultures were washed twice with phosphate-buffered saline (PBS), followed by a final incubation in serum-deprived medium. During this final incubation, cardiac myocytes were treated with one of the following: 1) doxorubicin at 10⁻⁶ mol/l; 2) doxorubicin at 10⁻⁸ mol/l in the presence of amlodipine (10⁻⁹ to 10⁻⁵ mol/l); or 3) doxorubicin at 10⁻⁶ mol/l in the presence of nifedipine (10⁻⁶ mol/l). To study the effects of several antioxidants on doxorubicin-induced apoptosis, the myocytes were treated with doxorubicin (10⁻⁶ mol/l) in the presence of: 4) probucol (10⁻⁵ to 10⁻⁴ mol/l); 5) ascorbic acid (5 × 10⁻⁷ mol/l); 6) alpha-tocopherol (10⁻⁴ mol/l); 7) catalase (4 × 10⁴ U/l); 8) glutathione (15 × 10⁻³ mol/l); 9) N-acetylcysteine (NAC) (10⁻⁴ mol/l); 10) mannitol (2 × 10⁻² mol/l); or 11) superoxide dismutase (SOD) (1.2 × 10⁻⁵ U/l). Unless otherwise indicated, treatment was continued for 14 h, at which time we examined cell death and apoptosis-associated signaling cascades by histochemical and biochemical methods. Control myocytes were incubated in serum-deprived DMEM but were not treated with any chemical.

Histochemical determination of cell viability and apoptosis. The relative number of living and dead cells was determined using a viability/cytotoxicity kit (Molecular Probes, Eugene, Oregon) (19). Apoptotic cells were identified by the distinctive condensed or fragmented nuclear structure in cells stained with 0.5 μg/ml of Hoechst 33258 (Molecular Probes), as described previously (20). The cells were photographed with the use of fluorescence microscopy. An average of 800 to 1,000 nuclei from randomly selected fields was analyzed for each experiment, and the number of apoptotic cells was expressed as a percentage of the total number of nuclei counted.

Apoptotic cells were also assessed using annexin V labeling (21). The myocytes on coverslips were washed with ice-cold PBS. Then, 100 μl of diluted annexin V–fluroscein isothiocyanate (FTTC) solution (Sigma, St. Louis, Missouri) was added to the myocytes. The cells were then incubated at room temperature, in the dark for 1 h on a swirling base. The annexin V solution was removed and the myocytes were washed once with PBS. Annexin V binding was analyzed by fluorescent microscopy. For apoptosis quantification by annexin V, the myocytes were scraped and stained with annexin V–FTTC, according to the manufacturer’s instructions. After 15-min incubation on ice, apoptotic cells were measured by fluorescence-activated cell sorter analysis (Becton Dickinson, San Jose, California).

Measurement of adenosine 5′-triphosphate (ATP) content. The ATP content of myocytes was measured after treatment with doxorubicin in the presence or absence of either amlodipine or nifedipine. Following lysis with perchloric acid, cell supernatants were analyzed by high-performance liquid chromatography, as described previously (22).

Myocyte oxidative stress. 2′,7′-Dichlorofluorescin diacetate (H₂DCFDA) (Molecular Probes) was used to evaluate oxidative stress induced by doxorubicin. Peroxides can be detected with the use of the nonfluorescent dye H₂DCFDA, which can freely permeate cells. Once inside the cells, H₂DCFDA is hydrolyzed to 2′,7′-dichlorofluorescein (DCF) and entrapped intracellularly. Then, DCF interacts with peroxides, which convert it to fluorescent DCF, a compound readily detected by a fluorescence microscope (23,24). Activation of DCF is relatively specific for the detection of hydrogen peroxide and secondary and tertiary peroxides. One hour before doxorubicin treatment, H₂DCFDA was added to the cell cultures, and fluorescence from DCF was detected with excitation at 488 nm. H₂DCFDA was prepared in ethanol and diluted with myocyte culture medium to a final concentration of 5 mmol/l.

Measurement of mitochondrial membrane potential. After treatment of myocytes with doxorubicin (10⁻⁶ mol/l) in the presence or absence of amlodipine (10⁻⁶ mol/l) in

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**Abbreviations and Acronyms**

- **ATP**: adenosine 5′-triphosphate
- **Bax**: Bcl-2-associated X protein
- **DCF**: 2′,7′-dichlorofluorescein
- **DMEM**: Dulbecco’s modified Eagle’s medium
- **FITC**: fluorescein isothiocyanate
- **H₂DCFDA**: 2′,7′-dichlorofluorescin diacetate
- **JC-1**: 5,5′,6,6′-tetrachloro-1,1′,3′,3′-tetraethylbenzimidazolylcarbocyanine isode
- **MAPK**: mitogen-activated protein kinase
- **NAC**: N-acetylcysteine
- **PBS**: phosphate-buffered saline
- **SOD**: superoxide dismutase

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serum-deprived medium for 14 h, the myocytes on cover-slips were incubated in PBS containing 10−5 mol/l of 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Molecular Probes) at 37°C for 5 min. Fluorescence was viewed at 527 and 590 nm with excitation at 480 nm.

