Solvent Vapor Abuse Leukoencephalopathy.
Comparison to Adrenoleukodystrophy

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Abstract. Chronic organic solvent vapor inhalation can cause permanent damage to the central nervous system. Clinical features and radiologic abnormalities are well known, but pathology has not been definitively established. This study describes the gross, microscopic and ultrastructural changes and fatty acid composition of cholesterol esters in the brain of two chronic paint sniffers as well as the electron microscopic findings from a third, all with permanent neurological impairment. The abnormalities which were the same in all cases consisted of a demyelinating process which grossly manifested itself as brain atrophy and subtle discoloration of the cerebral and cerebellar white matter. Periodic acid-Schiff-positive macrophages in the absence of foamy macrophages were the histological hallmark of this process. Electron microscopy revealed oval membrane-bound cytoplasmic bodies filled with bundles of triaminar inclusions composed of 3 nm paired dense leaflets separated by a space 3–7 nm wide in macrophages. Biochemical analysis showed an increase of very long chain fatty acids in the white matter cholesterol esters. This study defines the morphologic substrate of solvent vapor abuse leukoen cephalopathy. The novel ultrastructural observations in conjunction with biochemical findings provide a link with adrenoleukodystrophy and raise the possibility of similar mechanisms of myelin degredation in both.

Key Words: Adrenoleukodystrophy; Leukoencephalopathy; Peroxisome; Solvent vapor abuse; Toluene; Triaminar inclusions; Very long chain fatty acids.

INTRODUCTION

Chronic exposure to vapors of volatile organic solvents particularly as it takes place under the circumstances of abuse can cause severe, often permanent damage to the central nervous system (CNS). Numerous clinical reports have produced evidence of an encephalopathy with manifestations of cerebellar involvement, cognitive impairment, dysfunction of corticospinal tract, visual and auditory system and abnormalities of ocular motility in solvent vapor abusers (1–18). Diffuse cerebral, cerebellar and brainstem atrophy as well as white matter disease have been documented by CT and MRI scanning (7, 10, 11, 13, 14). In contrast to the plentiful clinical information is the dearth of morphologic studies. There are only two detailed neuropathologic reports (16, 19) with partially disparate findings, each based on examination of postmortem material from a single patient. While loss of myelin in the cerebral and cerebellar white matter was present in both cases, diffuse cerebral and cerebellar cortical atrophy and giant axonal swelling was noted only in the first and small perivascular aggregates of periodic acid-Schiff (PAS)-positive macrophages only in the second. This study was undertaken to clearly delineate the pathology of solvent vapor abuse leukoencephalopathy (SVAL). It describes gross, light microscopic, electron microscopic and pertinent chemical abnormalities in the CNS of two solvent vapor abusers and also reports the ultrastructural observations in a third case, the light microscopic features of which were described previously (16).

MATERIALS AND METHODS

All tissue was obtained at autopsy and fixed in 10% neutral formalin. The following staining techniques were applied to paraffin sections: hematoxylin and eosin (H&E), Luxol Fast Blue/periodic acid-Schiff (LFB-PAS) and Bodian stain. Immunostaining with antibodies to glial fibrillary acidic protein (GFAP) and macrophage markers CD-68 and HAM-56 were carried out on selected sections using peroxidase-antiperoxidase or avidin-biotin techniques. Oil red O (ORO) and Sudan black (SB) stains were applied to frozen sections. In addition, several whole brain slabs were embedded in paraffin, cut in a Tetradicer giant microtome and the sections stained with LFB/PAS stain. Material for electron microscopy (EM) was washed in phosphate buffer, osmicated and embedded into Spurr low-viscosity embedding media (Polysciences, Inc., Warrington, PA); semithin sections were stained with methylene blue-azure II and the ultrathin sections with uranyl acetate and lead citrate.

Cholesterol esters were extracted and purified from white matter according to methods described in Theed et al (20). The cholesterol ester fatty acids were measured as methyl esters by capillary gas liquid chromatography by the method of Moser and Moser (21).

CASE HISTORIES

I) This 33-year-old male was found dead at home after being unable to eat and drink for 1 week. He had a history of paint
and glue sniffing since the age of 11. When seen as an outpatient 1 year before demise complaining of visual hallucinations, he was wheelchair bound. Neurological examination showed severe cerebellar dysfunction with disequilibrium, intention tremor and coarse nystagmus in all directions of gaze. A CT scan of the head showed generalized atrophy of the cerebrum and contents of the posterior fossa. General autopsy revealed bronchopneumonia.

2) A 41-year-old woman collapsed and died while inhaling paint fumes and ingesting an alcoholic drink. She had a longstanding history of addiction to paint sniffing. During a hospitalization for delivery 9 years previously, she was noted to have cerebellar tremor and a wide-based gait. Findings at general autopsy were unremarkable except for a fatty liver.

