PLASMOCID ENCEPHALOPATHY IN THE RHESUS MONKEY:
A STUDY OF SELECTIVE VULNERABILITY

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ABSTRACT

The effects of plasmocid (8-diethylaminopropylamino-6-methoxyquinoline) on the central nervous system of rhesus monkeys were studied by electron and correlative light microscopy. Light microscopic studies showed neuronal vacuolar lesions distributed selectively in the diencephalon and brain stem. In the affected nuclei, principally III, IV, VI and VIII, the brunt of the damage was consistently borne by large multipolar neurons. The cerebral and cerebellar cortices were normal. Electron microscopy showed the earliest effects to be an abnormality of neuronal mitochondria, which were enormously increased in number with incompletely formed transverse inner cristae. Later stages of degeneration showed dissolution of mitochondrial contents so that only their outer membranes remained. Neuroglia were morphologically normal, as were synapses in contact with the altered neurons. Neurons in the most advanced stage of degeneration exhibited complete destruction of cytoplasmic contents and disruption of the cell membrane with crenated nuclei remaining. The ultrastructural data confirm the highly selective vulnerability of brain stem nuclei to plasmocid and suggests that the primary effect of the drug is on neuronal mitochondria.

INTRODUCTION

Several 8-aminoquinoline compounds were studied as antimalarial agents in the 1940's. The clinical use of some of them, including plasmocid (8-diethylaminopropylamino-6-methoxyquinoline), was precluded because of severe toxic effects on the central nervous system (6). Studies of the toxicity of plasmocid in experimental animals showed that this compound produced a strikingly selective distribution of brain lesions (14, 17, 18).

Plasmocid given to rhesus monkeys in small doses (1–10 mg/kg per day)

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produced a distinctive neurological syndrome characterized by nystagmus, severe disturbances in equilibrium, paresis of extraocular muscles and abnormalities of the pupillary light reflexes (14, 17). The rate of development and the severity of the neurological abnormalities were dose-related. Histologically, the principal alterations were in nuclear groups containing large multipolar neurons in the diencephalon and brain stem. The structures most regularly affected were the oculomotor, trochlear, abducens and vestibular nuclei (14).

In the only electron microscopic study of the effects of plasmocid on the nervous system, D'Agostino demonstrated alterations of the rough endoplasmic reticulum and mitochondria in neurons of the trigeminal ganglia in rats (3).

In the present study the effects of plasmocid on the central nervous system of rhesus monkeys were examined by electron and correlative light microscopy. Our findings confirm the highly selective vulnerability of certain nuclear groups in the brain stem, and, at the ultrastructural level, they suggest that the primary toxic action of plasmocid is on the mitochondria of large multipolar neurons.

METHODS

Plasmocid, as the hydrochloride salt (M.P. 218-220°C), was administered to four rhesus monkeys; four normal rhesus monkeys matched for age and sex served as controls. The drug was given by nasogastric tube one to three times daily in a dose of 2.5-10 mg/kg per day. The animals were examined three times each day to assess the development of neurological signs. None of the monkeys were allowed to die as a consequence of plasmocid intoxication. Depending upon the rate of progression of the neurological syndrome, the monkeys were sacrificed four to seven days following the initial dose.

Following intravenous pentobarbital anesthesia, the intravascular perfusion procedure was begun by making large lateral incisions in the ribs and turning the entire chest wall anteriorly to expose the thoracic cavity. The pericardium was opened and an incision made in the apex of the left ventricle, through which an 8 gauge cannula was inserted and advanced until its tip could be palpated in the root of the ascending aorta. The cannula was clamped in place, the right atrium was incised, and the perfusion was begun at a pressure of 120-140 mm Hg. The descending thoracic aorta was cross-clamped to facilitate delivery of the fixative to the head. The animals were perfused first with 500 cc of a 608 mOsm glutaraldehyde-paraformaldehyde mixture in cacodylate buffer at 37°C. This was followed by 2000 cc of a 2000 mOsm glutaraldehyde-paraformaldehyde mixture (7, 22) at the same temperature. Calcium chloride was not added to the fixative. In order to assure optimum fixation, the time between opening the thoracic cavity and delivery of the initial fixative was kept to a minimum and ranged between 40-60 seconds. This technique produced optimal, uniform fixation for electron microscopy in the brains of all control and experimental monkeys.

