Radicicol activates heat shock protein expression and cardioprotection in neonatal rat cardiomyocytes

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Radicicol activates heat shock protein expression and cardioprotection in neonatal rat cardiomyocytes. Am J Physiol Heart Circ Physiol 287: H1081–H1088, 2004. First published April 29, 2003; 10.1152/ajpheart.00921.2003.—Heat shock proteins (HSPs) constitute an endogenous cellular defense mechanism against environmental stresses. In the past few years, studies have shown that overexpression of HSPs can protect cardiac myocytes against ischemia-reperfusion injury. In an attempt to increase the HSPs in cardiac tissue, we used the compound radicicol that activates HSP expression by binding to the HSP 90 kDa (HSP90). HSP90 is the main component of the cytosolic molecular chaperone complex, which has been implicated in the regulation of the heat shock response and a significant decrease in protection against ischemia-reperfusion injury in cardiomyocytes. Our experiments show that radicicol induces HSP expression and protect neonatal rat cardiomyocytes against simulated ischemia-reperfusion injury (7, 15). Studies from our laboratory and others have shown that geldanamycin and herbimycin A induce HSP expression and protect neonatal rat cardiomyocytes against simulated ischemia-reperfusion injury (7, 15). The macrocyclic antifungal antibiotic radicicol, isolated from an herbal remedy, has now been found to bind HSP90 with a 50-fold greater affinity than geldanamycin or herbimycin A (22). Therefore, we tested the hypothesis that radicicol might also induce HSP expression and protect against ischemia-reperfusion injury in cardiomyocytes. Our experiments show that radicicol does protect against ischemia-reperfusion injury by inducing the heat shock response. In addition, we find that the mechanism by which radicicol induces HSP expression is directly dependent on the amount of HSP90 present in the cell. An increase in HSP90 levels before radicicol treatment results in a reduction in the heat shock response and a significant decrease in protection against ischemia-reperfusion injury in cardiomyocytes. These results indicate that HSP90 modulates the level of cardioprotection in cardiomyocytes by directly controlling the amount of HSP expression through its ability to bind to HSF1.

MATERIALS AND METHODS

This study was conducted in accordance with animal care guidelines issued by the National Institutes of Health. All protocols were in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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approved by the Animal Care and Use Committee of Loyola University Medical Center.

**Neonatal cardiomyocyte preparation.** Cardiomyocytes were prepared from 1- to 2-day-old Sprague-Dawley rat hearts by enzyme digestion. Hearts were collected from four litters of pups (~50), trimmed of atria and excess vessels, and then rinsed in 1× ADS solution (in mM: 11.6 NaCl, 1.8 HEPES, 0.1 NaH2PO4, 0.5 KCl, 55 glucose, and 83 μM MgSO4). The ventricles were cut into four pieces and subjected to a series of digestions in collagenase (type 2; Worthington Biochemicals) and pancreatin (Sigma) at a concentration of 80 mg/ml and 62.5 μg/ml, respectively, in 1× ADS. The digestion, 5 min, was discarded and the subsequent 5–6 digestions, 20 min each, were collected into newborn calf serum. All were gently agitated in a 37°C shaking water bath. After tissue dissociation, cells were spun for 5 min at 1,750 g. The resulting pellet was resuspended in 5 ml of 1× ADS and layered over percoll gradients. The gradient was spun for 80 min at 30,000 g, 4°C. The resulting supernatant was used for ATP determination.

**Adenoviral construction.** Adenoviral vectors were constructed by using the Admax system (Microbix Biosystems; Ontario, Canada) following the manufacturer’s instructions. Briefly, the human HSP90β was introduced into the EcoRI/Sal sites of the pDC516 shuttle vector, which contains the frt sites required for FLP recombination. The resulting plasmid construct, pDC516/HSP90β was then cotransfected with pBHGfrtDE1,3FLP, a plasmid containing the adenoviral genome, as well as the FLP recombimase, into 293 cells. Cotransfection was performed by using 10 μg of pBHGfrtDE1,3FLP, 20 μg of herring sperm DNA, 500 μl of 0.25 M CaCl2, and 500 μl of N,N-bis(2-hydroxyethyl)-2-aminoethane-sulfonic acid. After proper mixing, 500 μl of this transfection mixture were added to the medium dropwise of a 22-mm plate of 293 cells and then left overnight in a 3% CO2 incubator. The next day, plates were overlaid with 1.5 ml of normal medium containing 0.65% agarose and maintained in a 5% CO2 incubator. Seven to ten days post-infection, cells were spun for 5 min at 1,750 g. The resulting supernatant was used for ATP determination, according to the manufacturer’s instructions. Viral titers were determined by optical density (3).

