Differential cardioprotective/cardiotoxic effects mediated by β-adrenergic receptor subtypes

Daniel Bernstein,¹ Giovanni Fajardo,¹ Mingming Zhao,¹ Takashi Urashima,¹ Jennifer Powers,¹ Gerald Berry,² and Brian K. Kobilka³

Departments of ¹Pediatrics, ²Pathology, and ³Molecular and Cellular Physiology, Stanford University, Stanford, California

Submitted 5 January 2005; accepted in final form 13 July 2005

β-adRENERGIC RECEPTORS (β-ARs) are members of the superfami-
ily of seven-transmembrane G protein-coupled receptors (30),
three subtypes of which (β1, β2, β3) have been identified in
the heart (6, 11, 13, 14, 25–27). Classically, β-ARs were
regarded primarily as regulators of cardiac function (inotropy,
lusitropy, and chronotropy). Recent evidence suggests that
β-ARs also play a role as regulators of cardiac remodeling in
response to stress. In vitro, stimulation of cardiomyocytes with
β-agonists induces apoptosis (36). In vivo, chronic stimulation
of the myocardium with β-agonists (29) or conditions associ-
ated with chronically increased sympathetic drive (5) leads
to the development of dilated cardiomyopathy. Many of the
adverse effects of increased sympathetic stimulation are
blocked by β-antagonists, both in vitro and in vivo, and
β-blocker therapy has become standard in the treatment of
patients with dilated cardiomyopathy (4, 8, 12, 34).

There is increasing evidence to support a differential role for
β1- vs. β2-AR subtypes in regulating both cardiac function and
alterations in cardiac structure. In vitro, the β2-receptor has
been shown to functionally couple to both the stimulatory (Gs)
and inhibitory (Gi) proteins (33) and to play a role in cardiac
remodeling through activation of MAPK pathways (10, 22).
β1-ARs have been linked to proapoptotic pathways (3, 9, 36),
whereas a dual modulation has been suggested for β2-ARs
(36). However, previous studies have been performed mostly
in vitro; several have utilized cardiomyocytes transfected to
express “nonphysiological” levels of signaling molecules of
interest; the pharmacological blockers used have only relative
specificity for each β-receptor subtype; and studies performed
on rat vs. mouse and neonatal vs. adult myocytes have often
yielded conflicting results. Confirmation of this dual role for
β-AR subtypes has been more difficult in the intact circulation.

The anthracycline group of anticancer agents have been
widely utilized as a model of toxic cardiomyopathy in both
mice and rats (15, 19, 24, 31). Their cardiovascular effects are
mediated by their ability to generate reactive oxygen species
(H₂O₂ and •OH), resulting in peroxidation of membrane lipids
and mitochondrial DNA (18). Previous studies have demon-
strated a role for MAPK pathways in this toxicity (35) as well
as alterations in β-AR signaling (24).

The purpose of the current study was to determine the role
of β1- and β2-AR subtypes in the pathogenesis of dilated
cardiomyopathy in vivo. Utilizing β-AR knockout mice and a
model of anthracycline toxic cardiomyopathy, we present evi-
dence suggesting that β1-ARs mediate cardiotoxicity, whereas
β2-ARs mediate cardioprotection. This subtype-specific toxic-
ity could have significant implications in the design of more
subtype-selective β-adrenergic antagonists for the treatment of
patients with heart failure.

METHODS

Generation of β-AR Knockout Mice

Homozigous β1-AR and β2-AR knockouts were generated using a
positive-negative selection strategy to effect homologous recombi-
nation in embryonic stem cells, as previously described (6, 25, 28).
Crosses were then carried out between homozigous β1−/− and
homozigous β2−/− mice to generate compound heterozygotes, which
were in turn intercrossed to generate mice homozigous for the double
knockout (β1−/−β2−/−) (28). Mice were genotyped for both β1-AR and
β2-AR disruptions using the polymerase chain reaction. All proce-

The costs of publication of this article were defrayed in part by the payment
of page charges. The article must therefore be hereby marked “advertisement”
in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpheart.org 0363-6135/05 $8.00 Copyright © 2005 the American Physiological Society

