Original article

CLINICAL TOXICOLOGICAL INVESTIGATIONS ON ACUTE CARBOFURAN INTOXICATION IN DOGS

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Summary

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The present studies were conducted to evaluate the toxic effects of the carbamate insecticide carbofuran (Carbosan 35 CT) after experimental acute intoxication in dogs. The experiments included one control and five experimental groups (total number of 42 dogs), treated with increasing single doses of the preparation via an oesophageal tube as followed: experimental group I: 0.525 mg/kg, experimental group II: 1.05 mg/kg; experimental group III: 2.1 mg/kg; experimental group IV: 3.5 mg/kg and experimental group V: 5.25 mg/kg, corresponding to $1/20$ LD_{50}, $1/10$ LD_{50}, $1/5$ LD_{50}, $1/3$ LD_{50} and $1/2$ LD_{50} − oral doses for albino rats, respectively. The clinical status of all grous was followed out three consecutive days prior to the treatment (hours –48, –24 and 0) and 1, 3, 5, 7, 24 and 48 hours thereafter to evaluate rectal body temperature, heart and respiratory rates, faeces and urine excretion, locomotion, and perception. It was found out that the first toxic effects were observed at a dose of 1.05 mg/kg ($1/10$ of LD_{50} for albino rats), the first lethal issues occurred at 5.025 mg/kg ($1/2$ of LD_{50} for albino rats) and the dose provoking 100% lethality was 10.5 mg/kg, which was equal to LD_{50} for albino rats. The toxicity of the tested carbamate insecticide was clinically expressed by hyperthermia, tachycardia and polypnea hypersalivation, staggering, wandering gaze, bruxism, vomiting, faecal and urine incontinence, tremor, convulsions, seizures, clonic spasms, paresis and paralysis of limbs.

Key words: carbamate insecticide, carbofuran, clinical signs, dogs, intoxication

INTRODUCTION

The extensive use of carbamate anticholinesterase pesticides as insecticides, nematocides and acaricides (Wang *et al.,* 2007; Eddleston *et al.,* 2008; Vlcek & Pohanka, 2012) contaminates waters, air and soils entailing an important risk for animal and human health (Motas-Gusman *et al.,* 2003, Fleischli *et al.,* 2004; Tall *et al.,* 2010; Goud *et al.*, 2012). The lack of specific odour and the long-term persistence of carbamate insecticides in the environment are the reason for the numerous reports for mass intoxications in wild animals (Martin & Tucker, 2002; Kwon *et al.,* 2004; Wobeser *et al.* 2004; Berny, 2007; Eddleston *et al.,* 2008; Vlcek &

Pohanka, 2012). The frequent cases of intoxications of domestic animals in the clinical practice (Fleischli *et al.,* 2004; Martinez-Haro *et al.,* 2008; Modra & Svobodova, 2009; Aleksic *et al.*, 2011) are attributed to these features and to the relatively high carbofuran toxicity ($LD_{50}<$ 50 mg/kg).

Carbamate compounds provoke an acute intoxication via reverse inhibition of the enzyme acetyl cholinesterase (ACE) resulting in excessive accumulation of acetylcholine in synapses causing overstimulation of acetylcholine receptors (muscarinic and nicotinic). This mechanism is responsible for the variety of clinical signs – frequent defecation and micturition, miosis, vomiting, salivation, convulsions, seizures etc. (Eddleston *et al.,* 2008; Bernya *et al.,* 2010). Depending on the dose of the ingested toxic substance and the animal species, most researchers (Berny, 2007; Martinez-Haro *et al.,* 2008; Vlcek & Pohanka, 2012) reported that acute intoxication occurred rapidly (for about 5 h) with most obvious clinical signs during the first minutes or hours after the ingestion of carbamate organic compounds.

The reported data are mainly from spontaneous carbofuran intoxication incidents (Wang *et al.* 2007; Tall *et al.,* 2010; Aleksic *et al*., 2011). In Bulgaria, experimental studies on carbamate toxicity have been conducted in pigs (Zhelev, 2004), sheep (Yotsev *et al.,* 1990; 1997) and chickens (Yotsev *et al.,* 1997), but not in dogs.

The purpose of the present research was to evaluate the toxic effect of the carbamate insecticide carbofuran in dogs through monitoring of clinical status changes after administration of doses between 0.525 and 10.5 mg/kg.

MATERIALS AND METHODS

Experimental animals

The experiments were carried out on 42 dogs, at the same age and weight. Thirty days prior to the study they were kept in individual cages under the same conditions in compliance to hygienic microclimatic and feeding norms. The animals were fed canine food for adults (Lyubimets) and had free access to water.