**Cellular ATP determination assay.** After treatment, cells were harvested by scraping into PBS and spun for 2 min at 1,750 g. Supernatants were discarded and the pellets were resuspended in 100 μl of solution B and vortexed hard at the start and at 5–6 min intervals for 20 min. Supernatants were spun again, and supernatants were reserved. One microliter of each was fixed to filter paper by 5% trichloroacetic acid solution before scintillation count. Samples were loaded on 8% SDS-PAGE gels at 1 × 106 counts/ lane. After the run, gels were fixed in 40% methanol/10% acetic acid for 30 min and then amplified (Amplify, Amersham) for 30 min before drying on filter paper and exposing to X-ray film.

**Western blot analysis.** After appropriate treatment and conditions, cells were harvested by scraping into PBS and spun for 2 min at 14,500 revolutions/min (Minispin Plus; Eppendorf), and pellets were resuspended in 100 μl of solution B and vortexed hard at the start and at 5–5 min intervals for 20 min. Extracts were spun again, and supernatants were reserved. Protein concentrations were determined by Bio-Rad protein assay on a microplate reader. ATP concentration was measured by kit (Molecular Probes), according to the manufacturer’s instructions.

**Simulated ischemia protocol.** After treatment, culture plates were divided into sets. One set was changed to control buffer (in mM: 1.13 CaCl2, 5 KCl, 0.3 KH2PO4, 0.5 MgCl2, 0.4 MgSO4, 128 NaCl, 0.3 Na3HPO4, 4 NaHCO3, 10 glucose, 10 HEPES, pH 7.2), and the other was changed to ischemic buffer (in mM: 1.13 CaCl2, 5 KCl, 0.3 KH2PO4, 0.5 MgCl2, 0.4 MgSO4, 128 NaCl, 4 NaHCO3, 10 HEPES, pH 7.2), which had been bubbled for 30 min with argon. The control set was left in a 5% CO2 incubator, whereas the ischemic buffer was set in an oxygen-controlled chamber (Pro-Ox; Reming Bioinstruments), and maintained at 0.03% O2 for 16 h within the same incubator. Supernatants were collected and labeled as such, when needed. Cell extracts were made by scraping attached cells into PBS.

**Protein concentration determination.** Protein concentration was determined by Bio-Rad or Pierce protein assay according to the manufacturer’s instructions for microplate readers.

**Creatine kinase assay.** Supernatant and cell extracts were sonicated for 10 s each. Creatine kinase (CK) reagent (Sigma) was reconstituted in 10 ml. Two parts of each sample were mixed with one part CK reagent and read for 7 min at 340 nm by microplate reader (Molecular Devices). CK was determined as a fraction of CK release versus total sample. Results are presented as percent total CK compared with control. All points were normalized for protein by bicinchoninic acid assay (Pierce).

**Cytotoxicity assays.** Cells were cultured in 96-well plates. After appropriate treatment and conditions, medium was replaced with reconstituted Na2[3,2-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] (XTT) solution according to the manufacturer’s directions. Plates were returned to the incubator for 4 h and then read on a microplate reader at 450 nm. A reference wavelength of 690 nm was subtracted to obtain final measurement; or alternatively, after treatment and conditions, medium was replaced with reconstituted 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution according to the manufacturer’s directions. Plates were returned to the incubator for 4 h. After incubation, plates were aspirated of solution and replaced with MTT solubilization solution in an amount equal to the original culture volume, shaken for 2 min on an orbital shaker to dissolve formazin crystals, and read on a microplate reader at 570 nm. The reference wavelength of 690 nm was subtracted to obtain final measurement.