H2441

Differential Cardioprotective/Cardiotoxic Effects of β-Receptors

Model of Doxorubicin Cardiotoxicity

Mice were injected with a single dose of 15 mg/kg of doxorubicin (NovaPlus, Bedford, OH) (200–300 μl) via the dorsal tail vein. Genotypes studied included β1−/−, β2−/−, β1β2−/−, and wild-type mice. All mice studied were males and were 3 mo old. β1−/− and β2−/− mice were on a congenic FVB background. Experiments with β1−/− and β2−/− mice were compared with wild-type FVB littermates. β1β2−/− mice were on a mixed FVB/C57/129/DBA background, as attempts to breed these mice on a congenic background have not been successful. Because of this, all experiments with β1β2−/− mice were compared with wild-type littermates of the same mixed strain.

Hemodynamic Monitoring

Electrocardiography. Unanesthetized ECG recordings were obtained with an implantable telemetric unit (PhysioTel, Data Sciences, St. Paul, MN) in four wild-type and four mice. ECG recordings were also performed during light anesthesia in six additional β2−/− mice performed in conjunction with echocardiograms (see below). Mice were anesthetized with 2% isoflurane, and an incision was made over the back. Two smaller incisions were made on the opposite sides of the pectoral region for suturing of the leads, which were tunneled subcutaneously cranially and sutured into the pectoral muscles.

Blood pressure. Mice were anesthetized with inhaled isoflurane (3%) induced in a closed chamber and maintained via nose cone (1.5–2%) throughout surgery. With the mouse supine, a 1-cm midline neck incision was made from just below the mandible to the thoracic inlet. Under a dissecting microscope (Nikon USA, Melville, NY), the left carotid artery was identified and a stretched Intramedic PE-10 inlet. Under a dissecting microscope (Nikon USA, Melville, NY), the left carotid artery was identified and a stretched Intramedic PE-10 catheter (Clay Adams, Parsippany, NJ) was advanced to an approximate depth of 1 cm and secured in place with 4-0 surgical silk suture (Ethicon, Somerville, NJ). The catheter was then flushed with heparinized saline (100 U/ml saline), sealed with cyanoacrylate glue, tunneled to the back of the neck, and tucked into a subcutaneous pocket. Intravenous ampicillin (100 mg/kg) was administered after skin closure. The mice were then allowed to recover for a minimum of 3 days after surgery. ECG signals were recorded from the telemetric unit using a receiver mounted under the cage (DataSciences International), digitized at a sampling rate of 1 kHz and fed into a microcomputer-based data acquisition system (MacLab System, AD Instruments, Milford, MA).

Blood pressure. Mice were anesthetized with inhaled isoflurane (3%) induced in a closed chamber and maintained via nose cone (1.5–2%) throughout surgery. With the mouse supine, a 1-cm midline neck incision was made from just below the mandible to the thoracic inlet. Under a dissecting microscope (Nikon USA, Melville, NY), the left carotid artery was identified and a stretched Intramedic PE-10 polyethylene catheter (Clay Adams, Parsippany, NJ) was advanced to an approximate depth of 1 cm and secured in place with 4-0 surgical silk suture (Ethicon, Somerville, NJ). The catheter was then flushed with heparinized saline (100 U/ml saline), sealed with cyanoacrylate glue, tunneled to the back of the neck, and tucked into a subcutaneous pocket. Intravenous ampicillin (100 mg/kg) was administered after skin closure. The mice were then allowed to recover for food and water available ad libitum for 24 h before study. Two wild-type and two β2−/− mice had awake blood pressure measurements performed.

Echocardiography. Six β2−/− mice that had previously undergone implantation of ECG telemetry units were anesthetized with avertin (250 mg/kg) and had carotid arterial catheters placed. Echocardiographic studies were performed using a Siemens Sequoia system with a 15-MHz linear array transducer (Siemens, Mountain View, CA). M-mode echocardiographic measurements were obtained from the parasternal short axis view, just below the level of the mitral valve. Baseline measurements included left ventricular internal dimension at end-diastole (LVIDd) and left ventricular internal dimension in systole (LVIDs). Left ventricular fractional shortening (%FS) was calculated. Measurements of mean blood pressure, ECG, and echocardiographic parameters were made at baseline and at 1-min intervals after administration of 15 mg/kg doxorubicin via tail vein. Echocardiograms were also performed in three wild-type mice after 15 mg/kg doxorubicin.