Western blot analysis. Myocyte cell fractions were used (25). For cytochrome c determination, mitochondrial and cytosolic fractions were subjected to electrophoresis and blotting, and the blots were reacted with anti-cytochrome c monoclonal antibody (Pharmingen, San Jose, California), followed by horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Amersham, Buckinghamshire, UK). Laser scanning densitometry was used for semiquantitative analysis of the data.

Caspase-3 activities. The activities of caspase-3 were determined with a CPP32 assay kit (MBL, Nagoya, Japan) by detection of chromophore p-nitroanilide after cleavage from the labeled substrate Asp-Glu-Val-Asp (DEVD)-p-nitroanilide, as described previously (26).

Statistical analysis. Data are expressed as the mean value ± SEM of at least from six samples derived from six separate experiments. Differences were analyzed by one-way analysis of variance combined with the Bonferroni test, and p values of <0.05 were considered statistically significant.

RESULTS

Detection of doxorubicin-induced cell injury. The effects of doxorubicin treatment on cell viability (necrosis) and apoptosis are illustrated in Figure 1A. Under control conditions, the fraction of viable cells (fluorescent green) was consistently >95%. Treatment of myocytes with doxorubicin at 10−6 mol/l for 14 h did not increase either the fraction of nonviable cells (red fluorescent nuclei) or creatine kinase activity in the medium (data not shown). In contrast, doxorubicin significantly increased the frequency of apoptotic cells with typical fragmented nuclei and condensed chromatin on histochemical nuclear staining with Hoechst 33258, as compared with control myocytes.

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Figure 1. (A) Histochemical determination of cell viability and apoptotic myocytes. Cardiac myocytes were treated with serum-free DMEM alone (a and e), 10−6 mol/l of doxorubicin (b and f), 10−6 mol/l of doxorubicin in the presence of 10−6 mol/l of amlodipine (c and g), or 10−6 mol/l of doxorubicin in the presence of 10−6 mol/l of nifedipine (d and h) for 14 h. The cells were then labeled with calcein acetoxymethyl ester and ethidium homodimer-1 (a–d) or stained with Hoechst 33258 (e–h) and visualized by fluorescence microscopy, as described in the Methods section. Photomicrographs are representative of at least three experiments for each experiment (acetoxymethyl ester and ethidium homodimer-1: magnification × 200; Hoechst 33258: magnification × 400). (B) The effect of calcium channel antagonists on myocyte apoptosis. Myocytes were treated with 10−6 mol/l of doxorubicin in the presence or absence of either amlodipine (10−9 to 10−5 mol/l) or nifedipine (10−6 mol/l) for 14 h. Fluorescent-stained nuclei of apoptotic myocytes were analyzed morphologically and expressed as the percentage of total nuclei, as described in Methods. Control myocytes were incubated in serum-deprived DMEM without any chemicals (n = 6). *p < 0.0001 vs. control. †p < 0.0001 vs. doxorubicin.
Doxorubicin at 10⁻⁶ mol/l thus significantly increased the percentage of apoptotic myocytes, estimated by Hoechst 33258 staining, in a time-dependent fashion.

**Effect of amlodipine on doxorubicin-induced apoptosis.** Amlodipine at 10⁻⁹ to 10⁻⁶ mol/l markedly decreased the number of apoptotic myocytes with fragmented nuclei induced by doxorubicin (Fig. 1A). Figure 1B shows the effect of either amlodipine (10⁻⁹ to 10⁻⁶ mol/l) or nifedipine (10⁻⁶ mol/l) on doxorubicin-induced myocyte apoptosis. Amlodipine inhibited myocyte apoptosis in a dose-dependent fashion, and the percentage of myocytes showing doxorubicin-induced apoptosis was significantly inhibited to 16.4 ± 1.2% and 15.4 ± 0.7% with 10⁻⁷ mol/l and 10⁻⁶ mol/l of amlodipine, respectively, whereas nifedipine at 10⁻⁵ mol/l did not significantly decrease apoptotic myocytes any further. In contrast, the calcium channel antagonist nifedipine at 10⁻⁶ mol/l did not inhibit doxorubicin-induced myocyte apoptosis.

