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ELECTRON MICROSCOBIC EVALUATION OF BRAIN EDEMA IN RATS WITH CARBONTETRACHLORIDE-INDUCED HEPATIC FAILURE

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Karbontetraklorürle Hepatik Yetmezlik Oluşturulmuş Sıçanlardaki Beyin Ödeminin Elektron Mikroskobik Değerlendirilmesi

ÖZET

Karaciğer yetmezliğin en büyük komplikasyonu beyin ödemidir ve hasta ölümlerinin büyük bir yüzdesinden sorumludur. Bu çalışmada, karaciğer yetmezliğine bağlı olarak, beyin dokusunun elektron mikroskobik yapısı incelenmiştir.

Karbontetraklorüre bağlı karaciğer yetmezliğinde, sıçan beyin dokusunda sıvının aşırı birikimini açıkca gözlemledik. Astrositlerin perivasküler uzantılarının sitoplazmasında görülen şişkinlikler belirlendi. Beyinin diğer hücresel bileşenleri normal morfolojiye sahipti. Kapiller endotel hücrelerinin bazal laminaları kalınlaşmıştı. Beyin ödeminin histolojik olarak kanıtı olan, sıvı birikimi ve astrositlerin şişmesi bu modelde gözlendi.

Astrosit alanlarının ödemli olduğu görüldü. Endotel hücrelerinin bazal laminası kalınlaşmıştı. Bazı miyelinli lifler dejeneratif değişiklikler gösterdi. Bu bulgular hepatik yetmezlikte, beyinde ödemin gelişimini destekler. Astrositlerin zedelenmesi veya işlevlerinin kaybolması, hepatik ensefalopatinin patogenezisinin ortaya çıkmasına neden olabilir.

Anahtar Kelimeler: Beyin, Karbontetraklorür, Mikroskopi.

SUMMARY

Brain edema is a major complication of hepatic failure and responsible for death in a large percentage of patients. In this study, we reported the electron microscopic structure of brain tissue in hepatic failure.

We previously demonstrated the progressive accumulation of water in the rat brain with carbontetrachloride (CCl4) -induced hepatic failure. Marked swelling of the cytoplasm, perivascular processes of astrocytes were noted. The other cellular components of the brain had normal morphology. The basal lamina of the capillary endothelial cells had thicked. Histologic evidence of brain edema is seen in this model, with swelling of astrocytes as the primary manifestation of accumulation of water. Astrocytes areas were marked to be edema. Some myelinated fibers were showed degenerative changes. These findings support that develops edema in the brain in hepatic failure. Damage to astrocytes or inhibitions of their function may contribute to the pathogenesis of hepatic encephalopathy in this model.

Key Words: Brain, Carbontetra chloride, Microscopy.

INTRODUCTION

Hepatic failure results in a complex clinical syndrome with profound encephalopathy (1). A major complication is development of brain edema, a finding which has frequently been implicated as a cause of mortality (2). Brain edema and its relationship with encephalopathy have not been definitively established.

Reports suggest that the brain edema with fulminant hepatic encephalopathy induced by the spesific hepatotoxin carbontetrachloride, ammonia, aminoacids, neurotransmitters, other possible toxins is responsible for brain edema (3).

Histologically, varying degrees of cerebral edema are found in 25-50 % of patients who have died of fulminant hepatic failure (2).

In the patients dying after chronic or recurrent encephalopathy have been seen hypertrophy and hyperplasia of astrocytes in the cerebral cortex. In the experimental animals with hepatic encephalopathy has shown abnormalities the microtubules in astrocytes. Microtubules are active in intracellular transport. Similarly but less has marked changes occured in oligodendrocytes (3).

Reese and Karnovsky (4) attributed the impediment to the passage of horse radish peroxidase (HRP) across the cerebral parenchyma following its injection into the blood stream of the adult mouse to 2 characteristic features of the endothelium of cerebral capillaries. The first was the presence of endothelial tight junctions which did not appear to be involved in transendothelial transport from blood to brain. This wiew confirmed by Reese (1967) who showed that endothelial and epithelial tight junctions occluded the spaces between blood and brain parenchyma or cerebral ventricles, thereby constituing a structural basis for the blood-brain and blood-cerebrospinal fluid barriers. Pericytes associated with the capillary probably play a role as phagocytes in maintaining the function of the blood-barrier by trapping any serum derived foreign substances with astrocytes having a regulatory role in the formation of the barrier (4). GFAP immunostaining showed that, outer surface of the pericytes are covered by the astrocytic end-feet (5).

