3. 脳卒中一般の発症予防

3-1. 脳卒中一般の危険因子の管理

(2) 糖尿病

推 奨

1. 糖尿病患者では血糖のコントロールが推奨される(グレードC1)。
2. Ⅱ型糖尿病患者では血圧の厳格なコントロールが推奨される(グレードA)。

●エビデンス

糖尿病は脳梗塞の確立された危険因子である[1-4](Ib)。Ⅱ型糖尿病では血糖のコントロールにより細小血管症(網膜症、腎症、末梢神経障害)は減少するものの、大血管症である脳梗塞は減少しない。しかし、血圧の厳格な管理により脳卒中の発症率を減少させることができる[5, 6](Ib)。

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3-1．脳卒中一般の危険因子の管理
(3) 高脂血症

推　奨

冠動脈疾患を伴う高脂血症患者にはHMG-CoA還元酵素阻害薬(スタチン)の大量投与が脳梗塞発症予防に有効である(グレードA)。

●エビデンス

海外の研究では高コレステロール血症は脳梗塞の危険因子であることが報告されている[1,2](Ⅱb)。本邦では高コレステロール血症は脳梗塞一般の確立された危険因子とは言われていない[3]。しかし、低HDL血症や低HDL／LDL比についてはアテローム血栓性脳梗塞[4-6]や脳梗塞全体[7-9]の危険因子であることが日本および海外の研究により報告されている(Ⅱb)。冠動脈疾患を対象として行われた大規模臨床試験では、Scandinavian Simvastatin Survival Study(4S)[10]、Cholesterol Recurrent Events(CARE)Study[11]、Long-Term Intervention with Pravastatin in Ischemic Disease(LIPID)Study[12]のいずれにおいても、事後解析によりスタチン大量投与(本邦の常用量の2～4倍)による高脂血症治療により脳卒中発症予防効果が認められた(Ⅰb)。また、これまでに行われたスタチンの大規模臨床試験をメタアナリシスにより解析した成績ではスタチンによる30%前後の脳卒中予防効果が示されている[13,14](Ⅰa)。しかし、4Sではシンバスタチン20～40mg、CAREではプラバスタチン40mg、LIPIDではプラバスタチン40mgが用いられており、本邦での通常の臨床用量より多く、しかも、冠動脈疾患患者が対象であった。一方、Medical Research Council(MRC)／British Heart Foundation(BHF)Heart Protection Study(HPS)[15]、Pravastatin in Elderly Individuals at Risk of Vascular Disease(PROSPER)[16]、Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial(ALLHAT-LLT)[17]では対象を冠動脈疾患の既往のある患者に限らず、冠動脈疾患以外の閉塞性血管疾患(末梢血管疾患，脳卒中など)の既往、糖尿病または高血圧症など冠動脈疾患の危険因子を有する患者も対象としている。HPSではシンバスタチン40mg投与群での全脳卒中発症の相対危険度低下率は25%で、この効果は出血性脳卒中では認められず、虚血性脳卒中では30%の低下を認めた(Ⅰb)。PROSPERでは冠動脈疾患による死亡、非致死性心筋梗塞、致死性および非致死性脳卒中が一次エンドポイントとされたが、プラバスタチン40mg投与群では一次エンドポイント全体の発症率は有意に低下したが、脳卒中の発症は有意な低下を示さなかった(Ⅰb)。ALLHAT-LLTでは高血圧症および他の1つ以上の冠動脈疾患の危険因子と中等度高コレステロール血症の両者を合併した患者を対象としたが、プラバスタチン40mg投与群では全脳卒中発症の相対危険度が9%減少したものの有意ではなかった(Ⅰb)。また、本邦で行われたKyushu Lipid Intervention Study(KLIS)[18]では脳梗塞と心筋梗塞の既往のない高脂血症患者を対象としており、プラバスタチン10～20mgにより有意ではなかったが、22%の脳梗塞相対危険度の低下を認めた(Ⅰb)。また、同じく本邦で行われたPravastatin Anti-atherosclerosis Trial in the Elderly(PATE)[19]では高脂血症の高齢者を対象としてプ