To confirm the results from Hoechst 33258 staining, an annexin V–FITC binding assay was performed. As shown in Figure 2A, doxorubicin at 10⁻⁶ mol/l increased the number of annexin V–FITC–positive cells. Amlodipine at 10⁻⁶ mol/l decreased the number of doxorubicin-induced apoptotic myocytes stained with annexin V–FITC, whereas nifedipine at 10⁻⁶ mol/l did not. When myocyte apoptosis was quantified by flow cytometry (Fig. 2B and 2C), the percentage of apoptotic myocytes significantly increased to 20.6 ± 0.9% after 10⁻⁶ mol/l of doxorubicin treatment, compared with control. Amlodipine at 10⁻⁶ mol/l significantly inhibited the percentage of doxorubicin-induced apoptosis to 12.5 ± 0.6%. In contrast, nifedipine at 10⁻⁶ mol/l did not inhibit doxorubicin-induced myocyte apoptosis.

**Myocyte ATP content.** The ATP content was 21.6 ± 2.9 nmol/mg protein under control conditions. Treatment of myocytes with 10⁻⁶ mol/l of doxorubicin for 14 h significantly decreased the ATP content to 65.1% of control. Amlodipine (10⁻⁹ to 10⁻⁶ mol/l) inhibited doxorubicin-induced ATP depletion in a dose-dependent fashion.
fashion, and the ATP content was significantly increased by co-treatment with 10^{-6} mol/l of amlodipine to 83.0% of control. In contrast, nifedipine did not increase the ATP content of doxorubicin-treated myocytes.

**Effect of antioxidants on myocyte apoptosis.** To compare the antiapoptotic activity of amlodipine with that of other antioxidants, the effect of probucol, ascorbic acid, or alpha-tocopherol on doxorubicin-induced myocyte apoptosis was examined, as shown in Figure 3A. There was no cardioprotective effect after treatment with 5 \times 10^{-5} mol/l of ascorbic acid. Although 10^{-4} mol/l of probucol and 10^{-4} mol/l of alpha-tocopherol significantly attenuated apoptosis to 21.2 \pm 1.0% and 20.1 \pm 1.2%, respectively, these treatments were significantly less effective than 10^{-6} mol/l of amlodipine.

**Reactive oxygen species produced by doxorubicin.** Figure 4 shows the effects of the antioxidants catalase, glutathione, NAC, mannitol, and SOD on doxorubicin-induced apoptosis. Treatment of myocytes with catalase, glutathione, and NAC, but not mannitol and SOD, significantly decreased apoptosis induced by doxorubicin to 18.7 \pm 1.2%, 19.1 \pm 1.7%, and 18.7 \pm 0.6%, respectively.

Doxorubicin at 10^{-6} mol/l increased the number of cells with elevated peroxides, as histochemically estimated by H_2DCFDA (Fig. 3B). Co-treatment with amlodipine at 10^{-6} mol/l, probucol at 10^{-4} mol/l, or alpha-tocopherol at 10^{-4} mol/l markedly inhibited the intensity of DCF fluorescence. In contrast, neither nifedipine at 10^{-6} mol/l nor ascorbic acid at 5 \times 10^{-5} mol/l affected DCF fluorescence.

**Mitochondrial membrane potential and cytochrome c release.** The fluorescent green JC-1 exists as a monomer at low membrane potential. However, at higher potentials, JC-1 forms fluorescent red “J-aggregates.” The emission of this dye can therefore be used to monitor mitochondrial membrane potential in apoptotic cardiac myocytes (27). Under control conditions, the myocytes showed red-orange mitochondrial staining, indicative of normal high membrane potentials (Fig. 5A). In contrast, myocytes treated with doxorubicin at 10^{-6} mol/l showed green fluorescence, indicating a loss of mitochondrial membrane potential, and
Co-treatment with amlodipine at $10^{-6}$ mol/l increased the fluorescent red intensity. As shown in Figure 5B and C, cytochrome $c$ was detected only in the mitochondrial fraction under control conditions. However, after treatment with doxorubicin at $10^{-6}$ mol/l, immunoreactivity of cytochrome $c$ in the mitochondria significantly decreased to 27.0 ± 1.0% and simultaneously increased to 368 ± 21% of control levels in the cytosolic fraction. Furthermore, this doxorubicin-induced translocation of cytochrome $c$ was significantly inhibited by co-treatment with amlodipine at $10^{-6}$ mol/l.

