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TOXIC EFFECT OF LANNATE ON CELLULAR DEFENCE SYSTEM IN ERYTHROCYTE OF RAT

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التأثير السام للآنيت على وسائل الدفاع الخلوي في كرات الدم الحمراء في الجرذان

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أجريت هذه الدراسة على كرات الدم الحمراء للجرذان بوضعها مع تركيزات مختلفة من المبيد الحشري للآنيت وقد تم تعيين تركيز الجلوتاثيون وتقدير نشاط انزيم السوبر اكسيد ديسميوتيز وانزيم الجلوتاثيون المختزل بعد نصف ساعة و١٦ ساعة من اضافة الآنيت إلى خلايا الدم الحمراء .

أظهرت النتائج أن هناك نقص في نشاط انزيم السوبر اكسيد ديسميوتيز وانزيم الجلوتاثيون المختزل بعد نصف ساعة وكذلك بعد ١٦ ساعة من اضافة اللآنيت إلى خلايا كريات الدم الحمراء كما اوضحت النتائج وجود نقص ذو دلاله معنوية في تركيز الجلوتاثيون .

يستخلص من هذه النتائج أن هبوط نشاط تلك الانزيمات بالإضافة لمستوى تركيز الجلوتاثيون – أحد اضرار اللآنيت – قد يكون من مسببات تحلل خلايا الدم الحمراء وأكسدة الهيموجلوبين وظهور حالات الانيميا المصاحبة للتعرض لمثل هذه المبيدات الحشرية .

Key Words : Superoxide dismutase-Glutathione reductase - Reduced glutathione

ABSTRACT

Lannate l - methomyl was incubated with erythrocytes of rats at different concentrations. It has been observed that there was a decrease in the activities of superoxide dismutase and glutathione reductase after 1/2 and 16 hour of Lannate addition. There was also a decrease in the amount of glutathione. The inhibition of enzyme activities and the decrease in glutathione concentration were dose independent. The results suggest that Lannate toxicity might lead to haemolysis, oxidation of haemoglobin and finally induced erythropenia.

INTRODUCTION

The use of insecticides and pesticides in agriculture and in insect control has been creating a potential danger to human health. Lannate, like other insecticides compounds, acts as neurotoxin due to its ability to block neurotransmission by inhibiting acetyl-cholinesterase as well as producing several changes in blood chemistry. (1).

Erythropenia and leukopenia are among the major effects observed in animals intoxicated with different insecticides and pesticides (2) and (3).

Superoxide dismutase (SOD) is widely distributed in oxygen-metabolising cells and it has been supposed to protect such cells against the deleterious actions of superoxide radical (O^-_2) (4) and (5). Under certain conditions O^-_2 is a precursor of hydrogen peroxide (H_2O_2) and it has been found that the presence of SOD prevents the detection of O^-_2 and stimulates the production of H_2O_2 (6). The generation of large amounts of H_2O_2 that occur in vivo, could be important pathophysiologically from several aspects. Erythrocytes are particularly suitable for such studies because of their accessibility, limited life-span, and relative simplicity.

The glutathione (GSH), glutathione reductase (GR) and superoxide dismutase belong to systems which protect cells against damage by superoxide radicals (4). It is necessary to enquire after the potential source of O^-_2 within RBC's. The purpose of this study was to investigate the influence of Lannate on glutathione, glutathione reductase and superoxide dismutase as defence system against oxidative substances in red cells of rats.

MATERIAL AND METHODS

Insecticides: The insecticides used in the present study was Lannate; L-methomyl [S-methyl-N- (methyl carbamyl) oxy thioacetimidate].

Erythrocytes: Blood was collected from Albino rats in heparinized tubes. The plasma and the buffy coat were removed after centrifugation. The erythrocytes were washed three times with saline phosphate buffer pH 7.4.

