Gene Transfer of Calcitonin Gene–Related Peptide Prevents Vasoconstriction After Subarachnoid Hemorrhage

Kazunori Toyoda, Frank M. Faraci, Yoshimasa Watanabe, Toshihiro Ueda, Jon J. Andresen, Yi Chu, Shoichiro Otake, Donald D. Heistad

Abstract—We sought to determine whether adenovirus-mediated gene transfer in vivo of calcitonin gene–related peptide (CGRP), a potent vasodilator, ameliorates cerebral vasoconstriction after experimental subarachnoid hemorrhage (SAH). Arterial blood was injected into the cisterna magna of rabbits to mimic SAH 5 days after injection of AdRSVCRGP (8×10⁹ pfu), AdRSVβgal (control virus), or vehicle. After injection of AdRSVCRGP, there was a 400-fold increase in CGRP in cerebrospinal fluid. Contraction of the basilar artery to serotonin in vitro was greater in rabbits after SAH than after injection of artificial cerebrospinal fluid (P<0.001). Contraction to serotonin was less in rabbits with SAH after AdRSVCRGP than after AdRSVβgal or vehicle (P<0.02). Basal diameter of the basilar artery before SAH (measured with digital subtraction angiogram) was 13% greater in rabbits treated with AdRSVCRGP than in rabbits treated with vehicle or AdRSVβgal (P<0.005). In rabbits treated with vehicle or AdRSVβgal, arterial diameter after SAH was 25±3% smaller than before SAH (P<0.0005). In rabbits treated with AdRSVCRGP, arterial diameter was similar before and after SAH and was reduced by 19±3% (P<0.01) after intracisternal injection of CGRP-(8-37) (0.5 nmol/kg), a CGRP, receptor antagonist. To determine whether gene transfer of CGRP after SAH may prevent cerebral vasoconstriction, we constructed a virus with a cytomegalovirus (CMV) promoter, which results in rapid expression of the transgene product. Treatment of rabbits with AdCMVCRGP after experimental SAH prevented constriction of the basilar artery 2 days after SAH. Thus, gene transfer of CGRP prevents cerebral vasoconstriction in vivo after experimental SAH. (Circ Res 2000;87:818–824.)

Key Words: gene therapy ▪ cerebral vasospasm ▪ vasodilation ▪ neuropeptide ▪ rabbit

Cerebral vasospasm is a delayed, prolonged arterial constriction that follows subarachnoid hemorrhage (SAH), which can lead to brain ischemia and infarction.¹ Vasospasm occurs in 30% to 40% of patients after SAH¹ and is the leading cause of mortality (28%) and morbidity (39%) in patients after SAH.² Several mechanisms may contribute to vasospasm after SAH, including impaired endothelium-dependent relaxation; production of endothelium-derived contracting factors, including endothelin; and impaired activity of potassium channels in cerebral blood vessels.³ Cerebral vascular muscle is depolarized after SAH, most likely as a result of inhibition of potassium channels, and this depolarization is thought to contribute to vasospasm.⁴ Although several dilator mechanisms, including NO and nitrovasodilators, are impaired after experimental SAH in most studies,³–⁷ dilation of large cerebral arteries is not impaired in response to synthetic (aprikalim and cromakalim) and endogenous (calcitonin gene-related peptide [CGRP]) openers of potassium channels.⁶–⁸ Thus, we speculated that openers of potassium channels in vascular muscle may prevent vasospasm after SAH.

CGRP is an extremely potent vasodilator, which hyperpolarizes arterial muscle at least in part via opening of potassium channels.⁹,¹⁰ The peptide is abundant in perivascular nerve fibers surrounding cerebral blood vessels,¹¹ and depletion of CGRP may have functional effects in the cerebral circulation after SAH.¹²,¹³ Intrathecal and intravenous injection of exogenous CGRP increases arterial diameter in animals with vasospasm after experimental SAH.¹⁴–¹⁶ Thus, it seemed likely that, if a sufficient amount of CGRP is released around cerebral arteries after SAH, vasospasm might be prevented.