ラバスタチン5mg(低用量)もしくは10～20mg(標準用量)の効果が検討されたが、血管イベント全体の発生率は低用量群に比して標準用量群で有意に低かったものの，脳梗塞の発生率は両群で有意差を認めなかった(Ib)。したがって、現時点では冠動脈疾患の既往を有さない患者におけるスタチンの脳卒中発症予防効果については確立されているとは言えない。

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3. 脳卒中一般の発症予防

3-1. 脳卒中一般の危険因子の管理

(4) 心房細動

推 奨

1. 脳卒中の危険因子として、脳卒中・一過性脳虚血発作(TIA)の既往、高齢(70～75歳以上)、心不全、高血圧の既往、冠動脈疾患、糖尿病のいずれかを合併した非弁膜症性心房細動(NVAF)患者にはワルファリンが推奨される(グレードA)。
2. ワルファリン療法の強度は、一般的にはinternational normalized ratio(INR) 2.0～3.0が推奨されるが、高齢のNVAF患者ではINRを1.6～2.6にとどめることが推奨される(グレードA)。
3. 脳卒中の既往や危険因子がないNVAF患者、もしくはワルファリンが禁忌のNVAF患者にはアスピリンが推奨される(グレードB)。

●エビデンス

NVAFは脳梗塞の危険因子である。NVAF患者の脳梗塞発症率は平均5％／年であり、心房細動のない人々の2～7倍高い[1-3](Ⅱb)。平成12年度厚生科学研究費による脳梗塞急性期医療の実態に関する研究によれば、発症後7日以内に入院した脳梗塞患者の20.8%に心房細動を合併していた[4](Ⅲ)。これまでにNVAF患者を対象に脳卒中の予防を目的として行われた抗血栓療法の大規模臨床試験をメタアナリシスした成績によれば、用量調節法によるワルファリン療法はきわめて有効であり、プラセボに対して61%(95%信頼区間47～71%)の脳卒中予防効果がある[5](Ⅰa)。アスピリンはワルファリンよりも劣るが、24%の脳卒中予防効果がある[5, 6](Ⅰa)。NVAF患者における脳卒中の危険因子は、脳卒中または一過性脳虚血発作の既往、高血圧の既往、うっ血性心不全、加齢、糖尿病、冠動脈疾患であり、これらのうち、いずれかの危険因子を有するNVAF患者ではアスピリンによる脳卒中予防効果は期待できないので、ワルファリンを投与すべきであるとされた(Ⅰa)[7-10]。ワルファリン療法は脳卒中の予防効果があり、なおかつ重篤な出血合併症を最小限にしうる強度を目標値として設定すべきであるが、虚血性脳卒中と出血性脳卒中を合計した全脳卒中を最小限にしうるワルファリンの強度はINR2.0～3.0の範囲なので、一般的にはこの範囲の強度のワルファリン療法が推奨される[11, 12](Ⅰa)。しかし、高齢者ではワルファリンによる重篤な出血合併症(頭蓋内出血と頭蓋外の大出血)のリスクが大きいので、ワルファリンの強度をINR 1.6～2.6に下げたほうがよいと考えられる[8-10, 13, 14](Ⅰa-Ⅱa)。本邦においても日本循環器学会研究班による非弁膜症性心房細動におけるアスピリンによる症候性脳梗塞および一過性脳虚血発作の予防効果に関する大規模臨床試験(JAST：Japan Atrial fibrillation and Stroke Trial)が行われ、最終結果はまだ論文として発表されていないが、脳梗塞の発症率はアスピリン投与群と非投与群で差がなく、重篤な出血合併症はアスピリン投与群で非投与群より有意に多く、虚血性イベントと出血性イベントを合計した発症率もアスピリン投与群で非投与群より多かった[15]。