**Metabolic 35S labeling of proteins.** After appropriate treatment and conditions, cells were labeled with 50 μCi of 35S-Translabel methionine-cysteine per plate for 4 h in methionine-cysteine-deficient medium (GIBCO-BRL). Cells were rinsed and scraped in PBS and spun for 2 min at 14,500 revolutions/min (Minispin Plus; Eppendorf), and pellets were resuspended in 100 μl of solution B. Samples were left on ice and vortexed hard at the start and then at 10-min intervals for 20 min. Samples were spun again and supernatants were reserved. One microliter of each was fixed to filter paper by 5% trichloroacetic acid solution before scintillation count. Samples were loaded on 8% SDS-PAGE gels at 1 × 106 counts/lane. After the run, gels were fixed in 40% methanol/10% acetic acid for 30 min and then amplified (Amplify, Amersham) for 30 min before drying on filter paper and exposing to X-ray film.
EMSA. Electrophoretic mobility shift assay (EMSA) was performed as previously described by us (7). Briefly, after treatment and conditions, cells were rinsed and then scraped in cold PBS, spun at 14,500 revolutions/min (Minispin Plus; Eppendorf), and the liquid aspirated. Nuclear fractions were obtained from the pellets by following manufacturer’s instructions for extraction (NE-PER; Pierce). The resulting fractions were flash frozen in liquid nitrogen and stored at −80°C. The DNA probe used was the double-stranded synthetic oligonucleotide containing the heat shock element 1 (HSE1) of the rat inducible HSP70i gene (12). The synthetic oligonucleotide was radioactively labeled by filling in the protruding 5′ ends using a DNA polymerase I large fragment (Klenow) in the presence of [α-32P]dCTP, -dTTP, -dGTP, and -2′-deoxythymidine 5′-triphosphate. The radioactively labeled oligonucleotide was purified from the free nucleotides by two 2.5-M ammonium acetate/ethanol precipitations. Binding reactions (15 μl) were done by incubating 20 ng of the radiolabeled DNA probe (10⁶ counts/min) with 3 μg poly(dI-dC), and 1 μg of heat-denatured herring sperm DNA in a buffer containing 4% Ficoll, 20 mM DTT, 0.25 μg/ml BSA (Sigma), and 15 μg of nuclear extract, which was added last. The mixture was then incubated at room temperature for 30 min before loading buffer was added (4% glycerol, 0.5× Tris-boric acid-EDTA solution, 10 mM EDTA, 0.2 μg/ml xylene cyanol, and 0.2 μg/ml bromophenol blue) and performing electrophoresis in a 5% acrylamide gel at 100 V. Gels were then soaked in 5% glycerol for 20 min at room temperature, dried onto a 3M filter paper, and exposed to X-ray film.

Statistical analysis. Results are expressed as means ± SE. Statistical significance was assessed by ANOVA followed by a Bonferroni t-test. Results were interpreted to be significantly different when P < 0.05.

RESULTS

To determine whether radicicol is able to induce the HSPs in the same fashion as herbimycin A and geldanamycin, we treated neonatal rat cardiomyocytes with either herbimycin A (0.8 μM), geldanamycin (0.1 μM), and radicicol (5 μM) for 4 h, or heat shocked them for 1 h at 42°C. Cardiomyocytes were then labeled with 35S-Translabel in methionine-cysteine deficient medium for 4 h. Protein extracts prepared from these cardiomyocytes were fractionated by 8% SDS-PAGE, fixed, dried onto filter paper, and exposed to X-ray film. Figure 1A represents the experimental results. As observed, radicicol is able to induce a heat shock response in neonatal rat cardiomyocytes similar to herbimycin A, geldanamycin, or heat shock. A similar experiment was performed to determine the optimal radicicol concentration that induces the HSPs (Fig. 1B). As shown, a concentration of 5 μM radicicol is sufficient to achieve a heat shock response in neonatal rat cardiomyocytes. It should be noted that all of these compounds, geldanamycin, herbimycin A, and radicicol, strongly induce the expression of the HSP90β isoform (lower band of the HSP90s shown in Fig. 1) compared with induction by a heat shock. This may indicate that the HSP90β isoform is the one primarily involved in the molecular chaperone complex.

As mentioned, studies have indicated that pretreatment of cardiomyocytes with herbimycin A or geldanamycin not only induces a heat shock response but also confers protection against ischemia-reperfusion injury. Therefore, to assess whether radicicol pretreatment can protect the cardiomyocytes against ischemia-reperfusion injury, we treated the cardiomyocytes with geldanamycin or radicicol before submitting them to simulated ischemia-reperfusion injury. Cellular damage was measured by a cytotoxicity assay kit (XTT; Sigma) that detects mitochondrial dehydrogenase activity as a measure of cell viability. The results in Fig. 2A show that radicicol imparts protection to cardiomyocytes during simulated ischemia-reperfusion injury as demonstrated by significant mitochondrial dehydrogenase activity preservation.

