Gene Transfer of Extracellular Superoxide Dismutase Reduces Arterial Pressure in Spontaneously Hypertensive Rats

Role of Heparin-Binding Domain

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Abstract—Oxidative stress may contribute to hypertension. The goals of this study were to determine whether extracellular superoxide dismutase (ECSOD) reduces arterial pressure in spontaneously hypertensive rats (SHR) and whether its heparin-binding domain (HBD), which is responsible for cellular binding, is necessary for the function of ECSOD. Three days after intravenous injection of an adenoviral vector expressing human ECSOD (AdECSOD), mean arterial pressure (MAP) decreased from 165±4 mm Hg (mean±SE, n=7) to 124±3 mm Hg (n=7) in adult anesthetized SHR (P<0.01) but was not altered in normotensive Wistar-Kyoto rats. Cardiac output was not changed in SHR 3 days after AdECSOD. Gene transfer of ECSOD with deletion of the HBD (AdECSODΔHBD) had no effect on SHR MAP, even though plasma SOD activity was greater after AdECSODΔHBD than after AdECSOD. Immunohistochemistry revealed intense staining for ECSOD in blood vessels and kidneys after AdECSOD but not after AdECSODΔHBD. Impaired relaxation of the carotid artery to acetylcholine in SHR was significantly improved after AdECSOD. Cumulative sodium balance in SHR was reduced by AdECSOD compared with AdECSODΔHBD. Gene transfer of ECSOD also reduced MAP in conscious SHR, although the effect was not as profound as in anesthetized SHR. In summary, gene transfer of ECSOD, with a strict requirement for its HBD, reduces systemic vascular resistance and arterial pressure in a genetic model of hypertension. This reduction in arterial pressure may be mediated by vasomotor and/or renal mechanisms.

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Key Words: hypertension ■ gene therapy ■ oxidative stress ■ superoxide ■ spontaneously hypertensive rats

Increased levels of reactive oxygen species, particularly superoxide, have been demonstrated in blood vessels and in several tissues in experimental models of hypertension, including spontaneously hypertensive rats (SHR), Dahl salt-sensitive rats, hypertension induced by angiotensin II, and hypertension associated with obesity. Increased superoxide may contribute to hypertension by inactivation of vascular NO and by formation of peroxynitrite, which may produce further impairment of vasorelaxation. Thus, reduction of superoxide levels might be expected to attenuate hypertension.

In some but not all studies, antioxidants appear to attenuate hypertension. The explanation for variability in the effects of antioxidants on blood pressure is not clear, but this variability may be due to several factors, including differences in cellular binding and/or cell permeability of the antioxidants. For example, copper/zinc superoxide dismutase (CuZnSOD) protein given intravenously, which does not bind to cells and is not cell permeable, does not reduce blood pressure in SHR, in angiotensin II–induced hypertensive rats, or in patients with essential hypertension. In contrast, CuZnSOD protein, with fusion of a heparin-binding domain (HBD), which is able to bind to cells, or in cell-permeable liposomes, and tempol (a cell-permeable SOD mimetic) reduce blood pressure in experimental animals with hypertension.

Extracellular SOD (ECSOD), which is the only isoform of SOD that is expressed extracellularly, binds to tissues. There has been no study that has examined the effects of ECSOD on arterial pressure in experimental hypertension, and no study has examined the effects of gene transfer of an antioxidant enzyme on arterial pressure in a model of hypertension. The first goal of the present study was to examine the effects of gene transfer of ECSOD on arterial pressure in anesthetized and conscious SHR.

ECSOD contains a positively charged HBD, which mediates the binding of ECSOD to cells and the interstitium. ECSOD differs from CuZnSOD in that ECSOD is a glycosylated high molecular weight homotetramer (155
performed to examine the effects of gene transfer of ECSOD on superoxide and vasomotor function and on sodium balance in SHR.

Materials and Methods

Animals
Male SHR and Wistar-Kyoto (WKY) rats (332 to 346 g, ~20 weeks old, from Harlan, Indianapolis, Ind) were used. Procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH) and approved by the Animal Care and Use Committee of the University of Iowa.

Recombinant Adenoviral Vectors
Replication-deficient adenoviruses that express human ECSOD (Ad-ECSOD) and ECSOD with deletion of the HBD (AdECSODΔHBD), both driven by the human CMV promoter/enhancer, were constructed (please see online data supplement, available at http://www.circresaha.org) using standard procedures. The ratio of particle to plaque-forming unit was ~50:1 in all preparations used in the present study except in AdECSOD used in conscious SHR, in which the ratio was 100:1.

