Biological Optimization of Polymer-based Anti-HIV Drug Delivery towards the Gut Associated Lymphoid Tissue (GALT)

Arkajyoti Paul [1*], Caroline Garcia [1], Armando Garces[1], Deepa Roy[1], Matthew J Haney [2], Elena V Batrakova [2] and Upal Roy[1]

[1] Department of Health and Biomedical Sciences, University of Texas Rio Grande Valley, Brownsville, Texas, USA

[2] Department of Molecular Pharmaceutics, Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA.

ABSTRACT

According to the Global HIV statistics, in 2020, about 37.7 million people globally were living with HIV and 680,000 people died from HIV infection-related illnesses. Currently, combination antiretroviral therapy (cART) has significantly decreased the disease mortality associated with HIV-1. However, current cART therapy does not efficiently reach the HIV reservoir organs such as gut-associated lymphoid tissue (GALT) due to the lack of drug penetration in the GALT which results in other significant side effects in patients. In this regard, the present study has established a polymer-based nanoformulations using F127 and L61 with three clinically available cART drugs such as Emtricitabine (FTC), Tenofovir (TNF), and Dolutegravir (DLG). The selected formulations were studied for their cytotoxicity towards human cell lines viz. Caco-2, HMC-3 and mouse macrophage cell. Through our preliminary characterization, an optimized concentration of polymers F127 and L61 were used for the formulation manufacturing purpose. F127 alone and in combination with L61 were used to encapsulate FTC, TNF, and DLG. The safety study of all these formulations indicated that the combination of F127 and L61 formulation showed no significant toxicity, and the majority of all three formulations were observed to be in the size range of nanoparticle. The overall study indicated that all three F127 and L61 based nanoformulations are safe for further *in vitro* characterization. The future study will be to focus on the amount of drug taken into the cell through cell uptake study in different cell lines.

INTRODUCTION

HIV-1 (Human Immuno Deficiency Virus -1) and cART: According to the WHO, in 2020, about 37.7 million people were living with HIV, where about 36 million were adults. The cART is a treatment that uses a combination of three or more drugs to treat HIV infection.

Limitation of cART: cART cannot cure HIV infection due to the existence of the HIV dormant reservoir. It is a lifelong treatment procedure. If any patient discontinues the therapy, the virus can rebound.

Nanomedicine in HIV-1 treatment: The important advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, the feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation.

HIV reservoirs and GALT: There are many HIV reservoirs in the human body like the brain, testis, Gut-associated lymphoid tissue (GALT), etc. GALT works in the immune system to protect the body from invasion into the gut. The microfold cells (M-cells) in the GALT are the specialized cells that act as the gateway of the mucosal immune system.

MATERIALS AND METHODS

Formulation Preparation: We selected the most promising candidates of Pluronic block copolymers such as P84, L61, and F127 and characterized them by spectrophotometry with the fluorescent dye, pyrene for critical micellization concentration, (CMC), as well as Nanoparticle Tracking Analysis (NTA) for size and polydispersity of the micellar nanocarriers and Dynamic Light Scattering analysis (DLS) for size polydispersity. This observation was important to design and develop a nanodrug formulation that can hold both hydrophobic and hydrophilic anti-HIV drugs. The Pluronic F127 solution was sonicated with a probe sonicator for 60 sec. at count rate 405.2 kcps, and size distribution was assessed by DLS. Unexpectedly, the second pick with larger micelles increased after the sonication up to 375.0 ± 80.57 nm. We hypothesized that the increase in temperature solution upon sonication resulted in further aggregation of Pluronic micelles that are known for these block copolymers. Furthermore, incubation at RT for 4 days did not significantly affect the size distribution of the Pluronic micelles. We plan to further characterize Pluronic micellar nanocarriers with or without antiviral drugs and optimize formulations for lower size polydispersity and high drug loading capacity. The objective is to synthesize and characterize McART drugs for their drug loading efficiency, sustained drug release profile, and efficacy.

