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Roles of oxidative stress and Akt signaling in doxorubicin cardiotoxicity

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Abstract

Cardiotoxicity is a treatment-limiting side effect of the anticancer drug doxorubicin (DOX). We have now investigated the roles of oxidative stress and signaling by the protein kinase Akt in DOX-induced cardiotoxicity as well as the effects on such toxicity both of fenofibrate, an agonist of peroxisome proliferator-activated receptor-α, and of polyethylene glycol-conjugated superoxide dismutase (PEG–SOD), an antioxidant. Mice injected intraperitoneally with DOX were treated for 4 days with fenofibrate or PEG–SOD. Fenofibrate and PEG–SOD each prevented the induction of cardiac dysfunction by DOX. Both drugs also inhibited the activation of the transcription factor NF-κB and increase in lipid peroxidation in the left ventricle induced by DOX, whereas only PEG–SOD inhibited the DOX-induced activation of Akt and Akt-regulated gene expression. These results suggest that fenofibrate and PEG–SOD prevented cardiac dysfunction induced by DOX through normalization of oxidative stress and redox-regulated NF-κB signaling.

Keywords: Doxorubicin; Heart failure; Oxidative stress; Apoptosis; Angiogenesis; Fenofibrate; Superoxide dismutase

Doxorubicin (DOX) is one of the most widely used drugs in the treatment of a variety of human neoplasms [1]. However, cardiotoxicity often develops in individuals treated with this drug and is a major limiting factor in its further use [2]. Long-term treatment with DOX can thus result in the development of cardiomyopathy and congestive heart failure in a process that involves multiple factors including the generation of free radicals that damage cellular membranes [3,4], disturbance of adrenergic function, alterations in intracellular Ca^{2+} homeostasis [5], myocardial cell apoptosis [6], and selective inhibition of the expression of cardiac muscle-specific proteins [7]. Treatment with *n*-acetylcysteine or antioxidant drugs such as probucol has been found to protect against DOX-induced cardiotoxicity in animal models [8,9], as has overexpression of manganese-dependent superoxide dismutase (Mn–SOD) or catalase [10,11]. These observations thus indicate that free radicals play an important role in DOX-induced cardiotoxicity, but the molecular mechanisms that underlie the associated myocardial impairment have remained unclear.

Fenofibrate, a ligand and activator of peroxisome proliferator-activated receptor-α (PPAR-α), is used clinically for the treatment of dyslipidemia. Treatment with fenofibrate also reduced the extents of myocardial inflammation and collagen deposition in rats infused with angiotensin II [12] as well as ameliorated cardiac dysfunction by suppressing inflammatory responses associated with redox-regulated transcription factors in rats with salt-sensitive hypertension [13]. Polyethylene glycol-conjugated superoxide dismutase (PEG–SOD) had succeeded to prolong plasma half-life of SOD [14], and pretreatment with this

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conjugate was shown to protect against reperfusion-induced arrhythmias as well as myocardial ischemia-reperfusion injury in animal models [15,16]. We have now investigated the mechanism of DOX-induced cardiotoxicity as well as whether fenofibrate or PEG–SOD protects against such toxicity in mice.

Materials and methods

Experimental animals. Male C57BL/6J mice were obtained at 7 weeks of age and were maintained under constant environmental conditions. At 8 weeks of age, they were randomly divided into two groups: those injected intraperitoneally with physiological saline (control group, $n = 6$) and those injected intraperitoneally with DOX (15 mg/kg of body weight). The latter animals were further divided into five groups: those treated with vehicle (DOX group, $n = 6$), those treated with a low dose (30 mg/kg per day, $n = 6$) of fenofibrate [DOX + PPAR (low) group], those treated with a high dose (50 mg/kg per day, $n = 6$) of fenofibrate [DOX + PPAR (high) group], those treated with a low dose (300 U/kg per day, $n = 6$) of PEG–SOD [DOX + SOD (low) group], and those treated with a high dose (1000 U/kg per day, $n = 6$) of PEG–SOD [DOX + SOD (high) group]. Fenofibrate (Grelan, Tokyo, Japan), PEG–SOD (Sigma–Aldrich, St. Louis, MO), and the corresponding vehicle (3% gum arabic) were administered orally by gastric gavage once a day for 4 days. All experimental procedures were performed in accordance with institutional guidelines for animal research.

Assessment of LV structure and function. Four days after the onset of treatment, mice were anesthetized by intraperitoneal injection of sodium pentobarbital (10 mg/kg) and subjected to transthoracic echocardiography with a SONOS 5500 system (Philips Medical Systems, Bothell, WA) as described previously [17]. The thickness of the interventricular septum as well as left ventricular (LV) end-diastolic and end-systolic diameters were obtained from a short-axis view at the level of the papillary muscles, and LV fractional shortening was calculated.

