Adenosine-Dependent Induction of Glutathione Peroxidase 1 in Human Primary Endothelial Cells and Protection Against Oxidative Stress

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Abstract—Cellular glutathione peroxidase (GPx-1), a selenocysteine-containing enzyme, plays a central role in protecting cells from oxidative injury. GPx-1 is ubiquitously expressed in eukaryotic cells where it reduces hydrogen and lipid peroxides to alcohols. Adenosine, which is released from stressed or injured cells, protects against ischemia/reperfusion injury and apoptosis. In this study, we hypothesize that the cytoprotective effect of adenosine involves an increase in the activity of GPx-1. Treatment of human primary pulmonary artery endothelial cells (HPAECs) with 50 μmol/L adenosine in the presence of 10 μmol/L erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an adenosine deaminase inhibitor, for 48 hours increased GPx-1 mRNA levels 2-fold. GPx-1 protein and enzyme activity also increased ~2-fold after treatment. The induction of GPx-1 expression was found to be a consequence of increased mRNA stability and not an increase in transcription. Bisindolylmaleimide I (BIM), a protein kinase C signaling pathway inhibitor, significantly attenuated the induction of GPx-1 mRNA by ~36%. The adenosine/EHNA-treated cells were more resistant to hydrogen peroxide stress. Both pharmacological inhibition and siRNA knockdown of GPx-1 attenuated the protective affect of adenosine/EHNA treatment, indicating that the adenosine-induced increase in GPx-1 contributes to an increase in cellular protection against oxidative stress. These data suggest that adenosine may protect the cardiovascular system from ischemia/reperfusion injury, in part, by enhancing the expression of the central intracellular antioxidant enzyme, GPx-1. (Circ Res. 2005;96:831-837.)

Key Words: adenosine ■ antioxidant ■ cellular glutathione peroxidase ■ endothelial cells ■ RNA stability

A denosine is a ubiquitous molecule with a wide distribution in many tissues and numerous physiological actions in the cardiovascular, nervous, renal, pulmonary, and immune systems. Exposure of rabbit heart to adenosine increases tolerance of the myocardium to subsequent ischemia,1 similar to ischemic preconditioning. In isolated hearts, adenosine enhanced recovery of contractile function after no-flow ischemia compared with control hearts.2 The protective role of adenosine against myocardial ischemia/reperfusion injury has also been observed in situ3 as infarct size is significantly reduced by adenosine infusion during the reperfusion period. The protective role of adenosine in ischemia/reperfusion injury has also been documented in human studies. In a prospective clinical trial, the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial, adenosine given within 6 hours of the onset of acute myocardial infarction resulted in a significant reduction in infarct size.4 Although the myocardial protective role of adenosine is widely recognized, the underlying mechanism still remains unclear. Owing to an important role of reactive oxygen species (ROS) in ischemia/reperfusion injury in myocardium as well as in the brain,5 it is likely that adenosine may increase resistance to oxidative stress. In fact, studies in cultured chick cardiomyocytes showed that the adenosine agonist, AMP579, directly protects these cells by attenuating intracellular oxidant stress during reoxygenation.7 Adenosine attenuates oxidant injury in human proximal tubular cells,8 and in vivo, adenosine treatment before or after ischemia protects the kidney from ischemia/reperfusion injury.9,10 Adenosine also provides protection against oxidant injury in neural cells11 and plays a role in cerebral ischemic preconditioning.12 Glutathione peroxidases (GPxs) are selenocysteine-containing proteins that serve an important role in the cellular defense against oxidant stress by reducing H₂O₂ and a wide range of organic hydroperoxides. GPx-1, cellular glutathione peroxidase, is the most abundant isoform within eukaryotic cells and is a major intracellular antioxidant enzyme.13 GPx-1 transcription is induced by oxidant stress,14 and levels of GPx-1 increase during ischemia/reperfusion.15,16 Previously, we have found that GPx-1–deficient mice have endothelial dysfunction, an increase in the oxidant stress products iPF₂α,3-III isoprostanes, and abnormal cardiac function after ischemia/reperfusion injury.17,18 Other studies suggest a protective role for GPx-1 in ischemia/reperfusion as increased expres-

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sion of GPx-1 in transgenic mice decreases tissue damage after cerebral or myocardial ischemia/reperfusion. In this report, we examine the relationship between adenosine and GPx-1 in mediating cytoprotection against oxidant stress.

