DESIGN AND PRODUCTION OF GENE THERAPY VECTORS FOR THE TREATMENT OF HYPERAMMONEMIA

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Introduction.

Gene therapy is a promising therapeutic alternative that consists in the delivery of a gene that provides a lost or needed function to a tissue. Viral vectors are the most effective and versatile gene delivery tools. However, one of the main challenges for the use of viral vectors is to produce them in large volumes with high titers in order to satisfy the requirements for clinical use. The insect cell-baculovirus expression system has increasing popularity for gene therapy applications, as baculovirus (BVv) and adeno-associated virus vectors (AAVv) are produced in it at a high titer and at a low cost. In this work, strategies for the efficient production of AAVv and BVv in insect cells are presented. The use of such vectors to contend with hyperammonemia in an animal model will be presented.

Methods.

BV coding for the therapeutic gene and for the control green fluorescence protein (GFP) gene were constructed. Sf9 insect cells were used to amplify BV and BVv in shaking flasks. AAVv were produced in High Five® insect cells in shaking flasks by coinfection with BV containing the AAV Cap and Rep genes and a BV containing the therapeutic gene under the CMV promoter and flanked by the AAV inverted terminal repeats (ITR). Various cell concentrations at the time of infection (CCI) and multiplicities of infection (MOI) were tested. The elements required for AAVv production were quantified by real time PCR, Western blotting and ELISA. BV were quantified as previously described[5]. An animal model of hyperammonemic rats was developed to test the efficiency of the vectors.

Results.

The conditions that resulted in the highest BV productivities were investigated. Results are listed in Table 1. Low MOI (0.1 pfu/cell) and CCI ($1x10^6$ cell/mL) resulted in the highest volumetric and specific BV productivities. During AAVv production, it was found that the concentration of vector genomes containing the gene of interest limits AAVv concentration. Strategies for increasing the concentration of AAVv will be proposed.

Table 1. BV titers obtained at different CCI and MOI.

CCI, $x10^6$ cel/mL	BV titer, $x10^8$ pfu/mL		Specific BV titer, pfu/cell	
	MOI 0.1 pfu/cell	MOI 1 pfu/cell	MOI 0.1 pfu/cell	MOI 1 pfu/cell
1	3.8±2.0	1.6±0.7	153±82	89±63
2	1.7±0.0	1.1±0.3	30±10	33±5

Rats with induced hyperammonemia were treated with the BVv containing the therapeutic gene. A significant decrease in serum ammonia concentration was found.

Conclusions.

Strategies for the production of AAVv and BVv were developed. Bottlenecks were identified and strategies to overcome them were proposed. Treatment with BVv resulted in the decrease of ammonia concentration in hyperammonemic rats. The strategy proposed here is promising for the treatment of encephalopathies caused by hyperammpnemia, which has an increasing impact in health.

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