Tested substance

The experimental intoxication was done with the commercial preparation Carbosan 35 CT (Agro Science – USA), containing 350 mg carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) in 1 mL, with oral LD_{50} 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

The dogs were divided into 7 groups: one control and six experimental (with 6 dogs in each), treated on hour 0 with increasing single doses as followed: experimental group I: 0.525 mg/kg; experimental group II: 1.05 mg/kg; experimental group III: 2.1 mg/kg; experimental group IV: 3.5 mg/kg; experimental group V: 5.25 mg/kg, and experimental group VI: 10.5 mg/kg corresponding to 1/20 LD_{50}, 1/10 LD_{50}, 1/5 LD_{50}, 1/3 LD_{50}, 1/2 LD_{50} and LD_{50} – oral doses for albino rats, respectively.

The complete clinical status included rectal body temperature (by a digital thermometer GT 2038 Geratherm Medical, Germany); heart and respiratory rates, defecation, micturition, locomotion and perception (using routine clinical diagnostic approaches) was monitored in all groups of dogs three days before the

treatment (hours –48, –24 and 0) and on post treatment hours 1, 3, 5, 7, 24 and 48.

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

The experiments aimed at establishing the tolerance of dogs to carbofuran showed that the dose of 0.525 mg/kg (1/20 of LD_{50} for albino rats) (experimental group I) was not toxic. Treated dogs did not exhibit any signs of intoxication.

The changes observed in dogs treated at 1.05 mg/kg and 2.1 mg/kg correspond-

ing to 1/10 and 1/5 of LD_{50} for albino rats – group II and III respectively, were of similar extent and time course. All treated animals were with arrhexia, wandering gaze, restlessness, moderate salivation, diarrhoea, polydipsia, vomiting or vomiturition, tremor, colic tonic spasms commencing from hindlimbs and reaching the cervical muscles, and reddened conjunctivas. The described clinical signs appeared about one hour after treatment and persisted up to hour 3, fading and disappearing up to hour 5.

Dogs treated with a single carbofuran dose of 3.5 mg/kg (1/3 of LD_{50} for albino rats; IV experimental group showed clinical signs of intoxication as early as the 15th min – depression, salivation with thick saliva discharge and miosis. By the 20th min, staggering, wandering gaze, bruxism, vomiting or vomiturition, faecal and urine incontinence have occurred.
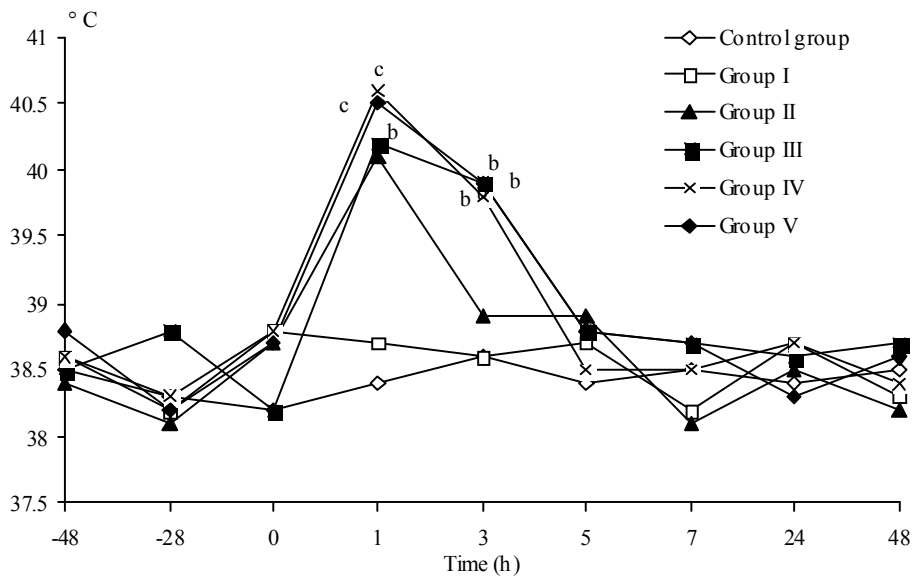
Fig. 1. Changes in rectal body temperature in dogs – untreated (control group) and treated with carbofuran at doses of 0.525 mg/kg (1/20 LD_{50} for albino rats, Group I), 1.05 mg/kg (1/10 LD_{50} for albino rats, Group II), 2.1 mg/kg (1/5 LD_{50} for albino rats, Group III), 3.5 mg/kg (1/3 LD_{50} for albino rats, Group IV) and 5.25 mg/kg (1/2 LD_{50} for albino rats, Group V); $^bp<0.01$; $^cp<0.001$ vs hour 0.
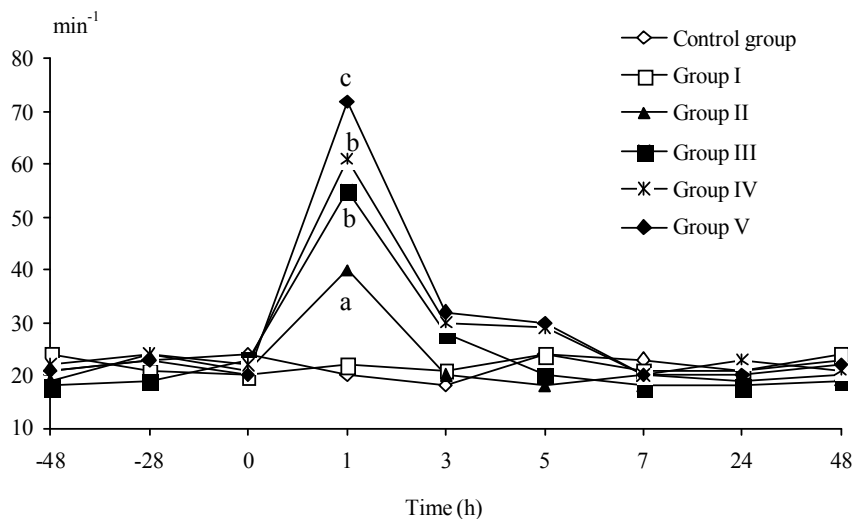
Fig. 2. Changes in respiratory rate in dogs – untreated (control group) and treated with carbofuran at doses of 0.525 mg/kg (1/20 LD_{50} for albino rats, Group I), 1.05 mg/kg (1/10 LD_{50} for albino rats, Group II), 2.1 mg/kg (1/5 LD_{50} for albino rats, Group III), 3.5 mg/kg (1/3 LD_{50} for albino rats, Group IV) and 5.25 mg/kg (1/2 LD_{50} for albino rats, Group V); [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$ vs hour 0.