Quantitative RT-PCR analysis of cardiac gene expression. The left ventricle was separated from the atria and right ventricle, weighed, and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Total RNA was subsequently extracted from the LV tissue and subjected to quantitative reverse transcription and polymerase chain reaction (RT-PCR) analysis as described [13] with primers specific for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and cardiac adriamycin response protein (CARP) genes. The abundance of each target mRNA was normalized by that of β-actin mRNA.

Assay of HEL and glutathione. Frozen LV tissue was homogenized on ice and assayed for hexanoyl-lysine (HEL) with an enzyme-linked immunosorbent assay (ELISA) as described [18]. The assay was performed in duplicate, and absorbance at 450 nm was measured with a microtiter plate reader. The amount of total glutathione [reduced (GSH) plus oxidized (GSSG)] in homogenates of frozen LV tissue was determined as described [13] with a recycling assay based on glutathione reductase and 5,5′-dithiobis-(2-nitrobenzoic acid). The amount of GSSG was determined by Griffith's method [19] after the addition of 2-vinylpyridine to the assay mixture.

Analysis of NF-κB activity and IκB expression. Nuclear extracts were prepared from homogenates of LV tissue by standard procedures and were assayed for the DNA binding activity of nuclear factor-κB (NF-κB) with the use of a Trans AM NF-κB ELISA Kit (Active Motif, Carlsbad, CA). Cytosolic fractions of LV tissue homogenates were subjected to immunoblot analysis with goat polyclonal antibodies to the NF-κB inhibitor IκB (1:200 dilution; Santa Cruz Biotechnology, Santa Monica, CA) and the mouse monoclonal antibodies to β-actin (1:500 dilution; Chemicon International, Temecula, CA). Immune complexes were detected with enhanced chemiluminescence (ECL) reagents (GE Healthcare Bio-Science, Piscataway, NJ). Band intensity was quantified with the use of Quantity One Image software (Bio-Rad, Hercules, CA).

Assay of caspase-3 activity. The activity of caspase-3 in cytosolic fractions of LV tissue homogenates was measured by detection of the cleavage of the colorimetric substrate *N*-acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-*p*NA) with an assay kit (MBL, Nagoya, Japan).

Analysis of HIF-1α and VEGF expression and Akt phosphorylation. Nuclear extracts of LV tissue homogenates were subjected to immunoblot analysis with rabbit polyclonal antibodies to hypoxia-inducible factor-1α (HIF-1α) at a dilution of 1:200 (Santa Cruz Biotechnology) as well as with mouse monoclonal antibodies to lamin (1:500 dilution, Zymed Laboratories, South San Francisco, CA). Cytosolic fractions of LV tissue were also subjected to immunoblot analysis with goat polyclonal antibodies to vascular endothelial growth factor (VEGF) at a dilution of 1:200 (Santa Cruz Biotechnology), rabbit polyclonal antibodies to Akt (1:200 dilution, Santa Cruz Biotechnology), as well as mouse monoclonal antibodies to phosphorylated Akt (1:1000 dilution; Cell Signaling Technology, Danvers, MA) and to β-actin (1:500 dilution, Chemicon International).

Statistical analysis. Data are presented as means ± SEM. Differences among the six experimental groups were evaluated by one-way analysis of variance followed by Dunnett's post hoc test. A P value of <0.05 was considered statistically significant.

Results

Physiological findings

Four days after the onset of treatment, body weight was smaller in the DOX group than in the control group, and this difference was abolished by treatment with PEG–SOD at the low or high dose (Table 1). Heart and LV weight were also significantly smaller in the DOX group than in the control group, and heart weight was significantly greater in the DOX + SOD (high) group than in the DOX group. The thickness of the interventricular septum was significantly smaller in the DOX group than in the control group and was significantly greater in both of the DOX + PPAR and DOX + SOD groups than in the DOX group. The LV end-systolic diameter was significantly greater in the DOX group than in the control group and was significantly smaller in both of the DOX + PPAR and DOX + SOD groups than in the DOX group. Finally, LV fractional shortening was significantly smaller in the DOX group than in the control group and was significantly greater in both of the DOX + PPAR and DOX + SOD groups than in the DOX group.

Cardiac gene expression

The abundance of ANP, BNP, and CARP mRNAs in the left ventricle was significantly reduced in the DOX group compared with that in the control group at 4 days after the onset of treatment (Fig. 1A). The DOX-induced decrease in the amount of ANP mRNA was prevented by fenofibrate or PEG–SOD at the high dose. Treatment with fenofibrate or PEG–SOD at the low or high dose did not affect the DOX-induced changes in the amounts of BNP or CARP mRNAs.

