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

Cloning and expression of pdgf-bb gene in eschrishia coli

F. Nouri[1,*], M. Ghoshoon[2], Y. Ghasemi[2]

[1]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background and Aims: Platelet-derived growth factor (PDGF) is an important mediator in wound healing. PDGF is a potent mitogen for fibroblasts and other cells of mesenchymal origin . It is chemotactic for neutrophils, monocytes, fibroblasts, and smooth muscle cells . PDGF stimulates production of several connective tissue molecules and supports angiogenesis. A gel formulation of recombinant human PDGF (rhPDGF) is currently approved to treat full-thickness ulcers in diabetic patients.
Methods: coding sequence of PDGF-BB gene after optimization was synthesized, PDGF-BB gene and PET15b vector were digested with restriction enzymes. The gel-purified digested PDGF-B gene was cloned into pET15b. Successful construction of the pET15b- PDGF-B clone was confirmed by restriction digestion and PCR amplification. For protein production, host E. coli strain BL21(DE3) was transformed by insertion of a recombinant PDGF-B plasmid. After Growth and harvesting of E. coli BL21(DE3)/pET15-hPDGF-B in Luria-Bertani (LB) medium containing 100 µg ampicillin/ml at 37ºC until the OD600 reached 0.6, after which 1 mM IPTG was added and culture continued for another 4 h. Analysis of the expression samples was carried out using SDS-PAGE method.
Results: After cell culture and induction of expression with IPTG , SDS-PAGE was done & The gel shows increasing expression of gene product following induction with IPTG.
Conclusions: The construction of recombinant prokaryotic expression vector pET-PDGF-BB and the preparation of PDGF-BB protein provide a foundation for further study of the function of PDGF-BB and producing biological PDGF-BB Protein.

Keywords: Cloning; PDGF-BB; *E. coli*