·Basic Research·

Electrophysiological evaluation of the safety of injection of tissue plasminogen activator into optic nerve in rabbits

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Abstract

• AIM: To investigate the safety of tissue plasminogen activator (tPA) intra optic nerve injection in rabbits.

• METHODS: Group 1 and 2 (6 eyes in each group) received injection of tPA 25μ g and 12.5μg in 0.1mL balanced saline solution (BSS). Group 3 (6 eyes) received injection of 0.1mL BSS. Six eyes in Group 4 as a normal control received no injection. The eyes were examined with slit lamp biomicroscope, indirect ophthalmoscope, visual evoked potentials (VEP) and electroretinography (ERG) at 1, 3, 7, 14 and 28 days after injection.

• RESULTS: No evidence of optic nerve or retinal toxicity or physical damage were revealed by ophthalmoscopy, VEP, and ERGs after the injection of tPA into the optic nerve. The means of the latency of the first peak of the VEP were 24.6± 1.5, 24.1± 1.9, 24.0± 2.0 and 24.6± 1.3mS respectively for the above specified groups (P=0.4112). The means of the amplitude of the first peak of the VEPs were 124± 42, 145± 41, 132± 48 and 117± 29μV respectively (P=0.0649). The means of the latency of a-waves were 6.0± 0.4, 5.9± 0.4, 5.9 ± 0.5 and 5.8 ± 0.3mS respectively (P=0.6279). The means of the amplitude of a-waves were 110± 14, 112± 15, 110± 16 and 108 11μV respectively (P=0.7248). The means of the amplitude of b-waves were 151± 12, 148± 14, 144± 16 and 141 20μV respectively (P=0.0957).

• CONCLUSION: Injection of tPA upto 25μg in 0.1mL into optic nerve is well tolerated.

• KEYWORDS: retinal vein occlusion; tissue plasminogen activator; intra optic nerve injection; VEP; ERG

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INTRODUCTION

Central retinal vein occlusion (CRVO) has long been recognized as one of the most common causes of severe visual loss, but the treatment of CRVO is still a challenging task [1-3]. There is no proven effective treatment for CRVO so far. Medical treatment strategies have mostly aimed to manage the sequelae of CRVO. The only established management, laser photocoagulation, targets the neovascular complications of CRVO. Grid laser photocoagulation and the current pharmacological intervention such as Avastin application mainly focus on macular edema with limited visual acuity improvement. Laser-induced chorioretinal anastomosis attempting to bypass venous obstruction has been performed with limited success and significant complications. The unsatisfactory results of medical treatment led to the development of surgical treatment focus on the occluded retinal vein. However, radial optic neurotomy remains a controversial method of treatment for CRVO[4-8].

The increasing understanding of hypercoagulability in patients with CRVO promotes the use of antithrombotic drug in the treatment of this disease [9]. The systemic administration of tPA to treat RVO has shown some beneficial effects, but was associated with vitreous hemorrhage and serious systemic complications, including patient mortality [10]. Systemic administration of tPA, therefore, is currently not recommended as a treatment for RVO. Intravitreal administration of tPA, which avoids systemic drug exposure, has been performed with no serious side effects [11]. However, an experimental study in rabbits

showed that intravitreal tPA does not diffuse through the neural retinal in the absence of a retinal damage, and no study proofed that intravitreal tPA can diffuse into the retinal vein in RVO animal model. A recent study showed that retinal toxicity associated with intravitreally injected tPA in a dose-dependent manner and was exacerbeted in CRVO eyes[12]. Vitrectomy with retinal vein cannulation and infusion of tPA was performed with some success, but the procedure involves very difficult surgical skill as well as instrumentation and was also reported with complications, such as vitreous hemorrhage, retinal detachment and some other potential complications [13-15]. We recently have shown that injection of tPA into optic nerve increased the incidence of recanalization of the occluded vessels in an experimental rabbit model of RVO, suggesting that injection of tPA into the optic nerve may have a potential benefit in the treatment of CRVO [16]. The purpose of this study is to investigate the safety, by electrophysiological methods, of injection of tPA into the optic nerve in rabbits.

MATERIALS AND METHODS

Materials A total of 24 Japanese albino rabbits (Hokusetsu Co. LTD, Japan), weighing 2.0 to 2.5kg, were used in this study. All experiments were performed in the accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the policies in the Guide to the Care and Use of Laboratory Animals by the National Institute of Health.

