MICROVESSELS OF LIVER AND BRAIN AT CHRONIC STRESS

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The system of microcirculation is the first link that is involved in pathological process at different extremely situations. Stress is followed with disturbance of microcirculation, development of organs' hypoxia and oxidative stress. The aim of this study is to show the type of angiogenesis in liver and brain under the influence of chronic variable physical stressors. In this study the influence of different stressors such as forced swimming inhalation, restraint stress, cold stress, orthostatic shock and food deprivation on the microcirculatory stream of liver and brain was observed. Investigation of sections of liver and brain yielded proof of changes of microvessels on chronic stress (CS). Thus CS influences a reorganization of blood vessels of liver and brain and it causes angiogenesis of lever sinusoids and brain capillaries.

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INTRODUCTION

Stress is a non-specific general mobilized answer of organism to any kind of irritant that disturbs its homeostasis. Stressor means all irritants that really threaten homeostasis such as pain, hypoxia, hunger, infection and many other extreme factors.

Blood vessels are intricate networks of hollow tubes that transport blood throughout the entire body. The system of microcirculation is the first link that is involved in pathological process at different extreme situations.[1] Stress is followed with disturbance of microcirculation, development of hypoxia of organs and oxidative stress.[17] Hypoxia is thought to be a primary trigger of angiogenesis. Vicarious angiogenesis sprouts[21] as an answer to hypoxia through hypoxia-inducible factor and following induction as a result of appropriate gene expression of the main growth factor - vessel endothelium growth factor (VEGF).

Angiogenesis is a process of growth of new capillaries from the existing ones through capillary sprouting or intussusception.[4,18] Non-sprouting angiogenesis by means of intussusception (growth within itself) is an important mode of capillary formation and it is termed intussusceptive microvascular growth.[2,6,16] Comprehension of the structural and functional characteristics of hepatic microcirculation can help to improve the design, planning and practice of image-guided treatment for hepatic tumors and for portal vein embolization (PVE).[23] Brain vessels are the most important structures in the brain to deliver energy and substrates to neurons.[7] Brain vessels are composed of a complex interaction between endothelial cells, pericytes and astrocytes, controlling the entry of substrates into the brain. Damage of brain vessels and vascular impairment are general pathologies observed in different neurodegenerative disorders including e.g., Alzheimer's disease.[1]

The aim of this study is to show the type of angiogenesis in liver and brain under the influence of variable physical stresses.

EXPERIMENTAL

General

Powdered paraformaldehyde, OsO_4, sodium cacodylate trihydrate, 96 % ethyl alcohol, acetone, Epon 812, Epon Hardener MNA, Epon Hardener DDSA, Epon accelerator DNP-30, uranyl acetate, sodium citrate, lead nitrate and all other reagents used were of analytical grade and purchased from Sigma Chemical Co. (USA).

All procedures involving animals were approved by the Institutional Review Board\ Institutional Animal Care and Use Committee (H. Buniatian Institute of Biochemistry, Yerevan, NAS RA) and Ethics Committee of the National Academy of Sciences, the Republic of Armenia, and they were conformed to the European Communities Council's directives (86\609\EC).

After overnight fasting, rats were subjected to chronic variable physical stress (CVS). Animals underwent CVS regimen for 14 days consisted some times of twice a daily exposures to alternating stressors.[9] Animals were divided into three groups: one group served as control and the other two as CVS-exposed groups - after CVS (stress group) 6 animals and four days later (post stress group) 6 animals.

Treatment of material

At the end of the experiment, small pieces of the liver and brain have immediately put in cold 4 ºC mix of paraformaldehyde in a sodium cacodylate buffer and glutaraldehyde for 12 h with post fixation in 1% OsO_4 solution for 2 h, dehydration in ascending series of spirits, saturation in a mixture of acetone and Epon resins of different proportions and pouring in gelatinous capsules into Epon.

Observation under light microscope

Semithin epoxy sections thickness to up1 μm were made using ultracut LKB (Swedish) and Reichert (Austria). The semithin epoxy sections were stained with Azur 2 and observed under the light microscope with resolution by volume x40 x10 ocular lens.

Results

In this study the influence of different stressor such as forced swimming, inhalation, restraint stress, cold stress, orthostatic shock and food deprivation on the microcirculatory stream of liver and brain was observed. Samples of tissues of control, first and second group of experimental animals were analyzed.

As have shown the results of our study visually the condition of animals in two groups was different. In the first group just after the termination of the experiment the fell? of rats was white and in normal state, liver was a little red and the brain of all animals was with hemorrhage. In the second group of animals after four days of termination of the experiment the fell? looked crumpled and not very white. The liver was bright red and the brain looked normal outwardly. Investigation of semi thin epoxide sections of liver and brain, stained by Azur 2 by our method,[11] revealed morphological changes in microvessels induced by chronic stress. It must be mentioned that the use of semi thin sections of up to 1 μm thickness allowed us getting deeper picture of bloodstream without investigating liver and brain cells' parenchyma.

