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Thiodicarb Induced Changes in the Detoxification Enzymes in the Brain of Albino Rat

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ABSTRACT

Carbamates are used as insecticides, fungicides, nematocides, accaricides, molluscides very extensively. The present study is aimed to evaluate the oxidative stress markers MDA (end product of lipid peroxidation) and reduced glutathione. In the present study the effect of $1/10^{th}$ LD_{50} of thiodicarb (a carbamate) was studied in different regions of ***Albino*** rats which were exposed to 10, 20 and 30 days respectively. The results of the present study clearly indicate that oral administration of thiodicarb caused a significant elevation in LPO and XOD in all the regions of the brain. A gradual decline in SOD, CAT, GPx and GR was noticed in experimental rats. From the results it can be concluded that the regular dosing of carbmate may lead to gradual depletion in the activities of SOD, CAT, GPx and GR, thus resulting in the production of free radicals in the rats. The present study indicates that oxidative stress of the brain is region specific. Disturbances in the antioxidant cascade in the brain regions also indicate an adaptive biochemical response to the thiodicarb induced oxidative stress.

INTRODUCTION

The Oxidative stress results when there is an imbalance between biochemical processes and it leads to the production of reactive oxygen species (ROS). The antioxidant cascade contains the enzymes and other metabolites responsible for the removal of ROS. A large body of information suggests that oxidative stress is observed in a number of diseases. Various biomolecules and central nervous system are vulnerable to oxidative stress on account of high rate of oxygen utilization. Oxidative stress alters the enzymes and other metabolites of antioxidant cascade. Oxidative stress leads to the alterations of enzymes which results in neurodegenerative diseases where damage to the neurons can result in an increase in oxidative processes and a decrease in antioxidant defenses. Determining the levels of oxidative stress markers establishes an association of oxidative stress during pesticide toxicity. Generation of free radicals and ROS are considered as diagnostic index in pesticide poisoning[1]. Aruoma[2] reported that free radicals and ROS promises a new age of health and disease management. Throughout the world several billions of tons of pesticides are used to kill pests and unfortunately residual amount of these compounds enter human beings and other non-target organisms[3]. The most employed insecticides for indoor and agricultural purposes belong to organophosphorus

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compound, carbamates or pyrethroids. The chemical structures of these three groups correspond to carbamic, carboxylic and triphosphoric esters. Technical monographs suggest that the hydrolysis of ester bonds of carbamates plays an important role in the detoxification of these compounds. These compounds are neurotoxic to mammals and insects. In the present study we report the effect of thiodicarb on the detoxification enzymes in different regions of the brain of ***Albino*** rats exposed for thirty days.

MATERIALS AND METHODS

Test Chemical: Thiodicarb (99.9%) pure in supplied as an off white powder was obtained from Nagarjuna Agrichem limited, Hyderabad, A.P. India.

Animal and Experimental Design: The protocol was approved by the Institutional Animal Ethics Committee, S.V.University (10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/PJD/ Dt.19-04-2012). Male adult ***Albino*** rats of 7 weeks old and aged 200 ± 10 10g was obtained from Indian Institute of Science (I.I.Sc.), Bangalore. They were housed in an ambient temperature 28 ± 2ºC in a 12 h light/dark cycle and a minimum humidity of 40%. The animals had free access to commercial pellet diet supplied by Sai Durga Feeds and Foods, Bangalore, India and water ***ad libitum***. All the male healthy adult male ***Albino*** rats were randomly divided into four groups having six rats per group. The first group animals were considered as control animals. Second group of animals was treated with Thiodicarb via oral gavage (1/10th of i.e. 39mg/kg body weight) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively. Different regions of the brain namely cerebral cortex (CC), hippocampus (HC), cerebellum (CB) and medulla oblongata (MO) were used in the present investigation to study the effect of thiodicarb.

The Determination of antioxidant enzymes As a measure of malondialdehyde (MDA) formation, levels of thiobarbituric acid reactive substances (TBARS) were estimated following the method of Ohkawa[4]. LPO activity was expressed as MDA/mg protein/min. Xanthine oxidase activity was estimated by the dye reduction method[5]. Superoxide dismutase activity was determined according to the method of Beachamp and Fridovich[6]. Catalase activity was measured by Aebi[7]. Se-Dependent Glutathione Peroxidase was determined by Flohe and Gunzler[8] (1984) at 37˚C. GR activity was determined by Carlberg and Mannervik[8].

