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REVIEW

Gene therapy for cerebral vascular disease: update 2003

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Gene therapy is a promising strategy for cerebrovascular diseases. Several genes that encode vasoactive products have been transferred via cerebrospinal fluid for the prevention of vasospasm after subarachnoid hemorrhage. Transfer of neuroprotective genes, including targeting of proinflammatory mediators, is a current strategy of gene therapy for ischemic stroke. Stimulation of growth of collateral vessels, stabilization of atherosclerotic plaques, inhibition of thrombosis, and prevention of restenosis are important objectives of gene therapy for coronary and limb arteries, but application of these approaches to carotid and intracranial arteries has received little attention. Several fundamental advances, including development of safer vectors, are needed before gene therapy achieves an important role in the treatment of cerebrovascular disease and stroke.
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Abbreviations: bFGF, basic fibroblast growth factor; CBF, cerebral blood flow; CGRP, calcitonin gene-related peptide; CSF, cerebrospinal fluid; HGF, hepatocyte growth factor; HSP, heat-shock protein; HVJ, hemagglutinating virus of Japan; MCA, middle cerebral artery; MCP-1, monocyte chemoattractant protein-1; NO, nitric oxide; NOS, nitric oxide synthase; SAH, subarachnoid hemorrhage; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor

Introduction

Seven years have passed since we reviewed the promise and problems associated with gene therapy for stroke and several cerebrovascular diseases (Heistad & Faraci, 1996) (Figure 1). Since then, a large number of studies have demonstrated feasibility of gene transfer to alter the cerebral circulation in experimental animals. Clinical application of this strategy to human disease states, however, has not yet begun, although clinical trials of gene therapy for other cardiovascular diseases, including coronary artery disease and limb ischemia, are promising (Baumgartner *et al*., 1998; Grines *et al*., 2002; Losordo *et al*., 2002; Makinen *et al*., 2002). Special challenges are presented by cerebral circulation, including the limited therapeutic time window for the treatment of acute stroke. In this review, we summarize progress in experimental gene therapy of cerebrovascular diseases in recent years and propose possible future applications to patients.

Therapeutic framework

What to treat

We proposed previously (Heistad & Faraci, 1996) several possible targets for cerebral vascular gene therapy: (1) prevention of vasospasm after subarachnoid hemorrhage (SAH), (2) stimulation of growth of collateral blood vessels in the area at risk of ischemia, and (3) stabilization of atherosclerotic plaques, inhibition of thrombosis, and prevention of restenosis after angioplasty of the carotid and vertebrobasilar arteries. It is now clear that several transgenes are promising for the first target (Onoue *et al*., 1998; Stoodley *et al*., 2000; Toyoda *et al*., 2000c; Watanabe *et al*., 2002). For the latter two targets, clues about appropriate transgenes may emerge from experimental and clinical studies of coronary and limb circulations.

In the past few years, it has also become evident that gene transfer to brain parenchyma for cerebrovascular diseases holds considerable promise (Betz *et al*., 1995). Neuroprotection during acute stroke, for example, by inhibition of inflammatory mediators, may be useful (Yang *et al*., 1997; Ooboshi *et al*., 2002). Gene therapy for risk factors for atherosclerosis, including hypertension and diabetes mellitus, may also prove to be useful for indirect prevention of cerebrovascular diseases (Table 1).

Where to treat

In the treatment of cerebral vascular disease, there are several important sites for gene transfer: cerebral blood vessels and brain parenchyma.

Since the first successful studies (Nabel *et al*., 1989; Lin *et al*., 1990) of gene transfer to blood vessels *in vivo*, the most common approach has been intraluminal delivery of vectors with transgenes. Using this approach, nitric oxide synthase (NOS) and other vasoactive transgene products have been expressed in vascular endothelium to alter vascular reactivity (Von der Leyen *et al*., 1995). The intraluminal approach, however, has important limitations for gene transfer to