Oxidative stress and NF-κB signaling

The concentration of HEL in the left ventricle at 4 days after the onset of treatment was significantly increased in

Table 1
Physiological and echocardiographic findings for mice in the six experimental groups at 4 days after the onset of treatment

Parameter	Control	DOX	DOX + PPAR (low)	DOX + PPAR (high)	DOX + SOD (low)	DOX + SOD (high)
Body weight (g)	23.34 ± 0.46	19.54 ± 0.63*	21.09 ± 0.77*	21.49 ± 0.66	22.54 ± 0.69†	22.58 ± 0.53†
Heart weight (mg)	109.7 ± 2.4	89.5 ± 3.3*	97.6 ± 3.3*	100.3 ± 3.4	100.8 ± 3.2	103.5 ± 3.0†
LV weight (mg)	78.1 ± 1.7	63.5 ± 3.5*	72.0 ± 3.6*	72.6 ± 3.8	74.5 ± 2.0	76.5 ± 2.5
LV/body weight ratio	3.41 ± 0.09	3.29 ± 0.12	3.37 ± 0.07	3.46 ± 0.04	3.35 ± 0.10	3.44 ± 0.05
IVST (mm)	0.69 ± 0.01	0.61 ± 0.02*	0.67 ± 0.01†	0.69 ± 0.01†	0.69 ± 0.02†	0.71 ± 0.01†
LVDd (mm)	3.38 ± 0.05	3.56 ± 0.03*	3.47 ± 0.03	3.42 ± 0.04	3.51 ± 0.07	3.46 ± 0.04
LVDs (mm)	2.30 ± 0.03	2.70 ± 0.06*	2.47 ± 0.04*,†	2.39 ± 0.04†	2.52 ± 0.04*,†	2.47 ± 0.03*,†
FS (%)	32.0 ± 0.43	24.1 ± 1.05*	28.6 ± 0.97†	30.2 ± 0.99†	28.2 ± 0.91†	28.6 ± 1.13†

Abbreviations not defined in text: IVST, thickness of the interventricular septum; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; FS, left ventricular fractional shortening. Data are means ± SEM of values from six mice per group.
* $P < 0.05$ vs. control group.
† $P < 0.05$ vs. DOX group.