Gene transfer to vascular adventitia via cerebrospinal fluid (CSF) is an appealing strategy to modulate cerebral vascular function.¹⁷,¹⁸ We made a recombinant adenovirus that encodes prepro-CGRP and reported that gene transfer via CSF in normal rabbits expresses biologically active CGRP, increases the level of cAMP (a second messenger for the vasodilator response to CGRP) in the basilar artery, and attenuates contraction of the artery in vitro.¹⁷ Exposure of canine cerebral arteries to blood in vivo does not diminish, or may even augment, efficiency of expression after gene

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From the Departments of Internal Medicine and Pharmacology (K.T., F.M.F., Y.W., J.J.A., Y.C., D.D.H.), Cardiovascular Center, and Department of Radiology (T.U., S.O.), University of Iowa College of Medicine and Veterans Affairs Medical Center, Iowa City, Iowa
Correspondence to Donald D. Heistad, MD, Department of Internal Medicine, University of Iowa College of Medicine, 200 Hawkins Dr, Iowa City, IA 52242. E-mail donald-heistad@uiowa.edu
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transfer in vivo and in vitro. Thus, we speculated that perivascular application in vivo of a virus that expresses CGRP might prevent constriction of cerebral arteries after SAH. The goal of this study was to test the hypothesis that gene transfer of CGRP in vivo ameliorates cerebral vasoconstriction after SAH.

**Materials and Methods**

We made a replication-deficient adenovirus encoding prepro-CGRP (AdCGRP) or β-galactosidase (Adβgal) with a Rous sarcoma virus (RSV) promoter. We also constructed an adenovirus with a cytomegalovirus (CMV) promoter encoding prepro-CGRP (AdCMVCGRP) and used AdCMVβgal. Male New Zealand White rabbits weighing 2.4 to 3.2 kg were anesthetized intramuscularly with xylazine and ketamine. A needle was inserted into the cisterna magna, 250 μL of CSF was withdrawn, and 250 μL of viral suspension (PBS with 3% sucrose with 3×10⁹ pfu/mL virus) or vehicle (PBS with 3% sucrose) was infused.

Experimental SAH was produced in rabbits 5 days after injection of virus (AdRSVCGRP or AdRSVβgal) or vehicle. The anesthetized rabbit was placed in a prone position, and a needle was inserted into the cisterna magna. After withdrawal of 1 mL of CSF, 1 mL/kg of fresh, nonheparinized autologous arterial blood was injected.

Two days after experimental SAH (day 7), rabbits were anesthetized with pentobarbital IV and exsanguinated. Basilar arteries were removed and cut into rings (3 mm in length) for recording of isometric tension to examine vascular reactivity. Contraction to KCl, serotonin, and histamine was examined. Contraction was expressed as a percentage of response to 40 mmol/L KCl. Relaxation to acetylcholine and sodium nitroprusside was examined after vasoconstriction with 1 to 3 μmol/L of histamine. Relaxation was expressed as percentage of precontraction. In separate experiments, arterial rings were examined for relaxation to synthetic CGRP 2 days after injection of arterial blood or artificial CSF (without gene transfer).

Digital subtraction angiography was performed immediately before and 2 days after experimental SAH in rabbits treated with virus. The anesthetized rabbit was placed in a supine position, and an angiocatheter was introduced through the exposed femoral artery into the thoracic aorta to measure systemic blood pressure. Then the catheter was advanced into the left vertebral artery, and angiography into the thoracic aorta to measure systemic blood pressure. Then the catheter was withdrawn, and 250 μL of viral suspension (PBS with 3% sucrose with 3×10⁹ pfu/mL virus) or vehicle (PBS with 3% sucrose) was infused. Then the catheter was placed in the aorta to measure systemic blood pressure.

Before studying rabbits after experimental SAH treated with adenoviral vectors, we performed experiments to determine effects of experimental SAH or gene transfer alone on expression of CGRP in CSF. CGRP immunoreactivity was measured in CSF from rabbits after injection of blood into CSF without gene transfer (Figure 1A). Peak concentration of CGRP 30 minutes after injection of blood was 0.17±0.10 (mean±SEM) nmol/L. Concentration of CGRP in serum from the same rabbits was 0.44±0.09 nmol/L.

CGRP concentration was measured in CSF from rabbits treated with AdRSVβgal without experimental SAH (Figure 1B). Between 3 and 7 days after injection of the virus, concentration of CGRP exceeded 1 nmol/L, with peak expression of CGRP 5 days after injection of the virus (2.2±0.9 nmol/L). This course of expression of the transgene product in CSF was similar to the previous finding of expression in other tissues after injection of virus with the RSV promoter. On the basis of this result, we injected blood into the cisterna magna of rabbits 5 days after injection of virus in the following study.