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3. 脳卒中一般の発症予防

3-1. 脳卒中一般の危険因子の管理

(5) 喫煙

推 奨

喫煙者には禁煙が推奨される(グレードA)。

●エビデンス

喫煙は欧米において脳卒中の危険因子であることが報告されており[1,2]、日本を含む各国で行われた32の研究のメタアナリシスでも喫煙は脳卒中の有意な危険因子であることが示されている[3](Ⅱb)。また、このメタアナリシスの病型別解析によれば、喫煙は脳梗塞とクモ膜下出血の有意な危険因子であり、脳出血の有意な危険因子ではなかった[3](Ⅱb)。本邦においても、男性では20本／日以上の喫煙が脳梗塞の危険因子である[4]ことや、ラクナ梗塞の危険因子である[5]ことが報告されている(Ⅱb)。脳卒中のリスクは喫煙本数が多いほど大きくなり[1-3](Ⅱb)、禁煙によりリスクは低下する[2,6](Ⅱa-Ⅱb)。

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3. 脳卒中一般の発症予防

3-1. 脳卒中一般の危険因子の管理

(6) 飲酒

推 奨

脳卒中の予防には大量の飲酒を避けるべきである(グレードB)。

●エビデンス

出血性脳卒中(脳出血やクモ膜下出血)の発症率と飲酒量との間は直線的な正の相関関係がある[1-4](Ⅱb)。一方、虚血性脳卒中の発症率は非飲酒者に比べて小量飲酒者では低く、大量飲酒者では高い[3-5](Ⅱb)。したがって、出血性脳卒中と虚血性脳卒中の共通の危険因子である大量飲酒は避けるべきであるとされた[6]。

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3. 脳卒中一般の発症予防

3-2. 脳卒中ハイリスク群の管理

(1) 無症候性脳梗塞

推　奨

無症候性脳梗塞患者では高血圧の管理が推奨される(グレードB)。

●エビデンス

無症候性脳梗塞を認めた脳ドック受診者は認めなかった受診者よりも脳卒中発症率が高い[1] (IIa)。無症候性脳梗塞はラクナ梗塞と脳出血の危険因子と考えられ、脳卒中の発症は高血圧合併例で非合併例より多い[1] (IIa)。縦断的コホート研究であるRotterdam Studyでは無症候性脳梗塞および脳室周囲高信号域、白質病変が脳梗塞の発症を有意に増加させた[2] (IIa)。さらに最近では明らかな無症候性脳梗塞のみならず、無症候の白質病変も脳卒中の予知因子となるという報告が本邦からも散見される。

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3-2. 脳卒中ハイリスク群の管理
(2) 無症候性頸動脈狭窄

推　奨

高度(60%以上)の無症候性頸動脈狭窄には抗血小板療法をはじめとする最善の内科療法に加え、手術および周術期管理に熟達した施設では頸動脈内膜剥離術(CEA)が推奨される(グレードA)。

●エビデンス

狭窄率60%以上の無症候性頸動脈狭窄例では抗血小板療法をはじめとする最善の内科的治療のみよりも内科的治療に加えてCEAを行ったほうが脳卒中発症率が低い[1](Ib)。ただし、無症候性頸動脈狭窄例に対するCEAの適応には周術期合併症が3%未満の高い治療技術水準が要求される[2,3](Ib)。

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Gene therapy for cerebral arteries

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Abstract

Cerebrovascular disease is the leading cause of disabled people in Japan and Western countries. Gene transfer technique is a promising approach for the treatment of severe types of cerebrovascular disease. Cerebral blood vessels could be targeted by gene therapy in several pathological conditions. Perivascular approaches of gene transfer to the cerebral arteries through the subarachnoid space may prevent brain ischemia caused by vasospasm after subarachnoid hemorrhage. In the setting of brain ischemia, post-ischemic gene therapy could be effective in the attenuation of ischemic damage in both global and focal brain ischemia. Thus, gene transfer to cerebral blood vessels and ischemic brain tissues would be one of the future therapeutic approaches to cerebrovascular disease.

