CHANGES IN BLOOD ENZYME ACTIVITIES AFTER EXPERIMENTAL ACUTE INTOXICATION OF QUAILS (COTURNIX COTURNIX) WITH THE CARBAMATE INSECTICIDE CARBOFURAN

R. BINEV1, I. VALCHEV1, K. STOYANCHEV1, R. MIHAYLOV2 & Y. NIKOLOV1

1Department of Internal Non-Infectious Diseases, Faculty of Veterinary Medicine; 2Department of Animal Morphology, Physiology and Nutrition, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria

Summary


Carbamate anticholinesterase pesticides are widely used for plant protection. Their common application together with the high toxicity, are responsible for the increasing number of intoxication accidents with wild animals (mammals and birds). The present experiment aimed to determine the changes occurring in blood enzyme activities of quails after experimental acute intoxication with the carbamate insecticide carbofuran (Carbosan 35 CT). Quails were divided into 5 groups: one control and 4 experimental. They were treated by increasing single doses of the tested pesticide: 1.05 mg/kg (experimental group I), 2.1 mg/kg (experimental group II), 5.25 mg/kg (experimental group III) and 10.5 mg/kg (experimental group IV), corresponding to 1/10 LD50, 1/5 LD50, 1/2 LD50 and LD50 – oral doses for albino rats, respectively. Prior to the treatment (hours –48, –24 and 0) and 1, 3, 5, 7, 24 and 48 hours thereafter, blood was sampled from v. subcutanea ulnaris for analysis of activities of serum acetylcholinesterase (AChE), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), creatine kinase (CK) and gamma glutamyltransferase (γ-GT). The tested carbamate insecticide caused reduction in AChE activities, along with increased ASAT, ALAT and CK concentrations. There were no changes in γ-GT concentrations.

Key words: blood enzymes, carbamate insecticide, carbofuran, intoxication, quails

INTRODUCTION

Carbamate anticholinesterase pesticides are widely used organic compounds with mainly insecticide, herbicide and fungicide effect (Mineau & Tucker, 2002; Wang et al., 2007). Some of them are used in everyday’s life of man for disinfection of homes (against cockroaches) or are included as active ingredient in flea repellent
collars (Tall et al., 2010; Vlcek & Pohnka, 2012).

Carbofuran is among the most employed carbamate pesticides – a broad-spectrum systemic insecticide, nematocide and acaricide. Its common use and high toxicity have increased the cases of intoxication in both domestic and wild mammals (Berny, 2007; Reljic et al., 2012) and birds (Mineau & Tucker, 2002a; Brasel et al., 2007; Berny, 2007; Lehel et al., 2010).

The treatment of seeds or plants during the vegetation are the main causes for ingestion of these compounds by animals. Numerous spontaneous intoxication accidents have been reported in wild birds such as California gulls, Canadian geese, vultures, swans, hawks, herons and eagles (Elliott et al., 1998; Mineau & Tucker, 2002b; Fleischli et al., 2004; Wobser et al., 2004; Brasel et al., 2007), including quails (Modra & Svobodova, 2009; Tall et al., 2010).

It is known that intoxications with cholinesterase inhibitors such as organic carbamate compounds result in substantial reduction of serum acetylcholinesterase (AChE) activities. According to some authors, (Padilla et al., 2007), AChE activity reduction could attain up to 80% in acute intoxication. The inhibition of the enzyme occurs by ester bond blockage, which is reversible unlike intoxications provoked by organophosphate compounds (Soler-Rodriguez et al., 1998; Eddleston et al., 2008; Scholtz et al., 2009). Reported data on the influences of carbamate pesticides on AChE are obtained mainly in spontaneous cases (Gupta, 2004; Eddleston et al., 2008).

In Bulgaria, experiments for evaluation of the toxic effect of carbamate insecticides in birds have been conducted with chickens (Yotsev et al., 1997), but no data are available from wild birds, which are the commonest victims of accidental intoxication in the nature.

The gaps in available knowledge and increasing incidence of mass intoxications of game species with carbamate insecticides at both national and global scale motivated the present investigation on alterations in some blood enzymes in quails after experimental acute intoxication with carbofuran. Additionally the study aimed to throw light on some toxicological aspects of acute carbamate toxicity in wild birds, their diagnostics, treatment and prevention.

MATERIALS AND METHODS

Experimental animals

The experiments were carried out with 30 quails with uniform gender, age (10–12 weeks) and weight. They originated and were kept in the Stara Zagora City Zoo. One month before the trial, the birds were housed under uniform conditions compliant with hygienic norms. All quails were fed a ration corresponding to their species and age, and had free access to drinking water.