Particle size and PDI: Dynamic laser scattering (DLS) was used to evaluate the hydrodynamic radius, size distribution, and surface charge of nanoformulations (90Plus Particle Size Analyzer). At a temperature of 25°C, scattered light was recorded at a 90° angle. The mean ± SEM of triple data was used to calculate hydrodynamic size.

Cytotoxicity test: The cytotoxicity of the nanoformulations in Caco-2 cells, HMC 3 and GFP RAW were assessed using the MTS assay (G3582; Promega Corporation). The cells were grown in 96-well plates for 24 hours at 37°C, 5%CO2. After 24 hrs. of growth, the cells are treated with different dosages of nanodrug. The treated cells were then incubated for 2 hours. The cells were then washed with PBS and cultured in fresh growth media. After 70 hours of incubation at 37°C, the cells were treated with 20 µL of MTS reagent (CellTiter 96® AQueous One Solution; Promega, Madison, WI, USA). The absorbance at 490 nm was measured using a BioTek plate reader after 1hr incubation (BioTek). As a control, untreated cells were cultured with fresh medium alone. The net absorbance (A) was used to determine the viability of the cells. The cell viability was determined by the following formula: Cell viability= $\frac{Sample}{Control}$ X 100%. Nontoxic nanoformulations were those that did not induce a 10% decrease in cell viability after a minimum of 24 hours of exposure.

Statistical analysis: For statistical analysis we used two-way anova in GraphPad prism software.

RESULTS

Formulations	PDI	Size (nm)
F127+FTC (Formulation-1)	0.3096 ± 0.1677	26.443 ± 22.924
F127++L61+FTC (Formulation-3)	0.2523 ± 0.0350	7.124 ± 0.375
F127+TNF (Formulation-5)	0.213 ± 0.0823	16.926 ± 14.735
F127+L61+TNF (Formulation-7)	0.2613 ± 0.0288	7.434 ± 0.563
F127+DLG (Formulation-9)	0.8793 ± 0.1066	1284.333 ± 198.444
F127+L61+DLG (Formulation-10)	0.9296 ± 0.0766	830.333 ± 423.896

Table 1: PDI (Polydispersity index) and particle size of the nanoformulations.

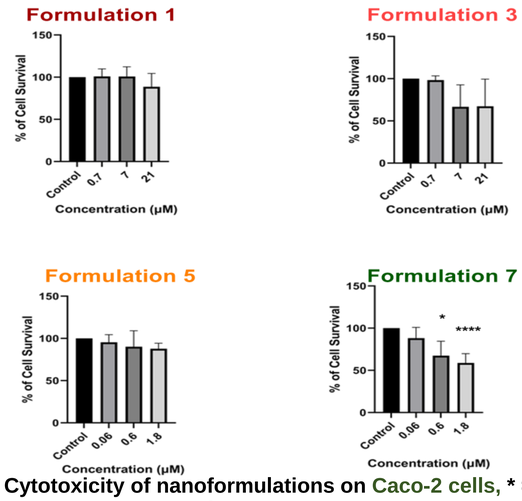

Fig 1. Cytotoxicity of nanoformulations on Caco-2 cells, * = p<0.05 and **** = p<0.0001.

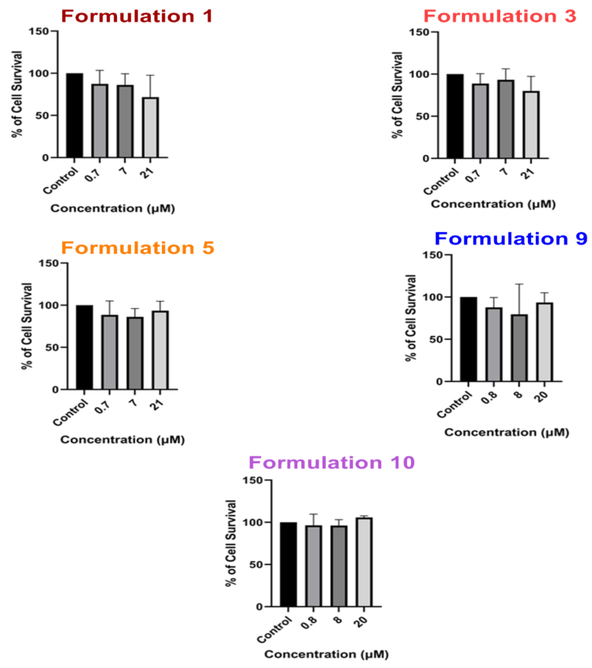

Fig 2. Cytotoxicity of nanoformulations on mouse macrophage cell (GFP RAW), * = p<0.05.