Brain edema can be classified into two major types: cellular (cytotoxic) and vasogenic. Cellular edema occurs when there is swelling of cellular elements due to impaired osmoregulation; water accumulates predominantly in the brain tissue. Vasogenic edema, there is break down of the blood-brain barrier with entry of plasma into the interstitial space and water accumulation predominantly (6). In many clinical situations, there is combination of both mechanism. Morphologic examination of the brain with electron microscopy following infusion of electron dense tracers is an important step in defining the mechanism (7). Traber et. al. Shown that cortical gray matter was the main site of water accumulation in the rabbit with hepatic failure induced by galactosamine. They noted that ischemic injury to the brain can result in astrocyte swelling of all cell types in the brain, including endothelial cells (8).

The aim of this study was to determine ultrastructural alterations in the brain on rats with CCl4-induced hepatic failure.

In conclusion, the brain edema which occurs in the rat with CCl4 –induced hepatitis appears to develop primarily as a result of the accumulation of intracellular water in astrocytes. Similar abnormaiaties noted in astrocytes in models of chronic encephalopathy raises the possibility that brain edema in hepatic failure may be related to degree elevation of rate of accumulation of a circulating toxins.

MATERİAS AND METHOD

Hepatic failure was induced in 10 male Wistar rats, weigthing approximately 180-200 g and housed in wire-floored cages with free access to food and water. Rats divided into two groups, control and test group.

CCl4 which was prepared with mixing of olive oil (4:3), was injected subcutaneously with 0.15 ml/100g three times per week. Controls were similarly injected with oliveoil during the same periods. After injection, animals were sacrificed under anesthesia and perfused by gluteraldehyde solution in phosphate buffer. The brain was rapidly extracted from the skull. The brains were

removed, tissue samples were post fixed in osmium-tetroxide and processed according to routine electron microscopic prosedures. The semi-thin sections were stained with Tıluidine Blue, and thin sections were stained with Lead citrate-Uranyl acetate. They were photographed with a Jeol 100 CX II electron microscope.

RESULTS

Brain tissue was showed normal structure in the control group examination of light and electron microscopy (Figure 1,2).

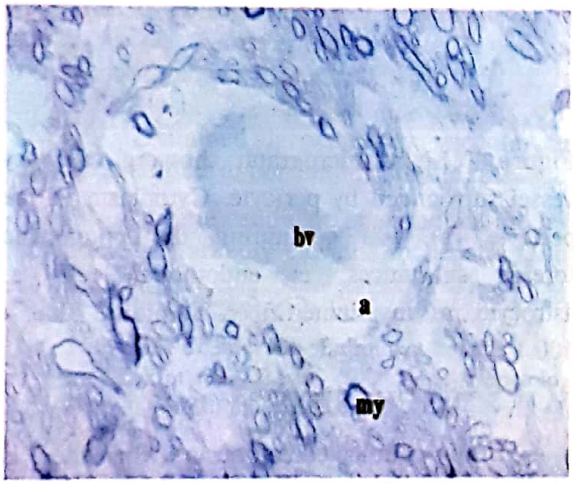

Figure 1. . The brain tissue of the control groups. A:astrocyte, B:Blood vessel, my: myelinated fiber. Toluidine Blue. x 100.

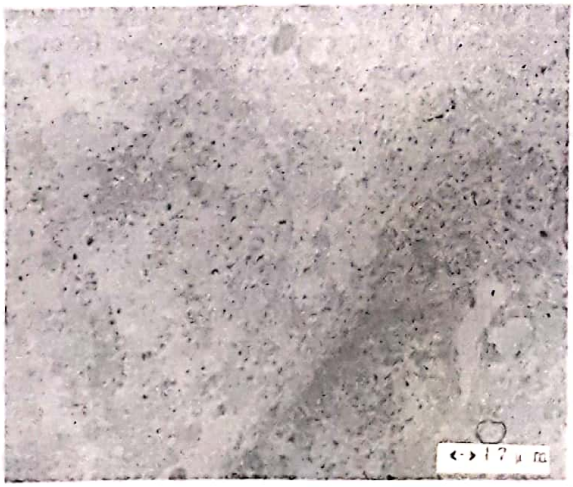

Figure 2. Electron micrograph of the control brain tissue in the normal structure. Lead citrate_Uranyl acetate. x 7000.

Following the injection CCl4 three times a week (0.15 ml/g i.p.), rats developed encephalopathy. The ultrastructural abnormalities were confined to astrocytes in the CCl4 groups.

There were marked swelling of the pericapillary foot processes of astrocytes (Fig 3).

