6-Aminonicotinamide-Induced Hydrocephalus in Suckling Mice

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Abstract. Following a single intraperitoneal injection of 6-aminonicotinamide (6-AN, 50 mg/kg of body weight) into newborn mice of the Institute of Cancer Research strain, hydrocephalus consistently developed nine days after injection, with rapid progression. All of these mice died before reaching adulthood. The most striking early histologic change in these mice was cytoplasmic vacuolation of ependymal cells, which was observed as early as 24 hours after injection. Vacuolation of subependymal astrocytes appeared during the next few days. After day seven, the aqueduct was obliterated by swollen vacuolated ependymal cells and subependymal astrocytes. The aqueduct remained obliterated even after the vacuolation of the ependymal cells subsided after day nine, when vacuolation of subependymal astrocytes was still pronounced. These morphological observations reveal that, in newborn mice, the ependymal cells are the most sensitive to the toxic action of 6-AN and suggest that the pathogenesis of 6-AN-induced hydrocephalus is likely to be due to the combination of ependymal cell damage and compression of the lumen by the edematous periaqueductal gray matter. This is a highly reproducible animal model of drug-induced hydrocephalus.

Key Words: 6-Aminonicotinamide; Aqueductal stenosis; Ependyma; Hydrocephalus; Suckling mice.

INTRODUCTION

The agent 6-aminonicotinamide (6-AN) is an antagonist of nicotinamide and its toxic effect on the nervous system is well documented in the literature. The neuropathologic lesions in 6-AN-treated animals are characterized by diffuse spongy changes in the spinal anterior horns and brainstem nuclei, due to the hydropic swelling, and vacuolar degeneration of the cytoplasm of oligodendroglia and astroglia (1–21).

It is also known as a potent teratogenic compound when administered to pregnant animals. Hydrocephalus, cleft palate and skeletal anomalies are frequently reported (22–29). During our investigation on experimental pellagra using suckling mice, we noted that hydrocephalus was consistently produced, in addition to pellagra-like skin lesions, by a single intraperitoneal injection of 6-AN.

In this communication, we report a chronological study of the morphological alterations in the development of hydrocephalus and discuss possible mechanisms of 6-AN-induced hydrocephalus.

MATERIALS AND METHODS

A total of 121 newborn mice of ICR strain (Institute of Cancer Research, Philadelphia) were used for this study. Initially, to determine the effective dosage, 59 newborn mice were...
used. They were divided into five groups shortly after birth. Group I (eight mice), Group II (30 mice) and Group III (six mice) received a single intraperitoneal injection of 10, 50 and 100 mg of 6-AN (in physiological saline)/kg of body weight (BW), respectively, on day one. Those in Group IV (eight mice) received physiological saline only. The seven mice of Group V were injected with 6-AN (50 mg/kg BW) after five postnatal days. All animals were weighed every day and examined only for the clinical study.

For the precise morphological study, 62 mice (39 experimental and 23 control) were used. All mice of the experimental group were injected with 50 mg/kg BW of 6-AN at birth since this dosage was the most effective in producing hydrocephalus in the initial study. Mice from both the experimental and control groups were killed on each day from day two to day 13 postinjection. Fifteen mice (eight experimental and seven control) were used for the light microscopic study alone. For the light and electron microscopic investigations, 25 experimental and 13 control mice were anesthetized with Nembutal and perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer. The cerebrum, brain stem and cerebellum were sliced coronally. The sections of the midbrain and the walls of the lateral and third ventricles were thinly sliced, postfixed in 2% osmium tetroxide in phosphate buffer, dehydrated in a series of graded ethanol and embedded in Epon. Semithin sections were stained with toluidine blue for light microscopic examination. Selected areas were thin sectioned, stained with uranyl acetate and lead citrate, and examined with a Siemens 101 electron microscope.

To test the patency of the aqueduct of Sylvius, six hydrocephalic and three control mice received a single injection of 0.05 ml of a 1% toluidine blue solution into the right lateral ventricle. Two of the former and one of the latter were injected on the fifth, seventh and ninth days after 6-AN injection. These brains were sectioned horizontally to demonstrate the entire ventricular system.