In Vivo Gene Transfer to SHR and WKY Rats
Rats were anesthetized intraperitoneally with methohexital sodium (50 mg/kg). Adenovirus (1 × 1012 particles/mL in 3% sucrose in PBS, vehicle) or vehicle was injected into the penile vein. The usual dose of adenovirus was 0.5 mL (5 × 1011 particles). Rats began to awaken within half an hour after injection. Plasma ECSOD proteins were identified by zymography (see online data supplement).

Measurement of MAP
Mean arterial pressure (MAP) was measured in 6 groups (online data supplement). Rats were anesthetized with sodium pentobarbital (50 mg/kg IP) 3 days after injection. A tracheostomy was performed, and the rats were ventilated. The femoral artery was cannulated, and arterial pressure was recorded directly. After 30 to 60 minutes of equilibration, MAP value was taken as an average of a 30- to 60-minute recording. Body temperature and blood gases were monitored and were normal in all groups. No heparin was used during the recording of arterial pressure.

To study the effects of gene transfer on MAP in conscious rats, anesthetized SHR and WKY were instrumented with an indwelling catheter in the aorta, which was inserted through the femoral artery. Two days after insertion of the catheter, we injected AdECSOD or Adβgal as virus control. MAP was then measured once daily (7:00 AM to 12:00 PM) for 3 or 4 days, and values were obtained as an average of the last 15 minutes of a 30- to 60-minute recording.

Echocardiography
Echocardiography was performed on age-matched SHR (n=7) and WKY rats (n=10) before and 3 days after AdECSOD (online data supplement).

Immunohistochemistry
Immunostaining for ECSOD and ECSODΔHBD is described in the online data supplement. We found that it is important to avoid using biotin-avidin techniques because endogenous biotin produced nonspecific staining. The antibody detected ECSOD and ECSODΔHBD equally well by immunoblotting (data not shown).

Vasomotor Function and Detection of Superoxide With Lucigenin
Vasomotor function of carotid arteries was measured as described in the online data supplement. Levels of superoxide were obtained by the lucigenin method, as previously described.
Detection of Nitrotyrosine by Immunoblotting
Levels of nitrotyrosine in protein extracts of the aorta and kidney were detected by immunoblotting and quantified by densitometry (online data supplement). Data were presented, with the density of nitrotyrosine in SHR after AdECSODΔHBD set at 100%.

Sodium Balance
Male SHR were placed in individual metabolic balance cages, and daily measurements were made after 1 week of adaptation\(^1\) (online data supplement).

Statistical Analysis
A Student \(t\) test (unpaired) was used to compare MAP in treated versus control SHR and WKY rats and cardiac output in SHR and WKY rats before versus after AdECSOD. ANOVA with repeated measures and the Scheffé test were used for comparison in the vasomotor function and sodium balance studies. The significance level was set at \(P<0.05\) (2-sided). All values are presented as mean±SE.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

Effects of Gene Transfer of ECSOD and ECSODΔHBD
SOD activity of the transgene products, ECSOD and ECSODΔHBD, was observed in the culture medium of A549 cells (not shown) and in the plasma of rats 3 days after intravenous injection of AdECSOD and AdECSODΔHBD (\(5\times10^11\) particles) (Figure 1B). Because there is a deletion of 13 amino acid residues (per monomer) at the carboxy terminal in ECSODΔHBD (Figure 1A), its mobility is faster than that in intact ECSOD. Vehicle or Adβgal did not produce an ECSOD band in plasma from SHR or WKY rats. AdECSODΔHBD produced a more intense band than did AdECSOD in both SHR and WKY rats, probably because ECSOD, which binds to cells, was sequestered in tissues. Human ECSOD with and without the HBD moved more slowly than endogenous rat ECSOD (a homodimer), suggesting that the transgene products are homotetramers, as expected.