Erythrocytes Superoxide Dismutase Extraction: Washed erythrocytes are haemolyzed by adding 1.5 volumes of distilled water. Cell membrances are removed by centrifugation. The haemoglobin is adjusted to 10g/100ml. To 0.5 ml of haemolyzate, 3.5 ml of ice-cold distilled water is added followed by 1.0 ml of ethanol and 0.6 ml of chloroform. After each addition, the contents are well mixed and finally shaken for 1 minute using vortex mixer. The tube is centrifuged for 10 minutes at 3000 r.p.m. The enzyme is contained in the clear top layer (7).

Determination of SOD Activity: The ability of SOD to inhibit the phenazine methosulafate (PMS)- medicated reduction of nitroblue terazolium (NBT) dye was varified by the procedure of Nishikimi et al. (8)

Determination of Glutathione Reductase Activity: The activity of glutathione reductase was determined by the method of Beutler (9). The principle depends on the ability of glutathione reductase to reduce the oxidized glutathione (GSSG) to reduced glutathione (GSH).

Determination of Reduced Glutathione: Red blood cells glutathione was estiamated by the method of Beutler (10) using 5,5'-dithiobis-2 nitro benzoic acid. (DTNB).

RESULTS

As shown in Figure (1), the addition of low concentration of Lannate to erythrocyte suspension produced a decrease in SOD activity as evidenced by remarkable percent inhibition compared to the control value. At high doses of Lannate used in the present experiment, the % inhibition of SOD acitvity was not remarkable after 1/2 hr of Lannate treatment and was close to the control value. However, the % inhibition was more eveident but unremarkable after 16 hr of Lannate addition than after 1/2hr of treatment.

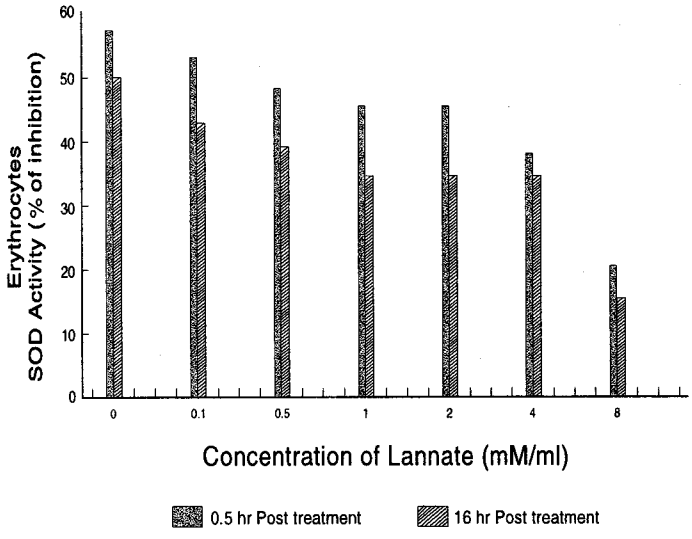

Figure 1. Activity of SOD in erythrocytes after addition of Lannate

Figure (2) illustrates the decrease in glutathione reductase (GR) activity in erythrocytes after the incubation with different concentration of Lannate. It is evident that the inhibition of GR activity was not concentration dependent. There was no effect of the incubation time as it is observed from the unsignificant difference in the enzyme activity level after 1/2 and 16 hr incubation times.

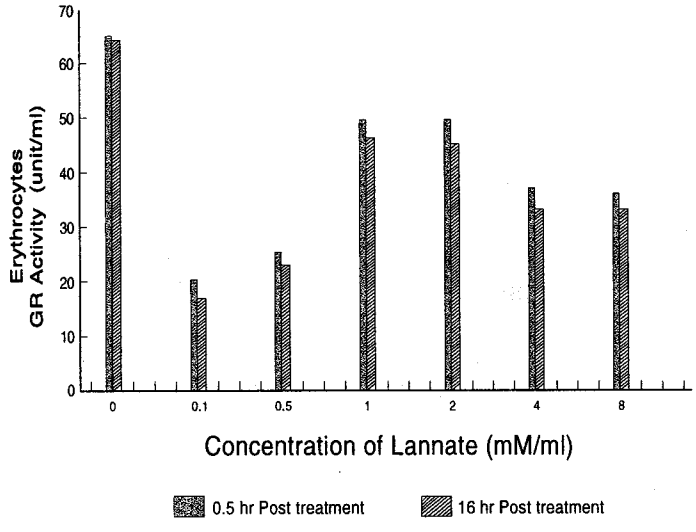

Figure 2. Activity of GR in Erythrocytes after addtion of Lannate

There was a significant decrease in the glutathione concentration after 1/2 hr of Lannate addition, Figure (3). The decrease of GSH was Lannate concentration independent. Glutathione concentration was drastically low after 2 hr of Lannate exposure.