RESULTS

Clinical Study

Hydrocephalus was noted only in 25% (two of eight) of the mice in Group I, which received 10 mg/kg BW, while almost all the mice (29 of 30) that received 50 mg/kg BW (Group II) developed hydrocephalus. Those that received 100 mg/kg BW (Group III) had severe anorexia and died within a week. Only two of the seven in Group V, which received 50 mg/kg BW after five postnatal days, developed hydrocephalus. No abnormalities were found in littermate controls (Group IV).

The weight gain of 6-AN-treated mice was retarded throughout the experimental period, as seen in Figure 1. Several hours following the injection, the newborn mice became less active and weakness of the hindlimb became apparent one day after injection. Around day three neurological signs such as paresis of the hindlimbs, ataxia and trembling became conspicuous, and some developed anorexia and diarrhea. Growth of the hair was delayed in the experimental group and the skin surface became scaly around day seven as noted in pellagra. However, the skin lesions gradually became less noticeable with subsequent growth of the fur. Enlargement of the head, particularly the forehead, was first noticeable around day nine (Fig. 2) and became prominent after day 11. These mice did not survive to adulthood and died before reaching day 20.

Pathology

Dilatation of the lateral and third ventricles was not too apparent until day seven, when narrowing of the aqueduct was observed in some mice. The toluidine blue dye test revealed occlusion of the aqueduct of Sylvius on day nine, when dye injected into the lateral ventricle could not be detected in the fourth ventricle (Fig. 3). Marked hydrocephalus and thinning of the cerebral cortex were noted after day 11. The
Fig. 1. Weight gain curves of 6-AN (50 mg/kg BW) treated newborn mice compared to littermate controls. Each point represents the mean of three to six mice.

Fig. 2. In this coronal section of the cerebrum on day nine, the lateral ventricles are markedly dilated. ×4.
lateral ventricles were markedly dilated, especially the posterior horns. The rostral portion of the third ventricle was also dilated, but no dilatation was observed in the fourth ventricle. Due to the markedly enlarged posterior horns of the lateral ventricles, the midbrain was compressed medially and the cerebellum was displaced caudally.

The most striking histological change was cytoplasmic vacuolation of the ependymal cells (Fig. 4). The cytoplasm of ependymal cells appeared watery and dilatation of the perinuclear cisterns and the formation of numerous small clear vacuoles were conspicuous features. These changes could be observed in the aqueduct as early as 24 hours after injection. The vacuolation of the cytoplasm of ependymal cells reached its maximum by day five, when almost all ependymal cells on the ventricular walls were vacuolated with the exception of those in the ventral portion of the third ventricle (Fig. 5) and the high columnar ependymal lining cells in the aqueduct. In the lumen of the aqueduct, many vacuolated foamy cells were also noted. The epithelial cells of the choroid plexus did not show vacuolar changes (Fig. 4), although slight edematous changes of the stroma were noted in some mice after day nine. Edematous arachnoidal cells, some of which were undergoing mitotic division, were also observed in some animals as early as day three (Fig. 6).

Cytoplasmic vacuolation was apparent in the subependymal astrocytes around the lateral ventricles, especially in the occipital region, on day one. However, in the aqueductal region, such astrocytic changes were not apparent until around day five. On day seven, edematous changes in subependymal astrocytes and vacuolation of the ependymal cells became equally pronounced and the lumen of the aqueduct was obstructed (Fig. 7). After day seven, the periaqueductal gray matter revealed a diffusely spongy appearance. After day nine, edematous changes in the ependymal cells were less pronounced in the aqueduct, while spongy changes became severe in the periaqueductal region. Some ependymal cells had pyknotic nuclei and shrunken atrophic cytoplasm. The sequence of the pathological findings in the aqueduct is summarized in Figure 8.

