Peripheral and Central Nervous System Lesions Caused by Triethyl- and Trimethyltin Salts in Rats*

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ABSTRACT

Both trimethyltin and triethyltin salts are known to produce toxic lesions in the central nervous system. Triethyltin intoxication has been associated with central intramyelin edema, while trimethyltin has been shown to produce neuronal necrosis in selected limbic and sensory regions of the brain. Only scant attention has been paid to peripheral nerves of animals treated with alkyltins. In this study, we have treated rats with 6 or 8 mg/kg trimethyltin, and 1, 2, 4, 6, or 8 mg/kg triethyltin (single or multiple exposure), and evaluated in detail at the light microscope level both central and peripheral nervous system lesions. In addition to the central neuron necrosis or myelin edema described previously, both compounds produced peripheral axon degeneration and chromatolysis of large spinal cord and brain stem neurons. Chromatolysis was seen in reticular neurons of the brain stem and ventral horn or spinal cord in rats receiving high doses (6 or 8 mg/kg) of triethyltin, and in these same areas plus mesencephalic trigeminal nucleus in animals treated with trimethyltin. Wallerian-like degeneration of peripheral axons was seen in sciatic and tibial nerve and ventral roots of animals receiving 3 injections of 4 mg/kg or single or multiple injections of 6 or 8 mg/kg triethyltin. Axon degeneration was also seen in sciatic and tibial nerves 21 days after a single exposure to 8 mg/kg trimethyltin. Since myelin edema is believed to be reversible, the axonal changes described here may be of greater clinical significance in relation to human exposure.

INTRODUCTION

Organotin compounds have been widely used as plastic stabilizers, preservatives, and biocidal agents (29). In the early 1950s, over 100 deaths in France were attributed to the neurotoxic properties of a proprietary treatment for furunculosis which may have contained a proportion of triethyltin (22). Studies (in experimental animals) revealed that triethyltin compounds produced severe edema of cerebral white matter (21, 24). The edema fluid, representing a plasma ultrafiltrate (2, 21, 27), collected in vacuolar spaces formed by splitting of myelin at the intraperiod line, especially outer lamellae (1, 2, 20–22, 25, 28–30, 32). No predilection for fiber diameter was noted (21). There have been only a few reports of axonal damage including edema and “dense bodies” in central axons (29, 31) and occasional Wallerian degeneration in peripheral nerves (15–17). Axon degeneration was described in an in vitro spinal cord system (18).

In contrast, trimethyltin has not been found to produce damage to axons or myelin in rodents. Triethyltin has been associated with a syndrome of tremor, aggression, and seizures (4, 13) and with morphological damage including necrosis of neurons in limbic and sensory regions including olfactory tubercle, olfactory cortex, subiculum, Ammon’s horn, and dentate gyrus (4, 6, 7, 13). Chromatolysis was also reported in spinal cord ventral horn (8) and reticular and mesencephalic trigeminal nuclei (9). Loss of cells in special sensory organs has been reported in retina and inner ear (6). Reports of occasional Wallerian degeneration with triethyltin exposure and the presence of central chromatolysis in ventral horn neurons after trimethyltin treatment are suggestive of peripheral nerve lesions. We therefore performed a study to define lesions to the central and peripheral nervous systems.

METHODS

Animal Management. A series of experiments were conducted to compare the distribution of le-
sions caused by trimethyltin and triethyltin over a wide range of doses. Male Sprague-Dawley rats (Charles River, Canada) weighing approximately 120 g were housed individually in suspended wire cages with Purina Certified Rat Chow and tap water ad libitum. After 2 weeks acclimation, rats were randomized by body weight to treatment groups. Clinical condition was checked daily, and any animals found to be moribund were sacrificed for humane reasons.

Trimethyltin Experiments. Three groups of 6 animals each were treated with a single intraperitoneal injection of trimethyltin chloride in 5 ml/kg saline at dose levels of 0 (control), 6 mg/kg, or 8 mg/kg...
Triethyltin Experiments. Six groups of rats were treated with single or multiple injections of triethyltin bromide in 5 ml/kg saline at dose levels of 0, 1, 2, 4, 6, or 8 mg/kg/injection. All groups consisted of 6 animals except the 6 mg/kg and 8 mg/kg groups which consisted of 4 rats each. Dose and sacrifice schedules were as follows: all groups were treated on Days 0, 5, and 10, and sacrificed on Day 14. Some animals from Groups 5 and 6 (6 and 8 mg/kg) became severely weakened and unable to eat and were sacrificed prior to Day 14. These included 1 animal from each of Groups 5 and 6 on Day 4 (single exposure only), and 2 of Group 5 and 3 of Group 6 on Day 9 (2 exposures). Therefore, only 1 animal from Group 5 and none from Group 6 was sacrificed on Day 14.

