Acute Lead Encephalopathy in the Guinea Pig*

Thomas W. Bouldin and Martin R. Krigman

Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, U.S.A.

Received June 10, 1975; Accepted August 20, 1975

Summary. Acute lead encephalopathy was induced in adult guinea pigs with daily oral doses of lead carbonate. Cerebral capillaries were examined by electron microscopy, and the blood-brain barrier (B-BB) evaluated with Evans blue and horseradish peroxidase. Brain lead levels were also determined during the developing encephalopathy. There was no cerebral capillary alteration or demonstrable B-BB dysfunction. Brain lead concentrations increased over the 5-day period.

The encephalopathy in the absence of any vascular alteration suggests that lead can produce a primary toxic effect at the neuronal level.

Key words: Guinea pig — Lead — Encephalopathy — Blood-brain barrier.

Introduction

The neurologic effects of acute lead toxicity are well known, yet the mechanisms by which lead enters the nervous system and produces a characteristic encephalopathy remain obscure. To elucidate these mechanisms, several investigators have studied the suckling rat model of acute lead encephalopathy and have noted capillary alterations, cerebellar hemorrhages, and brain edema. Such observations have prompted Pentschew, Lampert, Thomas, Clasen, Goldstein et al. to conclude that acute lead encephalopathy is a disease in which a dysfunction of the blood-brain barrier (B-BB) plays a primary pathogenic role.

Since it has been proposed that one of the primary lesions in acute lead encephalopathy is at the small blood vessel level, we evaluated the structure of the cerebral microvasculature and the integrity of the B-BB of adult guinea pigs during the development of acute lead encephalopathy by means of light and electron microscopy. In the guinea pig model of acute lead encephalopathy (Powow; Weller), seizures and death are regularly produced after 5 daily oral doses of lead. Despite the severity of the encephalopathy, these intoxicated animals show few morphologic alterations in the nervous system except for congestion and hemorrhages in the choroid plexus. The acute onset of seizures and the paucity of morphologic findings in this model resemble more closely the disease observed in man than the acute encephalomyelopathy produced in suckling rats.

Materials and Methods

Adult, male, random-bred guinea pigs, weighing 475—800 g, were maintained in individual plastic cages at 70° F on tapwater and guinea pig chow, ad libitum. The experimental animals

* This study was supported in part by USPHS Grants ES01104-01 and GM-92.
received a single daily oral dose of 155 mg of lead carbonate in a gelatin capsule and were sacrificed 24 hrs after 2 (3 animals), 3 (4 animals), 5 (6 animals), or 6 (2 animals) consecutive daily doses. The animals were anesthetized with intramuscular pentobarbital, 3.6 mg/100 g body weight. 10--15 min after an intravascular injection of Sigma type II horseradish peroxidase (HRP), 25 mg/100 g body weight, dissolved in 3 ml physiologic saline, the animals were sacrificed by either decapitation or vascular perfusion. Two additional animals were injected intraperitoneally with 40 mg of Evans blue (5 mg/ml saline) after 5 daily oral doses of lead carbonate; 24 hrs later, the animals were anesthetized and received intravenously administered horseradish peroxidase 15 min prior to vascular perfusion. The distribution of the Evans blue was studied by fluorescence microscopy in frozen sections. For perfusion, the animals were intubated and respiration maintained with room air, the ascending aorta was cannulated via the heart, and then the brain was perfused with Karnovsky’s diadehyde fixative (Karnovsky), diluted (1:3) with 0.1 M sodium cacodylate buffer (pH 7.4), for 15 min at a pressure of 100 cm of fixative. The perfused brains were removed, coronally sectioned through the neostriatum, mamillary bodies, and midpons and cerebellum, and fixed for an additional 2--4 hrs in full-strength Karnovsky’s fixative. Brains from decapitated animals were rapidly removed, coronally sectioned, and fixed by immersion in full-strength Karnovsky’s fixative for 2--4 hrs. All of the fixatives were maintained at ambient temperatures. Perfused and immersion-fixed tissues were washed overnight in 0.1 M sodium cacodylate buffer at 4°C. For electron microscopy, 70 μm slices of cerebral cortex and cerebellum were cut with a Smith-Farquhar tissue chopper (Sorvall TC-2); and for light microscopy, 20 μm coronal sections of brain were cut on a freezing microtome. Peroxidase activity was identified in the tissue slices and frozen sections using the method of Graham and Karnovsky. Following incubation, the 70 μm slices were postfixed in 1% cacodylate-buffered osmium tetroxide for 1 hr, stained en bloc with 1% uranyl acetate in 0.1 M maleate, pH 5.4, for 1 hr, dehydrated initially with graded ethanol and finally with propylene oxide, and embedded in Epon 812. Survey “thick sections” were cut, stained with toluidine blue, and areas of the block were selected for ultrathin sectioning. “Thin sections” of a silver interference color were cut and examined either unstained or stained with uranyl acetate and lead citrate in a JEM-T7 electron microscope. Tissue slices were also prepared as above for electron microscopy but without incubation in the Graham-Karnovsky media. Selected coronal sections were also processed for light microscopy by embedding in paraffin, sectioned at 6--8 μm, and stained with hematoxylin and eosin or Luxol fast blue-periodic acid-Schiff stains.

Control guinea pigs were not given lead carbonate, but did receive intravenous horseradish peroxidase 15 min prior to vascular brain perfusion. Tissue sections and slices were processed for light and electron microscopy as described above.

Tissue lead was determined in a separate study. Four guinea pigs were sacrificed by cervical dislocation 24 hrs after the 5th dose of lead. Four age-matched, nontreated animals were used as controls. Lead levels were determined in blood, brain, kidney, and liver by atomic absorption spectroscopy utilizing a modified Delves cup method (Ediger and Coleman). Three determinations were averaged for each tissue value.

Results

The lead-treated animals began showing clinical signs of intoxication after 2–3 doses. Food consumption dropped and the guinea pigs lost 10–15% of their body weight after 5 doses of lead. After 2 doses, the animals were easily startled and irritated by noise or visual stimuli. These signs became more striking during the course of the intoxication and included occasional seizures, obtundation, and an abnormally heightened startle response. Tonic seizures were noted in 3 animals on the 5-dose regime, appearing in 2 animals after 4 doses of lead and in one after 5 doses.

There were no macroscopic distinctions between the brains of the control and intoxicated animals, irrespective of the number of lead doses. Light microscopic