Review

Acrylamide Encephalopathy

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Abstract: Acrylamide Encephalopathy: Hideki Igisu, et al. Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health—Although the toxic effects of acrylamide on the peripheral nervous system have been well established, less attention has been paid to its effects on the brain. Nevertheless, (1) ultrahigh field magnetic resonance imaging revealed enlarged cerebral ventricles and cisterns in rats treated with acrylamide 50 mg/kg/d for 8 d, (2) creatine kinase (CK) activities were suppressed in the brain from such rats and mice, (3) CK activities in the brains of mice intoxicated with acrylamide changed in parallel with neurological dysfunction, (4) in human cases of acrylamide intoxication due to occupational exposure, symptoms suggesting brain involvement were seen in a few, and (5) cases of acrylamide intoxication due to contaminated well water showed unquestionable signs of brain dysfunction including mental confusion with hallucinations. These indicate that acrylamide can cause encephalopathy in animals as well as in humans, and that the spectrum of impairment of the nervous system can vary greatly depending on the mode of exposure.

Key words: Acrylamide, Neurotoxicity, Brain, Magnetic resonance, Creatine kinase

Acrylamide (CH$_2$=CHCONH$_2$) and its derivatives have been used widely, including in water and sewage treatment, paper and pulp manufacture, grouting, and in mining and mineral processing. In biotechnology, acrylamide is indispensable for electrophoretic separation of proteins and nucleic acids. Polymerized acrylamide is not toxic, but monomeric form is neurotoxic and can penetrate into the body through the lungs, skin and gastrointestinal tract. Its neurotoxicity was recognized soon after commercial production was started in 1954 and acrylamide has been classified as a specified chemical (2nd class) in Japan. Nevertheless, the precise mechanism involved in the neurotoxicity has not been clarified.

Since Fullerton and Barnes demonstrated that acrylamide morphologically damages peripheral nerves in rats, it has become one of the most widely used chemicals to produce experimental neuropathies in animals. Indeed, acrylamide is now considered to be a reliable and convenient means of producing distal axonopathies in experimental animals. Moreover, these are compatible with clinical pictures of most human cases of acrylamide intoxication observed in workers handling acrylamide.

In contrast, less attention has been paid to changes caused by acrylamide in the brain, and acrylamide is sometimes regarded as a mere peripheral nerve toxin. Here we present data, mainly ours, showing that acrylamide can cause encephalopathy in animals and in humans.

Animal Study

Morphology

The earliest studies on the toxicity of acrylamide focused on its effects on the brain because cats intoxicated with acrylamide developed seizures at the highest doses and ataxia at slightly lower doses but no morphological abnormalities were found in the brain.

Rats treated with acrylamide 50 mg/kg i.p. per day for 8 d show ataxia and weakness in the hindlimbs (Fig. 1). Ultrahigh-field magnetic resonance (MR) imaging in such rats disclosed enlarged ventricles and cisterns in the brain (Fig. 2). Quantifying structures, the size of the cerebral cortices, especially that corresponding to the primary motor area, decreased while that of the caudate-putamen did not (Fig. 3).

With stereological methods, Tandrup and Braendgaard have shown that acrylamide (500 mg/kg in total) causes a decrease in the volume of the neocortex in rats. Nevertheless, since their methods were based on conventional histological examination, shrinkage of the samples was unavoidable during dehydration and staining and had to be corrected by calculation. In this regard, examination with MR is straightforward because the brain can be examined in situ in living rats. Therefore, although no localized intracerebral lesions were seen even with...
Fig. 1. Rat treated with acrylamide 50 mg/kg/d for 8 d (B) and control (A). Note abnormal posture of the hindlimbs in B.

Fig. 2. T2-weighted images of the rat brain recorded with a 4.7 T magnetic resonance spectrometer. Images shown in the upper row are from a body weight-matched control and those in the lower row from a rat treated with acrylamide 50 mg/kg/d for 8 d. Although 11 slices were obtained from each rat, only slices 3 through 10 are shown (from Archives of Toxicology 2000; 74: 487–489, with permission of Springer-Verlag).

4.7 T MR, it did show that acrylamide alters the brain size, most notably in the regions corresponding to the primary motor area.

Biochemistry

With the same animal model of acrylamide intoxication (50 mg/kg i.p. per d for 8 d), it has been found that creatine kinase (CK) activities are suppressed in the rat brain (Fig. 4)\(^9,10\). CK catalyzes the reaction: Phosphocreatine + ADP → ATP + creatine. The concentration of phosphocreatine in the brain is higher than that of ATP and exquisitely sensitive to changes in oxygenation, providing \(-\text{P}\) for ADP phosphorylation\(^{(1)}\). In addition, the phosphocreatine/CK system seems to connect intracellular sites of ATP production (mitochondria) with sites of ATP consumption whereas intracellular distribution of mitochondria may be largely heterogeneous as typically seen in neural axons\(^{(2)}\). Therefore, CK appears to be a key enzyme not only in temporal replenishment of ATP but also in its spatial distribution in tissues, especially in the nervous system for which a constant supply of energy is an absolute requisite for normal functioning. Furthermore, in experiments comparing the clinical course and changes in enzymatic activities in the brain in mice, CK activity was suppressed by acrylamide in parallel with the neurological dysfunction measured by landing foot-spread (Fig. 5a, b)\(^{(3)}\). No clear changes were found in other enzyme activities including those that had been reported to be suppressed by acrylamide, i.e., glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and neuron-specific enolase (NSE) over the experiment period (eight days for exposure and 43 d for recovery) (Fig. 5b)\(^{(3)}\).