Necropsy. Animals were sacrificed under deep sodium pentobarbital anesthesia by intracardiac perfusion with Ringer's lactate solution followed by 4% phosphate buffered glutaraldehyde. All gross lesions were noted. The following tissues were retained in neutral buffered formalin for paraffin sections: olfactory bulb, right hemisphere of brain, spinal cord, sciatic nerves, Gasserian ganglia, eyes, liver, kidney, spleen, thymus, heart, gastrocnemius muscle, urinary bladder, stomach, jejunum, mid-colon, salivary glands, femurs, and mesenteric lymph nodes. In addition, thin slices of spinal cord, sciatic and tibial nerve, optic nerve, and dorsal root ganglia were postfixed in 1% phosphate buffered osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide, and embedded in epoxy resin. Short segments of sciatic and tibial nerves from TET-treated rats were teased to individual or groups of 2 to 3 fibers in liquid resin, mounted on glass slides, and polymerized under cover slips. Paraffin sections (7 μm) were stained with H&E, Klüver-Barreta or a silver impregnation method. Epoxy sections (1.0 or 0.5 μm) were stained with 1% borax-buffered toluidine blue.

RESULTS

Triethyltin

Clinical Signs. Within 3–5 minutes following each injection of 6 or 8 mg/kg triethyltin, animals became stuporous or immobile and their signs lasted for approximately 6 hr. By 24 hr after injection, the rats became irritable, developed hindlimb paraparesis, piloerection, and exaggerated kyphosis. After 2 injections of 8 mg/kg or 3 injections of 6 mg/kg, the paraparesis progressed to paraplegia. Animals receiving 4 mg/kg/treatment or less showed no clinical signs.

Neuropathological Findings. A predominant microscopic finding in paraffin sections was severe and extensive spongy vacuolation of white matter in all areas of brain and spinal cord, and was found in rats sacrificed after a single injection of 6 or 8 mg/kg and to a lesser extent after 3 injections of 4 mg/kg (Fig. 1). Neuronal changes were not extensive and consisted of cytoplasmic vacuolation in ventral thalamus in animals receiving a single injection of 6 or 8 mg/kg. Central chromatolysis of large neurons in the reticular formation raphe nuclei and spinal cord ventral horn was observed in animals receiving a single injection of 6 or 8 mg/kg. Central chromatolysis of large neurons in the reticular formation raphe nuclei and spinal cord ventral horn was observed in animals sacrificed after 2 injections of 6 or 8 mg/kg or 3 injections of 4 or 6 mg/kg (Fig. 2). No necrosis of neurons was observed.

In semithin epoxy sections the white matter was....
vacuolated due to ballooning of split myelin sheaths (Fig. 3). Myelin splitting was seen in virtually all fibers at the highest doses, but, in animals treated with 4 or 2 mg/kg, smaller-diameter fibers were more affected than large fibers. This pattern was most apparent in the dorsal column of the spinal cord where smaller-caliber fibers of the dorsal corticospinal and gracile tracts showed significantly more vacuolation than the larger fibers of the cuneate tract (Fig. 1). In severely vacuolated areas of spinal cord, swelling suggestive of intra-axonal edema was seen. Some myelin sheaths contained nucleated phagocytic cells, possibly microglia (Fig. 4).

In semithin epoxy sections and in teased fibers of sciatic and tibial nerve from animals receiving 3 times 4 mg/kg or single or multiple exposures to 6 or 8 mg/kg, numerous degenerating axons were seen. Large-diameter ("A") fibers were predominantly affected. Changes included endoneurial edema, Schwann cell proliferation, axon degeneration and phagocytosis, and collapsed myelin sheaths (Fig. 5). Macrophages were seen within and between myelin sheaths, and some fibers had large empty-appearing vacuoles (Fig. 5). In teased fiber preparations, degenerating fibers appeared as linear rows of myelin balls and ovoids (condition E of Dyck) (Fig. 6). No paranodal swelling was observed. However, in ventral nerve roots some swelling and paranodal myelin thinning were observed (Fig. 7). Occasional fibers in ventral roots were undergoing Wallerian-like degeneration. Dorsal root ganglion cells appeared normal.

Trimethyltin

Clinical signs were not observed at all doses. In animals given 8 mg/kg TMT and sacrificed 7 or 21 days after treatment, histopathological changes in the brain consisted of neuronal necrosis, characterized by homogeneous, eosinophilic cytoplasm and nuclear pyknosis or karyorrhexis. In animals sac-
rified 7 days after treatment, neuronophagia and
glial proliferation were seen. By Day 21 necrotic
neurons were less frequent, but there was pro-
nounced astrocytosis (Fig. 8). Areas affected in-
cluded the lateral anterior olfactory nucleus, olfactory
tubercle, olfactory cortex, entorhinal cortex, and
Ammon’s horn. The CA3–CA4 region of the dorsal
and CA1 in the ventral hippocampus were severely
affected. In addition to neuronal necrosis, chro-
matolysis was observed in mesencephalic trigeminal
nucleus, reticular raphe neurons, and occasionally
in spinal cord ventral horn neurons (Fig. 9). The
lesions at the lower dose (6 mg/kg) were of similar
nature but of lesser severity. In particular, the extent
of damage in Ammon’s horn was reduced with only
CA3c and CA4 in the dorsal hippocampus and CA1
in the ventral hippocampus strongly affected. Chro-
matolysis was only rarely seen at this lower dose.

