N-Benzyladriamycin-14-valerate (AD 198): A Non-Cardiotoxic Anthracycline That Is Cardioprotective through PKC-ε Activation

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ABSTRACT

N-Benzyladriamycin-14-valerate (AD 198) is one of several novel anthracycline protein kinase C (PKC)-activating agents developed in our laboratories that demonstrates cytotoxic superiority over doxorubicin (Adriamycin; DOX) through its circumvention of multiple mechanisms of drug resistance. This characteristic is attributed at least partly to the principal cellular action of AD 198: PKC activation through binding to the C1b (diacylglycerol binding) regulatory domain. A significant dose-limiting effect of DOX is chronic, dose-dependent, and often irreversible cardiotoxicity ascribed to the generation of reactive oxygen species (ROS) from the semiquinone ring structure of DOX. Despite the incorporation of the same ring structure in AD 198, we hypothesized that AD 198 might also be cardioprotective through its ability to activate PKC-ε, a key component of protective ischemic preconditioning in cardiomyocytes. Chronic administration of fractional LD₅₀ doses of DOX and AD 198 to mice results in histological evidence of dose-dependent ventricular damage by DOX but is largely absent from AD 198-treated mice. The absence of significant cardiotoxicity with AD 198 occurs despite the equal ability of DOX and AD 198 to generate ROS in primary mouse cardiomyocytes. Excised rodent hearts perfused with AD 198 prior to hypoxia induced by vascular occlusion are protected from functional impairment to an extent comparable to preconditioning ischemia. AD 198-mediated cardioprotection correlates with increased PKC-ε activation and is inhibited in hearts from PKC-ε knockout mice. These results suggest that, despite ROS production, the net cardiac effect of AD 198 is protection through activation of PKC-ε.

The clinical antitumor efficacy of doxorubicin (DOX) is limited by the development of well defined, life-threatening cardiotoxicities, including cardiomyopathy and congestive heart failure (CHF) (Frishman et al., 1996). CHF has been reported to occur in 20% of those patients given 600 mg/m² DOX with subsequent 50% mortality after 2 years without cardiac transplantation (Frishman et al., 1996; Jensen et al., 2002). In retrospective analyses of phase III clinical trials using anthracycline-based combination therapies, the frequency of CHF escalates dose-dependently: 5% at 400 mg/m²; 26% at 550 mg/m²; and 48% at 700 mg/m² (Swain et al., 2003). Cardiotoxicity is exacerbated when DOX is administered with the humanized anti-erbB-2 antibody trastuzumab (Herceptin) and cyclophosphamide (Slamon et al., 2001). In addition to overt cardiotoxicity, the association of subclinical cardiac effects with DOX remains largely unquantified but is a contributory factor to subsequent cardiovascular disease in surviving patients (Schultz et al., 2003). Evidence suggests that DOX-related cardiotoxicities are the result of DOX-mediated generation of reactive oxygen species (ROS) and of interference with myocardial Ca²⁺ homeostasis (Saeki et al., 2002).

Numerous therapeutic strategies have been explored to reduce DOX cardiotoxicity, including the design of anthracycline-based combination therapies, the development of well defined, life-threatening cardiomyopathies and congestive heart failure (CHF) (Frishman et al., 1996). CHF has been reported to occur in 20% of those patients given 600 mg/m² DOX with subsequent 50% mortality after 2 years without cardiac transplantation (Frishman et al., 1996; Jensen et al., 2002). In retrospective analyses of phase III clinical trials using anthracycline-based combination therapies, the frequency of CHF escalates dose-dependently: 5% at 400 mg/m²; 26% at 550 mg/m²; and 48% at 700 mg/m² (Swain et al., 2003). Cardiotoxicity is exacerbated when DOX is administered with the humanized anti-erbB-2 antibody trastuzumab (Herceptin) and cyclophosphamide (Slamon et al., 2001). In addition to overt cardiotoxicity, the association of subclinical cardiac effects with DOX remains largely unquantified but is a contributory factor to subsequent cardiovascular disease in surviving patients (Schultz et al., 2003). Evidence suggests that DOX-related cardiotoxicities are the result of DOX-mediated generation of reactive oxygen species (ROS) and of interference with myocardial Ca²⁺ homeostasis (Saeki et al., 2002).