Keywords: Gene therapy; Gene transfer; Brain; Ischemia; Adenovirus

1. Introduction

Gene transfer is an attractive method for studies of vascular and neuronal biology [1,2]. Development of the technique has provided clinical usefulness of gene therapy for cardiovascular disease [3]. Furthermore, recent studies suggest that cerebrovascular disease, the leading cause of disable persons in Japan and Western countries, could be treated by gene therapy [4]. Cerebral blood vessels are important targets of treatment of the disease. In this article, we describe possibilities of gene therapy for cerebrovascular disease, with special focus on the cerebral arteries.

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2. Adenoviral vectors

Several vectors have been used in the experimental studies of gene therapy, and each vector has advantages and disadvantages. One vector which is commonly used in the cardiovascular clinical trials is the recombinant adenovirus [5]. The adenoviral vectors consist of 36 kb DNA, accommodate a cDNA insert up to ~ 8 kb, and can be grown to high titers. Advantages of this vector are a wide host range, low probability of mutagenesis, and a high degree of successful gene transfer [6,7]. Therefore, we mostly describe recent works on the adenovirus-mediated gene transfer to the cerebral circulations.

3. Gene transfer to cerebral blood vessels

Because the primary lesion of cerebrovascular disease is the vasculature, cerebral arteries are important targets in acute and chronic treatment of the disease. As for the gene transfer to cerebral arteries, the intravascular administration of vectors would be an ideal approach as used in the peripheral vessels [8]. However, there is a concern about the needs of transient cessation of blood flow during gene transfer to cerebral

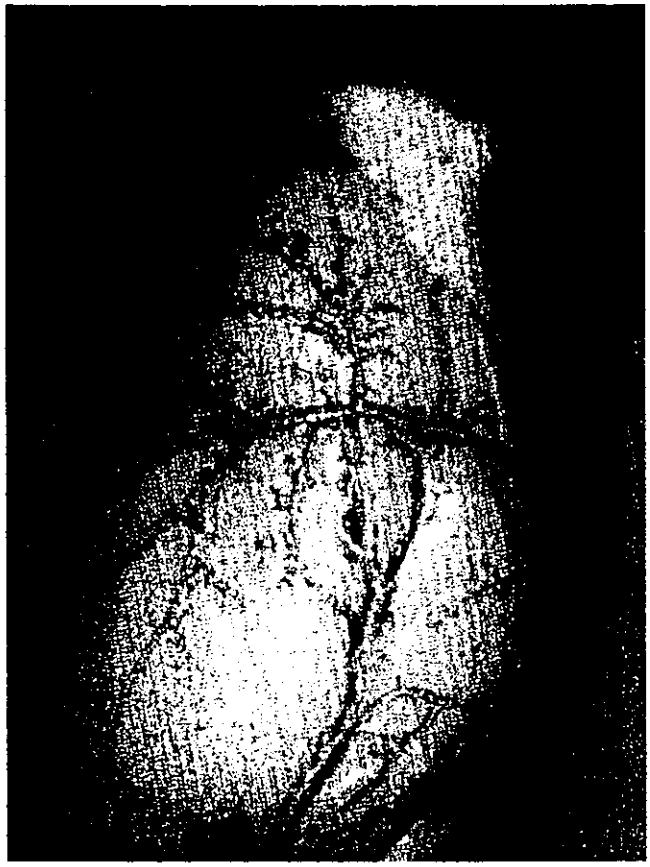

Fig. 1. Histochemical examination of the rat brain 1 day after the adenoviral vector encoding β-galactosidase gene was injected into the cisterna magna. Marked expression of the reporter gene was observed on the surface of the brain, especially along the middle cerebral artery.

arteries in order to contact vectors with vessels. Therefore, we have worked on the strategy to minimize such problems. One approach is the perivascular approach [9,10]. When vectors were administered via the cisterna magna, transgene expression was obtained around the major cerebral arteries (Fig. 1) and the adjacent tissue [11,12]. Therefore, these perivascular approaches may be feasible when the target of gene transfer is the major cerebral vessels.