Statistical Treatment The data was subjected to statistical treatment. One way analysis of variance (ANOVA), and S-N-K tests were performed using SPSS (ver. 20) in the personal computer and $p<0.01$ was considered as statistically significant.

RESULTS

The results of the present study are presented in the tables (1). From the results it is clear that XOD and lipid peroxidation activities showed a steady increase in all the regions of the brain with maximum increase in 30 days exposed animals, while SOD, Catalase, Glutathione peroxidase and Glutathione reductase showed a steady decline, clearly indicate that the effect of Thiodicarb was more when the animals are repeatedly exposed to this chemical.

DISCUSSION

The Carbmate pesticides show low toxicity to non-target animals–especially mammals and are easily biodegradable. However, it is possible that during carbamate toxicity there is a generation of ROS and carbamates may induce oxidative stress in mice, hence the present study was undertaken. From the results it is clear that administration of thiodicarb causes alterations in the antioxidant enzymes in all the regions of the brain. The toxic effect was more pronounced in the animals which were exposed for longer duration of time. Uner[9] and Isik and Celik[10] reported ROS are produced during the detoxification process and they damage cellular macromolecules like lipids, proteins and DNA of the cells resulting in serious physiological disturbances[11]. Li [12] reported that cellular antioxidant responses can be used as potential biomarkers for monitoring the pesticide impact on organism. Oxidative stress, generated by xenobiotics, induces disturbances in antioxidant enzyme systems[13]. Several authors have suggested that oxidative stress is an important component of the mechanism of toxicity of many pesticides. All types of pesticides are capable of inducing oxidative stress leading to a generation of free radicals and alterations in antioxidants or reactive oxygen species (ROS) scavenging enzymes[14]. Alteration in the XOD activity leads to increased cellular damage which may be due to non-availability of iron during the toxic period. A decrease in the XOD activity in pesticide exposed animals is reported by several authors. Rathore[15] observed a decrease in the catalase activity was observed in Isoproternol administered rats. Seth[16] reported a decline in SOD activity in Hexachlorohexane exposed immature