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choline congeners with reduced cardiotoxic potential. Epirubicin (4’-epidoxorubicin) induces cardiotoxicity only after higher cumulative doses than DOX (Frishman et al., 1996). However, clinical studies have revealed that the 850 to 1000 mg/m² dose of epirubicin used to treat breast cancer results in a 59% incidence of significantly reduced left ventricular function within 3 years of treatment, with 20% developing severe cardiomyopathy after 5 years (Jensen et al., 2002).

Other nuclear-targeted congeners, such as 4-demethoxydaunorubicin (idarubicin), 3'-deamino-3'-hydroxydoxorubicin (hydroxyrubicin), 4'-deoxydoxorubicin (esorubicin), and the disaccharide anthracycline MEN 10755 (sabarubicin), reportedly produce less acute cardiotoxicity than DOX, with long-term effects that are not yet well established (Speyer and Wasserheit, 1998; Binaschi et al., 2001). Of the clinically approved anthracyclines, only valrubicin (Valstar), an N'-trifluoracetamide derivative of adriamycin-14-valerate, is devoid of cardiac toxicity (Sweetman and Israel, 1996).

In addition to these anthracyclines, the novel anthracycline N-benzyladriamycin-14-valerate (AD 198) shows promise in demonstrating reduced cardiotoxicity based upon its mechanism of action. AD 198 exhibits no significant organ-specific toxicities at therapeutic doses and is less myelosuppressive than DOX at comparable doses (Sweetman and Israel, 1996). Its principal mechanism of cytotoxicity in proliferating cells is the direct, rapid induction of apoptotic signaling through protein kinase C (PKC) activation in a manner that circumvents multiple mechanisms of cellular drug resistance (Lothstein et al., 1998, 2001, 2006; Barrett et al., 2002; Bilyeu et al., 2004). The three-dimensional structure of the 5-carbon alkyester group of AD 198 combined with portions of the chromophore A ring bears a striking similarity to diacylglycerol (DAG), an endogenous ligand for the C1b regulatory domain of PKC (Fig. 1) (Roaten et al., 2001, 2002). AD 198 also competitively inhibits phorbol ester binding to the C1b domain (Roaten et al., 2001, 2002).

Within the context of cardiac damage control, PKC signaling plays a critical protective role. Ischemia-induced myocardial damage is attenuated by short duration and transient ischemia (ischemic preconditioning, IPC) (Murry et al., 1986). IPC is associated with receptor-mediated actions of multiple ligands in combination with ROS generated via production of nitric oxide by Ca²⁺-dependent nitric oxide reductase (Cohen et al., 2000). This, in turn, activates phospholipase, which generates DAG (Cohen et al., 2000) and activates PKC-ε, an essential nexus of signaling for cell surface receptors involved in both the early and late phases of IPC (Liu et al., 1999; Bolli, 2000; Cohen et al., 2000). However, a role for PKC-δ activation in promoting preconditioning has also been reported (Kawamura et al., 1998).

Multiple events downstream of PKC activation may feed back to enhance cell surface receptor-mediated signaling (Oldenburg et al., 2002) or may signal further downstream events, such as the activation of p38-MAPKβ (Yue et al., 2002), p42/p44 ERK, or c-Jun N-terminal kinase (Baines et al., 2002). Recent evidence suggests that PKC-ε forms a complex or “signaling module” with MAPKs following translocation of PKC-ε to mitochondria and subsequent activation (Baines et al., 2002). This PKC-ε/MAPK-active complex results in the inhibition of mitochondrial-dependent apoptosis (Baines et al., 2002). In addition, PKC-ε is reported to associate with no less than 36 structural, signaling, and stress-activated proteins in cardiomyocytes (Ping et al., 2001) that ultimately function to maintain homeostasis. Given the ability of AD 198 to target and activate PKC in a variety of cell lines, we determined in the study whether AD 198 activates PKC-ε in cardiomyocytes and, subsequently, induces cardioprotective signaling in intact hearts.