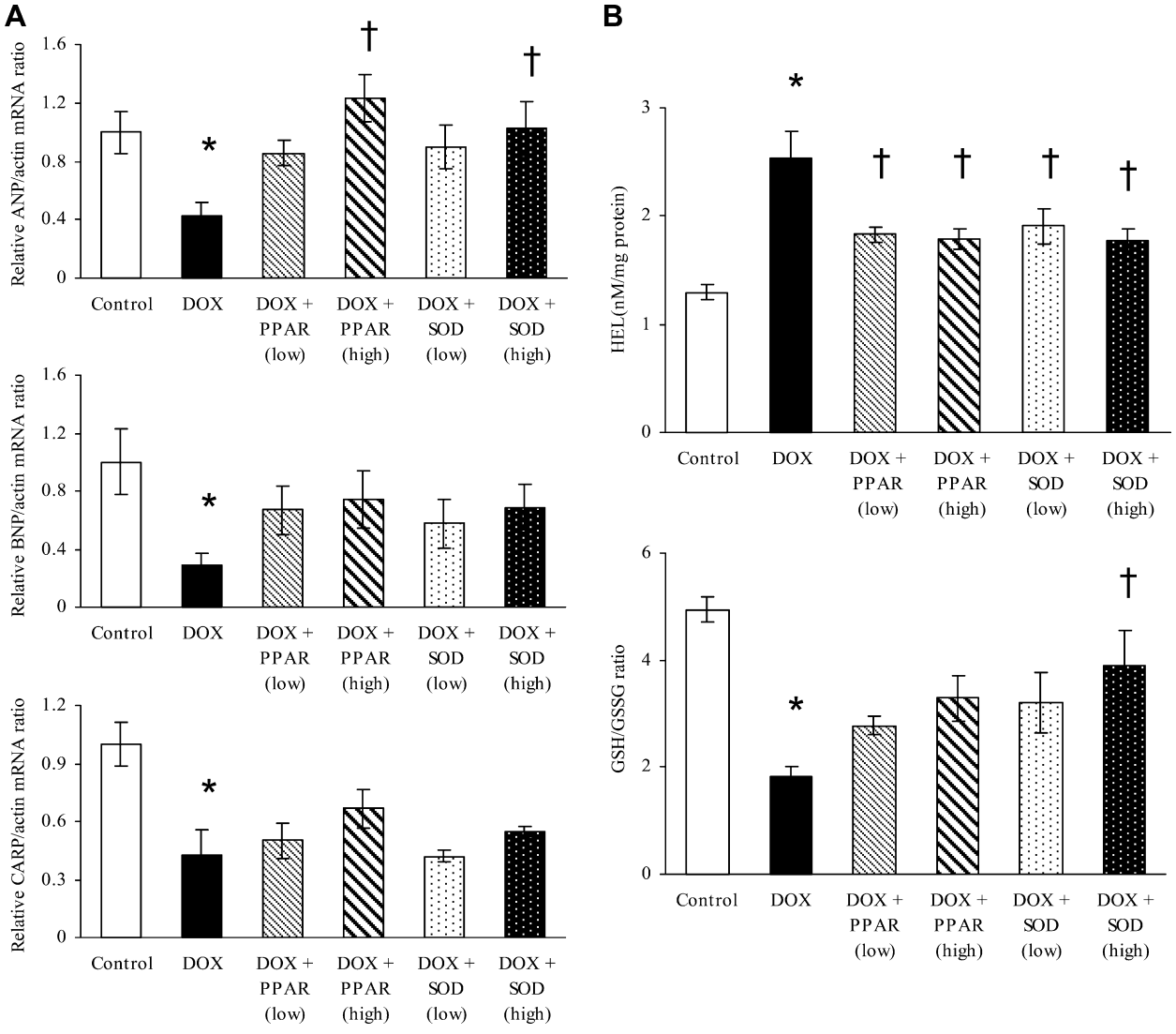

Fig. 1. Cardiac gene expression and oxidative stress in the left ventricle of mice in the six experimental groups at 4 days after the onset of treatment. (A) The abundance of mRNAs for ANP, BNP, and CARP in the left ventricle was determined by quantitative RT-PCR analysis and normalized by the amount of β-actin mRNA. The normalized values are expressed relative to that for the control group. (B) The concentration of HEL and the GSH/GSSG ratio were determined in homogenates of LV tissue. All data are means ± SEM of values from six mice per group. *$P < 0.05$ vs. control group; †$P < 0.05$ vs. DOX group.

the DOX group compared with that in the control group, and this effect of DOX was prevented by treatment with fenofibrate or PEG–SOD at either dose (Fig. 1B). The GSH/GSSG ratio in the left ventricle was significantly smaller in the DOX group than in the control group, and treatment with PEG–SOD at the high dose prevented this effect of DOX (Fig. 1B). The DNA binding activity of the redox-regulated transcription factor NF-κB in nuclear extracts of LV tissue was significantly greater for the DOX group than for the control group, and this effect of DOX was prevented by fenofibrate or PEG–SOD at the low or high dose (Fig. 2A). The abundance of IκB in the cytosolic fraction of LV tissue was significantly reduced in the DOX group compared with that in the control group, and this effect of DOX was blocked by fenofibrate or PEG–SOD at either dose (Fig. 2B).

Activity of caspase-3, expression of HIF-1α and VEGF, and phosphorylation of Akt

The activity of the apoptotic protease caspase-3 in the left ventricle at 4 days after the onset of treatment was significantly increased in the DOX group compared with that in the control group, but the activities in the DOX + SOD groups did not differ significantly from that in the control group (Fig. 3A). The amounts of the hypoxia-induced transcription factor HIF-1α and the angiogenic factor VEGF in the left ventricle were also markedly increased in the DOX group compared with those in the control group; these effects of DOX were inhibited by PEG–SOD at both doses but not by fenofibrate. Finally, the abundance of the phosphorylated (activated) form of the protein kinase Akt in the left ventricle was increased in the DOX group compared with that in the control group, and this effect of DOX was inhibited by PEG–SOD at both doses (Fig. 3C). The total amount of Akt did not differ among the six experimental groups.

Discussion

Our results suggest that cardiotoxicity induced by DOX is associated with increased oxidative stress, apoptosis, and impaired angiogenesis in the left ventricle of mice. Treatment with either the PPAR-α activator fenofibrate or the antioxidant PEG–SOD ameliorated the cardiac dysfunction induced by DOX. The DOX-induced up-regulation of NF-κB signaling and increase in the abundance of HEL, a marker of free radical-induced lipid peroxidation, in the left ventricle were inhibited by both fenofibrate and PEG–SOD, whereas DOX-induced activation of Akt and the expression of HIF-1α and VEGF were inhibited only by treatment with PEG–SOD.