Materials and Methods

Chemicals and Reagents

Adenosine and EHNA [erythro-9-(2-hydroxy-3-nonyl)adenine] were obtained from Sigma. PD98059, SB203580, bisindolylmaleimide I (BIM), and LY294002 were obtained from Calbiochem. FuGENE 6 transfection reagent was obtained from Roche Molecular Biochemicals. Anti–human GPx-1 monoclonal antibody was purchased from Medical & Biological Laboratories Co, anti–β-actin antibody was obtained from Santa Cruz Biotechnology, and peroxidase-conjugated anti-mouse and anti-goat antibody were purchased from Sigma.

Cell Culture

Primary human pulmonary artery endothelial cells (HPAECs) were purchased from Cambrex and were used up to passage 10. Cells were grown in endothelial growth medium-2-MV (EGM-2-MV, Cambrex) to 90% to 95% confluence, and then treated with reagents as supplements to endothelial growth medium-2 (EGM-2, Clonetics), as indicated.

Northern Blot Analysis

HPAECs were grown to 90% confluence in EGM-2-MV medium in 60-mm dishes and treated with different reagents in the presence of EGM-2. Total RNA was extracted with Trizol (Invitrogen); 5 μg total RNA from each sample was fractionated on 1.2% formaldehyde-agarose gels and transferred onto GeneScreen Plus membranes. Human GPx-1 or glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA fragments were labeled with α-32PdCTP using a random priming kit (Roche Molecular Biochemicals), and hybridization was performed for 3 hours at 65°C in Quickhyb solution (Strategene), followed by serial washes for 15 minutes each in 0.1X SSC, 0.1% SDS at room temperature, 45°C, and 65°C. Membranes were stripped and rehybridized with the G3PDH probe. The quantitative analysis of hybridization signals was performed using ZeroDscan software or after scanning with a Versadoc imager. Analysis was performed with the QuantityOne software from Biorad.

To correct for gel loading, GPx-1 band densities were normalized to G3PDH band densities.

GPX-1 mRNA Stability

HPAECs were grown to 90% confluence in EGM-2-MV medium and then in EGM-2 medium with or without supplementation with 50 μmol/L adenosine/10 μmol/L EHNA for 48 hours. Actinomycin D at a concentration of 5 μg/mL was then added, and cells were harvested at 2, 4, 6, 9.5, and 11 hours. Total RNA was subjected to Northern blot analysis for GPx-1 expression.

GPx-1 Promoter Activity Analysis

A human GPx-1 promoter fragment (~580/+47) cloned into the pGL-3 basic vector (Promega) was used to assay promoter function. HPAECs were seeded into 12 well plates at a density of 5×10^3/mL in EGM-2-MV medium. The human GPx-1 promoter construct (0.5 μg) was mixed with 3 μL of FuGENE 6 and 25 ng renilla luciferase control reporter vector, pRL-CMV (Promega), for each well, and incubated with the cells for 6 hours. Medium was then replaced with fresh EGM-2 medium supplemented with various reagents. After 24 hours, cells were lysed, and the luciferase and renilla luciferase activities were measured with a luminometer (Turner Designs, TD-20e). Luciferase activity was corrected with renilla luciferase activity to normalize for transfection efficiency.

Western Blot Analysis of GPx-1

For determination of GPx-1 protein expression levels, HPAECs were lysed with ice-cold RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) supplemented with a protease inhibitor cocktail (Pierce Biotechnology). Protein samples (30 μg) were separated on 10% SDS-polyacrylamide gels (BioRad) and transferred to nitrocellulose membranes. The membranes were probed with 10 μg/mL monoclonal anti-GPx-1 antibody, followed by a 1:2000 dilution of peroxidase-conjugated anti-mouse IgG antibody (Sigma). Detection was accomplished with an ECL reagent (Amer sham). The membranes were then stripped and reprobed with 1 μg/mL anti-β-actin antibody.

