Purkinje Cell Injury Caused by Acrylamide

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Abstract

We encountered an unusual case of fatal acrylamide intoxication. A 23-year-old woman obtained the acrylamide (ACR) solution from the laboratory and consumed it in her room. ACR was detected in heart blood, urine and gastric contents for toxicological examinations. High-dose ACR intoxication cause selective Purkinje cell injury in cerebellum. Most Purkinje cells express the fast and slow forms of the calcium buffer protein calbindin. Some serial sections of samples were immunohistochemically stained with calbindin (CB-955). Populations of intense calbindin-immunoreactive Purkinje cells showed no positive reactions. We describe the histopathological findings of high dose ACR-induced Purkinje cell damage in the cerebellum.

Keywords: Acrylamide intoxication; Purkinje cell injury; Histological findings; Calbindin

Introduction

Acrylamide (ACR) appears to be a contaminant generated during the preparation of certain foods. Although the polymer is nonneurotoxic, exposure of human and laboratory animals to monomeric ACR produces ataxia, skeletal muscle weakness, and weight loss [1]. Early morphological studies indicated that this neurotoxic syndrome was associated with nerve damage characterized by multifocal paranodal swelling of preterminal distal myelinated axons. These swellings contained an abundance of tubulovesicular profiles, neurofilaments, and degenerating mitochondria and, as ACR intoxication progressed, axonal regions below these swellings degenerated [1]. However axonopathy developed only during low-dose / long term exposure conditions [2]. Axon degeneration did not play an important role in the expression of ACR neurotoxicity [3]. Axon degeneration might be abundant in the central nervous system (CNS) of rats exposed at this higher ACR dose rate. High-dose ACR intoxication produced primarily CNS nerve damage [4]. In the cerebellum, the spatiotemporal pattern of argyrophilia in the cortex and white matter was consistent with selective ACR-induced Purkinje cell injury [5]. In rats intoxicated at the higher ACR dose-rate, argyrophilic neurons were evident in the Purkinje layer but not in the molecular or granule layers. The spatial distribution of axon and nerve terminal degeneration in the cerebellum of rats intoxicated at the higher dose rate was indicative of selective Purkinje cell death. Degenerating axons could be traced from argyrophilic Purkinje neurons in all cerebellar cortical regions through the white matter to region-specific terminations in deep cerebellar and vestibular nuclei [5]. ACR caused selective Purkinje cell injury in cerebellum and axon degeneration is a component of the general injury response of Purkinje neurons.

Case Report

A 23-year-old woman obtained the 29% acrylamide (ACR) solution from the laboratory and consumed it in her room. The ingested dose was estimated to be exceeding a fatal level of ACR. She was drowsy on arrival to the emergency department and said that she had swallowed the ACR solution for the purpose of suicide. She was admitted to the intensive care unit (ICU) but despite invasive ventilation support, aggressive fluid resuscitation and administration of activated charcoal,

her condition deteriorated and death occurred 18 h after swallowing the ACR.

Autopsy Findings

The autopsy was performed on the day following death. The body was 154 cm in height and 56 kg in weight. The brain weighed 1270g and brain edema was noted. Heart blood, urine and gastric contents were collected for toxicological examinations and ACR was detected in these samples. Acrylamide was analyzed according to the method described by Ono et al. in which the analyte was detected as a dibromo derivative by GC-MS with following modifications [6]. 2 ml of analytical samples from blood, urine or fluid stomach content were added to 40ml of distilled water. After centrifuging 2 ml of supernatant of each sample was washed with hexane, and adjusted under pH 1.0 by 5mMol of sulfuric acid, and 10 g of potassium bromide was added absolutely to dissolve the samples. 6 ml of 0.1mol brominating regent were added and the samples were gently mixed, and then allowed to react in a refrigerator for 90 min. Several drops of 1N sodium thiosulfate were added to each sample to decompose any remaining bromine. Each sample was extracted by 10ml of ethyl acetate, and then centrifuged for 10 min. After dehydration by sodium sulfate, ethyl acetate was removed in an evaporator. The residual samples were resolved with 2ml of hexane containing 10% of acetone and sample solution was filtered in florisil column with hexane. Eluted material was concentrated in a centrifugal concentrator, and after drying samples and adding 0.5ml of acetone to each sample, 20μl of tri-ethyl amine was added to make an analysis solution. The qualitative analysis was performed by GC 2010 plus (Shimazu, Kyoto, Japan) with selected ion monitoring. The quantitative analysis was performed by GC-2010 plus–FTD.