One of the most suitable applications of perivascular gene therapy is the prevention of vasospasm after subarachnoid hemorrhage. Because vasospasm occurs several days after bleeding and the following brain ischemia is severe, gene therapy seems suitable for this disorder. The efficacy of gene transfer to the cerebral blood vessels after subarachnoid hemorrhage was acceptable even in the presence of subarachnoid clotting [13]. Currently, transfer of the gene for vasodilatation, quenching of superoxide, or inhibition of inflammatory process using the perivascular approach has been reported as effective in preventing vasospasm [14–17]. Therefore, perivascular gene therapy to the cerebral blood vessels may be useful in treatment of subarachnoid hemorrhage.

Another alternative method for gene transfer to the cerebral circulation is the shortening of the contact period by improving efficiency of gene transfer. Combination of specific materials with vectors has enhanced the efficiency in vitro and in vivo [18]. Technological progresses in this area, including modulation of vectors, would lead to practical uses of the intravascular approach for gene transfer to cerebral arteries.

4. Gene transfer to ischemic brain

Recently, brain infarction has been proposed for a target of gene therapy [4]. Because most gene transfer approaches depend on the host machinery of gene expression and protein synthesis is impaired in ischemic conditions, it is important to clarify how gene transfer is affected by ischemia in the brain. Previous reports revealed that protein synthesis began to decline when cerebral blood flow (CBF) fell to less than 50% [19]. Therefore, we examined effects of focal brain ischemia on transgene expression with the photothrombotic ischemia model occluding the distal middle cerebral artery of spontaneously hypertensive rats with a platelet-rich thrombus. Our model has produced a consistent size of brain infarction [20]. When the adenoviral vector was directly injected into the ischemic and non-ischemic regions after induction of focal ischemia [21], transgene expression in the ischemic core was poor, but the peri-ischemic area, the presumable ischemic penumbra in our model, revealed the relatively good expression of the transgene. Analysis of blood flow revealed that CBF threshold for transgene expression was approximately 40% of the resting value. Our results suggest that gene transfer to the ischemic penumbra area may be feasible for treatment of brain infarction.

Although several gene transfer approaches were reported to protect against brain ischemia in different models (Table 1), usefulness of post-ischemic gene therapy for brain ischemia has not been established. Because direct gene transfer to the ischemic core seems inefficient, one alternative approach would be the gene transfer to the non-ischemic area where the transgene expresses releasable or diffusible products [22,23].

Table 1
Protection of brain ischemia by gene transfer

Gene	Vector	Ischemic model	Authors	Year
IL-1 ra	Adenovirus	Focal ischemia	Betz et al.	1995
bcl2	Herpes virus	Focal ischemia	Linnik et al.	1995
glucose transporter	Herpes virus	Focal ischemia	Lawrence et al.	1996
NAIP	Herpes virus	Focal ischemia	Xu et al.	1997
hsp72	Herpes virus	Focal ischemia	Yenari et al.	1998
GDNF	Adenovirus	Focal ischemia	Kitagawa et al.	1999
SAG	Adenovirus	Focal ischemia	Yang et al.	2001
HGF	HVJ-liposome	Global ischemia	Hayashi et al.	2001
TGF-ss1	Adenovirus	Focal ischemia	Pang et al.	2001
calbindin D28K	Herpes virus	Focal ischemia	Yenari et al.	2001
COX-1	Adenovirus	Focal ischemia	Lin et al.	2002

IL-1 ra: interleukin-1 receptor antagonist, NAIP: neuronal apoptosis inhibitory protein, hsp: heat shock protein, GDNF: glial cell line-derived neurotrophic factor, SAG: sensitive to apoptosis gene, HGF: hepatocyte growth factor, TGF: transforming growth factor, COX: cyclooxygenase, HVJ: hemoagglutinating virus of Japan.