Preserving ATP synthesis levels is one way that the HSPs may protect against ischemia-reperfusion injury. Therefore, we
shown in Fig. 2, where we observed that radicicol significantly preserves ATP levels after simulated ischemia-reperfusion injury. As mentioned, radicicol binds to HSP90, the main component of the cytosolic molecular chaperone complex, with a 50-fold higher affinity as herbimycin A or geldanamycin. Binding to HSP90 by these compounds inhibits or neutralizes the function of HSP90 within the molecular chaperone complex. This results in the release of client proteins from the complex, among which is HSF-1. We then reasoned that if we increased the amount of HSP90 present in the cardiomyocyte before radicicol treatment, we should be able to inhibit the effect of radicicol on the HSP90 complex.

Therefore, we utilized an adenoviral vector containing a copy of the human HSP90 (AdHSP90) to overexpress this protein in cardiomyocytes. Figure 3A shows an autoradiogram of metabolically labeled (35S) protein extracts of neonatal rat cardiomyocytes infected with either a control adenoviral construct or a construct containing the human HSP90 gene at two different multiplicities of infection (MOIs). As observed, infection with our AdHSP90 construct results in overexpression of HSP90 in a dose-dependent manner. We then used this adenoviral construct in cardiomyocytes, subsequently treated with radicicol, to establish whether HSP90 overexpression would inhibit the induction of HSPs. Figure 3B shows a representative Western blot of such an experiment. Neonatal rat cardiomyocytes were infected with either our control adenoviral construct or the AdHSP90 at an MOI of 10:1. After 48 h, cells were left untreated or treated with radicicol (5 μM) for 4 h. Protein extracts from these cardiomyocytes were analyzed by Western blot reacted with a polyclonal antibody that recognizes both the constitutive and inducible HSP70. As seen in Fig. 3B, the increased presence of HSP90 blunts the ability of radicicol to induce the expression of the inducible HSP70. Therefore, we can assume that with more HSP90 present in the cardiomyocyte to bind to radicicol, less of the HSP90 in the molecular chaperone complex is being sequestered. This results in a diminished amount of the HSF-1 activation and thus a weaker heat shock response.

To assess whether having excess HSP90 present in the cardiomyocytes is truly interfering with the activation of HSF-1 by radicicol treatment, we tested the level of HSF-1 activation by EMSA. Neonatal rat cardiomyocytes infected with either our control adenoviral construct AdSR or AdHSP90 were subsequently treated with radicicol (5 μM) for 4 h. Nuclear protein extracts were prepared from these cardiomyocytes as well as from untreated and heat shocked (42°C for 60 min) cardiomyocytes that were used as controls. The HSE1 binding activity {Na[2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide] (XTT) assay; Sigma}. Results are presented as %cell survival

tested whether HSP induction by radicicol or geldanamycin is able to preserve ATP levels after simulated ischemia-reperfusion injury (ATP assay kit; Molecular Probes). Results are shown in Fig. 2B, where we observed that radicicol significantly preserves ATP levels as well as geldanamycin compared with untreated cardiomyocytes.

These results indicate that radicicol not only induces a heat shock response in neonatal rat cardiomyocytes but also protects against simulated ischemia-reperfusion injury. As mentioned, radicicol binds to HSP90, the main component of the cytosolic molecular chaperone complex, with a 50-fold higher affinity as herbimycin A or geldanamycin. Binding to HSP90 by these compounds inhibits or neutralizes the function of HSP90 within the molecular chaperone complex. This results in the release of client proteins from the complex, among which is HSF-1. We then reasoned that if we increased the amount of HSP90 present

Fig. 3. A: autoradiograph of 8% SDS-PAGE with 35S-labeled protein extracts from neonatal rat cardiomyocytes infected with our control adenoviral construct and our adenoviral construct containing the HSP90 gene (AdHSP90) at two different multiplicities of infection (MOIs); 1:1 and 10:1. Arrow indicates position of the exogenously introduced and expressed HSP90. B: Western blot of protein extracts from neonatal rat cardiomyocytes that were either infected with our control adenoviral construct and left untreated (control) or treated with radicicol (5 μM) for 4 h or were infected with AdHSP90, or treated with radicicol (Rad + AdHSP90). The blot was reacted with a rabbit polyclonal antibody that recognizes both the constitutive HSP70 (HSP70c) and inducible HSP70 (HSP70i).
from the inducible rat HSP70 (12) was radioactively $^{32}$P labeled and used as a probe for the EMSA experiments.

