BRAIN EDEMA IN RATS CAUSED BY HIGH-DOSE 2,5-HEXANEDIONE TREATMENT

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Abstract: High dose (500 mg/kg) and low dose (300 mg/kg) of 2,5-hexanedione were orally administered to female Sprague-Dawley rats for 4 and 8 weeks, respectively, and the influence of these treatments on the nerve tissue was examined histologically and ultrastructurally. Abnormal gait was observed in the high dose group at the 4th week of treatment, but no neurological signs could be detected in the low dose group during 8 weeks of treatment. In the histopathological examination, the brain of animals belonging to the high dose group showed edema and necrosis in the thalamus and vacuolation in the cerebellar and vestibular nuclei. Small foci of hemorrhage were rarely observed in the thalamus. In the low dose group, the histopathological changes were characterized by axonal swelling in the cerebellar white matter, fasciculus gracilis of the cervical cord, and peripheral nerves. The swollen axons contained numerous neurofilaments with disorganized arrangement. Edema and necrosis in the thalamus or vacuolar change in the cerebellar and vestibular nuclei were not detected in the low dose group. These results indicated that the abnormal gait in the high dose group could be ascribed to the changes in the thalamus or in the cerebellar and vestibular nuclei. (*J Toxicol Pathol* 1: 165~170, 1988)

Key words: Edema, 2,5-Hexanedione, Thalamus, Vestibular nuclei, Neuropathology

Introduction

Neurotoxin, 2,5-hexanedione (2,5-HD), has been found to be a common metabolite of neurotoxic hexacarbons such as *n*-hexane and methyl-*n*-butyl ketone¹. Major outbreaks of hexacarbon neurotoxicity have been attributed to industrial exposure to *n*-hexane, abuse of *n*-hexane, and industrial exposure to methyl-*n*-butyl ketone. They were used as solvents and their excessive intake results in numbness, foot drop, and paralysis. The characteristic neuropathological feature was axonal swelling with accumulation of neurofilaments in the distal parts of the peripheral nerves and in the rostral regions of the ascending tracts and the caudal regions of the descending tracts of the spinal cord and has been referred to as dying back neuropathy or distal axonopathy^{2,3}.

In this study, the effects of high dose and low dose of 2,5-HD treatment in female rats were examined neuropathologically. A feature slightly different from the well-known hexacarbon neuropathy was observed.

Materials and Methods

Four-week old female Sprague-Dawley rats (Crj: CD) purchased from Charles River Japan, Atsugi, Kanagawa-ken were housed separately in a wire-bottom metal cage and kept in a barrier sustained animal room which was maintained at $24 \pm 1^\circ\text{C}$ in temperature and $55 \pm 5\%$ in relative humidity and had a 12 hour artificial light cycle. The room air was refreshed 15 times per hour. The animals were fed with commercial standard diet (CE-2, Japan Clea Co.). Drinking water and diet were made available *ad libitum*. After three weeks of observation for quarantine, the animals were divided into three groups. The first group consisting of 4 animals served as the control group and the second group consisting of 2 animals was orally administered 300 mg/kg/day of 2,5-HD

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(Wako Pure Chemical Industries, Ltd.) for 8 weeks. The third group consisting of 4 animals was treated with 500 mg/kg/day of 2,5-HD for 4 weeks. The animals were weighed every week. The test chemical was dissolved in distilled water and administered with a stomach tube 6 times per week in a volume of 0.5 ml per 100 g body weight. Control animals were given distilled water. Sodium pentobarbital-anesthetized rats were perfused with phosphate buffered saline followed by 0.1 M phosphate buffered 2% paraformaldehyde-1.25% glutaraldehyde fixative (pH 7.4) through the ascending aorta.

Four parts of the transverse sections of the brain were stained by luxol fast blue-hematoxylin eosin. The cervical and lumbar parts of the spinal cord, spinal ganglions of the lumbar part, peripheral nerves of the right hind legs (ischiatric, tibial, sural and plantar nerve), and gastrocnemius were sampled for electron microscopic examination. These tissues were additionally fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 hours, postfixed with 2% osmium tetroxide, dehydrated with graded series of ethanol and then embedded in epoxy resin. One micrometer epon sections were stained with toluidine blue and examined by light microscopy, and thin sections of the representative areas selected for electron microscopy were stained with uranyl acetate followed by lead citrate. The thin sections were examined with a Hitachi HS-9 electron microscope.

Results

The weight gain of the treated animals was lower than the controls (Table 1). Animals in the high dose group showed weakness in their hind legs and abnormal gait at the 4th week of treatment (Fig. 1). The low dose group showed no apparent toxic signs during 8 weeks of treatment.

Histopathological examination of the brain of the high dose group showed edema and necrosis in the thalamus without distinct glial cell proliferation, and the nerve cells were degenerated with empty cytoplasm (Fig. 2). Large and small vacuoles were present in the cerebellar and vestibular nuclei (Fig. 3). The large vacuoles were encircled by thin myelin sheath and contained an axon in the vacuole. The small vacuoles were present around the nerve cells, and in one case there were eosinophilic granules around the nerve cells in the vestibular nuclei. A few large vacuoles were also detected in the olivary nucleus. Small foci of hemorrhage were rarely observed in the thalamus.

Axonal swelling with accumulation of neurofilaments was rarely observed in the peripheral nerves and nerve fibers in the gastrocnemius of the high dose group. No changes were detected in the spinal ganglion.