**Gene Transfer In Vivo**

We injected adenovirus into CSF of rabbits, then injected blood to mimic SAH 5 days later (day 5), and performed final assays 2 days later (day 7). All rabbits tolerated injection of adenovirus without detectable neurological deficits. Immediately after injection of blood, almost all rabbits developed transient apnea. Seven of 53 rabbits (13%) died within a few minutes after injection of blood. Data from those rabbits were excluded. Rectal temperature was 39.3±0.1°C before injection of virus, 39.4±0.1°C before injection of blood (day 5), and 39.4±0.1°C on day 7. Leukocyte count in CSF increased from 3±1/mm³ to 327±59/mm³ 5 days after injection of AdRSVβgal (before injection of blood) and 417±76/mm³ 5 days after AdRSVβgal. We did not measure leukocyte count on day 7, because contamination with blood affected the counts.

In rabbits treated with AdRSVβgal or vehicle, baseline concentration of CGRP on day 0 was minimal (0.006±0.003
Effect of Gene Transfer on Vasoconstriction In Vitro After Experimental SAH

Contraction to 40 mmol/L KCl was similar in arteries exposed to blood and treated with vehicle (1.48±0.14 g), AdRSVβgal (1.40±0.11 g), and AdRSVCGRP (1.50±0.12 g), and in arteries from rabbits that received an injection of artificial CSF (instead of blood) into the cisterna magna and treated with vehicle (control rabbits, 1.58±0.23 g).

The contractile response to serotonin (0.01 to 3 μmol/L) was greatly augmented in arteries from rabbits after experimental SAH that were treated with vehicle, compared with control rabbits without SAH (P<0.001 for the entire concentration-response curve; maximal response [R\text{max}] was 98±7% versus 48±11%, P<0.0005, Figure 2A). Contraction was less after AdRSVCGRP (R\text{max} 70±11%) than after vehicle or AdRSVβgal (R\text{max} 93±8%) (P<0.02 for entire curve; P<0.05 for R\text{max}).

Contractile responses to histamine (0.03 to 30 μmol/L) were similar in arteries from rabbits after experimental SAH (treated with vehicle) and from control rabbits without SAH (Figure 2B). In arteries treated with AdRSVCGRP, contraction to 3 and 10 μmol/L histamine was less than after vehicle or AdRSVβgal (P<0.05).

Relaxation to high concentrations of acetylcholine (1 to 10 μmol/L) were attenuated after SAH (in rabbits treated with vehicle) compared with control rabbits (R\text{max} 78±8% versus 99±1%, P<0.05, Figure 2C). After SAH, relaxation to acetylcholine did not change after AdRSVCGRP compared with vehicle or AdRSVβgal. Relaxation to nitroprusside was similar among the 4 groups (Figure 2D).

In separate experiments, relaxation to synthetic CGRP (0.03 to 10 nmol/L) was virtually identical between the arteries from rabbits 2 days after experimental SAH and after injection of artificial CSF instead of blood (without gene transfer, Figure 2E).

Thus, vasoconstriction to serotonin, which was augmented after SAH, and vasoconstriction to histamine, which was not augmented, were both attenuated by gene transfer of CGRP. The response to synthetic CGRP was preserved after SAH.

Effect of Gene Transfer on Vasoconstriction In Vivo After Experimental SAH

We tested the hypothesis that gene transfer of CGRP prevents constriction of arteries in vivo. First, we determined effects on resting diameter of the basilar artery 5 days after gene transfer (Figure 3A). Injection of AdRSVβgal did not change diameter on day 5 (899±19 μm) compared with vehicle (887±13 μm). In rabbits treated with AdRSVCGRP, diameter on day 5 (1000±25 μm) was 13% greater than in rabbits treated with vehicle or AdRSVβgal (P<0.005). Thus, gene transfer of CGRP increased resting diameter of the basilar artery.
Arterial diameter was compared on day 7 (2 days after SAH) and on day 5. In rabbits treated with vehicle, diameter was $25 \pm 6\%$ smaller on day 7 (670 $\pm$ 62 mm) than on day 5 ($P < 0.001$, Figures 3A, 4A, and 4B). In rabbits treated with AdRSV$\beta$gal, diameter was $23 \pm 3\%$ smaller on day 7 (696 $\pm$ 31 mm) than on day 5 ($P < 0.001$). Vertebral arteries and vessels of the circle of Willis, in addition to the basilar artery, were constricted on day 7 (Figures 4A and 4B). In rabbits treated with AdRSVCGRP, diameter was similar on days 5 and 7 (963 $\pm$ 10 mm, $P > 0.1$, Figures 3A, 4D, and 4E).

