Comparison of Central and Peripheral Nervous System Lesions Caused by High-Dose Short-Term and Low-Dose Subchronic Acrylamide Treatment in Rats*

DONALD J. O'SHAUGHNESSY AND GEORGE J. LOSOS

Division of Pathology, Bio-Research Laboratories Limited, 87 Senneville Rd., Senneville, Quebec, Canada H9X 3R3

ABSTRACT

The effects of high-dose subacute acrylamide treatment of up to 50 mg/kg/day for 4 or 10 d were compared to those of subchronic exposure, up to 12 mg/kg/day for 90 d. In the subacute study, Purkinje cells, long ascending tracts of the spinal cord, optic tract terminal or preterminal regions in superior colliculus, sensory ganglion cells, and distal large-caliber peripheral axons were severely affected. Purkinje cells and fasciculus gracilis changes were the earliest lesions.

In the subchronic study, the dominant lesion was confined to the distal peripheral axon, with only minor changes occurring in spinal cord and medulla. Paranodal swellings with the characteristic appearance of neurofilament aggregations were not seen. This morphological study suggests a significant difference between high- and low-dose acrylamide-induced lesions. If there is a reduced tendency for long-term low-dose acrylamide exposure to produce CNS lesions, the risk of irreversible nervous system damage would be less than that predicted from subacute studies.

INTRODUCTION

Acrylamide is known to induce a “dying back” (4) type of axonopathy also seen in intoxication by hexacarbons (17, 18), carbon disulfide (1), organophosphates (2), or other compounds (3). Because of the initially distal site of axonal degenerative changes, Spencer and Schaumburg have proposed the term “central-peripheral distal axonopathy” (18) to describe this type of axon lesion. In subchronic or chronic exposure, the characteristic morphological feature of acrylamide-induced lesions is axon degeneration proceeding distally from paranodal swellings composed mostly of accumulations of neurofilaments (13, 14). They are located at the proximal side of the most distal nodes of Ranvier and the incidence is seen to gradually ascend proximally resulting in “dying back” of the nerve fibers.

Recent studies with short-term, high-dose schedules have pointed to a slightly different view of acrylamide neurotoxicity (5, 6, 21). In addition to the distal axon degeneration in peripheral nerves and high cervical ascending tracts, ganglion cell “remodelling” (20), axon terminal swelling in optic tract (5), and selective necrosis of cerebellar Purkinje cells (6) have been reported.

The purpose of this study was to characterize and compare the neurotoxicity of acrylamide in subacute or subchronic exposure. Rats intoxicated with acrylamide over a range of doses were examined after 2 or 14 days of exposure to high doses of acrylamide, and the morphological effects were compared to those of rats treated with lower doses over a subchronic schedule (90 d).

METHODS

Two experimental designs were utilized to evaluate subacute effects of high-dose acrylamide. In the first experiment, male Sprague-Dawley rats (Charles River Canada) were randomized according to body weight into 4 groups of 6 rats with mean body weight of approximately 200 g. Treatment groups received 10 intraperitoneal injections once daily, and 5 days per week. Dose levels were 50 mg/kg, 25 mg/kg, or 12.5 mg/kg acrylamide in 0.9% saline and were selected by prior range-finding study. Controls received daily i.p. injections of saline. Postmortem examination was undertaken 1 day after the tenth injection. In the second experiment, 2 groups of 8 rats were treated with 4 i.p. injections once daily of
50 mg/kg acrylamide, or saline. Four animals per group were sacrificed 3 or 14 days after the fourth injection.

In the subchronic study, 3 groups of 9 animals were used. Two groups were treated by i.p. injection daily, 5 days per week, using 12 mg/kg/day or 8 mg/kg/day in 1 ml normal saline for a total period of 13 weeks. The control group received injections of 1 ml saline on the same schedule. Thus, the cumulative high dose was approximately 50% higher than in the subacute study. In all experiments clinical observations were recorded daily. Rats were anesthetized with sodium pentobarbital and perfused through the heart with lactated Ringer's solution followed by 4% glutaraldehyde in 0.06 M phosphate buffer, pH 7.3, at a pressure of 100 cm H$_2$O. The following tissues were retained in 10% neutral-buffered formalin for subsequent paraffin or epoxy plastic embedding: brain (right hemisphere), spinal cord with dorsal root ganglia, olfactory bulbs, Gasserian ganglion, pituitary, gastrocnemius muscle, kidney, bladder, liver, and testes. Sciatic and tibial nerves were dissected carefully from the sciatic notch to the medial malleolus, stretched slightly on strips of card, and immersed in the glutaraldehyde fixative for an additional 45 min before being placed in formalin.