Both ECSOD and ECSODΔHBD produced in vitro and in vivo bind to concanavalin A (data not shown), which indicates that the proteins are glycosylated, as expected. Thus, recombinant adenoviruses likely produce the native forms of ECSOD, a glycosylated homotetramer with and without the HBD, respectively. Both forms of ECSOD have expected enzymatic activity as demonstrated in SOD activity gel electrophoresis, which is consistent with a previous study on mutants of human ECSOD.\(^1\)

Effects of Gene Transfer of ECSOD and ECSODΔHBD on Arterial Pressure in Anesthetized SHR and WKY Rats
In WKY rats, ECSOD had no significant effect on arterial pressure 3 days after intravenous injection of AdECSOD (\(5\times10^11\) particles), MAP was 57 mm higher in SHR than in WKY rats, and it decreased by 72% (41 mm Hg), approaching that of normotensive WKY rats, after AdECSOD (\(P<0.01\), Figure 2). Injection of a control virus (Adβgal) had no effect on MAP in SHR (Figure 2).

Gene transfer of AdECSODΔHBD (\(5\times10^11\) particles) did not alter MAP in SHR (Figure 2). Thus, the HBD is necessary for ECSOD to reduce blood pressure in SHR.

Doses of AdECSOD 10 or 50 times lower than the dose described above also reduced MAP in SHR (139±6 mm Hg, \(n=7\); 26 mm Hg lower than that in SHR). Thus, gene transfer of ECSOD reduced arterial pressure in adult anesthetized SHR in a dose-dependent manner.

Six days after gene transfer of ECSOD (\(5\times10^11\) particles), MAP in adult SHR tended to be lower (155±5 mm Hg, \(n=5\); \(P=0.07\)) than that in untreated SHR. After 6 days, ECSOD activity was lower in plasma than it was 3 days after gene transfer\(^2\) (data not shown). Plasma ECSOD activity remained detectable by SOD activity gel electrophoresis for \(\approx10\) days after AdECSOD or AdECSODΔHBD (data not shown).

Because the major target after intravenous injection of adenovirus is the liver,\(^2\) liver function was examined. Three days after AdECSOD (\(5\times10^11\) particles), plasma alanine aminotransferase (35±12 U/L, \(n=4\)) was not significantly different from that in untreated SHR (45±14 U/L, \(n=4\)). In addition, histological examination of the liver did not reveal gross differences in rats after AdECSOD compared with untreated rats. Thus, intravenous injection of AdECSOD did not produce detectable acute hepatotoxicity.
Effect of Gene Transfer of ECSOD on Cardiac Output

Cardiac output, assessed by echocardiography, was not altered in WKY rats \((P=0.85)\) or SHR \((P=0.58)\) 3 days after AdECSOD \((5\times10^11\) particles\) (Figure 3). Therefore, reduction of arterial pressure by ECSOD gene transfer in SHR is not produced by a decrease in cardiac output and thus results from a decrease in systemic vascular resistance.

Effects of Gene Transfer of ECSOD and ECSOD\(\Delta\)HBD on Blood Vessels and Kidneys

Immunostaining for ECSOD was performed in SHR after AdECSOD and AdECSOD\(\Delta\)HBD to confirm differential tissue affinity based on the HBD status. There was intense staining for ECSOD in endothelium and less intense but positive staining in the media of the carotid artery and aorta after AdECSOD, with no detectable staining after AdECSOD\(\Delta\)HBD (Figure 4). There was intense staining for ECSOD in renal glomeruli, large preglomerular vessels, and medullary papillary interstitium in all sections (Figure 4), with staining in macula densa in some but not all sections (not shown) after AdECSOD but no detectable staining after AdECSOD\(\Delta\)HBD. In contrast, staining for ECSOD was similarly intense in the liver after AdECSOD and AdECSOD\(\Delta\)HBD (Figure 4), which suggests that transduction efficiencies of the 2 viruses and the reactivity of the antibody to the 2 forms of ECSOD are both similar, as expected.

Vasomotor responses were compared in SHR after gene transfer of ECSOD and ECSOD\(\Delta\)HBD, with Ad\(\beta\)gal as an additional virus control. Contraction of the carotid artery to phenylephrine and relaxation to sodium nitroprusside were not different in WKY rats and in SHR after the viruses (Figures 5A and 5C). Relaxation of the carotid artery to acetylcholine was impaired in SHR after Ad\(\beta\)gal or AdECSOD\(\Delta\)HBD and was significantly improved after AdECSOD (Figure 5B). These findings suggest that gene transfer of ECSOD, but not ECSOD\(\Delta\)HBD, improves endothelial function in SHR.

