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Araştırma Notu / Short Note

Kinetic Analysis of the *in vivo* Inhibition of Liver AChE in Air Breathing Fish *Clarias batrachus* (Linnaeus, 1758)

*Mahira Parveen, Santosh Kumar, Pramila Singh

Department of Biosciences, Barkatullah University, Bhopal, 462026 (M.P), India
**E mail: mahira_pp@yahoo.co.in*

Özet: Clarias batrachus *(Linnaeus, 1758) karaciğerinde AChE inhibisyonunun* in vivo *kinetik analizi.* Karaciğerdeki AChE dağılımından dolayı, nörotoksik pestisit karbaril ile zıt bir etkileşim gözlenmektedir. Bu çalışma, karbaril'in karaciğer AChE üzerindeki toksik etkilerini değerlendirmek amacıyla gerçekleştirilmiştir. Çalışmada, balıklar 96 saat boyunca karbarilin subletal konsantrasyonuna maruz bırakılmış ve karaciğerdeki AChE enzim kinetiği üzerindeki etkileri araştırılmıştır. Subletal konsantrasyon 0.04 ppm olarak, karbarilin LC50 değeri bazında (0.137 ppm, Ramana Rao vd., 1991) alınmıştır. Karaciğer çıkartılmış ve asetilkolinesteraz enzim kinetiği kontrol ve deney gruplarında gözlenmiştir. AChE için Km, 1.46x10-3 M olarak tespit edilmiştir. Karbaril uygulamasından sonra Km artarak 2.0 x 10-3 M değerine yükselmiştir. Karbaril uygulanmış ve kontrol karaciğeri için Vmax 0.9A /mg protein/30 min değerinde sabit kalmıştır. Kontrol ve karbamat uygulanmış balık dokularındaki AChE kinematik çalışması, karbaril inhibisyonunun rekabetçi doğasını göstermiştir..

Anahtar Kelimeler: Karbaril, kinetik, AChE, balık

Abstract: Because of the distribution of AChE in liver it is affected adversely by the neurotoxic pesticide carbaryl. To evaluate the toxic effects of carbaryl on liver AChE , the work is carried out. In the present investigation, the fishes were intoxicated with sublethal concentration of carbaryl for 96 hours period and its effects were studied on AChE enzyme kinetics in liver. Fishes were exposed to carbaryl for 96 hours period. The sublethal concentration was taken as 0.04 ppm on the basis of LC_{50} of carbaryl i.e. 0.137 ppm (Ramana Rao et al 1991). The liver was removed and acetylcholinesterase enzyme kinetics was observed in control and experimental groups. The K_m for AChE in control liver was observed as $1.46x\ 10^{-3}$ M. After the carbaryl treatment the K_m was increased and became $2.0\ x\ 10^{-3}$ M. The V_{max} for carbaryl treated and control liver was constant at 0.9A /mg protein/30 min. Kinetic study of AChE in control and carbamate exposed fish tissues show competitive nature of inhibition by carbaryl.

Key Words: Carbaryl, kinetics, AChE, fish

Introductıon

The frequent uses of pesticides in agriculture practices as well as pest control pollute the soil, hydrosoil and water bodies thus reaching the aquatic ecosystem get enriched in the aquatic food chain organisms like fishes. The pharmacological action of carbamate pesticides is described to an inhibition of acetylcholinesterase enzyme activity in fishes in acute lethal and sublethal concentrations. Liver is one of the major tissue in fish body which work as a necessary digestive gland and is the main site for important metabolic reactions. Because of the distribution of AChE in liver it is affected adversely by the neurotoxicant carbaryl. To evaluate the toxic effects of carbaryl on liver AChE, the work is carried out. In the present investigation, the fishes were intoxicated with sublethal concentration of carbaryl for 96 hours period and its effects were studied on AChE enzyme kinetics in liver.

Materials and Methods

Fish, *Clarias batrachus* of 10-12 cm in length were collected from fish farm Bhel Lake Bhopal. They were acclimatized in glass aquaria for one week and were regularly fed with a mixture of rice bran and groundnut oil cake in a equal proportion. The toxicant used is carbaryl manufactured by Poulenc Agrochemicals Bombay, India Ltd.

Fishes were exposed to carbaryl for 96 hours period. The sublethal concentration was taken as 0.04 ppm on the basis of LC_{50} of carbaryl i.e. 0.137 ppm (Ramana Rao *et al.,* 1991). The liver was removed and acetylcholinesterase enzyme kinetics was observed in control and experimental groups. The tissue homogenate preparation, enzyme kinetics and kinetic constants were determined as described by Parveen and Kumar (2002).

Results

Fishes after carbaryl treatment showed behavioral changes in the form of lethargic behavior and most of the time were sitting at upper corner of glass aquarium. Black and dark brown spots were also observed on the skin, indicating poisonous effect of carbaryl on integument. The K_m for AChE in control liver was observed as $1.46x10^{-3}$ M. After the carbaryl treatment the K_m was increased and became $2.0x10^{-3}$ M. The

V_{max} for carbaryl treated and control liver was constant at 0.9A/mg protein/30 min.

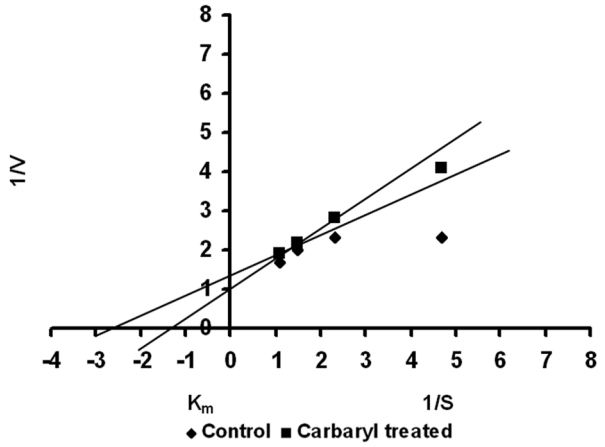

Figure 1. Effect of carbaryl toxicity on AChE kinetics showing K_m and V_{max} values for control and carbaryl intoxicated fishes. *Clarias batrachus.*

Discussion

Kinetic study of AChE in control and carbamate exposed fish tissues show competitive nature of inhibition by carbaryl. Toxicity of another carbamate pesticide dieldrin caused higher residues in liver than tissues of *Clarias batrachus* (Lamai 2003). Physostigmine analogs inhibit the AChE enzyme, where second order rate constant $K_{(on)}$ of the enzyme inhibitor complex correlates with the conformational positioning of aromatic residues especially Trp 84, in the transition state complex (Gavuzzo and Pomponi, 2002). The AChE enzyme inhibited by the eseroline and eserine indicated that kinetic rates for association and dissociation of eseroline with electric eel AChE were two grades of magnitude higher than those of eserine.(Golicnik and Stojan, 2003).Kinetic parameters observed between interspccies IC_{50} differences indicated that carps were more sensitive to eserine than human (Jeantly *et al.,* 2002). Kinetics of AChE inhibition in fish tissues by other than carbamates showed that tarcine and huprinexbind more tightly to Torpedo fish than to human AChE (Dvir *et al.,* 2002). Most of the 17 coumarin and 2 chromone derivatives inhibit AChE noncompetitively (Bruhlman *et al.,* 2001). Whereas, TPPs (White and Harmon, 2002) and profenofos (Venkateswara Rao *et al.,* 2003) inhibit fish AChE competitively. The kinetic mechanism of carbaryl induced AChE inhibition gives the evidences that this action is competitive as evaluated from kinetic constants.

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