Electron microscopic examination confirmed the presence of vacuoles within the cytoplasm of ependymal cells (Fig. 9). Some vacuoles were formed by the dilatation of the perinuclear cisterns and the cisterns of the rough endoplasmic reticulum. The Golgi apparatus was slightly dilated but other cellular organelles such as mitochondria, cilia and microvilli were well preserved. Even in the vacuolated ependymal cells, junctional complexes between the ependymal cells were identified. Dilatation of the extracellular space became apparent after day seven, and vacuolation of the subependymal astrocytes became pronounced after day nine.

There were many supra-ependymal cells within the lumen of the aqueduct, which probably correspond to the foamy cells seen with the light microscope. Some had

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**Fig. 3.** Horizontal section of the brain on day nine. Toluidine blue dye stains the wall of the lateral ventricles but is not detected in the aqueduct or in the fourth ventricle (arrow). × 3.

**Fig. 4.** All the ependymal cells in the fourth ventricle are vacuolated on day five, but the choroid plexus is unremarkable. Neurons and the blood vessels are well preserved. Epon 1 μm section. Toluidine blue stain. L—lumen of the fourth ventricle; C—choroid plexus; E—ependymal cells. × 270.

**Fig. 5.** Coronal section of the third ventricle through the median eminence of the hypothalamus on day five. Vacuolar changes are observed in the ependymal lining cells in the
dorsal portion of the ventricular wall (arrows), but not in those (arrowheads) in the ventral portion near the arcuate nucleus. Vacuolar changes are also noted in the meninges. Epon 1 μm section. Toluidine blue stain. ×96.

Fig. 6. Vacuolated arachnoid cells (arrows) are prominent in the leptomeninges in the interhemispheric fissure on day three. Epon 1 μm section. Toluidine blue stain. C—cortex; V—vessel. ×420.

Fig. 7. Aqueductal lesion near the obstructive site on day nine. Ependymal cells (arrows) are vacuolated and severe spongy changes are observed in the periaqueductal gray matter. Epon 1 μm section. Toluidine blue stain. ×106.
numerous vacuoles and the overall appearance was that of a macrophage, but cilia-like processes could be identified in some (Fig. 9, inset).

DISCUSSION

This is a new experimental model of hydrocephalus induced by postnatal administration of the drug 6-aminonicotinamide (6-AN) which is also a well-known teratogenic agent (22–29). With a single maternal injection of 6-AN, 8 mg/kg BW, on gestational day 13, congenital hydrocephalus was produced in all of the rat fetuses by term (26). In those hydrocephalic brains, vacuolation of neuroblasts and cellular rarefaction were observed. Foci of hemorrhage in the cerebral parenchyma and intraventricular hemorrhage were frequent findings (26, 27). Unlike the hydrocephalus in our study with newborn mice, however, the aqueduct of the affected fetuses was not narrowed and the enlargement of the ventricles was considered to be at the expense of the brain tissue (hydrocephalus ex vacuo). The cerebellum was often hypoplastic or absent.

Hemorrhage and necrosis of cells were not observed in our hydrocephalic mice that received 50 mg/kg BW of 6-AN on day one. The hydrocephalus was clearly obstructive in nature and the hydropic changes in ependymal cells were the first pathological changes observed in these mice. Such changes in ependymal cells have not been observed in 6-AN-treated animals before.

The early reports of the neuropathology of 6-AN described degenerative changes in the anterior horn cells of the spinal cord and brain stem neurons in adult rats (1–5). However, subsequent studies by many investigators (11, 12, 14, 15, 17, 19) have shown that glial cells were more susceptible to 6-AN than neurons and that affected cells showed hydropic swelling of the cytoplasm with numerous vacuoles, some of which were clearly formed by dilatation of the cisterns of the endoplasmic reticulum. Both astrocytes and oligodendroglia were affected in the central nervous system (CNS). In the peripheral nervous system, hydropic degeneration of Schwann cell cytoplasm was reported (20, 21). Neuronal chromatolysis, similar to that of pellagra in humans, was reported only in aged rats (13).

