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INFLUENCE OF AGRIYA 1050 ON BLOOD PARAMETERS IN LAMBS

M. P. POPOVA[1] & N. S. NIKOLOVA[2]

[1]Faculty of Agriculture, [2] Faculty of Veterinary Medicine,
Trakia University, Stara Zagora; Bulgaria

Summary

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The study aimed to observe in dynamics the influence of the organophosphorus preparation Agriya 1050 containing fenitrothion as active substance [O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate], applied at various doses and durations of treatment on some enzymatic and non-enzymatic blood indices in lambs.

The data showed that the acute intoxication (single oral dose of 150 mg/kg) with the insecticide did not result in an immediate response. The peak deviations in observed parameters occurred by post treatment day 6 and thereafter a normalization followed. The changes observed following repeated treatment (orally at 50 mg/kg four times at 7-day intervals) and their comparison to the acute experiment allowed us to summarize that the organism of lambs succeeded to become accustomed to challenge doses and duration with the organophosphorus preparation Agriya 1050, used in the present experiments.

Key words: ceruloplasmin, lambs, lipid peroxidation, total cholesterol, total lipids, total protein,

INTRODUCTION

Several investigations demonstrated that organophosphorus compounds, used widely as pesticides, have an adverse effect on the organism of higher animals. Many among them cause deviations in the activities of enzymes and enzymatic systems, located in blood cells, blood plasma and various organs. The inhibitory effect of organophosphorus compounds on cholinesterase activity is the most thoroughly studied (Feyereisen, 1995; Banerje *et al.,* 1999; Kale *et al.,* 1999). The information about the effects of those compounds on enzymes of endoplasmic reticulum, participating as components of the monooxigenase system (Popova & Popova, 1981; Lundgren & de Pierre, 1987; Kale *et al.*, 1999), on mitochondrial enzymes catalyzing the bioenergetic reactions (Popova & Popova, 1981), on adenylate cyclase (Crisk *et al.,* 1980) etc. is less complete. It is known that lindane and malathion cause increase in lipid peroxidation and higher activity of superoxide dismutase, catalase, γ-glutamyl transferase, glutathione peroxidase and glutathione-S-transferase. The reduced activity of acetylcholine esterase was accompanied with a simultaneous decrease in the levels of reduced glutathione (Banerje *et al.*, 1999).

Organophosphorus compounds exert their effect on multiple cellular structural

parameters as well: change the stability of hepatic and renal subcellular membranes (Ivanov *et al.,* 1981), impair membranic fluidity of erythrocyte membranes and haemolysis (Klezczyńska *et al.*, 2001). Also, it was recognized that the organophosphorus insecticide diazinon hindered the metabolism of L-tryptophan to NAD^+ via inhibition of the amino acid utilization (Henderson & Kitos, 1982). A number of metabolic and membrane disturbances are caused by organophosphorus compounds through altering the activity of various enzymes and enzymatic systems, located in different cellular pools. We determined that the inhibition of bioenergetic reactions with Agriya 1060 was related to *in vivo* changes in membrane stability (Ivanov *et al.*, 1981; Popova & Popova, 1981).

The specialized literature about organophosphorus compounds contains some contradictory data, probably because of the variety of used doses and durations of treatment. Furthermore, the individual effects of the members of the group due to their chemical structure and the route of administration are most likely also important. On the basis of the foresaid and our previous results with Agriya 1060 on functional and stability deviations in rats, we undertook studies in lambs with the preparation Agriya 1050, applied at different doses and for variuos periods of time.

MATERIALS AND METHODS

The study was performed with the organophosphorus insecticide Agriya 1050 containing fenitrothion [O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] as active substance (Fetfadjieva *et al.*, 1994). Twenty individual studies were performed with 13 half-merino male lambs at the age of 60 days weighing 15–20 kg. A cross-over experimental design was used (all the animals served as controls –autocontrols and part of them thereafter – as experimental subjects). The animals were reared in a common premise of 56 m^2 and fed a commercial standard starter ration for weaned lambs. The trials were preceeded by a 7-day period of adaptation and acclimatization. Two experiments were performed: acute intoxication via a single treatment at 150 mg/kg and repeated treatment (4 times at 50 mg/kg) at 7-day intervals (Simeonov, 1989). The preparation Agriya 1050 (50% fenitrothion solution) was dissolved in water prior to the challenge to a concentration of 1.5% and was applied orally via a gastric tube in the morning. At the end of the various time intervals, heparinized blood samples were obtained in the morning from *v. jugularis* using sterile needles. A part of sampled blood was used to obtain blood plasma.