Cellular GPx Activity Assay

HPAECs were grown on 100-mm dishes until 80% confluent. Cells were treated with reagents dissolved in EGM-2 for 2 days, with fresh medium and reagents added daily. Cellular extracts were prepared by sonication in ice-cold buffer (50 mmol/L Tris-HCl, pH 7.5, 5 mmol/L EDTA, and 1 mmol/L DTT). After sonication, lysed cells were centrifuged at 10,000g for 20 minutes, and GPx-1 activity was measured in the supernatant. Cellular GPx activity (nmol NADPH/min/mL) was measured using an assay kit (Calbiochem) that measures the coupled oxidation of NADPH during glutathione reductase (GR) recycling of oxidized glutathione from GPx-1-mediated reduction of t-butyl peroxide. In the assay, excess GR, glutathione, and NADPH were added. The activity was normalized to protein concentration.

Trypan Blue Exclusion

HPAECs were treated with or without 50 μmol/L adenosine/10 μmol/L EHNA for 48 hours. Cells were then transferred into 100-mm plates for an additional 4 hours of treatment with adenosine/EHNA and/or 10 mmol/L mercaptopropicosuccinate (MMA), an inhibitor of GPx-1. After removal of this media, cells were then treated with 500 μmol/L H2O2 for 2 hours, after which they were trypsinized and prepared for trypan blue exclusion. Briefly, cells were resuspended in HBSS buffer (Cambrex), mixed 1:1 with 0.4% trypan blue (Sigma), and incubated for 5 minutes, after which stained and unstained cells were counted separately with a hemocytometer. Cells stained blue were considered nonviable, whereas unstained cells were considered viable. The percentage of stained cells was used as an index of cell death.

Transfection With siRNA

Flasks (75 cm²) of nearly confluent HPAECs were transfected for 4.5 hours with a 10-mL solution of OptiMEM1 (Invitrogen) containing 36 nmol/L Stealth siRNA (Invitrogen) and 7.6 μL lipofectamine (Invitrogen). After transfection, cells were then incubated in 0.1X SSC, 0.1% SDS at room temperature, 45°C, and 65°C. Membranes were stripped and rehybridized with the G3PDH probe. The quantitative analysis of hybridization signals was performed using ZeroDscan software or after scanning with a Versadoc imager. Analysis was performed with the QuantityOne software from Biorad.
treat cells for 48 hours, after which cellular extracts were analyzed for GPx-1 protein and for GPx-1 enzyme activity. Western blot analysis showed that adenosine/EHNA increased GPx-1 protein levels 2.3/0.3-fold (P<0.05) (Figure 2A), under conditions where mRNA levels increased ~2-fold (P<0.02) (Figure 1A). There was no effect of EHNA or adenosine alone on GPx-1 protein expression (data not shown). Cellular GPx-1 activity was also increased 2.2/0.2-fold (P<0.05) (Figure 2B) with adenosine/EHNA treatment. Similar results were also observed in human coronary artery endothelial cells (data not shown).

Adenosine/EHNA Decreases GPx-1 mRNA Degradation
To determine the mechanism by which adenosine/EHNA up-regulates GPx-1 mRNA, the human GPx-1 promoter construct was transfected into HPAECs. After 24-hour treatment with adenosine, EHNA, or adenosine/EHNA, luciferase activity was measured (Figure 3A). Adenosine alone had no significant effect on luciferase activity. EHNA caused a small (18%) but significant (P<0.05) increase in luciferase activity. By contrast, adenosine/EHNA decreased the activity of the GPx-1 promoter by ~35% (P<0.005), indicating that the upregulation of mRNA levels is a not a result of enhanced GPx-1 gene transcription. In the presence of actinomycin D, GPx-1 mRNA levels decreased by almost 50% after 11 hours of treatment (Figure 3B). Pretreatment with adenosine/EHNA increased GPx-1 mRNA stability such that no change in GPx-1 mRNA was detected in this time frame. Longer treatments with actinomycin D appeared to cause cell death. These data suggest that adenosine/EHNA increased expression of GPx-1 by increasing GPx-1 mRNA stability and not by increasing GPx-1 gene transcription.