This approach may overcome the current limitations by releasing sufficient amount of products in spite of the limited area of transfection. Gene transfer to the lateral ventricle provides prominent gene expression at the ventricular wall [9], and gene transfer of interleukin-1 receptor antagonist to the ventricular wall was reported to reduce infarct size [24]. Therefore, the gene transfer of anti-inflammatory cytokines or growth factors with this approach may be a promising treatment of brain ischemia. We have recently obtained a reduction of brain infarction by the post-ischemic gene transfer of anti-inflammatory cytokine, interleukin-10 [25]. Other recent reports have also suggested protective effects against ischemic damages in focal ischemia models [26,27]. Therefore, post-ischemic gene therapy may be effective in the attenuation of ischemic damage.

5. Conclusion

Gene transfer approaches for the treatment of cerebrovascular disease, including subarachnoid hemorrhage and brain ischemia, have been shown as promising in the experimental settings. Although safety issues must be cautiously evaluated in several conditions, it appears that cerebrovascular disease is a potential target of gene therapy.

Acknowledgements

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Adenovirus-mediated gene transfer to ischemic brain

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Abstract

Gene therapy may be a promising approach for treatment of brain ischemia. Expression of transgene is affected by several factors, including host conditions and intervals after gene transfer. Therefore, we studied how those factors affect adenovirus-mediated gene transfer to the ischemic brain. The expression after gene transfer to the lateral ventricles was augmented by brain ischemia. Moreover, transgene expression in the transfected brain tissue in aged rats was coordinated or augmented as compared with that in adult rats. Thus, brain ischemia is a potential target of gene therapy.

Keywords: Gene transfer; Ischemic brain; Adenovirus

1. Introduction

Gene transfer is a promising method for the treatment of cardiovascular disease. Several clinical gene transfer trials have been undertaken, some of which have shown good outcomes [1]. Ischemic stroke is a major cause of death in elderly populations. Although recent experimental studies suggest that stroke could be treated by gene therapy [2–4], there are no enough number of studies that clarify efficiency of gene transfer to the ischemic brain. Therefore, we examined several factors that would affect adenovirus-mediated gene transfer to the ischemic brain of rats.

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2. Methods

Spontaneously hypertensive rats (SHR) were used in the present study. Surgical procedures used in this study were performed as previously described [5]. Rats were anesthetized with halothane. The right femoral artery and vein were cannulated for monitoring mean arterial pressure (MAP) or sampling blood, and for the injection of agents, respectively. The rats were endotracheally intubated and mechanically ventilated after intravenous injection of pancuronium bromide. Rectal and head temperatures were maintained at 37 °C and 36 °C, respectively, by means of a heating pad and a warming lamp. Rats were mounted on a stereotaxic head holder, and burr holes were drilled in the bilateral parietal and the right temporal skull bone. Cerebral blood flow (CBF) was measured by laser Doppler flowmetry. Brain ischemia was produced by photochemical occlusion of the distal middle cerebral artery (MCA), as described previously [6]. In the present gene transfer study, we used a replication-deficient recombinant adenovirus carrying bacterial β-galactosidase (AdCMVβGal) as a reporter gene. Ninety minutes after the right distal MCA occlusion, the viral suspension was injected into the contralateral ventricle, or the bilateral parietal regions. The burr holes were then covered with bone wax, and the scalp was sutured. The rats were carefully weaned from the respirator, then were returned to their home cages. After the designated survival periods, the rats were anesthetized with amobarbital (i.p.) and perfused transcardially with 2% paraformaldehyde and 0.2% glutaraldehyde in PBS. Then, the brain was removed. Transgene expression in the brain tissue was analyzed by histochemical assay, X-Gal staining and by biochemical assay, Aurola GAL-XE.

3. Results

3.1. Effect of ischemia on gene transfer to brain

We compared the time course of transgene expression after the delivery of vectors injection into the lateral ventricle between sham group and ischemia group. The expression was observed in the bilateral ventricle from 6 h after ischemia, and reached the peak 12 h after ischemia in both groups. In the ischemia group, expression of the reporter gene was detected until 7 days after ischemia in some rats, whereas the expression almost disappeared within 7 days in the sham group (Fig. 1). In the quantitative biochemical assay, focal brain ischemia augmented adenovirus-mediated transgene expression in the bilateral periventricular area, where ischemia was not induced in our model. Therefore, it was suggested that gene transfer to the ependyma might be a promising approach for the treatment of brain ischemia.

