PEGylated asparaginase does not impair fatty acid oxidation, increase lipid synthesis or decrease VLDL secretion in HepG2 cells

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INTRODUCTION

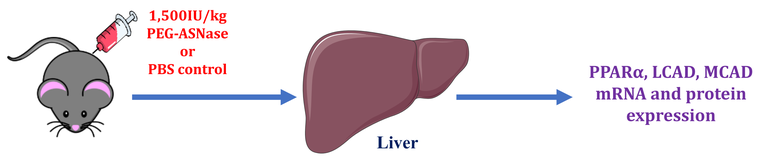
Acute Lymphoblastic Leukemia (ALL) is the malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow and blood, as well as extramedullary sites. PEGylated asparaginase (PEG-ASNase) is an antineoplastic, bacteria-derived agent that has become an integral part of remission induction and intensification treatment in pediatric regimens but avoided in adult regimens due to the risk of liver injury. As of now, there has been little research investigating the mechanism of the toxicity. Preliminary data involving a murine model of PEG-ASNase fatty liver confirms the clinical toxicity and shows that PEG-ASNase leads to the development of microvesicular hepatic steatosis and extensive white adipose tissue (WAT) lipolysis in mice. Clinical studies have shown that this defect is more common in adults characterized by elevated plasma levels of bilirubin, ALT, and AST, while previous case reports suggest it is due to a defect in liver fatty acid oxidation.[1-2]

OBJECTIVES

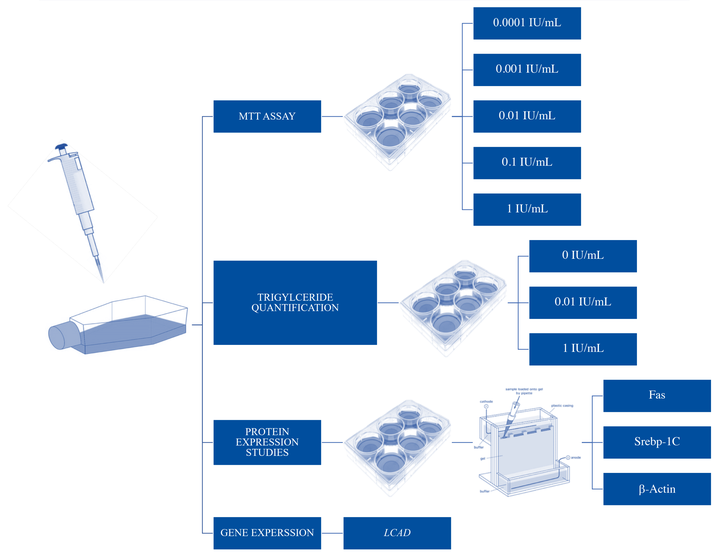
This study aims to determine the effects of PEG-ASNase on intracellular triglyceride levels, the protein levels of genes involved in lipid synthesis, and *in vitro* VLDL secretion.

METHODS

***IN VIVO* STUDY IN MALE BALB/C MICE**



***IN VITRO* STUDY USING HUMAN HEPATOMA *HEPG2* CELL LINES**



RESULTS

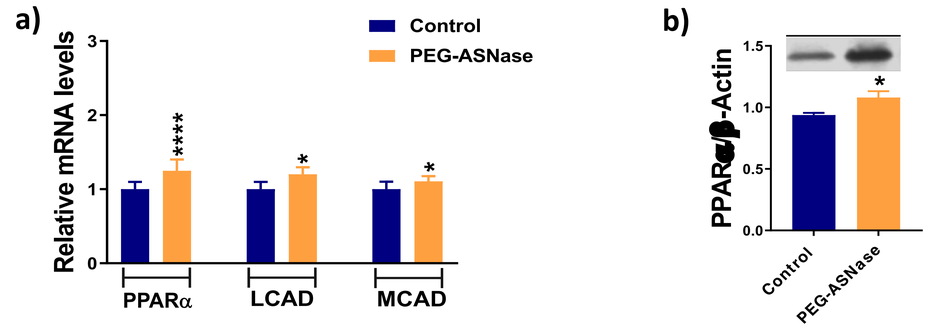


Figure 1: The expression of genes involved in fatty acid oxidation are increased in mice after PEG-ASNase treatment. A) The hepatic mRNA levels of Pparα, Lcad, and Mcad are increased after PEG-ASNase treatment. Consistent with the gene expression, hepatic Pparα protein levels are increased after PEG-ASNase treatment.

