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Influence of Some Commercial Pesticides on Human and Bovine Erythrocyte Carbonic Anhydrase Enzymes (*in Vitro*)

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Abstract: In this study, the effects of Deltamethrin, Pyrazophos, Chlorpyrifos-methyl, Dimethoat, Metamidophos, Dichlorvos, Diazinon, Captan and Malathion, which are pesticides, were investigated on human erythrocyte carbonic anhydrase (HCA) and bovine erythrocyte carbonic anhydrase (BCA). It was found out that the chemicals have inhibition effects on the enzymes. The I_{50} values of the chemicals caused inhibition were determined by means of activity percentage- [I] diagrams. The values of I_{50} were 2.6×10^{-3} M, 6.9×10^{-2} M, 3.7×10^{-2} M, 7.7×10^{-2} M, 3.6×10^{-1} M, 2.6×10^{-1} M, 9.7×10^{-3} M, 6×10^{-2} M and 1.1×10^{-2} M, for HCA respectively. The values of I_{50} same chemicals for BCA were 4.1×10^{-3} M, 6.9×10^{-2} M, 6.3×10^{-2} M, 5.6×10^{-2} M, 1.8×10^{-1} M, 2.1×10^{-1} M, 3.1×10^{-2} M, 7.6×10^{-2} M and 2×10^{-2} M, respectively. The results showed that Deltamethrin was the strongest inhibitor for both HCA and BCA. While the pesticide having the least inhibition effect on HCA was Metamidophos, on BCA was Dichlorvos.

Key words: Carbonic anhydrase, inhibition, pesticides

Bazı Ticari Pestisitlerin İnsan ve Sığır Eritrosit Karbonik Anhidraz Enzimi Üzerine Etkileri (in-Vitro)

Özet: Bu çalışmada, insan eritrosit karbonik anhidraz (HCA) ve sığır eritrosit karbonik anhidraz (BCA) enzimi üzerine pestisitlerden; Deltamethrin, Pyrazophos, Chlorpyrifos-methyl, Dimethoat, Metamidophos, Dichlorvos, Diazinon, Captan ve Malathion'un etkileri araştırıldı. Bu kimyasalların enzimler üzerinde inhibisyon etkilerine sahip olduğu bulundu. İnhibisyona sebep olan kimyasalların I_{50} değerleri, % aktivite-[I] grafikleri yardımıyla belirlendi. İnsan CA'sı için I_{50} değerlerinin sırasıyla: 2.6×10^{-3} M, 6.9×10^{-2} M, 3.7×10^{-2} M, 7.7×10^{-2} M, 3.6×10^{-1} M, 2.6×10^{-1} M, 9.7×10^{-3} M, 6×10^{-2} M ve 1.1×10^{-2} M olduğu bulundu. Sığır CA'sı için aynı kimyasal maddelerin I_{50} değerleri ise sırasıyla: 4.1×10^{-3} M, 6.9×10^{-2} M, 6.3×10^{-2} M, 5.6×10^{-2} M, 1.8×10^{-1} M, 2.1×10^{-1} M, 3.1×10^{-2} M, 7.6×10^{-2} M ve 2×10^{-2} M idi. Bu sonuçlara göre; Deltamethrin'in insan ve sığır CA'sı için en güçlü inhibitör etki yaptığı tespit edildi. HCA üzerinde en az inhibisyon etkisi gösteren pestisit Metamidophos iken, BCA üzerine Dichlorvos idi.

Anahtar kelimeler: Karbonik anhidraz, inhibisyon, pestisitler

Introduction

Environmental pollution by pesticide residues is a major environmental concern due to their extensive use in agriculture and in public health programs (Waliszewski et al., 1996). The environmental impact of pesticide use is related to several fundamental properties, essential to their effectiveness as pesticides. Firstly, pesticides are toxicants, capable of affecting all taxonomic groups of biota, including non-target organisms, to varying degrees depends on physiological and ecological factors. Secondly, many pesticides need to be resistant to environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced. This property also promotes long-term effects in natural ecosystem (Christensen and Tucker, 1977).