These findings suggest that the inhibition of CK activities may be closely related to the genesis of the neurotoxicity in the above animal models of acrylamide intoxication. Should it not be related, it appears that CK...
Fig. 3. Size of the cerebral cortex and caudate-putamen determined in magnetic resonance images obtained as shown in Fig. 2. The values (mean ± SD, n=6) (%) are the distances across each structure relative to those across bilateral cerebral hemispheres. A, B, C and D correspond to the primary motor area, primary sensory area, secondary sensory area, and caudate-putamen, respectively. *p<0.05, **p<0.01 vs control (based on the data in Table 1 in Archives of Toxicology 2000; 74: 487–489).

Fig. 4. Creatine kinase (CK) activities in the Cc (cerebral cortex), St (striatum), Hyp (hypothalamus), Hip (hippocampus), Md (midbrain), Mo (medulla oblongata), Ch (cerebellar hemisphere), and Cv (cerebellar vermis) from rats treated with isotonic saline (control), acrylic acid and acrylamide. Acrylamide was dissolved in isotonic saline. Acrylic acid was diluted with isotonic saline and neutralized with 5 N NaOH. The chemicals were given i.p. 50 mg/kg/day, and isotonic saline 3.3 ml/kg/d, for 8 d. CK activities were suppressed by acrylamide but not by acrylic acid which is not neurotoxic (based on the data in Table 2 in Archives of Toxicology 1994; 68: 67–70).

Fig. 5. a: Landing foot-spread (LFS, cm) in mice intoxicated with acrylamide. Mice were injected with acrylamide from d 0 to 7 (total dose; 400 mg/kg), and then allowed to recover from d 8 to d 50. The number of mice examined by LFS was eight except for d 50 when five mice were examined (it should be noted that some symbols are completely overlapped). Regression lines were drawn with a linear regression analysis, separating the experimental period into d 0 to 8 (y=0.18x + 2.67, r=0.79, p<0.0001) and d 8 to 50 (y= -0.04x + 3.87, r= -0.77, p<0.0001) (from Occup Environ Med 1996; 53: 468–471). b: Creatine kinase (CK), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), enolase and neuron-specific enolase (NSE) activities (mean ± SD) in the soluble fractions from brains of mice intoxicated with acrylamide as indicated in Fig. 5a; n=6 (d 0, 5, 8, 15, and 23), 5 (d 11), and 4 (d 36 and 50), *p<0.001 vs control (d 0) (based on data reported in Occup Environ Med 1996; 53: 468-471).

Activity in the brain can be a sensitive indicator of intoxication with acrylamide.

On the other hand, no definite inhibition of CK activities was found in the sciatic nerve from rats treated with acrylamide 50 mg/kg/d for 8 d; 1.21 ± 0.37 and 1.42 ± 0.25 µmol/min/mg protein (mean ± SD, n=6) in the treated and control group, respectively. Lehning et al.6 found...
changes neither in rubidium transport nor in histology in the tibial nerve from rats given acrylamide 50 mg/kg/d for 11 d and showing “classic signs of neurotoxicity”.

Therefore, rats (and possibly mice) treated with acrylamide 50 mg/kg i.p. per d for 8 d may represent, not only structurally but also biochemically, an animal model of acrylamide encephalopathy rather than peripheral neuropathy.

Exposure to lower doses (10 to 30 mg/kg) per d over 10 to 14 d caused changes in neurotransmitter receptors14, 15), and immunoreactivities of microtubule-associated proteins16) in the rat brain. It remains to be determined whether or not these changes are related to the effects of acrylamide on CK activities.

**Human Study**

To our knowledge, no pathological or MR (even CT) examinations of the brain have been carried out in humans with acrylamide intoxication. This may be partly because the prognosis of acrylamide intoxication is not poor17), and partly because severe intoxication has not occurred recently in industrialized countries. It is therefore important to analyze clinical pictures of human cases of acrylamide intoxication based on the reported records.

### Occupational exposure

Almost all human cases of acrylamide intoxication were due to occupational exposure17). Among 42 such cases, 25 subjects were engaged in the preparation of the polymerized form such as flocculator from acrylamide monomer, 10 in the synthesis of acrylamide from acrylonitrile, and 7 in grouting operation. Exposure varied in duration from 4 wk to 8 yr.