MAPK Activation and Inhibition

Expression and activation of the three major MAPK family members, p38, p42/44, and JNK, were quantified using anti-MAPK and anti-phospho-MAPK antibodies specific to each kinase by Western immunoblotting. Heart tissue was harvested and homogenized in lysis buffer (final concentration: 80 mM NaCl, 20 mM HEPES, 0.05% Triton X, 1 mM DTT, 0.5% sodium deoxycholate, 20 mM β-glycerophosphate, 50 mM Na3VO4, 4 μg/ml leupeptin, 1.0 mM EDTA, 10 μg/ml benzamidine, 2 μg/ml aprotinin, and 0.1 mM PMSF) on ice. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Collected supernatant was assayed to quantify protein concentration. Samples were then loaded on a 10% polyacrylamide gel. After one-dimensional separation, the protein was electrophoretically transferred to nitrocellulose membranes. Blots were sequentially probed with anti-phospho- and non-phospho-p38 and p44/42 MAPK polyclonal antibodies (Cell Signaling technology kit, Beverly, MA) and anti-JNK antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) and a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody. Secondary antibodies were then detected by incubation with LumiGLO chemiluminescence for 1 min before exposure to autoradiography film (Kodak, Rochester, NY) and then quantified using a Bio-Rad Imaging Densitometer GS 710 (BioRad, Hercules, CA). Phospho-MAPK signals were normalized to both non-phospho-MAPK as well as to GAPDH expression.

Confirmation of p38 activation was also performed by immunoprecipitation with anti-p38 antibodies and kinase assay using activating transcription factor-2 (ATF-2) as a substrate. Heart tissue was harvested as above. Active p38 kinases were selectively immunoprecipitated using an immobilized phospho-p38 monoclonal antibody (Cell Signaling Technology, Beverly, MA) overnight at 4°C. After the beads were washed, the kinase assay was initiated by the addition of ATF-2 fusion protein (substrate of active p38 kinase) and ATP and incubated 30 min at 30°C. The phosphorylated substrate ATF-2 was separated by SDS-PAGE. Blots were probed with anti-phospho-ATF-2 primary antibody and then horseradish peroxidase-conjugated secondary antibody and visualized by autoradiography.

There are two isoforms of p38 (α and β) expressed in the myocardium (32). To determine which isoform was activated in our experiments, we used both p38 (N-20):sc-728 (Santa Cruz Biotechnology, Santa Cruz, CA), which is significantly more specific for the α-p38 isoform (although there is some cross-reactivity), and p38β (E-20): sc-6187 (Santa Cruz Biotechnology), which is specific for the β-p38 isoform.

Further investigation of the role of p38 activation in the enhanced doxorubicin toxicity in β2−/− mice was achieved by using the p38-selective inhibitor SB-203580 (Calbiochem, La Jolla, CA). These studies were performed using the 50% lethal dose (LD50) of doxorubicin in β2−/− mice, determined by previous experiments to be 8 mg/kg. Sixteen β2−/− mice were treated with 8 mg/kg iv doxorubicin alone and 15 β2−/− mice received 1 mg/kg SB-203580 ip 30 min before administration of 8 mg/kg iv doxorubicin. The dose of SB-203580 was chosen after dose-ranging studies performed in myocardium demonstrating a near total reduction in p38 activation after 1 mg/kg of SB-203580 (see Fig. 6A). Assessment of the possible crossover inhibition of JNK by SB-203580 was performed using an immunoprecipitation (IP) kinase assay for JNK activity, similar to that described for p38, but using e-Jun as the phosphorylated substrate.