After intracisternal injection of CGRP (0.1 nmol/kg) to rabbits treated with vehicle on day 7, diameter of the constricted basilar artery returned to resting values ($1 \pm 3\%$, n=5, Figure 4C). After intracisternal injection (0.5 nmol/kg) of a CGRP$_1$ receptor antagonist, CGRP-(8-37), to rabbits treated with AdRSVCGRP on day 7, the basilar artery constricted by $19 \pm 3\%$ (n=5, $P < 0.01$, Figures 3B and 4F). These findings suggest that either gene transfer of CGRP or an intracisternal bolus injection of synthetic CGRP can prevent vasoconstriction after experimental SAH by an effect on CGRP$_1$ receptors.

Systemic arterial pressure and arterial blood gases during angiography were not significantly different between treatment groups or at the time of the 2 angiograms in each group (Table). Thus, these physiological factors did not account for differences in arterial diameter.

In the studies described above, we examined effects of treatment with AdRSVCGRP before SAH. In other studies, we examined effects of gene transfer of CGRP after experimental SAH. For these studies, we constructed a recombinant virus with a CMV promoter, because maximal expression is much more rapid with a CMV than an RSV promoter. In rabbits treated with vehicle or AdCMV$\beta$gal, diameter of the basilar artery was $21 \pm 2\%$ smaller 2 days after than before SAH (Figure 5, control). Reduction in diameter after SAH was similar in rabbits treated with vehicle ($23 \pm 3\%$, n=4) or AdCMV$\beta$gal ($18 \pm 2\%$, n=4, $P > 0.1$). In rabbits treated with AdCMVCGRP, diameter was similar before and 2 days after SAH (Figure 5). Systemic arterial pressure and arterial blood gases during angiography were not significantly different between treatment groups or at the time of the 2 angiograms in each group (data not shown).

**Discussion**

This is the first successful use of gene transfer of a vasoactive protein or peptide to prevent cerebral vasoconstriction in vivo after experimental SAH. There are 2 major new findings. First, gene transfer of CGRP in vivo prevented excessive contraction of the basilar artery to serotonin in vitro after experimental SAH. Second, gene transfer of CGRP prevented vasoconstriction after experimental SAH by an effect on CGRP$_1$ receptors.

<table>
<thead>
<tr>
<th>Physiological Parameters During Angiography</th>
<th>Vehicle or Ad$\beta$gal</th>
<th>AdCGRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>63 $\pm$ 6</td>
<td>64 $\pm$ 3</td>
</tr>
<tr>
<td>Arterial blood gas</td>
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<td>pH</td>
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<td>$P_{O_2}$, mm Hg</td>
<td>66 $\pm$ 7</td>
<td>61 $\pm$ 4</td>
</tr>
<tr>
<td>$P_{CO_2}$, mm Hg</td>
<td>38 $\pm$ 1</td>
<td>38 $\pm$ 2</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ SEM.
constriction of the basilar artery in vivo after experimental SAH both when the virus was applied before SAH and after SAH. Thus, gene transfer of CGRP is a promising approach for prevention of vasospasm after SAH.

**Gene Transfer to Cerebral Arteries**

A unique implication of the present study is for gene therapy for cerebrovascular disease. Although clinical trials for patients with coronary and peripheral vascular disease are under way,27 gene transfer has not received extensive study for even animal models of cerebrovascular diseases. A reason for this lag may be that intravascular gene delivery, which is feasible for coronary and peripheral arteries, has not yet been accomplished for intracranial cerebral arteries, because intravascular approaches require interruption of blood flow to the brain.