Examination of semithin epoxy sections of pe-
ripheral nerves revealed Wallerian-like degenera-
tion of axons very similar to that described for tri-
ethyltin, characterized by rows of myelin ovoids
and macrophages (Fig. 10). Segmental demyelination
was rarely observed. Paranodal axon swelling was not
seen. Peripheral axon degeneration appeared to be
dose-related, with degenerating axons most frequent
21 days following a single injection of 8 mg/kg, less
frequent 7 days after this dose, and only rarely ob-
erved in animals treated with 6 mg/kg.

In the spinal cord, some axonal changes were not-
ed especially in large-diameter axons in lateral and
ventral columns (Fig. 11), including a few distended
myelin sheaths containing shrunken axons, and ac-
cumulation of dark staining profiles in other axons.
These lesions were not frequently observed in any
group but appeared to be most numerous 21 days
after treatment with 8 mg/kg.

**DISCUSSION**

In these experiments, triethyltin produced central
myelin edema as previously described (1, 2, 20–22,
25, 28–30, 32). It was noted that smaller-caliber
fibers were predominantly affected at lower doses
producing lesions less severe than in these earlier
studies. Myelin splitting is known to occur rapidly
(21, 29) and in this study appears to be related to
the single dose rather than to the cumulative dose.
For example, 3 times 4 mg/kg TET produced sig-
ificantly less edema than a single dose of 8 mg/kg.

Trimethyltin did not produce myelin edema but
rather neuronal necrosis in limbic and olfactory areas
of the brain and chromatolysis in spinal cord, re-
ticular neurons, and mesencephalic trigeminal neu-
rons as previously reported (3, 4, 6–9). These changes
were dose-related and were more severe 21 days
after acute treatment than 7 days after treatment.
This may be due to the prolonged retention of tri-
methyltin in brain (12); the concentration does not
Fig. 8.—Neuronal necrosis and gliosis resulting from a single injection of trimethyltin chloride, 8 mg/kg. (a) Neuronal necrosis is prominent in CA3b-c region of dorsal hippocampus (×30). (b) higher-power view demonstrates marked gliosis 21 days following injection of 8 mg/kg trimethyltin chloride (×105); (c) marked neuronal loss and gliosis in olfactory cortex of the same rat; (d) higher-power view illustrating necrosis, karyorrhexis (arrow), and glial proliferation (mitosis, arrowhead). (H&E. ×105).

decline, but rather increases in brain homogenates up to 24 hr, and levels at 73% of the peak 24 hour concentration 5 days after dosing (10). Karyorrhexis and eosinophilia of neurons, signs of relatively recent damage, were still seen 21 days after injection along with extensive astrocytic scarring.

To date, relatively little attention has been paid to neuropathological effects of alkyltins other than the most prominent lesions discussed above. In trimethyltin intoxication, not only neuronal necrosis but also chromatolysis of certain groups of neurons have been described, including mesencephalic trigeminal nucleus, raphe reticular neurons, and spinal ventral horn cells (8). No chromatolysis was reported after triethyltin intoxication, although myelin clefts and occasional Wallerian degeneration were noted in peripheral nerves (17, 18). Central chromatolysis is a response of the neuron to axotomy (11) and is also seen in severe toxic neuropathies of the dying back type (5). Thus, the finding of central chromatolysis of ventral cells after either trimethyltin or triethyltin intoxication is likely to be related to the axon degeneration which was seen at levels as high as proximal nerve roots. The chromatolytic reaction in brain and spinal cord after alkyltin intoxication has been regarded as primary neuron damage (7, 9) and has been compared to selective chromatolysis seen following brain ischemia (4). However, axon degeneration was seen in spinal cord after triethyltin treatment in this study, as well as in an in vitro system by Graham et al (19). While in the study presented here degeneration of axons of the reticular neurons was not directly demonstrated, it is possible that the chromatolysis in these neurons is a response to central axon damage.

Clinical results of earlier studies have suggested the existence of peripheral axon damage in triethyltin intoxication. In one electrophysiologic study (17) sciatic nerve conduction velocity was reduced by 33% without demyelination following triethyltin exposure. Hindlimb weakness has been considered a sign of peripheral neuropathy (7) and has been prominent in most studies of TET intoxication (1, 2, 15, 17, 18, 23, 27, 28, 30). Fechter and Young (14) found by reflex modulation audiometry that results of startle response experiments are more like-
This study examined differences in the central and peripheral nervous systems as well as similarities in the lesions induced by triethyltin or trimethyltin. Both compounds produce significant peripheral axon degeneration and central chromatolysis of specific neuron groups. Since myelin splitting and edema may resolve readily (23, 27, 29), while axons regenerate poorly, this axon degeneration may be of greater clinical significance in triethyltin intoxication.

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References


