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The Effect of Acrylamide on the Ultrastructures of Nervous System of the Mouse

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The effect of acrylamide on the nervous system has been morphologically studied using light and electron microscopes. The light micrographs on central and peripheral nervous tissues of mouse treated with acrylamide monomer showed total vacuolation of spinal cord, cell degradation containing neuron and neuroglia, and distal nerve fiber degeneration. The electron micrographs showed ultrastructural changes. Abnormal mitochondria in neuron, splitting of myelin sheath in lumbar ventral root nerve, partial disintergration of myelin sheath and axoplasmic degeneration in sciatic nerve, and overall polyneuropathies in nervous system were observed. These results suggest that acrylamide intoxicated mouse shows distal behavioral neuropathy as an earlist clinical sign, but the initial effect of acrylamide on the nervous system seems to appear at nearly the same time in both central and peripheral nervous systems.

KEY WORDS: Acrylamide, Neuropathy, Ultrastructure, Nervous system

Acrylamide ($CH_2 = CHCONH_2$), a crystalline powder, is a 3-carbon vinyl monomer. Monomeric acrylamide is in widespread use to make a nontoxic polymer. It is also used as a grouting agent to water proof tunnels and foundations, and by biochemists as the stationary phase during the electrophoretic separation of proteins.

The neurological features of monomeric acrylamide intoxication vary as a function of the type of intoxication (LeQuesne, 1980). Rapidly intoxicated humans have occasionally developed encephalopathy with confusion, discorientation, memory disturbance and hallucinations, ataxia, and a subsequent mild peripheral neuropathy. More characteristically, chronically exposed individuals in the work place complain of vagus CNS disturbances (O'Donghue, 1985).

While initial concern for acrylamide neurotoxicity on the central nervous system shifted to the peripheral effects, it is again shifting back to central effects (Fullerton, 1969; Mapp *et al.*, 1968; Takahashi *et al.*, 1971). Damaged to central axons may account for behavioral, visual and memory change (Schaumburg and Spencer, 1979).

The pathology of experimental acrylamide neuropathy is characterized by progressive centripetal axon degeneration of the distal ends of the longest and the largest nerve fibers, with preservation proximally (Cavanagh, 1969). This pattern of distal degeneration (dying-back), which typifies many diseases of the nervous system, results from progressive compromise of neuronal pericaria and their gradual failure to supply materials required by their axons (Fullerton and Barnes, 1966).

Dying-back axonbopathy is the most common pathologic reaction of the peripheral nervous system to environmental toxins (Schaumburg and Spencer, 1979). A large body of evidence suggested that toxins that cause dying-back disease of the axon act directly on the axon itself (Ashbury and Brown, 1980; Lowndes and Baker, 1980; Prineas, 1969; Spencer and Schaumburg, 1977; Spencer and Schaumburg, 1978). But the axon depends on the neuronal cell body for maintenance and repair, and outcome of neurotoxic exposure may depend in part on events in the cell

body.

Thus in the present study, to elucidate the time of onset of toxicological effect of acrylamide, both central and peripheral nervous tissues of the intoxicated mouse were morphologically investigated using a light and an electron microscope.

Materials and Methods

Animal and Treatment

10-week-old young male mice of an inbred strain of average body weights of 34 g were divided into two groups and housed individually in separate cages with wire-meshed floor in an air-conditioned room. Animals were allowed free access to food and water throughout the experimental period.

Acrylamide was dissolved in sterile 0.9% saline to diliver the desired dose, 50 mg/kg in a volume of 0.1 ml. Solutions were made fresh every other week and were stored in the dark in a refrigerator. Two groups, control and treatment, were injected intra-peritoneally with either saline or acrylamide once a day in the afternoon 6 days per week with no injection on Sunday for 2 weeks.

Tissues for Microscopic Analysis

Animals were perfused by total body fixation through the left ventricle with 4% paraformaldehyde, followed by 3% glutaraldehyde in a 100 ml cacodylate buffer (pH 7.4) using peristaltic pump (Gilson Co.). After the perfusion, lumbar 6(L_6) spinal cord and proximal part of sciatic nerve segment at the level of the triceps surae nerve were sampled as CNS and PNS neural tissues, respectively.

Light and Electron Microscopy

Small blocks of tissues were fixed for additional 20 min with 3% glutaraldehyde in 100 mM cacodylate buffer (pH 7.4) at 4°C. The tissue blocks were washed for 2 hrs in several changes with the same vehicle at 4°C, post-fixed in 1% osmium tetraoxide solution for 1.5 hrs at 4°C, dehydrated stepwise in graded concentrations of acetone, infiltrated and embedded in epoxy resin. Sections were cut with an ultratome (LKB 2188). Sections cut into 1 μm in thickness were stained with toluidine blue for light microscopic observation. For electron microscopy, sections were cut into 80-100 nm and stained with uranyl acetate and lead citrated (Millonig, 1961), and then examined with a transmission electron microscope (JEM 100SX).

