Toxic Effect of Pesticides on Aldose Reductase Enzyme

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Abstract

In this study, the effect of pesticides on the enzyme activity of Aldose reductase (AR) (EC 1.1.1.21), which is involved in polyol pathway and detoxification reactions, has been investigated. For this purpose, AR enzyme was purified from chicken liver and the IC50 values were plotted as % Activity-[I] for pesticides with inhibition effect and The maximum inhibitory effect was determined at 2,4,5-T with 162µM.

Keywords: Inhibitor, enzyme, aldose reductase

Introduction

The developments occuring from the technological field Started to feel its presence felt in the scientific world as much as it is in daily life. These developments in the field of science are manifested in studies carried out in biochemical fields (eg, ecology, toxicology). Enzymes in the protein structure are biological catalysts that accelerate metabolism reactions under appropriate conditions. The catalytic function of enzymes depends on the stability of their natural protein conformation [1, 2]. When enzymes are exposed to chemical compounds and agents, changes in their activity can affect the metabolic pathways as well as the entire metabolic system and lead to toxicity in the cell[3].

Received: 03.09.2018
Revised: 08.10.2018
Accepted:24.10.2018
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Cite this article as: V. Çomaklı, Ş.Adem and Ç. Güler, Toxic Effect of Pesticides on Aldose Reductase Enzyme, Eastern Anatolian Journal of Science, Vol. 4, Issue 2, 1-5, 2018.

Aldose reductase enzyme (AR) (EC 1.1.1.21) is a member of the aldo keto reductase family responsible for the reduction of aldehydes and ketones. As shown in figure 1, The AR enzyme, which uses NADPH as the cofactor, enables the reduction of monosaccharides and compounds containing many carbonyl groups to alcohol and the detoxification of metabolites formed by lipid peroxidation [4, 5].

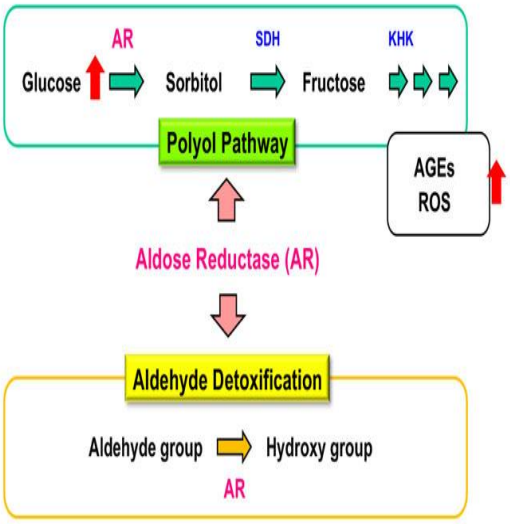

Figure 1. The role of aldose reductase enzyme under hyperglycaemia conditions and detoxification [5]

Under hyperglycemic conditions, rate limiting enzyme in the polyol pathway, AR converts the glucose to sorbitol [6]. Researchers, under the conditions of hyperglycemia, reduction of polyol pathway activation by using AR inhibitors could be a potential therapeutic treatment or prevention of diabetic complications [7-9] (7,8,9). The saturated aldehydes [e.g., malondialdehyde (MDA)] and unsaturated aldehydes [e.g., 4-hydroxy-trans-2-nonenal (HNE)] resulting from lipid peroxidation

cause the formation of cytotoxic products in living organism. The cytotoxic products occurring by lipid peroxidation contribute to the formation and spread of oxidative damage. This products are metabolized or removed by diverse enzymatic or nonezymatic processes, which are catalyzed by, aldehyde dehydrogenases, glutathione S-transferases and aldo-keto reductases (AKRs) using glutathione peptidyl antioxidants. Aldose reductase (AR), a member of the aldo keto reductase enzyme family, plays an important role in the metabolism of these cytotoxic products[10]. Due to this multifunctional property of the above-mentioned AR enzyme, the design of the inhibitor for the enzyme is gaining importance.

Pesticides are chemical substances used as a disinfectant, antimicrobial or biological agent against any pest. Some of these chemicals are persistent organic pollutants due to their effects on the soil and the environment. In many cases, pesticides can interfere into aquatic environment, plants, animals and may be toxic for specific enzymes. In particular, these chemicals may exert toxic effects on living organisms by increasing or decreasing the activity of enzymes that fulfill important metabolic functions in living systems[11]. In this study, purification from the chicken liver of AR enzyme, which is an important task in detoxification and it is aimed to investigate the toxic effect of some pesticides on enzyme activity.

Materials and Methods

Purification of enzyme

The 5 g chicken liver was first washed with isotonic NaCl, then cut into small pieces. The sample was placed in -80 refrigerator 3 times for freeze-thaw method. The sample thoroughly crushed in a porcelain mortar was treated with 30 ml 50 mM Tris-HCI buffer containing 1mM EDTA, 2 mM DDT, pH:7.5. Homogenate was centrifuged at 10 rpm, +4 C for 20 minutes. Purification steps were continued with supernatant. 2'5' ADP Sepharose 4B affinity column was prepared according to the method previously determined[12]. 25 ml homogenate was uploaded to the column, then, the column was washed with 50 mM phosphate buffer including 1mM DTT, 1mM EDTA, pH 7.35 until the absorbance of the wash solution was 0.02 at 280 nm. Aldose reductase was eluted with 80 mM phosphate buffer containing 80 mM KCl, 0.1 mM NADP + 1mM EDTA, pH 7.85. Active eluates pooled and dialyzed against 80 mM phosphate buffer, pH: 7.5 for ten hours.