Further investigation of the role of JNK activation was achieved by using the JNK-selective inhibitor SP-600125 (Calbiochem, La Jolla, CA). SP-600125 was dissolved in vehicle, modified to reduce toxicity from that previously described (2, 16), containing 25% DMSO-15%-cremophor-2.5%-ethanol-57.5% saline. SP-600125 was administered 30 min before doxorubicin at a LD50 dose of 8 mg/kg. Eight β2−/− mice were treated with vehicle (100–150 μl iv) 30 min before intravenous doxorubicin, and nine β2−/− mice received 3 mg/kg SP-600125 iv 30 min before intravenous doxorubicin. This dose was...
chosen based on a previous study showing inhibition of JNK activity in the central nervous system after intravenous administration (16). Surviving mice were killed 40 min after doxorubicin, and their hearts, as well as the hearts of those that died, were excised for assessment of JNK activity by IP kinase assay (Cell Signaling, Beverly, MA). Unlike the effect of SB-203580 on p38, the inhibition of JNK in myocardium was less consistent with SP-600125, possibly due to its poor solubility.

**Pathology**

Samples of left ventricular myocardium were obtained 30 min after administration of doxorubicin from all genotypes and placed in formalin and glutaraldehyde for both light microscopic (hematoxylin-eosin and trichrome stains) as well as electron microscopic analysis. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining (R&D Systems, Minneapolis, MN) for detection of cardiomyocyte apoptosis was performed at 30 min in β2−/− and wild-type littermates receiving 15 mg/kg doxorubicin. Because it is possible that β2−/− mice receiving the full dose of 15 mg/kg did not survive long enough for the detection of apoptosis, additional studies were performed at 48 h in mice receiving 3.75 mg/kg doxorubicin. To further evaluate whether the apoptotic pathway played a role in the enhanced cardiotoxicity, an assessment of caspase 3 activity was performed at 30 min in mice receiving 15 mg/kg, at 6 h and 4 days in mice receiving 7.5 mg/kg, and at 4 days in mice receiving 3.75 mg/kg doxorubicin. Caspase 3 was assayed using the ApoTarget-Caspase-3/CPP32 kit (BioSource International, Camarillo, CA).

**β-Antagonists**

Mice were administered β-adrenergic antagonists intravenously 10 min before doxorubicin, including the β2-specific antagonist ICI-118,551 (500 μg/kg), the β1-selective antagonist metoprolol (2.5 mg/kg), and the nonspecific β-antagonist propranolol (3 mg/kg, a dose previously demonstrated to block heart rate response to isoproterenol in wild-type mice). Additional mice received continuous propranolol (5 mg/kg) daily via a subcutaneous osmotic minipump (Alza, Palo Alto, CA) for 14 days before doxorubicin administration.
**Statistical Analysis**

Comparisons of mortality between different genotypes or between different treatments (β-blockers, p38 inhibitor) were performed by χ² analysis. Where multiple comparisons were performed, Bonferroni’s correction was utilized. Comparisons of MAPK expression under different conditions or hemodynamics at various time points was performed by analysis of variance with Fisher’s protected least significant difference post hoc testing. Statistical significance was considered achieved when \( P < 0.05 \).

**RESULTS**

**Mortality**

Wild-type mice receiving 15 mg/kg of the anthracycline doxorubicin did not manifest acute adverse cardiovascular effects, and none of the animals died (Fig. 1). Results were similar in \( β1^{-/-} \) mice. In contrast, the mortality in \( β2^{-/-} \) mice was 100%, occurring within 20–30 min of doxorubicin administration. Surprisingly, the additional deletion of the \( β1 \)-receptor (in \( β1/β2 \) double knockout mice) totally rescued the enhanced toxicity in the \( β2 \)-knockout.

**Hemodynamics and Pathology**

In \( β2^{-/-} \) mice, systolic function (echocardiographic fractional shortening) and mean blood pressure fell nearly simultaneously, starting within 2 min of doxorubicin administration. The decrease in fractional shortening reached statistical significance (\( P < 0.05 \) by ANOVA) by 6 min and the decrease in blood pressure by 7 min, at which point it had fallen to \( \sim 50\% \).
of baseline value (Fig. 2, top). Heart rate fell gradually within the first 2 min, reaching statistical significance by 12 min, then dropping again preterminally (Fig. 2, bottom). There were no acute changes in blood pressure or in left ventricular function in wild-type, β1/−/−, or β1/β2/−/− mice (data not shown).