RESULTS

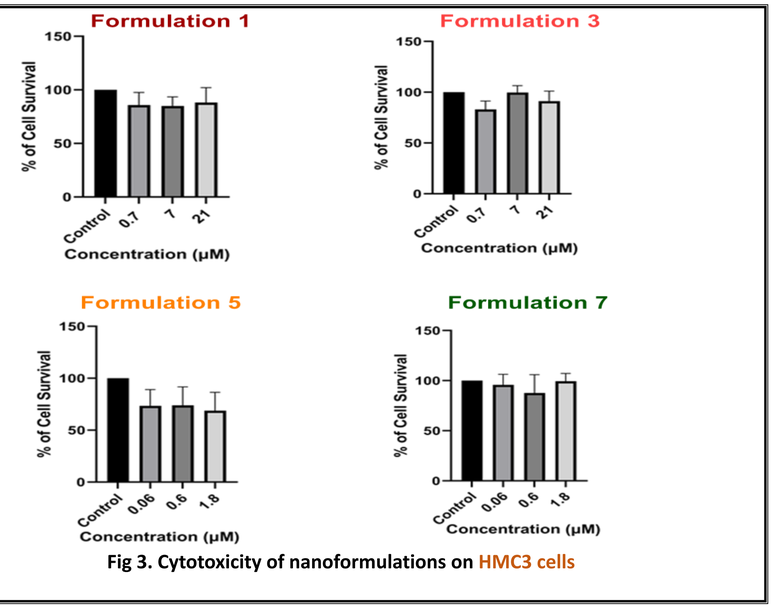

Fig 3. Cytotoxicity of nanoformulations on HMC3 cells

DISCUSSION

- Among the selected formulations 1,3,5 and 7 particle size range was from 7 – 26 nm which was within the standard range of nanoparticles. However, the formulation - 9 and 10 were relatively larger within the range of 830-1200nm.
- PDI of all the tested formulations within the range of 0.0-1.0 is the characteristics of the nanoformulations.
- The cytotoxicity assay of formulation 1,3,5 and 7 on Caco-2 cell showed that % of cell survival rate decrease with the highest concentration of emtricitabine formulations. The cell survival rate decreases gradually in both formulations of tenofovir, while formulation 5 was not significant and formulation 7 was significant (p<0.0001).
- In cytotoxicity assay of formulation on mouse macrophage cell (GFP RAW) presented that % of cell survival rate of formulations 1, 3, 9 and 10 were not significant. Whereas formulation 5 was significant (p<0.05).
- The percentage of cell survival rate of formulations 1, 3, 5 and 7 showed no significant.
- All the tested formulations observed to be safe on Caco-2, GFP Raw and HMC-3 cells.

CONCLUSION

The initial chemical and biological characterization of all formulations have revealed a promising result for nanodrug delivery of anti-HIV-1 drugs towards the GALT. Our future work will be on *In vitro* cell uptake, drug release and *in vivo* study with all formulations will be performed to maximize its potential use as a therapeutic agent for HIV-1 patients and its detailed metabolomics.

ACKNOWLEDGEMENT

❖ Dr. Upal Roy, Mrs. Deepa Roy, Armando, Andrea, Daphne, Jeo & Alen, Department of Health and Biomedical Sciences, University of Texas Rio Grande Valley, Brownsville, Texas, USA.

❖ The research work is supported by NIH (Grant no. 1R01AI147731-01A1).

BIBLIOGRAPHY

Roy, Upal, et al. International journal of nanomedicine 10 (2015): 5819. PMID: 26425084.