As an alternative approach, perivascular gene delivery via CSF can be used for overexpression in the cerebral arteries of vasoactive proteins and peptides, including CGRP17 and endothelial NO synthase (eNOS).18 An advantage of perivascular gene delivery by injection into CSF versus intravascular delivery is that expression of transgene products is not limited to small regions but is distributed over an expanded area. Because multiple cerebral arteries both in anterior and posterior circulations are at risk for vasospasm after SAH, perivascular gene delivery may be more beneficial than intravascular delivery for prevention of vasospasm. In a recent study, perivascular application of liposomes with oligonucleotides with high affinity for nuclear factor-kB to act as decoy DNA also prevented cerebral vasoconstriction after experimental SAH.21

**Vasodilators in SAH**

The early release28 and subsequent depletion of CGRP12,13 in and around cerebral arteries after SAH suggest that endogenous CGRP may be protective in the cerebral circulation after SAH and that depletion of CGRP may contribute to vasoconstriction. The findings in Figure 1A confirm a previous finding that, 30 minutes after experimental SAH, CGRP concentration in CSF reached a peak,29 possibly from release of endogenous CGRP after SAH. Because the peak concentration of CGRP in CSF in both the present and previous studies28 was lower than concentrations in serum, however, blood contamination may contribute substantially to increases in CSF levels of CGRP. Importantly, the increase in endogenous CGRP in CSF derived from SAH was far less than the enormous increase in transduced CGRP after gene transfer.

The present and previous findings in vitro indicate that relaxation of cerebral arteries to CGRP is similar with and without SAH.8 The findings are in contrast to the previous finding in vivo that dilatation of the basilar artery to CGRP after SAH is augmented as compared with arteries without SAH.6 One possible explanation for differences in findings in vivo and in vitro is that effects of SAH on arteries may be attenuated in vitro, because arteries are removed from clot remnants and products in CSF that may contain vasoconstrictor substances.

The present and previous findings indicate that endothelium-dependent vasorelaxation is impaired after SAH.5-7 Several mechanisms have been proposed to account for this vascular dysfunction after SAH, including a reduction in production or activity of NO, impairment of activation of soluble guanylate cyclase or production of cGMP, inhibitory effect of hemoglobin on NO, and adhesion and infiltration of leukocytes in cerebral arteries.3 Adenovirus encoding eNOS (AdeNOS) has been used extensively for gene transfer to blood vessels,18,29,30 AdeNOS is useful for improvement of impaired NO-mediated relaxation in vitro of arteries after experimental SAH.20 The virus, however, has not yet been shown to be effective in vivo in prevention of vasoconstriction after SAH.31 Differences in effects in vivo and in vitro may be due in part to absence of effects of hemoglobin on NO-mediated relaxation of arterial rings in vitro. A previous study in vitro supports this hypothesis; although relaxation of the rabbit basilar artery to acetylcholine was normal after SAH, it was greatly inhibited compared with the artery without SAH once oxyhemoglobin solution was added to the organ bath.32 Impaired responses to NO after SAH are a major reason that we used adenovirus that expresses CGRP for the present study in vivo instead of AdeNOS.

**Vasoconstrictors in SAH**

In this study, vasoconstriction in vitro to serotonin was potentiated, and vasoconstriction to KCl and histamine was not altered, after experimental SAH. Results were similar to those of previous studies in vitro of the basilar artery from rabbits.33-36 Serotonin has been used commonly to test vascular reactivity after SAH, and previous studies demonstrated that contraction to serotonin was increased 2-fold or more after SAH.33-36 Denervation hypersensitivity of cerebral arteries to serotonin and norepinephrine, from a marked reduction or alteration in perivascular adrenergic nerves, may contribute to altered vascular response to serotonin.37

In contrast, contraction of the rabbit basilar artery to KCl33,34 or histamine35 in vitro was not altered after experimental SAH. Contraction to histamine, however, was augmented in arteries after SAH when oxyhemoglobin was added to the organ bath.32 Thus, it is possible that, although SAH has the potential to alter vascular responses to histamine, augmented responses were not demonstrated in the present and previous35 studies in the organ bath because vasoconstrictor substances from clot remnants and products in CSF were not present in the bath.

