Expression and Function of Recombinant S1179D Endothelial NO Synthase in Human Pial Arteries

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Background and Purpose—Mutation of serine 1179 to aspartate on the endothelial NO synthase (eNOS) increases NO production in the absence of stimulation by agonists. The present study was designed to determine the effect of recombinant S1179DeNOS gene expression on the vasomotor function of human pial arteries.

Methods—Pial arteries were isolated from 28 patients undergoing temporal lobectomy for intractable seizures. Adenoviral vectors (10^10 pfu/mL) encoding β-galactosidase (AdCMVLacZ) or S1179DeNOS (AdCMVS1179DeNOS) were used for ex vivo gene transfer, and vasomotor function was evaluated in control and transduced arteries.

Results—Contractions to cumulative additions of U46619 were not affected by expression of LacZ or S1179DeNOS. Endothelium-dependent relaxations to bradykinin or endothelium-independent relaxations to Diethylaminodiazen-1-ium-1,2-dioate were significantly reduced in arteries expressing S1179DeNOS. A superoxide dismutase mimetic, manganese (III) tetrakis (4-benzoic acid) porphyrin chloride, failed to improve the reduced relaxations to bradykinin. The levels of cGMP were significantly elevated in arteries expressing S1179DeNOS.

Conclusions—Our results support the concept that high local production of NO in pial arterial wall causes adaptive reduction of vasodilator reactivity to NO. (Stroke. 2005;36:158-160.)

Key Words: free radicals ■ gene therapy ■ nitric oxide

The serine/threonine kinase Akt phosphorylates endothelial NO synthase (eNOS; at serines 1179 and 1177 for bovine and human eNOS, respectively). Phosphorylation on this residue is associated with an increase in NO production and activation of the enzyme at lower calcium/calmodulin concentrations compared with nonphosphorylated eNOS. Mutation of serine 1179 to aspartate (S1179DeNOS) increases NO production in the absence of stimulation by agonists. In a recent study, expression of recombinant S1179DeNOS increased the basal production of NO in canine basilar arteries. The present study was designed to determine the effect of recombinant S1179DeNOS gene expression on the vasomotor function of human pial arteries.

Materials and Methods

The procedures and handling of human tissue were reviewed and approved by the institutional review board of the Mayo Foundation. Human pial arteries (OD 500 to 1000 μm) were isolated from temporal lobe specimens of patients undergoing surgery for intractable lobe epilepsy. There were a total of 28 patients (13 males and 15 females) from whom viable tissue could be obtained (age 29±4 years). Arteries were harvested within 15 minutes of removal as described previously. Rings obtained from the same patient were randomly assigned for gene transfer and were transduced with adenoviral vectors (10^10 pfu/mL; AdCMVS1179DeNOS) or AdCMVLacZ (10^10 pfu/mL). Rings obtained from the same patient were randomly assigned for gene transfer and were transduced with adenoviral vectors (10^10 pfu/mL; AdCMVS1179DeNOS) or AdCMVLacZ (10^10 pfu/mL). For ex vivo gene transfer, expression of recombinant proteins and vasomotor function was evaluated in control and transduced arteries.

40 minutes; 37°C) for AdCMVLacZ or AdCMVS1179DeNOS or vehicle (control) as described previously. Twenty-four hours after gene delivery, expression of recombinant proteins and vasomotor functions were studied. Vascular reactivity in the pial arteries was evaluated by determining the concentration-response curves to different agonists in the isolated arteries connected to an isometric force transducer as described previously. In a separate set of patients, rings from the same patients were transduced with AdCMVS1179DeNOS, and relaxations to bradykinin were studied in the absence and in the presence of a superoxide dismutase mimetic, manganese (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBA); 10^-3 M). Radioimmunoassay kits (Amersham) were used to measure the basal levels of intracellular cGMP from nontransduced or LacZ- or S1179DeNOS-transduced arteries as described previously.

Statistical Analysis

Results of this study are expressed as the means ± SEM. Relaxations are expressed as percentage of maximal relaxations produced by 3×10^-4 mol/L papaverine. The concentration-response curves and multiple comparisons were analyzed by ANOVA followed by Fisher’s least significant difference post hoc test. Statistical significance was accepted at P<0.05.

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158
Results

Contractions to U46619 (10⁻⁹ to 10⁻⁶ mol/L) were not different in control (nontransduced), AdCMVLacZ, or AdCMVS1179DeNOS-transduced arteries (data not shown).

In AdCMVS1179DeNOS-transduced arteries, endothelium-dependent relaxations to bradykinin (10⁻⁹ to 10⁻⁶ mol/L) were significantly reduced (Figure 1A; P<0.05), and MnTBAP did not improve these relaxations (Figure 1B). Endothelium-independent relaxations to DEA-NONOate (10⁻⁹ to 10⁻⁵ mol/L) were also significantly reduced in arteries transduced with S1179DeNOS (Figure 2; P<0.05).

In AdCMVS1179DeNOS-transduced arteries, basal levels of cGMP were significantly elevated compared with either nontransduced control or AdCMVLacZ-transduced arteries (Figure 3).