Activity assay

Aldose reductase activities were measured following absorbance change in the 5 minutes according to Beutler's method at 340 nm [13]. 200 µL of phosphate (0.1 M, pH = 6.2), 600 µL of pure water, 0.11 mM NADPH and enzyme solution and 30 µL of enzyme were pipetted with automatic pipette and incubated for 5 min at 30°C. The reaction was initiated with the addition of 40 µL of the 15 mM DL-glyceraldehyde to the mixture.

Inhibition studies

Pesticides were dissolve as 1mg/ml. Different volumes were added to reaction mixture by reducing the volume of water in the same amount. The concentration that reduces the enzyme activity by 50% was determined by preliminary testing. To determine the IC50 values, enzyme activity was tested at 5 different concentrations of pesticides.

Results and Discussion

Here, we isolated the AR enzyme chicken liver tissue for the first time. We achieved to purify with 2'5' ADP Sepharose 4B afinity choromatography. In addition to purification of the enzyme the inhibitory effects of some pesticides on AR enzyme activity have been examined. For this purpose, the I50 parameters of methomyl, carbofuran, simazine, tebuconazole, atrazine, propoxur, 1-naphthol, 2,4-D and 2,4,5-T pesticides with inhibitor effect were determined The maximum inhibitory effect was determined at 2,4,5-T with 162µM (Figure 2).

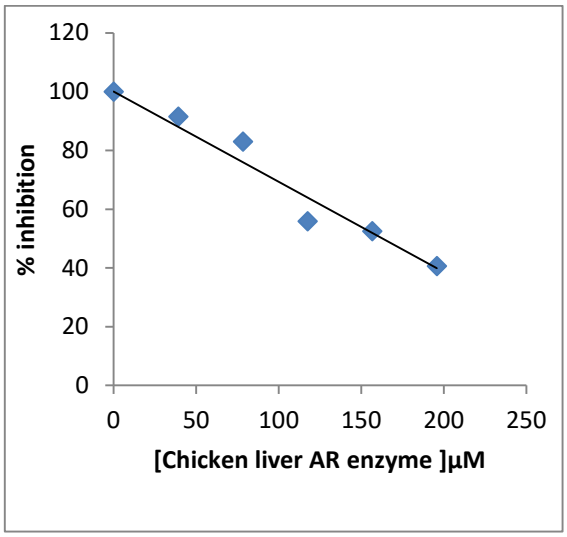

Figure 2. Activity of Chicken liver AR (%) in concentration of 2,4,5-T Pesticide.

Aldose reductase enzyme is an important enzyme responsible for the metabolism of aldehydes [9]. Because this enzyme is responsible for the development of diabetic complications, especially because of its activity on the polyol pathway, researchers have been tempted to make efforts to identify AR inhibitors [3, 8, 9]. But, Studies to determine the effect of AR enzyme on detoxification have shown that AR enzyme inhibition increase pathological conditions such as the modification of proteins in inflamed arteries[14], atherosclerotic lesions[15], ischemic hearts [16], elderly hearts[17] diabetic hearts[18]. Therefore, we suggest that this enzyme, which exhibits different functional properties, should be thought of as tissue dependent and that the inhibition of the enzyme should be assessed correctly for the cell's benefit / harm relationship.

Today, many toxic substances such as pesticides, have been passing into the soil, water, plants, and then from there to animals and people. For this reason, today, in particular enzyme activity works made by using these substances remains its popularity [11, 19]. It has been reported by researchers in the literature that pesticides showed toxic effect for POD, CA, G6PD, PON1, GST, TrxR and GR enzymes purified from different organisms[20-24].

In study, IC50 values were determined as 162, 163, 203, 216, 220, 232, 290, 309 and 340 µM, for 2,4,5-T, 2,4-D, propoxur, carbofuran, simazine, tebuconazole, atrazine, 1-naphthol and methomyl, respectively (Table 1). Our results showed that pesticides inhibit chicken liver AR enzyme activity with rank order 2,4,5-T > 2,4-D > propoxur > carbofuran > simazine > tebuconazole > atrazine > 1-naphthol > methomyl, in in vitro conditions.

Table 1. Values of IC50 obtained from regression analysis graphs for AR from chikhen liver tissue of pesticides.

Pesticide name	IC_{50} concentration(µM)
2,4,5-T	162
2,4-D	163
propoxur	203
carbofuran	216
simazine	220
tebuconazole	232
atrazine	290
1-naphthol	309
methomyl	340

Consequently, our findings indicate these pesticides are potent inhibitors for AR enzyme, and might cause undesirable results by disrupting detoxification. For this reason, the usage of pecticides must be well controlled. These results are consistent with the literature in that the overuse of pesticides is detrimental to the metabolic processes of living things.

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