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Carbosulfan exposure during embryonic period can cause developmental disability in rats

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ABSTRACT

Carbosulfan, a wide spectrum pesticide is used to improve crop productivity. During their application, they disperse in the environment exerting harmful consequences on human health. We speculated that exposure to carbosulfan, a carbamate insecticide during early development can affect neurogenesis and synaptic development. In order to test this, pregnant dams were exposed to carbosulfan in four doses (0.5, 1, 2, and 4 mg/kg) during the embryonic period (ED 1–15). Offspring were evaluated for neurobehavioral changes, oxidative markers, acetylcholinesterase levels, and formation of carbonylated proteins. Histopathology of the cerebellum was carried out. Carbosulfan exposure produced alteration in sensorimotor tasks, motor function and elevated anxiety in pups. Carbosulfan affected growth rate of pups in a dose dependent manner. A significant increase in malondialdehyde, a lipid peroxide marker, carbonylated proteins and a dose dependent decrease in the levels of glutathione and glutathione peroxidase were observed. Carbosulfan produced a decline in acetylcholinesterase levels which might contribute to poor exploratory behavior. Distinct changes in the Purkinje cells were observed as the dose of carbosulfan increased. Largely, alteration in behavior can be due to oxidative damage, thereby, affecting neurogenesis, synaptogenesis and myelination. Therefore the propensity of carbosulfan to induce developmental disability is high and should be cautiously avoided during embryonic development.

1. Introduction

Organochlorine and organophosphorous pesticides are utilized the world over to improve crop productivity. Despite their availability, there was a constant search for newer and effective pesticides that led to the development of carbamates. Carbosulfan, a carbamate derivative and a broad spectrum insecticide, [2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamino)thio]methylcarbamate] belongs to the benzofuranyl methyl carbamate group. Carbamates destroy fungi, nematodes, acaricides and pyrethroid resistant mosquitoes (Guillet et al., 2001).

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Pesticides improve crop production but their dispersal into the environment is a cause of concern (U.S. EPA, 1996). Importantly, 99.9% of the applied pesticides contaminate the environment and less than 0.1% is actually consumed to destroy pests (Pimentel, 1995). Carbosulfan contaminates fruits and vegetables (Parveen et al., 2004); however, the probability of carbosulfan toxicity could be high in regions heavily relying on rice as the staple food and in areas extensively using spices. Although determination of maximum residue limits of pesticide on a variety of agricultural products (EU, Commission Directive 2001) is a prerequisite, it has been practically seen that their limits often surpass the desired range. Adding to the burden, pesticides undergo degradation yielding toxic metabolites which along with the parent molecule can have far reaching consequences on human health.

The process of neurogenesis, synapse formation and neurotransmitter development takes place during the prenatal period (Acosta et al., 2002). On the other hand, synaptic growth and dendritic arborization occur during the postnatal phase. Neuronal dysfunctioning due to defects in proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis could ensue on exposure to pesticides both in humans and animals (Barone et al., 2000). Pesticide exposure during pregnancy or in early postnatal life could exert deleterious consequences on the physiological system. As neurodevelopment in the fetus begins during gestation, exposure to carbamates might derange this process leading to impaired motor activity, cognition, and associated behavioral changes (Segerstrom and Miller, 2004).

Neuronal development and plasticity are vital for adequate performance of an individual in adult life. The extensive utilization of carbosulfan as a pesticide creates an increasing need to ascertain its impact on human health, particularly during gestation and early postnatal life. Carbosulfan is found to be mutagenic and genotoxic; however, the impact of carbosulfan on neurodevelopment has not yet been elucidated. The present study, therefore, was designed to investigate the role of carbosulfan exposure on the developing brain; its propensity to induce alterations in behavior, exert neuronal damage leading to neurodevelopmental disability.

2. Methods

2.1. Drugs and chemicals

Technical grade carbosulfan (Marshal, 92% purity) was obtained from FMC Pvt. Ltd., Tamil Nadu, India. Thiobarbituric acid was purchased from S.D. Fine Chemicals, Hyderabad, India. Bovine serum albumin, o-pthalaldehyde and 5,5′-dithiobis (2-nitro benzoic acid) were procured from Sisco Research Laboratories, Mumbai, India. All other chemicals and solvents used were of analytical grade.

2.2. Animals and treatments

Male and female Wistar rats were purchased from the National Institute of Nutrition, Hyderabad. The rats were housed in a temperature controlled room and maintained on a 12–12 h light–dark cycle (07:00 h lights on). The rats were provided with standard chow (National Institute of Nutrition, Hyderabad) and water *ad libitum*. Animal experiments were started after an acclimation period of 6 days. All procedures were approved by the Institutional Animal Ethical Committee and conducted as per the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Non-sibling rats were housed together in polypropylene cages (3 females and 1 male per cage) and allowed to mate. Animals were examined for the presence of a vaginal plug and the next morning was considered as embryonic day (ED) 0. Each pregnant animal was individually housed and randomly assigned to one of the following treatment groups (6 dams per group): Control received the vehicle orally (0.5% (w/v) aqueous polysorbate 80); other groups received carbosulfan (0.5, 1, 2 and 4 mg/kg body weight) respectively from ED 1 to ED 15 by the oral route. The dams were allowed to deliver, and two days after delivery, each litter was culled to 8 pups per dam to improve lactation efficiency. Weaning was done on PND 24. Pups were selected from each of the 6 litters and were examined. Body weight of pups was monitored at regular intervals.

2.3. Behavioral testing procedures

2.3.1. Surface righting

On postnatal days 5–9, each animal was placed on its back and gently held with all four limbs extended outward at which time it was released. Time taken to right such that all four paws were touching the surface was recorded. A cut off time of 30 s was provided for the animal to right (Kihara et al., 1991).

2.3.2. Negative geotaxis

Negative geotropism was tested on PND 13–17 of life by placing pups on a 45° inclined plane. The ability of the animal to turn around 180° with the head facing up the plane was recorded for a total of 20 s (Kahne et al., 2002).

2.3.3. Mid-air righting

On PND 13–17, pups were grasped by the scruff of the neck with the ventral side up and all the four paws extended 30 cm above a padded surface. Ability to right was scored positive if the pup landed on all four paws. A score of two out of three successful mid-air righting attempts was recorded as ability to right on each day (Cheh et al., 2006).

2.3.4. Hanging wire grip strength

On PND 13–19, pups were placed on a grid wire surface (30 cm × 18 cm divided into 1.2 cm grid squares), the plane was inverted and held 30 cm above a padded surface. Latency to fall was recorded with a maximum of 30 s for each trial.

2.3.5. Elevated plus-maze

The elevated plus-maze consisted of four arms (50 cm × 10 cm), two enclosed by walls 40 cm high, and two exposed, elevated 50 cm above the ground. Behavior was tested in a dimly lit room with a 40 W bulb hung 60 cm above the central part of the maze. The time spent in the open arms, and the number of open arms entries were used as anxiety measures during the 5-min test. This test was conducted