

[PS1.34]

Recombinant Neutrophil Gelatin Aseassociated Lipocalin (NGAL/Lcn2) effectively inhibits platelets- contaminating bacteria

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Bacterial contamination is a major problem with transfusion of platelet (PLT) products that can lead to serious morbidity and mortality. Lipocalins constitute a broad but evolutionally conserved family of small proteins; however, the functions of many lipocalins remain unclear to date. Neutrophil gelatin aseassociated lipocalin (NGAL; also known as lipocalin 2 or human neutrophil lipocalin) is a 25-kDa glycoprotein that was initially purified from neutrophil granules. Isolation, cloning and expression of recombinant Lcn-2 (NGAL) and the study of its antibacterial property were the aim of this study.

Lcn-2 gene was isolated from HepG2 cell line by RT-PCR and then it was cloned to pet28a vector. Recombinant vector was transformed into E.coli. Recombinant colonies were screened in the presence of ampicillin. Recombinant Lcn2 was induced in E.coli BI21 strain in the presence of IPTG. Expression of Lcn2 was determined by RT-PCR , ELISA and western blot analysis. Recombinant Lcn2 was purified by nickel resin. platelets- contaminating bacteria such as Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis were cultivated in the presence or absence of recombinant NGAL.

Expression of Lcn2 was confirmed by RT-PCR , ELISA and western blot analysis. Approximately single band, 25KD, was observed after purification. When the bacteria were cultivated in the presence of recombinant NGAL/Lcn2 , their growth was effectively inhibited especially Escherichia coli, Pseudomonas aeruginosa and Bacillus cereus.

Findings of this study revealed that NGAL/Lcn2 acts as an antibacterial agent against platelets- contaminating bacteria suggests that NGAL/ Lcn2 might have the potential application to prevent platelets contamination from bacteria.

Keywords: Lipocalin 2, Bacteria, platelet, Blood