LIPID PEROXIDATION LEVELS IN THE BRAIN AND CEREBROSPINAL FLUID, FOLLOWING BILATERAL INTERNAL JUGULAR VEIN OCCLUSION AND RECIRCULATION

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SUMMARY : Temporary internal jugular vein ligation was employed to mimic surgical procedures carried out in operations involving the basis of cranium or neck, and clinical pictures like thrombosis of venous sinuses. Our purpose was to evaluate whether lipid peroxidation of the brain and cerebrospinal fluid (CSF) increased following bilateral internal jugular vein ligation and recirculation.

Bilateral jugular vein ligation was performed in anaesthetised guinea pigs. Lipid peroxidation activity quantified as the thiobarbituric acid reactive material (malondialdehyde, MDA) level was assessed in the brain tissue spectrophotometrically and in the cerebrospinal fluid fluorometrically. Samples of similar contralateral brain structures were investigated with light microscopic histological techniques.

Lipid peroxidation activity of the brain decreased after ligation when compared with the sham operated group, and it increased up to the control values after recirculation. On the contrary, lipid peroxidation activity of the cerebrospinal fluid increased following ligation and decreased after recirculation.

These results suggested that, such an one and two hours indirect ischemic model based on increased intracerebral pressure and extravasation did not cause an increase of the lipid peroxidation, probably due to the impaired circulation resulting in low PO_2 levels. However, following recirculation, there was a moderate increase of lipid peroxidation, due to increased PO_2 levels. Where as MDA contents of the CSF increased following one and two hours ligation, because it couldn't transport away by the venous circulation; it decreased by two hours recirculation.

***Key Words :** Jugular Vein Ligation, Cerebral Ischemia, Malondialdehyde, Cerebrospinal Fluid.*

INTRODUCTION

Bilateral jugular vein ligation (BJL) model should be reserved to mimic those cases in which sinus thrombosis (18), and septicemia do not respond to initial surgery and intravenous antibiotics (19). Occasionally there may also be occlusion in some major cerebral veins or dural sinuses. These maladies block cerebral venous outflow (8), increase intracranial pressure (ICP) (24) and decrease the absorption of cerebrospinal fluid (SCF); resulting in vasogenic brain edema (10, 12). However, in the development of vasogenic brain edema, there are also some other factors that must be taken into conside-

ration like enhanced vascular permeability and metabolites such as bradykinin, histamine, serotonine, arachidonic acid and free oxygen radicals (FOR) (9).

Radicals are species containing one or more unpaired electrons. They are formed during the metabolism of all aerobic cells (9) and are rather useful especially in protective mechanisms. For example microglial FOR generation could serve as a protection against infective organisms in the CNS (4). Any type of tissue trauma (including ischemia) leading to arachidonic acid release, can amplify the production of FOR (3, 13, 14). An important consequence of FOR formation is the lipid peroxidation (21). With lipid peroxidation the membrane viscosity and rigidity are increased (5) which enhance permeability (21) and give rise to brain swelling-edema (2, 7). The purpose of this study was to evaluate whether an overt peroxidation of membrane lipids was induced due to secondary ischemia induced by venous ligation.

MATERIAL AND METHODS

The experiments were performed in Gazi University, Faculty of Medicine, Department of Neurosurgery, on 50 guinea pigs of either sex, each weighing 300-400 gr. Seven of them had died during anaesthesia induction or operation. Anaesthesia was induced with urethane, 700 mg/kg, IP. The animals were tracheotomized and bilateral internal jugular vein occlusion was performed (under Zeiss, OPMI 9-FC microscopy) for one and two hours, using an atraumatic clamp in the experimental groups 1 and 2 consecutively. These animals constituted the ligated groups. In groups 3 and 4, the clamp was removed away after one or two hours of occlusion to let the brain get recirculated for two hours. These animals constituted the recirculated groups. The controls had no occlusion but were sham operated.

Immediately following one and two hours occlusion and two hours recirculation, cisterna magna was punctured with a 19 gauge needle and clear CSF was withdrawn. Then, the animals were decapitated after a high dose of urethane anaesthesia. The brains were immediately harvested and after being washed in physiological saline solution, specimens were obtained from one hemisphere by coronal sections contining tissue from cortical, subcortical, basal ganglia and brain stalk regions. Then, these were frozen by pouring liquid nitrogen over them in a plastic container so as to terminate the ischemic effects (23).