3) This patient had a history of 20 years of inhalant abuse. For details, see reference 16.

RESULTS

Brain weight in Cases 1 and 2 was reduced to 1,100 and 980 grams, respectively. In Case 1, gross examination revealed thinning of the corpus callosum and mild dilatation of the lateral ventricles (Fig. 1A). The central, and sometimes the convolutional, white matter of all cerebral lobes had a mottled appearance due to a gray
patchy discoloration which was ill-defined toward the periventricular white matter but sharply delineated toward the U-fibers where these were spared. Often, however, the abnormalities abutted on the cerebral cortex and in places the white matter was granular. Similar but more marked mottling was present in the cerebellar white matter, particularly in the vermis and adjacent parts of each hemisphere (Fig. 1B). In Case 2, the corpus callosum was only slightly thinner than normal, and the ventricles were of normal size. An irregular continuous zone of mild, poorly defined, pale gray discoloration was seen to extend through the central white matter of the posterior frontal, parietal and temporal lobes. The cerebellum showed a patchy discoloration similar to that in Case 1, though less intense. The brainstem was grossly normal in both cases. Light microscopy in Case 1 revealed the main abnormalities in the white matter of the cerebrum and cerebellum which was characterized by an uneven pallor in the H&E-stained slides. The LFB-PAS stain demonstrated a patchy loss of myelin in the central as well as convolutional white matter (Fig. 2). The patchiness was in part the result of severe or sometimes complete loss of myelin around blood vessels with milder involvement of more distant myelin. However, zones of myelin destruction not apparently related to vasculature also contributed to this appearance. Myelin was best preserved in
the convolitional white matter of an occasional gyrus, but even here perivascular sleeves or large zones of myelin loss were usually present. Better preservation of myelin was noted in the superficial zones of the corpus callosum, occasional U-fibers and subependymal white matter around the frontal horns of the lateral ventricles. In areas of partial myelin loss, disintegrating fibers were intermixed with well-preserved fibers. Bodian stain demonstrated a rarefaction of the axonal network in totally or subtotally demyelinated areas. The degree of axonal loss lagged behind that of myelin loss. Marked loss of myelin was accompanied by a reduction in numbers of oligodendrocytes and mild gliosis. The latter was accentuated in areas of perivascular myelin loss. Also present throughout the cerebral white matter were irregularly rounded mononuclear cells 5–15 μm in diameter. Their cytoplasm was pale hematoxyphilic or amphophilic in H&E stain and loaded with fine or occasionally courser granules which were strongly PAS-positive (Fig. 3A), argentophilic and isotropic in polarized light, and in part stained with ORO and SB. Such cells occurred singly within the parenchyma and as aggregates in the perivascular spaces where they were occasionally associated with sparse infiltrates of lymphocytes. In general, the perivascular aggregates were most prominent in areas of the most severe myelin loss while the intraparenchymal cells predominated in areas with better-preserved myelin. Irrespective of their location, the mononuclear cells were

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**Fig. 3.** A: A perivascular aggregate of large mononuclear cells loaded with PAS-positive granules in frontal white matter. Case 1. (Paraffin, LFB-PAS ×365). B: One large macrophage and a sparse lymphocytic infiltrate are located in the perivascular space. Adjacent parenchyma contains two small mononuclear cells (arrow). Cerebellum. Case 2. (Paraffin, H&E ×500).

**Fig. 4.** A: Tightly packed, pale mononuclear cells surround a cerebellar blood vessel. The white matter shows a near complete loss of myelin. Case 1. (Spurr embedding media, methylene blue-azure II ×250). B: A small group of macrophages next to a blood vessel (arrow). A number of myelinated fibers are preserved. Cerebellum. Case 2. (Spurr embedding media, methylene blue-azure II ×580).
marked with antibodies CD-68 and HAM-56. Abnormalities of the kind observed in the central and convolutional white matter were also present in the bundles of white matter fibers in the striatum, in the mammillothalamic tract and optic tract. The internal capsule was more involved in the posterior than in the anterior limb. While the network of cortical axons appeared intact in Bodian stain, LFB stain revealed only sparse myelin sheaths. The neuronal population of the cerebral cortex was intact. Occasional mononuclear cells with PAS-positive granules were found in the middle and deep layers of the cortex. In the cerebellum, there was a profound loss of myelin in the central white matter and a number of foci. This was accompanied by numerous PAS-positive mononuclear cells, moderate loss of axons and gliosis. The hilum and amidulla of the dentate nucleus were spared. In addition, a moderate dropout of Purkinje cells was noted. The brainstem showed a near total loss of myelin in the lateral and medial aspects of the peduncles, central tegmental tract and amigdala of the inferior olive. Partial myelin loss was present in the lateral lemnisci, corticospinal tracts and transverse fibers of the pons. Even in tracts with well-preserved myelin, scattered PAS-positive macrophages were present. The optic nerves were depleted of myelin in their central parts but in the superficial areas the myelin density was only moderately reduced. Cranial nerves 3, 5, 9 and 10 were histologically normal. No axonal swellings were found in any portion of the CNS. The spinal cord was not available for examination. The adrenal cortex contained no ballooned cells with striated, granular or hyaline cytoplasm. In the atrophic testes, no Leydig cells with cytoplasmic striations were found. The liver contained no PAS-positive macrophages.