Tested substance

The experimental intoxication was provoked with carbofuran (Carbosan 35 CT, Agro Science – USA), containing 350 mg 2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate in 1 mL, with oral LD₅₀ for albino rats = 10.5 mg/kg. The preparation was applied once orally via an oesophageal probe, two hours before feeding (at 6.00 AM).

Experimental design

Quails were divided into 5 groups: one control and 4 experimental (6 birds in
They were treated on hour 0 with different single doses of the tested pesticide: 1.05 mg/kg (experimental group I), 2.1 mg/kg (experimental group II), 5.25 mg/kg (experimental group III) and 10.5 mg/kg (experimental group IV), corresponding to 1/10 LD$_{50}$, 1/5 LD$_{50}$, 1/2 LD$_{50}$ and LD$_{50}$ – oral doses for albino rats, respectively.

Three consecutive days prior to the treatment (hours –48, –24 and 0) and at post treatment hours 1, 3, 5, 7, 24 and 48, blood was sampled from v. subcutanea ulnaris for analysis of activities of serum acetylcholinesterase (AChE), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), creatine kinase (CK) and gamma glutamyltransferase ($\gamma$-GT) using Cyba Corning commercial kits (Bayer Diagnostics Ltd, Germany) on an automated biochemical analyzer Olympus AU 600 (Japan).

**Statistical analysis**

All results were processed with statistical software (Statistica 6.0 for Windows, Stat Soft Inc. USA, 1993). The significance of differences between treated groups and untreated controls were evaluated by ANOVA. The level of statistical significance was P<0.05.

**RESULTS**

Serum acetylcholinesterase (AChE, Fig. 1) was reduced in experimental groups II, III and IV one hour after the treatment – 1152±52 U/L (P<0.05), 914±48 U/L (P<0.01) and 883±44 U/L (P<0.01) vs control group levels (2213±164 U/L). At this time interval, the analyte values were the lowest recorded. Enzyme activity was restored by the 5$^{th}$ hour in Group II, and by the 7$^{th}$ hour in Groups III and IV.

Blood aspartate aminotransferase (ASAT) increased after 1 hour in all treated groups (Fig. 2). The peak activities were attained by post treatment hour 3 – 222.3± 8.8 U/L (P<0.05) in Group I, 288.8±9.2 U/L (P<0.01) in Group II, 366.3±10.5 U/L (p<0.001) in Group III and 752.4± 22.8 U/L (P<0.001) in Group IV as compared to controls – 124.8±5.9 U/L. ASAT was normal by the 24$^{th}$ hour.

**Fig. 1.** Changes in blood acetylcholinesterase activity in quails – untreated (◊– control group) and treated with carbofuran at doses of 1.05 mg/kg (1/10 LD$_{50}$ for albino rats, □– Group I), 2.1 mg/kg (1/5 LD$_{50}$ for albino rats, ▲– Group II), 5.025 mg/kg (1/5 LD$_{50}$ for albino rats, ■– Group III) and 10.5 mg/kg (LD$_{50}$ for albino rats, ×–Group IV); *P<0.05; †P<0.01; ‡P<0.001 vs hour 0.
Changes in blood enzyme activities after experimental acute intoxication of quails.

Alanine aminotransferase (ALAT) was also increased in all intoxicated groups (Fig. 2) reaching maximum activities as followed: 10.6±0.9 U/L (P<0.01), 16.7±0.9 U/L (P<0.001), 26.5±1.4 U/L (P<0.001) and 29.7±1.8 U/L (P<0.001) vs controls (3.2±0.8 U/L). The restoration of normal activity occurred by the 5th hour in Group I, by the 7th hour in Group II and after 24 hours in Groups III and IV.

One hour after treatment with carbofuran, alkaline phosphatase (ALP) was elevated in all groups– 735.3±22.4 U/L (P<0.001) in Group I, 815.8±19.1 U/L (P<0.001) in Group II, 1120.3±38.8 U/L (P<0.001) in Group III and 1740.9±42.2 U/L (P<0.001) in Group IV as compared to control group (289.6±9.4 U/L) (Fig. 2). These were also the maximum determined concentrations. Blood ALP attained normal levels by the 7th hour in Group I and by the 24th hour in the other treated groups.

Creatine kinase concentrations (CK) increased in all carbofuran-treated quails by the 1st hour – 2860±96 U/L (p<0.01), 3214±90 U/L (P<0.01), 4265±38.8 U/L (P<0.001) and 4925±138 U/L (P<0.001) in Groups I, II, III and IV respectively as compared to untreated controls (555±29 U/L). After attaining maximum activities by hour 1, CK declined and regained its normal values by hour 5 (Group I) and hour 24 (Groups II, III and IV) (Fig. 2).

In all treated groups, gamma glutamyltransferase activities varied insignificantly near the control range.