Tissue blocks for electron microscopy, approximately 1 mm on a side, were dissected with the aid of a dissecting microscope. Specimens were selected from the cerebral cortex, the cerebellar cortex and dentate nuclei; from the thalamus, including nuclei ventralis posterior lateralis and medialis, lateralis posterior, dorsomedialis and the pulvinar; and from the following nuclei of the brain stem: oculomotor, trochlear, abducens, lateral and inferior vestibular, motor and sensory trigeminal, hypoglossal, and the dorsal motor nuclei of the vagus. All tissue was further fixed in cacodylate-buffered osmium tetroxide and aqueous uranyl acetate (8). Following rapid dehydration in graded methanols and propylene oxide, the tissue was embedded in Durcupan, sectioned at 1.5μ (for light microscopy) and at 400-700Å (for electron microscopy). The 1.5μ sections were stained with toluidine blue-O; thin
sections were mounted on uncoated 75×300 mesh copper grids, stained with both warm uranyl acetate in 50% methanol and lead citrate, and examined in a Philips 200 electron microscope at 60 kv.

The remaining cerebral hemispheres, thalami, cerebellum and brain stem, from which the small blocks of tissue had been removed, were embedded in celloidin and stained with cresyl violet and luxol fast blue for light microscopic study. This method afforded precise anatomic orientation and verification, since the small blocks were taken unilaterally from paired structures.

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RESULTS

All monkeys were healthy and active prior to administration of the drug. Within 36–72 hours, depending on the dose of plasmodic, the animals developed an unmistakable neurological syndrome. This was characterized by conspicuous disturbances of equilibrium and balance which developed as the earliest sign and progressed with increasing severity. Initially, the intoxicated monkeys were reluctant to move about the cage and would cling tenaciously to the sides for support. As progression occurred they were unable to stand or walk and in the most severely affected monkeys there were no positive supporting or checking reactions. Vertical and horizontal nystagmus appeared early and was temporarily related to the disturbances of equilibrium. With severe neurological impairment, dysmetria and ataxia became evident in the extremities. In the late stages, abnormalities of the pupillary light reflexes usually appeared, together with restriction of ocular movements. One animal, receiving the highest dose, developed a complete ophthalmoplegia with absent oculocephalic reflexes. Throughout the course of the encephalopathy, it was not possible to elicit abnormalities of sensation, reflexes, or pyramidal tract function.

A. Light Microscopy

The distribution of plasmodic-induced lesions was nearly identical to that described by the Schmidts (17) and Richter (14). The cerebral and cerebellar cortices of intoxicated monkeys were anatomically normal. The basal ganglia and the deep white matter of the cerebral hemispheres were also relatively spared; a few scattered microscopic lesions consisting of early central chromatolysis were seen in some large neurons in the globus pallidus and putamen. Small neurons of the lenticular nuclei were uninvolved.

In the dorsal thalamus and hypothalamus, more conspicuous neuronal alterations were evident. These ranged from early central chromatolysis to complete dissolution and degeneration of the neuronal perikarya with only small fragments of nuclear membrane remaining visible. Thalamic nuclei showing the most consistent involvement were ventralis posterior lateralis, lateralis posterior and medialis dorsalis. The subthalamic nuclei also invariably exhibited especially advanced neuronal destruction and proliferation of neuroglia.

The most well developed lesions were present in cranial nerve nuclei III, IV, VI, VIII and in the dentate nucleus of the cerebellum. The earliest alterations to be seen in epoxy-embedded sections stained with toluidine blue-O were
numerous clear vacuoles in the neuronal cytoplasm. Later stages were marked by complete replacement of neuronal cytoplasm by these clear vacuoles (Figure 1B). In the most advanced stage only a faintly staining nucleus with peripherally dispersed chromatin remained after presumed dissolution of cytoplasmic contents (Figure 1C). Only in the advanced lesions were other cellular elements involved. In such instances there was some proliferation of darkly staining perivascular phagocytic cells together with astrocytic proliferation and apparent spongiform breakdown of the neuropil (Figure 1D). No pathological alterations were seen in control animals (Figure 1A).

