Mouse cerebellar Purkinje cell damage induced by diphenylhydantoin acute intoxication

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ABSTRACT: Twenty one days old Swiss albino mice that received diphenylhydantoin (25 mg/kg, i.p., daily for 15 days) progressively developed gait alterations, changes of behavior and cerebellar ataxia. Cerebellar slices were processed by conventional transmission electron microscopy. The body of Purkinje cells exhibited fragmented limiting plasma membranes, dilated nuclear envelopes, swelling and disassembly of nuclear pores, enlargement of rough and smooth endoplasmic reticulum and a notable detachment of membrane associated ribosomes, together with distorted vacuoles of smooth endoplasmic reticulum, bizarre shaped and swollen mitochondria with dilated cristae, as well as disrupted limiting lysosomal membranes. Degenerated axosomatic synapses apparently corresponding to basket cell axonal endings were recognized. Degenerated Purkinje cell axon initial segments exhibited vacuolar degeneration of myelin sheath, dilated axoplasmic tubular bundles, fragmented axonal membranes, swollen mitochondria, and disassembly of cytoskeletal structures. Some edematous and clear secondary and tertiary dendrites exhibited areas of dilated cisterns of smooth endoplasmic reticulum, clear and dark multivesicular bodies, and coated vesicles. Other dendritic ramifications exhibited an electron dense dendroplasm. Degenerated and large climbing fiber endings were observed making axodendritic synapses with edematous Purkinje dendrites. These presynaptic endings appeared depleted or containing few synaptic vesicles. These synapses did not exhibit pre- and postsynaptic densities. At the molecular layer, the edematous synaptic varicosities of parallel fibers containing pleomorphic synaptic vesicles and dense extravesicular substance were observed making asymmetric synaptic contacts with swollen Purkinje dendritic spines. These findings are postulated as pathogenic mechanisms of mouse cerebellar ataxia.

Introduction

Purkinje’s cell density after diphenylhydantoin intoxication in rats was early reported by Dam in experimental animals and epileptic patients (1970 a,b,c). Later, the effects of diphenylhydantoin on activity of rat cerebellar Purkinje cells was described by Puro and Wooward (1973). Takeichi (1981, 1983) reported neurobiological and electron microscopic investigations of the rat cerebellum with chronic diphenylhydantoin intoxication. The oral administration of diphenylhydantoin for a period of more than nine months produced ataxia and muscle weakness in rats (Tashiro et al., 1982). Chronic administration of diphenylhydantoin in the daily diet of mice induced on presynaptic segments of Purkinje cell axons massive enlargement and swelling in the deep cerebellar nuclei due to accumulation of spherical particles and tubular structures in the axoplasm (Volk and Kirchgässner, 1985). Kiefer et al. (1989) reported Purkinje cell axonal swellings in deep cerebellar nuclei of mice following orally administered phenytoin from 3 to 46 days.

Imamura et al. (1992), Villa and Sica (1994) and Pulliainen et al. (1997) subsequently described that diphenylhydantoin yields cerebellar ataxia in chronically treated epileptic patients due to cerebellar atrophy with loss of Purkinje cells. Later, Awada et al. (1999) reported that high doses of phenytoin can be toxic to human cerebellar cortical cells and induce mild cerebellar atrophy. Altered synaptic organization in the cerebellum and ataxia was observed in pogo/pogo mutant mice. In these mutant mice, parallel fiber varicosities were larger than normal and a single fiber often established synaptic contacts with up to four dendritic spines of a Purkinje cell (Jeong and Hyun, 2000).

However, a detailed submicroscopic study of pathological changes of Purkinje cell body, dendritic ramifications and their synaptic connections in Swiss albino mouse with diphenylhydantoin acute intoxication has not been carried
out up to the present time. Such study is basically important for further understanding the pathogenic mechanisms of experimental and human cerebellar ataxia.

**Material and Methods**

Swiss albino mice 21 days postnatal received intraperitoneal injection of diphenylhydantoin (Dilantin) at 25 mg/kg daily for 15 days developing progressively gait alterations, changes of behavior and cerebellar ataxia. Mice were decapitated after intraperitoneal injection of sodium pentobarbital. The cerebellar cortex was first fixed in 0.1M glutaraldehyde-buffer phosphate solution by two hours, and later fixed by 1 hour in similarly buffered 1% osmium tetroxide solution. The samples were dehydrated and embedded in Epon. Ultrathin sections were obtained in MT-2B Sorvall ultramicrotome, and stained with uranyl salts and lead hydroxide. Observations were made in a JEOL 100B at 80 kV at intermediate magnifications (20,000 to 60,000 X) (Castejón, 2012).