Light microscopy in Case 2 revealed a pathological process which showed the same characteristics noted in Case 1, namely a loss of myelin accompanied by accumulation of PAS-positive mononuclear cells (Fig. 3B). The distribution of lesions was similar with the centrum semiovale, gyral cores and cerebellum bearing the brunt of damage. Again the various fiber tracts of the brainstem were involved, and the optic nerve was the only cranial nerve with pathology. No axonal swellings and no cerebrocortical atrophy were present. The adrenal did not contain any ballooned cells, and no PAS-positive macrophages were present in the liver. Case 2, however, differed from Case 1 in three points: 1) The loss of myelin in Case 2 was milder and more uniform, resulting in diffuse pallor with a very poor definition between severely depleted and moderately affected regions and between the latter and the normal areas. Still, the preferential damage of perivascular myelin was apparent. 2) The intensity of cellular response was much milder. Even in the regions of maximal myelin loss, the PAS-positive cells were present in numbers considerably lower than in Case 1. Also, the lymphocytic infiltrates were sparser. 3) Gliosis was more marked.

Case 3 was histologically similar to Case 2, with ill-defined demyelination of the cerebral and cerebellar white matter and collections of PAS-positive macrophages (16).

In the semithin sections of resin-embedded material, the large mononuclear perivascular and parenchymal cells had very pale cytoplasm with barely detectable granules. Also demonstrable was a variable loss of myelin (Fig. 4A, B). The large mononuclear cells in the white matter of the cerebrum and cerebellum in all three cases had an identical ultrastructural appearance. Their cytoplasm was filled with lamellar aggregates almost to the exclusion of any other cytoplasmic constituents (Figs. 5–7). Only exceptionally was a droplet of fat found among the aggregates (Fig. 6A). Under high magnification the basic unit presented as a trilaminar structure composed of two electron-dense leaflets 3 nm thick and separated by a 3–7 nm wide electron-lucent space (Fig. 7 inset). Straight or slightly curved stacks of such units were compacted into oval or angulated bodies enclosed by a membrane. In a number of bodies the membrane was only partially preserved or absent altogether. Cells in the perivascular spaces differed in no respect from those found in the parenchyma. The adrenal cortical cells in Cases 1 and 2 and the Leydig cells in Case 1 were free of lamellar inclusions.

Biochemical analysis showed an increase in the total amount of very long chain fatty acids (VLCFA) in cholesterol esters of brain from Cases 1 and 2. The increase was more marked in Case 1 (Table 1). Cholesterol ester fatty acid composition was normal in the adrenal of patient 1; it was not studied in patient 2. No material was available for biochemical analysis from patient 3.

Fig. 5. A: A portion of a mononuclear cell flattened between two layers of basement membrane in a cerebral blood vessel. Stacks of trilaminar inclusions are compacted into oval bodies. Cerebellum. Case 1. (EM ×13,100). B: The cytoplasm of an intraparenchymal mononuclear cell is filled with trilaminar inclusions aggregated into irregular bodies. Cerebellum. Case 1. (EM ×31,000).

Fig. 6. A: A plump perivascular macrophage is loaded with clusters of straight or wavy lamellar inclusions. A few fat droplets and lysosome-like bodies are also present. Cerebellum. Case 2. (EM ×10,500). B: A macrophage with clusters of lamellae in the vicinity of the blood vessel has only partially visible cell membrane. Cerebellum. Case 2. (EM ×14,700).

Fig. 7. Clusters of trilaminar inclusions distend the cytoplasm of an intraparenchymal macrophage. The cell membrane is poorly preserved. Cerebellum, Case 3. (EM ×17,550). Inset: Detail of trilaminar inclusions. (EM ×94,500).

**TABLE 1**

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All values as % of total fatty acids.
XALD = x-linked adrenoleukodystrophy; 18:0 = unsaturated C18 fatty acids; VLCFA = very long chain fatty acids (longer than C22:0).

