THE INHIBITORY EFFECTS OF SOME PESTICIDES ON HUMAN ERYTHROCYTE CARBONIC ANHYDRASE ACTIVITY (*IN VITRO*)

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ABSTRACT

The effects of Folidol™ [O,O-dimethyl O-(4-nitrophenyl) phosphorothionate; methyl parathion], Amin'a™ [dimethylamine salt of 2,4-D; 2,4-dichlorophenoxy acetic acid dimethylamine], Trimidal™ [a-(2-chlorophenyl)-a-(4-fluorophenyl)-5-pyrimidine-methanol], Fusilade™ [fluazifop-p-butyl; R-2-[4-((5-trifluoromethyl)-2-pyridinyl)oxy) phenoxy)propanate] and Rubigan™ [a-(2-chloro-phenyl)-a-(4-chlorophenyl)-5-pyrimidinemethanol; Fenarimol], which are commonly used in agricultural fields, have been investigated on human erythrocyte carbonic anhydrase isoenzymes (HCA-I, HCA-II) *in vitro*. Isoenzymes employed in the study were purified by using Sepharose-4B-L-tyrosine-sulphanylamide affinity gel. All the pesticides evaluated inhibited the activity of isoenzymes to various degrees. I_{50} values of chemicals caused inhibition were determined by means of activity percentage-[I] diagrams. These values of Folidol™, Amin'a™, Trimidal™, Fusilade™, Rubigan™ for CA-I and CA-II were 5.25 x 10^{-4} and 3.60 x 10^{-4}, 4.74 x 10^{-4} and 2.65 x 10^{-4}, 1.84 x 10^{-3} and 1.36 x 10^{-5}, 1.18 x 10^{-4} and 5.89 x 10^{-5}, 8.91 x 10^{-4} and 6.99 x 10^{-5}, respectively. Trimidal™, Fusilade™ and Rubigan™ were the most effective inhibitors for CA-II isoenzyme. The inhibition of Trimidal™ was quite higher to CA-II than CA-I, although Folidol™ and Amin'a™ showed similar inhibition effects on CA-I and CA-II activities.

KEYWORDS: Carbonic anhydrase, Pesticide, Inhibitor.

INTRODUCTION

The amount and variety of pesticides used have increased tremendously in recent years. This increase has caused a positive effect on crop production, however, certain pesticides, their residues, metabolites and/ or contaminants have created many unforeseen adverse effects on the environment. Under some conditions, pesticides may be present in very low concentrations which have no immediate detectable effect. These small amounts of chemicals can cause sublethal (chronic) damage to organisms and this is more insidious and difficult to define than acute toxicity. Sublethal effects may be further enhanced by persistent pesticides which are accumulated in the organisms and magnified in the food chain. Many chemicals at relatively low dosages affect the metabolism of biota by altering normal enzyme activity (1-6). In some of these interactions there is high reactivity involving a high degree effect on the whole animal or plant. On the other hand, many chemicals affect the activity of many enzymes only to a moderate degree and it is presumed that the ultimate debilitating effect on the whole organism develops from a variety of nonspecific biochemical functions (7,8).

The enzyme carbonic anhydrase (EC 4.2.1.1) catalyzes the reversible hydration of CO_2 to HCO_3^- and H^+ and is present in nearly all organisms. So far six isoenzymes have been described in mammals (9). The only known physiological function of the carbonic anhydrase isoenzymes is to facilitate the interconversion of CO_2 and HCO_3^- so that they play key roles in diverse processes such as physiological pH control and gas balance, calcification and photosynthesis (10).

In this present study, effects of some pesticides on human erythrocyte carbonic anhydrase isoenzymes were investigated.

EXPERIMENTAL

Materials

Analytical grade chemicals and solvents were supplied by BDH. Sepharose-4B, TEMED, standard bovine serum albumin, dialysis bag, p-aminobenzene sulfonamide, L-tyrosine, sodium carbonate, sodium bicarbonate, sodium acetate, sodium sulfide, sodium citrate, cyanogen bromide, and trizma base were bought from Sigma. All the above-mentioned pesticides (technical grade) employed in the investigation were purchased locally from companies licensed to sell.

