A possible new treatment for Alzheimer's disease and stroke using nanoparticles

Alzheimer’s disease (AD) and stroke are two neurological disorders that share the common feature of neuronal death. The peptide under investigation here is IRL-1620, which has been shown in previous studies to reverse neuronal death as well as increase blood flow to the brain by dilating, or widening, blood vessels. However, in order for IRL-1620 to exert its effect on its target area in the body (brain tissue) it must first make its way across the blood brain barrier (BBB). Furthermore, IRL-1620 has a half-life of around 7 minutes in the body, which means that 7 minutes after reaching the blood stream, 50% of the peptide is already excreted.

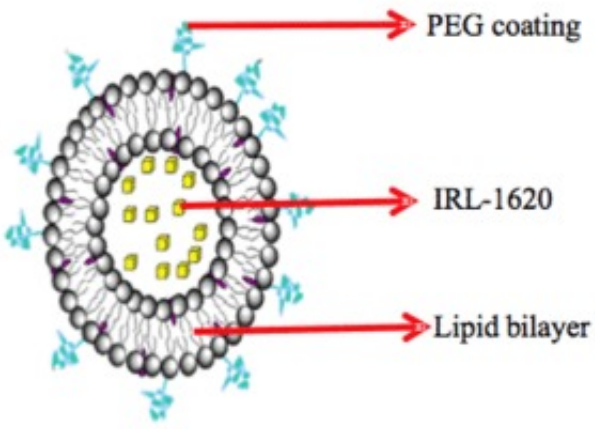

Fig. 1. Representation of IRL-1620 Loaded PEGylated liposomes

In order to help IRL-1620 cross the BBB and increase its half-life, we have packaged the peptide into liposomal nanocarriers (Fig. 1). These liposomes act as little vehicles that transport the peptide throughout the body. They are less than 200 nm in diameter and are made up of phospholipids and cholesterol, the same components that make up our cell membranes. The liposomes are regularly tested for stability.

In order to test the efficacy of our liposomal formulation in vitro, rat pheochromocytoma (PC-12) cells were chosen. The reason why PC-12 cells are great candidates for studying neurological diseases is because of their ability to differentiate into neuron-like cells. In the first experiment, we wanted to determine the most effective dose of IRL-1620 in terms of preserving cell viability. The PC-12 cells were transformed into neurons by administration of nerve growth factor (NGF). After several days NGF was removed from the cells. Because the cells had now committed to being neurons they could not replicate anymore, nor could they survive without continuous NGF administration. In order to determine IRL-1620’s ability to protect the neurons, it was added to the

cells in place of NGF. The 1 nM dose of IRL-1620 resulted in the highest amount of viable PC-12 cells after NGF withdrawal.

In the next experiment, we wanted to determine if loading the peptide into liposomes would somehow further increase PC-12 cell viability after removing NGF from the treatment. As it turns out, the liposomal IRL-1620 showed increase cell viability as compared to administering the free drug alone (Fig. 2). This suggests that the liposomes were able to deliver the peptide more effectively into the PC-12 cells.

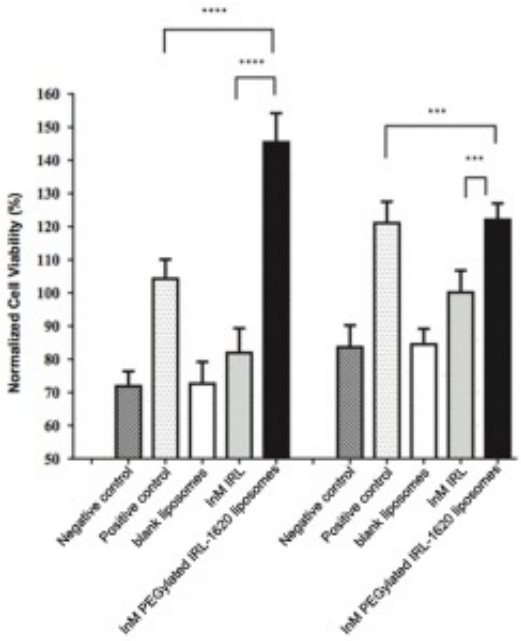

Fig. 2. Serum deprinted, NGF differentiated neuronal PC-12 cell viability after treatment with 1 nM and 10 nM free and liposomal IRL-1620

In order to gain insight into how IRL-1620 was actually maintaining PC-12 cell viability, the amount of NGF in the cells was determined. High NGF levels were only detected in the positive control, which was expected since this group was continuously being administered NGF. However, the groups receiving IRL-1620 showed a lesser amount of NGF present. These results are important because they indicate that IRL-1620 administration does not trigger the cells to produce their own NGF in order to maintain cell viability. Rather, cell survival is likely due to other, unknown factors and IRL-1620 is behaving like NGF.

Neuronal extensions, neurites, can be measured under a microscope to check if a cell is differentiating into a neuron as well as the health of that neuron. To show that IRL-1620 administration did not caused undesired retraction of neurites, PC-12 cell neurite lengths were

measured after IRL-1620 administration. The results indicated that IRL-1620 did not cause neurite retraction but rather maintained them as does NGF.

Lastly, PC-12 cells treated with liposomal IRL-1620 were evaluated in terms of production of BAX, a pro-death protein, and Bcl-2, a pro-survival protein. Here, BAX levels decreased after liposomal IRL-1620 administration suggesting that less cell death was occurring while, Bcl-2 levels increased to levels significantly higher than the positive, negative, as well as free IRL-1620, indicating a higher drive in terms of cell survival and possible reversal of cell death.

Liposomal IRL-1620 has been shown to be more effective in maintaining PC-12 cell viability compared to the free drug due to enhanced drug delivery when using nanocarriers allowing the differentiated PC-12 cells to utilize the drug more effectively.

Medha D. Joshi, Benjamin M. Oesterling, Christopher Wu2, Natalie Gwizdz, Gwendolyn Pais, Seema Briyal and Anil Gulati

Chicago College of Pharmacy, College of Health Sciences, Midwestern University, Downers Grove, IL, USA

Publication

Evaluation of liposomal nanocarriers loaded with ETB receptor agonist, IRL-1620, using cell-based assays.
Joshi MD, Oesterling BM, Wu C, Gwizdz N, Pais G, Briyal S, Gulati A
Neuroscience. 2016 Jan 15