Results

Clinical Signs

In the present study, with a dose of 50 mg/kg i. p., animal were developed the first neurological sign at 14 days after commencing the injections. Mild ataxia of the hindlimbs was the earlist sign. There were no deaths in the 50 mg/kg group after 14 days of acrylamide administration (12 doses = 600 mg/kg commulative dose). There were also no deaths in the saline injected control group.

Toxicity at Nervous Tissues

The effects of acrylamide treatment on the CNS and PNS were morphologically examined and were compared with the untreated control group. At the time of onset of earlist clinical sign, the effects of acrylamide treatment on the central and peripheral tissues were as follows:

LM Study

In the specimens obtained from lumbar spinal cord of acrylamide administrated mice, there was total vacuolation in the white matter, particularly in the ventral columns, with the exception of one area in the dorsal column (Fig. 1). Cells containing neuron and neuroglia in the gray matter showed a little pathological signs that included the eccentric nucleus, inner perinuclear zone with pigmented granules (Fig. 2). Also, some degenerative features containing irregularity of nuclear membrane were observed in the ependyma cells of central canal (Fig. 3). The ependyma cell of central canal has a simple cuboidal shape under normal condition. In

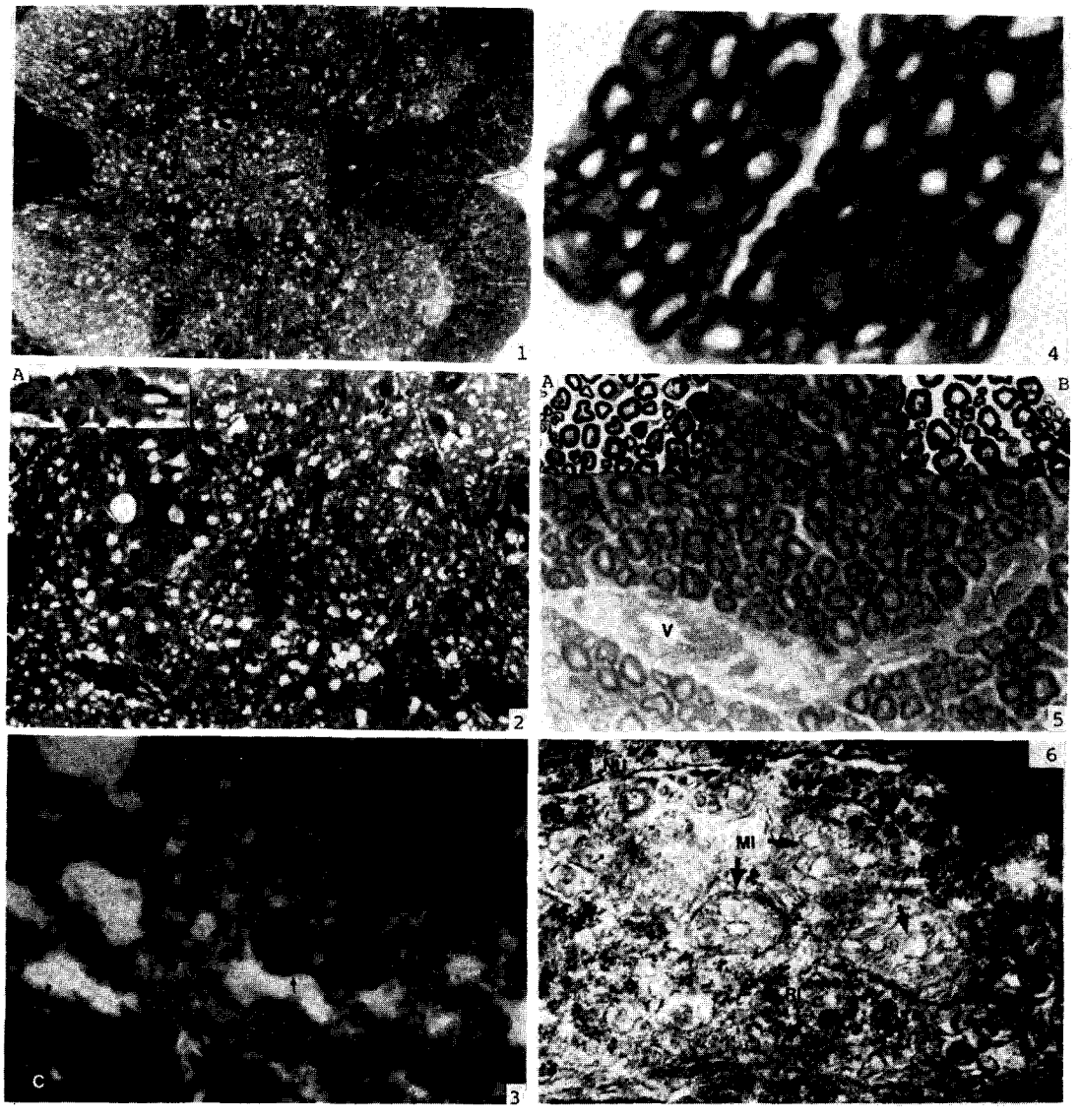

Fig. 1. The lumbar spinal cord of the treatment group. There is total vaculolation of the white matter with the exception of one aera in the dorsal column (arrow). This aera represent the corticospinal tract. Toluidine blue. Magnification, × 40

Fig. 2. The gray matter of the lumbar spinal cord showing degenerative signs. The eccentric, irregular nuclei and abnormal pigmented granules in the perinuclear zone are observed. C: Central canal. Toluidine blue. Magnification, × 100. inset A; degenerative features in the ependyma cell of central canal. magnification, × 200

Fig. 3. Many neurons and ependyma cells show cell degradations. C: Central canal. Toluidine blue, magnification, × 1,000

Fig. 4. The axonal degenerations containing axonal swelling in the lumbar ventral root nerve fibers. Toludine blue. Magnification, × 1,000

Fig. 5. The sciatic nerve of the treatment group showing axonal degeneration. V: Blood vessle. Toluidine blue. Magnification, × 400 inset A, B; many small myelinated nerve fibers are intact. Magnification, × 1,000

Fig. 6. Electron micrograph of the neuron in the lumbar spinal cord of the treatment group showes subcellular degradations. NU: Nucleus, MI: Mitochondria, RI: Ribosome. Magnification, × 40,000