DOX-induced cardiotoxicity has previously been suggested to be attributable mostly to the cardiomyocte damage caused by reactive oxygen species [3,4]. In the present study, we found that the GSH/GSSG ratio was decreased and the concentration of HEL was increased in the left ventricle of mice injected with DOX. These observations are consistent with previous results showing that the extent of protein nitration was correlated with that of cardiac dysfunction in mice treated with DOX [20]. The expression of HIF-1α was also previously shown to increase with the progression of heart failure in rats with myocardial infarction or hamsters with cardiomyopathy [21,22]. HIF-1α

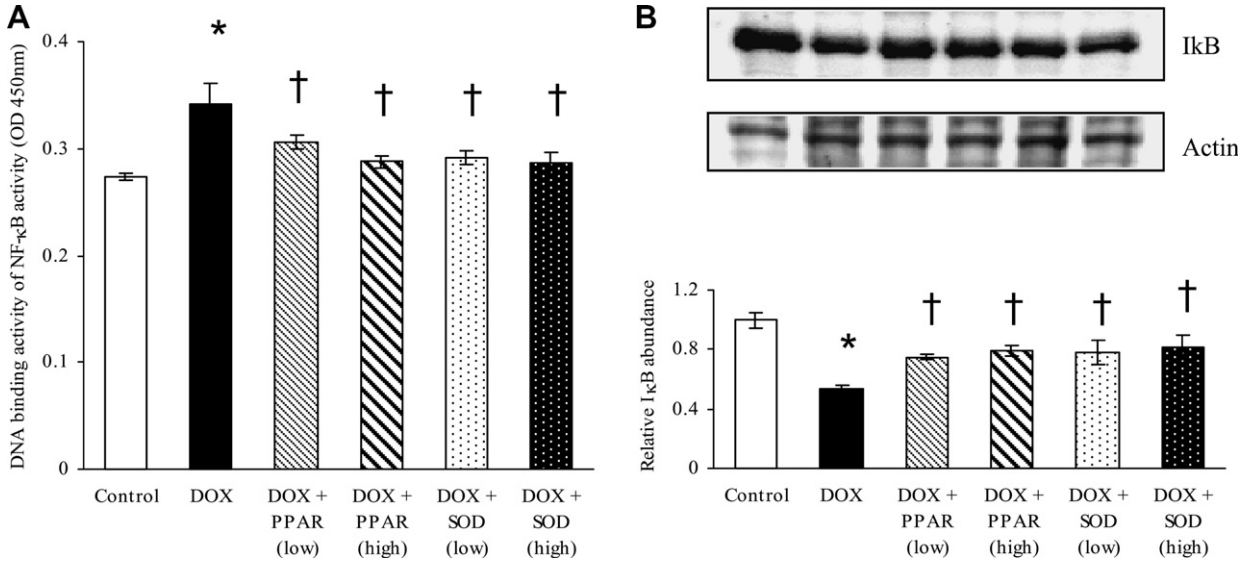

Fig. 2. DNA binding activity of NF-κB and abundance of IκB in the left ventricle of mice in the six experimental groups at 4 days after the onset of treatment. (A) The DNA binding activity of NF-κB in nuclear extracts of LV tissue was determined with an ELISA. Absorbance was measured at 450 nm (A_{450}). (B) Representative immunoblot analysis of cytosolic fractions of LV homogenates with antibodies to IκB and to β-actin (loading control) is shown in the upper panel. The abundance of IκB was quantified and expressed relative to the value for the control group (lower panel). All quantitative data are means ± SEM of values from six mice per group. $^{*}P < 0.05$ vs. control group; $^{\dagger}P < 0.05$ vs. DOX group.

