·Basic Research·

A feasibility study of recombinant adeno-associated virus as a vector for transferring a target gene to retina

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Abstract

• AIM: To study the feasibility of recombinant adeno-associated virus (rAAV) as a vector to transfer the green fluorescent protein (GFP) gene as a target gene into rabbit retina.

• METHODS: Intravitreal injection of rAAV-gfp was performed in either eye for each rabbit with the other eye taken as control. At the 3rd, 7th, and 14th day after injection, the eyeballs were removed, and the retinas were flat-mounted on glass slides to inspect the retinal fluorescence, respectively.

• RESULTS: After intravitreal injection of rAAV-gfp, the presence of fluorescent spots in the cytoplasm of retinal cells indicated that GFP gene was efficiently transferred and expressed in the rabbit retina.

• CONCLUSION: Recombinant adeno-associated virus is a reliable and simple vector for transferring target gene, e.g., GFP gene, to the retina.

• KEYWORDS: recombinant adeno-associated virus; green fluorescent protein gene; gene transfer; retina

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INTRODUCTION

Optic nerve lesion is an important factor which influences the therapeutic efficacy of glaucoma. As an alternative to drugs, gene therapy has been used in treating some kinds of the human diseases and may be equally effective in the treatment of glaucoma. Gene therapy fulfills two distinct purposes. First, it serves to replace the abnormal (diseased) gene *in vivo* with the normal gene constructed *in vitro*. Second, it increases the expression of a special gene that can not otherwise express enough corresponding protein so that therapeutic effect is achieved. Moreover, it has been demonstrated that genes extrinsic to the human body can be introduced by special vectors. Recombinant adeno-associated virus (rAAV) is such a gene vector [1]. Its molecular weight is small, and it has no pathogenicity. It can transfect cells that are in both the mitotic and quiescent stages. The gene carried by the adeno-associated virus (AAV) vector can be transferred into the chromosome of the target cell. In this way, the target gene can be expressed for a long time, particularly since rAAV has been proved to be a safe and effective vector [1-3]. To determine whether rAAV can carry and transfer a target gene, e.g., green fluorescent protein (GFP) gene, we injected rAAV-gfp into the vitreous body of rabbits and observed the expression of the GFP gene in the retina.

MATERIALS AND METHODS

Titer of rAAV -gfp rAAV-gfp was supplied by Xi'an Huaguang Bioengineering Company Limited (Xi'an, China). The titer of the rAAV-gfp was 2.25×10^{10}cfu/mL. Experimental Animals and the Procedure: Eighteen New Zealand white rabbits, either male or female and each weighing 1.8-2.5kg, were supplied by the Experimental Animal Center of Xi'an Jiaotong University. One of the bilateral eyes of each rabbit was used as the test eye, and the other eye as control. After shearing the upper-temporal bulbar conjunctiva from 2mm behind the corneal limbus, a 6G needle was injected into the vitreous body at an angle of 40-60 degrees. 10μL rAAV-gfp was injected into the vitreous body in front

of the posterior pole of the central retina by microsyringe, while 10μL physiological saline was injected into the vitreous body of control eyes. Antibiotic eye drops were used after the procedures for 3 days. With each time course consisting of a group of 6 rabbits, bilateral eyes were excised and flat-mounted on glass slides to detect the retinal fluorescence intensity at the 3rd, 7th, and 14th day post-injection, respectively.

Retina Whole Flat-mount Preparations and Fluorescence Observation Intramuscular anaesthesia was performed by using 50mg/kg ketamine hydrochloride. Saline was perfused via ascending aorta into left ventricle after thoracotomy. 40g/L paraform perfusion was done replacing saline perfusion after clear liquid flowed out from the heart. Bilateral eyes were enucleated after the rabbit expired. Cornea was cut at 2mm posterior to the corneal limbus. The lens and vitreous body were removed. The residual eye cups were fixed in 40g/L paraform for 24 hours. After separation from the eye wall, the retina was partly cut radially from the edges at four positions: 1:30, 4:30, 7:30, and 10:30. Then the whole retina flat-mount preparations were made on the slide and sealed with glycerine. The retina flat-mount slides were observed and imaged with both fluorescence microscope (Leica Q550cw) and laser confocal scanning microscope (Leica Tcs SP2). The mean grey of the whole retina fluorescence was detected by the Leica Q550cw image analysis system.