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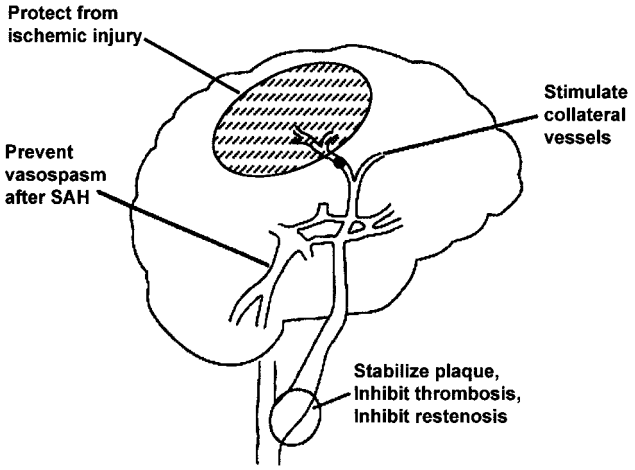

Figure 1 Potential applications of gene therapy for cerebral vascular disease. Revised from Heistad and Faraci (1996).

cerebral circulation. First, interruption of blood flow for several minutes, which is needed for effective gene delivery to cells in the vessel wall, would produce cerebral ischemia. Second, intraluminal gene transfer usually requires occlusion of a vessel and injection of the vector between ligatures, so that transfection is limited to only the small segment of the vessel into which the vector is instilled. Third, the intraluminal approach results in low efficiency of gene transfer beyond the endothelium, unless the endothelium is denuded or damaged.

An alternative approach is perivascular delivery of transgenes with vectors. Gene transfer to the intracranial circulation can be achieved by intracisternal injection of vectors into the cerebrospinal fluid (CSF) (Ooboshi *et al*., 1995; Christenson *et al*., 1998). Gene transfer to the extracranial carotid artery can also be accomplished by the injection of vectors into the periarterial sheath (Rios *et al*., 1995). Overexpression of vasoactive transgene products in the vascular adventitia, after perivascular gene transfer, alters nitric oxide (NO)-dependent vascular responses, which normally are endothelium dependent (Chen *et al*., 1997; Ooboshi *et al*., 1997b; Toyoda *et al*., 2000b). The perivascular approach has been used in human cerebral arteries *ex vivo* (Khurana *et al*., 2000; Gunnett *et al*., 2002).

Gene transfer to brain parenchyma has been mainly achieved by direct administration of vectors with transgenes into the parenchyma, including the striatum and cortex (Davidson *et al*., 1993; Le Gal La Salle *et al*., 1993). The approach can be used to overexpress transgene products in several cell types, including neurons and astrocytes. After the injection of vectors into cerebral ventricles, transgene products are overexpressed in the leptomeninges and ependymal cells that line the ventricles (Akli *et al*., 1993; Bajocchi *et al*., 1993). Overexpressed genes in the leptomeninges and ependymal cells had therapeutic effects for the ischemic lesion in brain parenchyma (Betz *et al*., 1995).