Signaling Pathways Mediating the Adenosine/EHNA-Dependent Induction of GPx-1
To study the signaling pathway involved in the induction of GPx-1 by adenosine, we performed Northern blot analysis of GPx-1 mRNA from nontreated and adenosine/EHNA-treated HPAECs in the presence or absence of inhibitors of mitogen-activated protein kinase (MAPK), protein kinase C...
Northern blot analysis showed that pretreatment with the PKC inhibitor BIM blocked GPx-1 induction by adenosine by 36% (P < 0.05), whereas the MAPK inhibitor SB203580 augmented GPx-1 induction by 38% (P < 0.05). These results indicate that adenosine-mediated GPx-1 induction is partially mediated by PKC-dependent pathways and suggest that MAPK pathways may suppress GPx-1 expression.

**Adenosine/EHNA Treatment Protects HPAECs From H₂O₂-Induced Cell Death**

To determine whether the increase in GPx-1 expression resulted in an increase in intracellular antioxidant capacity, adenosine/EHNA-treated and untreated cells were challenged with 500 μmol/L H₂O₂ for 2 hours and assayed for cell death by trypan blue exclusion (Figure 5). Cells not treated with adenosine/EHNA and exposed to H₂O₂ (Figure 5A and 5B) showed a significant, 2-fold increase in cell death (P < 0.05). By contrast, cells treated with adenosine/EHNA and exposed to H₂O₂ showed only a small, nonsignificant increase in cell death (Figure 5A and 5B). These data suggest that adenosine/EHNA treatment can improve the antioxidant capacity of endothelial cells. Pretreatment of cells with MSA, a GPx-1 inhibitor, led to an 8-fold increase in cell death in both untreated cells and cells treated with adenosine/EHNA, abolishing the protective effects of adenosine/EHNA treatment. To confirm that GPx-1 levels contribute to cellular protection against oxidants, siRNA was used to knockdown the expression of the enzyme, followed by adenosine/EHNA treatment and H₂O₂ exposure. siRNA transfection significantly increased cell death in response to H₂O₂ treatment (P < 0.0005), whereas adenosine/EHNA cells transfected with the scrambled 310 siRNA showed no significant increase in cell death in response to H₂O₂ treatment. Importantly, the level of cell death in the siRNA transfection after treatment with H₂O₂ is significantly greater than that measured in the control transfected treated with H₂O₂ (P < 0.02).

**Discussion**

In this study, we found that adenosine/EHNA can upregulate GPx-1 expression and activity by decreasing GPx-1 mRNA degradation in endothelial cells. Adenosine is released in small amounts at a constant basal rate during normoxia in myocardium. During ischemia, the release of adenosine can increase 50-fold, and, therefore, endogenously generated adenosine released during acute myocardial ischemia is likely sufficient to induce GPx-1 expression.

Ischemic preconditioning has two phases, an early phase and a delayed phase. The early phase involves receptor-mediated signaling events that include the opening of potassium channels, the activation of kinases, and, in endothelial cells, the activation of vasodilatory responses. It has been speculated that the delayed phase of preconditioning involves de novo synthesis of cardioprotective proteins and posttranslational protein modification. The data presented in this study show that adenosine

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**Figure 3.** Adenosine/EHNA increases GPx-1 mRNA stability. A, HPAECs cultured in 12-well plates were cotransfected with a GPx-1 promoter luciferase reporter construct and a renilla luciferase control reporter construct, pRL-CMV. After transfection, cells were treated with 50 μmol/L adenosine plus 10 μmol/L EHNA for 24 hours. Graph shows the ratio of luciferase/renilla activities in treated and untreated cells (n=36). *P<0.001 compared with adenosine/EHNA treatment; #P<0.05 compared with no treatment. B, HPAECs were grown with or without 50 μmol/L adenosine/10 μmol/L EHNA for 48 hours. Actinomycin D at a concentration of 5 μg/mL was then added, and the cells were harvested at 2 to 11 hours for GPx1 mRNA analysis. Total RNA was subjected to Northern blot analysis.