**Results**

Clear and edematous Purkinje cell bodies exhibited fragmented limiting plasma membrane, dilated nuclear envelope, swollen and disassembly of nuclear pores, enlargement of rough endoplasmic reticulum and a notable detachment of membrane associated ribosomes, smooth enlarged cisterns and distorted vacuoles of the smooth endoplasmic reticulum, bizarre shaped and swollen mitochondria with dilated mitochondrial cristae, lysosomes with disrupted limiting membrane, and swollen Golgi apparatus, with fragmentation and vacuolar necrosis (Figs.1 and 2). Areas of dilated smooth endoplasmic reticulum surrounding small islands of Purkinje cell cytoplasm containing numerous vesicular profiles and free ribosomes were observed (Fig. 3). Fine and coarse electron dense precipitate of dense matrix lysosomes was observed as well as disrupted lysosomal limiting membranes (Fig. 4). Degenerated axosomatic synapses presumably corresponding to basket cell axonal endings were recognized on Purkinje cell bodies, which appeared surrounded by the edematous Bergmann glial cell cytoplasm (Fig. 5).

The degenerated Purkinje cell axonal initial segment exhibited vacuolar degeneration of myelin sheath, dilated axoplasmic tubular bundles, fragmented axonal membranes, swollen mitochondria, and disassembly of cytoskeletal structures (Fig. 6). At the molecular layer, some edematous and clear Purkinje primary dendritic trunks (Fig. 7) exhibited areas of dilated cisterns of smooth endoplasmic reticulum,
FIGURE 3. Swollen Purkinje cell cytoplasm (PC) depicting a cytoplasmic island (CI) surrounded by vacuolated smooth endoplasmic reticulum cisterns (SER). A swollen mitochondrion (M) shows its outer surface fused with a short rough endoplasmic reticulum cistern (arrow).

FIGURE 4. Swollen Purkinje cell cytoplasm (PC) showing irregularly shaped lysosomes (L) with dark matrix granular deposit, bizarre-shaped mitochondria (M), and surrounded by the swollen perineuronal Bergmann glial cell cytoplasm (BG).

In the present paper we have found edematous changes of mouse Purkinje cell body, axonal and dendritic processes and degenerated intracortical synaptic contacts induced by diphenylhydantoin intoxication. Similar electron microscopic findings in altered Purkinje cells (edematous cisternae of endoplasmic reticulum, swollen mitochondria and cytoplasmic bodies, along with the synaptic and axonal changes) were earlier reported by Breiden-Arends and Gullotta.

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progressive accumulation of proliferating membranous material arranged in an increasingly complex fashion. However, they presumably represent edematous enlargement of these substructures since bundles of tubular structures in the axoplasm of Purkinje cell initial axonal segment are also found in normal mice (Castejón, 2012).

Ohmori et al. (1997) reported that phenytoin induces neurotoxic damage to granule cells and Purkinje cells in the developing cerebellum and impairs selected aspects of motor coordination ability. Tauer et al. (1998) also described in adult mice focal swellings along the Purkinje cell axon correlated with ataxia and incoordination of movements following administration of phenytoin. Later, Fonnum and Lock (2000) found that Purkinje cells are very sensitive to ischemia, bilirubin, ethanol and diphenylhydantoin. We have also reported similar findings in severe and complicated human traumatic brain injuries (Castejón 1985, Castejón et al. 1995, 1997), suggesting a common and non-specific Purkinje cell reaction.

(1981) in two epileptic patients who had been treated over years with diphenylhydantoin. Biggiogera et al. (1983) studied under the electron microscope the effect on Purkinje cells of diphenylhydantoin administration on postnatal ontogenesis in the rat, and reported signs of neuronal immaturity, both at cytoplasmic and nuclear levels, particularly evident between the 4th and 17th day of postnatal life. Volk and Kirchgässner (1985) earlier reported in male C57/BL6J mice the occurrence of marked dystrophic changes in Purkinje cell axons in the cerebellar vermis after chronic phenytoin administration. According to these authors, the presynaptic segments of Purkinje cell axons in the deep cerebellar nuclei showed massive enlargement and swelling due to accumulation of spherical particles and tubular structures in the axoplasm. They suggested that tubular structures represent a proliferation of smooth endoplasmic reticulum. Kiefer et al. (1989) postulated that phenytoin-induced axonal pathology of Purkinje cells is a dynamic process characterized by the

FIGURE 5. Swollen Purkinje cell body showing a partial nuclear (N) pole surrounded by a dilated nuclear envelope (long arrow), notably swollen mitochondria (M), and a fragmented and vacuolar Golgi apparatus (GA). A degenerated presynaptic ending (DPE), surrounded by swollen Bergmann glial cell cytoplasm, exhibits few dark presynaptic vesicles (short arrow), and depleted clear synaptic vesicles. Note the absence of pre- and postsynaptic densities.