Table 1 Some approaches to cerebrovascular gene therapy

Gene	*Vector*	*Administration*	*References*
Vasospasm after SAH			
Endothelial NOS	Adenovirus	Cisterna magna	Onoue *et al*. (1998) Stoodley *et al*. (2000) Khurana *et al*. (2002)
ECSOD	Adenovirus	Cisterna magna	Watanabe *et al*. (2002)
Antisense preproendothelin-1	Oligodeoxynucleotide	Cisterna magna	Onoda *et al*. (1996) Ohkuma *et al*. (1999)
Prepro-CGRP	Adenovirus	Cisterna magna	Toyoda *et al*. (2000a, b, c)
Decoy of NFκB	Oligodeoxynucleotide	Cisterna magna	Ono *et al*. (1998)
Intracranial collateral circulation			
Basic fibroblast growth factor	Adenovirus	Ventricle	Yukawa *et al*. (2000)
Hepatocyte growth factor	HVJ–liposome complex	Cisterna magna	Yoshimura *et al*. (2002)
Arterial disease (regression of atherosclerosis or restenosis)			
Apolipoprotein E	Adenovirus	Systemic injection	Desurmont *et al*. (2000)
Anti-MCP-1	HVJ–liposome complex	Femoral muscle	Egashira *et al*. (2000) Mori *et al*. (2002)
Tissue factor pathway inhibitor	Adenovirus	Carotid artery	Atsuchi *et al*. (2001)
Hirudin	Adenovirus	Carotid artery	Rade *et al*. (1996)
Tissue plasminogen activator	Adenovirus	Femoral artery	Waugh *et al*. (1999)
Decoy of transcription factor E2F	Oligodeoxynucleotide	Carotid artery	Morishita *et al*. (1995)
Brain ischemia			
Calbindin D28K	Herpes simplex virus	Striatum	Yenari *et al*. (2001a, b)
Glucose transporter	Plasmid	Striatum	Lawrence *et al*. (1996)
HSP72	Herpes simplex virus	Striatum	Hoehn *et al*. (2001)
Interleukin-1 receptor antagonist	Adenovirus	Lateral ventricle	Yang *et al*. (1997)
Interleukin-10	Adenovirus	Lateral ventricle	Ooboshi *et al*. (2002)
Transforming growth factor-β1	Adenovirus	Lateral ventricle	Pang *et al*. (2001)
Glial cell line-derived neurotropic factor	Adenovirus	Cortex	Kitagawa *et al*. (1999)
Hepatocyte growth factor	HVJ–liposome complex	Cisterna magna	Hayashi *et al*. (2001)
bcl-2	Adeno-associated virus	Hippocumpus	Shimazaki *et al*. (2000)
Neuronal apoptosis inhibitory protein	Adenovirus	Hippocumpus	Xu *et al*. (1997)
Cyclooxygenase-1	Adenovirus	Lateral ventricle	Lin *et al*. (2002)
Redox-inducible antioxidant protein	Adenovirus	Caudate putamen	Yang *et al*. (2001)

In addition to local gene transfer, systemic administration of vectors may be an important approach if the transgene product is secretable protein. After intravenous injection of adenoviral vectors, viruses are cleared by the liver, and transgene products are mainly overexpressed in the liver and secreted into the systemic circulation (Drazan *et al*., 1995; Balague *et al*., 2000). Binding of the circulating transgene product to the cerebral or systemic vascular endothelium may require special targeting. For example, extracellular superoxide dismutase (ECSOD) contains a positively charged heparin-binding domain (HBD), which mediates binding of ECSOD to cells and the interstitium (Oury *et al*., 1996). Preliminary studies suggest that intravenous administration of an adenoviral vector encoding ECSOD produced overexpression of ECSOD in the aorta and carotid artery, reduced systemic vascular resistance, and reduced arterial pressure in an animal model of hypertension, but administration of an adenoviral vector encoding ECSOD with deletion of its HBD did not reduce arterial pressure (Chu *et al*., 2003).

Injection of vectors into skeletal muscle can also be used for expression of transgenes, and release of transgene products into the systemic circulation, to affect remote organs (Ueno *et al*., 2000). For example, attenuation of atherosclerosis in carotid and coronary arteries was accomplished after intramuscular injection of a vector that expresses soluble FLT-1 gene, which acts as a decoy to suppress the normal function of vascular endothelial growth factor (VEGF), or antimonocyte chemoattractant protein-1 (MCP-1) gene, a mechanism that is discussed later (Mori *et al*., 2002; Zhao *et al*., 2002).

How to treat

The principal goal of gene therapy is either to express a gene that is deficient or to overexpress a therapeutic gene. Selection of a vector is challenging. Naked plasmid DNA is effective as a delivery system in skeletal muscle, but naked DNA does not effectively pass into vascular or neuronal cells (Wolff *et al*., 1990). Viral vectors or carrier molecules (nonviral vectors) can augment the efficiency of gene transfer to cells.