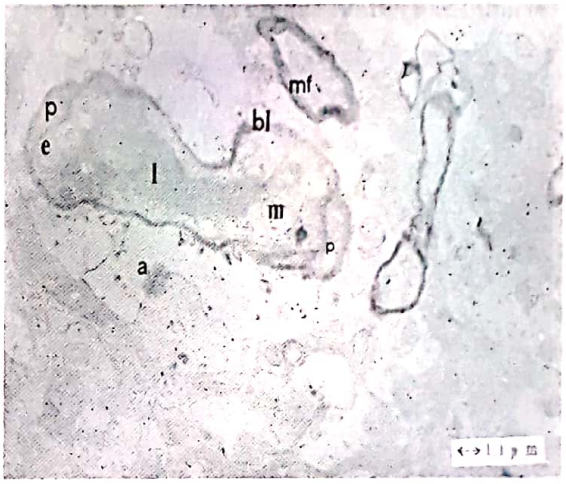

Figure 3. Electron micrograph showing a capillary vessel surrounded by greatly dilated astrocytic foot proses (a). p:pericyt, bl: basal lamina, e: endothelial cell, l:lumen. M: mitocondrium, mf: myelinated fiber. Lead sitrate-Uranyl acetate. x 7000.

However, the changes were not limited to this area of cell. In the initial phase of astrocytic swelling, vacualisation could be best observed in the perinuclear area of the cytoplasm even in astrocytes at a distance from vessel walls. The nuclear morphology of astrocytes was normal (Fig 4).

Some of the axons were seen presenting mild floccular changes in their axoplasm. In the myelin sheath surrounding axons there were distent (Fig 5).

These changes may be by development of the demonstrated edema. The basal lamina of the pericytes and the endothelial cells lining capillaries had thicked. There are vacuolles (v) in the astrocyte foot processes (Fig 6).

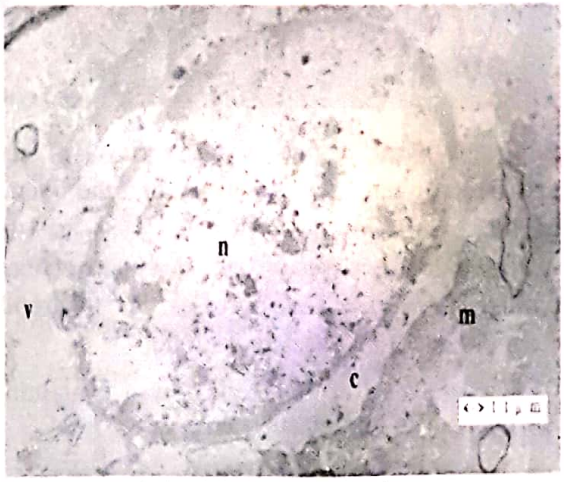

Figure 4. Electron micrograph showing an astrocyte with normal nucleus (n) and distended

watery-appearing portion of cytoplasm (c) around the nucleus. Membranous material is present in the abnormal cytoplasm. m: mitochondrium. v: vacuol. Lead citrate-Uranyl acetate. x 7000.

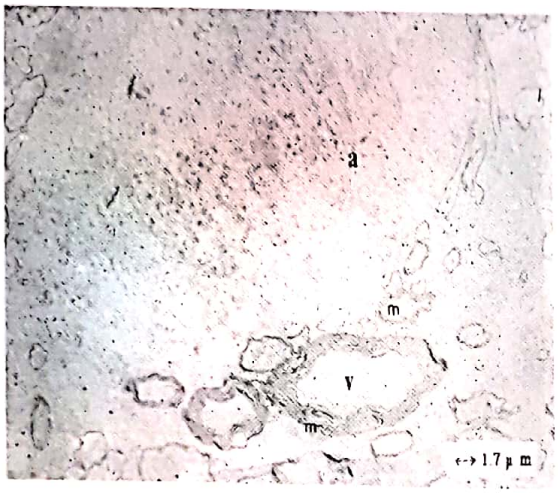

Figure 5. Electron micrograph showing an astrocyte (a). Myelinated axons (m) are present in the lower sides of this photograph. Both of the myelin and axon are appearing degenerative changes in content. There are vacuolles (v) in the axons. In the myelinated sheaths (m) are present distents. Lead citrate-Uranyl acetate. x 4400.

