Optimized Pretreatment Method for Long-Term Transduction Efficiency of AAV Into Rat Bladder

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Research Article

Keywords: AAV, bladder urothelium, transduction efficiency, method

Posted Date: March 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1360426/v1>

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Abstract

Objective

We sought the optimal preprocessing method to improve the long-term transfer efficiency of adeno-associated viral (AAV) vectors of rat bladder urothelium.

Methods

Rat bladders were pretreated by eight different chemical reagents including PBS, trypsin, poly-L-lysine (PLL), protamine sulfate (PS), sodium dodecyl sulfate (SDS), n-dodecyl- β -D-maltoside (DDM), HCl or NaOH followed by transurethral instillation with AAV2/9-CMV-zsGreen. The transfection efficiency was evaluated by the percentage of urothelium with green fluorescence after 4 weeks.

Results

We employed eight different chemical components and eleven pretreatment methods. The rat bladder was harvested and evaluated without any mortality at 4 weeks post-infection. Compared with other treatment groups, the rate of urothelium transfection is comparatively high in the groups of rats treated with HCl or NaOH. In addition, rats treated with 0.1N NaOH 4 min showed significantly highest transfection efficiency (about 3%). The histopathologic results showed regular morphology of the bladder treated with HCl or NaOH and no significant damage was observed.

Conclusion

Pretreatment with 0.1N NaOH 4 min could improve the transfection efficiency of AAV in bladder urothelium and maintain the transfection efficiency for a long time without pathological damage.

Importance

We found that pretreatment of the bladder with HCl and NaOH can improve the transfection efficiency of AAV for a long time compared to control group. In addition, rats treated with 0.1N NaOH 4 min could have the most significant effect among these strategies, which was discovered for the first time. This could be valuable for future bladder gene therapy.

Introduction

Gene therapy has the advantages of accurate therapeutic targets and changing the development of diseases at the molecular level, so it has been widely studied and explored. However, the clinical

application of gene therapy is limited by many factors, including immunogenicity of gene vector, low transfection efficiency, possible tumorigenesis and limited efficacy[1]. The key step of gene therapy is selecting and constructing vectors for transmitting therapeutic nucleic acids to express or silence a gene[2]. At present, as an effective vector for gene therapy, adeno-associated virus(AAV) is considered to have great development potential in the clinic. Due to its low immunogenicity and the ability of specific site integration, AAV gradually shows its application value in the clinic[3]. Up to now, FDA has approved some AAV-based gene therapy drugs for the treatment of hereditary retinal diseases and spinal muscular atrophy[4, 5].

As we all know, the main target of adenovirus infection is located in the hollow organs of the respiratory tract and gastrointestinal tract. However, some studies have found that adenovirus infection can also occur in human bladder tissue[6, 7]. Therefore, we speculated that the urinary bladder system may also be an effective target of an adenovirus to accurately treat local lesions in the bladder and reduce the impact on other parts of the human body. Based on this hypothesis, many studies have reported that transurethral virus perfusion can target the key genes of bladder cancer cells *in vivo*[8, 9]. However, it is still a major challenge to achieve a good transfection rate of AAV in bladder urothelium. previous studies had identified that sulfated polysaccharide glycosaminoglycan(GAG), as a layer of nonspecific antiadhesion factor, is attached to the surface of bladder luminal surface, which can be used as a first-line isolation barrier to interfere with the interaction of bladder urothelial cells and many harmful substances in urine[10, 11]. To solve this problem, some studies pretreated the bladder mucosa with some chemicals, trying to break the protective barrier to improve the transfection efficiency of AAV[12–14]. However, no stable and unified pretreatment scheme can make AAV effectively transfect into the bladder urothelium and achieve the purpose of gene therapy. In addition, chemical compound stimulation and bladder urothelial barrier injury may lead to cystitis, affecting the transfection efficiency.

Based on this research background, we hope to obtain a stable method to enhance the effectiveness of adenovirus transfection by comparing transfection efficiency after pretreatment of bladder epithelium with different kinds and concentrations of chemical compounds.

Materials And Methods

Animals

60 female Sprague-Dawley rats (200-250 g) used in this study were allowed free access to food and water. All animal studies were approved by the Animal Care and Use Committee of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine (SJTUSM; Shanghai, China). All methods were carried out in accordance with institutional guidelines and regulations and reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>) for the reporting of animal experiments.

Animal model

We anesthetized 8-week-old rats with isoflurane. A 24-gauge intravenous catheter was inserted into the bladder through the urethra of rats. Subsequently, the bladder was emptied and washed three times using 100 μ L of phosphate-buffered saline (PBS). Chemical compounds were instilled into the bladder to damage the GAG layer. And then, the bladder was washed with PBS three times again, and 200 μ L AAV vector were instilled. The adeno-associated virus (AAV), HBAAV2/9-CMV-zsGreen, was constructed and synthesized by HANBIO (1.4×10^{12} viral genome/ μ L, Shanghai, China). The urethra was bound with suture to retain the AAV in the bladder and released after 4 hours. Rats were turned over at 2h from start to ensure that the bladder had full access to virus. The operation process is shown in Figure 1.