Replication-deficient recombinant adenovirus is a widely used vector both in experimental gene transfer to cerebral blood vessels and in clinical cardiovascular trials of gene therapy (Isner *et al*., 2001). Adenoviral vectors can transduce efficiently both dividing and nondividing cells. The virus has room for fairly large cDNA inserts, and can be prepared in higher titers than most other viral vectors. Several studies reported successful adenovirus-mediated gene transfer to carotid arteries (Lee *et al*., 1993; Lemarchand *et al*., 1993) and intracranial arteries and perivascular tissue (Ooboshi *et al*., 1995; Christenson *et al*., 1998). Adenovirus-mediated vascular gene transfer has been performed in disease states, including hyperlipidemia and atherosclerosis (Rios *et al*., 1995; Kozarsky *et al*., 1996; Ooboshi *et al*., 1997a, 1998; Lund *et al*., 1998; Tangirala *et al*., 1999), diabetes (Lund *et al*., 2000; Zanetti *et al*., 2001), hypertension (Alexander *et al*., 1999; Gelband *et al*., 2000; Nakane *et al*., 2000; Phillips, 2001; Li *et al*., 2002), and after SAH (Muhonen *et al*., 1997).

An advantage of adenoviral vectors is that viral proteins facilitate some steps of gene transduction, including escape from the endosome and entry into the nucleus. A disadvantage of the virus, however, is that binding to vascular cells is limited by a paucity of adenoviral fiber receptor activity in vascular cells (Seth *et al*., 1994; Wickham *et al*., 1996). Approaches to overcoming this limitation include the use of complexes of adenovirus with cationic polymer or lipids (Toyoda *et al*., 1998, 2001), or the use of complexes of adenovirus with calcium phosphate precipitates (Toyoda *et al*., 2000a). These complexes are easy to prepare. For augmentation of adenovirus-mediated gene transfer to specific cells without widespread effects on other cells, tissue-specific ligands are useful (Wickham, 2000). Improvement of adenoviral vectors to deliver genes selectively, safely, and with little immune response is necessary before application of the vectors to human gene therapy becomes widespread (Thomas *et al*., 2001).

Viral vectors that have been used for vascular and neuronal gene transfer include adeno-associated virus, herpes simplex virus, retrovirus, and lentivirus (Hu & Pathak, 2000; Buchschacher and Wong-Staal, 2001; Kay *et al*., 2001; Yenari *et al*., 20001b; Lowenstein, 2002). Nonviral vectors, or carrier molecules, include cationic polymer and liposomes.

An alternative to transfer of genes that produce enzymes and other proteins is gene transfer using oligonucleotides that regulate transcription of endogenous genes and inhibit their expression. For example, antisense oligodeoxynucleotides are widely used (Simons *et al*., 1992). Double-stranded oligodeoxynucleotide *cis*-elements act as a decoy to bind transcription factors and block the expression of genes that require the transcription factor to be transcribed (Morishita *et al*., 1995). Small interfering RNA (siRNA) may emerge as a particularly promising strategy to inhibit specifically gene expression (Xia *et al*., 2002). These tools are potentially useful for gene transfer to cerebral circulation.

Advances toward cerebrovascular gene therapy

Vasospasm after SAH

Vasospasm is a catastrophic complication after SAH for which no effective prevention has yet been demonstrated, and gene therapy may hold great potential. Several features of vasospasm after SAH suggest that it may be amenable to gene therapy. First, vasospasm typically occurs several days after SAH, which allows sufficient time after SAH to administer the vector and for expression of a transgene product. Second, the period of risk of vasospasm is only 2–3 weeks at most, and thus prolonged expression of a transgene is not needed. Third, intracisternal administration of vectors allows expression of transgene products around vessels at the base of the brain, and a hematoma at the site of the SAH does not prevent access of the virus or its product to target blood vessels (Muhonen *et al*., 1997). Proliferation of adventitial fibroblasts and resultant fibrosis after SAH may alter transgene delivery to vascular adventitia (Onoue *et al*., 1998).

Several mechanisms may contribute to vasospasm after SAH, including (1) impaired endothelium-dependent vasorelaxation, (2) production of endothelium-derived contracting factors, including endothelin, and (3) impaired activity of potassium channels in cerebral blood vessels (Harder *et al*., 1987; Faraci & Heistad, 1998). To address the first mechanism, gene transfer *in vivo* of endothelial NOS improved NO-mediated relaxation *in vitro* of basilar arteries after experimental SAH (Onoue *et al*., 1998). Gene transfer of endothelial NOS, however, failed to protect against vasospasm *in vivo* in

one study, presumably due in part to scavenging of NO by hemoglobin in CSF (Stoodley *et al*., 2000) and to reduced activity of soluble guanylate cyclase (Sobey *et al*., 1996). Another study reported partial attenuation of constriction of the basilar artery *in vivo* after SAH, using gene transfer of endothelial NOS before SAH (Khurana *et al*., 2002). Since the vector was administered 1 day before SAH, the approach as described would not be clinically useful.