Between the 45th and the 60th min, nervous signs as generalised tremor, tonic-clonic spasms and convulsions have appeared. The animals fell on the ground and bit the bars of the cage. The described clinical signs persisted with decreasing intensity until post treatment hour 5, and 2 hours later the dogs regained their normal behaviour.

Dogs treated once with 5.25 mg/kg carbofuran (1/2 of LD_{50} for albino rats; experimental group V) showed similar signs of toxicity, but the initial signs appeared between the 5th and 7th min after the treatment. The recovery period was the same – between hours 5 and 7. Two of treated dogs (33.3 %) died within one hour.

All dogs treated with 10.5 mg/kg (LD_{50} for albino rats; group VI) showed signs of intoxication after the 5th min and of similar grade as those treated with the twice lower dose (5.25 mg/kg). Between the 30th and the 45th min, all treated dogs died.

The investigations on the changes in rectal body temperature (Fig. 1) showed that it increased after the administration of the carbamate insecticide. The highest values in dogs from group II to V were attained 1 hour after administration – 40.1 ±0.3 °C ($P<0.01$), 40.2±0.2 °C ($P<0.01$), 40.6±0.3 °C ($P<0.001$) and 40.5±0.3 °C ($P<0.001$), as compared to control group (38.4±0.2 °C). Except for group II, fever persisted by hour 3 in all other groups. Five hours after the treatment, the studied clinical parameter regained initial values.

By the first hour after carbofuran treatment, polypnea was established in experimental groups II to V (Fig. 2) – 40±5 min^{-1} ($P<0.05$), 55±6 min^{-1} ($P<0.01$), 61±6 min^{-1} ($P<0.01$) and 72±7 min^{-1} ($P<0.001$), respectively, vs control respiratory rate of 20±4 min^{-1}.

There were no significant changes in measured heart rates.

DISCUSSION

The experimental studies on acute intoxication with the carbamate insecticide carbofuran in dogs, treated with increasing doses of the compound (1/20 LD_{50}, 1/10 LD_{50}, 1/5 LD_{50}, 1/3 LD_{50}, 1/2 LD_{50} and LD_{50} oral doses for albino rats) showed that, the pesticide was highly toxic for dogs in agreement with data reported for laboratory, wild and productive animals (Prakash *et al.*, 2002; Adhikari *et al.*, 2004; Vidair, 2004).

The observed clinical signs in dogs were similar to those specific for intoxication with carbamate insecticides.

The studies on the total tolerance of dogs to carbofuran showed that the preparation had no significant toxic effect at a dose of 0.525 mg/kg (1/20 of LD_{50} oral dose for albino rats, group I), due to the lack of clinical signs of intoxication. This allowed assuming that the tolerated dose for dogs among all doses tested in this study was 0.525 mg/kg.

According to our experiments, the 1.05 mg/kg (1/10 of LD_{50} for albino rats; experimental group II) carbofuran provoked clinical signs (moderate salivation, diarrhoea, polydipsia, vomiting or vomiturition, tremor, tonic-clonic spasms beginning from hindlimbs and attaining the neck muscles) that were in agreement with those reported in spontaneous intoxications in dogs by Hovda & Hooser (2002), Shapiro & Hoff (2002), Srebosan *et al.* (2003), Modra & Svobodova (2009), Bernya *et al.* (2010) and Aleksic *et al.* (2011).