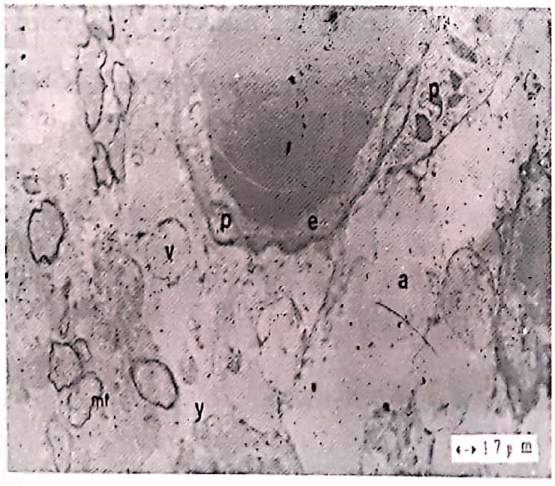

Figure 6 Basal lamina of the pericyt (p) and endothelial cell (e) thicked in the some capillaries. There are vacuolles (v) in the astrocyte foot processes. mf: myelinated fiber, a:dilated astrocytic foot prosses. n: neutropil. Lead citrate-Uranyl acetate. x12000.

Cytoplasm of the pericyt was containing the phagocytic foreign substances (Fig 7).

As a result, it is observed that hepatic failure induced with CCl4 by present dose does not disturb blood-brain barrier significantly.

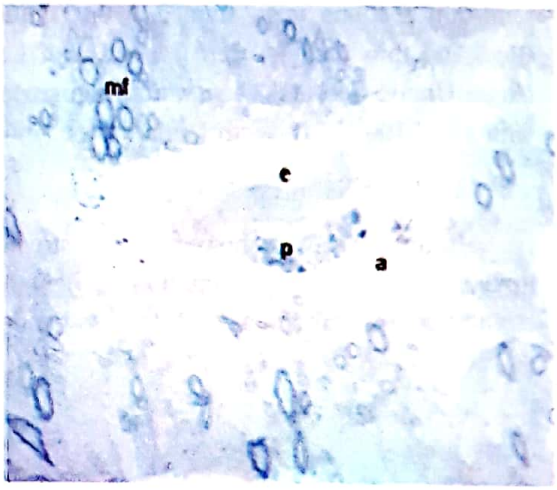

Figure 7 Light micrograph showing capillary vessel surrounded by pericyte. Cytoplasm of the pericyte (p) was containing the phagocytic foreign substances. e: endothelial cell. a: astrocyte, mf: myelinated fiber. Toluidine Blue. x 100.

DİSCUSSİON

Brain cellular edema occurs when there is swelling of cellular componenets due to spoiled osmoregulation; water accumulates predominantly in gray matter areas (9). In vasogenic edema; there is breakdown of the blood-brain barrier, with entry of plasma into the interstitial space and water accumulation predominantl in the white matter (9). In many clinical situations, there is a combination of both mechanism; however, determination of the primary disturbance can lead to understanding into mechanism and therapy. Histologic examination of the brain with electron microscopy is an important step in defining the mechanism (10).

The regional difference in astrocyte swelling may be due to originally differences in the astrocytes from the two areas, which has been shown by Norenberg 10).

In this study, we demonstrated marked swelling of astrocytes in the white matter, with no differences in gray matter areas. It should be noted that CCl4 injury to the brain can also result in astrocyte swelling.

The blood brain-barrier did not impaire but the basal lamina of the some brain endothelial cells were thicked. The swelling in the cytoplasm and other processes distant from capillaries

indicates that the entire astrocyte was affected. These observations confirm the occurrence of brain edema. There is swelling of all cell types in the brain, including endothelial cells (12). The selective effect on astrocytes in this model appears to be single. The changes on brain, histology in animal models of hepatic failure were most not observe separations between tight junctions at endothelial cells.

Norenberg and Lapham (13) and Traber et al. (8) have studied a putative model of chronic encephalopathy. In rats with mild encephalopathy, there was an increase in mitochondria marked and included swelling of astrocyte cytoplasm and foot processes. In our study, we did, rough endoplasmic reticulum and cytoplasmic matrix in the gray matter astrocytes. With the onset of coma, the astrocytes become swollen, contained large empty vacuoles and appeared similar to our findings in the rat.

A prime candidate is ammonia, the level of which is markedly elevated in hepatic failure (15). Other potential toxins or metabolic products include mercaptans, phenols, free fatty acids and glutamate (16).

We found marked swelling of astrocytes areas and endothelial cells with thicked basal lamina. Further, myelinated fibers were showing degenerative changes. These findings support that brain edema develops by cellular swelling in this model of hepatic failure.

In conclusion, the brain edema which occurs in the rat with CCl4-induced hepatitis appears to developed primarily as a result of the accumulation of intracellular water in astrocytes. Similar abnormalities noted in astrocytes in models of chronic encephalopathy raises the possibility that brain edema in hepatic failure may be related to degree elevation of rate of accumulation of a circulating toxins.

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