Superoxide may contribute to vasospasm after SAH (Kajita *et al*., 1994; Shishido *et al*., 1994), and superoxide dismutase (SOD) is a candidate for the prevention of vasospasm after SAH. Transgenic mice that overexpress CuZnSOD or ECSOD have attenuated cerebral vasoconstriction after experimental SAH (Kamii *et al*., 1999; McGirt *et al*., 2002). We recently demonstrated a partial protective effect against vasospasm after SAH by injection into CSF of an adenovirus that expresses ECSOD (Nakane *et al*., 2001; Watanabe *et al*., 2003). Antisense preproendothelin-1 oligodeoxynucleotide, which reduces production of endothelin peptide, attenuates vasospasm after SAH following intracisternal injection of the antisense alone (Onoda *et al*., 1996) or together with tissue plasminogen activator to lyse the subarachnoid thrombi (Ohkuma *et al*., 1999).

Calcitonin gene-related peptide (CGRP) is an extremely potent cerebral vasodilator, which may prove to be useful for the prevention of vasospasm after SAH. The peptide opens potassium channels, hyperpolarizes arterial muscle, and dilates arteries (Nelson *et al*., 1990; Kitazono *et al*., 1993). After SAH, CGRP is depleted from nerves to cerebral arteries (Nozaki *et al*., 1989a; Edvinsson *et al*., 1991). Intracisternal or systemic administration of exogenous CGRP increases cerebral arterial diameter *in vivo* after experimental SAH (Nozaki *et al*., 1989b; Toshima *et al*., 1992). Systemic administration of exogenous CGRP to patients with SAH also transiently reduced cerebral vasoconstriction and neurological deficits, but efficacy of intravenous CGRP in increasing cerebral blood flow was limited by its hypotensive effect (Johnston *et al*., 1990; Juul *et al*., 1994). Thus, we speculated that intracranial overexpression of CGRP for longer periods by a gene transfer technique might be effective for the prevention of vasospasm after SAH. We prepared a recombinant adenoviral vector encoding prepro-CGRP, and demonstrated that it modulates cerebrovascular tone after intracisternal gene transfer (Toyoda *et al*., 2000b). Treatment of rabbits with this vector after SAH prevented vasospasm (Toyoda *et al*., 2000c) (Figure 2). This was, to our knowledge, the first successful transfer of vasoactive genes *in vivo* for the prevention of vasospasm after SAH. The potential of this strategy was also demonstrated using a dog model of SAH in which vasoconstriction is greater than in rabbits, and thus more closely represents clinical vasospasm (Satoh *et al*., 2002).

Vasospasm after SAH may be related in part to an inflammatory vasculitis (Peterson *et al*., 1990; Aihara *et al*., 2001), and inhibition of inflammation by gene transfer appears to attenuate vasospasm (Ono *et al*., 1998). The transcription factor NFκB plays an essential role in the activation of inflammatory cytokines and adhesion molecules. Intracisternal administration of a decoy oligodeoxynucleotide of NFκB is reported to be useful for the prevention of vasospasm (Ono *et al*., 1998).

Collateral circulation

Stimulation of growth of collateral blood vessels may be useful for the preservation of cerebral circulation and prevention of cerebral infarction. Growth factors, including VEGF (Baumgartner *et al*., 1998; Rosengart *et al*., 1999), hepatocyte growth factor (HGF) (Hayashi *et al*., 1999), and basic fibroblast growth factor (bFGF) (Udelson *et al*., 2000), have been reported to have angiogenic effects in experimental ischemia of myocardium and limbs, and are being used for clinical trials of gene therapy. Gene delivery of these growth factors with vectors into CSF induced angiogenesis on the brain surface in animal models (Yukawa *et al*., 2000; Yoshimura *et al*., 2002). It is not yet clear whether this strategy will be useful in focal stimulation of angiogenesis to regions of the cerebrum that are at risk for infarction.

