

## **Loading of L-asparaginase therapeutic enzyme in human red blood cells by recombinant listeriolysin O**

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Listeriolysin O (LLO) is one of the members of cholesterol-dependent cytolysin family of protein toxins. Large pores of LLO toxin molecules in target membranes can facilitate the entrance of larger macromolecules into the cells. In the present study loading of L-asparaginase enzyme in human RBCs as new drug delivery vehicles was investigated by recombinant LLO protein which expressed in *BL21 DE3* strain and purified by affinity chromatography. SDS-PAGE and western blot analyses were performed to confirm the recombinant protein. Biological activity of recombinant protein was investigated in an RBC hemolysis assay. Controlled pore-formation, limited lysis of RBCs (<10%) and the best enzyme loading were examined turbidimetrically in different buffers and conditions. Drug loading was evaluated by western blotting and residual enzymatic activity. Stability of the toxin-treated RBCs was analyzed for 10 days. Appropriate concentration of the recombinant toxin for efficient L-asparaginase loading with the least RBC breakdown has been determined. The results of western blot and residual enzymatic activity indicated that loading of L-asparaginase in human RBC could be achieved using LLO toxin. Half-life of toxin-treated RBCs has not been statistically different from control RBCs after 10 days of treatment. Several loading strategies have been investigated for the efficient and safe loading of therapeutics into RBCs. Fast loading procedure and stable RBCs achieved in the presented method can introduce an alternative method for loading of macromolecular drugs.

Keywords: listerilysin O, drug-delivery, L-asparaginase