Levels of superoxide were examined in carotid arteries from SHR and WKY rats. Levels of superoxide in SHR after Ad\(\beta\)gal were \(\approx 2\)-fold higher than levels in WKY rats. AdECSOD reduced the levels of superoxide in SHR to the levels in WKY rats, whereas AdECSOD did not reduce the levels of superoxide further in WKY rats (Figure 5D). These findings are consistent with previous studies,\(^2,3\) in which

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**Figure 4.** Immunostaining for ECSOD in carotid artery, kidney, and liver of SHR 3 days after intravenous injection of AdECSOD or AdECSOD\(\Delta\)HBD \((5\times10^11\) particles per rat\) in left panels, endothelium is at top. In kidney, staining (brown color) was observed in glomeruli but was not observed in other parts of the kidney (except for preglomerular vessels and macula densa, in which staining was found in some but not all sections). Staining was not observed in any samples from untreated SHR or when anti-ECSOD antibody was not used. These findings are representative of sections from 3 different animals for each treatment.

**Figure 5.** Effects of gene transfer on vasomotor responses (A through C) and levels of superoxide (D) in carotid arteries. For panels A through C, *\(P<0.05\) for SHR treated with AdECSOD \((n=7)\) or WKY rats \((n=5)\) vs Ad\(\beta\)gal-treated \((n=7)\) or AdECSOD\(\Delta\)HBD-treated \((n=7)\) SHR; **\(P<0.05\) for AdECSOD-treated SHR vs WKY rats \((n=4)\). For panel D, *\(P<0.05\) for SHR treated with Ad\(\beta\)gal \((n=8)\) vs SHR treated with AdECSOD \((n=8)\). Values are also for WKY rats \((n=5)\) and WKY rats treated with AdECSOD \((n=5)\).
vascular levels of superoxide were found to be increased in SHR compared with WKY rats. These findings suggest that gene transfer of ECSOD improves endothelial function by reducing vascular levels of superoxide.

Nitrotyrosine, which can be produced by peroxynitrite, the product of the reaction of NO and superoxide, was measured in SHR and WKY rats after AdECSOD and AdECSOD/H9004HBD. With the nitrotyrosine density in SHR after AdECSOD/H9004HBD set at 100%, levels of nitrotyrosine were 64% lower in kidney protein extract (36±2%, n=3) and 40% lower in aorta protein extract (60±6%, n=3) in SHR after AdECSOD than after AdECSOD/H9004HBD (100±2%, n=3 for kidney; 100±17%, n=3 for aorta). Levels of nitrotyrosine in WKY rats were 21±3% (n=3, kidney) and 39±4% (n=3, aorta), respectively. The findings suggest that cell-bound ECSOD may reduce tissue levels of superoxide and the formation of peroxynitrite and, thus, protect NO from degradation.

Sodium Balance in SHR
We tested the hypothesis that reduction of arterial pressure after gene transfer of ECSOD is due in part to a renal mechanism. The cumulative daily sodium balance was significantly reduced after AdECSOD compared with after AdECSOD/H9004HBD, starting 2 days after injection of the virus and continuing for the duration of the study (Figure 6). The finding suggests that a renal action is associated with reduction of arterial pressure in SHR after AdECSOD.

Effect of Gene Transfer of ECSOD on Arterial Pressure of Conscious SHR
To test whether ECSOD reduces arterial pressure in conscious SHR, rats were instrumented with an indwelling catheter in the aorta. MAP was measured at approximately the same time of day at baseline (the day of injection) and at 3 days after injection of the virus. MAP was 55 mm Hg higher in SHR than in WKY rats (120±2, n=5) and decreased by 27% (15 mm Hg), approaching that in WKY rats, after AdECSOD (Figure 7). Injection of Adβgal did not alter arterial pressure in conscious SHR. There was no significant change in heart rate (from 405±16 to 380±14 bpm for SHR after AdECSOD) or body weight (from 322±4 to 313±4 g for SHR after AdECSOD) in any group.

Discussion
The major findings of the present study are as follows: (1) gene transfer of ECSOD greatly reduces arterial pressure in anesthetized SHR by reducing systemic vascular resistance; (2) the HBD is necessary for the antihypertensive effect of ECSOD; (3) the antihypertensive effect of ECSOD, but not ECSOD/H9004HBD, is associated with improved vasomotor responses to acetylcholine and reduced vascular levels of superoxide and nitrotyrosine; (4) a renal effect, with reduced sodium balance and reduced renal levels of nitrotyrosine, may contribute to the reduction of arterial pressure by ECSOD in SHR; and (5) gene transfer of ECSOD reduces arterial pressure in conscious SHR, although the effect is not as profound as in anesthetized SHR.