**Materials and Methods**

**Test Agents.** DOX was obtained from Sigma-Aldrich (St. Louis, MO), whereas AD 198 hydrochloride salt was prepared in these laboratories according to previously described procedures (Lothstein et al., 1998). For in vitro and ex vivo use, both drugs were dissolved in DMSO and diluted with aqueous media where indicated. For in vivo use, DOX was formulated in sterile saline as usual, whereas AD 198 was formulated in 20% NCI Diluent 12 (polyhydroxylated castor oil/ethyl alcohol, 1:1 by volume), 80% saline.

**Cardiotoxicity Assessment.** The murine chronic anthracycline cardiotoxicity model system developed by Bertazzoli et al. (1979) was used in this study. In this system, dose levels of test drugs for chronic administration are based upon the single dose LD₅₀ (30 days) value of the drug. Preliminary studies in mice of the same strain and sex as used for the cardiotoxicity assessment ultimately gave a tightly titrated i.v. single dose LD₅₀ (30 days) value for DOX (23 mg/kg) and for AD 198 (46 mg/kg). For chronic administration, high, middle, and low dose levels corresponding to 0.2, 0.1, and 0.05 of the i.v. single dose LD₅₀ (30 days) values of each drug were used.

Female CD1 mice (PAPIPLUS-virus free; Charles River Labora-
tories, Wilmington, MA) were divided into dose groups of 10 animals/group. AD 198 or DOX was administered at 0.2, 0.1, and 0.05 of the respective single dose LD sub{50} via the caudal vein. Mice were treated two times per week on weeks 1, 2, 5, 6, and 7 as described by Bertazzoli et al. (1979); no treatments were administered on weeks 3 and 4 (total 10 injections) to avoid acute adverse effects of drug administration and to limit myelosuppression. Four weeks after last treatments, animals were sacrificed, and hearts were excised immediately. Intact ventricular myocardia were fixed for a minimum of 24 h in 10% formalin phosphate buffered to pH 7.0 and then carefully sectioned into 2 to 3-mm thick slices before being dehydrated in graded ethanol and cleared in xylene prior to embedding in paraffin at 58°C. Sections (4-μm thick) were mounted on glass slides, deparaffinized in xylene, and stained in the routine fashion with Mayer's hematoxylin and eosin. Slide labels were blinded to evaluator. Myocardial lesions were evaluated by routine light microscopy and scored with regard to the severity and extent of damage (Bertazzoli et al., 1979):

- Degree of severity (S)—1, sarcomplasmic microvacuolization and/or inclusions (interstitial or cellular edema); 2, as in 1 plus sarcomplasmic macrovacuolizations or atrophy, necrosis, fibrosis, endocardial lesions, and thrombi.
- Degree of extension (E)—0, no lesions; 0.5, less than 10 single altered myocytes on the whole-heart section; 1, scattered single altered myocytes; 2, scattered small groups of altered myocytes; 3, spread small groups of altered myocytes; 4, confluent groups of altered myocytes; 5, most of cells damaged.

Total cardiotoxicity score/animal = S × E, and mean total score (MTS) for each treatment group was MTS = Σ(S × E)/number of animals.