We have established that the first lethal outcomes in this study occurred in animals treated at 5.25 mg/kg, equal to 1/2 of LD_{50} for albino rats. All dogs treated with 10.5 mg/kg (LD_{50} for albino rats) (group VI) showed signs of intoxication after the 5th min and died within post treatment min 30 to 45.

Carbofuran intoxication signs in dogs were observed about 15 to 30 min after the application of the preparation and lasted 3 to 5 hours. Depending on the used dose, the most obvious clinical signs were noticed by the first hour after treatment. The effect of carbofuran was faster, stronger and shorter compared to that of other carbamate compounds, for instance carbaryl and methiocarb. In our view, the differences among the influence of carbamate pesticides could be explained by the more rapid metabolism of carbofuran in the body of poisoned animals compared to Sevin (carbaryl) and methiocarb (Berny, 2007; Wang *et al.* 2007; Eddleston *et al.*, 2008; Vlcek & Pohanka, 2012).

The body temperature curve in treated dogs showed that the low doses of carbofuran (1/20 of LD_{50} for albino rats) had no effect. With increasing of doses, it was elevated with peak by the 1st hour and then was normalised within post treatment hours 3 to 5. Observed hyperthermia is of diagnostic value between the 1st and the 3rd hour after carbofuran intoxication. Similar results are communicated by Motas-Gusman *et al.* (2003), Kwon *et al.* (2004), Gupta (2004) and Modra & Svobodova (2009). Hyperthermia probably corresponds to enhanced basal metabolism under the influence of carbamate pesticides (Hovda & Hooser, 2002; Srebosan *et al.,* 2003). On the other side, impaired thermoregulation, functionally due to the neurotoxic effect of this carbamate pesticide, could be also involved (Santo *et al.,* 2002; Kwon *et al.,* 2004; Bernya *et al.,* 2010; Aleksic *et al.*, 2011).

In our experiments, there were no significant alterations in heart rates of carbofuran-treated dogs. Some authors (Motas-Gusman *et al.,* 2003; Adhikari *et al.,* 2004) reported bradycardia consequently to the anticholinesterase effect of carbamate insecticides. Our study however did not confirm these findings. The lack of bradycardia could be explained by the haematological changes (hypochromaemia and erythropaenia) reported by Modra & Svobodova (2009) and Goud *et al.* (2012), which result in impaired oxygenation and compensatory acceleration of heart rate. These two opposite effects – the triggering of cholinergic receptors by acetylcholine clinically manifested by bradycardia on one hand, and compensatory tachycardia on the other, could explain at a certain extent the lack of heart rate changes in our study.

During the course of the acute intoxication with carbofuran, respiratory rates were higher only by the first hour. This is in agreement with the data of Adhikari et al. (2004), obtained with other carbamate compounds. Treated animals recovered very rapidly (for one hour) and restored their normal respiratory rate by the 3rd hour. The polypnea and dyspnea were due to the inhibition of cholinesterase, and irritation of choline receptors by non-metabolised acetylcholine causing rapid breathing (Wobeser *et al.,* 2004; Wang *et al.* 2007).

The changes in the behaviour and general condition observed by us and other researchers (Vidair, 2004; Modra & Svobodova, 2009; Tall *et al.,* 2010; Vlcek & Pohanka, 2012) are also attributed by cholinergic receptor stimulation by high acetylcholine concentrations (Srebocan *et al.,* 2003; Aleksic *et al.,* 2011).

The performed toxicity tests in dogs with the carbamate insecticide carbofuran revealed the following clinical signs of intoxication: hyperthermia, polypnea, arrhexia, diarrhoea, polydipsia, vomiting or vomiturition, tremor, tonic-clonic spasms, depression, hypersalivation with discharge of thick saliva, miosis, uncoordinated movements, inability to stand, staggering, wandering gaze, bruxism, faecal and urine incontinence, generalised tremor, convulsions, biting cage bars, myoclonus episodes, moving into a circle, limb paresis and paralysis alternating with depression and somnolence, reduced perception and olfaction, turning of the head, reduced reflexes and sensory perception.

The experimental intoxication with carbofuran applied once orally at increasing doses (0.525 mg/kg, 1.05 mg/kg, 2.1 mg/kg, 3.5 mg/kg, 5.25 mg/kg and 10.5 mg/kg corresponding to 1/20, 1/10, 1/5, 1/3, 1/2 and LD$_{50}$ oral doses for albino rats) demonstrated that dogs were more sensitive to the toxic effects of carbofuran than albino rats.

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