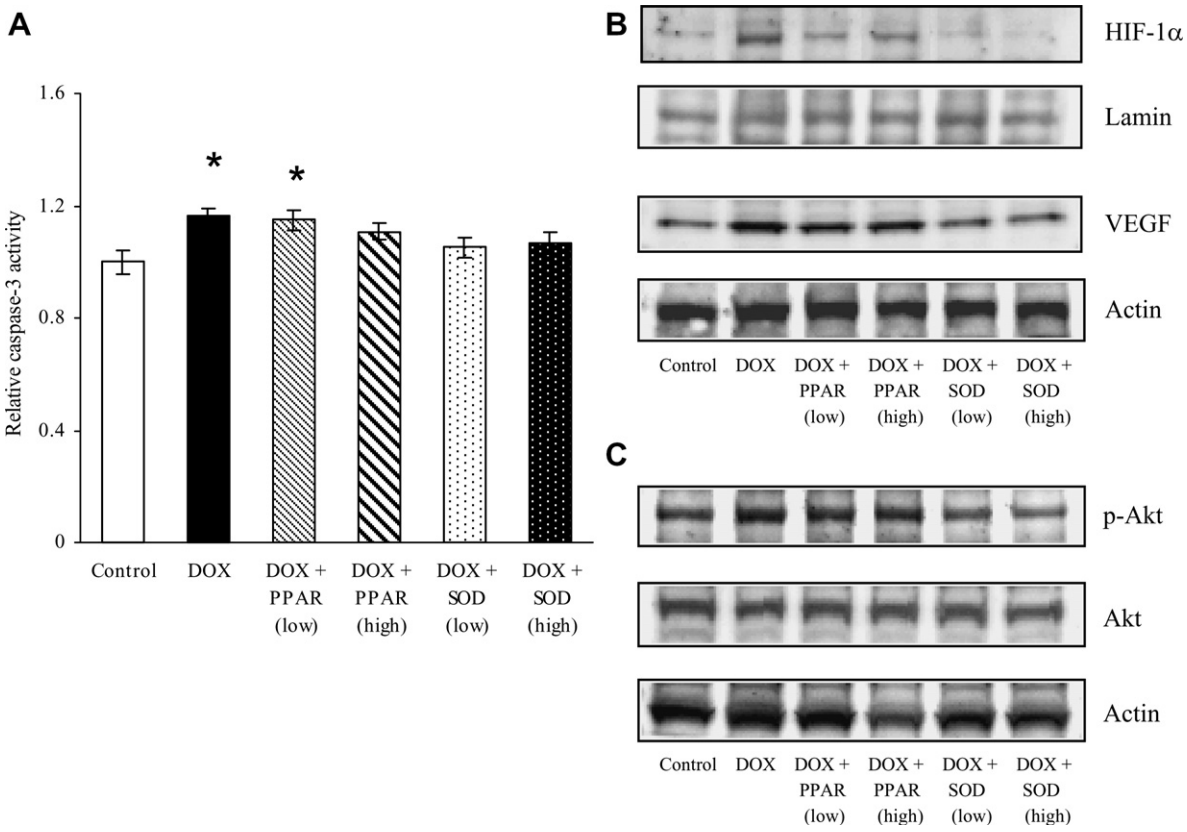

Fig. 3. Caspase-3 activity, expression of HIF-1α and VEGF, and phosphorylation of Akt in the left ventricle of mice in the six experimental groups at 4 days after the onset of treatment. (A) The activity of caspase-3 in LV homogenates was determined with a colorimetric assay and expressed relative to the value for the control group. Data are means ± SEM of values from six mice per group. $^{*}P < 0.05$ vs. control group. (B) Representative immunoblot analysis of HIF-1α and lamin (loading control) in nuclear extracts and of VEGF and β-actin (loading control) in cytosolic fractions of LV tissue. (C) Representative immunoblot analysis of phosphorylated (p-) and total Akt as well as of β-actin in cytosolic fractions of LV tissue. Data in (B) and (C) are representative of results obtained with six mice of each group.

regulates the expression of genes whose products affect the generation of reactive oxygen species [23] or contribute to cardiac metabolism, including those for glycolytic enzymes, a glucose transporter, and lactate dehydrogenase [24]. We have now shown that DOX increased the abundance of HIF-1α in the left ventricle, suggesting that a switch in principal ATP source from β-oxidation to glycolysis may be important in the pathophysiology of DOX-induced cardiotoxicity.

DOX has been shown to induce activation of the serine-threonine kinase Akt [25]. The phosphoinositide 3-kinase-Akt signaling pathway inhibits the function of Bcl-2 family proteins that trigger apoptosis [26] as well as promotes angiogenesis by inducing VEGF expression through activation of HIF-1α [27]. The induction of cardiomyocyte apoptosis was observed in rats injected with DOX [6], suggesting that this effect contributes to DOX-induced cardiotoxicity. Furthermore, an increase in myocardial Akt signaling was found to ameliorate DOX-induced heart failure [28], and transplantation of bone marrow mononuclear cells ameliorated DOX-induced cardiac dysfunction through acceleration of angiogenesis [29]. In the present study, DOX induced the activation of both caspase-3 and Akt in the left ventricle, with the latter effect likely reflecting a protective response to counteract the induction of cardiomyocyte apoptosis. The increase in the expression of HIF-1α and VEGF elicited by DOX in the left ventricle might also reflect a protective response to counteract impairment of angiogenesis by this drug.