The *acute intoxication* was performed in 4 lambs. Blood samples were obtained prior to the treatment and 1, 6 and 8 days after the single administration of the insecticide. All experimental data were compared to control ones (the baseline 13 lambs). The experimental design of *multiple intoxication* was developed according to the data from the acute trial. The animals received 4 treatments with 50 mg/kg Agriya 1050 at 7-day intervals. The blood samples were obtained by days 1, 6, 8, 13, 15, 20 and 28 after the first treatment. The experiment was performed in 3 lambs.

In both designs, the determined blood parameters were identical. The lipid peroxidation products in whole blood and blood plasma (via malone dialdehyde – MDA) were determined according to the procedure of Popov and Pavlova (1977) for tissue fractions. In this trial, the lipid

peroxides were determiend as followed: 0.5 mL blood or blood plasma was mixed with 2.5 mL 10% CCl_3COOH solution. After staying 10 min at 0–4° C, the precipitated protein was removed by filtration. One mililiter of the filtrate was mixed with equal volume of 0.5% thiobarbituric acid. The samples were put in a boiling water bath for 10 min and then, their optical density was measured at 535 nm. The lipid peroxides were assayed also in blood plasma stored for 24 h at 0–4° C. The MDA concentrations were calculated using a molar extinction coefficient of 1.56×10^5 mL.mmol^{-1} (Wills, 1969).

The activity of ceruloplasmin (EC 1.16.3.1.) in blood plasma was determined immediately after obtaining the sample and after storage for 24 h at 0–4° C by the method of Ravin (1961). The observed non-enzymatic parameters were total protein (Biuret reaction, modification of Popov, 1972), total lipids (by the sulfo-phospho-vanillin reaction) and total cholesterol (Ilk's method, based on the Liberman-Burchardt reaction) (Kolb & Kamyshnikov, 1982).

The statistical significance of results obtained in treated animals vs baseline was evaluated by the Student's t-test.

RESULTS AND DISCUSSION

Acute intoxication

The obtained results are presented in Table 1. The activity of ceruloplasmin, an acute phase protein (Eckersall, 1988) involved in the degradation of reactive oxygen species (Goldstein *et al.*, 1979), did not respond immediately, but increased by post treatment day 6. By day 8, it regained the normal values. Similar data were obtained after storage of samples at low temperature. Therefore ceruloplasmin maintained its activity 24 h after the sampling as well.

The changes in lipid peroxidation products followed a biphasic pattern of change. The values by day 6 were signifi-

Table 1. Blood parameters in lambs prior to the treatment (controls) and after treatment – orally with a single dose Agria 1050 at 150 mg/kg (means ± SEM)

Parameters	Sample storage at 0–4° C (h)	Prior to treatment (controls) (n=13)	Days post treatment (n=4)		
			1	6	8
Ceruloplasmin, mg/L	0	142±6	148±11	158±23	141±25
	24	130±6	131±12	148±20	145±13
Lipid peroxidation products, nmol MDA/L					
Blood	0	53.4±4.2	39.4±1.8*	67.6±9.0*	71.4±5.4*
Plasma	0	21.2±2.6	19.2±2.4	42.0±3.8*	37.6±3.8*
Plasma	24	35.2±3.4	45.4±7.6	44.0±3.6	35.0±1.6
Total protein, %	0	8.13±0.15	7.98±0.15	8.66±0.44	8.69±0.40
Total lipids, mg%	0	498±23	416±15*	281±14*	314±21*
Total cholesterol, mmol/L	0	2.15±0.10	1.95±0.07	1.51±0.18	1.73±0.09

MDA= malone diladehyde; * $p<0.05$ vs controls.