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Amount of detected acrylamide was 0.110μg/ml in the blood, 0.116μg/ml in urine and 0.096μg/ml in stomach fluid, respectively. Slices from areas of the cerebellum were formalin fixed, paraffin embedded, and sectioned at 5μm. One slice from each location was stained with hematoxylin and eosin for histological observation (Figure 1). Immunohistochemistry was carried out using the Strept-Avidin-Biotin-Peroxidase complex (SABC) method (ABC histofine, SAB-PO kit, Nichirei, Japan) Some serial sections of samples were immunohistochemically stained with calbindin (CB-955) (Clone: sc-58699, Santa Cruz Biotechnology, U.S.A) at a dilution of 1:400. Populations of intense calbindin immunoreactive Purkinje cells showed no positive reactions (Figure 2a).

Discussion

The short-term effects of harmful food components can cause structural damage to various systems and organs from the direct toxic tissue effects the cause functional failure [7]. Genotoxic and non-genotoxic pathways have been suggested for the carcinogenic effect of acrylamide. Some studies with short-term, high-dose schedules have indicated a slightly different view of acrylamide (ACR) neurotoxicity [8,9]. The spatial distribution of axon and nerve terminal degeneration in the cerebellum of rats intoxicated at a higher dose-rate was indicative of selective Purkinje cell death. Degenerating axons could be traced from
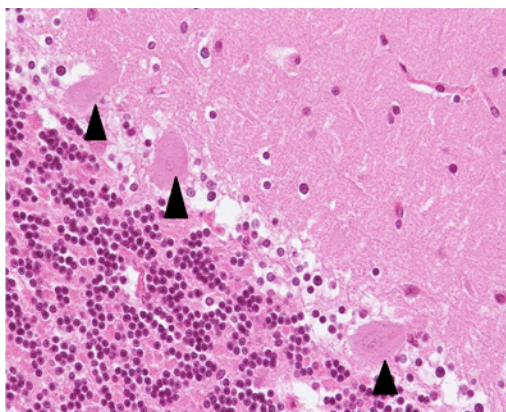

Figure 1: Cerebellar cortex of showing lower Purkinje nuclei and cell density (arrowhead). (Hematoxylin Eosin stain ×200).
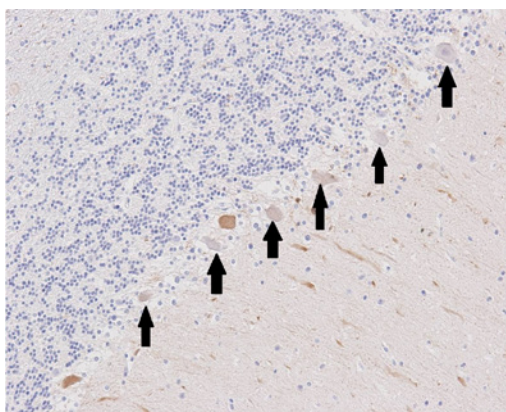

Figure 2a: Some Purkinje cells showing negative reaction (arrow). ACR-intoxicated present case (Calbindin antibody stain×200).
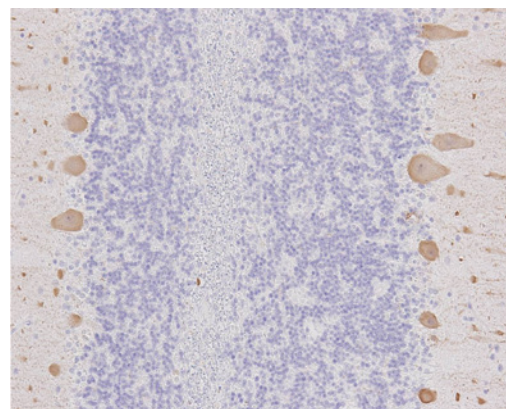

Figure 2b: Purkinje cells showing positive reaction. Contrast (Calbindin antibody stain×200).