Monitoring of ROS Generation. Primary murine cardiomyocytes from female C57 mice (Harlan, Indianapolis, IN) isolated as described previously (Lester et al., 1996) were preloaded with 5-(and-6)-chloromethyl-2′,7′-dichlorodihydro-fluorescein diacetate, acetyl ester (CM-H{22}DCFDA; Molecular Probes, Eugene, OR) in PBS for 1 h, followed by removal of PBS and replacement with warm RPMI 1640 medium with 10% fetal bovine serum. After a 15-min incubation to allow cellular esterases to cleave acetate groups and make the dye sensitive to oxidation, 2.0 μM DOX, 2.0 μM AD 198, or 3.0 μM H{22}O{2} was added to the cells in suspension for 2 h, followed by incubation in drug-free medium for 23 h. Cellular fluorescence was quantified by flow cytometry at 488-nm excitation and 525-nm emission.

Alternatively, NAD(P)H oxidase activity in cell lysates was assayed by the formation of H{22}O{2}. Murine C2 myoblasts, obtained from the ATCC (Manassas, VA), were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. To prepare lysates, cells grown in confluent monolayers were washed in PBS and scraped with a rubber policeman into 1 ml of PBS (10{6} cells/ml). Cells were sonicated and centrifuged (12,000g, 10 min, 4°C). Supernatants were assayed directly for redox cycling and H{22}O{2} formation in NAD(P)H oxidase assays using 10-acetyl-3,7-dihydroxyphenoxazinie (Amplex Red) as the substrate. In this assay, the substrate was converted to the highly fluorescent resorufin by H{22}O{2} and horseradish peroxidase (Zhou et al., 1997). Reaction mixtures in a 0.1-ml volume contained 8 μg of cell lysate and 0.5 mM NADPH in 50 mM phosphate buffer, pH 7.4. Reactions were initiated by the addition of 20 nmol of 10-acetyl-3,7-dihydroxyphenoxazinie, 0.2 mg of horseradish peroxidase, and increasing concentrations of either DOX or AD 198. Accumulation of resorufin was measured using an HTS 7000 Plus BioAssay Reader (PerkinElmer Life and Analytical Sciences, Beaconsfield, Buckinghamshire, UK) at 540-nm excitation and 570-nm emission. Reactions were performed at 37°C for 15 min.

Perfused Heart Studies. Perfusion studies were performed as described by Pyle et al. (2000). In brief, hearts were removed from methoxyflurane-anesthetized adult female Wistar rats, female C57 mice, or the progeny of C57Bl16J x129SvJae F1 heterozygous mice (generous gift of Dr. Robert O. Messing, University of California, San Francisco, CA) bred to produce homozygous PKC-ε−/− mice (Jin et al., 2002). Isolated hearts were cannulated in ice-cold modified Krebs-Henseleit buffer (4.7 mM KCl, 118 mM NaCl, 1.2 mM MgSO4, 1.3 mM CaCl2, 25 mM NaHCO3, 11 mM glucose, 1.2 mM KH2PO4, 0.05 mM EDTA, and 2 mM lactic acid, pH 7.4) and then mounted on a Langendorff perfusion apparatus. While mounted, hearts were placed in a 100-ml bath of oxygenated (95% O2/5% CO2), 37°C modified Krebs-Henseleit buffer and perfused with the same at a pressure equal to 100 cm of H2O. A pressure transducer was inserted through the left atrium into the left ventricle. A cellophane balloon on the end of the pressure transducer was inflated until left ventricular end-diastolic pressure (EDP) was 5 to 15 mm Hg. Pacing at 300 beats per min was initiated 10 to 15 min after instrumentation. Pacing voltage was set at twice the threshold value. Preischemic LVDP and EDP were averaged from the first 10 min of baseline perfusion, and only those hearts with an LVDP between 80 and 150 mm Hg and an EDP between 5 and 15 mm Hg were included in data analysis. Preischemic LVDP was calculated as the pressure difference between peak systolic pressure and EDP. Baseline perfusion or drug perfusion in modified Krebs-Henseleit buffer was carried out for 20 min in rats and 10 min in mice followed by a 2 to 5-min washout of the perfused heart with modified Krebs-Henseleit buffer plus vehicle. Global ischemia was induced for 15 min in rats and 35 min in mice, during which time pacing was discontinued. Reperfusion was for 60 min. Postischemic LVDP was determined by averaging the last 10 min of postischemic reperfusion. Hearts were excluded from data analysis if irreversible postischemic dysrhythmias were evident after 20 min of reperfusion. Test compounds were diluted from concentrated stock solutions in DMSO into modified Krebs-Henseleit buffer before perfusion.