**Figure 4.** Adenosine/EHNA induction of GPx-1 mRNA involves PKC-dependent pathways. HPAECs were pretreated with the MAP kinase kinase (MEK) inhibitor PD98059 at 20 μmol/L, the p38 MAPK inhibitor SB203580 at 3 μmol/L, the PKC inhibitor BIM at 5 μmol/L, or the PI3K inhibitor LY294002 at 10 μmol/L, each for 30 minutes. After pretreatment, 50 μmol/L adenosine/10 μmol/L EHNA was added for 48 hours. Total RNA was subjected to Northern blot analysis. *P<0.05 compared with adenosine/EHNA treatment.
upregulates GPx-1 mRNA levels and that GPx-1 enzyme activity increases in parallel. This upregulation is the result of adenosine’s prolonging the half-life of the GPx-1 mRNA rather than facilitating gene transcription; the relatively long half-life of GPx-1 mRNA accounts for the response to adenosine requiring 24 hours of treatment.

GPx-1 expression and activity are enhanced by ROS and hyperoxia, and reduced by selenium depletion and TGF-β. The effects of ROS and hyperoxia have been shown to be transcriptional, whereas selenium alters mRNA stability and translation of this selenocysteine-containing protein. The unique mechanism of selenocysteine incorporation at a UGA codon involves several translational cofactors, including proteins that bind to the GPx-1 mRNA and stabilize interactions among the mRNA, a specific selenocysteine elongation factor, and a selenocysteine tRNA at the ribosome. The stability of the GPx-1 mRNA for these motifs and identified one site for nucleolin binding. Published reports have shown that nucleolin can be targeted by some PKC isoforms. Additional studies are necessary to determine the precise role of PKC, and nucleolin in GPx-1 expression.

BIM-1 treatment partially blocks the adenosine-mediated increase in GPx-1 mRNA. This pharmacological inhibitor was used because it is most effective in targeting all PKC-subtypes; however, it has also been found to interfere with the activity of glycogen synthase kinase. Adenosine-mediated signaling is known to result in PKC-activation in many different cell types including endothelial cells; therefore, in our cell system it is most likely that the adenosine-mediated increase in GPx-1 mRNA that is sensitive to BIM 1 treatment is mediated by PKC.

RNA turnover, in some transcripts, is influenced by specific protein binding to AU-rich elements in the 3′ untranslated region (UTR) of the mRNA followed by subsequent deadenylation and exonuclease degradation. Additional RNA binding factors, such as the hnRNP factors, which recognize UCCCA motifs, and nucleolin, which recognizes U/G CCG A/G motifs, can stabilize mRNA. We analyzed the sequence of the 3′ UTR of the GPx-1 mRNA for these motifs and identified one site for nucleolin binding. Published reports have shown that nucleolin can bind to the GPx-1 3′ UTR, and others have reported that nucleolin can be targeted by some PKC isoforms. Additional studies are necessary to determine the precise role of PKC, and nucleolin in GPx-1 expression.

Other factors may influence the expression and activity of GPx-1. Recent analysis has suggested that nitric oxide (NO) can posttranslationally modify selenocysteine, thereby inactivating GPx-1; however, this effect requires high levels of NO generated from NO-donors or LPS-activation of iNOS. Treatments with inhibitors, such as L-buthionine sulfoximine, that decrease glutathione (GSH) production also decrease GPx-1–mediated protection. Theoretically, oxidation of GSH to GSSG would also decrease GPx-1 activity. Increased GSSG levels have been measured after oxidant stress; however, these changes reflect GSH oxidation by GPx-1. Levels of reduced GSH are restored and kept virtually unchanged under most cellular conditions by the adaptive responses of glutathione reductase and other enzymes involved in GSH biosynthesis.