Figure 4A shows a representative EMSA autoradiogram of one such experiment. As observed, nuclear extracts from heat-shocked cardiomyocytes exhibit a shifted band corresponding to the formation of a HSE1/HSF-1 complex. This indicates that in heat-shocked cells, the HSF-1 has been activated and binds to its target DNA sequence (HSE1). Infection with our control adenoviral construct (AdSR) does not affect HSF-1 activation by radicicol, as seen by the presence of a shifted HSE1/HSF-1 complex, whereas infection with our AdHSP90 construct results in a significant decrease in HSE1/HSF-1 complex formation, indicating a lack of HSF-1 activation after radicicol treatment. In addition, Fig. 4A shows the specificity of our shifted HSE1/HSF-1 complex. The presence of a 100-M fold excess of the noncompetitor nonlabeled oligonucleotide Octomer-1 (Oct-1) does not eliminate the complex, whereas the presence of the competitor oligonucleotide HSE1 at 100-M fold excess eliminates the shift of the HSE1/HSF-1 complex. Figure 4B presents an EMSA experiment performed with the same nuclear extracts as in Fig. 4A but this time using a $^{32}$P[oligo]nucleotide for the ubiquitous Oct-1 binding site. This demonstrates that all nuclear extracts have similar binding ability.

Because we now know that the excess HSP90 is able to blunt the activation of HSF-1 and consequently the induction of the HSPs by radicicol treatment, we investigated whether this directly affects protection against simulated ischemia-reperfusion injury. Figure 5A presents the results of such a study in which neonatal rat cardiomyocytes were infected with AdHSP90 or left untreated and were subsequently treated with or without radicicol (5 μM) for 4 h before simulated ischemia-reperfusion. Cell damage was assessed by measuring the release of the cytosolic enzyme CK. Our results show that pretreatment with radicicol in cardiomyocytes infected with our control adenoviral construct AdSR confers protection against ischemia-reperfusion injury, whereas cardiomyocytes infected with AdHSP90 lose the protective effect against ischemia-reperfusion injury as induced by radicicol pretreatment. To further confirm our results, we also assessed cellular damage after simulated ischemia-reperfusion injury by using a cytoxicity assay (MTT assay; Sigma). Figure 5B presents the results obtained in this series of experiments. As shown, cardiomyocytes treated with radicicol (5 μM) for 4 h in the absence of any exogenous HSP90 are better protected against ischemia-reperfusion injury than cardiomyocytes that overexpress HSP90. These results indicate that the increased presence of HSP90 in the cardiomyocyte interferes with the protective effect generated by radicicol pretreatment.
DISCUSSION

Over the past decade, a series of studies have shown that HSPs are able to protect cardiomyocytes against ischemia-reperfusion injury. Studies involving whole body heat stress pretreatment in animals to induce the HSPs (13), HSP gene overexpression in transgenic mice (10, 20, 21), or using viral vectors have clearly shown that HSPs play a protective role against ischemia-reperfusion injury (14). Compounds, such as the tyrosine kinase inhibitors geldanamycin and herbimycin A have also been used to induce the HSP response and, in this manner, protect neonatal rat cardiomyocytes against ischemia-reperfusion-induced cellular damage (7, 15). Newer compounds, such as radicicol and its derivatives, as well as derivatives of geldanamycin, have also been found to induce the heat shock response and produce lesser cytotoxicity in different mammalian cell lines (23–25). It is thought that these different compounds induce the heat shock response through their ability to bind to the HSP90 molecular chaperone complex. The HSP90 molecular chaperone complex is made up of a HSP90 dimer at its core and a series of cochaperones, such as HSP70, HSP40, the immunophilin FK binding protein (FKBP)52, FKBP51, cyclophilin-40, the E3 ubiquitin ligase COOH-terminus of HSP70 interacting protein (CHIP), etc. (for complete list see Ref. 18). This molecular chaperone complex has been found to interact with a large number of client proteins, such as steroid hormone receptors and kinases and transcription factors (see Ref. 18 for review). Among the transcription factors that bind the molecular chaperone complex, we find the heat shock transcriptional factor-1 (HSF-1). Therefore, the binding of geldanamycin, herbimycin A, or radicicol to HSP90 produces the disassociation of the molecular chaperone complex, resulting in the release of the inactive monomeric HSF1, which is then free to trimerize and migrate to the nucleus. Once in the nucleus, the homotrimeric HSF1 is phosphorylated and binds to its target DNA sequences located on the promoters of the heat shock genes and initiates transcription of these genes.