Fig. 2. Changes in blood aspartate aminotransferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP) and creatine kinase (CK) activity in Japanese quails – untreated (◊ control group) and treated with carbofuran at doses of 1.05 mg/kg (1/10 LD$_{50}$ for albino rats, ▼ Group I), 2.1 mg/kg (1/5 LD$_{50}$ for albino rats, ▲ Group II), 5.25 mg/kg (1/5 LD$_{50}$ for albino rats, ■ Group III) and 10.5 mg/kg (LD$_{50}$ for albino rats, × Group IV); *P<0.05; **P<0.01; ***P<0.001 vs hour 0.
DISCUSSION

The performed experiments with acute intoxication of quails with different doses of the carbamate insecticide carbofuran (1/10, 1/5, 1/2 of LD50 and LD50, oral doses for albino rats) revealed significant changes in the blood activities of studied enzymes – AChE, ASAT, ALAT, CK, AP.

Serum acetylcholinesterase decreased in all treated groups by the 1st hour regardless of the dose. Our data for the time course of this enzyme corresponded to those in spontaneous intoxications of mammals (Srebocan et al., 2003; Padilla et al., 2007; Reljic et al., 2012) and wild birds (Mineau & Tucker, 2002a; Berny, 2007; Eddleston et al., 2008; Tall et al., 2010; Vlcek & Pohanka, 2012). Our studies showed that AChE was reduced proportionally to the dose of the insecticide (carbofuran), with most pronounced changes on the first hour. The observed recovery on the 3rd hour after the treatment and the complete reactivation on hour 7 supported data that carbamate-induced AChE inhibition was a reversible process. The mechanisms of inhibition and reactivation depend not only on the dose of applied carbamate pesticides, but are also species-dependent (Padilla et al., 2007; Scholtz et al., 2009). Through carbachylation, carbamate compounds reduce cholinesterase activity. The observed inhibition however, unlike intoxications with organophosphorus compounds, is reversible (Soler-Rodriguez et al., 1998).

Acute experimental intoxication with carbofuran was accompanied by substantial changes in blood aminotransferases (ASAT and ALAT) and ALP. The activities of all enzymes were elevated in all treated groups of quails. Changes occurred as early as the 1st hour, and maximum levels were attained by the 3rd hour. The simultaneous elevation of transferases could be related to enhanced synthesis or reduced degradation rate in agreement with liver parenchyma morphological changes reported by de Lavaur et al. (1991), Padilla et al. (2007) and Lehel et al. (2010). Hepatocytic dystrophy and necrosis along with cell wall damage and cytolysis caused a significant influx of these mitochondrial enzymes in the blood circulation. According to Gupta (2004) altered liver enzymes concentrations could be associated with vasodilation ensuing from β2-adrenoceptor effect of higher catecholamine concentrations. Others (Zaahkouk et al., 2000) explain enhanced transaminase activities following carbamate intoxication with enhanced permeability of cell membranes consequently to cellular ATP depletion and potentiated liver function. After oral administration, carbamate compounds reach initially the liver via the portal vein stimulating the synthesis of liver enzymes. Damaged cells release enzymes into blood circulation; other factors (altered cell membrane permeability, enhanced synthesis or delayed degradation of enzymes) could be also involved in these events (Zaahkouk et al., 2000). As ALP is the most abundant enzyme in liver epithelial cells (de Lavaur et al., 1991; Scholtz et al., 2009), higher enzyme concentrations in our view could be attributed also to gastro-enterotrophic effect of carbamate compounds, manifested clinically with diarrhoea, salivation, vomiting and other signs as reported by Mineau & Tucker (2002b), Berny (2007), Brasel et al. (2007) and Lehel et al. (2010) in spontaneous carbofuran intoxication. The researchers reported that in carbamate intoxications, neuromuscular signs – convulsions, myoclonus, seizures, paralysis – were predominant, which could explain the increased CK concentrations as this enzyme is mostly located in...
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muscle and nervous tissues (Scholtz et al., 2009). The lack of statistically significant changes in gamma glutamyltransferase could be attributed to the rapid course of intoxication, and the slow reaction of γ-GT to hepatobiliary damage compared to ASAT and ALAT.

The analysis of results from blood enzyme activities in quail treated with different doses of the carbamate insecticide carbofuran showed reduced serum acetylcholinesterase and higher activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatine kinase. Most pronounced changes occurred in the beginning of intoxication (hours 1–3), followed by gradual restoration of studied analytes until their usual levels by the 24th hour.

REFERENCES


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**Correspondence:**

Assoc. Prof. Roumen Binev
Department of Internal Non-Infectious Diseases,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria
e-mail: binew@abv.bg