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Gene Delivery by Subconjunctival Injection of Adenovirus in Rats: A Study of Local Distribution, Transgene Duration and Safety

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Abstract

Subconjunctival injection is a minimally invasive route for gene delivery to ocular tissues, but has traditionally been limited to use in the cornea. The accurate ocular distribution of virus has not, however, been previously investigated. Adenovirus is an attractive gene vector as it can deliver large genes and allow for short-term gene expression, but how safe it is when delivered via subconjunctival injection remains to be established. We have characterized the bio-distribution and safety of subconjunctivally administered adenovirus in Brown Norway rats. The bio-distribution and transgene duration of adenovirus carrying luciferase gene (Ad-Luci) at various time intervals were evaluated via bioluminescence imaging after subconjunctival injection. Adenovirus carrying a reporter gene, β-galactosidase (Ad-LacZ) or hrGFP (Ad-hrGFP) was administered subconjunctivally and the viral distribution in various ocular tissues was assessed by histological analysis and quantitative PCR (qPCR). Hepatic damage was assessed by biochemical and immunohistological analysis with TUNEL stain. Systemic immunogenicity was assessed by measuring serum level of TNF-α via ELISA, 2 hours and 14 days after administration of adenovirus. Retinal function was examined by electroretinography. Subconjunctival injection of Ad-Luci induced luciferase expression in the injected eyes within 24 hours, for at least 64 days. Histological analysis showed adenovirus distributed across anterior and posterior ocular tissues. qPCR demonstrated different amounts of adenovirus in different ocular tissues, with the highest amounts closest to the injection site Unlike the intravenous route, subconjunctivally delivered adenovirus did not elicit any detectable hepatic injury or systemic immunogenicity. Retinal function was unaffected by adenovirus irrespective of administration route. In conclusion, an adenoviral vector administered subconjunctivally can infiltrate into different ocular tissues and lead to short-term ocular transgene expression, without causing hepatic injury and immune activation. Therefore, subconjunctivally administered adenovirus may be a promising gene delivery approach for managing anterior and posterior segment eye diseases requiring short-term therapy.

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Introduction

Gene therapy is an emerging and powerful modality in the treatment of eye diseases. It is unique in its ability to manipulate the expression or coding of a dysfunctional gene, allowing correction of the underlying pathological mechanism of the disease with prolonged benefits. [1] In recent experimental studies, gene therapy has been shown to have exciting therapeutic potential in many ocular applications, including treatment of currently incurable genetic eye diseases, as well as providing more effective treatments for common diseases affecting anterior and posterior segments of the eye.

Successful gene therapy is particularly dependent upon the suitable vector and route of administration that ideally has low toxicity, a high safety profile, and results in efficient therapeutic gene expression of the therapeutic gene product in target cells [1]. Currently, Adeno-Associated Virus (AAV) is the most commonly used vector in registered gene therapies for ocular disorders, but it lacks the ability to carry larger genes and are not ideal for situations when only short-term gene expression is desired.

Adenovirus is an attractive viral vector as it can produce large amounts of highly purified recombinant virus, which efficiently infects a wide variety of dividing and non-dividing cells [2, 3]. It can also deliver a large-sized foreign gene of up to 10kb. These features make adenovirus a suitable vector for delivering genes to target sites both *in vitro* and *in vivo*, and indeed, its development has