Thiodicarb Alterations in the Central Nervous System of Albino Mice

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ABSTRACT: Carbamates are organic compounds derived from carbamic acid. A carbamate group, carbamate ester, and carbamic acids are functional groups that are inter-related structurally and often are interconverted chemically. The carbamate insecticides are widely used as a replacement for its more persistent organochlorine counterparts. The present study is aimed to explore the toxicological effect of a predominantly used carbamate i.e. on different regions of the brain. In the present investigation we have studied the effect of thiodicarb on cholinergic mechanisms in the different regions of brain. For the present study the male mice were exposed to 1/10th LD_{50} of carbamate via oral gavage (i.e. 0.21 mg/kg body weight). All the experimental animals received the dose on alternate days. After the stipulated period of time different regions of the brain of control and experimental mice were dissected and acetylcholine and acetylcholinesterase activity were measured. The results of the present study indicate a steady decline of acetylcholinesterase (AChE) activity in all the regions of the brain of thiodicarb exposed animals. An increase in acetylcholine (ACh) activity was noticed in all the regions of the experimental mice. We suggest that cholinergic system is seriously affected by the intoxication of thiodicarb and the effect was more in the animals which were exposed to longer duration of time. As Thiodicarb is widely used on crops, they may pose several environmental and health concerns and therefore sufficient caution should be taken while using this compound so that non target animals are not affected.

KEY WORDS: Thiodicarb, ACh, acetylcholinesterase, Albino mice.

INTRODUCTION

Carbamate pesticides are extensively used in agriculture due to their rapid degradability and low toxicity to non-target species and are used annually on a large scale worldwide[1]. Therefore, indiscriminate use of carbmates is poisoing serious health hazard to humans and animals[2]. Acute carbamate poisoning episodes were described among pesticide sprayers due to inadequate personal protection[3,4]. Clinical manifestations for carbamates result from accumulation ACh in the synapses and over stimulation of muscarinic and nicotinic receptor throughout target organs. The carbamate insecticides are widely used as a replacement for its more persistent organochlorine counterparts.

Thiodicarb (dimethyl N,N- thiobis (methyl amino) carbonyloxy bisethamimido thioate is a new carbamate compound having broad spectrum of activity being extensively used for the crop protection. It is moderately toxic and belongs to class II category compounds. Thiodicarb acts by inhibiting acetylcholinesterase activity. Various carbamate compounds have been reported to cause biochemical Changes in different species.

MATERIALS AND METHODS

Test Chemical:

Thiodicarb (99.9%) pure in supplied as an off white powder was obtained from Samrudhi Agro Centre, Banglore , Karnataka, India.

Animal model: Male Albino mice

Healthy Male Albino mice of the same age group 6 weeks and weighing 30 ± 82 g. They were kept in well cleaned and sterilized cages. Mice were maintained in laboratory conditions in the animal house at 25±2°C with a photoperiod of 12hrs light and 12hrs darkness throughout the course of the present study. The mice were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water adlibitum.

Experimental Design:

Healthy adult Male Albino mice divided into four groups having ten animals each.

Group-I : The group I animals were considered as control animals.

Group-II : The group II animals were treated with thiodicarb for 10 days.

Group-III : The group III animals were treated with thiodicarb for 20 days.

Group-IV : The group IV animals were treated with thiodicarb for 30 days with 48 hr intervals through oral gavage.

Toxicity Evaluation

Toxicity of thiodicarb was evaluated by static bioassay method of [4] and the LD_{50} of thiodicarb in albino mice was found to be as 70mg/kg bw for 48hr. LD50 of Albino mice 2.1mg/30gms/body weight, $1/10^{th}$ of LD_{50} value of thiodicarb (i.e.0.21mg/kg bw.) was selected as sub lethal dose.

Determination of ACh and AChE

The ACh was estimated by[5] and AChE was estimated by the method of[6] .

STATISTICAL ANALYSIS

The data was subjected One way analysis of variance (ANOVA), two way ANOVA and S-N-K tests using SPSS (ver. 20) in the personal computer and $p < 0.05$ was considered as statistically significant.

RESULTS

The results of the present investigation are presented in Table 1. Exposure to carbamates resulted in signs of carbamate toxicity to all the regions of the brain. The toxic effect was more in animals which were exposed for a longer duration of time i.e.,20 days to 30 days. A steady decline in the AChE activity was observed in all the regions of the brain of thiodicarb exposed animals. ACh showed a steady increase in all the regions of the brain of thiodicarb exposed mice.

Table-1: Alterations in the levels of acetylcholine in different regions of the brain in albino mice exposed to Thiodicarb.

Brain Region	Control	10 days	20 days	30 days	F ratio
Cerebral cortex ± SD (% Change)	26.392 2.165	29.419 1.702 (11.47)	32.426 3.1 (22.86)	39.153 5.577 (48.35)	10.172
Hippocampus ± SD (% Change)	35.438 3.681	41.008 2.513 (15.71)	47.308 4.134 (33.49)	55.468 2.955 (56.52)	38.9
Cerebellum ± SD (% Change)	23.541 1.94	26.98 4.664 (14.39)	30.73 4.42 (30.55)	33.23 4.66 (41.16)	6.509
Medulla oblongata ± SD (% Change)	16.394 1.809	19.872 3.061 (21.21)	23.381 2.496 (42.61)	26.05 3.92 (58.94)	12.36

ANOVA

Source of Variation	SS	df	MS	F
Between different time intervals	6884.982	3	2294.994	188.1801
Between regions	2254.548	3	751.516	61.62124
Error	258.8706	9	28.7634	2.358481
Within groups	975.6568	80	12.19573	
Total	10374.06	95		

Table 2: Alterations in the acetyl cholinesterase activity in different regions of the brain in albino mice exposed to Thiodicarb.