Purification of carbonic anhydrase isoenzymes from erythrocytes

Blood samples were obtained, in tubes including anticoagulant citrate-dextrose, from government hospital in Balikesir and stored at 4 ^{0}C. The samples were centrifuged at 1500 g for 20 min at 4 ^{0}C, then plasma and buffy coat were removed. Erythrocytes were washed three times with NaCl (0.9 %), after which they were hemolysed with cold water. The debris and intact cells were removed by centrifuging at 20000 g for 20 min at 4 ^{0}C. The hemolysate was adjusted to pH 8.5 by addition of solid Tris, then was applied to the affinity column packed with Sepharose 4B-L tyrosine-sulfonylamide and equilibrated with 25 mM Tris-HCl/ 0.1 M Na_2SO_4 (pH 8.5). The affinity gel was washed with 25 mM Tris-HCl/ 22 mM Na_2SO_4 (pH 8.5) solution. HCA-I and HCA-II isoenzymes were eluted with the solutions of 1 M NaCl/ 25 mM Na_2HPO_4 (pH 6.3) and 0.1 M $NaCH_3COO$/ 0.5 M $NaClO_4$ (pH 5.6), respectively. Protein concentration was determined calorimetrically by the method of Bradford (11) and purities of the isoenzymes were controlled with SDS-PAGE (12).

Determination of the enzyme activity

Activity of isoenzymes was determined according to the method of Rickli *et al*. (13). This is a colorimetric assay, which involves the hydration of CO_2, with bromothymol blue as indicator. CO_2-Hydratase activity was calculated as the enzyme unit by the equation (to-tc/ tc), where to and tc are the times for pH changes of the nonenzymatic (buffer) and the enzymatic reactions, respectively.

Determination of I_{50} values

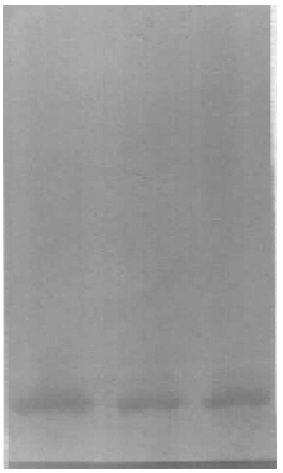
The values of I_{50} (inhibitor concentration reduces the enzymatic activity by 50 %) have been determined graphically, by means of activity percentage-[I], using six different concentrations of each pesticide.

RESULTS

Human erythrocyte carbonic anhydrase I and II were purified by using the affinity gel with the elution buffers of 1 M NaCl / 25 mM Na_2HPO_4 (pH 6.3) and 0.1 M $NaCH_3COO$ / 0.5 M $NaClO_4$ (pH 5.6), respectively. Purity of the isoenzymes were confirmed with SDS-polyacrylamide gel electrophoresis (Fig. 1).

Results are shown in Fig. 2 and listed in Table 1, in terms of molarity of the test chemicals causing a 50 % reduction of the enzymatic activities.

FIGURE 1
SDS-Polyacrylamide Gel Electrophoresis of carbonic anhydrase isoenzymes purified by affinity chromatography (1 bovine serum albumin; 2 HCA-I and 3 HCA-II).



1 2 3

TABLE 1 -The inhibitory effects of some pesticides on carbonic anhydrase isoenzymes (CA-I vs. CA-II).