For paraffin embedding, the brain right hemisphere was sliced into 10 evenly spaced slabs with a calibrated adjustable device. Two slabs of spinal cord, approximately 3 mm thick, were taken from each of cervical, thoracic, and lumbar segments. Midcervical cord, 0.5–1.0 mm slices, dorsal root ganglia, midcerebellar nerve, and distal tibial nerve were post-fixed in phosphate-buffered 1% osmium tetroxide, dehydrated in ethanols, cleared in propylene oxide, and embedded in epoxy resin. Paraffin sections were cut at 7 µm and stained with either hematoxylin and eosin or a modification of Holmes silver-impregnation method combined with cresyl-fast-violet. Epoxy sections were cut at 1 µm on glass knives and stained with toluidine blue.

**Results**

**Clinical Signs and Gross Observations.** In the subacute study, hindlimb weakness and wide-splayed gait were apparent in animals after 5–6 administrations of 50 mg/kg. At the time of sacrifice, this group also had severe hindlimb weakness or paralysis and porphyrin staining around the eyes. Animals receiving 25 mg/kg doses showed only mild hindlimb splay after 9–10 administrations. There were no clinical signs in animals in the subchronic study. The only gross pathological changes noted at necropsy were distended urinary bladders in high-dose animals.

**Microscopic Observations. a) Subacute studies.** In paraffin sections stained with H & E, lesions were confined to the cerebellar cortex and consisted of extensive necrosis of Purkinje cells and vacuolation of the molecular layer and necrotic, "ischemic"-looking Purkinje cells (arrows). b) Silver impregnation of same brain. Argentophilic swellings (arrowheads) are seen in the molecular layer.

**Fig. 1.—Paraffin sections of cerebellum of rat treated with 10 injections of 50 mg/kg acrylamide. ×260. a) H&E-stained section showing vacuolation of molecular layer and necrotic, "ischemic"-looking Purkinje cells (arrows). b) Silver impregnation of same brain. Argentophilic swellings (arrowheads) are seen in the molecular layer.**
induced peripheral axonopathy with Wallerian-like degeneration characterized by myelin ovoids and lipid-laden macrophages, occasional paranodal axon swelling with myelin retraction and Schwann cell hypertrophy and hyperplasia (Fig. 4). Some swollen paranodal axon profiles were filled with dark-staining granular material, and usually had a channel of light-staining axoplasm passing through the swelling and node of Ranvier (Fig. 4). The paranodal myelin was often markedly thinned. Occasionally, dark-staining demyelinated axon profiles of large diameter were seen with no visible light-staining core. Some axons were reduced in caliber within an enlarged, empty myelin sheath. Occasional profiles resembling the false intussusceptions described by Jones and Cavanagh (9) were seen (Fig. 4). Schwann cells were hypertrophied, and there were occasional mitoses. Swellings similar to those seen in peripheral nerves were seen in cervical spinal cord, primarily in the outermost portions of the anterior or lateral columns (Fig. 5).

Rats receiving 10 injections of 12.5 mg/kg or 4 injections of 50 mg/kg showed relatively few axon changes, and these included myelin tomacula (redundant folds of myelin) and Schwann cell hypertrophy. Spinal ganglion cells showed cytoplasmic remodelling which resembled central chromatolysis, with an eccentric nucleus, location of Nissl substance around the periphery of the soma, and accumulation of granular cytoplasmic features in the center of the cell. This reaction was seen frequently after 10 injections of 50 mg/kg, less frequently after 10 injections of 25 mg/kg and was not noted after the 10 injections of 12.5 mg/kg or 4 injections of 50 mg/kg. The distribution of these lesions is presented in Table I. There were no lesions noted in other organs sampled.

b) Subchronic studies. There were no lesions observed in H&E-stained paraffin sections. Purkinje cell density in cerebellum appeared normal. In high-dose (12 mg/kg/day) animals, a few argentophilic swellings were noted in silver-impregnated sections of ascending tracts of the spinal cord and nucleus gracilis. Axon terminals at neuromuscular junctions were intensely argentophilic and showed nodular swellings (Fig. 6a). Axon terminals around lower motor neurons of the lumbar ventral horn were also

Fig. 2.—Silver-stained paraffin sections of CNS areas affected by 10 injections of 50 mg/kg acrylamide. a) Nucleus gracilis shows numerous large argentophilic swellings. Some of these strongly resemble retraction bulbs (arrowheads). ×260. b) Argentophilic swellings in the optic layer of the superior colliculus (arrowheads). ×400. c) Intensely argentophilic axon terminals adjacent to lumbar ventral horn motor neurons (arrowheads). ×400.
intensely argentophilic and enlarged. No lesions were noted in paraffin sections of the low-dose (8 mg/kg) group.

In semi-thin epoxy sections of tibial and sciatic nerve from high-dose (12 mg/kg) animals, occasional axons were seen undergoing Wallerian-like degeneration (Fig. 6b). Paranodal swellings of neurofilamentous or dark-staining granular type were not seen. A few degenerating axons were seen in ascending tracts of cervical spinal cord and nucleus gracilis (Fig. 6c). No alterations were noted in spinal ganglion cells. There were no lesions noted in other organs sampled.