Since many of these previous studies were carried out on adult rats with a relatively lower dosage of 6-AN, these differences in pathology could be due to a variety of
Fig. 9. Electronmicrograph of two vacuolated ependymal cells in the aqueduct on day five. Junctions (arrows), cilia (C) and microvilli (M) are well preserved. Perinuclear cistern (PC) and the endoplasmic reticulum (E) are dilated. Supra-ependymal cell (S) has a large number of mitochondria and lysosomes. ×6,500. Inset: Higher magnification of the area indicated (*) showing cilia in this supra-ependymal cell. ×22,000.
reasons: species differences, different stages of brain maturation and differences in dosage of 6-AN, among others. Although the early target cells were different in newborn mice, the basic neuropathological cellular changes, namely dilatation of the rough endoplasmic reticulum and perinuclear cisterns, were similar, if not identical, to those reported in astrocytes and oligodendroglia (6, 11, 12, 14, 19).

Six-AN is a metabolic inhibitor which blocks the activity of nicotinamide adenine dinucleotide phosphate (NADP)-dependent enzymes, such as 6-phosphogluconate and glucose-6-phosphate dehydrogenases, in the pentose-phosphate pathway (30–32). The almost exclusive localization of NADP-dependent dehydrogenase activity in astrocytes and oligodendroglia, as demonstrated histochemically in adult rats, may explain the sensitivity of these glial cells to 6-AN toxicity (33). Susceptibility of ependymal cells in newborn mice to 6-AN may indicate that during an early stage of development NADP-dependent enzymes may be required in ependymal cells.

Ependymal cell changes were noted throughout the entire ventricular system in the mouse. However, the ependymal cells lining the ventricular surface of the ventral portion of the third ventricle (i.e., tanyocytes) did not show vacuolar degeneration. Tanyocytes are morphologically distinct from ependymal cells (34, 35). A different susceptibility to 6-AN also suggests some differences in cellular metabolism as well.

Hydrocephalus can be produced experimentally in fetuses by the maternal administration of many toxic substances, such as trypan blue (36) and cytosine arabinoside (37), and in maternal deficiency states, e.g., galactoflavin (38), vitamin B12 (39, 40) and folic acid (41). Edematous changes in ependymal cells have not been noted in any of these hydrocephalic animals. Degeneration of ependymal cells with subsequent obstruction of the aqueduct has been reported in viral infections (42–47). In this virus-induced hydrocephalus, ependymal cell damage is considered to be the primary lesion and periaqueductal edema is assumed to have followed as a result. On the other hand, hydrocephalus induced by Cuprizone treatment was thought to be the result of compression of the aqueduct by the surrounding edematous tissue (48). Mice fed a diet containing Cuprizone, a copper chelating agent, developed diffuse status spongiosus due to hydropic and vacuolar degeneration of glial cells and intramyelinic edema (49). The status spongiosus with severe edematous vacuolation of the brain stem preceded hydrocephalus in these mice. Although no lesions were described in ependymal cells with Cuprizone toxicity, the spongy state in 6-AN-induced hydrocephalus was quite similar to that of Cuprizone-induced hydrocephalus.

Several papers have suggested that aqueductal stenosis is a result of hydrocephalus (50, 51). Raimondi et al (52) believed that in the congenital hydrocephalic (hy-3/ hy-3) mouse, aqueductal stenosis was secondary to brain stem compression resulting from ventricular dilatation due to a block in the subarachnoid space.

Although there was no evidence that hydrocephalus preceded the obstruction of the aqueduct in 6-AN-induced hydrocephalus, we cannot rule out the possibility that edematous changes in arachnoid cells and ependymal cells might have contributed, to some extent, to the development of hydrocephalus before complete obstruction of the aqueduct, by disturbing the circulation and absorption of cerebrospinal fluid.

The aqueductal stenosis in 6-AN-induced hydrocephalus could have resulted from a combination of ependymal cell degeneration and dysfunction, compression of the brain stem by the dilated lateral ventricles and periaqueductal edema.

Cuprizone-induced hydrocephalus appeared to be the only postnatal animal model of drug-induced experimental hydrocephalus. We may now include 6-AN-induced
hydrocephalus as another highly reproducible animal model of drug-induced hydrocephalus.

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