Gene transfer of endothelial nitric oxide synthase but not Cu/Zn superoxide dismutase restores nitric oxide availability in the SHRSP

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Abstract

Objective: Previous studies from our group have shown a deficit in nitric oxide (NO) bioavailability and an excess production of the superoxide anion (O$_2^-$) in the stroke prone spontaneously hypertensive rat (SHRSP) compared to the normotensive Wistar Kyoto (WKY) strain. This present study has investigated whether adenoviral-mediated gene transfer of human eNOS or Cu/ZnSOD can alter the NO/O$_2^-$ balance, thereby improving endothelial function. Methods: A recombinant adenovirus, Ad/Hu/eNOS, containing the human eNOS cDNA fragment was generated by homologous recombination in 293 cells. Ad/Hu/eNOS or Ad/Cu/ZnSOD was delivered into SHRSP carotid arteries in vivo, using a titre of $2 \times 10^9$ to $2 \times 10^{10}$ plaque forming units (pfu)/ml, and the effect on gene expression was observed 24 h later. Results: Western blotting confirmed increased enzyme levels of eNOS and Cu/ZnSOD in the viral-infused vessels. Ex vivo, the pressor response to phenylephrine (PE) in the presence of L-NAME was increased in the eNOS-infused arteries relative to the contralateral controls, indicating restoration of basal NO availability to that observed in untreated control WKY rats. Infusion of the SOD virus produced a statistically insignificant increase in NO bioavailability. Conclusions: Our results support our previous findings obtained using a bovine eNOS recombinant adenovirus, that recombinant adenoviral gene transfer of human eNOS has a significant effect on NO bioavailability. In contrast, AdCu/ZnSOD gene transfer does not elicit an effect in our model. These results indicate that short-term overexpression of a recombinant eNOS, but not Cu/ZnSOD gene, in carotid arteries of the SHRSP is an effective means of locally increasing NO bioavailability to improve endothelial function.

Keywords: Endothelial function; Free radicals; Gene therapy; Gene expression; Hypertension; Nitric oxide; Vasoconstriction/dilation.

1. Introduction

The endothelium is an important organ involved in the control of vascular homeostasis through its ability to produce a range of factors which induce contraction and relaxation [1]. Endothelial dysfunction is the term used to describe the impaired endothelium-mediated vasodilation which occurs when the balance of these factors is disrupted. It is a common characteristic of patients with essential hypertension [2,3] and of many animal models of hypertension, where it is thought that the main contributing factor is a deficit in NO availability [4,5]. Recent studies in angiotension II-induced hypertension [6] and in the stroke-prone spontaneously hypertensive rat (SHRSP) [7,8], suggest that an increased production of the superoxide anion (O$_2^-$) could play a role in the reduced bioavailability of nitric oxide (NO) in these models of hypertension. Increased production of O$_2^-$ may also contribute to decreased NO bioavailability in the SHR [9], hypercholesterolemic rabbits [10] and diabetic rats [11]. Endothelial NOS is a 140-kDa protein and its activity accounts for endothelium-dependent vasorelaxation by catalysing the conversion of arginine to citrulline with the release of NO and consequently is implicated in blood pressure regulation [1]. The deficit observed in the SHRSP may come about through a reduction in its production, which is unlikely, as
it has been shown by northern and western analysis that eNOS mRNA and protein levels are in fact increased [4]. Alternatively, the reduction in bioavailability is more likely to be caused by an increased degradation, brought about by the excess O$_2^-$ observed in this model. Since NO production has both beneficial and detrimental consequences, understanding the molecular mechanisms that regulate NOS expression is critical to the control of NO release in physiological and pathophysiological conditions.

Cu/ZnSOD is an enzyme that dismutates O$_2^-$ into oxygen and hydrogen peroxide, thereby providing a protective effect on NO [12]. The scavenging of O$_2^-$ by SOD competes with NO for O$_2^-$ [13] and plays an important role in the regulation of NO bioavailability. One mechanism to regulate endothelial function may be to manipulate the balance between the generation of O$_2^-$ and NO. However, it is not clear how much O$_2^-$ contributes to modulating vascular tone and therefore in the present study, we propose to overexpress the SOD enzyme to scavenge O$_2^-$ in the vasculature and investigate whether gene transfer of eNOS or Cu/ZnSOD could increase the bioavailability of NO, thereby improving endothelial function. NOS gene transfer has been used efficiently to transduce cells of the vasculature in various models of endothelial dysfunction, including hypertension [14], atherosclerosis [15±17], arterial vasospasm [18], vein graft failure [19], and coronary artery restenosis [20]. In this study, the effect of adenovirus-mediated gene transfer of the human eNOS and Cu/ZnSOD genes to the SHRSP rat carotid artery is examined.

