

ABSTRACT

Sáva Klabačková

Nuclear factor kappa B and inflammatory reaction

Bachelor thesis

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Medical laboratory technician

Background: Proposed of LNA probe-based PCR systems for study of NF- κ B family-related molecules will make possible a comparison of differences between activation of immune systems of groups of germ-free and conventional piglets. This requires: a purification of total RNA and synthesis of cDNA, PCR, relative quantifications of transcriptions of NF- κ B-related molecules and a statistical evaluation of possible differences between piglet groups.

Methods: 1) Germfree and conventional piglets, 2) a proposal of PCR systems with LNA probes for genes of NF- κ B family and related molecules, 3) a column-based isolation of total RNA from ileum, 4) spectrophotometric evaluation of RNA purity and concentration, 5) a reverse transcription, 6) real-time PCR, 7) an electrophoretic confirmation of amplicons sizes, 8) a relative mRNA expression normalized against reference genes.

Results: The LNA probes-based PCR systems for RelA-85, RelB-1, cRel-63, NF- κ B1-63, NF- κ B2-21, NF κ BIA-27, Bcl3-9, IKK1-7, IKK2-3, NEMO-27 of predicted amplicons molecular sizes. Differences in gene transcriptions between germ-free and conventional piglets were found, but they were in some cases non-significant.

Conclusions: Several LNA probes-based PCR systems for detection of transcription of NF- κ B genes and related molecules were established and evaluated. NF- κ B is a key molecule of the activation of the immune system resulted in expressions of signaling and activation molecules as cytokines. The regulation of NF- κ B-related molecules is complex system. Its better understanding requires to work also on a protein level that should be the topic of our future work.