Arterial disease

Carotid endarterectomy and angioplasty with stenting are in wide use for the treatment of stenotic atherosclerotic lesions. An important goal is the prevention of progression of arterial stenosis. Regression of atherosclerotic lesions in mouse aorta has been documented after adenovirus-mediated gene transfer of human apolipoprotein E (Desurmont *et al*., 2000). MCP-1 recruits monocytes into the arterial wall and contributes to atherogenesis. Anti-MCP-1 gene transfer suppressed coronary and carotid hyperplasia after chronic inhibition of NOS

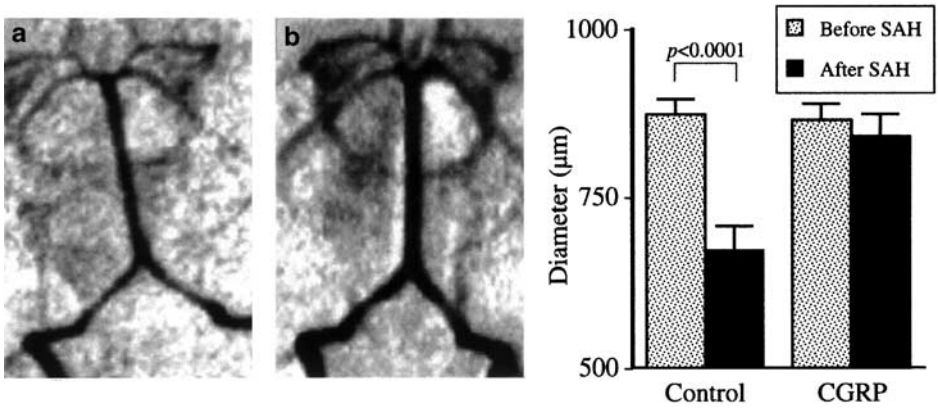

Figure 2 Gene transfer to prevent vasospasm after experimental SAH. Revised from Toyoda *et al*. (2000c). Arteriogram of the vertebrobasilar arteries in rabbits 2 days after experimental SAH. Rabbits were treated with vehicle (a) or adenovirus encoding CGRP gene (b).

(Egashira *et al*., 2000; Mori *et al*., 2002). Thrombosis and proliferativc changes after vascular injury were inhibited in the carotid artery by gene transfer of tissue factor pathway inhibitor (Atsuchi *et al*., 2001) and in other arteries by gene transfer of hirudin (Rade *et al*., 1996) and tissue plasminogen activator (Waugh *et al*., 1999).

After arterial bypass and angioplasty, restenosis is produced in part by intimal hyperplasia. Restenosis is inhibited by gene transfer of several inhibitors of cell cycle, intracellular signal transducers, transcription factors, cytokines, growth factors, NO, and Fas ligand (Kibbe *et al*., 2000). Among them, the transcription factor E2F transactivates cell cycle regulatory genes. Decoy oligodeoxynucleotides of E2F modulate gene expression and inhibit the proliferation of smooth muscle in the injured carotid artery *in vivo* (Morishita *et al*., 1995). Efficacy of the E2F decoy has been tested in clinical trials of restenosis after vascular bypass grafts (Mann *et al*., 1999) and after angioplasty (Morishita *et al*., 2001).

Ischemic stroke

Damage in the ischemic core starts very soon after the onset of stroke. Reduction in cerebral blood flow (CBF) reduces energy availability and, as a result, membrane ionic gradients fail rapidly, which result in excessive influx of calcium into cells within seconds to minutes after the onset of ischemia. Increased intracellular calcium causes release of excitotoxic neurotransmitters including glutamate within an hour, and thereby increases damage to neuronal cells. Cellular damage may be augmented during reperfusion, which implies that damage might be reduced by the prevention of reperfusion injury. The therapeutic time window appears to be (at most) only a few hours after the onset of stroke. Since expression of transgene products requires hours using current techniques of gene transfer, the initial damage after stroke is not a promising target for gene therapy, except when a direct effector (with no need of expression) including oligonucleotide is used.