2. Methods

2.1. Construction of the shuttle plasmid and generation of recombinant adenovirus

The cDNA for human eNOS (Accession no. M95296) was a generous gift from Dr P.A. Marsden (University of Toronto). The human eNOS cDNA was cloned into the EcoRI site of a shuttle plasmid pCA13 (Microbix Biosystems Inc., Canada). The resulting plasmid pCA13/human/eNOS was co-transfected with a plasmid containing the entire adenovirus genome, namely, pJM17 (Microbix) into HEK293 cells (human embryo kidney cells expressing the E1 region of Ad2) [21], by conventional calcium phosphate precipitation [22] and AdCMVeNOS was obtained by in vitro homologous recombination. Individual Ad/Hu/eNOS plaques were expanded in 293 cells [24] and viral DNA was isolated as previously described [23] followed by restriction analysis, Southern blotting and PCR using primers specific for the human eNOS and SOD cDNAs (Table 1) [25]. Before use, plaque isolates were evaluated for their potential to overexpress NOS activity in endothelial cells using the Griess assay [26].

The human Cu/ZnSOD viral vector was purchased from the Gene Transfer Core Group in University of Iowa. Infectious titre of all viral stocks were determined by duplicate plaque assays on 293 cells using standard techniques [22].

2.2. Experimental animals and rat aortic endothelial cell culture

Stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar Kyoto (WKY) rats were maintained as previously described [27] with systolic blood pressures measured by tail-cuff plethysmography according to a standard protocol [27]. Aortae were harvested from male rats and primary cultures of aortic endothelial cells were prepared, characterised, maintained and infected with virus as previously described [23]. For in vivo studies, SHRSP males at 12 weeks of age (mean systolic blood pressure (BP) of 165.25±2.53 mmHg) and WKY males of similar age (mean systolic BP of 127±2.31 mmHg) were used.

2.3. Carotid artery gene transfer in vivo

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication No 85-23, revised 1996) and carried out under the project licence from the UK Home Office. Rats were anaesthetized using halothane and carotid arteries were infused with 30 µl viral lysate, Ad/Hu/eNOS or Ad/Cu/ZnSOD at a titre of 2×10$^9$–2×10$^{10}$ pfu/ml [23]. Three groups of animals were used for isometric tension recording ($n$=24), seven SHRSP infused with the Ad/Hu/eNOS ($2×10^9$ pfu/ml); eight SHRSP infused with AdCu/ZnSOD ($2×10^9$ pfu/ml) while three SHRSP were infused with AdCu/ZnSOD at a titre of $2×10^{10}$ pfu/ml. Six WKY rats received infusions of Ad/Hu/eNOS ($2×10^9$ pfu/ml). The right contra-lateral carotid arteries from these animals were used as controls. At 24 h

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*Position relative to transcription start site.
after infusion, rats were killed by an overdose of halothane and the carotid arteries were removed. Additional carotid arteries from virus-infused animals were frozen in liquid nitrogen for western blot analysis, or taken for measurement of O$_2^-$ levels.

2.4. Localisation of superoxide using hydroethidine

In situ localisation of O$_2^-$ in the SHRSP and WKY animals was investigated using hydroethidine (HEt), a dye which, in the presence of O$_2^-$ is oxidised to ethidium bromide. Fluorescence can then be assessed microscopically after exciting at a 585-nm wavelength [28,29]. HEt was made up in dimethylsulphoxide (DMSO) as a 2 × 10$^{-4}$ M solution and diluted to 2 × 10$^{-6}$ M in PBS before use. The carotid arteries were removed from the experimental animals and embedded in OCT and 20-μm thick sections were placed on coverslips and the dye topically applied. The sections were then incubated at 37°C for 30 min before visualising the fluorescence under a microscope. Photomicrographs of both SHRSP and WKY animals were taken, and the intensity and localisation of the oxidised HEt, which reflects O$_2^-$ production, could be observed and compared between the rat strains.