**DISCUSSION**

The pathologic abnormalities documented in this study in the CNS of each of the three chronic abusers of solvent vapors displayed all the attributes of a demyelinating process: widespread though variably severe loss of myelin with relatively mild loss of axons, reduction in numbers of oligodendroglia, and gliosis. A constant and characteristic feature was the occurrence of macrophages loaded with PAS-positive granules in the absence of any foamy macrophages. Ultrastructurally, instead of the polymorphic contents found in the cytoplasm of macrophages engaged in breakdown of the previously normal myelin, these cells exhibited an accumulation, probably intralysosomal, of trilaminar structures composed of paired leaflets. This is a novel finding in SVAL. Macrophages of this type are known to occur in various forms of adrenoleukodystrophy (ALD) (22–25) and some related disorders (26–30). Indeed, the biochemical analysis of the white matter prompted by the similarity of macrophages in SVAL and ALD revealed in Cases 1 and 2 an increase of VLCFA, with higher values recorded in Case 1. The differences in severity of chemical abnormalities between
the two cases correlate well with the morphological findings which, though qualitatively identical in both, were more intense in the first than in the second. In spite of the strong similarities between SVAL and ALD there are differences between these two entities which allow for their separation on morphological grounds. While the loss of myelin in ALD is uniformly profound and with a sharp border toward the preserved normal areas, in SVAL the loss is variable, seldom complete and ill-defined. The lymphocytic infiltrates in SVAL are sparse and scattered while in ALD they are massive and concentrated at the active border of the lesion. While lipid-laden macrophages are present in addition to PAS-positive macrophages in ALD, in SVAL such cells are absent altogether. In ALD the abnormality of lipid metabolism is generalized resulting in changes not only in the nervous system but also in the viscera; in contradistinction, pathology of SVAL is limited to the CNS. Finally, the predilection for perivascular myelin breakdown characteristic of SVAL is not seen in ALD.

The patchy appearance of myelin in SVAL is somewhat reminiscent of the speckled myelin loss seen in Grinker’s myelinopathy (GM). Since drug abusers are at increased risk of hypoxia, it is important to stress that the differences between the two myelinopathies are such as to render improbable any role of hypoxia in causation of SVAL. Firstly, the speckled loss of myelin in GM is usually due to persistence of myelin around blood vessels (32) in contrast to the preferential perivascular myelin loss in SVAL. Secondly, foamy macrophages occur in GM (31) but not in SVAL. Thirdly, the lamellar intracytoplasmic inclusions, the ultrastructural hallmark of SVAL, do not develop in GM (33). In addition, neither the pallidal necrosis nor subtle cortical lesions which often accompany GM were noted in any case of this study (31, 34).

The consistency of pathological changes in all three cases of this study strongly suggests that they represent the substrate of SVAL which has been clinically well established for some time. The findings of neuroimaging such as brain atrophy, thinning of the corpus calosum or white matter pallor correlate well with the neuropathological observations. Similarly, the neurological deficits are well explained by the distribution and severity of the white matter changes. One might wonder why such a relatively frequent disorder with a characteristic morphology has not been observed previously more than once. The most likely reason is that although the pathology of SVAL is characteristic it is rather subtle. The mostly partial loss of myelin is as easily overlooked grossly as it is in H&E-stained sections in which the macrophages are difficult to detect unless the PAS reaction is carried out.

Toluene, the main constituent of glues, paints, thinners, lacquers and similar products, is widely held responsible for SVAL. However, other ingredients are present as well. In most instances, including the cases in this study, the exact composition of the product is not known and might differ from one abuser to another or vary during the course of exposure of one individual. Moreover, many solvent vapor abusers are addicted to other substances as well. Simultaneous exposure to several chemicals could conceivably potentiate or diminish the toxic effects of toluene, as was demonstrated in the experimental animal (35–38) and man (39–41), and modify the basic pathologic process. The minor differences among the cases of this study could perhaps be explained this way. The axonal swelling observed in a single case of chronic solvent vapor abuse (19) which was consistently absent from the material of this study was, as noted previously (16), almost certainly due to an ingredient not contained in the product used by our patients.

The mechanism of toluene neurotoxicity is unknown. The sparsity of information about the pathology of the nervous system resulting from chronic toluene exposure was one of the major impediments to the exploration of this mechanism. Not only has the morphological substrate in the human been uncertain until now, the experimental studies have also failed to disclose any characteristic abnormalities (42–44). Having established that the damage to the myelin sheath and myelin-forming cell is the essential feature of SVAL, this study gives direction to future investigations. The morphologic and chemical similarities of SVAL and ALD raise the possibility of similar mechanisms of myelin degradation in both. Specifically implicated by this association is the peroxisome, an organelle with a variety of catabolic and anabolic functions which include some of critical importance for the stability of myelin.

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REFERENCES


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