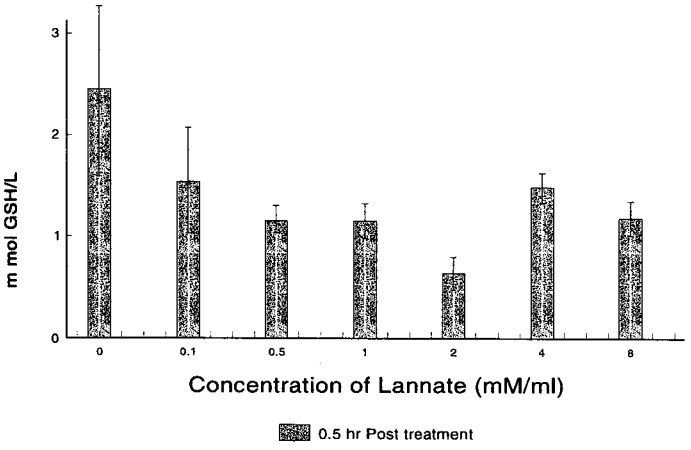

Figure 3 Concentration of GSH in erythrocytes after addition of Lannate

DISCUSSION

Some of the oxygen metabolite produced by poisoning material are powerful oxidizing agents and accordintly oxidized several molecules in cells. Superoxide radical (O^-_2) is formed by one-electron reduction of oxygen, and has been identified as a product in a number of biological reactions (11). It is particularly likely to be formed in the red cell and has been shown to be produced when oxyhaemoglobin is autooxidized to methoemoglobin (12).

Erythrocytes are particularly suitable for the study of the action of superoxide radical and H_2O_2. Assuming that the autooxidation of haemoglobin or myoglobin by Lannate would predict that superoxide radical could be produced during these autooxidation. The superoxide radical is responsible for the induction of the superoxide dismutase enzyme, since the SOD is an inducible enzyme (13). consequently , the production of O^-_2 is capable of attacking the erythrocytes stroma and thus limiting the life-span of these cells. Finally, we could predict the correlation between the concentration of Lannate and the levels of SOD activity in red cells. In another study, a haemolytic anemia was observed due to the high concentrations of such insecticides (3).

The data presented in this study describe the ability of Lannate to inhibit the activity of glutatione reductase in erythrocytes. The glutathione reducatase is an encyme responsible for maintaining normal cellular level of glutothione. A marked depletion in cellular concentration of GSH was observed in the erythrocyte treated with different doses of Lannate. It appears that the Lannate-related decrease in erythrocyte GSH concentration (Figure 3) was direct manifestations of depression in the biosynthesis of GSH. Although the direct interaction of Lannate with GSH or its metabolizing enzymes also could have contributed to the depletion of cellular GSH content after insecticide treatment. Moreover, the possibility of altered levels of cellular oxidized glutathione (GSSG) and mixed disulfides in response to Lannate treatment can't be ruled out. It is known that an increase in the concentrations of these two variables can result in a decreased cellular GSH concentration (14).

The role of GSH in cells is determined by its chemical properties. It playes a role in decreasing the toxicity of several drugs by facilitating their metabolism to less active compounds. It acts as a nucleophilic agent towards a wide variety of electrophilic agents leading to so-called GSH conjugates. This irreversible loss of GSH requires synthesis of GSH to restore the cellular GSH level (15). The present study suggested that Lannate might have similar irreversible impact on GSH and its enzymes.

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