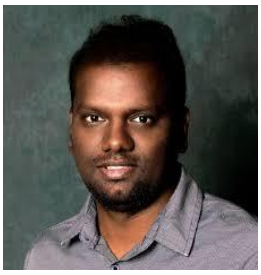
Synergistic Application of Metabolic Glycan Labelling and Click Chemistry to create a Novel Exosome-Based Therapeutic Against AZT Toxicity



Anand Krishnan*, Pon Yazhine Tamilselvan, Swantish Singh, Mamello Sekhoacha, Sandhya Tantuan,

Precision Medicine and Integrated Nano Diagnostics (P-MIND) Research Laboratory, Department of Haematology and Cell Biology, School of Pathology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa,
Department of Pharmacology, School of Pathology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa, (3) School of Biomedical Sciences, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa.
(Email : krishnana1@ufs.ac.za)

Abstract

Zidovudine (AZT) remains essential for HIV prevention in mother-to-child transmission (PMTCT). However, oxidative stress and mitochondrial dysfunction remain key adverse effects associated with AZT contributing to its limited clinical use. Adipose-derived mesenchymal stem cells (ADSCs) exosomes offer an approach to offset these adversities due to their endogenous cargos. This study utilised glycan engineered ADSC-derived exosomes and conjugated them to AZT via copper-catalysed azide–alkyne cycloaddition (CuAAC) to form a biomolecule to attenuate AZT-induced oxidative stress in HepG2 cells. ADSCs underwent metabolic glycan labelling (MGL) with Ac4GalNAl, and exosomes were isolated by size-exclusion chromatography (SEC) and ultracentrifugation (UC). The zeta-potential shifted from −2.471 mV (pure exosomes) to −7.789 mV (labelled exosomes) confirming surface modification. Ultraviolet-visible spectroscopy and high-performance liquid chromatography analyses yielded AZT concentrations of ~21 µg/mL in the biomolecule. Bioconjugate efficacy was then assessed using lipid peroxidation (MDA), glutathione (GSH),

and superoxide dismutase (SOD) assays. Compared with AZT alone, the bioconjugate reduced intracellular MDA from 0.1807 ± 0.0036 μM to 0.1409 ± 0.0029 μM (22% reduction) and extracellular MDA from 0.6292 ± 0.0054 μM to 0.05735 ± 0.0028 μM (90.9% reduction). Intracellular GSH increased to 186.93 ± 0.03 μM versus 25 AZT (173.22 ± 0.01 μM), and extracellular GSH increased from 157.27 ± 0.01 μM to 173.49 ± 0.04 μM. SOD activity increased from 2.54 ± 0.05 U/mL to 5.88 ± 0.01 U/mL. These findings demonstrate that ADSC-derived, glycan-engineered exosomes conjugated to AZT mitigated AZT-induced oxidative injury and enhanced antioxidant capacity, supporting their potential as therapeutic and drug-delivery platform.

Keywords: Zidovudine (AZT); Adipose derived mesenchymal stem cells (ADSCs); Exosomes; Metabolic glycan labelling (MGL); Click Chemistry