Glial Fibrillar Acidic Protein in Hepatic Encephalopathy
An Immunohistochemical Study

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Abstract. Basal ganglia, thalamus, cerebral cortex, and subcortical white matter were studied in ten cases of hepatic encephalopathy (HE), including three cases of acquired hepatocerebral degeneration (HCD), and in thirteen age-matched controls using the peroxidase-antiperoxidase immunohistochemical staining technique for glial fibrillar acidic (GFA) protein. HE cases all had pronounced Alzheimer type II astrocytosis. The perikarya and processes of Alzheimer type II glia did not stain for GFA protein. Staining of perivascular endfeet was evaluated by first selecting blood vessels throughout the gray and white matter in hematoxylin and eosin-stained slides to eliminate bias. The vessels were then identified in sections stained for GFA protein and graded as to complete circumferential, partial circumferential, or absence of staining. Both the degree and frequency of staining in the basal ganglia, thalamus, and cerebral cortex were significantly decreased in cases of HE; no statistically significant differences were found for the white matter. There were no significant differences in staining between HCD and other HE cases. These findings show that the Alzheimer II change is associated with a loss of immunohistochemically detectable GFA protein in cerebral gray matter.

Key Words: Alzheimer II cells, Astrocytes, GFA protein, Hepatic encephalopathy, Liver disease.

INTRODUCTION

The Alzheimer type II alteration, a vesicular enlargement of the nuclei of protoplasmic astrocytes with margination of the chromatin, is the principal light microscopic feature of hepatic encephalopathy (HE) (1, 2, 3), and is present in both acquired non-Wilsonian hepatocerebral degeneration (HCD) (4), and in Wilson's disease (2, 5). Alzheimer type II cells do not contain demonstrable glial fibers as determined by Cajal gold chloride impregnation (1, 3), and glial filaments are not conspicuous in ultrastructural studies of human and experimental hepatic and portocaval encephalopathy (6, 7, 8) or Wilson's disease (5). Several lines of evidence indicate that the astrocyte-specific protein, glial fibrillar acidic (GFA) protein, is the principal subunit of glial filaments (9, 10). In this study, paraffin sections from brains of patients who died with HE and HCD

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625
and from age-matched controls were stained for GFA protein by the peroxidase-antiperoxidase (PAP) immunocytochemical method of Sternberger. Alzheimer type II glia did not stain for GFA protein and perivascular astroglial endfeet in cerebral gray matter had markedly reduced or absent staining.

MATERIALS AND METHODS

Case Material

Ten cases with clinical and pathological diagnoses of cirrhosis and HE with numerous well-developed Alzheimer II cells throughout the gray matter were selected. Three of these had clinical HCD, with vacuolation of the deep cerebral cortex and basal ganglia. Thirteen age-matched controls did not have clinical or pathological evidence of liver disease.

The mean age of patients in the HE group was 55.9 years (range 48 to 70 years) and in the control group 55.8 years (range 41 to 64 years). One in each group was a female. The mean interval between death and autopsy was 16 hours (range 2 to 43 hours) in the HE group and 18 hours (range 3 to 68 hours) in the controls. Three cases in each group had hypertensive cardiovascular disease. Status cribrorum was present in the basal ganglia in two cases each of the control and HE groups. Ferrugination of vessels in the globus pallidus was present in two of the HE and three of the control cases. Rare clumps of hemosiderin pigment were noted around vessels in the basal ganglia in both groups.

All brains were fixed by immersion in formalin for a minimum of two weeks and embedded in paraffin according to standard histological methods.

Staining Methods

Six-micron-thick sections were obtained from frontoparietal cortex and subcortical gray matter. The Sternberger PAP technique (11), as modified by Taylor and Burns (12), was used to obtain specific staining of tissues, as described previously (13). The preparations of specific antisera to GFA protein (13) and myelin basic protein (14) have been described. Antisera dilutions were as follows: anti-GFA, 1:250; anti-myelin basic protein, 1:250; preimmune rabbit control serum, 1:100.

Evaluation of Staining and Statistics

The cell bodies and processes of Alzheimer type II glia were evaluated for GFA protein staining. Perivascular astrocytic endfeet lent themselves to quantitative as well as qualitative analysis. To quantitate the latter, vessels were first selected from hematoxylin-and-eosin-stained sections to eliminate sampling bias. Forty vessels were marked at random at all levels of the cortical gray matter in each case, and 20 vessels were marked in each caudate, putamen, globus pallidus, and thalamus. Forty vessels were also marked at random in the subcortical white matter. A larger proportion of capillaries was selected in the cortical and subcortical white matter, since fewer large-caliber vessels were present in those locations, as compared to the basal ganglia and thalamus. The vessel marks were then traced to the corresponding serial section stained for GFA protein. Each marked vessel was evaluated for perivascular endfoot staining and the resulting data were analyzed by two different statistical tests. First, perivascular staining was classified as either completely circumferential, partially circumferential, or absent, and the number of vessels in each category was counted. The statistical significance of this type of data was tested by ridit analysis (15). For the second method of analysis, the number of vessels that stained (completely and partially circumferential) was calculated as a proportion of the total and Student's t test (16) for small samples was
used to determine significance. The data obtained for each subcortical nuclear group were analyzed individually and as a combined "basal ganglia and thalamus."