2.5. Superoxide measurement

O$_2^-$ levels were measured in 4-mm rings of carotid artery by lucigenin chemiluminescence as previously described [8]. O$_2^-$ was quantified against a standard curve of O$_2^-$ generated by xanthine/hypoxanthine. Tissue O$_2^-$ was expressed as nmol/mg wet wt/min.

2.6. Western blot and immunohistochemical analysis of human eNOS and Cu/ZnSOD recombinant gene expression

For western blotting, crude protein extracts from the harvested arteries were subjected to polyacrylamide gel electrophoresis and transferred to Hybond P membranes as previously described [23]. Supernatant protein (10 μg) was separated on 7.5% SDS polyacrylamide gels in the case of eNOS and 12% in the case of Cu/ZnSOD. For immunostaining, sections were treated as previously described [23]. In both cases, eNOS was detected by a mouse anti-human monoclonal antibody (Transduction Laboratories) while Cu/ZnSOD was detected using a primary polyclonal sheep anti-human Cu/ZnSOD antibody (Calbiochem). The secondary antibody was a sheep anti-mouse IgG-peroxidase (Sapu) in the case of eNOS and donkey anti-sheep IgG-peroxidase in the case of SOD. Enhanced chemiluminescence (Amersham) was used to visualise the protein bands for western analysis. For the histochemistry, binding was visualised using 3',3'-diaminobenzidine (DAB) and 0.01% hydrogen peroxide as a chromogen. Sections were briefly air-dried and counterstained with hematoxylin and examined for positive staining of SOD (brown staining) by light microscopy.

2.7. Determination of NOS and SOD activity in endothelial cells

Aortic endothelial cells were infected with increasing titre of Ad/Hu/eNOS or AdCu/Zn SOD for 1 h, MOI of 10–200 particles of virus/cell. The cells were then incubated in serum-free media for 24 h. In the case of eNOS, the supernatant was removed to determine the activity of the NOS enzyme. This was assessed according to the Griess method [26] which measures nitrite concentration after conversion of nitrate to nitrite with nitrate reductase. Nitrite concentrations were determined at an optical density of 554 nm by comparison to standard solutions of sodium nitrite. In the case of SOD, cells were removed from flasks by scraping, washed and pelleted. Then, 100 μl water was added and samples were lysed by freeze/thawing. SOD activity was measured in 20-μl aliquots of supernatant using a kit (Calbiochem, 574600). The assay is based on the oxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[e]fluorene (THBF) and is measured as the rate of increase in optical density at 525 nm.

2.8. Vascular studies

Carotid arteries from the experimental animals were infused, harvested and suspended in organ baths as previously described [23]. Krebs buffer in which the carotid artery rings were maintained contained indomethacin (0.02 mM) to inhibit any prostanoid-mediated effects. Isometric tension studies were performed using a force transducer and recorded using a MacLab dedicated computer. Cumulative dose–response curves to phenylephrine (PE) (10$^{-9}$ to 10$^{-5}$ mol/l) were constructed, first in the absence, and again after washout, in the presence of 100 μM N$^G$-nitro-L-arginine methylester (L-NAME) to inhibit NO synthase. The increase in tension in the presence of L-NAME provided a measure of the effect of NO on basal tone [4]. The increase in tension in the presence of L-NAME was calculated for each ring over the full dose–response curve and expressed as an area under the curve (AUC).

2.9. Statistical analysis

In each experiment, n refers to the number of animals from which the carotid arteries were harvested. The increase in tension in the presence of L-NAME in the infused rings (human eNOS or Cu/ZnSOD) was compared to that obtained in the contralateral untreated control rings using Student’s t-test. Data are expressed as mean ± S.E.M. Difference among data sets were evaluated by performing
analysis of variance (ANOVA). A level of $P<0.05$ was accepted as statistically significant.