The extent of lipid peroxidation activity in the brain tissue was estimated spectrophotometrically (Shimadzu, UV-1208 spectrophotometer) using the thiobarbituric acid (TBA) test for malondialdehyde (MDA), utilizing the technique described by Uchiyama (20) and in CSF fluorometrically (Jasco FP-550 spectrofluorometer) using the modified technique of Yagi (1). Data were analysed by Student's t test.

Specimens from the homologous regions of the other hemisphere were obtained consequently and immediately fixed in a 10 % formaldehyde solution for light microscopic investigations.

RESULTS

MDA levels of the brain are shown in table 1. MDA levels were lower than the controls (sham) in the occluded groups (both 1h BJVL and 2h BJVL), and higher than the occluded groups in the recirculated groups (Table 1). MDA levels of the CSF are shown in Table 2. Because there were no significant differences between the one hour and two hours ligated groups; both were pooled into one ligated and one recirculated group. MDA levels of the CSF samples were high in the occluded groups and low in the recirculated groups.

Histological findings observed in the brains of the BJV occluded and recirculated groups are shown in the figures 1, 2, 3, 4. In the one hour occluded group there was no signs of edema except some arteriolar dilatation (Fig. 1) and in the one hour occlusion followed by two hours reperfusion group, edema signs were minimize degree and were only around the capillaries (Fig. 2). There were various signs of edema in the two hours occluded group, (Fig. 3) while these signs were scarce in the two ho-

Groups	n	Mean ± SD
Sham	6	90.00 ± 4.69^{a}
1h BJVL	10	79.80 ± 2.82^{b}
1h BJVL & Recir	13	81.77 ± 4.62^{c}
2h BJVL	7	80.43 ± 4.82^{d}
2h BJVL & 2h Recir	7	85.71 ± 3.64^{e}

Table 1 : MDA levels of brain, following sham operation, bilateral jugular vein ligation (BJVL) and recirculation (nmol/g).

a-b, a-c, a-d $p < 0.05$; a-e $p > 0.05$

Groups	n	Mean ± SD
Sham	6	62.70 ± 3.65^{a}
BJVL	6	75.68 ± 6.10^{b}
Recir	6	47.12 ± 7.67^{c}

Table 2 : MDA levels of CSF, following sham operation, BJVL and recirculation.

a-b, a-c, b-c $p<0.05$

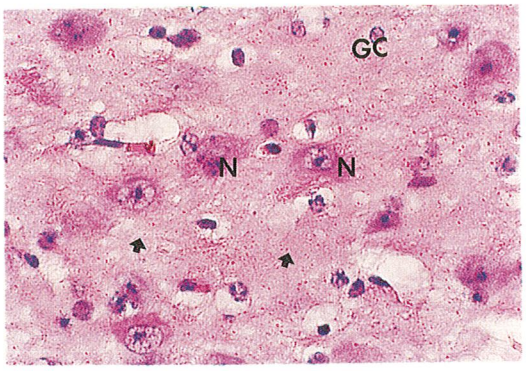

Fig - 1 : In the group of one hour occlusion, neurons (N), intercellular area (arrows), and glial cells (GC) are shown; demonstrating that arteriolar dilatation has started. Toluidin blue x400.

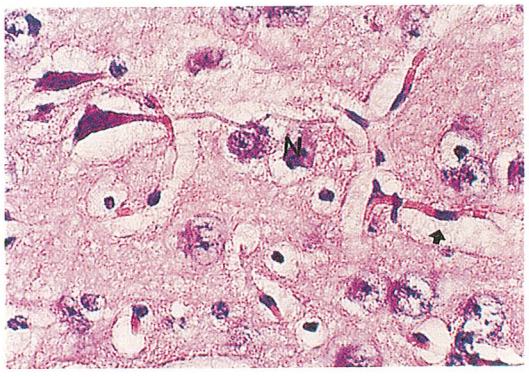

Fig - 2 : In the group of one hour occlusion followed by two hours reperfusion. A minimize degree edema is observed prominently around the capillaries (arrow) and the interstitial tissue seems to be almost normal. Toluidin blue x400.

urs ligated and two hours recirculated group (Fig. 4).