Brain Region	Control	10 days	20 days	30 days	F ratio
Cerebral cortex ± SD (% Change)	14.617 1.737	12.159 1.799 (-16.81)	10.872 1.189 (-25.62)	9.654 0.870 (-33.95)	12.843
Hippocampus ± SD (% Change)	14.121 1.300	11.800 1.585 (-16.43)	9.983 0.797 (-29.30)	8.114 1.412 (-42.54)	23.128
Cerebellum ± SD (% Change)	14.234 1.809	12.400 1.616 (-12.88)	10.325 1.020 (-27.46)	8.200 1.233 (-42.39)	19.308
Medulla oblongata ± SD (% Change)	14.067 1.983	12.170 1.789 (-13.48)	11.158 2.039 (-20.67)	10.915 1.794 (-22.40)	3.398

ANOVA

Source of Variation	df	SS	MS	F
Between different time intervals	3	17.27171	5.757237	2.4108
Between regions	3	336.9381	112.3127	47.0301
Error	9	21.94493	2.438326	1.021031

Within groups	80	191.0482	2.388103	
Total	95	567.203		

Values expressed in µmoles of ACh hydrolyzed/mg protein/min.are Mean ± SD of six individual animals. Values in parenthesis indicate percent change over control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. $^{*}p< 0.01$

DISCUSSION

Carbamates have the ability to reversibly inhibit AChE. The onset of clinical effects subsequent to carbamate exposure depends on the dose, route of exposure, type of carbamate involved, use of protective gear and the premorbid state of the victim[7]. The potency of inhibition depends on the carbamate as well as on a combination of alpha and beta subunit properties. It is concluded that carbamate pesticides affect different subtypes of neuronal nicotinic receptors independently of acetyl cholinesterase inhibition[8]. Acute carbamate poisoning episodes were reported among pesticide sprayers due to inadequate personal protection[9]. Satpal[10] reported that Thiodicarb caused significant inhibition of plasma and brain acetyl cholinesterase (AChE) activity in rats when they were exposed to $1/10^{th}$ LD_{50} dose for a period of 28 days. They further reported that plasma AChE activity decreased at higher dose of thiodicarb[11]. Ahmed[12] reported that carbofuran was found inhibit AChE of Snail when compared to methomyl. Clinical manifestations for carbamates result from accumulation of ACh in the synapses and overstimulation of muscarinic and nicotinic receptors throughout target organs. Sreelatha[13] reported elevated levels of ACh content and decreased activity of AChE in different brain regions of exposed to sublethal dose of cartaphydrochloride, a Carbamate compound. Umakanthi[14] reported that ACh and AChE were severely altered in all the regions of the brain when rats were exposed to Profenofos. Similar results were reported in different regions of the brain of Dimethoate and Lambda cyhalothrin exposed animals[14,15].

Changes in the AChE activity is frequently used as a biomarker for pesticide induced toxicity. AChE breaks down the neurotransmitter ACh at the synaptic cleft. In the present study, AChE was decreased in all the regions of the brain of thiodicarb exposed rats and the effect was more pronounced in thirty days exposed animals indicating that when the animals exposed for longer duration, maximum toxic effect is observed. A number of neurological disorders are observed on fall of AChE activity. Several organo phosphroous compounds cause degeneration of long axons in the spinal cord and peripheral nerves, a syndrome known as OP-induced delayed neuropathy. Olmos[16] reported that dichlorvos enhances long-term potentiation through a postsynaptic mechanism that involves the inhibition of enzyme acylpeptide hydrolase and the modulation of alpha nicotinic receptors. A decline in the AChE activity in different regions of the brain in Acephate exposed mice was observed.

Thiodicarb is extremely toxic because carbamate exposed animals showed an increment in the ACh content while AChE was inhibited in all the regions of the brain. Thiodicarb exposed mice showed typical signs of carbamate toxicity in all the brain regions. Maximum accumulation in the ACh content was observed in the animals which were exposed for longer duration indicating that toxic effect was more in 30 days when compared to 20 days and 10 days. Erythrocyte acetyl cholinesterase was inhibited at 30 and 6mg/kg doses of dimethoate exposure to rat brain[17]. The elevated levels of Ach activity when carbamate compound. Thiodicarb exposure in different brain regions of Albino rat[18]. Demonstrated the decreased levels of AChE activity during exposure in different brain regions of Albino rat for 10, 20, 30 days respectively[19]. Demonstrated decrease of AChe activity levels in brain of Cat fish due to Pesticide Envoy 50 exposure over a period of time[20].

From the foregoing results it is clear that thiodicarb is extremely toxic to non-target animals like mice and these pesticides should be used with great caution.

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