3. Results

3.1. Recombinant viral DNA analysis

A standard strategy was used to generate the recombinant human eNOS virus as described in the methods. To confirm the recombinant status and purity of the newly generated viral vector carrying the human eNOS cDNA, restriction analysis with *HindIII* was carried out on DNA extracted from virus-infected 293 cells. Fig. 1A shows a comparison of the eNOS recombinant adenoviral DNA with the eNOS insert illustrated in lane 1 and the wild-type parental dl309 DNA showing the presence of the E1 region in lane 2. These DNAs were subsequently transferred to Hybond N$^+$ and probed with an eNOS-specific probe confirming the presence of the eNOS fragment shown in Fig. 1b, lane 1 with the absence of this fragment in the wild-type adenoviral DNA, lane 2. The AdCu/ZnSOD virus, purchased from the Gene Transfer Core in the University of Iowa, was propagated and checked by restriction analysis shown in Fig. 1B, lane 1. The characteristic E1 is present as part of a doublet in the wild-type dl309 DNA in Fig. 1B, lane 2 but absent in the Cu/ZnSOD recombinant. The viral vectors were routinely characterised by PCR analysis (Fig. 1C) to confirm the presence of the eNOS and SOD genes using eNOS- and SOD-specific primers shown in Table 1.

Fig. 1. (A) Restriction analysis of DNA extracted from virally-infected cells. Recombinants were screened by extracting the adenovirus DNA from the virus-infected cells, digesting it with *HindIII* followed by fractionation on a 0.8% agarose gel shown in A. Lane 1a shows Ad/Hu/eNOS, lane 2, wild-type parental Ad5 dl309 DNA. These DNAs were subsequently transferred to Hybond N$^+$ and analysed by Southern blotting using an eNOS-specific probe shown in b. (B) Restriction analysis of DNA extracted from cells infected with Ad/Cu/ZnSOD, lane 1, compared to wild-type dl309 DNA, lane 2. (C) DNA extracted from virally-infected cells and subjected to PCR analysis using human eNOS- and Cu/ZnSOD-specific primers (Table 1) as described in Methods. Positive plaques yield an 841-bp eNOS fragment shown in lane 1, and a 407-bp Cu/ZnSOD fragment shown in lane 2, a 1-kb marker (Gibco) is shown in lane 3.

Fig. 2. Basal superoxide production in carotid arteries from SHRSP and WKY rats. Carotid arteries were taken from male 3-month-old SHRSP ($n=5$) and WKY rats ($n=5$) which had not undergone gene transfer procedures, in order to determine basal levels of superoxide production. The vessels were cleaned of connective tissue, weighed and placed in a scintillation vial containing 2 ml of Krebs buffer for determination of superoxide by lucigenin chemiluminescence. Results are expressed as nmol superoxide/mg wet weight/min.

3.2. Superoxide measurement by lucigenin chemiluminescence

Levels of $O_2^-$ in carotid arteries from male SHRSP and WKY rats were $3.75\pm0.41$ and $0.70\pm0.11$ nmol/mg wet weight/min, respectively, ($n=5$ for both SHRSP and WKY) (Fig. 2). No reduction in $O_2^-$ levels were found in SHRSP vessels which had been infused with the AdCu/ZnSOD gene 24 h earlier, $O_2^-$ levels being $4.31\pm0.78$ and $3.50\pm0.47$ nmol/mg wet weight/min in the infused ves-
sels and the contralateral control vessels, respectively (n = 6).

3.3. Superoxide localisation

The localisation of O$_2^-$ within the carotid artery of the SHRSP and WKY rat was examined using the oxidative fluorescent dye hydroethidine which is freely permeable across the cell membrane, and in the presence of O$_2^-$, is oxidised to EtBr. The EtBr then intercalates with the DNA in the nucleus and can be visualised by microscopy as red fluorescence. Since the EtBr cannot diffuse across the cell membrane, fluorescence can be taken as an indicator that reduction of HE by O$_2^-$ occurred within the cell. This experiment was carried out to compare levels and location of superoxide in the SHRSP and WKY rat carotid arteries and to add further strength to the results obtained in Fig. 2 using chemiluminescence. As can be seen in Fig. 3, tissue sections from the SHRSP showed marked increase in O$_2^-$ compared to WKY consistent with our findings shown in Fig. 2 using lucigenin-enhanced chemiluminescence. This increase was observed throughout the endothelium and media layer.