3.2. Effect of aging on gene transfer to brain

We determined transgene expression after gene transfer to contralateral control area (C), ipsilateral peri-ischemic area (I-p) and ischemic core area (I-c) in the parietal cortices. The expression in the aged rats was coordinated as compared with that in adult

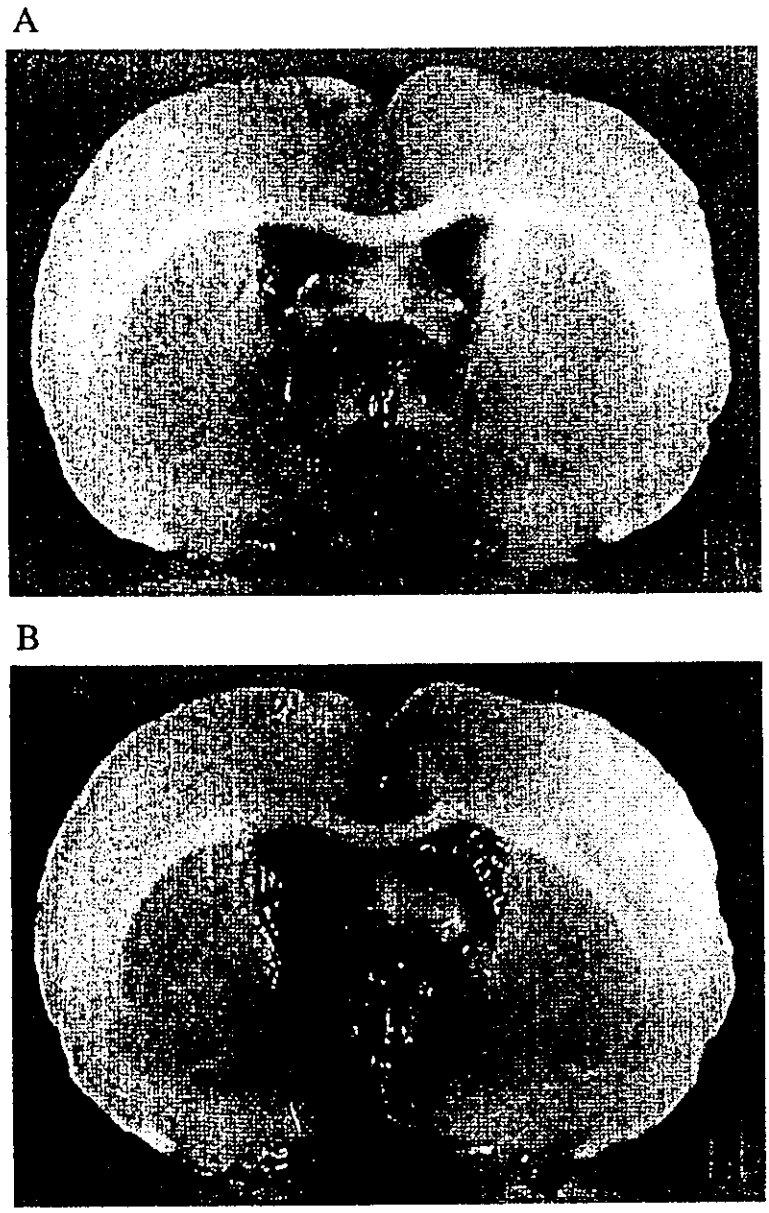

Fig. 1. Histochemical staining of the rat brain after brain ischemia and gene transfer to ventricles. (A) Sham rat; (B) ischemia rat. R: right (ischemic) side. Transgene expression in the ischemia group (B) was augmented as compared with that in the sham group (A).

rats at C and I-p from 1 to 7 days after ischemia (Fig. 2). Moreover, quantitative analysis revealed that the expression at I-c in the aged rats was increased as compared with that in the adult rats 7 days after ischemia. Therefore, it was suggested that adenovirus-mediated gene transfer to the ischemic brain would provide effective transgene expression in aged rats.