Chemical pretreatment

We examined the optimal chemical compounds for pretreatment prior to AAV infection. Chemical compounds all brought in sigma used and treatment times were as follows: 0.25% trypsin for 15 min [15], 10 mg/mL protamine sulfate (PS) for 20 min [16], 0.11% poly-L-lysine (PLL) for 15 min [15], 40 IU/mL Hyaluronidase for 20 min [17], 0.1% sodium dodecyl sulfate (SDS) for 15 min [13], 0.1% n-dodecyl- β -D-maltoside (DDM) for 15 min [13], 0.1 N hydrochloric acid (HCl) for 4 min [23], 0.1 N sodium hydroxide (NaOH) for 5 min [18] or PBS for 15 min as a control. All pretreatments were in a total volume of 200 μ L. These chemical compounds were previously shown to disrupt the negatively charged GAG layer of the bladder. AAV vectors were instilled following each pretreatment and the transduction efficiency was evaluated. The numbers of rat in each treatment group were as follows: Trypsin (n=5), PS (n=5), PLL (n = 5), Hyaluronidase (n=5), SDS (n = 5), DDM (n = 5), Hyaluronidase (n=5), HCl (n=5), NaOH (n=5) and PBS (n = 5).

Optimization of PS, HCl and NaOH pretreatment regimen

We investigated the optimal conditions for PS, HCl and NaOH pretreatment. PS concentrations and treatment times were as follows: 10mg/ml for 20 min (n = 5), 50mg/ml for 20 min (n = 5). HCl concentrations and treatment times were as follows: 0.1 N for 1 min (n = 5), 0.1 N for 4 min (n = 5). NaOH concentrations and treatment times were as follows: 0.1 N for 1 min (n = 5), 0.1 N for 4 min (n = 5).

Detection of transduction efficiency

Four weeks following intravesical instillation of AAV vectors with a different chemical compound, whole rat bladders were harvested and fixed in 4% paraformaldehyde for 48 h. Samples were dehydrated with 10 %, 20 % and 30 % sucrose. Tissue sections were mounted with 4',6-diamidino-2-phenylindole (DAPI). AAV transduction efficiency was evaluated for all rats (n = 60). Images were captured and analyzed using a fluorescence microscope.

Identify the damage of bladder urothelium

Histopathological examination of the bladder was conducted in rats treated with PBS, HCl or NaOH.

Statistical analyses

All statistical analyses were conducted using GraphPad Prism. All tests were two-sided, and values of $P < 0.05$ were considered statistically significant.

Results

Effect of pretreatment of urothelium with different chemical compounds on AAV transduction efficiency by Immunofluorescence

We compared 8 different bladder pretreatment modalities with chemical compounds using Immunofluorescence shown in Fig. 2. We found that other treatment modalities had no obvious transfection except for HCl and NaOH compared to the PBS-treated group. The highest transfection efficiency occurred in the bladder urothelium after 0.1N NaOH 4 min treatment of the bladder in rats. And in Fig. 2, we also found that 0.1N NaOH 4 min treatment group had the highest proportion of positive cells (3%) compared to the other groups.

Observe The Injury Of Hcl And Naoh Pretreatments On Bladder Urothelium

To observe the effect of HCl and NaOH pretreatment on the urothelium in a rat's bladder, we performed histopathological staining of the bladder. We found no significant pathological difference among the preconditioning groups compared to the control group in Fig. 3.

Discussion

AAV is a small, non-enveloped parvovirus with a ~4.7 kbp genome size of 20 – 25 nm capsid[19, 20]. Due to the specific structural characteristics of adenovirus, adenovirus has no pathogenicity and will hardly cause any disease. In addition, it could not cause an obvious immune response and can well adapt to the human body[15]. Another advantage of AAV is that it can be used as an important pillar of the carrier. AAV can infect active replication cells and effectively infect static and stable cells[21]. The bladder is a hollow organ. AAV can enter the bladder tissue as a gene therapy carrier through transurethral perfusion and achieve the purpose of gene therapy, which means that the bladder is an ideal target organ for local AAV-gene therapy. However, due to the existence of GAG, the infective effect of AAV is largely low. Therefore, applying different kinds of chemical compounds to break the ring or interfere with the formation of this mucosa provides a safe and effective way to enhance the transfection efficiency of AAV. We hoped to explore some suitable chemicals that can destroy the GAG of urothelium without affecting normal bladder structure and function. The target gene carried by AAV can be successfully transfected into the urothelial cells to play a role in treating bladder diseases. In this study, 8 different chemical components that we selected for pretreatment of rat bladder, based on the analysis of

Immunofluorescence, it was found that in 0.1N NaOH 4 min pretreatment group, AAV had the highest transfection efficiency (3%) and did not affect the bladder mucosa and urothelium.