Since pesticides are offered for plant protection, there has been improvement in the control of pest population and spread of infection born disease vectors.

Public health programs in many developing countries including Turkey also utilize these studies as pesticides of

choice to control disease-transmitting organism (Arslan et al., 1997).

There is abundant evidence that many pesticides produce their acute toxic action by inhibiting enzymes. In addition, chemicals via food chain have harmed physiological mechanisms in man.

In general, enzyme activity was strongly reduced by heavy metal inorganic cations, but less strongly by organometallic cations programs (Waliszewski et al., 1996).

On the other hand, many chemicals at even relatively low dosages disturb the metabolism of biota by altering normal enzyme activity (Arslan et al., 1997; Chang and Stockstad, 1975; Çelik et al., 1996a; Çelik et al., 1996b; Çelik and Kara, 1997; Çelik et al., 1997; Kara and Çelik, 1997; Şekeroğlu et al., 1997; Türkoğlu et al., 1999). The study done by Çelik (Çelik et al., 1997) can be given as an example.

In that study, the influence of Penncozeb, Dithane, Cupravit, Bayleton, Baythroid, Mavrik, Talstar and

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Endosulfan have been investigated on human erythrocyte carbonic anhydrase isozymes (HCA-I, HCA-II) and bovine erythrocyte carbonic anhydrase (BCA) in vitro. Among the chemicals; Baythroid, Talstar, Mavrik were determined to have inhibition effect, on both BCA and HCA isoenzymes, but Dithane only on BCA.

In Turkey, farmers and cattle, including goat extensively use the mentioned compounds and sheep, freely grazed near the agricultural fields. Thus, non-judicious application may lead to accumulation of the pesticides in the cattle, thereby causing undesirable metabolic effects. Unfortunately, there is no information available on the effect of mentioned pesticides in the cattle and other animals.

The present study was conducted to assess the in vitro effect of well-known pesticides Deltamethrin, Pyrazophos, Chlorpyrifos-methyl, Dimethoat, Metamidophos, Dichlorvos, Diazinon, Captan and Malathion on the activity of human and bovine total carbonic anhydrase.

Materials and Methods

Materials

The chemicals in the present study were obtained from the indicated sources: Sodium carbonate, sodium bicarbonate, sodium citrate dehydrate, NaCl, Citric acid were obtained from E. Merck.

All pesticides and CO₂ gas were provided from the market. The blood samples were obtained from humans and bovines.

Methods

Preparation of hemolysates containing CA activity from human and bovine red blood cells

Human blood samples were obtained by using bottles with anticoagulator (ACD = Acid citrate-dextrose) from Blood Centre of Special Polyclinic. Bovine blood samples were obtained from Van Slaughterhouse, and were centrifuged at 1500 rpm for 20 min., the plasma was removed. After the packed red cells were washed with NaCl (0.9 %) for three times, the erythrocytes were hemolysated with cold water. The cell membranes were removed by centrifugation at 4 °C, 14000 rpm (20.000xg) for 30 min (Çelik et al., 1997). The hemolysate was used for the determination effect of chemicals on total activity of erythrocyte carbonic anhydrase.

Determination of effect of chemicals on total activity of erythrocyte carbonic anhydrase

The effects of chemicals were assayed by hydratase method basing on hydration of CO₂ (Rickli et al., 1964).

CO₂-Hydratase activity as enzyme unit (EU) was calculated by the equation $(t_0 - t_c/t_c)$ where t_0 and t_c are the times for pH change of the non-enzymatic (buffer) and the enzymatic reaction, respectively. In order to determine I₅₀ values of the enzymes, regression analysis graphs were drawn by using % inhibition values by a packing program (Microsoft Excel 5.0) of a computer. The concentrations causing up to 50 % inhibition were determined from the graphs.

Results and Discussions

The method used in this study was hydratase to show the influence of chemicals. The reason of preference of this method was based on dehydration of CO₂ as the role of carbonic anhydrase in animals. It can be assumed that the color of hemoglobin can effect color reaction. But the hemolysate obtained from erythrocytes was over-diluted therefore, the effect of hemoglobin on color reaction is not significant.