A number of structural changes of liver vessels of 1 group are seen as compared to the liver of control rat (Figure 1).

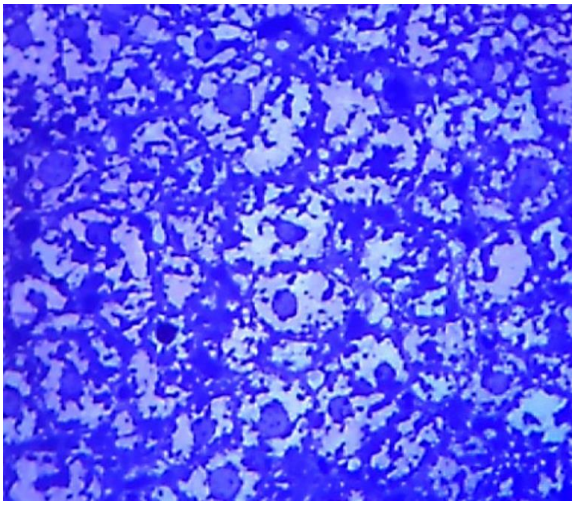
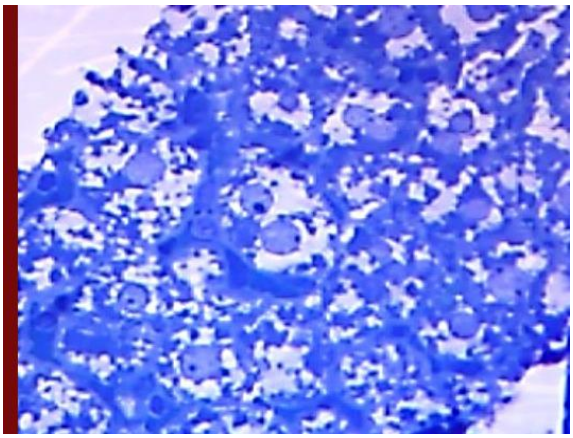


Figure 1. The blood vessels of the liver of control rats. X400

In the lobules the central vein has been changed in size and in quantity. The lobules are different. There are present as very small-sized lobules without central vein and quite big lobules with 3 or 4 small central veins. The last ones were obtained by means of bridge formation in the big vein. The vessels surrounding the lobules have also changed. They have widened for most of their length and bridges from one side of vessel to the other side are also seen (Figures 2a and 2b).

a)



b)

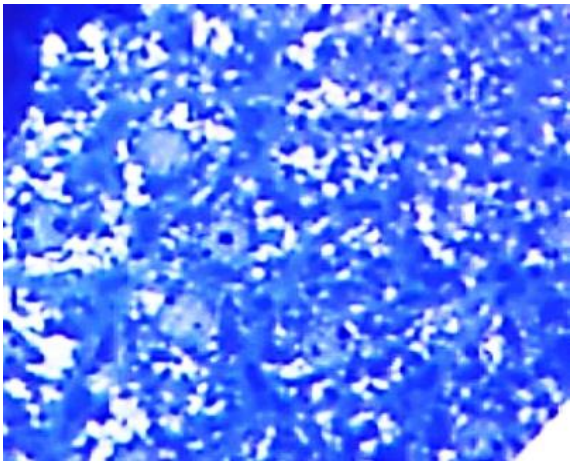


Figure 2a and 2b. Stress group. Rat liver. The increasing of the quantity of central venous. Intususception growths of blood vessels by translumenal bridge formation. X400

Sinusoids are widened with plenty of bridges. Their quantity has increased. In the 2 group animals, after four days of termination of the experiment, plenty of branches of sinusoids as well also formation of the bridges and their separations have been observed (Figures 3a and 3b).

During the investigation of microvessels of the brain in the first group of the animals, the vessels with wide lumen are observed as the ones with narrow lumen. Different inosculations between vessels have become evident In the first and the second group of the animals considerable number of capillaries with the formation of the bridges are observed (Figure 4). There is a large number of newly-formed capillaries.