**B. Electron Microscopy**

The ultrastructure of the brain stem neurons of the normal monkeys conformed, in general, to that described in the rat by Peters et al (11). The large multipolar neurons in cranial nerve nuclei contained prominent large nuclei with reticulated nucleoli. These cells all contained abundant granular endoplasmic reticulum in large clusters (Nissl bodies) (Figure 2). In all neurons, the Golgi apparatus appeared as a complex of broad flattened cisternae dispersed throughout the cytoplasm. The neuronal mitochondria were small rounded structures or slender rodlets 0.1–0.2 µ in diameter. Neuronal cytoplasm contained occasional primary lysosomes, scattered lipofuscin granules and the normal complement of microtubules and neurofilaments. The surrounding neuropil was a tightly compacted feltwork of intermingled processes including dendrites, axons, synapses and glial processes. We observed no perikaryal features in the neurons from various brain stem motor nuclei that distinguished one from another, though, as is true in light microscopy, the sensory neurons were easily recognized.

Electron microscopic study of the plasmocid lesions showed the earliest effect to be a universal abnormality of neuronal mitochondria, which were enormously increased in number and altered in form. They were greatly enlarged and ballooned, and transverse inner cristae were fragmented or lacking entirely (Figure 3). Later stages of degeneration showed dissolution of mitochondrial contents such that only their outer unit membranes remained (Figure 4). Although degenerating neurons contained severely altered mitochondria, synaptic structures making contact with these cell bodies were morphologically normal (Figure 3). The neuropil surrounding the degenerating neurons was entirely normal except in the regions of most advanced destruction.

Alteration of other subcellular organelles was apparent, but never as prominent as in the mitochondria. A proliferation of neurotubules and membrane-bound dense bodies resembling primary lysosomes was an early and consistent finding (Figure 3). The rough endoplasmic reticulum was dispersed amid the large numerous mitochondria, though the Golgi apparatus appeared intact. Neural processes of involved cells, either axonal or dendritic, also exhibited the characteristic mitochondrial lesion. Many myelinated axons, in addition, developed disruption of the normal myelin lamellar pattern and proliferation of dense bodies in the axoplasm (Figure 5).
Fig. 1A) Normal control trigeminal motor neuron with surrounding compact neuropil; × 780. (1B) Pronounced cytoplasmic vacuolation in a plasmocid-damaged oculomotor neuron; × 600. (1C) Plasmocid-induced cytoplasmic disintegration, oculomotor nucleus. A remnant of the cell’s nucleus is present, near cytoplasmic debris. The surrounding neuropil is normal; × 950. (1D) Most advanced stage of plasmocid-induced neuronal and parenchymal degeneration; × 720. All toluidine blue-O stained epoxy sections.
Fig. 2. Normal trigeminal motor neuron with usual complement of small mitochondria, prominent Nissl bodies (granular endoplasmic reticulum), small primary lysosomes, Golgi apparatus and a normal complement of neurotubules; × 12,500.
Fig. 3. Oculomotor neuron, with plasmocid-induced swelling and proliferation of mitochondria. Transverse inner cristae are disrupted. Note the normal mitochondria in adjacent myelinated axons; × 10,000.

Fig. 4. Severely involved neuron (adjacent to a normal capillary) in same stage of degeneration as illustrated by light microscopy in Figure 1B. Neuropil between the neuron and the endothelial cell is normal; × 10,400.
Fig. 5. Myelinated axon (tectal nerve nucleus) shows disruption of the normal myelin lamellar pattern and proliferation of abnormal axoplasmic mitochondria identical to those in involved neuronal perikarya. Again, adjacent neuropil is intact; × 10,300.

Fig. 6. Disintegrated neuron with cytoplasmic debris and a tattered nuclear membrane. Adjacent neuropil still intact. This neuron is in same stage of degeneration as illustrated by light microscopy in Figure 1C; × 11,300.
Fig. 7. Higher power view of an end-stage neuron showing fragmentation of its cell membrane and a remnant of its nuclear membrane. Adjacent neuropil is still largely intact; \( \times 40,400 \).

Fig. 8. Perivascular phagocyte with numerous cytoplasmic dense bodies (lysosomes), from an area of severe destruction in the lateral vestibular nucleus. This electron micrograph correlates with the light microscopic appearance as illustrated in Figure 1D; \( \times 10,300 \).
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Neurons in the most advanced stages of regression were characterized by dissolution of cytoplasmic contents and segmental disruption of the cell membrane (Figures 6 and 7). Additionally, there was often a centrifugal dispersion of clumped nuclear chromatin bounded by a folded, disintegrating nuclear membrane. In the most severely involved regions there was considerable activity of phagocytic cells. These macrophages were insinuated between the involved processes in the neuropil. In the most severe areas of tissue destruction the extracellular space was expanded.

