Exploring the Role of Glycosylation Inhibitors in Lymphoma and Drug Therapy

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INTRODUCTION

- Background: Lymphoma is a cancer of the lymphatic system, with two main types: Hodgkin and non-Hodgkin.[1] Mutations can start in the lymphatic system and then metastasize to the liver, brain, and bone marrow. Conventional treatments for lymphoma, include chemotherapy, radiation, or monoclonal antibodies. Since this is a systemic infection, these treatments are aggressive and may be difficult for patients to tolerate clinically.[1] The potential solution is to utilize nanoparticles with increased specificity, decreased toxicity, and increased circulation time.
- Hypothesis: Glycosylation is a cellular barrier to liposomal drug delivery and glycosylated cancer cells will develop more resistance to cancer drug therapies compared to those without glycation mesh.[2]

OBJECTIVES

1. Explore glycosylation inhibitors for lymphoma therapy.
2. Identify novel glycosylation inhibitors for lymphoma treatment.
3. Unravel mechanisms of glycosylation inhibition in lymphoma.

Glycosyl residues on lymphoma cells are hypothesized to be a cellular barrier in nanotherapeutic drug delivery, and eliminating such residues increases the cellular uptake of lipid nanoparticles.

METHODS

- The PubMed database (1946-July 29, 2023) was searched using the keywords “Lymphoma”; “Glycosylation Inhibition”; and “U937” using the Boolean operator “AND” resulting in 6 articles. The resulting 6 articles were examined to see if a particular inhibitor was used to decrease overall glycosylation patterns on the self surface. The article by Dwivedi et al. 2018 was considered.
- A second search was done using the keywords: “Lymphoma”, “Glycosylation Inhibitor”, and “O-glycosylation”, using the Boolean operator “AND”. This resulted in 6 articles. Articles from the last 10 years were excluded, leaving 1 article by Suzuki et al. 2015 to be considered.
- A third and final search was done to see if glycosylation could be inhibited at the transferase level instead of the biosynthetic level. The keywords were “galactosaminyl transferases inhibitor” and “uridine”, using the Boolean operator “AND”. This yielded 3 articles, the article by Hang et al. 2004 was considered due to its mention of lymphoma.

RESULTS

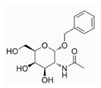
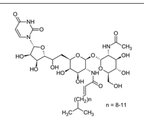
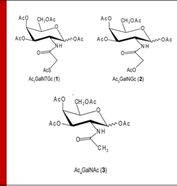
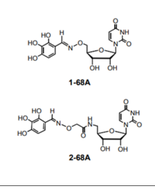
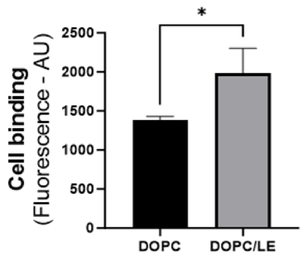
Inhibitor	Structure	Type	In Vitro/In Vivo: Cell Lines	Findings	Price	Author
Benzyl-α-GalNAc[3]		Mucin-type O-glycosylation inhibitor.	In Vitro: HBL-8, H-ALCL	Desalylation of O-glycans increased lymphoma cell adhesion to fibronectin.	Sigma: $504/100mg	Suzuki et al. 2015
Tunicamycin[3]		Type N-glycosylation inhibitor.	In Vitro: HBL-8	Tunicamycin decreased lectin-reactive oligosaccharide surface expression and enhanced cell adhesion to fibronectin.	Sigma: $920/50mg	
Peracetylated GalNAc analogues[4]: Ac5GalNTGc Ac5GalNGc Ac4GalNAc.		Mucin-type O-glycosylation inhibitor.	In Vitro: U937, Jurkat, K562.	The efficacy of Ac5GalNTGc varied depending on the cell line, with U937 cells showing more pronounced glycosylation inhibition. Additionally, treatment with Ac5GalNTGc induced homotypic clumping in U937 cells.	Not commercially available. Synthesized from N-acetyl-galactosamine (Sigma: $316/1g)	Dwivedi et al. 2018
1-68A and 2-68A[5]		Mucin-type O-glycosylation inhibitor.	In Vitro: Jurkat.	Uridine-based competitive inhibitors of N-acetyl-α-galactosaminyltransferases (ppGalNAcTs), bound to the enzyme approximately 2x more effectively than UDP-GalNAc and 30x more effectively than UDP alone. This hindered glycosylation and induced apoptosis in T-lymphocyte (Jurkat) cells.	Not commercially available. Synthesized from 2,3,4-Trihydroxybenzaldehyde (Sigma: $64.60/5g) and their corresponding uridine linkers.	Hang et al. 2004

Table 1: Evaluation of different glycosylation inhibitors across literature.