The thickness of the interventricular septum was decreased and systolic function was impaired 4 days after DOX injection. Treatment with the low or high doses of fenofibrate or PEG–SOD attenuated these effects of DOX. DOX has previously been shown to selectively inhibit ANP, BNP, and CARP gene expression in cultured neonatal rat cardiac myocytes [7,30]. In the present study, the abundance of ANP, BNP, and CARP mRNAs in the left ventricle was also reduced by DOX. Treatment with the high dose of fenofibrate or PEG–SOD prevented the decrease in ANP gene expression induced by DOX. These results are consistent with the previous observations that inhibition of ANP expression by DOX was reversible by antioxidant treatment whereas that of BNP expression was virtually unaffected [30]. In addition, DOX-induced Akt activation and the expression of HIF-1α and VEGF were inhibited by treatment with PEG–SOD but not by

fenofibrate. Previous observations that apoptosis was induced only by low concentrations of DOX and peaked 24 h after DOX injection in rat models [6,31] suggest that adaptive responses to DOX-induced apoptotic death of cardiac myocytes were predominant 4 days after a single injection of DOX in the present study. Although inhibition of DOX-induced Akt activation and expression of HIF-1α and VEGF was observed only in mice treated with PEG–SOD, fenofibrate and PEG–SOD had similar protective effects against the cardiac dysfunction triggered by DOX. These results thus suggest that the cardiac dysfunction induced by a single dose of DOX was mainly attributable to increased oxidative stress.

In conclusion, treatment with the PPAR-α activator fenofibrate or PEG–SOD attenuated the cardiac dysfunction induced in mice by injection of DOX. These beneficial effects were associated with a reduction in the level of oxidative stress in the left ventricle and inhibition of the DOX-induced increase in the DNA binding activity of the redox-regulated transcription factor NF-κB.