Since a detailed history was not available in all cases, the frequency of symptoms (Fig. 6a) and signs (Fig. 6b) may be underestimated17), but skin changes such as rash or peeling, especially on the hands were noted in more than 20 cases, and excessive sweating in more than 10. Weakness in hands or feet was noted in 7 cases, and unsteadiness in 12. The most common symptom was disturbance of sensations such as numbness or “ant-crawling” sensation.

On physical examination (Fig. 6b), muscle atrophy and weakness were observed in distal parts of the extremities, in 7 and 13 cases, respectively. The most common sign was a decrease in sensation in the extremities. Romberg sign was positive in 8 cases. No pathological reflexes were described in any case. Deep tendon reflexes were hypoactive or absent in many cases. Diminished deep tendon reflexes were more frequent in the lower limbs than in the upper.

These indicate that the major clinical pictures in occupational acrylamide intoxication, which is usually caused by repeated exposures to relatively low doses of acrylamide over a long period, are those of sensory dominant polyneuropathy. These are consistent with the well-established pathological findings in animal experiments. These also seem to be compatible with findings by Lapin et al.18) who observed decreased CK activity in distal sciatic nerves from rats given acrylamide at a relatively low dose per d (25 mg/kg/d, 3 d per wk to 550 mg total dose).

However, some had symptoms and signs that cannot be explained solely by peripheral nerve impairment, such as difficulty in going to sleep or sleepiness (4 cases) and hyperactive deep tendon reflexes especially in lower limbs were often found on physical examination (from J UOEH 1988; 10 suppl: 219–227).
In this regard, cases described below show unquestionable signs of brain involvement.

**Intoxication with contaminated well water**

The accident occurred in 1974 in a town located to the north-east of Fukuoka City and all 5 members of one family were affected. On February 18, chemical grouting with acrylamide was carried out for sewerage construction in a road facing the patients’ home. The family used both well water and tap water but they used well water only from March 12 to 18 while the tap water was not supplied. The well water sampled on March 23 was found to contain 400 ppm acrylamide monomer. The illness was most severe in the housewife who stayed at home and was apparently most exposed to the well water, as follows.

A 40-yr-old housewife noted rhinorrhea and dizziness on March 15. Three d later, she became unsteady on her legs. On March 20, she began to show irrational behavior and was unable to walk and urinate. On admission (March 22), she was confused and hallucinated (visual, auditory, and tactile). Orientation and memory were poor, and a tendency to confabulate was noted. The cranial nerves were intact except for mild slurred speech. Neither apparent weakness nor ataxia was found in the limbs. Deep tendon reflexes were mildly hyperactive without any pathological reflexes. Truncal ataxia was so severe that she could not stand alone. A catheter was needed for urination, and an enema for defecation.

The complete blood count, serum electrolytes, total cholesterol, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase were normal. Blood urea nitrogen was slightly high (32 mg/dl). The cerebrospinal fluid cell count, glucose and protein were normal. The electroencephalogram on the fifth hospital day suggested excessive sleepiness but no abnormalities were found. The motor nerve conduction velocity of the ulnar nerve and the tibial nerve were normal. The sural nerve sensory conduction velocity on the sixth hospital day was 37.1 m/s (normal: more than 38 m/s).

Thiamine (500 mg) was injected daily for 20 d. The mental symptoms, dysarthria, truncal ataxia, and dysuria disappeared within one month but mild difficulty in defecation persisted. Two wk after admission, she began to complain of numbness in the distal parts of the extremities. Decreased touch, pain, and vibration sense was found in the extremities, more prominent distally. The ankle jerks were lost. The sensory conduction velocity of the sural nerve on the 20th hospital day was apparently lowered (35.9 m/s), though the ulnar and tibial nerve motor conduction velocities were normal. Four months later, neurological examination revealed no abnormalities except for mild dysesthesia in the feet and slight difficulty in defecation. The sural sensory conduction velocity had returned to normal.

In this case, marked central nervous system dysfunction dominated the clinical picture and signs of polyneuropathy appeared later.

Among the manifestations in other family members (Fig. 7), the upper respiratory tract infection-like symptoms in 3 members might be due to irritation of the mucous membrane by acrylamide. Although the degree varied greatly, mental symptoms were noted in all members. And in 4 members truncal ataxia was observed, suggesting the involvement of cerebellar vermis. After these symptoms and signs subsided, sensory impairment in distal parts of the extremities, which is consistent with sensory dominant polyneuropathy, appeared in 3 members.

**Conclusions and Implications for Further Study**

It is now clear that acrylamide can affect not only the peripheral but also the central nervous system in animals and humans. These manifestations can vary greatly depending on the mode of exposure (doses and duration). This suggests that subjects suffering from acrylamide intoxication especially from acute intoxication should be examined for possible involvement of the brain and that MR imaging may be indicated in such cases because MR imaging revealed structural changes in the brain in rats intoxicated with acrylamide. With those examinations, clues may be obtained to clarify lesions responsible for acrylamide encephalopathy. Furthermore, although it requires very advanced apparatus, determination of CK activities with MR may be considered because CK activities in the human brain can be measured with MR, utilizing magnetization transfer.
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References

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