Importantly, in our studies, the increase in mRNA stability correlated with an increase in GPx-1 protein levels and a significant

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**Figure 5.** Adenosine/EHNA treatment protects HPAECs from hydrogen peroxide–induced cellular injury. A, HPAECs treated with or without 50 μmol/L adenosine/10 μmol/L EHNA for 2 days were seeded in 100-mm dishes for 4 hours in the presence or absence of 10 mmol/L mercaptosuccinate (MSA), a GPx-1 inhibitor, after which 500 μmol/L H2O2 was added for 2 hours. Cells were trypsinized, then incubated with trypan blue, and the percentage of blue cells was determined by counting cells on a hemocytometer. Graph shows the fold increase in dead cells under each condition relative to untreated cells. Baseline trypan blue staining in the untreated cells averaged 10.3% in 3 experiments. Overall, cell death from BMSA pretreatment followed by 500 μmol/L H2O2 was ~50% in both untreated and adenosine/EHNA–treated cells. B, Cells were mock transfected or transfected with siRNA 310 to knockdown GPx-1 expression or a control (scrambled) 310 siRNA, followed by no additional treatment or adenosine/EHNA treatment for 2 days, and then H2O2 treatment as above. Cell death was determined by counting cells on a hemocytometer. Graph shows the fold increase in dead cells under each condition relative to untreated cells. Baseline trypan blue staining in the untreated cells averaged 10.3% in 3 experiments. Overall, cell death from BMSA pretreatment followed by 500 μmol/L H2O2 was ~50% in both untreated and adenosine/EHNA–treated cells. B, Cells were mock transfected or transfected with siRNA 310 to knockdown GPx-1 expression or a control (scrambled) 310 siRNA, followed by no additional treatment or adenosine/EHNA treatment for 2 days, and then H2O2 treatment as above. Cell death was determined by counting cells on a hemocytometer. Graph shows the fold increase in dead cells under each condition relative to untreated cells. Baseline trypan blue staining in the untreated cells averaged 10.3% in 3 experiments. Overall, cell death from BMSA pretreatment followed by 500 μmol/L H2O2 was ~50% in both untreated and adenosine/EHNA–treated cells. B, Cells were mock transfected or transfected with siRNA 310 to knockdown GPx-1 expression or a control (scrambled) 310 siRNA, followed by no additional treatment or adenosine/EHNA treatment for 2 days, and then H2O2 treatment as above. Cell death was determined by counting cells on a hemocytometer. Graph shows the fold increase in dead cells under each condition relative to untreated cells. Baseline trypan blue staining in the untreated cells averaged 10.3% in 3 experiments. Overall, cell death from BMSA pretreatment followed by 500 μmol/L H2O2 was ~50% in both untreated and adenosine/EHNA–treated cells. B, Cells were mock transfected or transfected with siRNA 310 to knockdown GPx-1 expression or a control (scrambled) 310 siRNA, followed by no additional treatment or adenosine/EHNA treatment for 2 days, and then H2O2 treatment as above. Cell death was determined by counting cells on a hemocytometer. Graph shows the fold increase in dead cells under each condition relative to untreated cells. Baseline trypan blue staining in the untreated cells averaged 10.3% in 3 experiments. Overall, cell death from BMSA pretreatment followed by 500 μmol/L H2O2 was ~50% in both untreated and adenosine/EHNA–treated cells. B, Cells were mock transfected or transfected with siRNA 310 to knockdown GPx-1 expression or a control (scrambled) 310 siRNA, followed by no additional treatment or adenosine/
increase in GPx-1 enzyme activity. It is most likely that the increase in enzyme activity in this system is solely a result of an increase in protein levels as the magnitude of these changes is similar. Concurrent with the increase in GPx-1 levels and activity, there was an increase in protection against H₂O₂-induced cell damage. MSA, a pharmacological inhibitor of GPx-1, blocked protective effects of adenosine/EHNA treatment on endothelial cells; however, MSA may have other effects on the cells. Importantly, specific targeting of GPx-1 with siRNA that resulted in decreased levels of GPx-1 protein also abrogated the protective effects of adenosine/EHNA treatment on endothelial cells.