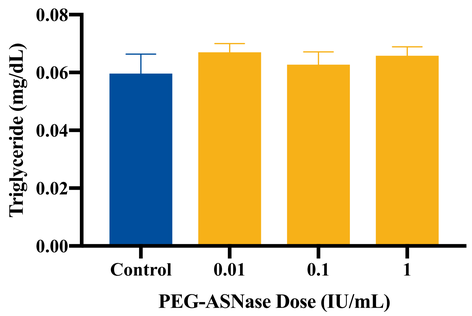


Figure 2: Triglyceride Quantification via Stanbio TG Screening Assay. Figure 1 displays that the intracellular triglyceride levels were not increased in PEG-ASNase (0.01, 0.1, and 1 IU/mL) treated groups in comparison to the control.

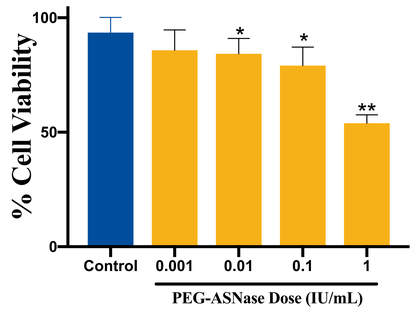


Figure 3: Cell Viability Results via MTT Assay Treatment of HepG2 cells at 0.01, 0.1 and 1 IU/mL of PEG-ASNase resulted in 15, 20 and 46% cytotoxicity, respectively.

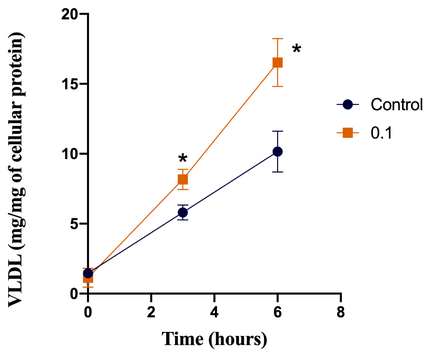


Figure 4: VLDL Secretion Assay. The VLDL secretion assay indicates that secretion is increased in HepG2 cells after treatment with 0.01 IU/mL of PEG-ASNase.

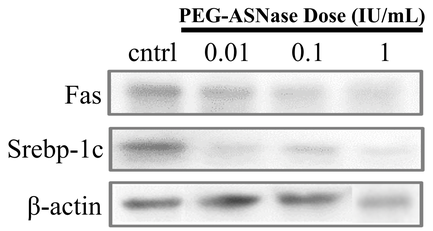


Figure 5: Protein Expression of Fas and Srebp-1c, and β-Actin. Protein expression of Fas and Srebp-1c are decreased in HepG2 cells after PEG-ASNase treatment.

CONCLUSION

The present study suggest that the abnormal lipid accumulation within the liver may not be due to changes in de novo synthesis of fatty acids, nor in defective hepatic very-low density lipoprotein secretion after PEG-ASNase treatment. Thus, our *in vitro* study using HepG2 cells supports that other mechanisms are responsible for the onset of PEG-ASNase-induced hepatic steatosis.

FUTURE DIRECTION

Preliminary data shows that the livers from mice receiving PEG-ASNase do not develop increased de novo lipid synthesis, decreased fatty acid oxidation, or decreased VLDL secretion, which have been identified as methods capable of inducing hepatic steatosis. The present study confirms some of the data from our preliminary *in vivo* studies and supports our hypothesize that PEG-ASNase hepatic steatosis occurs through a novel mechanism involving mobilization of fatty acids from the white adipose tissue. Our future studies include analysis of Pparα signaling and measurement of fatty acid oxidation in HepG2 cells, primary hepatocytes, and *in vivo* in mice after PEG-ASNase treatment.

REFERENCES

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2. Bodmer M, Sulz M, Stadlmann S, et al. Fatal liver failure in an adult patient with acute lymphoblastic leukemia following treatment with L-asparaginase. *Digestion*. 2006;74(1):28-32.

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