argyrophilic Purkinje neurons in all cerebellar cortical regions through the white matter to region-specific terminations in deep cerebellar and vestibular nuclei [10]. Purkinje cells are large, GABAergic neurons, which serve as the sole output of the cerebellar cortex. Purkinje cells are typically located in a single row at the border of the granular and the molecular layers. Their myelinated axons terminate on neurons of the cerebellar nuclei trees, are flattened and oriented perpendicular to the parallel fiber. Spatiotemporal analysis of argyrophilic changes in the cerebellum suggested that Purkinje cell dendrites and nerve terminals degenerate as an early consequence of high dose-rate intoxication and that somal and axonal argyrophilic changes developed subsequently [10]. ACR produced selective degeneration of Purkinje cell dendrites, axons, and nerve terminals, which is consistent with toxic neuron damage. Gas chromatographic tandem mass spectrometry has been proven to be a sensitive method for the detection of ACR [11]. In the case described here, ACR was detected in heart blood, urine and gastric contents. 50% lethal dose in mice is reported 170mg/kg [12]. There are no relevant data in human, but 50% lethal dose in 29% solution is 29.3ml for 50kg body weight on an exchange table. In present case, we don't know how much the deceased drank, but thought that she drank the ACR solution exceed in lethal dose from view of the histological findings. In paraffin sections stained with hematoxylin and eosin, the population of Purkinje cells was low and Purkinje nuclei and cell density were lowered. Purkinje cells showed extensive necrosis (Figure 1).

Nerve terminal swelling is often the first observed morphological effect of ACR and nerve terminals are the primary sites of ACR action. Axon degeneration is associated with subchronic ACR, but acute intoxication is not associated with axonopathy [4]. Research by Cavanagh and associates using conventional fixation techniques or Glees and Marsland's silver stain showed that ACR intoxication of rats (30 mg/kg body weight/day ×5days/week ×3weeks, ip) produced a progressive and selective loss of cerebellar Purkinje cells. The earliest (after 5 injections) noted morphological change was swelling of Purkinje neuron dendrites. Somatodendritic degeneration began after 7 injections, whereas axonal argyrophilia was observed after 14 injections [7]. ACR causes primary Purkinje cell injury and degeneration of corresponding axons is a secondary phenomenon [10]. The molecular mechanism of Purkinje neuron damage during ACR

intoxication is not known. ACR might cause Purkinje cell injury by an indirect mechanism.

Neuronal calcium may play a role in neuronal survival as well as in apoptosis, pathological neuronal, degeneration and necrosis [13]. Sustained increase in intracellular calcium levels, such as those observed after excitatory amino acid treatment or attributed to reduced transport activity, have been shown to correlate with increased protein and lipid degradation, which progresses to cell death [14]. In the cerebellum, where rapidly firing neurons experience high levels of calcium influx, controlled calcium regulation is essential to avoid rapid neuronal death owing to excessive excitatory stimulation [14]. Intracellular calcium is mainly regulated by calcium binding proteins (CaBPs). Interaction of calcium with these proteins represents one of the mechanisms by which a second messenger controls many biological processes [14]. ACR-induced Purkinje cell damage might involve a direct effect on intracellular Ca^{2+} regulation or membrane targeting processes. Relative to other neurons, maintenance of Ca^{2+} homeostasis in Purkinje neurons is critically important to their survival and function [15]. Purkinje cells contain copious amounts of smooth endoplasmic reticulum (SER) and relatively large concentrations of cytoplasmic Ca^{2+} binding proteins such as calbindin [16].

Calbindin is a CaBP highly expressed in Purkinje cells. Calbindin is a marker of Purkinje cells in normal and degenerative cerebellar tissue [17,18]. In Purkinje cells, calbindin expression is neuroprotective against degeneration in a variety of acute and chronic disorders [14]. All normal Purkinje cells are calbindin immunopositive [19], but histological studies confirmed the loss of Purkinje cells in natural and experimentally intoxicated animals [20]. Immunohistochemistry of the slices was performed using a monoclonal primary antibody, anti-calbindin. The present case confirmed a loss of Purkinje cells in ACR intoxication (Figure 2b). Calbindin plays a protective role against calcium-mediated excitatory amino acid neurotoxicity by reducing the levels of intracellular free calcium [18]. Disruption of the calcium-buffering system may result in calbindin depletion and loss of immunoreactivity. Calbindin may be associated with differentiation and dendritic synapse formation and stabilization. Synaptogenesis may result in a more complex calcium regulation, perhaps requiring increased neuronal level of calbindin.

Conclusion

High-dose ACR intoxication cause selective Purkinje cell injury in cerebellum. We describe the histopathological findings of high dose ACR-induced Purkinje cell damage in the cerebellum.

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