Astrocytes and oligodendrocytes were not abnormal, even in the zone of most severe and widespread neuronal changes. Specifically, glial mitochondria were not affected.

The microvesselature was entirely normal, even when near severely altered neurons (Figures 4 and 8). In the regions of severe parenchymal destruction, dark cells with multiple dense bodies and secondary lysosomes were present in an abnormal pericapillary space. These cells were interpreted as macrophages or histiocytes (Figure 8), and were easily correlated with those seen in the light microscopic preparations.

DISCUSSION

Our ultrastructural findings indicate that the most conspicuous and the earliest discernible lesion in plasmodial encephalopathy in the rhesus monkey is degeneration of neuronal mitochondria. This is restricted to the large multipolar neurons of those nuclei of the brain stem and diencephalon that were shown in the earlier studies of the Schmidts (17) and Richter (14) to be selectively vulnerable to plasmocidal toxicity. It would appear reasonable to assume that the ultimate degeneration and loss of nerve cells in plasmodial intoxication is a direct result of these severe alterations in mitochondria.

The mechanism by which plasmodial produces these effects on the central nervous system is unknown. The 8-aminoquinoline antimalarial compounds have been shown to inhibit coenzyme Q and succinooxidase systems (20), and their antimalarial activity is thought to be due, at least in part, to interference with coenzyme Q in the electron transfer process in the mitochondria of the malarial parasite. Another possibility, suggested by Pentschew (10), is that the 8-aminoquinolines are vitamin E antagonists, since an encephalopathy similar to that induced by plasmodial occurs in vitamin E-deficient animals. Schochet has described mitochondrial changes in the axonal dystrophy associated with vitamin E deficiency (19), but these appear to be different morphologically from those we have observed in plasmodial intoxication.

The only other electron microscopic study of the effect of plasmodial on the nervous system has been that of D’Agostino, who examined the trigeminal ganglia of intoxicated rats (3). He found prominent mitochondrial alterations not unlike those we have observed, together with reduction of endoplasmic reticulum and an apparent proliferation of neurofilaments. Changes in myelinated axons, which were quite similar to those in our material, were also found.

Cardiac muscle of plasmodial intoxicated rats also shows early and severe
degeneration of mitochondria, with loss of cristae, swelling and dense inclusions (2). The mitochondria of skeletal muscle (diaphragm) were more resistant, despite severe degeneration of the contractile elements (2). There is evidence that mitochondrial changes are more pronounced in metabolically more active skeletal muscle (21).

The selective character of the effects of plasmocid on the nervous system remains as unexplained as its mechanism of action. Whether the localized effects of the compound are a consequence of local differences in vascular permeability, which seems unlikely, or of selective uptake by, or unique metabolic features in certain cell types, is a question which remains to be elucidated by further investigation.

Richter pointed out the similarity in the localization of plasmocid-induced lesions in experimental animals to that of the necrotizing lesions of Leigh's encephalopathy in human infants (15). On re-examining the material from Richter's three cases of Leigh's disease, however, we were impressed as much by the differences in the histopathology of the two conditions as by their similar localization. Preservation of nerve cells in the midst of severely degenerated neuropil and vascular proliferation are typical of Leigh's disease, while plasmocid preferentially affects neurons. Although a specific metabolic defect has been proposed in Leigh's disease (1, 12), many intoxications and deficiency states associated with similarly selective and focal cerebral lesions, such as carbon disulfide encephalopathy (13), thiophen-induced granule cell destruction (4) and thiamine deficiency (16), remain incompletely understood. Plasmocid encephalopathy is another example of this group of conditions, which brings the lack of homogeneity of brain metabolism into sharp relief, and emphasizes the importance of biochemical investigation of the brain region by region.

One case of encephalopathy in man from intoxication with an 8-aminoquinoline derivative (pamaquine) has been studied pathologically (9). The principal alterations were neuronal, with preferential localization in the third, fourth and sixth cranial nerve nuclei. Minor changes were described in the pallidum and cerebral cortex. While the histological changes were less severe than those we observed in our animals from plasmocid, the findings in this case suggest that the effect of plasmocid on the human brain would be quite similar to that in the rhesus monkey.

REFERENCES