Targeted Nitric Oxide Donors for Enhanced Drug Delivery and Treatment of Glioblastoma Multiforme

Shahana Safdar, Lakeshia J. Taite
Chemical and Biomolecular Engineering, Georgia Institute of Technology

Statement of Purpose: Glioblastoma Multiforme (GBM) is the most common malignant central nervous tumor in adults. The mean survival rate of GBM patients is only about one year. The ability of glioma cells to rapidly disperse and invade healthy brain tissue coupled with their high resistance to chemotherapy and radiotherapy have resulted in extremely poor prognosis among patients. Viable tumor cells extend even into areas with minimal or no abnormalities on imaging studies, adding further complexity to surgery. Additionally the blood-brain barrier prevents most therapeutic agents from accumulating within the tumor in cytotoxic concentrations.

Nitric oxide is a small, easily diffusible molecule which has shown to increase the permeability of the BBB. Studies have shown that at sufficient concentrations nitric oxide (NO) is able to induce apoptosis as well as increase radiosensitization in tumor cells

The aim of this work is the development of controlled release nitric oxide donors for the treatment of gliomas. In order to effectively target tumor cells as well as cross the blood brain barrier, short peptides sequences and small proteins that were able to specifically bind to tumor cells were used as the basis for this drug delivery system.

Methods: Proteins and peptides have a high number of surface amines which can easily be reacted with NO to form diazeniumdiolates. The peptide VTWTPQAWFQWV (VTW) and the protein chlorotoxin were chosen because of their high selectivity to glioma. Since VTW does not have any free amines available to react with NO, five lysine residues and a GGGS spacer sequence were added to the peptide sequence.

The biomolecules were dissolved in deionized water at room temperature and pH 7.4 and the atmosphere was evacuated with a vacuum pump. The solutions were than exposed to NO gas for 1 hour. After this the pH was adjusted to 7.4, the atmosphere evacuated, and the solution was exposed to NO gas for 24 hours. Subsequently a colorimetric Ninhydrin assay was performed in order to quantify free amines. NO release from the biomolecules at 37^{o}C was measured using an Apollo 4000 Free Radical Detector equipped with a nitric oxide microsensor.

Preliminary cell studies were performed in order to determine the effect of NO concentration on tumor proliferation. T98G cells were cultured in Minimum Essential Media (MEM) with 10% fetal bovine serum, 1% L-glutamine-penicillin-streptomycin and 1% non-essential amino acids at 37^{o}C and 5%CO_2. The cells were seeded in 24-well plates at a density of 10,000 cells/cm^2. After allowing the cells to proliferate for 48 hours they were incubated for 1 hour with varying concentrations of trilysine which had been reacted with NO. Subsequently the media was changed and the cells incubated for 48 hours. After 48 hours the cell were removed from the culture surface using trypsin-EDTA and counted using an automated cell counter.

Results: The colorimetric Ninhydrin assay showed that after reacting the biomolecules with NO for 24 hours, no free amines could be detecting the formation of diazeniumdiolates. It was also found at physiological pH of 7.4 the peptides were able to release NO for over 6 days (Fig 1). When the pH was reduced to 5.5, similar to the local tumor microenvironment, the peptides have a sharp burst release of NO in the first 200 seconds after which there is a gradual release of NO for 4 days. The peptides reacted with NO were freeze dried for storage purposes. It was found that freeze drying did not affect NO release.

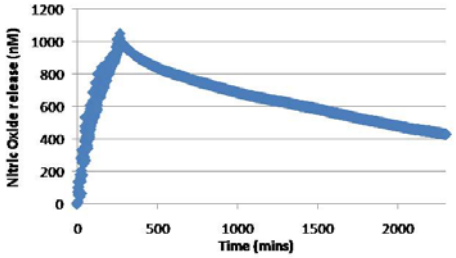

Figure 1: NO release from VTW at pH=7.4

The preliminary cell studies showed small concentrations of NO promote tumor growth but at higher concentrations tumor regression is induced. The studies also showed, at median concentrations tumor cell viability was more severely impacted in comparison to fibroblast cell viability.

Conclusion: In this study we were able to achieve repeatable NO release from both chlorotoxin and VTW. Cells incubated with FITC-labeled biomolecules were effectively and efficiently targeted by these peptides, as assessed by fluorescent microscopy. DNA damage assays will be used to further study the effect of our NO delivery system on brain tumor cells. Coupling the targeting ability of the biomolecules with their NO releasing capabilities will provide a more efficient NO delivery method and reduce damage of glioma therapies to surrounding brain tissue.

References:

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