Intra-optic-nerve tPA Injection Rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (32mg/kg) and xylazine hydrochloride (4mg/kg) for all procedures. Fundus examination was performed to exclude any abnormality and fundus photographs (TRC-50IX, Topcon, Japan) were taken to record the pretreatment fundus status. Then, the animals were randomly divided into three groups: Group 1 (6 eyes) received injection of tPA 25μg in 0.1mL balanced saline solution (BSS) into the optic nerve; Group 2 (6 eyes) received injection of tPA 12.5μg in 0.1mL BSS; Group 3 (6 eyes) received injection of 0.1mL BSS; Group 4 (6 eyes) received no injection as normal control. Either tPA or BSS was injected into the center of the optic disc with a 30 gauge needle (Becton Dickinson) through the pars plana and vitreous. The depth of the injection was about 1mm, and the needle stayed in the optic nerve for one minute before being drawn out.

Rabbits were examined before injection, and at 1, 3, 7, 14 and 28 days after injection. Ophthalmic examination included slit lamp biomicroscopy, indirect ophthalmoscopy and fundus photography.

Visual Evoked Potential Recording Under general anesthesia, recording cortical electrodes were implanted and VEP were recorded as described previously [17]. The skull was exposed through a skin incision at the top of head along the midline, and a hole was drilled over the visual cortex contralateral to the eye. The hole was 8mm anterior to the lambda suture and 7mm lateral to the midline. A screw-type stainless steel electrode, coated with silver, was screwed into the skull to contact the dura mater. The reference electrode was placed 16mm anterior to the lambda point in the midline. Grounded was provided by an electrode on the body. The eyes were dilated with topical 5g/L tropicamide (Mydrin-P; Santen Pharmaceutical Co., Japan), and the corneas were anesthetized with topical 5g/L proparacaine hydrochloride (Santen Pharmaceutical Co.). The stimuli were obtained from a stroboscopic unit (LS-704B: Nihon Kohden, Tokyo, Japan) controlled by a stimulator (SLS-3100: Nihon Kohden). The strobe unit was placed 15 cm in front of the rabbits' eyes. The energy of the light was set at 1.2 J, and 50 VEP elicited by a 1Hz light stimulation were averaged and displayed on a digital storage oscilloscope (Neuropack 2, MEB-7202, Nihon Kohden). Measurements were carried out 3 days before injection of tPA, and at 1, 3, 7, 14, and 28 days after injection. Before each recording, dark adaptation for 30 minutes was allowed. The latency and amplitude of the first peak were measured as an indicator of visual function.

Electroretinogram Recording Under general anesthesia, ERGs were recorded as described previously[18]. A gold-ring active electrode for recording ERGs, which was attached to a contact lens and placed on the cornea, was obtained from Nippon Contact Lens, Tokyo, Japan (base curve: 7.20mm, size: 12mm). The stimuli were obtained with the same unit used for VEP. The strobe unit was placed 15cm in front of the rabbits' eyes. The energy of the light was set at 2.0 J and recordings were made from the active electrode on the cornea by averaging four responses to the light stimulus at 0.1Hz. Measurements were carried out 3 days before injection of tPA, and at 1, 3, 7, 14, and 28 days after injection of tPA. Before each recording, dark adaptation for 30 minutes was allowed. The latency and amplitude of a-

Table 1 Amplitude of the first peak of VEP (mean±SD)

tPA	Latency						Mean
	BO	PO-1d	PO-3d	PO-7d	PO-14d	PO-28d	
Amplitude:25μg	125±50	122±42	142±47	116±40	118±51	120±32	124±42
12.5μg	138±40	133±33	146±40	144±53	152±48	162±50	145±41
BSS	134±45	123±36	147±57	126±54	141±60	122±56	132±48
Control	123±28	122±32	114±42	114±34	112±29	115±30	117±29
Latency:25μg	24.0±2.2	24.6±1.5	25.1±1.1	25.1±1.6	24.2±1.6	24.7±1.4	24.6±1.5
12.5μg	23.5±2.0	23.6±3.0	24.5±1.4	24.4±2.3	24.3±1.1	24.4±1.6	24.1±1.9
BSS	23.8±2.3	23.4±3.2	24.4±1.1	23.9±2.3	24.4±1.2	24.4±1.3	24.0±2.0
Control	25.4±1.5	24.6±1.6	24.4±0.5	24.3±1.8	24.6±0.8	24.3±1.3	24.6±1.3

