Immunological and Immunochemical studies on Phospholipase A_2 from two snake venoms of Egypt

A Thesis

Submitted to Zoology Department,
Faculty of Science, Cairo University

In fulfillment to the requirement for the Degree of Philosophy
Doctorate of Science (Immunology and Parasitology)

By

Amr El Said Mohamed El Hakim
M.Sc. (Immunology and Parasitology)
Assistant Researcher, Molecular Biology Department,
National Research Center, Cairo, Egypt

Prof. Dr. Abd El Hakim Saad Wahby
Professor of Immunology
Department of Zoology,
Faculty of Science,
Cairo University, Cairo

Prof. Dr. Ahmed Fekry
Professor of Immunochemistry,
Molecular Biology Department,
National Research Center, Cairo

Department of Zoology
Faculty of Science
Cairo University
2005

ABSTRACT

The PLA_2s of *Naja haje haje* and *Pseudocerastes persicus fieldi* venoms were purified, identified and their antigenicity and immunogenicity were investigated. The *P. fieldi* venom contained at least five PLA_2 isoenzymes of quite different pI values, while the *N. haje* contained tow or more isoenzymes of quite close pI values. The molecular weight of the PLA_2 variants of the tow venoms in SDS-PAGE were close to 14 KDa, whereas the major PLA_2s of the *N. haje* and *P. fieldi* were focused at 9.2, 8.4 *pIs* and 5.6, 6.9, 8, 8.8, 9.8 *pIs* respectively. The major hemolytic PLA_2 variant of the tow venoms were separated in four successive steps, which include gel filtration on Sephadex G-50, ion exchange chromatography on CM-Sephadex-CMS, preparative isoelectric focusing and reverse phase HPLC-C4 column. In the first tow steps, the hemolytic PLA_2s fractions, (the *N. haje*-CMS and *P. fieldi*-CMS) were separated from the *N. haje* and *P. fieldi* respectively. The PLA_2 variants of the *N. haje*-CMS and *P. fieldi*-CMS fractions were purified to homogeneity by preparative isoelectric focusing and reverse-phase HPLC chromatography on C4 column respectively. The protonated molecular ions $[M+H]^+$ were obtained at m/z 13396, for *N.haje*-PLA_2II; 13720, for *P. fieldi*-PLA_2I; 13722, for *P. fieldi*-PLA_2II; 13723; for *P. fieldi*-PLA_2III; and 13718, for *P. fieldi*-PLA_2IV, respectively. The complete amino acid sequence of *P. fieldi* FI, *P. fieldi* FII PLA_2s and the partial sequences of the *N. haje* FII, *P. fieldi* FIII, and *P. fieldi* FIV PLA_2s were obtained. The N-terminal amino acid sequences of the *P. fieldi*- FI, FII, III and IV PLA_2s are similar to that of the CbI alpha, the acidic subunit of the *P. fieldi* heterodimeric neurotoxin. The N-terminal amino acid sequence of the *N. haje* FII PLA2 is similar to the *Naja melanoleuca* PLA_2. The value of the *N. haje*- CMS and *P. fieldi*-CMS as immunogens for raising therapeutic antisera and as antigens for in vitro evaluation of the antisera potency was evaluated. The ELISA antibody titers to the *N. haje* and the *N. haje*-CMS in ten therapeutic equine antisera revealed poor correlation with the lethality neutralization. However, the rabbit antibodies to the *N. haje*-CMS and *P. fieldi*- CMS, neutralized the lethality of the corresponding venoms, which demonstrates its value as immunogens rather than diagnostic antigens. The isolated *N. haje*-PLA_2 was tested for its ability to cause pathological changes to myocardium, skeletal muscle and cardiac ganglia of albino mice. Electron microscopic study revealed myodegeneration in cardiac and skeletal muscles and reduction in synaptic vesicles population of

preganglionic nerve terminals in cardiac ganglia. Ultra structural changes in tissues were dose dependent. The lower dose (4 mg/kg) of PLA_2 produced mild myocardial changes, while higher dose (8 mg/kg) of PLA_2 caused myocardial degeneration. The skeletal muscle lesions were more severe than the myocardial changes. Some of the myofibrils were severely disorganized and lack typical striated appearance, sarcomeres disrupted, most of mitochondria were vesiculated and destroyed. Intramuscular injection of *N. haje*-PLA_2 induced elevation in IL-6 in muscles, but no elevation of TNF-α was detected. It is concluded that these cytokines do not have a significant role in the pathogenesis of the local pathological effects induced by *N. haje* venoms in mice, although this does not exclude the possibility that these cytokines play a role in other aspect of venom-induced local pathology, as well as in reparative and regenerative responses that takes place after the onset of tissue damage.

Key words: *Naja haje*; *Pseudocerastes fieldi*; Snake venom; Phospholipase A_2 ; Homogencity; Amino acid sequence; Chemical structure; HPLC, Mass spectrometry, Antivenom serum therapy; Histopathological changes; Myocardium; Skeletal muscles; Cardiac ganglia; Electron microscopy; TNF-α; IL-6; Hemorrhage.