Immunoblot Analysis of Cardiomyocyte Proteins. Rat ventricular myocytes were isolated as described previously (Liu and Hofmann, 2003). For translocation studies, total membrane fractions were isolated by differential centrifugation in the absence of nonionic detergent (Friszman et al., 1996). Identification of PKCs α, δ, and ε in cell fractions was performed by SDS-polyacrylamide gel electrophoresis and immunoblotting as described previously (Barrett et al., 2002).

Results

To determine the cardiac effects of chronic dosing with AD 198, we compared AD 198 with DOX in a murine chronic cardiotoxicity assessment model (Bertazzoli et al., 1979). CD1 mice were treated chronically with DOX over a 10-week period as described under Materials and Methods. Four weeks after the final drug dose, excised hearts were assessed for cardiac damage, using as a quantitative measure of the severity and extent of myocardial damage to yield MTS. As shown in Table 1, at a dose range of 1.15 to 4.6 mg/kg for DOX, MTS increased from 0.7 to 6.14, with overt cardiac damage observed in 90 to 100% of the animals. In contrast, 0.2, 0.1, and 0.05 of the LD sub{50} of AD 198 in the same chronic treatment regimen produced only marginal histological ef-

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fects with an MTS of 0.1 to 0.25 that were concentration-independent. These results indicate that, at chronic dosing levels sufficient to achieve DOX-induced cardiotoxicity, AD 198 is noncardiotoxic.

The lack of cardiac damage by AD 198 is evident despite the presence of the semiquinone ring structure on the AD 198 chromophore. Therefore, to determine whether AD 198 is, in fact, capable of generating ROS, we examined the relative abilities of AD 198 and DOX to produce the oxidation of the ROS detection reagent CM-H$_2$DCFDA (Fig. 2A). Primary rat ventricular cardiomyocytes were preloaded with CM-H$_2$DCFDA and then treated with either DOX or AD 198 for 2 h. Oxidation of CM-H$_2$DCFDA was monitored by increased fluorescence of the reagent due to oxidation by anthracycline-generated ROS. H$_2$O$_2$, a positive control, produced an approximately 26-fold increase in fluorescence over cells treated with either H$_2$O$_2$ or CM-H$_2$DCFDA alone. Both AD 198 and DOX treatments produced similar increases in CM-H$_2$DCFDA fluorescence at 44- and 37-fold, respectively, indicating comparable rates and levels of ROS generation by both compounds.

We have reported previously that the rate of intracellular uptake of AD 198 is significantly more rapid than DOX (Lothstein et al., 1992) and consequently may affect the relative levels of intracellular ROS generation. Therefore, we assessed the level of ROS generation in an NADPH oxidase-dependent manner in cell lysates treated with drug to eliminate the potential differential effect of drug uptake (Fig. 2B). Equal concentrations of DOX and AD 198 resulted in the generation of equal levels of H$_2$O$_2$ in cell lysates, indicating that ROS generation from the semiquinone rings of AD 198 and DOX occurs at equal rate per molar drug concentration. Further H$_2$O$_2$ generation by both AD 198 and DOX was similar from 1 to 100 μM drug concentration (data not shown).