Fig. 6. Potential model of radicicol action on the heat shock response. In cardiomyocytes, radicicol binding to the HSP90 dimer within the chaperone complex produces a conformational change or disruption of the complex resulting in the release of the monomeric HSF1 from the complex. The HSF1 is then trimerized and translocated into the nucleus in which it is phosphorylated and binds to the DNA heat shock elements (HSE) present on the promoters of the heat shock genes and initiates transcription of these genes. Increase in heat shock proteins in the cardiomyocyte results in protection against ischemia-reperfusion injury. HOP, heat shock protein-organizing protein; HIP, heat shock protein-interacting protein; FKBP52, binding protein; R, radicicol.
shock genes (1, 26). This process results in the rapid increase in expression of the HSPs or characteristic heat shock response of the cell and subsequent protection against ischemia-reperfusion injury (see Fig. 6 for model of potential mechanism).

Our present study has concentrated on the ability of radicicol to induce the heat shock response in neonatal rat cardiomyocytes. This radicicol-induced increase in HSP expression is directly related to its ability to activate the HSF1, as shown by the presence of HSF1-HSE complex formation in radicicol-treated neonatal rat cardiomyocytes. We also show that HSF1 activation by radicicol can be blocked by an increase in exogenously introduced HSP90 gene copies. This excess of HSP90 competes for radicicol and therefore permits the HSP90 molecular chaperone complex to remain associated with its client proteins, such as HSF1. It is important to mention that although some studies have implicated HSP90 overexpression as conferring cytoprotection (5, 9, 17), we did not observe any cardioprotection when we overexpressed HSP90 in rat neonatal cardiomyocytes (Fig. 5A). This discrepancy may be due to the difference in cell type and cellular damage against which HSP90 has been found to be protective in the studies compared with our present study. For example, the study by Heads et al. (9) overexpressed HSP90 in a stable myogenic cell line (H9c2) and not in primary cultured rat neonatal cardiomyocytes. In addition, these investigators found HSP90 to be protective against heat stress and hypoxia, whereas our study concentrated on protection against simulated ischemia.

The radicicol-induced HSP increase has also been shown to have important consequences for the neonatal rat cardiomyocytes, because it imparts a significant protective effect against subsequent ischemia-reperfusion injury. Therefore, cellular protein levels of functional HSP90 seem to modulate the ability of cardiomyocyte to sustain damage from an ischemia-reperfusion event. We should point out that binding of radicicol to the NH2-terminal ATP-site of HSP90 renders this protein nonfunctional and does not reduce the HSP90 protein level in the cell. A simple decrease in the total amount of HSP90 in the cell using antisense oligos directed to HSP90 does not result in a heat shock response and therefore does not produce a cardioprotective effect (data not shown).

Whereas inhibition of HSP90 function has received significant attention in cancer research due to its ability to retard or block tumor growth, it would seem that an additional application of these HSP90 inhibitors may be found in the cardiovascular research field where nontoxic derivatives of these compounds may offer ways to protect cardiac tissue against the ravages of ischemia-reperfusion-induced cellular damage.

In summary, our present results show that radicicol is able to induce HSP expression in the same manner as do geldanamycin and herbimycin A. This radicicol increase in HSP expression results in the protection against simulated ischemia-reperfusion injury in cardiomyocytes. We also show that both the level of HSP expression and protection against simulated ischemia-reperfusion injury are modulated by the protein level of HSP90 present. One major advantage of radicicol over geldanamycin and herbimycin A is its lower cytotoxicity, at least in cultured cells. This raises the possibility that nontoxic derivatives of radicicol may one day be useful as therapeutic agents for the treatment of cardiac ischemia-reperfusion injury.

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REFERENCES