In endothelial cells, hydrogen peroxide detoxification is primarily mediated by GPx-1 as catalase is reportedly not expressed or expressed at very low levels in these cells. Our cell culture studies showed that decreasing the levels of GPx-1 increases sensitivity to oxidants, whereas increasing levels of GPx-1 decreases sensitivity to oxidants. These findings are in agreement with other cell culture studies that used transfection methods to overexpress GPx-1, resulting in a protection against oxidative stress. In vivo studies have found that a reduction of GPx-1 expression by ~50%, as in heterozygous murine knockout of GPx-1, is sufficient to cause endothelial dysfunction and oxidative stress. Other studies in transgenic and overexpressing mice illustrate the importance of GPx-1 expression to protect against oxidant injury during ischemia/reperfusion or direct oxidant exposure. For example, GPx-1 null mice showed increased infarct size and augmented neuronal sensitivity to apoptosis in a cerebral ischemia/reperfusion model, whereas GPx-1 overexpression protected against ischemia/reperfusion injury. Similarly, GPx-1–deficient mice have impaired cardiac function after ischemia/reperfusion injury and an increased susceptibility to oxidants, such as paraquat and H₂O₂, whereas GPx-1 overexpression protected against myocardial ischemia/reperfusion injury. In this current study, the novel finding is that adenosine can mediate increased expression of this antioxidant enzyme, which results in enhanced cellular tolerance to H₂O₂. Thus, we speculate that augmented activity of GPx-1 may contribute to the cardioprotective role of adenosine in cardiac ischemia/reperfusion and preconditioning, especially late preconditioning, by increasing cellular tolerance to oxidant stress. Extracellular adenosine mediates physiological responses via activation of G-protein–coupled adenosine receptors, or it is transported into the cells via nucleoside transporters where it is metabolized by adenosine deaminase or adenosine kinase. Adenosine deaminase is the major enzyme that catabolizes this nucleoside. Previous studies have shown that adenosine receptors are rapidly desensitized after continued adenosine treatment. In our study, adenosine was replenished in the media daily, and EHNA, an adenosine deaminase inhibitor, was included to increase the half-life of adenosine.

Many studies demonstrate that adenosine has a protective role against ischemia/reperfusion injury and can promote preconditioning for subsequent ischemia. In cultured cells, adenosine was found to activate the cellular antioxidant defense system by enhancing the activities of several antioxidant enzymes, including GPx-1; however, unlike our results, this effect was detected within 1 hour. This study also differs from our study in that cells were first treated with adenosine deaminase to remove endogenous levels of adenosine: this pretreatment actually significantly lowered the levels of the antioxidant enzymes including GPx-1. A single dose of an A₁-selective adenosine receptor agonist 24 hours before coronary artery occlusion in rabbits led to protection from ischemic injury, suggesting a delayed, but long-acting, effect of adenosine receptor activation.

It appears that adenosine enhances GPx-1 expression partially though a PKC-dependent mechanism. By use of specific receptor agonists, both the A₁ and A₂B subtypes have been associated with protective preconditioning in the myocardium, whereas A₂A receptors have been shown to promote the vasodilatory effects of endothelial cells. It has been established that PKC is an obligatory mediator of delayed ischemic preconditioning, and A₁, A₂B, and A₂A have all been associated with mediating cytoprotection via PKC-dependent pathways. Owing to the timeframe for the upregulation of GPx-1 expression and the ability of the PKC inhibitor to block this increase, our findings suggest that adenosine-mediated increases in GPx-1 levels may play a role in delayed or late-phase ischemic preconditioning.

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