After gene transfer of AdRSVCGRP, contraction of the basilar artery to serotonin and histamine in vitro was attenuated in the present study and in a previous study in normal vessels.17 Attenuation of contraction was mediated by CGRP, receptors, because pretreatment with CGRP-(8-37), an antagonist for CGRP, receptors, restored the response to normal.17 AdRSVCGRP did not alter responses to KCl, possibly because depolarization from KCl may prevent hyperpolarization from CGRP. Thus, the present findings of altered vascular reactivity in vitro strongly suggested that gene transfer of CGRP might prevent vasoconstriction in vivo after SAH. Because studies in the organ bath have important limitations described above, however, confirmation in vivo was essential.
Altered Vascular Tone After Gene Transfer

The present angiographic findings demonstrated several effects of gene transfer on the basilar artery. First, arterial diameter was similar after treatment with AdGal and vehicle. Thus, although leukocytosis in CSF demonstrated a substantial inflammatory response after injection of adenovirus, the inflammation itself did not alter arterial diameter before or after exposure to blood. Responses of normal arteries to several stimuli in vitro in a previous study17 and in arteries after SAH in the present study also were virtually identical in arteries treated with AdGal and vehicle.

Second, gene transfer of CGRP increased resting diameter in vivo before SAH and prevented vasoconstriction after experimental SAH by an effect on CGRP1 receptors. Vaso-dilator effects of transduced CGRP were greater in constricted arteries on day 7 (44% increase in diameter compared with vehicle-treated rabbits) than in nonconstricted arteries on day 5 (13%). After SAH, because endogenous CGRP is depleted in cerebral arteries,12,13 sensitivity of the basilar artery to CGRP may be greater. This hypothesis is consistent with previous findings that dilatation of the basilar artery in vivo to CGRP after SAH is augmented as compared with arteries without SAH.6 Another possible reason for augmented dilator effects in constricted arteries may be that vessels that are submaximally constricted have greater capacity for dilation than do nonconstricted vessels.

Limitations of the Study

Vasospasm is a frequent and often fatal complication after SAH in patients.1,2 Novel strategies, including perhaps gene therapy, are needed to prevent vasospasm. An important limitation of this study, however, is that experimental SAH in rabbits does not replicate vasospasm after SAH in humans. The rabbit is commonly used, however, for experimental SAH, with intracisternal injection of autologous blood. Previous reports demonstrated the effect of intracisternal injection of autologous blood. Previous studies reported constriction of the rabbit basilar artery by 12% to 35% after injection of blood into CSF.16,21–23,38,39 Thus, the present finding of 25% reduction in diameter is comparable with those in previous studies. Constriction of cerebral arteries does not produce signs of ischemia unless constriction is much greater (perhaps 50% or more), at least in patients,40 so it is not clear whether the present strategy will also be useful for prevention of symptomatic vasospasm.

Another difference between vasospasm in rabbits and patients is the interval after onset of SAH. Previous reports in rabbits demonstrated that both angiographic evidence of vasospasm22,23 and augmented vasoconstriction to serotonin in vitro33 are maximal 2 days after SAH. In this study, we first pretreated rabbits with virus before injection of blood to match the peak of transgene expression with the peak of vasoconstriction after SAH, as in a previous study of gene transfer for brain ischemia.25 In application of this method in patients, it obviously will not be possible to pretreat patients with gene transfer before SAH. Thus, we constructed a recombinant virus with a CMV promoter, for which duration of 2 days seems to be appropriate for maximal transgene expression.26 The finding that AdCMVCGRP can prevent cerebral vasoconstriction when it is injected after SAH suggests that gene therapy may eventually be used for prevention of vasoconstriction when the virus is injected after SAH. Patients frequently develop vasospasm 4 to 14 days after SAH.3 Thus, it is possible that a virus with the RSV promoter may be preferable to the CMV promoter for use in patients. The inflammatory response to this recombinant virus, demonstrated in this study by an increase in leukocytes in CSF, would prevent use in patients. To minimize unwanted inflammatory or immune responses, coadministration of immunosuppressant drugs41 or immunomodulatory proteins42 may be useful. Newer adenoviral vectors may be less immunogenic and thus applicable to human use.43

Implications

Intravenous administration of synthetic CGRP to patients after SAH altered velocity of blood flow in cerebral arteries, suggesting dilatation of stenotic arteries,44 and appeared to improve neurological outcome.45,46 The use of intravenous CGRP, however, is limited by pronounced hypotension.45 Intracisternal gene transfer of CGRP is likely to be more useful than intravenous administration, because local gene transfer may avoid systemic effects of CGRP, and sustained release of CGRP is likely to be required for efficacy. Thus, we suggest that gene transfer has important potential advantages over usage of exogenous peptides for prevention of vasospasm. Although there are limitations in the current vectors and animal models, as discussed above, the present study provides evidence, or perhaps proof of principle, that gene transfer may be useful for prevention of vasospasm after SAH.

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