Table 2. Blood parameters in lambs prior to the treatment (controls) and after treatment – orally 4 times at 7-day intervals with Agria 1050 at 50 mg/kg (mean values)

Parameters	Sample storage at 0–4° C (h)	Controls (n=13)	Days post treatment (n=3)						
			1	6	8	13	15	20	28
Ceruloplasmin, mg/L	0	142	130	162	160	138	113	120	105
	24	130	107	145	149	134	140	125	119
Lipid peroxidation products, nmol MDA/L									
Blood	0	53.4	34.6	67.2	53.8	54.4	53.0	46.2	49.4
Plasma	0	21.2	11.8	23.8	21.8	18.2	12.0	26.6	22.0
Plasma	24	35.2	50.0	49.0	35.2	30.8	35.2	44.2	33.4
Total protein, %	0	8.13	7.57	8.83	8.24	8.60	8.68	10.23	9.02
Total lipids, mg%	0	498	363	281	395	273	201	233	456
Total cholesterol, mmol/L	0	2.16	1.48	1.25	1.53	1.44	1.09	1.00	1.29

MDA= malone diladehyde.

cantly higher than those in controls. The initial response of this parameter was manifested by obvious decrease or tendency toward reduction of its concentrations in fresh samples of whole blood or blood plasma (Table 1). Following 24-hour storage in a refrigerator however, an increase in lipid peroxidation products was noticed not only in samples obtained by the 1st day, but in those sampled by the 6th day of the intoxication. Eight days after the treatment, the concentrations were similar to those in controls. In previous studies of ours (Popova & Popova, 1981), we observed an activation of lipid peroxidation in hepatic samples in rats after treating them twice with high doses of Agriya 1060.

The lipid parameters (total lipids and total cholesterol) manifested considerably reduced levels by post treatment day 6 (statistically singificant for total lipids at $P<0.05$). This fact corresponded to the enhanced lipid peroxidation in this period after the acute treatment and could in part explain the reduced total lipids concentrations in blood. The deviations in total protein were insignificant and variable: intitially, there was a decrease and in the following terms – a slight increase, but within the reference range (Table 1).

All those data evidenced that the systemic response of experimental animals was not immediate, but after a certain period of time. The most noticeable deviations occurred 6 days after the treatment and in most cases, a tendency towards normalization was thereafter observed.

Multiple intoxication

The results of the repeated treatment are presented in Table 2. Compared to the data after the acute intoxication, the activity of ceruloplasmin was increased again by day 6, but then, despite the repeated treatment, the activity of this acute phase protein was not elevated, and after a certain period of adaptation it was inhibited.

The phasic pattern of changes in lipid peroxidation products in the early time intervals of the chronic intoxication was similar to that occurring throughout the acute experiment. Then, a period of restoration was observed with the exception of

day 15 after the first treatment (= the first day after the 3rd treatment) (blood plasma), when a transient reduction was seen (Table 2).

In samples, stored at 0–4° C for 24 h, the dynamics of lipid peroxidation products levels was similar. A significant increase was observed as early as one day after the first treatment. It could be explained by the fact, that lipid peroxidation takes some time to be displayed. In fact, the studied organophosphorus preparation provoked lipid peroxidation in vivo by day 6 and by day 1 but after being stored at 0–4° C for 24 h (Table 2).

The data for total lipids also manifested some resemblance in the early stages of both the acute and repeated intoxication. It seemed that the initial systemic responses were identical, and that the quantity and the number of administered doses were not of primary importance. Then, the biphasic pattern of the changes was repeated. The first reduction occurred by day 6 after the first treatment and the second one – by day 6 after the 2nd treatment, i.e. day 1 after the 3rd treatment. An accumulation of effects was therefore achieved, but by day 28 the levels were completely normalized.

The changes in total cholesterol were comparable, but occurred later in time. The total protein concentrations in both trials followed an identical pattern of changes. The significant increase in blood plasma protein values by day 20 after the 1st treatment should be mentioned, but thereafter the normal values were regained regardless of the repetitive treatments (Table 2).

Our data suggested a presence of regulatory mechanisms of adaptation. The data reported by Kale *et al.* (1999) also show a similar effect after administration of organophosphorus and organochlorine compounds.

On the basis of what is known about Agriya 1050 (oral LD_{50} in rats 500 mg/kg, rapid inital action and activity 14 days after the intoxication) (Fetfadjieva *et al.*, 1994) and the present data, it could be concluded that the organism of lambs most probably succeeded to become accustomed to used doses and duration of treatment with the organophosphorus compound Agriya 1050.

Other data of ours (Popova *et al.*, 1991; Popova and Popov, 1996) on changes in various blood plasma and tissue indices after continuous treatment with heavy metal salts, also support a certain adaptation of animal organisms to toxic agents. Thus, the existence of living organisms in the polluted environment, including the pollution resulting from the use of chemicals in agriculture without visible signs of intoxication, could be explained.

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Correspondence:

Assoc. Prof. M. Popova,
Facuty of Agriculture,
Trakia University,
Student's Campus
6000 Stara Zagora, Bulgaria