In β2/−/− mice, ECG changes developed either coincident with or subsequent to the decreases in contractility and blood pressure, ranging from 2 to 9 min after doxorubicin administration. These changes consisted initially of ST segment changes (Fig. 3), progressing to second- and third-degree heart block and eventually terminal severe bradycardia. Corrected QT interval (QTc), which has been shown to increase after doxorubicin administration, increased by ~30% within 3 min of doxorubicin, then plateaued (Fig. 2, bottom). There were no significant ECG changes in wild-type, β1/−/−, or β1/β2/−/− mice (data not shown).

Because of the rapidity of decompensation in β2/−/− mice, neither light nor electron microscopy demonstrated any significant ultrastructural changes in the myocardium. Importantly, there were no signs of acute ischemic damage, such as endothelial swelling in coronary arterioles. TUNEL staining of myocardium obtained from mice 30 min after 15 mg/kg doxorubicin failed to show an increase in the percentage of apoptotic nuclei compared with wild-type littermates and compared with mice that had not received doxorubicin. Electron microscopy and TUNEL staining performed 48 h after a sublethal dose of 4 mg/kg doxorubicin did not show any evidence of ultrastructural damage or apoptosis. Additionally, there was no

Fig. 4. Alterations in different MAPKs after doxorubicin. β2/−/− mice demonstrate a 20-fold increase in p38 MAPK activation in contrast to smaller increases in other genotypes. p44 (ERK1), p42 (ERK2), and JNK are also differentially activated in β2/−/− mice in response to doxorubicin (Dox), although less dramatically than for p38. *P < 0.005 vs. other genotypes by ANOVA. Cont, control.
evidence of caspase 3 activation at multiple time points after doxorubicin administration. Thus enhanced activation of pathways leading to programmed cell death is an unlikely mechanism of the enhanced cardiotoxicity in the β2−/− mice.

Role of Differential Activation of MAPKs

Activation of MAPK has been previously demonstrated in cultured cardiomyocytes exposed to anthracyclines, with ERK2 (p42 MAPK) thought to play an antiapoptotic role and p38 MAPK a proapoptotic role (35). Baseline expression of each MAPK (p38, p44/42, and JNK) was not altered in any of the knockout mice. However, baseline level of phospho-p38 was decreased in β2−/− mice compared with wild-type controls (0.065 ± 0.075 vs. 0.150 ± 0.075, ratio of phospho-p38/GAPDH, P < 0.05). None of the other genotypes showed alterations in phospho-p38 at baseline.

In wild-type mice treated with doxorubicin, there was a twofold increase in activation of p38, as assessed by level of phospho-p38. In contrast, in β2−/− mice, activation of p38 was increased 20-fold. The β1−/− mice showed a level of p38 activation that was not different from wild-type, whereas the level in β1/β2−/− was intermediate (Fig. 4). The marked activation of p38 in β2−/− mice was confirmed by IP-kinase assay (Fig. 5A), and the results were equivalent whether phospho-p38 was corrected for GAPDH or for total p38. Activation of p38 occurred within 1 min after administration of doxorubicin (Fig. 5B). Evaluation of p38 isoforms using isoform-specific antibodies showed that the dominant isoform activated in β2−/− mice with doxorubicin was α-p38, which has been shown to be the dominant form expressed in the heart and has been previously linked to myocyte death (32). There were also increases in activation of p44 (ERK1), p42 (ERK2), and JNK, primarily in β2−/− mice receiving doxorubicin, although to a much lesser extent than p38 (Fig. 4).

To examine whether p38 activation was persistently activated or was associated only with the rapid demise of the β2−/− mice, an additional group of three mice in each genotype was given a sublethal dose (3.75 mg/kg) and killed after 4 days. The level of phospho-p38 was still increased by ~7-fold over wild type, but only in the β2−/− mice (data not shown).

