Combinatorial Therapy of Rolipram and pNGF for Traumatic Brain Injury

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Statement of Purpose:

Traumatic brain injury (TBI) represents one of the leading causes of disability and death following injury[1]. The presentation of TBI involves both a primary and a secondary injury. The primary injury is a direct result of the traumatic event, and is accompanied by inflammatory response. The progression of inflammatory response is marked by the production of various cytokines that either act in neuroprotective or neurotoxic roles[2]. Several key neurotoxic cytokines are inhibited by physiologically normal levels of cyclic adenosine monophosphate (cAMP)[1]. Rolipram, a hydrophobic drug used in treatment of traumatic CNS injury, prevents the degradation of cAMP and is able to inhibit production of potentially neurotoxic cytokines[3]. Additional treatment for TBI is the administration of exogenous nerve growth factor (NGF), which has shown neuroprotective function and can reduce edema following primary injury[1]. For simultaneous delivery of rolipram and pNGF, we designed amphiphilic copolymers composed of poly(lactide-co-glycolide)-g-polyethylenimine (PgP), which has priviously demonstrated its ability for efficient nucleic acid delivery[5]. Here, we present the rolipram loading efficiency and transfection efficiency and duration of PgP/pNGF polyplexes in B-35 neuroblastoma cells.

Methods:

The PgP was synthesized and characterized by ^{1}H- NMR and physico-chemical properties were evaluated.[4] To evaluate the ability of PgP to deliver nucleic acids to neuronal cells, PgP/pBLAST44-hNGF (NGFB (InvivoGen), 2ug/mL) polyplexes were transfected in B35 neuroblastoma cells (ATCC® CRL-2754) in both non-serum and 10% serum conditions. Transfection was performed by complexing the pNGF with PgP at N/P ratios 25/1 and 30/1, which provided the highest degree of transfection with pGFP completed in our 4D lab. To evaluate the duration of transfection, time-course study was performed using PgP/pNGF at N/P ratio of 25/1 and 30/1. At pre-determined time point, culture media was harvested and the NGF concentration was determined via ELISA assay (R&D system), while measuring cytotoxicity using MTT assay. To evaluate the rolipram loading efficiency, varying amount of rolipram was dissolved in ethanol and then added in PgP (1 mg/ml) solution and incubated overnight to allow the ethanol evaporation. The amount of rolipram in PgP solution was measured by HPLC (Waters System) using a Waters Symmetry C18 column with mobile phase water:acetonitrile (60:40).

Results:

B35 neuroblastoma cells were transfected using PgP/pNGF in non-serum and 10% serum condition. Transfection results in both non-serum and 10% serum conditions, depicted in Figure 1, show that in non-serum conditions transfection efficiency of PgP/pNGF polyplexes at N/P ratios 25/1 and 30/1 was significantly higher than that of PEI at N/P 5/1. Furthermore, transfection efficiency of PEI/pNGF polyplexes shows a dramatic decrease compared to PgP/pNGF polyplexes in 10% serum condition. The duration of NGF expression after transfection with PgP/pNGF polyplexes persisted at values above control even when tested at 5 days post transfection. The rolipram loading efficiency of PgP was calculated as follows.

% Loading efficiency = (Amount of Rolipram loaded/amount of Rolipram added) X 100.

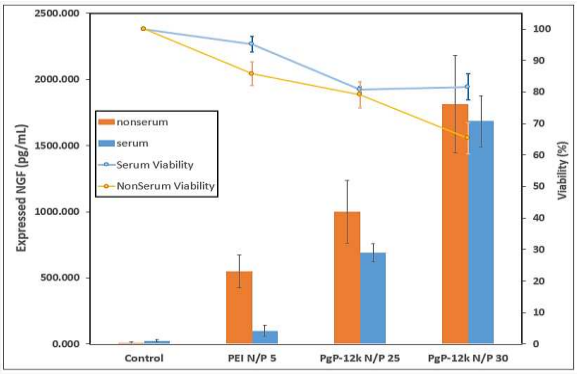

Figure 1: NGF expression (Bar) and cytotoxicity (line) 48 hours after transfection of PgP/ pNGF polyplex in B35 cells in non-serum and 10% serum condition.

The amount of Rolipram loaded to PgP solution (1 mg/ml) was about 0.86 mg ±0.029. This represents over 4 times higher than Rolipram's solubility in water (0.2 mg/ml), and loading efficiency was about 86%.

Conclusions: We demonstrated that PgP polymeric micelle can be a promising non-viral gene carrier for pNGF to B35 neuroblastoma cells in both non-serum and 10% serum condition. We also demonstrated persistent elevation of NGF expression over a course of 5 days. The hydrophobic drug rolipram was loaded in PgP the loading efficiency was about 86%. Currently, we are preparing to transfect PgP/pNGF polyplexes in microglia C8-B4 (ATCC® CRL-2540) cells to model transfection of immune cells that would be present at the site of injury. In the future, we will evaluate the synergistic efficiency of rolipram loaded PgP/pNGF in C8-B4 microglia cells in gaseous and $CoCl_2$ induced hypoxia condition in vitro.

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References:

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