In addition, no rats in this experiment died during the experiment. Although several previous studies had shown the effect of bladder pretreatment with different chemical compounds on virus transfection efficiency, their results were quite different. SIEMENS et al. used five different chemical compounds to pretreat the bladder in the mouse model and reported that they found only minimal increases in systemic activity[22]. While Ramesh et al. performed research in 2004, they suggested that pretreatment with 0.1% DDM or SDS for 5 min led to more than 90% transfection efficiency in a mouse model whose bladder only needed to be continuously exposed to adenovirus for 15 minutes[13]. LIN et al. considered that treatment with 60 mM HCl for 10 min in rat bladder resulted in approximately 80% of urothelial cells being successfully transfected by adenovirus[12]. Hamada et al. Published a methodological study to optimize the transfection efficiency of AAV in mouse bladder. They thought that mice treated with 0.1 N HCl for 4 min had the highest survival rates and had nearly 14.5% transfection efficiency[23]. We conducted an updated study and found that although the rat bladder treated with HCl could significantly improve the transfection efficiency compared with the control group, the transfection efficiency of AAV treated with 0.1N NaOH 4 min could get the best transfection effect. However, we were surprised to find that our transfection efficiency was only close to 3% compared with previous studies. Through comparison, we discovered that bladder samples were obtained for up to 7 days in these studies, and most studies chose mice for modeling. These differences brought great heterogeneity to the experimental results. Since it was necessary to maintain the cells successfully transfected with AAV for a long time to maintain the corresponding function, we hoped to improve the transfection efficiency of AAV and observe the long-term transfection efficiency by improving protocol in pretreatment the bladder. Since the urothelium had a certain cycle of cell proliferation and apoptosis, when we performed AAV transfection in only few hours, it was necessary to consider that some successfully transfected cells might undergo apoptosis due to self-senescence or exogenous damage, which affects the gene therapy of AAV in the long-term process. At present, there is no corresponding research to explore how to better maintain the long-term transfection effect of AAV. Our study tried to consider whether the transfection efficiency can be maintained and affected for a long time after bladder perfusion of AAV by different pretreatment chemical components. This may be more valuable for bladder gene therapy in the future.

Conclusion

In general, this study optimized the transfection efficiency of AAV by comparing 11 pretreatment methods of rat bladder for 4 weeks. We found that pretreatment of the bladder with HCl and NaOH can improve the transfection efficiency of AAV for a long time compared to control group. In addition, rats treated with 0.1N NaOH 4 min could have the most significant effect among these strategies.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

All authors agree to publish.

Availability of data and materials

All data and materials have been included in our article.

Competing interests

No competing financial interests exist.

Funding

The present research was funded by the National Natural Science Foundation of China (grant #81970657 to JQ).

Authors' contributions

Y Wu, J Zhou and H Chen: Project development, Data Analysis, Data Collection, Manuscript writing

J Ding and B Shi: Project development, Data analysis

J Qi: Project development, Manuscript editing

Acknowledgements

We thank all the authors for participating in this study.

All animal studies were approved by the Animal Care and Use Committee of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine (SJTUSM; Shanghai, China). All methods were carried out in accordance with institutional guidelines and regulations and reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>) for the reporting of animal experiments.

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Figures

Figure 1

24 G Vein Detained Needle
inserted through the urethra

Intravesical instillation of
different chemical pretreatments

Intravesical instillation of AAV

Keep the AAV vector
in bladder for 4 h

Figure 1

Experimental process of rat modeling

Figure 2

Figure 2

A. Photomicrographs of bladder tissue instilled with an AAV encoding IgtA-6 after treating with different chemical compounds. Tissue sections were stained with Immunofluorescence. Original magnification: 400 ×. Scale bars represent 20Mm. B. Transduction efficiencies in rat pretreated with PBS (n = 5), SDS (n = 5), PLL (n = 5), PS (n = 5), DDM (n = 5), hyaluronidase (n = 5), Trypsin (n = 5), 0.1N HCl 1 min (n = 5), 0.1N HCl 4 min (n = 5), 0.1N NaOH 1 min (n = 5), 0.1N NaOH 4 min (n = 5) .

Figure 3

Figure 3

Photomicrographs of the bladder of rat following intravesical AAV instillation and pretreated with PBS, 0.1 N HCl for 1 or 4 min, or 0.1 N NaOH for 1 or 4 min. Tissue sections were stained with hematoxylin and eosin. Original magnification: 100 ×. Scale bars represent 100 μM.