3.4. Griess and SOD assay for the determination of NOS and SOD activity in endothelial cells

To determine functional eNOS activity, nitrate and nitrite levels were measured in cell culture supernatants, after the cells had been infected with Ad/Hu/eNOS using the Griess assay [26]. Increased viral titre resulted in increased nitrate/nitrite production in three independent experiments (Fig. 4A). Infection of endothelial cells with the SOD gene resulted in a dose-related increase in the rate of oxidation of THBF as illustrated in Fig. 4B. In the presence of Ad/CuZnSOD 50, 100, 200 MOI, SOD activity was 325±65, 467±110, 720±168%, respectively, of that observed in untreated cells (n=4). No increase in SOD activity was observed in cells incubated with empty virus. These data indicate both viruses express functional genes.

3.5. Western analysis of the eNOS and SOD protein

Protein extracts were prepared from the infused arteries and subjected to western blot analysis using monoclonal and polyclonal antibodies reactive with human eNOS and Cu/ZnSOD, respectively. In western immunoblots, shown in Fig. 5A, lane 1, endogenous eNOS protein was observed in crude vessel lysate from the right contralateral control carotid arteries from the SHRSP strain showing cross-reactivity with the rat antigens using this procedure. However, after Ad/Hu/eNOS infection into the left carotid arteries of these animals, eNOS expression was clearly enhanced as shown in Fig. 5A, lane 2. Expression of eNOS was confirmed by the presence of a single band of 140 kDa, corresponding to the expected size of the eNOS protein. No cross-reactivity with other NOS isoforms was observed using this eNOS-specific antibody. Western blotting also demonstrated a significant increase in

![Fig. 3. Localisation of superoxide in unfixed, frozen cross-sections of SHRSP and WKY carotid arteries using hydroethidine and visualised at 585 nm wavelength using a confocal microscope. (A) Cross-section of an SHRSP carotid artery. (B) cross-section of a WKY carotid artery. E, endothelium; M, medial smooth muscle cells.](image-url)
Cu/ZnSOD expression in vessels infused with Ad/Cu/ZnSOD illustrated in Fig. 5B, showing protein from the right contralateral uninfused control artery in lane 1, and protein from the Cu/ZnSOD-infused artery in lane 2. The 15-kDa band representing the Cu/ZnSOD protein is illustrated.

3.6. Immunohistochemical analysis

The efficiency of adenoviral vector-mediated gene transfer in rat carotid arteries was estimated on transverse histological sections of the arteries transfected with Ad/CuZnSOD. No staining was found in the contralateral uninfused or control-infused artery (Fig. 6A) indicating a lack of cross-reactivity between the rat and human isoform of Cu/Zn SOD in these experiments. Antibody staining showed the transfer and expression of the human SOD gene was localised primarily in the endothelial layer with some staining in the adventitia consistent with our previous findings [23]. Since different blocking agents are used in the western and immunohistochemical analysis, this result is not surprising, as a more specific block has been used in the latter to prevent cross-reactivity with the rat antigens.

3.7. Effect of gene transfer on basal NO bioavailability in carotid arteries from SHRSP

In the SHRSP, the pressor response to phenylephrine in the presence of L-NAME was increased in the eNOS-infused vessels relative to the contralateral controls; the
areas under the curve (AUC) for phenylephrine in treated and control carotid arteries were 2.95±0.53 and 1.74±0.52, respectively, \( (n=7) \), \( P=0.0026 \); 95%CI 0.606–1.788 at a titre of \( 2\times10^9 \) pfu/ml (Fig. 7B). In contrast, in carotid arteries from WKY rats, the increase in the pressor response to phenylephrine in the presence of L-NAME was similar in the Ad/Hu/eNOS infused and contralateral control vessel (Fig. 7A). AUC being 3.12±0.51 and 3.19±0.64, respectively \( (n=6) \). Infusion of the AdCu/ZnSOD gene in SHRSP produced a slight improvement in NO availability which did not reach significance, illustrated in Fig. 7C, AUC for phenylephrine in treated and control carotids were 2.14±0.41 and 1.64±0.17, respectively \( (n=8) \), \( P=0.28 \); 95%CI -0.49 to 1.49. Titre is usually a critical factor in determining gene expression levels after gene transfer experiments. When no effect was observed in NO bioavailability at a titre of \( 2\times10^9 \) pfu/ml from the SOD virus, the titre of the SOD virus was increased by a log of 10 to determine if an improved response could be obtained at this higher titre. However, a
4. Discussion

The results of the present study support and confirm our previous findings that in vivo eNOS gene transfer into intact endothelium of SHRSP-carotid arteries can successfully restore NO bioavailability to a level observed in the WKY normotensive reference strain. In this study we used a human instead of a bovine eNOS gene as a first step towards future clinical applications.