**Caspase-3 activation.** Caspase-3 activity in doxorubicin-treated myocytes significantly increased by 1.9-fold, as compared with control myocytes. Amlodipine at $10^{-6}$ mol/l, probucol at $10^{-4}$ mol/l, and alpha-tocopherol at $10^{-4}$ mol/l significantly inhibited doxorubicin-induced caspase-3 activation to 1.3-fold, 1.6-fold, and 1.5-fold of control, respectively, whereas nifedipine at $10^{-6}$ mol/l and ascorbic acid at $5 \times 10^{-5}$ mol/l did not (Fig. 6).

**DISCUSSION**

To our knowledge, our study demonstrated for the first time that doxorubicin significantly increased the number of DCF fluorescent-positive cells and induced myocyte apoptosis by activating mitochondria-mediated apoptotic cascades. Amlodipine significantly attenuated DCF fluorescence, inhibited a mitochondrial apoptotic response, and finally decreased apoptosis in doxorubicin-treated myocytes. Other antioxidants, such as probucol and alpha-tocopherol, similarly decreased doxorubicin-induced myocyte apoptosis. In contrast, nifedipine did not inhibit apoptosis without affecting DCF fluorescence. Therefore, our data suggest that amlodipine inhibits apoptosis by suppressing oxidative stress, thereby protecting mitochondria.

**Doxorubicin and myocyte apoptosis.** We treated myocytes with $10^{-6}$ mol/l of doxorubicin, a concentration considered pharmacologically relevant and clinically achievable, for 14 h (28). This concentration of doxorubicin optimally induced myocyte apoptosis with typical fragmented nuclei or condensed chromatin in a time-dependent fashion, without necrosis. Doxorubicin neither increased myocytes showing a loss of membrane integrity and uptake of ethidium homodimer-1 nor induced creatine kinase release into the medium. The present data therefore suggest that morphologic and biochemical features of doxorubicin-treated cells are closely associated with apoptosis, but not with necrosis, under our experimental conditions.

Doxorubicin appears to stimulate a variety of intrinsic cascades leading to apoptosis of myocytes. Indeed, recent reports have indicated that activation of the mitogen-activated protein kinase (MAPK) superfamily (29), induction of Bcl-2-associated X protein (Bax) and activation of caspase-3 (30), ceramide generation (8), and the Fas/Fas ligand system (9) are involved in doxorubicin-induced apoptotic signals. Our study showed that $10^{-6}$ mol/l of doxorubicin induces the loss of mitochondrial membrane potential, translocation of cytochrome $c$ from the mitochondria into the cytosol, and concomitant activation of caspase-3, significantly increasing apoptotic myocytes. Therefore, our data essentially support and extend the results of Kotamraju et al. (11). Since we have confirmed that an inhibitor of mitochondrial permeability transition, cyclosporine A at $3 \times 10^{-7}$ mol/l, significantly inhibited doxorubicin-induced apoptosis to $17.6 \pm 0.8\%$ ($p < 0.001$ vs. doxorubicin alone) (data not shown), our data strongly suggest that doxorubicin can evoke mitochondrial permeability transition, as well as activate the mitochondrial apoptotic pathway in myocytes. Moreover, an inhibitor of extracellularly responsive kinase, PD98059 at $5 \times 10^{-5}$ mol/l, or an inhibitor of p38 MAPK, SB203580 at $10^{-5}$ mol/l, did not significantly decrease apoptosis induced by doxorubicin (data not shown), suggesting that the MAPK pathway does not play a principal role in apoptotic cascades under our experimental conditions.