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References

[1] R.H. Blum, S.K. Carter, Adriamycin. A new anticancer drug with significant clinical activity, Ann. Intern. Med. 80 (1974) 249–259.
[2] D.D. Von Hoff, M.W. Layard, P. Basa, H.L. Davis Jr., A.L. Von Hoff, M. Rozencweig, F.M. Muggia, Risk factors for doxorubicin-induced congestive heart failure, Ann. Intern. Med. 91 (1979) 710–717.
[3] H.G. Keizer, H.M. Pinedo, G.J. Schuurhuis, H. Joenje, Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity, Pharmacol. Ther. 47 (1990) 219–231.
[4] S. Rajagopalan, P.M. Politi, B.K. Sinha, C.E. Myers, Adriamycin-induced free radical formation in the perfused rat heart: implications for cardiotoxicity, Cancer Res. 48 (1988) 4766–4769.
[5] D.H. Kim, A.B. Landry 3rd, Y.S. Lee, A.M. Katz, Doxorubicin-induced calcium release from cardiac sarcoplasmic reticulum vesicles, J. Mol. Cell. Cardiol. 21 (1989) 433–436.
[6] O.J. Arola, A. Saraste, K. Pulkki, M. Kallajoki, M. Parvinen, L.M. Voipio-Pulkki, Acute doxorubicin cardiotoxicity involves cardiomyocyte apoptosis, Cancer Res. 60 (2000) 1789–1792.
[7] R. Jeyaseelan, C. Poizat, R.K. Baker, S. Abdishoo, L.B. Isterabadi, G.E. Lyons, L. Kedes, A novel cardiac-restricted target for doxorubicin. CARP, a nuclear modulator of gene expression in cardiac progenitor cells and cardiomyocytes, J. Biol. Chem. 272 (1997) 22800–22808.
[8] J.H. Doroshow, G.Y. Locker, I. Ifrim, C.E. Myers, Prevention of doxorubicin cardiac toxicity in the mouse by *n*-acetylcysteine, J. Clin. Invest. 68 (1981) 1053–1064.
[9] N. Siveski-Iliskovic, M. Hill, D.A. Chow, P.K. Singal, Probucol protects against adriamycin cardiomyopathy without interfering with its antitumor effect, Circulation 91 (1995) 10–15.
[10] H.-C. Yen, T.D. Oberley, S. Vichitbandha, Y.-S. Ho, D.K. St. Clair, The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice, J. Clin. Invest. 98 (1996) 1253–1260.
[11] Y.J. Kang, Y. Chen, P.N. Epstein, Suppression of doxorubicin cardiotoxicity by overexpression of catalase in the heart of transgenic mice, J. Biol. Chem. 271 (1996) 12610–12616.
[12] Q.N. Diep, K. Benkirane, F. Amiri, J.S. Cohn, D. Endemann, E.L. Schiffrin, PPARα activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats, J. Mol. Cell. Cardiol. 36 (2004) 295–304.
[13] S. Ichihara, K. Obata, Y. Yamada, K. Nagata, A. Noda, G. Ichihara, A. Yamada, T. Kato, H. Izawa, T. Murohara, M. Yokota, Attenuation of cardiac dysfunction by a PPAR-α agonist is associated with down-regulation of redox-regulated transcription factors, J. Mol. Cell. Cardiol. 41 (2006) 318–329.
[14] F.M. Veronese, P. Caliceti, O. Schiavon, M. Sergi, Polyethylene glycol-superoxide dismutase a conjugate in search of exploitation, Adv. Drug Deliv. Rev. 54 (2002) 587–606.
[15] Y. Tamura, L.G. Chi, E.M. Driscoll Jr., P.T. Hoff, B.A. Freeman, K.P. Gallagher, B.R. Lucchesi, Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardial ischemia/reperfusion injury in canine heart, Circ. Res. 63 (1988) 944–959.
[16] T. Yamakawa, Y. Kadowaki, M. Garcia-Alves, M. Yokoyama, Y. Iwashita, K. Nishi, Effects of polyoxyethylene-modified superoxide dismutase on reperfusion induced arrhythmia in isolated rat and guinea-pig hearts, J. Mol. Cell. Cardiol. 21 (1989) 441–452.
[17] S. Ichihara, T. Senbonmatsu, E. Price Jr., T. Ichiki, F.A. Gaffney, T. Inagami, Targeted deletion of angiotensin II type 2 receptor caused cardiac rupture after acute myocardial infarction, Circulation 106 (2002) 2244–2249.
[18] Y. Kato, Y. Mori, Y. Makino, Y. Morimitsu, S. Hiroi, T. Ishikawa, T. Osawa, Formation of N^{ε}-(hexanonyl)lysine in protein exposed to lipid hydroperoxide, J. Biol. Chem. 274 (1999) 20406–20414.
[19] O.W. Griffith, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine, Anal. Biochem. 106 (1980) 207–212.
[20] D.M. Weinstein, M.J. Mihm, J.A. Bauer, Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice, J. Pharmacol. Exp. Ther. 294 (2000) 396–401.
[21] Y. Kakinuma, T. Miyauchi, K. Yuki, N. Murakoshi, K. Goto, I. Yamaguchi, Novel molecular mechanism of increased myocardial endothelin-1 expression in the failing heart involving the transcriptional factor hypoxia-inducible factor-1α induced for impaired myocardial energy metabolism, Circulation 103 (2001) 2387–2394.
[22] C.-H. Kim, Y.-S. Cho, Y.-S. Chun, J.-W. Park, M.-S. Kim, Early expression of myocardial HIF-1α in response to mechanical stress: regulation by stretch-activated channels and the phosphatidylinostitol 3-kinase signaling pathway, Circ. Res. 90 (2002) e25–e33.
[23] P.J. Lee, B.H. Jiang, B.Y. Chin, N.V. Iyer, J. Alam, G.L. Semenza, A.M. Choi, Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia, J. Biol. Chem. 272 (1997) 5375–5381.
[24] Y. Huang, R.P. Hickey, J.L. Yeh, D. Liu, A. Dadak, L.H. Young, R.S. Johnson, F.J. Giordano, Cardiac myocyte-specific HIF-1α deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart, FASEB J. 18 (2004) 1138–1140.
[25] P. Deres, R. Halmosi, A. Toth, K. Kovacs, A. Palfi, T. Habon, L. Czopf, T. Kalai, K. Hideg, B. Sumegi, K. Toth, Prevention of doxorubicin-induced acute cardiotoxicity by an experimental antioxidant compound, J. Cardiovasc. Pharmacol. 45 (2005) 36–43.
[26] L. del Peso, M. Gonzalez-Garcia, C. Page, R. Herrera, G. Nunez, Interleulin-3-induced phosphorylation of BAD through the protein kinase Akt, Science 278 (1997) 687–689.
[27] E.L. Pagé, G.A. Robitaille, J. Pouysségur, D.E. Richard, Induction of hypoxia-inducible factor-1α by transcriptional and translational mechanisms, J. Biol. Chem. 277 (2002) 48403–48409.

[28] Y. Taniyama, K. Walsh, Elevated myocardial Akt signaling ameliorates doxorubicin-induced congestive heart failure and promotes heart growth, J. Mol. Cell. Cardiol. 34 (2002) 1241–1247.
[29] M. Ishida, S. Tomita, T. Nakatani, S. Fukuhara, M. Hamamoto, N. Nagaya, Y. Ohtsu, M. Suga, Y. Chikao, T. Yagihara, K. Yamada, S. Kitamura, Bone marrow mononuclear cell transplantation had beneficial effects on doxorubicin-induced cardiomyopathy, J. Heart Lung Transplant. 23 (2004) 436–445.
[30] S. Chen, M. Garami, D.G. Gardner, Doxorubicin selectively inhibits brain versus arterial natriuretic peptide gene expression in cultured neonatal rat myocytes, Hypertension 34 (1999) 1223–1231.
[31] D.A. Gewirtz, A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin, Biochem. Pharmacol. 57 (1999) 727–741.