chicks. Dorval[17] reported that Endosulfan causes alterations in oxidative stress and Lipid peroxidation. Hexacholorocyclohexane alters the antioxidant status in different regions of the brain of rats[18]. Pesticides alter malondialdehyde level and antioxidant enzyme activities in gut tissue of insects[19]. In the present study a change in SOD, CAT, GPx activities and MDA level was found to be decreased in all the regions of the brain and this change was time and dose dependent indicating that thiodicarb causes oxidative damage to different regions of the brain. A decrease in the GPx, GST and GR in thiodicarb exposed animals[20]. Decreased activities in the antioxidant enzymes were observed in the liver and kidney of pesticide exposed rats[21, 22]. Depletion of GSH is one of the major factors that permit lipid peroxidation and subsequent tissue damage[23]. GSH functions as a substrate for GPx and GST. GR and GPx activities were significantly reduced in experimental rats, which may be attributed to the unavailability of GSH. MDA, a major oxidation product of per oxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress[24]. In experimental rats an increase in the MDA level was seen in all the regions of the, which suggest that MDA levels could be used as a marker to study Thiodicarb injuries. Under physiological conditions, intracellular antioxidant enzymes, such as SOD, CAT, and GPx, eliminates ROS, thereby playing an integral role in the oxidative stress defenses of the cell[25]. Oxidative stress induces an efflux of GSSG from erythrocytes, which may decrease the red blood cell GSH and it may be possible that, the decreased GSH level observed in this study was not sufficient to combat with the enhanced production of MDA during increased lipid-peroxidation as a result of pesticides poisoning[26]. It has also been advocated that the cysteinyl residue of GSH offers a nucleophilic thiol which is important in the detoxification of electrophilic metabolites and metabolically produced oxidizing agents. Another important enzyme in the detoxification mechanism is SOD and an alteration in the SOD suggests that the pesticide studied in the present study induced superoxide radical. From the results obtained in the study, it is clear that SOD showed a steady decline in a dose dependent manner suggesting that SOD was stimulated by scavenging superoxide radical to protect the animals from pesticide stress. Similar toxicity studies were carried out[27, 28]. They reported an increase in XOD and LPO levels and decreased SOD, CAT, GPx and GR levels in different regions of the brain of Profenofos exposed ***Albino*** rats. A decrease in the SOD activity is probably a response towards increased ROS generation[29]. A decrease in SOD and CAT activity in different brain regions of ***Albino*** rats exposed to Sublethal dose of Chlorpyrifos and Cypermethrin respectively[30, 31]. In the present study a decrease in the CAT activity declined in all the regions of the brain of carbamate exposed rats. CAT protects cells from the toxic effects of H_2O_2 by catalyzing the H_2O_2 to H_2O and O_2. Alteration in the CAT activity is seen in the poisoning cases coupled with an increase in the lipid peroxidation level (MDA) suggests an insufficient antioxidant defense. The present study clearly indicates that the thiodicarb exposed rats were under severe oxidative stress. CAT is perfectly suited for reducing the high amount of H_2O_2 which resulted due to the decrease in SOD activity. Olga Lopez[32] reported that CAT protects the animal against oxidative stress and extend the lifespan of animals. GPx and GR activities showed a steady decrement in different regions of the brain in the present study and this indicates that the poor antioxidant defense mechanism exists in brain and this tissue is more prone to oxidative damage when compared to other tissues. Rai[33] reported that oxidative stress in the brain results in enhanced oxygen demand and lowers ATP/ADP ratio leading to neuronal membrane injury possibly via alterations in its fluidity and inactivation of transmembrane enzymes. Several authors have reported that pesticides severely damage blood brain barrier and membrane lipid contents under pesticide intoxication[34, 35]. Nagla Madkour Antioxidant enzymes are altered in the liver of pyrethroid exposed rats[36]. An increase in the LPO activities in cerebral cortex and cerebellum of pesticide exposed rats[37]. Production of ROS is associated with the synthesis of neurotransmitters and ROS induction in carbamate exposed animals could contribute to LPO. Differences in the fatty acid composition of the brain may also account for this pattern of LPO since white matter rich in myelin lowers polyunsaturated fatty acids than they grey matter. In the present investivation GPx activity significantly decreased in carbamate exposed rats. A decrease in GPx activity in gills, muscle, liver and brain of parathion exposed aquatic animals. GPx activity significantly decreased in carbamate exposed rats caused a significant increase in lipid peroxidation and XOD in a time and dose dependent manner indicating an enormous oxidative stress in pesticide exposed rats[38]. Decreased levels of CAT, SOD, GPx and GR suggest that rats were not able to cope up with pesticide toxicity. Induction of GR can be viewed as a mechanism for replenishment of GSH, an efficient antioxidant molecular which is important for cells to

withstand the toxic effect of thiodicarb or its metabolites. In the present investigation different regions of the brain responded differently to the carbamate toxicity. Regional differences in the action of carbamate on the brain could imply its differential distribution in the brain regions and / or its metabolism. Another possible reason for the regional differences in the oxidative stress observed in the present investigation could be due to variation of neurotransmitter profile in different brain regions. Caravallho[39] reported regional differences in the detoxification mechanism of brain in pesticide exposed animals. Verma[40] reported that the brain exhibits distinct variations in cellular as well as regional distributions of antioxidant defenses. In the present investigation also pesticide sensitivity was different in different regions of the brain. Hence it may be concluded that exposure to thiodicarb may lead to gradual depletion in the activities of SOD, CAT, GPx and GR, thus resulting in the production of free radicals in the rats. The present study indicates that oxidative stress of the brain is region specific and alterations in the antioxidant cascade in the brain regions also indicate an adaptive biochemical response to the carbamate induced oxidative stress.

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Table 1: Changes in the detoxification enzymes in different regions of the brain of Albino rats exposed to sub-lethal dose of Thiodicarb.