In the low dose group, edematous changes were not observed in the brain. The characteristic change of the low dose group was axonal swelling

Table 1. Total Doses of 2, 5-Hexanedione, Body Weight, and Abnormal Gait

Group	Rat No.	Total doses (mg/kg)	Autopsy (wk)	Body weight		Abnormal gait
				Initial (g)	Final (g)	
Control	1	0	(4)	153.4	198.3	—
	2	0	(7)	153.1	246.4	—
	3	0	(9)	163.9	265.2	—
	4	0	(11)	154.6	268.0	—
300 mg/kg	13	13,500	(8)	152.2	209.4	±
	14	13,500	(8)	155.1	162.4	±
500 mg/kg	17	14,000	(4)	147.7	185.0	#
	18	10,500	(4)	145.1	166.7	#
	19	14,000	(4)	161.2	174.6	#
	20	13,500	(4)	133.0	150.1	#

— : no change, ± : slight, # : severe

Fig. 1. Rat administered 500 mg/kg of 2, 5-hexanedione for 4 weeks developed severe neuropathy and showed hindleg weakness with foot-drop.

Fig. 2. Edema and necrosis in the thalamus of the rat administered 500 mg/kg of 2, 5-hexanedione for 4 weeks. Luxol fast blue-hematoxylin eosin stain. $\times 280$.

in the fasciculus gracilis of the cervical part of the spinal cord and in the cerebellar white matter. Ultrastructurally, these swollen axons contained many neurofilaments with disorganized arrangement (Fig. 4). Axonal swelling was also detected in the lateral and ventral funiculus of the cervical part and in the white matter of the lumbar part. A few myelin ovoids were observed in the dorsal funiculus in the cervical cord. In the peripheral

nerves and nerve fibers in the gastrocnemius of the low dose group, axonal swelling was observed and the change was slightly more prominent than in the high dose group.

Discussion

Pathological changes in rats administered high dose of 2, 5-HD were characterized by edema and necrosis in the thalamus and vacuoles in the

Fig. 3. Vacuolar change within the myelin sheath and around the neurons of the vestibular nucleus of the rat administered 500 mg/kg of 2, 5-hexanedione for 4 weeks. Luxol fast blue-hematoxylin eosin stain. $\times 350$.

Fig. 4. Axonal swelling containing numerous neurofilaments with disorganized arrangement in the fasciculus gracilis of the rat administered 300 mg/kg of 2, 5-hexanedione for 8 weeks. Thin epoxy section stained with uranyl acetate and lead citrate. $\times 20,000$.

cerebellar and vestibular nuclei. Large vacuoles in these nuclei were intramyelinic edema and myelin splitting. Small vacuoles around the neurons may be degeneration of the nerve endings of fibers which were distributed to these nuclei. As reported by many investigators¹⁻⁴, axonal swelling with accumulation of neurofilaments was detected in the fasciculus gracilis and in the peripheral nerves of the low dose group. Axonal swelling in the high dose group was more slight than in the low dose group.

Neurological signs in the high dose group were severe, making it difficult to move with their legs. Many investigators ascribed the abnormal gait of hexacarbon neuropathy to the accumulation of neurofilaments in axons and to the subsequent axonal degeneration. Spencer and Schaumburg^{2,3} showed that large myelinated fibers in tibial nerve branches supplying the calf muscle were especially sensitive in hexacarbon intoxicated rats. In the peripheral nerves within the gastrocnemius of our high dose group, axonal swelling was more slight than in the low dose group, but edema and necrosis in the thalamus, and vacuolar changes in the nuclei were prominent. These results in our study suggest that the abnormal gait observed in the high dose group may be ascribable to the changes in the thalamus or in the cerebellar and vestibular nuclei.

Edema in the nerve tissue was reported in animals treated with various compounds⁵, such as lead⁶, mercury⁷, hexachlorophene⁸, triethyltin⁹, misonidazole¹⁰ or pyridoxamine^{11,12}. Lead or mercury injures the endothelial cells and causes plasma extravasation which is followed by endothelial, microglial, astrocytic proliferation and edema. Since hemorrhagic foci in our experiment were rare and not distinct, vascular injury may not be the main cause of this edema.

The edema in hexachlorophene treatment is restricted to the myelin sheath and results from the separation of the intraperiod line of myelin lamellae⁸. Triethyltin, which is the most toxic agent among the organic tin, also induces similar changes as hexachlorophene and no abnormality is seen in the blood vessels⁹. Misonidazole, which was developed as a hypoxic cell sensitizer for cancer radiotherapy, induces spongy changes with splitting of myelin lamellae and necrosis in the

vestibular, cochlear and cerebellar nuclei of the medulla¹⁰. Pyridoxamine induces acute thiamine deficient encephalopathy. In histological examination of thiamine deficient animals, there were petechial hemorrhage and edematous necrosis of the brain tissue such as the thalamus and mammillary body^{11,12}. Electron microscopically, there were edematous swelling of astrocytes, oligodendrocytes, and myelin sheath, but the nerve cells and blood vessels were not altered.

The mechanism of these edematous changes with intact blood vessels is still not well elucidated. Although the pathogenesis of the changes observed in this study is also not defined, the result has indicated that edematous changes were the predominant findings in rats orally administered high dose of 2,5-HD. Hexacarbon neuropathy has been characterized by axonal swelling with accumulation of neurofilaments, but our result suggests the possibility of a different pathogenesis of hexacarbon neuropathy.

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