CURRENT RESEARCH

Figure 1: Uptake of U937 CLENs by U937 Cells at 15 Minutes Incubation



- The study compared the uptake of DOPC and DOPC/LE by U937 cells during a 15-minute incubation period. Utilizing an unpaired t-test with Welch's correction as the statistical method, assuming a Gaussian distribution using parametric tests, the analysis determined a p-value of 0.031. The results revealed a statistically significant enhanced uptake of DOPC/LE compared to DOPC alone.
- The next experimental phase entails exposing the U937 cells to a glycosylation inhibitor to assess further potential enhancements in uptake as well as assess the effects of cholesterol presence on the uptake.

CONCLUSIONS

- The literature confirmed that the N-acetyl-D-galactosamine (GalNAc) moiety is the most effective glycosylation inhibitor. As such, Benzyl-α-GalNAc is the most used inhibitor of O-glycosylation in lymphoma cell lines and the least expensive. The most effective GalNAc analog for inhibiting glycosylation was peracetyl N-thioglycolyl-d-galactosamine (Ac5GalNTGc). However, it is not commercially available. It is unclear if glycosylation poses a cellular barrier in drug delivery.
- The studies have also shown a lack of glycosylation inhibitors for lymphoma and much room for improvement, as shown in the development of various Peracetylated GalNAc analogs demonstrated by Dwivedi et al. 2018. Peracetylation of GalNAc analogs improves their permeability and, therefore, increases the incorporation of the inhibitor into the GalNAc Salvage Pathway, which was shown to decrease overall glycosylation of the cell.
- Given the lack of commercial availability of the more potent glycosylation inhibitors and the toxicity of tunicamycin, we believe that benzyl-α-GalNAc is the inhibitor of choice until there is enough data to show that glycosylation is indeed a cellular barrier for small drug molecules and nanotherapeutic agents. Until then, there is little need to acquire more potent inhibitors at this time.

REFERENCES

1. Le TV, Kim E, Togba-Bass T, Bom JC, Campbell RB. Therapeutic management of lymphoma: Conventional strategies andovercoming treatment barriers with Nanotherapeutics. Current Cancer Therapy Reviews. 2023;19(2):84-95. doi:10.2174/1573394718666220601124415
2. Wang X, Shah AA, Campbell RB, Wan K-t. Glycoprotein mucin molecular brush on cancer cell surface acting as mechanical barrier against drug delivery. *Applied Physics Letters*. 2010;97(26):263703.
3. Suzuki O, Abe M, Hashimoto Y. Sialylation and glycosylation modulate cell adhesion and invasion to extracellular matrix in human malignant lymphoma: Dependency on integrin and the Rho GTPase family. *Int J Oncol*. Dec 2015;47(6):2091-9. doi:10.3892/ijo.2015.3211
4. Dwivedi V, Saini P, Tasneem A, Agarwal K, Sampathkumar S-G. Differential inhibition of mucin-type O-glycosylation (MTOG) induced by peracetyl N-thioglycolyl-d-galactosamine (Ac5GalNTGc) in myeloid cells. *Biochemical and Biophysical Research Communications*. 2018;506(1):60-65. doi:10.1016/j.bbrc.2018.08.131
5. Hang HC, Yu C, Ten Hagen KG, et al. Small Molecule Inhibitors of Mucin-Type O-Linked Glycosylation from a Uridine-Based Library. *Chemistry & Biology*. 2004;11(3):337-345. doi:10.1016/j.chembiol.2004.02.023

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DISCLOSURE OF RELEVANT FINANCIAL RELATIONSHIPS

Nothing to disclose.