To examine whether p38 activation played a role in the enhanced cardiotoxicity encountered in the β2−/− mice, we administered the p38 inhibitor SB-203580, 1 mg/kg, to β2−/− mice 30 min before doxorubicin. We have shown this dose to significantly decrease p38 activation (Fig. 6A). When β2−/− mice were treated with the LD₅₀ dose of 8 mg/kg doxorubicin, the survival was 63% (10/16); all mortalities still occurred within 22 min postdoxorubicin (Fig. 6B). However, when these mice were pretreated with SB-203580, the survival was 93% (14/15) (P < 0.05 by χ² analysis). Although SB-203580 had been thought to be a specific inhibitor of p38, a previous report suggested that SB-203580 could also block JNK activation (7). Our studies have also determined that SB-203580 has some cross-reactivity with JNK (Fig. 6A) although the effect on p38 is significantly more dramatic. We attempted to eliminate the role of JNK by administering the JNK inhibitor SP-600125. Unlike SB-203580, SP-600125 did not rescue β2−/− mice from doxorubicin toxicity. Four of eight β2−/− mice receiving vehicle plus doxorubicin died vs. five of nine receiving SP-600125 plus doxorubicin [P = not significant (NS)]. However, the in vivo efficacy of SP-600125 in blocking JNK in the myocardium is less consistent than the activity of SB-203580 in blocking p38.

Effect of β-Blockade

Pharmacological blockade of the β2-AR in wild-type mice, either with the β2-specific antagonist ICI-118,551 or with the combined β1/β2-antagonist propranolol, recapitulated the effects of β2-AR gene disruption, markedly increasing mortality in response to doxorubicin (Fig. 7). p38 MAPK activity was increased eightfold in ICI-118,551-treated wild-type mice receiving doxorubicin compared with wild-type mice not treated with ICI-118,551. This activation of p38 MAPK is similar although less dramatic than the increase (20-fold) in β2−/− mice receiving doxorubicin.

In contrast to these results with β2-antagonists, pharmacological blockade of the β1-AR did not recapitulate the rescue effect we observed when the β1-AR was genetically deleted. When both β-ARs were genetically deleted (β1/β2−/−), 100% of mice survived; deletion of the β1-AR (β1−/− mice) also partially rescued mice from the combination of ICI-118,551 plus doxorubicin, with a 50% mortality rate vs. a 100%
mortality in wild types (P < 0.001 vs. β1−/− with no ICI-118,551 and P < 0.001 vs. β2−/−). In contrast, administration of the β1-specific antagonist metoprolol failed to rescue β2−/− mice from doxorubicin. This lack of effect of pharmacological blockade of the β1-AR is further evidenced by the effects of propranolol on wild-type or β2−/− mice, also failing to recapitulate the beneficial effects of genetically deleting the β1-AR. Administration of propranolol to mice lacking the β1-AR (both β1−/− and β1β2−/−) did not substantially increase doxorubicin-induced mortality [12.5% in β1−/− (P = NS); 0% in β1β2−/−]. Chronic administration of propranolol (5 mg·kg−1·day−1 for 14 days) also failed to rescue the β2−/− mice but had a markedly different effect on wild-type mice (20% mortality; P < 0.04 vs. wild type with no propranolol; P < 0.01 vs. β2−/− with propranolol) compared with acute propranolol (83% mortality).

**DISCUSSION**

The present study is the first comprehensive demonstration in vivo of a clear differential effect of β-receptor subtypes in mediating cardiotoxic vs. cardioprotective signaling in the heart. β2-ARs appear to modulate a cardioprotective role in anthracycline-induced cardiomyopathy. In contrast, β1-ARs appear to be responsible for at least some of the cardiotoxic effects. Previous studies in vivo have suggested a similar dual role for these β-receptor subtypes. Patterson et al. (23) showed enhanced cardiotoxicity in β2−/− mice after a chronic infusion of isoproterenol (23). Ahmet et al. (1) showed that a β2-AR-selective agonist exerted a beneficial effect on infarct size and ejection fraction in rats with ischemic cardiomyopathy.

The cardioprotective effect of the β2-AR was absent both in mice with genomic deletion of the β2-AR as well as mice with acute β2-AR blockade. In contrast, whereas genomic deletion of the β1-AR rescued the acute cardiac decompensation in β2−/− mice, this effect of β1-ARs was not duplicated with acute β1-AR blockade. We speculated that this cardioprotective effect could require chronic β1-blockade (as would be present in the β1−/− mice); however, chronic β1-blockade also...
failed to rescue β2−/− mice from enhanced anthracycline toxicity, although the toxicity to wild-type mice was reduced. We speculate that genomic deletion of the β1-AR may result in disruption of a downstream cardiotoxic signaling pathway, as yet to be elucidated.