the ventral root nerve fiber, axonal swelling, myelin debris, and other degenerative features were observed as signs of axonal degeneration (Fig. 4). The most notable fact in the gray matter of lumbar spinal cord was the morphological change of central canal epithelium. This may indicate acrylamide affects CNS at the time of onset of earlist peripheral neuropathy. In the sciatic nerve, acrylamide effects were the axonal degenerations of several myelinated nerve fiber. But there were no dramatic degenerations containing widespread intramyelinic edema, and many of the small axons were intact (Fig. 5).

EM Study

In the ultrastructural examination of the specimens from spinal cord of treatment group, many neuronal cell containing neuron and neuroglia showed pathological signs that included the irregularity of nuclear membrane, dilated or morphologically changed mitochondria in size and shape (Fig. 6). Occasionally, mitochondria containing a crystaline-like inclusion was observed (Fig. 7). The synapses in terminal button contained many dense neurosecretory granules and dense-cored mitochondria as shown in Fig. 8.

The degenerative features of several axons in the white matter of ventral culumn and of axons in the lumbar ventral root nerve fiber were observed. The affected myelinated nerve fibers in the white matter showed the irregularity of myelin sheath (Fig. 9). Among the affected axons in ventral root, the splitting and irregularity of arrangement of myelin lamellae were observed as common pathological signs (Fig. 10).

After acrylamide administration, the sciatic nerve also showed many pathological signs. Especially, the abnormal inclusions in the axoplasm and elongated or dense-cored axoplasmic mitochondria were observed (Figs. 11 and 12).

Discussion

The metabolic effects of acrylamide and other various neurotoxitants have been well reported in many studies. Kuperman (1958) made a first detailed physiological study of poisoned cats and concluded that the most likely site of action of acrylamide given in doses that produced acute poisoning was on the mesencephalic tegmentum of the brain stem. So he proposed the mesencephalic tegmentum as a primary target for chronic acrylamide poisoning. He did not, however, demonstrate any pathological change in his animal. McCollister *et al.* (1964) have a full account for the toxicity of acrylamide and shown that most species react in a similar way. He and his colleagues drew attention to the prominence of the neurotoxic effects in all species but had no evidence about the nature of the lesion.