Elder populations are susceptible to ischemic stroke [7], and the age-related vulnerability to brain ischemia has been reported [8–10]. Using a transient forebrain ischemia model, we have previously shown that the ischemic damages of the striatum and hippocampus in aged rats were significantly greater than those in adult ones [8], although the reduction of CBF was not different between aged and adult rats. The present study

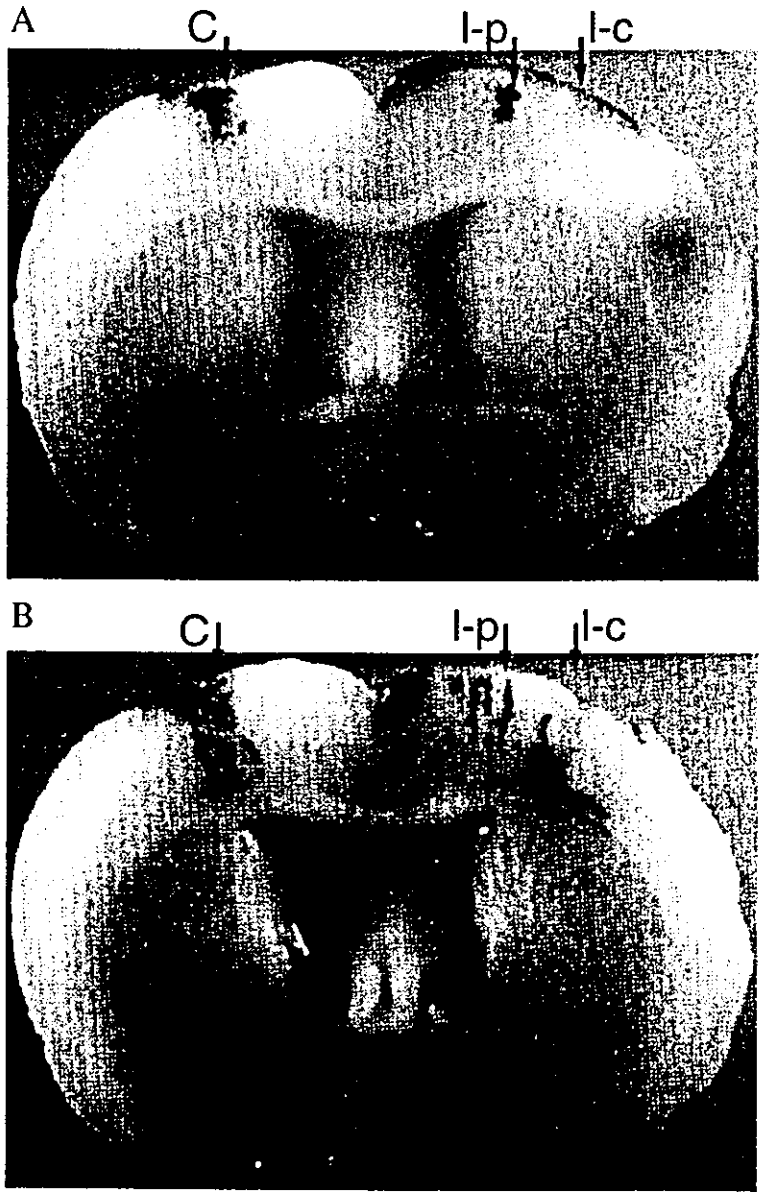

Fig. 2. Histochemical staining of the rat brain 7 days after brain ischemia and gene transfer to parietal cortices. (A) Adult rat; (B) aged rat. C: contralateral control area; I-p: peri-ischemic area; I-c: ischemic core area. Transgene expression at I-c in the aged group (B) was augmented as compared with that in the adult group (A).

provides useful information for the investigation of the pathophysiology of brain ischemia in the aged.

4. Conclusions

Brain ischemia is a potential target of gene therapy. Although safety issues must be evaluated and additional research should be performed, adenovirus-mediated gene transfer may provide a promising treatment for stroke.