Previous studies in vitro have implicated MAPKs in doxorubicin cardiotoxicity. In cultured cardiomyocytes, daunomycin activates proapoptotic signaling mediated via p38 MAPK and antiapoptotic signaling mediated via p42 MAPK (35). Differential activation of MAPKs has also been described in vitro as a mechanism for the subtype-specific cardiotoxicity of β-receptor agonists (36). We thus hypothesized that MAPK signaling represented a common pathway between these two cardiac stressors in vivo. Similar to in vitro, we found activation of multiple MAPK family members with doxorubicin. There was a dramatic (20-fold) activation of p38 in doxorubicin-treated β2−/− mice compared with a substantially lower activation in the other genotypes. This activation was of the α-p38 isoform, which has been implicated in cell death in myocytes in vitro (32). Doxorubicin also resulted in increased activity of p42, p44, and JNK in β2−/− mice, although the magnitude was not as great as p38. These data suggest that doxorubicin activates both pro- and antisuival pathways in the myocardium of β2−/− mice, similar to results in vitro (35). The MAPK inhibitor, SB-203580, rescued β2−/− mice from doxorubicin toxicity, whereas rescue was not achieved with the JNK inhibitor SP-600125. This confirms a role for p38 in this enhanced toxicity.

The rapidity of the cardiotoxic response suggests that apoptosis is not the dominant mediator, and our studies failed to show evidence of TUNEL staining or caspase 3 activation. Attempts to define the pathology of this toxicity have been limited by the fact that none of the animals lives long enough for the classical light or electron microscopic findings of doxorubicin cardiotoxicity to become manifest. However, our electron microscopy studies confirm the absence of ischemic damage (lack of endothelial swelling). The sequence of events leading to demise of the β2−/− mice is initiated by a decrease in contractile function (without cardiac dilation) accompanied by a decrease in blood pressure, with ECG changes occurring slightly later. There could be several additional mechanisms for the enhanced cardiotoxicity, including altered calcium fluxes, protein kinase C, Akt/GSK3β, and other regulators of cell death, mitochondrial energetics, or free radical processing. Compensatory up- or downregulation of other G protein-coupled receptors or components of their signaling pathways is also possible.

The acute cardiotoxicity we have described may be similar or quite different pathophysiologically from the chronic doxorubicin toxicity most commonly encountered in patients. Whether this enhanced acute toxicity is relevant to the clinical syndrome is not yet clear. Although most patients with doxorubicin cardiotoxicity manifest late heart failure, a minority suffer acute cardiotoxicity. More importantly, there is increasing recognition that so-called “late” clinical toxicity is almost always preceded by longstanding subclinical alterations in ventricular-vascular coupling (17, 21). Using echocardiographic indexes, up to 90% of children with anthracycline cardiomyopathy manifest some changes during their first year of therapy (20).

It is possible that our acute murine model does recapitulate the clinical syndrome, but with the “gain” of the toxicity markedly increased in terms of severity and time course. If this is the case, determining the mechanisms for this enhanced toxicity (and its rescue) may have important implications for patients receiving anthracyclines. Alternatively, our model may be mechanistically very different from the chronic clinical syndrome. In this case, we will still have shown, for the first time in an in vivo model, a marked difference in cardiotoxic/cardioprotective signaling mediated via different β-receptor subtypes. Although these differential effects have previously been demonstrated in cell culture, these studies have mainly used transfected cells expressing β-ARs at highly nonphysiological levels.

In patients with cardiomyopathy, state-of-the-art heart failure management includes the use of β-blocking agents, the most commonly used of which are nonspecific β-adrenergic blockers (e.g., carvedilol), although β1-selective antagonists have also been studied (e.g., metoprolol). Our results suggest the intriguing possibility that a combination of a β1-antagonist and a β2-agonist might further improve efficacy in heart failure management, similar to the findings by Ahmet et al. (1) in rats after myocardial infarction. These studies clearly support the need for further investigation of the subtype-specific cardiotoxic and cardioprotective effects of β-AR signaling.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-61535 to D. Bernstein. G. Fajardo was supported by a Postdoctoral Fellowship from the American Heart Association, Western States Affiliate.

REFERENCES


Differential cardioprotective/cardiototoxic effects of β-receptors