It has now been shown that the neurological abnormalities which develop during chronic poisoning are due to peripheral neuropathy. Impaired peripheral nerve function has been suggested by the observation of ultrastructural alteration in both motor and sensory nerves (Schaumburg *et al.*, 1974). Acrylamide induced neuropathy begin with the involvement of the distal limbs and slowly progresses to the proximal regions of the body. The neuropathy is usually reversible after the cessation of acrylamide exposure with the dose and duration of exposure being the limiting factors. Hopkins (1975), Davenport *et al.* (1976), and Schaumbur *et al.* (1974) showed that histologically, acrylamide caused a distal to proximal dying-back axonopathy of both sensory and motor nerves.

Teal *et al.* (1981) reported the behavioral effects of acrylamide in mouse. They showed that mice receiving 60 mg/kg subchronically had only a slight loss of body weight but developed a neuropathy within 3 weeks of dosing and the group reached a 50% mortality after 31 days (960 mg/kg cumulative dose). In the present study, mice received 50 mg/kg subchronically (600 mg/kg C. D.) showed no death and the clinical signs were mild ataxia of hindlimbs and a slight loss of body weight. These results support the previous reports.

Sterman (1982) demonstrated an array of cell body remodeling in large and small neurons in DRG when distal peripheral nerves showed few changes. Therefore, Spencer and Schaumburg (1978), Asbury and Brown (1980), Lowndes and baker (1980), and Spencer et al. (1980) reported that the necessity was needed to reexamine the

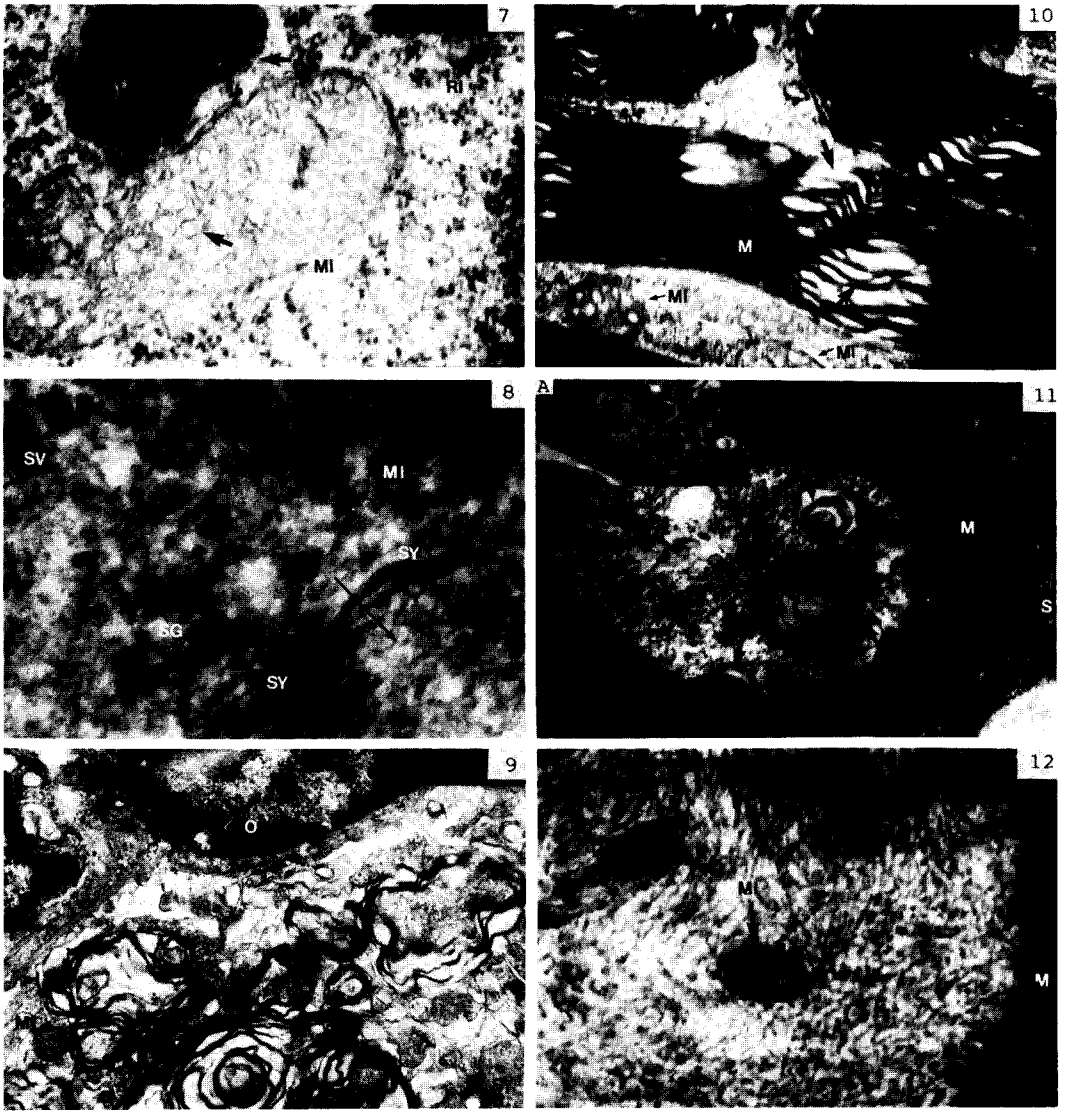

Fig. 7. The mitochondria showing crystaline-like inclusion and dilation. MI: Mitochondria, RI: Ribosome, Magnification, × 50,000