In the peri-ischemic area, or 'ischemic penumbra,' an inflammatory cascade has been characterized (Barone & Feuerstein, 1999) in the following order: gene expression of transcription factors (wave 1), heat-shock proteins (HSPs) (wave 2), cytokines, chemokines, adhesion molecules, and growth factors (wave 3), proteinases and proteinase inhibitors (wave 4), and delayed remodeling proteins (wave 5). The third wave, consisting of upregulation of many proinflammatory genes, begins about 1 h after stroke, peaks at about 12 h, and subsides after 2 – 5 days. The entire process is completed in about 2 weeks. The current strategy for gene therapy of stroke focuses on augmentation of neuroprotective genes or inhibition of genes that are believed to be harmful during the first 2 weeks, especially within about 5 days after the onset of stroke.

Protein synthesis begins to decrease when CBF declines to $<50\%$ of the resting value or about 30 mL/100 g per minute (Xie *et al*., 1989; Mies *et al*., 1993), which results in the inhibition of transcriptional and translational processes. Since most of the currently available viral and nonviral vectors use the expression mechanisms of host cells, a critical question about possible efficacy for gene therapy for brain ischemia is whether, despite cellular ischemia, effective transgene expression and protein synthesis can be expected in ischemic and peri-ischemic areas. After middle cerebral artery (MCA) occlusion and reperfusion, adenovirus-mediated transgene expression was observed to increase in the peri-ischemic area 7 days after ischemia/reperfusion to a level similar to that of the control, and diminished by 21 days (Abe *et al*., 1997). At 4 days after photochemical occlusion of the distal MCA and adenovirus-mediated gene transfer, transgene expression was minimal in the ischemic core, and moderate in the peri-ischemic area, where CBF decreased to about 40% (Ooboshi *et al*., 2001) (Figure 3). Thus, protein synthesis appears to be sufficient, at least in the peri-ischemic region, which suggests that gene transfer to ischemic penumbra may be a promising approach for the treatment of ischemic stroke.

Several experimental trials of gene therapy have been attempted for the treatment of poststroke disruption of ionic gradients, energy failure, and the inflammatory cascade. Calbindin D28K, which is an endogenous calcium-binding protein that inhibits excessive accumulation of intracellular calcium, attenuated brain damage by prestroke gene delivery (Yenari *et al*., 2001a). Prestroke administration of a glucose transporter gene also protected against ischemic brain injury presumably by increasing glucose uptake in neurons (Lawrence *et al*., 1996). HSP72 is neuroprotective, and is usually expressed within the first couple of hours and days after