All of the chemicals were determined to have inhibitory effects on both human CA and bovine CA enzymes. The human and bovine erythrocyte CA enzymes were strongly inhibited at the lower concentrations of Deltamethrin but were less sensitive to Metamidophos and Dichlorvos.

In the Table 1, the results were listed in terms of molarity of the chemical tested causing a 50% reduction of enzyme activity.

Individual species and organism in the natural environment differ widely in sensitivity to any pesticides. This variation in response means that pesticides can eliminate susceptible individuals from a population or an entire susceptible species from a community of organisms (Pimental and Goodman, 1974). A considerable literature exists describing the effects of pesticides on populations and communities of organisms under field conditions. Major effects of pesticides on animal and insect populations result primarily in significant changes in species abundance and associated shifts in population dynamics (Dempster, 1975). Thus, they have been resulted in an imbalance in the natural system.

Pesticides are commonly used agricultural chemicals. In our study, Deltamethrin Pyrazophos, Chlorpyrifos-methyl, Dimethoat, Metamidophos, Dichlorvos, Diazinon, Captan and Malathion were preferred because the reports concerning with their effects are very limited about the toxicological or biological results of these chemical on higher animals, and also they have been frequently used in our country. The effects of some pesticides, at the different concentration were investigated in our study.

It was found that they had inhibitory effect on the carbonic anhydrase enzymes of erythrocytes in human and bovine (Table 1).

Table 1. The values of I_{50} in terms of molarity of the chemicals tested causing a 50 % reduction of human CA and bovine CA enzyme activity

Chemicals	Human CA I_{50} [M]	Bovine CA I_{50} [M]
Deltamethrin	2.6×10^{-3}	4.1×10^{-3}
Pyrazophos	6.9×10^{-2}	6.9×10^{-2}
Chlorpyrifos-methyl	3.7×10^{-2}	6.3×10^{-2}
Dimethoat	7.7×10^{-2}	5.6×10^{-2}
Metamidophos	3.6×10^{-1}	1.8×10^{-1}
Dichlorvos	2.6×10^{-1}	2.1×10^{-1}
Diazinon	9.7×10^{-3}	3.1×10^{-2}
Captan	6×10^{-2}	7.6×10^{-2}
Malathion	1.1×10^{-2}	2×10^{-2}

Among the pesticides, Deltamethrin was found to have the strongest inhibitory effect on HCA. The decreasing order of inhibitory effect of pesticides for HCA was Diazinon, Malathion, Chlorpyrifos-methyl, Captan, Pyrazophos, Dimethoat, Dichlorvos, Metamidophos, respectively.

All of the chemicals tested were determined to have inhibitory effects on BCA enzyme. These results showed that Deltamethrin had also the strongest inhibitor effect on BCA, whereas the inhibitory effect of pesticides on BCA was slightly different from on HCA and in the decreasing order was Malathion, Diazinon, Dimethoat, Chlorpyrifos-methyl, Pyrazophos, Captan, Metamidophos and Dichlorvos.

No report was available in the literature concerning *in vitro* studies of the effect of these pesticides on carbonic anhydrase enzyme in human and bovine. Therefore, we were not able to compare our results with those of the previous studies. Because of variability in analyzing enzyme-inhibitor interaction (*in vitro*) due to inconsistent factors like incubation time, purity, species-tissue differences and temperature etc., it is difficult to compare these data to the result obtained in different laboratories regarding the ranking of test chemicals for inhibiting or activating effect.

Chemical compounds inhibited HCA and BCA activity exhibited their effect by binding to active centre of enzymes or to other part of enzymes. On the other hand, it can be thought that the reason of changing the values in respect to enzymes depend on the range, number and the kind of enzymes amino acids.

Conclusion

As a result, it is impossible to prohibit these kinds of chemicals, which are used against harmful insects and prevent the loose of crop under these conditions today. But the necessity of using pesticides should be decreased by improving resistant plants species to diseases and harmful agents. This gap led these researchers to investigate the toxic potential of these pesticides.

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