DISCUSSION

It It has been shown that, at the alarm stage of stress reaction i.e., free swimming in a cage (an hour after FSC), the structure of the liver underwent dystrophic changes of hepatocytes and blood flow in the liver lobules had increased. These changes was present at the resistance stage (48 hours after FSC), as well. At the exhaustion stage (FSC within 10 days) it caused greater dystrophy of hepatocytes, the occurrence of necrosis in them and microcirculatory disturbances in the lobules.[5]

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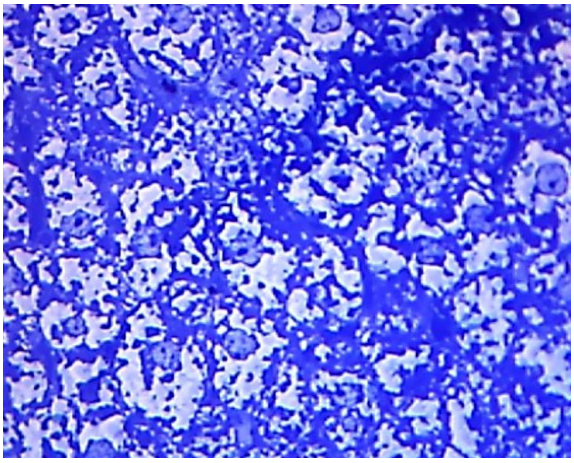
a)



b)

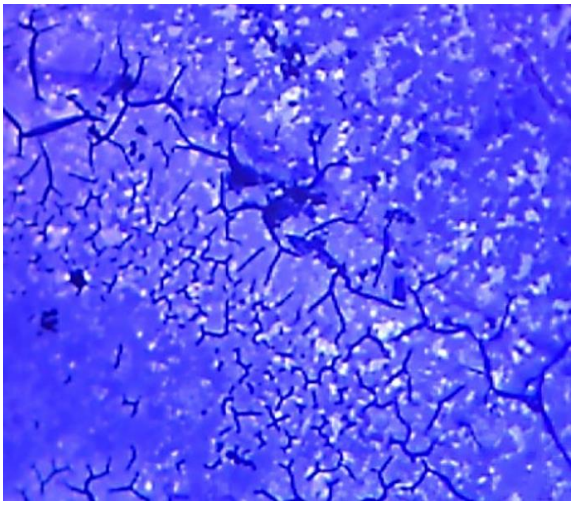


Figure 3a and 3b. Post stress group. Rat liver. It is characterized by sprouting angiogenesis. The transition from sprouting angiogenesis to intussusception angiogenesis. X400.

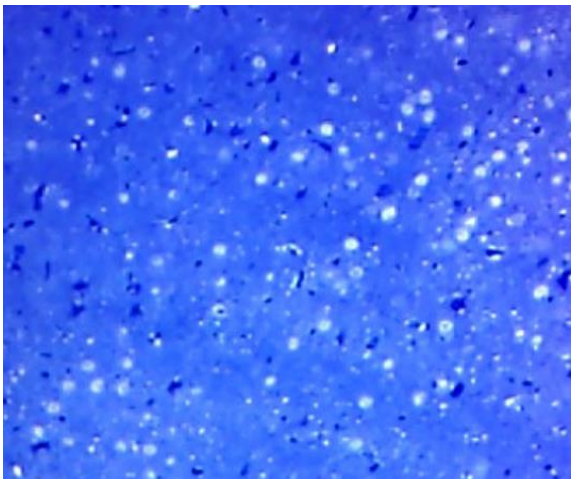


Figure 4. Post stress group. Rat brain. The intususception growths of blood vessels. X400

That agreed with our data concerning pathological remodelling of liver microcirculation structures when we dealt with multifactorial influence at CVS. Bigger vessels have shown changes like intussusceptive microvascular growth along with sinusoids. Typically the blood vessels showed the presence of the second type of angiogenesis i.e. sprouting angiogenesis, the major type of vasculature development in both liver regeneration and cancer development,[3,8,15,19] and intussusception angiogenesis like translumenal bridge formation as shown earlier by us.[10] The formation of new vessels and the establishment of an abnormal angioarchitecture of the liver are clearly related to the progressive fibrogenesis which finally leads to cirrohosis and liver cancer.[22]

In the brain of experimental animals pathogenic remodeling microvessel at CVS was also diagnosed. Brain microcirculation plays an important role in the pathogenesis of various brain diseases.[20] The brain is extremely sensitive to hypoxia, and brain edema is more dangerous than edema in other tissues. Brain vessels are part of the blood-brain barrier, which prevents the penetration of some of the substances in the blood into the brain tissue Increased vascular density at CVS has also been observed after stroke.

The density and architecture of capillary beds that are formed within a tissue depends on many factors, including local metabolic demand and blood flow.[12,13,14]

The formation of new vessels in brain at CVS intussusceptions of angiogenesis occurs just like in the liver by means of translumenal bridge formation.

CONCLUSION

CS influences the reorganization of blood vessels of liver and brain and it causes angiogenesis of liver sinusoids and brain capillaries.

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