Fig. 8. The synapses containing many neurosecretoric dense granules. SY: Synapse, SG: Synaptic granule, SV: Synaptic vesicle, MI: Mitochondria. Magnification, × 120,000

Fig. 9. The myelinated nerve fibers in the white matter showing irregulation and disintergration of the myelin sheath. 0: Oligodendrocyte, MI: Mitochondria, SY: Synapse. Magnification, × 15,000

Fig. 10. The myelinated nerve fivers of the lumbar ventral root showing axonal degenerations. The disintergration and splitting of axolamina are notable. M: Myelin sheath, MI: Mitochondria. Magnification, × 30,000

Fig. 11. Electron micrograph of the sciatic nerve showing axoplasmic abnormalities. The abnormal inclusions in the axoplasm are notable. M: Myelin sheath, S: Schwann cell. Magnification, × 30.000. inset A; axonal degenerations containing abnormal inclusion and dense cored mitochondria in the axoplasm. Magnification, × 6,000

Fig. 12. The alongated and dense cored mitochondria in the axoplasm. MI: Mitochondria. Magnification, × 50,000

prevailing view that the neuronal cell body displays either no change or minimal and nonspecific alterations in dying-back axonal diseases.

Two observations suggested that the neurotoxin may act diretly on the cell body, contrasting with axonopathy theory (Sterman, 1982). First, striking somal changes preceded the appearance of major degeneration in distal axons at the sites examined. Second, both large and small cell bodies showed early and pronounced changes. If the large long axons were the primary targets of the toxin, the large cell bodies associated with the largest and longest axons should be selectively involved. But neurons of all sizes were affected in DRG where the blood-nerve barrier is incomplete (Lieberman, 1976; Inoue *et al.*, 1979). In the present study, the results showed that both CNS and PNS are already affected at the time of onset of the earlist behavioral neuropathy. It may suggest that acrylamide acts directly on both CNS and PNS through blood-nerve barrier by systemic circulation.

In the mechanisms of action of acrylamide, protein alteration and inhibition of glycolysis have proposed as important mechanisms. Ochs and colleagues (Ochs and Smith, 1971; Ochs and Sabri, 1971; Ochs and Sabri, 1972) have demonstrated that fast axonal transport is dependent on ATP generation by glycolysis. Interference with glycolysis or energy metabolism has been proposed as a mechanism but with glycolysis or energy metabolism has been proposed as a mechanism but which serveral axonotoxic chemicals might exert their effects. In this study, the morphologically changed mitochondria indicates the inhibition of energy generation for fast axonal transport. The inhibition of energy production may be a cause of axonal dying-back degeneration, and the axonopathy may goes up to the proximal part of nerve (dying-back).

However, the results of this study and most previous reports do not show detailed acrylamide effects on the tissue proteins of affected organs. So, to investigate the protein alteration and to understand the detailed effects of the neurotoxin on nervous and non-nervous system, electrophoretic study using SDS-PAGE and two-dimesional electrophoresis is fervently needed.

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생쥐 신경계의 미세구조에 미치는 Acrylamide의 영향

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Acrylamide의 신경독성에 대해 조사하기 위하여 초기운동실조증이 유도된 생쥐의 신경계에 대한 Acrylamide의 영향을 관찰한 결과, 중추 및 말초신경조직에 있어서의 손상이 보여 척수의 전반적 공포현상, 신경원 및 신경교의 세포손상 그리고 말초신경섬유의 퇴행현상들이 보였다. 또한 이들에 대한 미세구조적 관찰에 의하여 신경원에서의 비정상적 미토콘드리아 및 척수전근섬유에서의 수초의 균혈화 현상등이 야기됨을 알았으며 좌골신경에서의 수초의 부분적 붕괴 및 축삭형질내의 퇴행현상들도 보였다. 위와같은 실험결과는 Acrylamide로 인한 중독의 영향이 말초신경장애의 증상으로 나타나나 최초의 신경독성효과는 중추 및 말초신경계에 대해 거의 유사한 시기에 작용함을 시사한다.