FIGURE 6. Degenerated Purkinje cell initial axonal segment (PIAS) showing the cross section of dilated axoplasmic tubular bundles (DT), edematous mitochondria (M), myelin sheath degeneration (DMS) with loss of myelin periodicity and myelin ovoids (MO). A swollen associated oligodendrocyte (Ol) with vacuolar degeneration of Golgi apparatus (GA) also is seen.
The alteration of the Golgi complex is apparently related with neuronal, axonal and synaptic degeneration (Castejón et al., 1995; Castejón et al., 1997; Castejón, 2011). The diphenylhydantoin-induced ischemic and anoxic conditions of cerebellar tissue also alter the post-translational modifications of proteins within the Golgi apparatus. Consequently, the insertion of integral membrane proteins, including ion channels and receptor molecules, into the plasma membrane is disturbed causing fragmentation and necrosis of the plasma membrane, as observed in the electron micrographs. The fragmentation of the mitochondrial cristae suggest that oxidative phosphorylation of ADP, the precursor of the high-energy phosphate bond of ATP, no longer occurs. In addition, we suppose an interruption of mitochondrial membrane intracellular transport, which causes respiration-dependent extrusion of H\(^+\) and accumulation of Ca\(^{2+}\) from the cytoplasm (Castejón, 2004a).

Diphenylhydantoin-induced coarse and dense granulation of lysosomal matrix and disruption of the limiting membrane were interpreted as abnormal protein aggregation of released lysosomal enzymes and adsorbed cytosolic proteins. In addition, the multiple factors involved in cytotoxic edema should be considered (Castejón, 2004b).

We observed cytoplasmic islands surrounded by smooth endoplasmic reticulum in Purkinje cells, suggesting diphenylhydantoin-induced proliferation of smooth endoplasmic reticulum. We also found a fine and coarse electron dense precipitates over the lysosomal dense matrix. This finding is probably due to diphenylhydantoin activation of lysosome-bound enzymes, and to a decrease of thiamine pyrophosphatase activity in Purkinje cells (Lettmann et al., 1987).

Hitchcock and Gabra-Sanders (1977) reported the effect of diphenylhydantoin upon gamma aminobutyric acid (GABA) and succinic dehydrogenase levels in rat Purkinje cells.
cells, and found a significant progressive loss of GABA with increasing dosage of diphenylhydantoin. These earlier studies are in agreement with the cytotoxic edema reported in the present study.

Bergmann glial cells showed a remarkable edema after diphenylhydantoin treatment, as illustrated in our electron micrographs, which indicates a severe disruption of the Purkinje cell-Bergmann glial cell unit. Bergmann glial cells contain a large repertoire of transmitter receptors, such as glutamate receptors and transporters (Kainate/Ampa receptors, NMDA receptors) and GABA receptors, allowing them to sense the activity of neighboring synapses (Somogyi et al., 1990; Chaudry et al., 1995, et al., Castejón, 2002). These receptors have distinct biophysical and pharmacological features activating second-messenger pathways in the Bergmann glial cells. These molecular pathways are altered by diphenylhydantoin action contributing to the pathogenic mechanisms of mouse cerebellar ataxia.

The degenerated climbing fiber and parallel fiber-Purkinje cell dendrite synaptic relationship, and the degenerated Purkinje cell axon reported herein suggest a diphenylhydantoin-induced decreased in excitability of Purkinje cells at the molecular layer. Cytotoxic edema, damage of Bergmann glial cell-Purkinje cell unit, synaptic degeneration of axodendritic and axospinodendritic junctions, and Purkinje cell axonal degeneration appear as essential components of the pathogenic mechanisms of mouse cerebellar ataxia.

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References


PURKINJE CELL DAMAGE INDUCED BY DIPHENYLHYDANTOIN


