Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

Cloning, extracellular expression, assay and purification of recombinant *Streptococcus pyogenes* streptokinase

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Background and Aims: Streptokinase is an important therapeutic enzyme that acts as a fibrinolytic agent and use in the treatment of multi serious and life threatening diseases such as pulmonary embolism and acute myocardial infarction. Cloning and extracellular expression of recombinant streptokinase from Streptococcus pyogenes was done by fusing the gene coding for streptokinase to an efficient vector (pET15b) with NdeI and BamHI as restriction enzymes and T4 DNA ligase. Streptokinase activity was determined using synthetic chromogenic substrate S-2251, and measuring the absorbance at 405 nm in a UV-spectrophotometer; and purification done under N-terminal 6× histidine tag complementation using affinity chromatography.

A 1323-bp of the structural streptokinase gene was amplified. The DNA and amino acid sequence alignments resulting from the BLAST search of streptokinase showed high sequence identity with the other strains of S. pyogenes. The recombinant enzyme had the same molecular weight as other reported streptokinase and the E. coli transformants showed high streptokinase activity.

Comparative clinical trials and cost-effectiveness considerations between several major available thrombolytic agents suggest that streptokinase is the drug of choice for thrombolytic therapy particularly in the poorer economies.

Keywords: *Escherichia coli*; Fibrinolytic enzyme; Gene cloning; *Streptococcus pyogenes*; Streptokinase