ECSOD and Hypertension: Role of HBD
Construction of AdECSOD/H9004HBD and comparison with AdECSOD allowed us to directly study the role of the HBD in the effects of ECSOD. The role of the HBD in hypertension (SHR) has been examined previously in a study of recombinant CuZnSOD.2 Intravenous injection of recombinant CuZnSOD protein with fusion of an HBD (HB-CuZnSOD, 25 mg/kg) reduced the systolic blood pressure of SHR by ~50 mm Hg (tail-cuff measurement in conscious rats). The present study differs from the previous study2 in important ways. First, the previous study fused an HBD to CuZnSOD in which (in contrast to the glycosylated tetrameric ECSOD in the present study) the fusion protein was a nonglycosylated...
dimeric synthetic CuZnSOD. The protein, containing 2 HBDS, which is similar to rat ECSOD, presumably has much lower affinity for heparan sulfate than the tetrameric ECSOD (form C), which contains 4 HBDS. Second, because we were able to obtain a reduction in arterial pressure for several days (in contrast to 40 minutes with the synthetic protein), it was possible to study vascular and renal mechanisms that account for the effect of ECSOD. Despite these differences, both studies indicate in a complementary manner that the HBD is necessary for SOD to reduce blood pressure in SHR. These findings may provide an explanation for the failure of intravenous administration of CuZnSOD protein (which does not have an HBD) to hypertensive patients to reduce arterial pressure. Indeed, injection of AdCuZnSOD did not alter arterial pressure in 2 SHR, as expected (data not presented).

The short duration of the effect of synthetic CuZnSOD (HB-CuZnSOD) on arterial pressure may have resulted from rapid clearance of the low molecular weight protein by renal filtration along with its lower affinity to heparan sulfate than the tetrameric ECSOD (form C). In contrast, the larger molecular weight glycosylated ECSOD has a much longer plasma half-life (18 hours) than CuZnSOD (4 minutes) or HB-CuZnSOD (1 and 8 minutes for the fast and slow phases, respectively). Modification at the protein level may prolong the half-life and enhance the targeting of SOD to vascular cells. In the present study, gene transfer effectively increased the duration of effect of ECSOD on arterial pressure, with a peak effect at 3 days and return to baseline pressure at 1 week.

An R213G polymorphism within the HBD has been described in normal humans, in whom there was a 10- to 20-fold increase in serum ECSOD activity, with decreased affinity of ECSOD for heparin and for tissues. These findings are analogous to our findings with AdECSODΔHBD. Subjects with this polymorphism did not have a significant increase in arterial pressure. Limitations of the study are that the number of subjects with the polymorphism is relatively low for a population study and that almost all of the subjects were heterozygotes.

In addition, arterial pressure is normal in ecso-d null mice at baseline. In ecso-d null mice, however, hypotension in response to an NO donor was attenuated, and the increase in arterial pressure in response to an NO synthase inhibitor was augmented. After considering these findings together, we speculate that ECSOD may be antihypertensive only when hypertension results from stimuli that reduce the levels of NO but not at (normotensive) baseline. It is not yet clear whether the HBD of ECSOD plays a role in hypertension in humans. Nevertheless, the present study strongly supports the hypothesis that intact, but not the HBD-deleted, ECSOD effectively reduces arterial pressure in established hypertension in SHR.

The findings with ECSODΔHBD may have broader clinical implications than in hypertension alone. For example, a clinical trial using CuZnSOD protein failed to demonstrate a reduction of infarction after myocardial ischemia and reperfusion. Although there are many possible explanations for the negative study, we speculate that the findings may have resulted in part from lack of an HBD in the SOD. Consistent with this hypothesis are the findings that overexpression of ECSOD preserved myocardial function and reduced infarct size after myocardial ischemia and reperfusion.

