Expression of Streptokinase (SK) Gene from Invasive Group A Streptococcus (NZ131): a New Gene for Recombinant SK Production

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Background & Objectives: Streptokinase (SK) a potent plasminogen activator protein produced by beta-haemolytic Streptococcus group A, C and G is currently being used as a thrombolytic agent in medical practice. Due to its ability to activate fibrinolytic system, SK is known as spreading virulence factor of streptococci. The main source of SK production is non-invasive group C streptococci strains. NZ131 is a well-documented invasive strain of group A Streptococci (GAS) that show more SK activity in vivo than non-invasive strains. The structure-function studies suggest that the degree of SK activity might be related to its structure (i.e: nucleotide sequences). The aim of this study was isolation, cloning and recombinant production of SK from invasive GAS strain (NZ131) in *E. coli* system.

Methods: The SK gene (ska) of NZ131 was amplified by specific primers, cloned into the pQE30 plasmid and expressed in E. coli (M15 strain). Expression of the recombinant SK (rSKNZ) was confirmed by western-blotting and purified by affinity chromatography using Ni-NTA resin.The biological activity of rSKNZ was determined through both semi-quantitative (caseinolysis) and colorimetric assays (using plasmin substrate; S2251). The activity of rSKNZ was compared with commercial SK (Streptase).

Results: The chimeric pQE30 plasmid containing the N-terminal fusion of His-tag with ska of NZ131 was successfully constructed. Expression of rSKNZ was confirmed by SDS-PAGE and WB analyses. Yield of the purified protein was 0.99 mg/ml. Semi-quantitative and quantitative activity assays indicated similar specific activities between rSKNZ and streptase (547000U/mg).

Conclusion: The results indicate that rSKNZ can be a potential candidate to be considered as a thrombolytic drug. However, amino acid sequence and immunogenicity of rSKNZ should be analyzed and compared with that of the streptase. In addition, further studies on the pharmacological and kinetic parameters of this new rSK (rSKNZ) are under consideration.

Keywords: Streptokinase (SK); Group A Streptococcus (NZ131)