DISCUSSION

In early studies on the neurotoxicity of acrylamide, the first clinical findings were staggering and uncertain gait, suggesting cerebellar ataxia, or other sensory deficits (10, 14). No morphologic changes were detected to explain the ataxia and the ensuing paraparesis or paralysis (11). Based on work with seizure-inducing doses of acrylamide, these effects were believed to be physiological at the level of the midbrain. In subsequent studies using osmium fixation of nerves, Fullerton and Barnes (7) demonstrated that acrylamide induced a severe distal degeneration of peripheral nerve fibers, and this was associated with the weakness, gait anomalies, and other sensorimotor disturbances. Further studies on long-term exposure followed, and a picture of a distal to proximal dying back axonopathy emerged (3, 4, 13). Work by Prineas (13) and Schaumburg et al (14) indicated that axon degeneration began with abnormal accumulation of organelles and neurofilaments at the proximal side of distal nodes of Ranvier and was followed by breakdown of axon and myelin distal to this paranodal swelling. This pattern was reported both in peripheral nerves and in long central tracts including the termination of spinal dorsal column axons in the medulla and spinocerebellar tracts in cerebellar vermis. Despite these reports of effects in CNS, attention has remained primarily focussed on the peripheral nervous system, and some toxicological publications still refer to acrylamide intoxication as causing only a peripheral neuropathy.

More recent studies with higher dose levels administered over short intervals have suggested some revision of the original view of acrylamide neurotoxicity. Sterman (21) and Sterman and Sheppard (22) in studies focussed on the peripheral nervous system have suggested that behavioral deficits precede peripheral axon lesions and that one of the earliest morphological responses is a cytoplasmic remodelling of spinal ganglion cells. A detailed study of lesions developing in the central and peripheral nervous tissue of subacute acrylamide intoxication

FIG. 4.—Epoxy sections of tibial nerve from rats treated with 10 injections of 50 mg/kg acrylamide. a) Typical view showing extensive degeneration of axons with macrophages laden with myelin debris. No paranodal swellings are seen. ×260. b) Paranodal swelling (arrowhead) consisting of dark-staining granular material at the periphery of the axon and a light channel extending through the node. The light channel has an appearance similar to normal axoplasm. Also seen (arrow) is a profile resembling the false intussusceptions described in hexacarbon intoxication. ×400. c) Cross-sectional view of similar swellings (asterisks). Also seen are folded myelin sheaths, attenuated axons in distended myelin (arrow), and proliferating Schwann cells (arrowheads). ×400.
in rats by Cavanagh (5) has indicated that among the earliest changes were Purkinje cell necrosis and argentophilic swellings in central fiber tracts including the spino-cerebellar tract, fasciculi gracilis and cuneatus, the optic tract and in axon terminals on lumbar and sacral ventral horn cells. In contrast to the peripheral nerve swellings, these central swellings appeared to be ultraterminal. That these CNS swellings could be associated with a functional deficit was demonstrated by Merigan et al (12), who found visual system deficiency by electrophysiological methods in acrylamide-treated monkeys.

The results of our subacute study extend those of Cavanagh (5). The earliest effect seen, 1 week after 4 successive doses of 50 mg/kg, was necrosis of cerebellar Purkinje cells, but no axon swelling or degeneration was seen at this time in either central or peripheral nervous system. Argentophilic swellings in the nucleus gracilis two weeks after 4 injections of 50 mg/kg was the feature most notable in this group. In animals receiving 10 injections of 25 mg/kg, argentophilic swellings were apparent in nucleus gracilis, spino-cerebellar tract and cerebellar vermis, and in the optic nerve layer of the superior colliculus. Wallerian degeneration and paranodal swelling of peripheral fibers was seen in this group but was not as severe as in the highest dose group where extensive degeneration of peripheral axons and some paranodal swelling was seen. The number of fibers degenerating in both distal (tibial) and more proximal (sciatic) nerves appeared to be considerably in excess of the number of swollen profiles.

The results in our subchronic study are consistent with the earlier reports of Princeas (13) and Schaumburg et al (14), in which peripheral axon degeneration was a predominant finding, and central lesions were less common. Our results differ, however, in that paranodal swellings were not seen in peripheral axons.

This comparison of subacute and subchronic neurotoxicity of acrylamide has indicated that the central nervous system is more affected than peripheral nerves in cases of high-dose exposure over a short time. Significantly higher cumulative doses administered over a subchronic schedule in contrast produced very few central nervous system lesions, while peripheral nerves were still significantly affected, al-
though to a lesser extent than in subacute studies. Since regeneration of central nervous system axons or neurons does not proceed readily under normal circumstances, early damage to CNS is likely to be irreversible and thus of greater clinical significance than damage to peripheral axons which can regenerate. If the human CNS is less sensitive to adverse effects of acrylamide in low-level exposure, as is that of rats, risk of CNS damage from chronic low-level exposure to acrylamide in the environment would be less than that predicted in short-term studies.

REFERENCES