Given the ability of AD 198 to generate levels of ROS comparable to DOX and yet remain noncardiotoxic, we next attempted to identify mechanisms by which AD 198 could reduce the cardiotoxic effects of ROS. The ability of AD 198 to activate conventional and novel PKC isoforms (Roaten et al., 2001; Barrett et al., 2002; Bilyeu et al., 2004) is significant, because PKC-ε and possibly PKC-δ activation are integral in cardioprotective signaling (Kawamura et al., 1998; Liu et al., 1999). Therefore, we determined whether AD 198 can induce cardioprotection in hearts through PKC activation. For this purpose, hearts were excised from anesthetized rats and subjected to a Langendorff perfused heart protocol to monitor recovery of LVDP during reperfusion after global ischemia following perfusion with AD 198, DOX, or solvent alone (Fig. 3). A 15-min perfusion of 1% DMSO in Krebs-Henseleit buffer alone did not reduce the extent of damage (22.5% recovery), whereas AD 198 and DOX treatment resulted in 84.2% and 7.5% recovery, respectively.
buffer (vehicle control) before 15 min of global ischemia resulted in a 22% recovery of LVDP to baseline levels after 60 min of reperfusion. Perfusion of 1 μM DOX before ischemia lowered LVDP recovery after 60 min of reperfusion to 7.5% (data not shown). Perfusion with 0.1 μM AD 198 before ischemia yielded LVDP recovery of 84.2%. These results indicate that AD 198 indeed confers protection against ischemic damage in rat hearts. Likewise, C57 mouse hearts pretreated with 0.1 μM AD 198 exhibited a 60% recovery of LVDP during postischemic reperfusion (Fig. 4). The effect of AD 198 is comparable to preconditioning ischemia composed of 3 1-min occlusions followed by a longer duration ischemia, which resulted in 56.6% recovery of LVDP.

Given the well-established role of PKC-ε signaling in preconditioning ischemia (Liu et al., 1999), we sought to determine whether AD 198 treatment of cardiomyocytes activates PKC-ε (Fig. 5). After a 5-min exposure of freshly isolated rat cardiomyocytes to 0.1 μM AD 198, we observed translocation of PKC-ε to the membrane, which was reduced by approximately half after 30 min of drug exposure. We observed only marginal translocation of PKC-δ within 5 min of AD 198 exposure, whereas no translocation of PKC-α was detected. In comparison, PMA, a known activator of cardioprotective signaling (Cohen et al., 2000), induced PKC-ε and -δ translocation to an extent similar to AD 198. Thus, AD 198-mediated cardioprotection correlates with its ability to induce rapid but transient translocation of PKC-ε in primary rat cardiomyocytes.

To determine whether PKC-ε is in fact the principal target of AD 198 in mediating cardioprotection, we assessed the cardioprotective effects of AD 198 in hearts excised from PKC-ε knockout mice (Fig. 6). In homozygous wild-type mice, pretreatment with vehicle (DMSO) before global ischemia resulted in a 23.4% recovery of LVDP, whereas perfusion with 0.1 μM AD 198 before global ischemia produced an increase in recovery of LVDP of 45.2%. Recovery of LVDP corresponds to PKC-ε translocation to membrane fractions in cardiomyocytes excised from treated hearts. Homozygous PKC-ε knockout mice perfused with DMSO before induced ischemia exhibited a 36.3% recovery of LVDP (p = 0.19 compared with wild type). In contrast, PKC-ε null hearts perfused with 0.1 μM AD 198 before ischemia showed only a 40.2% recovery in LVDP. These findings suggest that PKC-ε plays a significant role in cardioprotection by AD 198.