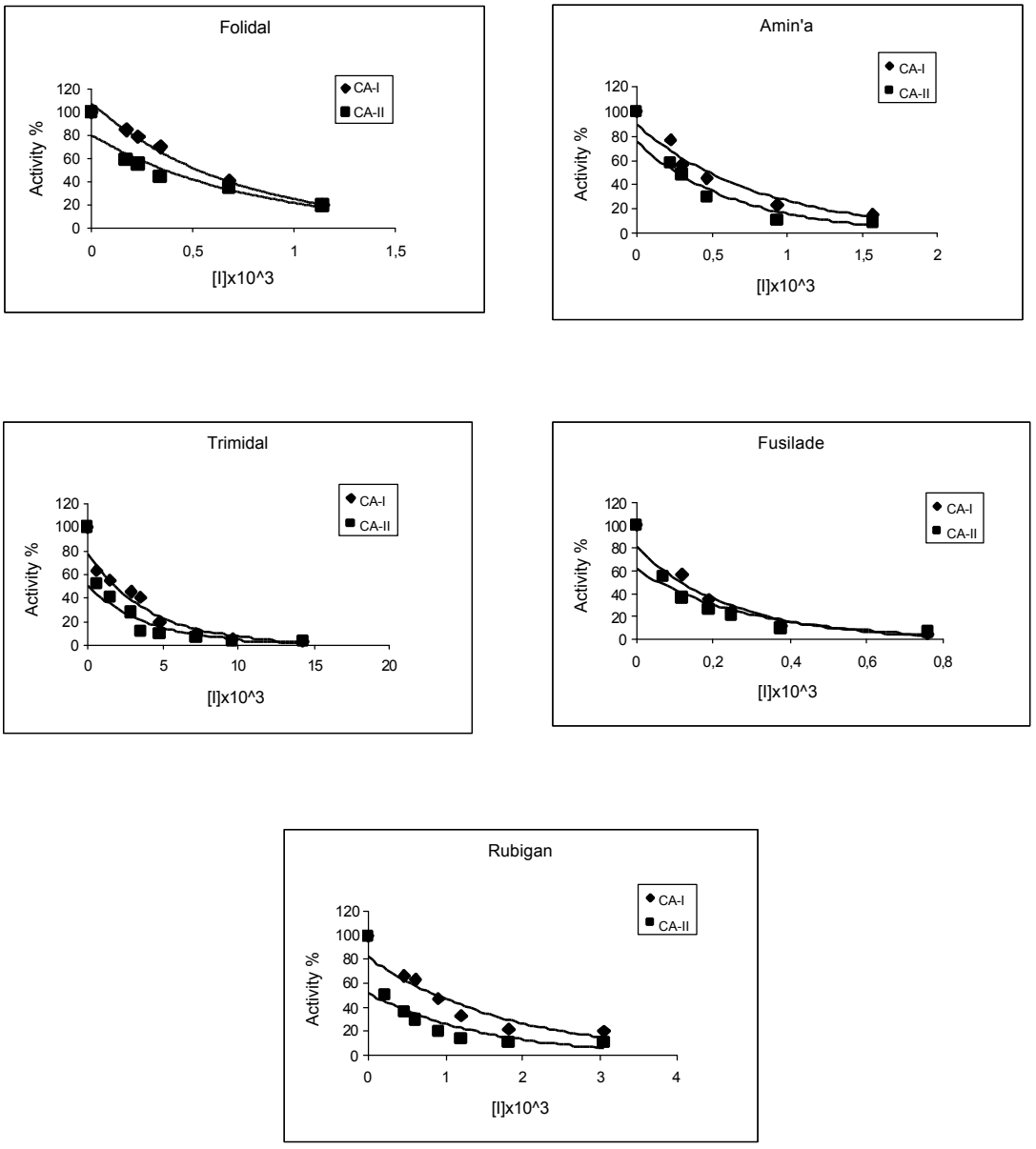
Pesticide	CA-I (I_{50} M)	CA-II (I_{50} M)
Folidol™	$5.25x10^{-4}$	$3.60 x10^{-4}$
Amin'a™	$4.74x10^{-4}$	$2.65 x10^{-4}$
Trimidal™	$1.84x10^{-3}$	$1.36 x10^{-5}$
Fusilade™	$1.18x10^{-4}$	$5.89 x10^{-5}$
Rubigan™	$8.91x10^{-4}$	$6.99x10^{-5}$

DISCUSSION

The pesticides employed in this present study, which are commonly used in agricultural fields of Turkey, showed inhibitory effects on the activity of human erythrocyte carbonic anhydrase isoenzymes I and II to various degrees. As can be seen in Table 1, I_{50} values of Folidol™, Amin'a™, Trimidal™, Fusilade™, Rubigan™ were $5.25x10^{-4}$, $4.74x10^{-4}$, $1.84x10^{-3}$, $1.18x10^{-4}$, $8.91x10^{-4}$ for CA-I and were $3.60x10^{-4}$, $2.65x10^{-4}$, $1.36x10^{-5}$, $5.89x10^{-5}$, $6.99x10^{-5}$ for CA-II, respectively. Fusilade™, Rubigan™ and, especially, Trimidal™ were the most effective inhibitors for CA-II isoenzyme which is very little in erythrocytes as amount. The inhibiting effect of Trimidal™ was rather less to CA-I than CA-II. Folidol™ and Amin'a™ also showed similar inhibitory effects to CA-I and CA-II activities.

The civilized population of human being is using natural resources inappropriately and also adding hundreds of pollutants in the forms of metals, acids, bases, aromatic-aliphatic hydrocarbons and phenolic compounds etc. Thus, an abnormal detrimental situation is being created in the balance of the natural system. In agriculture, pesticides are widely used against possible harmful factors in order to minimize the loss of crop. However, it is known that the pesticides that have a long half-life are a potential risk to animals and human health, since they can be taken into the organisms by various food chains. In most of the countries, the inappropriate use of pesticides make this issue more important to deal with. The results obtained in this work also confirmed the importance of the use of pesticides consciously under the control of specialists.

FIGURE 2
Activity (%) curves of CA-I and CA-II in different Folidol™, Amin'a™, Trimidal™, Fusilade™ and Rubigan™ concentrations.



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