RESULTS

The PAP technique for GFA protein stained many astrocytes in both the gray and white matter in control cases. Their processes, the subpial glia limitans, and perivascular endfeet stained strongly. The most intense and consistent perivascular staining occurred around lenticulostriate arteries and their branches (Fig. 1).

In cases of HE, a generalized loss of GFA protein was found throughout the gray matter but not in the white matter. Except for perinuclear lipofuscin pigment, Alzheimer type II astrocytes had no visible cytoplasm or fibrils in hematoxylin-and-eosin-, Holzer- or PTAH-stained sections and did not stain for GFA protein (Fig. 2). One HE case had a small focal cortical infarct in which prominent staining of the perikarya and processes of the surrounding fibrillary astrocytes occurred in the absence of staining of adjacent Alzheimer type II cells (Fig. 3). The loss of GFA protein staining from the gray matter was particularly evident in perivascular endfeet, as well as in the subpial glia lim-

Fig. 1. Control case. Lenticulostriate vessel showing complete circumferential staining of perivascular glial processes. GFA protein-PAP stain with hematoxylin. ×113.
Itans. Perivascular staining was not only less intense and more frequently absent in HE cases but, when present, was more often partially circumferential.

The two statistical methods of evaluating the frequencies of perivascular staining gave the same results. There was no statistically significant difference in staining between HCD and other HE cases. Mean and standard deviations of perivascular staining frequencies of control and encephalopathy groups, expressed as percentages of vessels evaluated in each case, are presented in Figure 4. Significantly lower mean staining frequencies were found in the basal ganglia, thalamus, and cerebral cortex in HE (p < .001) than in controls. Comparable differences between control and HE cases were found when nuclear groups were evaluated separately. The frequency and degree of perivascular staining were less in the cortex than in the basal ganglia and thalamus in both groups. There was no significant difference in staining of subcortical white matter (p > 0.1), although a slightly lower mean score was found in cases of HE (Fig. 4).

The Holzer and PTAH stains showed absence of fibrillary gliosis in both the HE and control groups except for delicate glial fibers in the region of large
blood vessels. This latter delicate gliosis was even less marked in the HE group.

The preimmune control serum did not stain astrocytes and gave only slight, nonspecific staining of connective tissue, especially the adventitia of vessel walls. Antiserum to myelin basic protein yielded prominent staining of myelinated fiber tracts of equal staining intensity in both groups.

DISCUSSION

Control and HE groups had nearly equal distributions of hypertensive arteriosclerotic disease, ferrugination of vessels in the globus pallidus, and status cribrosus. Cases with these findings did not have significantly different perivascular staining in the gray matter when compared to other cases within the two groups. These factors, therefore, did not account for the differences in GFA protein staining between HE and control groups.

The postmortem intervals were approximately the same in the two groups, so that postmortem autolysis would be expected to be the same. It is, however, possible that metabolic derangements in the HE cases might make the GFA
protein in Alzheimer II cells more susceptible to postmortem autolytic changes. Altered antigenicity, e.g., because of binding of ammonium ion to GFA protein, is a possible explanation for the difference in staining. However, astrocytes in the white matter showed the same range of staining in HE and control cases and strongly staining fibrillary astrocytes in a scar were found adjacent to non-staining Alzheimer II cells (Fig. 3). These findings militate against a generalized depression of GFA protein staining in the HE cases. Staining for myelin basic protein was the same in both groups; it is therefore unlikely that interference with the PAP reactions took place after the initial specific antigen-antibody step.

Increased permeability of the astrocytic cell membrane to horseradish peroxidase has been demonstrated in the rat portocaval anastomosis model of HE (17). A loss of cytoplasmic GFA protein might occur through similarly leaky membranes in the human condition. A general decrease in soluble brain
protein, particularly from areas of gray matter, has been described in human HE (18), although the authors felt that the loss was primarily neuronal. GFA protein has been found in the cerebrospinal fluid of alcoholic patients, some of whom may have had hepatic disease (19). Solubilization of GFA protein by ammonium ion, or other toxin which accumulates in the brain in HE, might enhance this loss.

It has long been known (1) that the astrocytic reaction in hepatic encephalopathy and Wilson's disease is very different from the astrocytic reaction leading to fibrillary gliosis in response to necrosis, demyelination, or nerve cell loss. This study shows that not only do Alzheimer II cells fail to form increased numbers of glial fibers, but they also display an actual decrease in GFA protein.

Normal protoplasmic and fibrous astrocytes, reactive fibrillary astrocytes, and neoplastic astroglia cells, which may or may not have demonstrable glial fibrils, all stain for GFA protein (9). Therefore, the loss of immunohistochemically detectable GFA protein from the perikaryon and processes of cerebral gray matter astrocytes in HE is a unique alteration and provides further support for the concept of compartmentalization of astroglial function (20, 21, 22).

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REFERENCES


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