**Discussion**

AD 198 is a semisynthetic anthracycline that was developed initially to circumvent multiple mechanisms of multidrug resistance that impede successful clinical treatment of cancer (Lothstein et al., 1998; Barrett et al., 2002; Bilyeu et al., 2004). In addition to the comparable tumor cytotoxicity of AD 198 and DOX both in vitro and in vivo, AD 198 has demonstrated improved efficacy and greater therapeutic potential than DOX and other nuclear-targeted anthracyclines (Sweatman and Israel, 1996). However, characterizing the clinical efficacy of any anti-tumor anthracycline requires an assessment of its cardiotoxic potential, given the serious cardiotoxic effects of nuclear-targeted anthracyclines such as DOX (Frishman et al., 1996). Using the standard Bertazzoli test to assess chronic cardiotoxicity, AD 198 demonstrates no significant dose-dependent cardiotoxicity in this murine model, despite the ability of AD 198 to generate ROS in primary murine cardiomyocytes to an extent comparable to DOX. Because the preservation of the semiquinone ring C within the chromophore allows the AD 198 to generate ROS, the lack of AD 198 cardiotoxicity then may be due to either differential compartmentalization of AD 198 and DOX or altered function of AD 198 within cardiomyocytes to counter...
PKC-Mediated Cardioprotection by AD 198

activates the enzyme, and triggers rapid apoptosis in proliferating cells by depolarizing mitochondria in a PKC-δ-dependent (Barrett et al., 2002) but Ca\textsuperscript{2+}-independent manner (Lothstein et al., 2006). However, the observed lack of organ toxicity of AD 198 at therapeutic levels in vivo in rats suggests that PKC activation by AD 198 may not necessarily lead to cell death in nondividing cells. The role of PKC activation in cardioprotective signaling in cardiomyocytes has been well established (Cohen et al., 2000). Consequently, phorbol esters, which bind to the C1b domain of PKC in a manner that competitively inhibits AD 198 binding (Roaten et al., 2001), directly activate the enzyme to achieve cardioprotection (Cohen et al., 2000).

The enhancement of LVDP recovery during reperfusion by AD 198 pretreatment before global ischemia in the Langendorff perfusion system is comparable to other mediators of preconditioning, such as brief ischemia (Yaguchi et al., 2003), hydrogen peroxide (Pyle et al., 2001), and opioid agonists (Gray et al., 1997). The recovery of LDVP during reperfusion in AD 198-treated hearts is preceded by PKC-ε translocation and, to a modest extent, PKC-δ translocation from cytosol to membrane, suggesting that one or both PKC isoforms are involved in AD 198-mediated preconditioning. As observed previously with PMA, AD 198-induced PKC translocation is transient (Gysembergh et al., 2000). Because PKC translocation and activation is modulated by the generation of DAG in the plasma membrane, these results are consistent with the transient generation of DAG observed after preconditioning ischemia (Ping et al., 1999).

The importance of PKC-ε in AD 198-mediated preconditioning is suggested by the ability of AD 198 to induce its membrane translocation and activation. Furthermore, elimination of PKC-ε has a significant effect on reducing the preconditioning activity of AD 198, suggesting that PKC-ε is the primary target for AD 198. However, the potential contribution of other PKC isoforms in contributing to the preconditioning pathway must also be considered. PKC-δ has been reported to play a comparable role in stimulating preconditioning in rat hearts independently of PKC-ε following preconditioning ischemia (Inagaki et al., 2000), although opposing roles for PKCs δ and ε have been reported in mice (Yang and Kazanietz, 2003) and as such would argue against a compensatory action of PKC-δ in PKC-ε knockout mice. Nevertheless, the modest translocation of PKC-δ by AD 198 warrants further investigation. Second, AD 198 may also target other components of cardioprotective signaling downstream of PKC activation. AD 198 cannot be narrowly classified as a PKC-activating agent but rather as a C1b domain-binding agent, capable of binding to the C1b domains of PKC and β2-chimerin (Roaten et al., 2002). However, as of yet, no C1b domain-containing protein other than PKC has an identifiable role in myocardial preconditioning.

In summary, the novel anthracycline PKC-activating agent AD 198 induces preconditioning in rodent hearts in an ischemia/reperfusion system through a mechanism that involves, at least in part, PKC-ε activation. AD 198-mediated cardioprotection is also associated with the absence of chronic in vivo cardiotoxicity, often associated with anthracyclines, despite the generation of abundant ROS by AD 198 in cardiomyocytes.


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