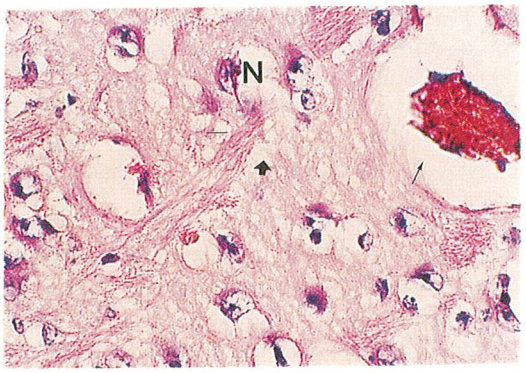

Fig - 3 : In the group of two hours occlusion, neuron sizes are small and due to the edema, they are separated from the interstitial tissue (N). Perivascular area is also strikingly edematous (arrow), while intercellular area is totally degenerated (thick arrow). Toluidin blue x400.

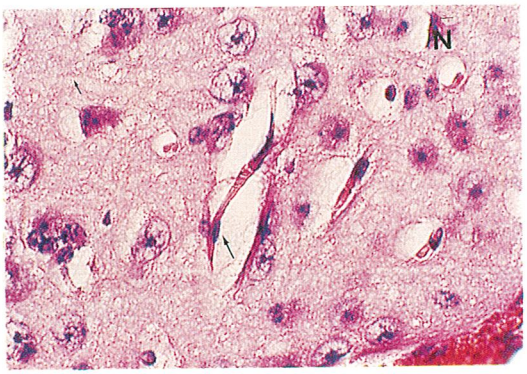

Fig - 4 : In the group of two hours occlusion followed by two hours reperfusion, neurons seem to be almost normal, while in the periphery of some neurons, edema can be observed (N). Interstitial tissue seems almost normal, with edema surrounding the vessels (arrow). Toluidin blue x400.

DISCUSSION

Jugular veins play a most important role as the route of venous outflow from the brain. Occlusion of them impair venous outflow, increase ICP and decrease the absorption of CSF. CSF malabsorption is related to the lowered pressure gradient between ICP and intracranial sinus pressure which leads to vasogenic edema (10).

In histological studies, we observed signs of

edema in the brain tissue of the ligation group (Fig. 3), consistent with previous reports (10, 12). However, the mentioned edema signs were scarce in the recirculated group (Fig. 2 and 4). MDA levels of the one hour and two hours occluded groups were lower than sham operated controls; although there was a significant increase up to the control levels after recirulation, again consistent with previous reports (1, 11, 15). Considering MDA levels of CSF; they were higher than the control levels in the occluded group while lower in the recirculated group.

These results suggest that, BJVL, impairing CSF reabsorbtion, caused brain edema but as mentioned before by Asano in 1987, there is scarce evidence that, edema is related to increased lipid peroxidation (1). Consistent with these data, MDA content of the brains in the occluded group was found to be decreased in our experiment; which may probably be due to the impaired circulation resulting in low PO_2 levels (17). Increased amount of MDA values of the CSF may be due to impaired absorption of CSF. It was proposed that in acute hypertension of the cerebral vasculature, release of arachidonic acid from phospolipids is the initial event, stimulated by activation of phospolipase A_2. The arachidonic acid then is metabolised by cyclooxygenase to PGG_2 and thence to PGH_2 by PG hydroperoxidase, producing O_2^-. Following recirculation, there was a moderate increment in MDA content of the brain, probably due to the increased PO_2 levels (11, 17). On the other hand the charged O_2^- molecules probably escape via an anion channel in the cell membrane to enter the CSF and brain extracellular fluid (17).

So it is found that, MDA levels were high in the CSF of the occluded group, but low after recirculation. Because the primarily one-way flow of CSF from the ventricular system, around the spinal cord into the subarachnoid space around the brain, and into the venous sinuses is a major way potentially harmful brain metabolites are removed (16).

Gaudet et al (1980) showed that, free radical scavengers such as ONO 3141 and AVS reduced edema and improved CSF absorption (6). However, Asano (1987) pointed out that the, results of various experiments were not consistent, considering the lipid peroxidation status during or following cerebral ischemia (1).

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