Brain Region	Control	10 days	20 days	30 days	F ratio
LPO					
CC	11.653 ± 0.954	13.459 ± 1.595 (15.50)	15.138 ± 0.980 (29.90)	17.368 ± 1.224 (49.04)	24.451*
HC	16.306 ± 1.190	18.911 ± 1.377 (15.98)	20.354 ± 1.387 (24.82)	26.039 ± 2.152 (59.69)	41.185*
CB	24.011 ± 1.682	26.549 ± 1.674 (10.57)	30.327 ± 1.281 (26.30)	35.407 ± 2.025 (47.47)	51.830*
MO	34.123 ± 1.543	37.380 ± 3.580 (9.55)	48.48 ± 4.520 (42.09)	52.118 ± 4.262 (52.74)	32.255*
XOD					
CC	0.519 ± 0.046	0.567± 0.031 (9.15)	0.638 ± 0.085 (22.84)	0.718 ± 0.088 (38.25)	10.023*
HC	0.551 ± 0.076	0.650 ± 0.011 (17.94)	0.671 ± 0.139 (21.76)	0.751 ± 0.132 (36.27)	2.958*
CB	0.820 ± 0.225	0.943 ± 0.097 (15.08)	1.002 ± 0.058 (22.23)	1.229 ± 0.140 (37.70)	4.763*
MO	0.829 ± 0.139	0.943 ± 0.097 (13.73)	1.002 ± 0.058 (20.79)	1.229 ± 0.140 (36.08)	7.208*
SOD					
CC	4.054 ± 0.193	3.600 ± 0.195 (-11.19)	3.238 ± 0.187 (-20.13)	2.760 ± 0.236 (-31.93)	43.558*
HC	3.655 **±** 0.127	3.263 ± 0.213 (-10.75)	2.760 ± 0.236 (-24.50)	2.541 ± 0.321 (-30.50)	27.492*
CB	3.263 **± 0.113**	2.864 **± 0.513 (-12.20)**	2.583 **± 0.381 (-20.82)**	2.281 **± 0.257 (-30.88)**	8.072*
MO	3.331 ± 0.125	2.760 ± 0.236 (-17.15)	2.541± 0.321 (-23.72)	2.120 ± 0.251 (-36.37)	20.380*
CAT					
CC	1.890 ± 0.155	1.652 ± 0.124 (-12.59)	1.480 ± 0.076 (-21.69)	1.245 ± 0.101 (-34.14)	32.057*
HC	1.619 **±** 0.158	1.414 ± 0.192 (-12.68)	1.206 ± 0.065 (-25.51)	1.032 ± 0.171(-36.28)	16.330*
CB	1.360 ± 0.217	1.189 ± 0.065 (-12.57)	1.015 ± 0.185 (-25.38)	0.826 ± 0.072 (-39.29)	13.908*
MO	1.390 ± 0.193	1.190 ± 0.099 (-14.40)	1.064 ± 0.261 (-23.46)	0.924 ± 0.083 (-33.53)	7.696*
GPx					
CC	2.998 ± 0.109	2.701± 0.261 (-9.89)	2.312 ± 0.374 (-22.87)	1.966 ± 0.118 (-34.41)	20.849*
HC	2.783 **±** 0.238	2.259 ± 0.049 (-18.82)	1.966 ± 0.118 (-29.35)	1.634 ± 0.230 (-41.28)	45.246*
CB	2.985 ± 0.143	2.776 ± 0.062 (-7.00)	1.966 ± 0.118 (-34.14)	1.640 ± 0.087 (-45.07)	215.678*
MO	2.749 ± 0.075	2.351± 0.461 (-14.50)	1.993 ± 0.102 (-27.51)	1.695 ± 0.160 (-38.34)	19.551*
GR					
CC	15.283 ± 1.224	12.659 ± 1.464 (-17.17)	11.170 ± 1.596 (-26.91	9.973 ± 0.341 (-34.75)	19.942*
HC	14.293 **±** 1.842	11.955 ± 1.410 (-16.36)	10.413 ± 0.488 (-27.15)	9.153 ± 1.006 (-35.95)	17.718*
CB	14.463 **± 1.622**	11.955 **± 0.887 (-17.34)**	10.583 **± 1.027 (-26.83)**	9.135 **± 0.683 (-36.84)**	20.265*
MO	14.460 ± 1.744	12.122 ± 1.724 (-16.17)	10.221 ± 0.724 (-29.31)	9.278 ± 0.900 (-35.83)	17.107*

Units:
LPO: MDA/mg protein/min.;
XOD: mmoles of formazon formed/mg protein/h
SOD superoxide anion reduced/mg protein/min;
CAT: µmoles of H_2O_2 degraded/mg protein/min
GPx, GR: µmoles of NADPH oxidized/mg protein/min

All the values 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. * $p < 0.01$