RESULTS

Fluorescence Observation of GFP Expression on the Whole Retina Flat-mount Preparations with the Fluorescence Microscope No fluorescence on the normal retina whole flat-mount preparations was observed by fluorescence microscopy. Scattered hypofluorescence was found near the injection site on the retina at the 3rd day after intravitreal injection of rAAV-gfp. GFP protein expression increased gradually 7 days later. Fluorescence spots were also more abundant during this period, and fluorescence intensity was stronger. At the 14th day after injection, fluorescence intensity of the GFP protein expression was strongest. The value of mean grey of GFP protein fluorescence on the whole retina is shown in Table 1.

Fluorescence Observation of GFP Expression on the Whole Retina Flat-mount Preparations with the Laser Confocal Microscopy 14 days after intravitreal injection of rAAV-gfp, the laser confocal scanning microscope identified obvious evidence of fluorescence spots in the cytoplasm of the retinal cells (the arrow in Figure 1).

Table 1 The mean grey value of the whole retina fluorescence (n=6)

	Control eyes	Test eyes
3 days	6.94±0.83	30.12±1.62[a]
7 days	6.79±1.56	40.14±2.07[a△]
14 days	6.07±0.79	55.85±2.06[a△▲]

[a]P <0.05 *vs* the control eyes. [△]P <0.05 *vs* the test eyes at 3rd day after injection. [▲] P <0.05 *vs* the test eyes at 7th day after injection

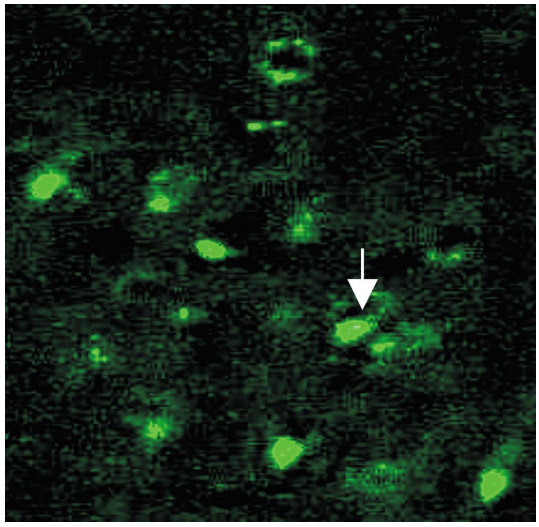

Figure 1 Fluorescence expression in retinal cells at the 14th day after intravitreal injection of rAAV-gfp under laser scanning confocal microscope (×600) Fluorescence was shown in the cytoplasm of retinal cells (shown by arrow)

DISCUSSION

Gene therapy is believed to have great potential for treatment of diseases in eyes. It was reported that brain derived neurotrophic factor (BDNF) could improve the survival of retina ganglion cells when the optic nerve was injured [4,5]. However, the clinical application of BDNF is limited because BDNF, a macromolecular protein, is unable to pass the blood-retina barrier. Therefore, researchers have made efforts to introduce BDNF gene into oculi using different gene vectors, include adeno-associated virus (AAV), adenovirus, retrovirus, among others [6-9].

AAV can be integrated into the human 19th chromosome long arm and can transfect proliferation cells and nonproliferation cells. AAV does not result in disease, and, compared with other vectors, AAV can make the target gene express stably over the long term. Therefore, it is necessary to investigate whether the recombinant AAV (rAAV) is an effective vector for transferring targeting genes for gene

therapy of retinal and optic nerve diseases.

In this study, we select GFP gene as a target gene since GFP, an irradiated protein, can be easily detected by showing green fluorescence after irradiation with blue or ultraviolet light. We found that fluorescent spots in the cytoplasm of retinal cells could be observed three days after intravitreal injection of rAAV-gfp, and fluorescence intensity significantly increased on day 7 and 14 post-infection. These results confirm that rAAV is an ideal vector that can effectively transfer the GFP gene into the rabbit retina, which suggests that rAAV can be used for transferring a target gene for gene therapy of diseases in eyes.

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