We have previously reported that in the SHRSP, as in a number of other models of hypertension, decreased NO bioavailability is associated with an increase in $O_2^-$ levels [4,6,7,30]. In our previous studies using aortae of SHRSP, it appeared that the $O_2^-$ was of endothelial origin [8]. In this study, we have confirmed the existence of increased $O_2^-$ levels in carotid arteries from SHRSP compared to WKY. In the carotid arteries using dihydroethidine, we have been able to show excess $O_2^-$ throughout the arterial wall. As the decreased NO bioavailability may be at least in part, if not primarily, a consequence of increased $O_2^-$ generation, we proposed that delivery of a recombinant adenovirus carrying and expressing a SOD gene may also be useful in the treatment of endothelial dysfunction by scavenging the excess $O_2^-$. In support of this hypothesis, inhibition of bovine coronary artery superoxide dismutase has been shown to attenuate endothelium-dependent vasodilation [31], while treatment with membrane-permeable superoxide dismutase mimetics improves endothelial function in cholesterol-fed rabbits and SHR [9,10]. However, in our study, intraluminal delivery of AdCu/ZnSOD to carotid arteries of SHRSP did not result in a significant improvement in NO bioavailability ex vivo, nor to a reduction in $O_2^-$ levels in the treated vessels.

There are a number of potential explanations for this. Increased $O_2^-$ has been found throughout the vessel wall in a number of models of atherosclerotic disease. In WHHL rabbits, a marked increase in $O_2^-$ levels in vascular smooth muscle has been reported [29], while in rats infused with angiotensin, increases in adventitial production of $O_2^-$ occurs [32,33]. Our results, showing intense fluorescence after hydroethidine staining in vascular smooth muscle of carotid arteries from SHRSP are consistent with these observations. If the majority of the $O_2^-$ is generated in the sub-endothelial layers of the vessel and the virus is delivered intraluminally, the SOD gene may be transferred primarily into endothelial cells, rather than vascular smooth muscle. Consistent with this hypothesis, Miller et al. [29] found that ex vivo SOD virus infection of rings of aorta from WHHL rabbits failed to improve endothelial function. They concluded that delivery of SOD to the media, as well as the endothelium is required to reduce oxidative stress and improve endothelial function in atherosclerosis. In contrast, Fang et al. [34] were able to show a reduction in LDL oxidation in endothelial cells overexpressing SOD. However, these in vitro studies are not directly comparable to the in vivo situation. In vivo, the most likely site of oxidation of LDL within the vessel wall is the sub-endothelial space. Overexpression of SOD within the endothelium has yet to be shown to inhibit LDL oxidation in vivo.

Another aspect of our study which needs to be considered is the choice of SOD isoform. Inhibition of Cu/ZnSOD has been shown to have deleterious effects on endothelial function [32,35], while lipid permeable Cu/ZnSOD restored endothelium-dependent vasorelaxation in cholesterol-fed rabbits [10] and enhanced vasorelaxation in B6C3F-1 mice [36] supporting our choice of Cu/ZnSOD for gene transfer. However, mitochondria are an important source of oxygen radicals generated as a by-product of electron transport. Future studies will address the issue of the effect of MnSOD and extracellular (ec) SOD on $O_2^-$ levels in carotid arteries from SHRSP although ecSOD is not normally present in rats at significant levels [37]. The relative importance of these two isoforms in this model of hypertension is uncertain.

Finally, overexpression of SODs alone may not improve endothelial dysfunction as this could lead to increased production of hydrogen peroxide. Hydrogen peroxide itself may be harmful, it may promote vascular smooth muscle hypertrophy, alter gene expression and activate matrix metalloproteinases [38]. Moreover, hydroxyl radicals ($OH^-$) may be produced from hydrogen peroxide via the Fenton reaction. OH$^-$ is several orders of magnitude more reactive towards cellular contents than superoxide and is considered to be highly toxic.

In conclusion, our data indicate that endothelial delivery of the human eNOS gene to SHRSP carotid arteries can correct the deficit in NO bioavailability in these animals. Cu/ZnSOD gene transfer had little effect. This may relate to the mode of gene delivery or could be a consequence of complexities in the SOD signalling pathways which have yet to be unravelled.

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