Discussion

The principal new finding of the current study is that expression of recombinant S1179DeNOS in pial arteries increases cGMP levels but attenuates vascular reactivity to endogenous and exogenous NO. Pharmacological analysis with a superoxide anion scavenger, MnTBAP, ruled out chemical antagonism between superoxide and NO as a mechanism underlying endothelial dysfunction in arteries transduced with S1179DeNOS. Consistent with the results of the present study, overexpression of eNOS in transgenic mice also reduced vasodilator effect of NO, although vascular cGMP levels were elevated causing systemic vasodilatation and hypotension.⁸⁻⁹ Similarly, high pro-

Figure 1. A, Endothelium-dependent relaxations to bradykinin in nontransduced (control; n=7), AdCMVLacZ (LacZ; n=12), and AdCMVS1179DeNOS-transduced (S1179DeNOS; n=13) human pial arteries. Relaxations were obtained during contractions to U46619 (10⁻⁸ to 10⁻⁷ mol/L) and are expressed as percentage of maximal relaxation induced by 3×10⁻⁴ mol/L papaverine, (100%=0.53±0.06 g in control, 0.59±0.1 g in LacZ, and 0.51±0.07 g S1179DeNOS-transduced arteries, respectively). Differences between S1179DeNOS compared with control and LacZ are statistically significant (*P<0.05). B, Effects of the superoxide dismutase mimetic MnTBAP on the relaxations to bradykinin in S1179DeNOS-transduced arteries (n=6). Relaxations were obtained during contractions to U46619 (10⁻⁸ to 10⁻⁷ mol/L) and expressed as percentage of maximal relaxations to papaverine (100%=0.59±0.08 g in the presence of MnTBAP, respectively).

Figure 2. Endothelium-independent relaxations to DEA-NONOate (10⁻⁹ to 10⁻⁵ mol/L) in nontransduced (control; n=7), AdCMVLacZ (LacZ; n=12), and AdCMVS1179DeNOS-transduced (S1179DeNOS; n=13) human pial arteries. Relaxations were obtained during contractions to U46619 (10⁻⁸ to 10⁻⁷ mol/L) and are expressed as percentage of maximal relaxation induced by 3×10⁻⁴ mol/L papaverine (100%=0.80±0.06 g in control, 0.56±0.11 g in LacZ, and 0.62±0.10 g in S1179DeNOS-transduced arteries, respectively). Differences between S1179DeNOS compared with control and LacZ are statistically significant (*P<0.05).

Figure 3. Basal levels of cGMP production in nontransduced (control; n=4), AdCMVLacZ (LacZ; n=8), and AdCMVS1179DeNOS-transduced (S1179DeNOS; n=8) human pial arteries. Differences between S1179DeNOS compared with control and LacZ are statistically significant (*P<0.05).
duction of NO in the rabbit, canine, and human cerebral arteries expressing recombinant inducible NOS (iNOS) resulted in a reduced vasodilator effect of NO.\textsuperscript{10,11} These findings support the concept that increased local NO production initiates adaptive downregulation of signal transduction mechanisms responsible for mediation of NO-induced vasodilatation.\textsuperscript{10} The results of our study expand this concept to expression of S1179DeNOS in the human cerebral arteries.

S1179DeNOS did not affect reactivity to vasoconstrictor thromboxane A\textsubscript{2} receptor agonist U46619. This observation is in agreement with reported normal vasoconstrictor reactivity to UTP in canine basilar artery expressing S1179DeNOS, as well as normal reactivity to histamine and serotonin in rabbit cerebral arteries transduced with iNOS.\textsuperscript{4,11}

It is difficult to predict effect of recombinant S1179DeNOS in diseased arteries. However, establishing pharmacodynamic profile of S1179DeNOS in human arteries adds important information needed for further development of therapeutic application. Our results demonstrate that in human cerebral arteries, expression of S1179DeNOS increases cGMP production. This is associated with adaptive reduction of vasodilator reactivity to NO.

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Editorial Comment

eNOS: Can We Exploit the Good?

One of the major functions of vascular endothelium is to regulate tone of underlying smooth muscle. This function is mediated in large part by release of diverse endothelium-derived relaxing factors (EDRFs).\textsuperscript{1,2} A major EDRF that regulates both cerebral vascular function and structure is nitric oxide (NO) produced by the endothelial isoform of NO synthase (eNOS).\textsuperscript{1,3} Most studies indicate that NO is mediated in large part by release of diverse endotheliopy and receptors. Reduced responses to NO in vessels that express S1179DeNOS probably reflects a compensatory response within vascular muscle because of increased basal levels of NO.

Reduced responses to NO in these experiments did not appear to be mediated by oxidative stress as a scavenger of the free radical superoxide had no effect on impaired responses. This observation is important as increased activity of eNOS could potentially deplete enzyme substrate (L-arginine) or cofactors (ie, tetrahydrobiopterin), resulting in ‘uncoupling’ of eNOS and production of superoxide rather than NO.

The use of viral-mediated gene transfer of NOS isoforms to study the impact of NO on the vasculature is a relatively recent experimental approach. The strategy allows testing of basic biology of the vasculature but is also attractive as it may lay the framework, or at least establish proof-of-principle, for future therapeutic applications using viral vectors. To my knowledge, only
two studies have used adenoviral vectors to express any gene in human cerebral arteries. The present work suggests that gene transfer of a constitutively active form of eNOS alters vascular function in these arteries. Because the vector used appears to increase basal levels of NO without increasing superoxide levels, it may have beneficial effects in models of cerebral vascular disease. This latter possibility remains to be tested.

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