An important mechanism of doxorubicin cardiotoxicity involves redox activation of doxorubicin to a semiquinone intermediate, which generates superoxide radicals on one-electron reduction of oxygen (31,32), as well as hydrogen peroxide and hydroxyl radicals (33,34). The increased DCF fluorescence in the doxorubicin-treated cells in our study is consistent with this oxidative mechanism of doxorubicin cardiotoxicity. Although the intracellular localization of reactive oxygen species formed by doxorubicin is still under investigation (1,35), recent studies suggest that mitochondria in which doxorubicin accumulates over time are the target organelle of doxorubicin-induced toxicity in myocytes (36,37). Moreover, mitochondrial enzymes have been demonstrated to activate doxorubicin to form semiquinone...
Figure 5. Loss of mitochondrial transmembrane potential and release of cytochrome c. A, Myocytes were treated with 10⁻⁶ mol/l of doxorubicin in the presence or absence of 10⁻⁶ mol/l of amlodipine for 14 h. The cells were then stained with JC-1, as described in the Methods section, and fluorescence was monitored at 527 and 590 nm (magnification × 400). Control myocytes were incubated in serum-deprived DMEM without any chemicals. (a) Control cardiac myocytes; (b) myocytes exposed to 10⁻⁶ mol/l of doxorubicin for 14 h; (c) myocytes exposed to 10⁻⁶ mol/l of doxorubicin in the presence of 10⁻⁶ mol/l of amlodipine for 14 h. B, Myocytes treated with 10⁻⁶ mol/l of doxorubicin in the presence or absence of 10⁻⁶ mol/l of amlodipine were harvested for detection of cytochrome c. Mitochondrial and cytosolic fractions were prepared, and aliquots containing 20 µg protein were subjected to Western blot analysis and probed with antibody for cytochrome c, as described in Methods. Photomicrographs are representative of at least three experiments for each experiment. C, Densitometric analysis of cytochrome c release. Levels of cytochrome c are shown as the percent change in the average from three independent experiments, as compared with control. * p < 0.01 vs. control. † p < 0.01 vs. doxorubicin.
Amlodipine and antioxidant property. Amlodipine is reported to have a very high affinity for lipid constituents of cellular membranes (i.e., amlodipine has a charged amineterminal structure by which amlodipine can easily combine with phospholipids in cellular membrane and stay in it for a long time) (40,41). Moreover, amlodipine has two hydrogen atoms within the dihydropyridine rings. The hydrogen atoms are thought to play an important role in quenching reactive oxygen species (42). Our study demonstrated that amlodipine significantly attenuated the intensity of DCF fluorescence, preserved ATP content, inhibited the mitochondrial apoptotic response, and finally decreased doxorubicin-induced myocyte apoptosis. These findings suggest that amlodipine decreased doxorubicin-induced oxidative stress and inhibited the mitochondrial death signal cascade. Other antioxidants such as probucol and alpha-tocopherol, but not nifedipine, similarly inhibited apoptosis and decreased DCF fluorescence. The antiapoptotic action of amlodipine is therefore attributed to antioxidant activity, although we have no good explanation for the reversal of this antiapoptotic effect at $10^{-5}$ mol/L. The molecular mechanism by which amlodipine inhibits the mitochondrial apoptotic cascade should be further examined in the future; however, our data provide evidence that amlodipine directly quenches reactive oxygen species and preserves mitochondrial membrane potential.

Our data also show that catalase, glutathione, and NAC, but not mannitol and SOD, significantly decreased doxorubicin-induced myocyte apoptosis, which suggests that hydrogen peroxide, but not superoxide nor hydroxyl radicals, plays an important part in the induction of doxorubicin-induced apoptosis. Therefore, our results agree with those of Kotamraju et al. (11), but are inconsistent with the findings of Sawyer et al. (10), as they showed that hydrogen peroxide was not involved in doxorubicin-induced apoptosis. We are unable to explain this apparent discrepancy. Moreover, we found that amlodipine inhibited apoptosis more effectively than did either probucol or alphatocopherol, although these antioxidants similarly decreased DCF fluorescence. We again have no plausible explanation for these results. However, we are interested in the possibility that amlodipine produces endogenous nitric oxide (43) and exerts additive antiapoptotic activity, as recent studies have demonstrated that endogenous nitric oxide can inhibit apoptosis by direct suppression of caspase-3–like activity (44) or inhibition of mitochondrial permeability transition (45). Further studies are needed to confirm these speculations.

The treatment of anthracycline-induced cardiotoxicity has received considerable attention for more than two decades. In the present study, we showed that amlodipine had higher antiapoptotic action than other antioxidants, such as probucol, ascorbic acid, and alpha-tocopherol. Although future studies will be needed to examine whether amlodipine exerts antiapoptotic action in vivo, our findings strongly suggest that amlodipine can be useful in the management of doxorubicin cardiomyopathy.

Conclusions. The present study shows that doxorubicin generates hydrogen peroxide and induces myocyte apoptosis. Amlodipine significantly inhibits doxorubicin-induced apoptosis by suppressing the mitochondrial apoptotic pathway through its antioxidant properties. Our data suggest that antioxidant therapy with amlodipine may clinically provide cardioprotection against doxorubicin cardiomyopathy.

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REFERENCES

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41. Mason RP, Campbell SF, Wang SD, Herberger LG. Comparison of location and binding for the positively charged 1,4-dihydropyridine calcium channel antagonist amlodipine with uncharged drugs of this class in cardiac membranes. Mol Pharmacol 1989;36:634–40.