Mechanisms for Antihypertensive Effect of ECSOD in SHR

Echocardiography indicated that AdECSOD does not reduce cardiac output in SHR. Thus, the reduction of arterial pressure in SHR by gene transfer of ECSOD is the result of a decrease in systemic vascular resistance. We considered 2 mechanisms (ie, vascular and renal) by which ECSOD may reduce vascular resistance and arterial pressure in SHR. Both mechanisms could potentially involve an increase in bioavailability of NO after ECSOD that is due to less inactivation of NO by superoxide.

We examined the effects of ECSOD and ECSODΔHBD on vasomotor function. ECSOD immunostaining was intense in endothelial cells of the aorta and carotid arteries after AdECSOD, with no detectable staining after AdECSODΔHBD. Relaxation of the carotid artery in response to acetylcholine was impaired in SHR after control virus and was significantly augmented after AdECSOD but not AdECSODΔHBD. ECSOD improved vasorelaxation in SHR to the level observed in normotensive WKY rats (except at the highest concentrations of acetylcholine tested). This finding is consistent with a recent study in which local gene transfer of ECSOD to carotid arteries reversed endothelial dysfunction in stroke-prone SHR.

Furthermore, we found that gene transfer of ECSOD reduced the increased level of superoxide in vessels of SHR to that in WKY vessels. In addition, we found a 40% reduction of nitrotyrosine, a marker for peroxynitrite, in the aorta after AdECSOD compared with AdECSODΔHBD. Taken together, these findings provide evidence that improved vascular function, through reduction of vascular superoxide by ECSOD but not ECSODΔHBD, may contribute to the reduction of vascular resistance and arterial pressure after the gene transfer of ECSOD.

A second mechanism for the antihypertensive effect of ECSOD in SHR may involve a renal action. Superoxide levels in the kidneys are increased in SHR compared with WKY rats, and treatment with tempol increases renal blood flow, specifically medullary blood flow, and the glomerular filtration rate through the reduction of superoxide and consequent increase of NO bioavailability. Reduced renal blood flow and glomerular filtration rate are associated with increased renal sodium retention in SHR compared with WKY rats. In the present study, we found that there is a significant reduction in cumulative sodium balance starting 2 days after the injection of AdECSOD and continuing for the duration of the study compared with AdECSODΔHBD. This effect is associated with intense staining for ECSOD in glomeruli and preglomerular vessels and with variable staining in the macula densa (but not postglomerular vessels or tubules) in the kidney after gene transfer of ECSOD but not ECSODΔHBD. We also found a 64% reduction in nitrotyrosine levels in the kidney after AdECSOD compared with AdECSODΔHBD. These findings strongly suggest renal involvement in the effect of ECSOD on arterial pressure in SHR.
Gene Therapy for Hypertension
Gene transfer of a variety of vasodilators or antisense to vasoconstrictors, generally in neonatal or young animals, has been reported to reduce arterial pressure in SHR and other models of hypertension. The present study demonstrates a reduction in MAP in anesthetized and conscious SHR after a single injection, which strongly supports an important role for increased superoxide in the pathophysiology of hypertension in SHR. Implications of the present study include the following: (1) gene transfer may prolong antihypertensive effects of a gene such as ecsod, and (2) intravenous injection is an excellent route of gene transfer when a secreted protein is the gene product, because transduction of hepatocytes is efficient, and there is effective production of the gene product by the transduced, metabolically active cells.

Antioxidants, including the SOD mimetic tempol, vitamin E, and vitamin C have not proven to be effective in the treatment of hypertension. This implies that ECSOD may be an excellent agent for gene therapy for hypertension.

Some major obstacles to the possibility of gene therapy for hypertension, based on the present approach, are as follows: (1) the inflammatory response to the adenoviral vector will prevent clinical application using the present vector, (2) there is a short duration of the effect of gene transfer of ECSOD (which peaks at 3 days and wanes in 10 days), and (3) repeat treatment is not possible with an adenoviral vector. By applying nonviral or other viral (eg, adeno-associated virus) vectors, the antihypertensive effect of gene transfer of ECSOD may be prolonged, and treatment may be repeatable.

Conclusions
The present study provides the first evidence that ECSOD reduces arterial pressure in a model of hypertension. This effect is mediated by a reduction in systemic vascular resistance and is associated with an improved vascular response to acetylcholine and a reduction in renal sodium retention. The HBD is necessary for the effect of ECSOD. We speculate that gene therapy with ECSOD has the potential to be a new treatment for hypertension and possibly for other cardiovascular diseases that are associated with oxidative stress.

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