BO: Before operation; PO: Post operation

Table 2 A & B waves of the ERG (mean±SD)

tPA	latency						Mean
	BO	PO-1d	PO-3d	PO-7d	PO-14d	PO-28d	
Latency of a-waves:25μg	6.1±0.4	6.0±0.3	6.0±0.4	5.8±0.3	6.0±0.6	5.9±0.6	6.0±0.4
12.5μg	5.8±0.5	6.1±0.6	6.0±0.3	5.8±0.3	5.7±0.2	5.8±0.3	5.9±0.4
BSS	5.8±0.4	6.1±0.6	6.0±0.5	5.9±0.6	5.7±0.2	5.8±0.5	5.9±0.5
Control	6.0±0.3	6.0±0.5	5.9±0.3	5.6±0.3	5.7±0.2	5.8±0.3	5.8±0.3
a-wave amplitudes:25μg	120±20	107±11	102±7	112±12	110±20	110±9	110±13.9
12.5μg	112±21	111±17	109±13	112±14	112±17	116±14	112±15
BSS	119±19	103±10	105±13	108±14	115±18	108±21	110±16
Control	109±16	108±12	99±2	116±6	111±13	102±7	108±11
b-wave amplitudes:25μg	158±11	155±10	140±8	154±8	146±12	152±14	151±12
12.5μg	147±17	150±18	143±9	140±11	152±11	153±13	148±14
BSS	158±12	137±14	139±19	146±15	147±21	140±15	144±16
Control	140±30	143±16	147±23	148±20	133±19	135±23	141±20

BO: Before operation; PO: Post operation

and b-waves were measured as an indicator of visual function.

Statistical Analysis Data were expressed as mean ±SD. Two-way interactions were analyzed by repeated-measures analysis of variance (ANOVA), and statistical comparisons between two groups were done with Student's *t*-test. $P<0.05$ was considered as statistical difference.

RESULTS

No clinical signs of toxicity after tPA injection into the optic nerve were observed during the follow-up. Slit-lamp biomicroscopy showed that the cornea, aqueous, and crystalline lens remained transparent. Indirect ophthalmoscopy showed that no morphologic or color changes of the optic disc and retina at all time points. A comparison of the VEPs recorded from the different groups shows very little difference in the latency and amplitude of the first peak (Latency: $P=0.4112$; Amplitude: $P=0.0649$, Table 1).

A comparison of the ERGs recorded from the different groups shows very little difference in the latency and amplitude of the a and b wave (A wave latency: $P=0.6279$; A wave amplitude: $P=0.7248$; B wave amplitude: $P=0.0957$, Table 2).

There were no severe complications such as retinal detachment, vitreous hemorrhage, or laceration of major retinal and optic vessels. The only complication that we experienced was a little hemorrhage located at the site of injection, but this disappeared spontaneously within 2 weeks after injection.

DISCUSSION

In this experiment, we evaluated the safety of tPA injection into the optic nerve. The data of our study showed that no apparent evidence of optic nerve or retinal toxicity or damage was observed with ophthalmoscopy, VEPs, ERG following injection of tPA into the optic nerve. There were no severe complications and the only complication that we experienced was a little hemorrhage located at the site of injection. These results suggest that injection of tPA into the optic nerve is a simple, safe and feasible procedure. Penetration with 30G needle causes apparently less traumatic damage to the optic nerve when compared with radial optic neurotomy (RON) procedure in which an incision is made in the scleral ring, cribriform plate, and adjacent sclera at the nasal edge of the optic disc. During the intra optic nerve injection, the needle stayed in the optic

nerve for one minute before being drawn out to reduce the back flow into vitreous.

In this study, the comparisons of the VEPs recorded from the different groups shows very little difference in the latency and amplitude of the first peak (Latency: P=0.4112; Amplitude: P=0.0649). Also, the comparisons of the ERGs recorded from the different groups shows very little difference in the latency and amplitude of the A and B wave (A wave latency: P=0.6279; A wave amplitude: P=0.7248; B wave amplitude: P=0.0957).

In summary, injection of tPA into the optic nerve seems to be a simple, safe and feasible procedure without obvious toxicity or other damage to optic nerve or retina, and may have a potential usage for the treatment of CRVO.

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