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Corbrotoxin Interacts with Proteins of NF- κ B Signal Pathway Resulted in Inhibition of NF- κ B Target Gene Expression

Mi Hee Park¹, Seong Ho Lee¹, Dong Ju Son¹, Ho Seub Song²,

Ki Hyun Kim² and Do Young Yoon³

¹College of Pharmacy, Chungbuk National University, ²College of Oriental Medicine, Kyungwon University, ³Korea Research Institute of Bioscience and Biotechnology

Cobrotoxin, a venom of *Vipera lebetina turanica*, is a group of basic peptides composed of 233 amino acids with six disulfide bonds formed by twelve cysteines. NF- κ B is activated by subsequent release of inhibitory I κ B and translocation of p50 via activation of a multisubunit I κ B kinase (IKK). Since sulfhydryl group is present in kinase domain of IKK α and IKK β as well as p50 subunit of NF- κ B, cobrotoxin could modify IKKs and NF- κ B activity by protein-protein interaction. We therefore examined effect of cobrotoxin on IKK and NF- κ B activities in lipopolysaccharide (LPS 100 ng/ml) or sodium nitroprusside (SNP 200 nM)-stimulated astrocytes and Raw 264.7 mouse macrophages. Cobrotoxin suppressed the LPS or SNP-induced release of I κ B and p50 translocation resulted in inhibition of DNA binding activity of NF- κ B and NF- κ B-dependent luciferase activity. Surface plasmon resonance analysis showed that cobrotoxin directly binds to IKK α (Kd = 3.94×10^{-9} M), IKK β (Kd = 3.4×10^{-8} M) and p50 (Kd = 1.54×10^{-5} M). Inhibition of NF- κ B resulted in reduction of the LPS or SNP-induced production of inflammatory mediators NO and PGE2 generation as well as target genes involving inflammation and cancer cell growth. The inhibitory effect of cobrotoxin on the NF- κ B activity, binding affinity of cobrotoxin to p50 and IKKs, and NO and PGE2 generation were blocked by addition of reducing agents; dithiothreitol and glutathione. In addition, cobrotoxin did not show inhibitory effect in the transfected Raw 264.7 and astrocytes with plasmid carrying dominant negative mutant p50 (C62S), IKK α (C178A) and IKK β (C179A), but not in IKK β (K44A) mutant transfected cells. These results demonstrate that cobrotoxin directly binds to sulfhydryl group of p50 and IKKs resulting in the reduction of translocation of p50 and I κ B release, thereby inhibits activation of NF- κ B. Three data suggest that pico

to nanomolar range of cobrotoxin could inhibit the expression of genes in the NF- κ B signal pathway.

Keyword : IKK β , IKK α , NF- κ B, p50, Cobrotoxin