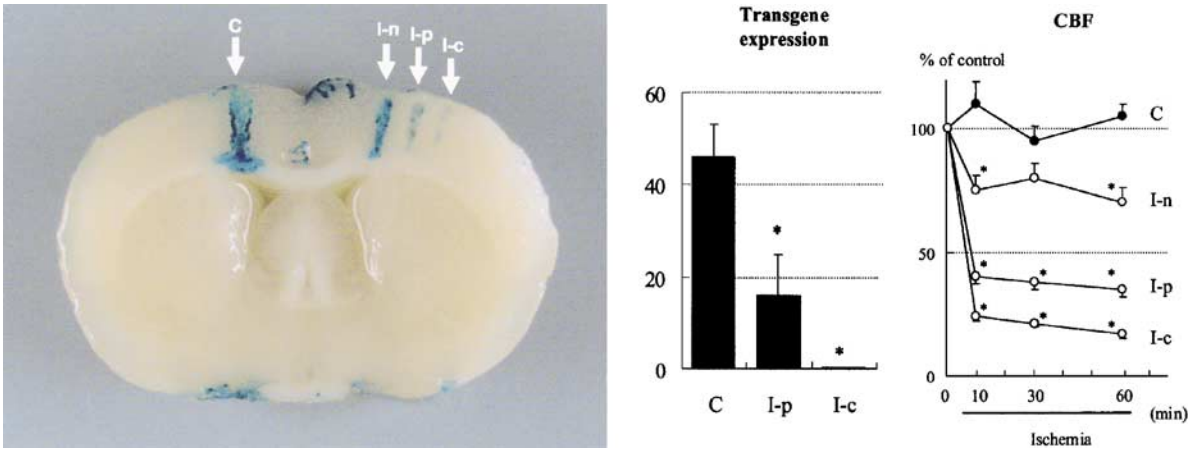

Figure 3 Adenovirus-mediated transgene expression in the ischemic brain. Revised from Ooboshi *et al*. (2001). (*Left*) X-gal staining of rat brain (coronal section) 4 days after brain ischemia and gene transfer of β-galactosidase. Arrows indicate sites of injection of vector. C, control (contralateral to ischemic side); I-n, nonischemic area; I-p, peri-ischemic area; I-c, ischemic core area. (*Center*) Quantitative analysis of transgene expression. $^*P<0.05$ vs C. (*Right*) Changes in CBF. $^*P<0.05$ vs C.

stroke. Gene transfer of HSP72 attenuated neuronal damage even when the vector was administered after the onset of stroke (Hoehn *et al*., 2001).

Proinflammatory genes during the third wave of the inflammatory cascade seem to be good candidates for gene therapy. Interleukin-1 receptor antagonist (Yang *et al*., 1997), interleukin-10 (Ooboshi *et al*., 2002), transforming growth factor-β1 (Pang *et al*., 2001), and neurotropic factors including glial cell line-derived neurotropic factor (Kitagawa *et al*., 1999) and HGF (Hayashi *et al*., 2001) all may have neuroprotective effects on brain ischemia and other mechanisms of neuronal damage. After transfer of these genes to brain parenchyma or subarachnoid space, ischemic brain injury was attenuated.

Gene transfer of antiapoptotic genes, including the proto-oncogene bcl-2 (Shimazaki *et al*., 2000), neuronal apoptosis inhibitory protein (Xu *et al*., 1997), and a redox-inducible antioxidant protein (Yang *et al*., 2001) are potentially useful for neuroprotection from ischemia. Gene transfer of cyclooxygenase-1 also is potentially useful for treating ischemic stroke, by augmenting synthesis of neuroprotective prostaglandins (Lin *et al*., 2002).

Future directions

Strategies that may lead to clinical use of cerebrovascular gene therapy include stimulation of growth of collateral vessels, and inhibition of atherogenesis, thrombogenesis, and restenosis for cervical arteries, because these approaches are already under study for coronary and limb arteries of patients. Since little information is available about the effects of these strategies on vascular morphology and function of the brain, application to intracranial arteries is unlikely in the near future. For example, based on the findings in peripheral vessels, a concern is that intracranial growth of collateral circulation might lead to vascular malformation and brain hemorrhage (Isner *et al*., 1996; Lee *et al*., 2000).

Protection against vasospasm is a very promising target for clinical use. Since experimental SAH in animals does not fully replicate vasospasm after SAH in humans, confirmation of findings in primates will be necessary.

Since there is currently no effective treatment to reduce significantly the size of strokes in humans, except possibly thrombolytic therapy, gene therapy for stroke is attractive. A serious limitation of several experimental gene therapy studies for brain ischemia, however, is that gene transfer was undertaken prior to stroke.

A critical concern for any gene therapy is safety of the vector. The death of Jesse Gelsinger during the adenovirus-mediated gene therapy trial (Teichler-Zallen *et al*., 2000) was a major setback for the area of research. The American Society of Gene Therapy (2002) announced that a child developed leukemia after gene therapy using a retroviral vector. It has been recognized that there is a potential risk of insertional mutagenesis when retroviruses are used for gene transfer. The risk of induction of malignancies by adenoviral gene transfer appears to be minimal, because the transgene remains episomal, not inserted into the chromosomes. Nevertheless, improvement of vectors is urgently needed.

It is not yet clear when gene therapy for cerebrovascular diseases will become clinically useful. It still appears likely that progress will be made in steps, and not in a great leap.

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