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Pathogenetic mechanisms of perinatal damage of the central nervous system in case of prenatal encephalitis at the fetuses

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ABSTRACT

We studied the paraventricular zone of thalamic area of a brain of 47 fetuses with signs of prenatal encephalitis in case of the term of a gestation of 26–27 weeks in comparison with 10 healthy fetuses in case of the same term. We showed that pathomorphologic changes in brain of human fetuses in case cellularity of prenatal encephalitis are characterized by a productive and necrotic inflammation. Reduction of cellularity of paraventricular zone, increase in amount of neurons and glial cells in thalamic area, and also the cells he expressing glial fibrous protein, the increased proliferative activity of cells of paraventricular zone indicated the accelerated maturation of its cells in case of prenatal encephalitis. Reduction of number of vessels in paraventricular zone was caused by decrease in expression of vascular endothelial factor of growth and transforming growth factor in cells.

Introduction

Prenatal encephalitis is a widespread enough pathology causing a high lethality of fetuses and newborn infants, development of disability during the subsequent periods of life that is caused by perinatal defeat of the centra l nervous system (CNS). The morphological picture of inflammatory process in brain is characterized by considerable polymorphism that is explained both by multifactorial etiology of encephalitis and heteroformed reaction of neurons under conditions of pathology.

One of pathogenetic links in development of perinatal lesion of CNS is the condition of paraventricular zone (PVZ) which contains neuroepithelial cells of germinal type. In the course of embryogenesis during migration of cells from PVZ the neuroblasts are differentiated in neurons and glioblasts create glial cells. There are two types of migration of neuroblasts: radial and tangential. Migration of neuroblasts from PZV a cortical plate is ended to the 32–33rd week. Then it begins migration of glioblasts which come ripe into astrocytes and oligodendrocytes [1]. Moreover PVZ is a richly vascularized area having the unripe vascular and capillary network which is being reconstructed into mature one after disappearance of PVZ. Deficiency of essential mechanical support [2, 3] makes the PVZ vessels sensitive to increasing of pressure and a local hypoxia, leads to subependymal hemorrhages and to burst of blood in side ventricles of brain [4, 5]. So, it was shown that under prenatal hypoxia the processes of cellular differentiation and mi-

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gration are changed in PVZ [6]. At premature births the neurogenesis in newborn infants is broken and migration of cells from PVZ [7] is suppressed.

Significance of formation of the vasculature of PVZ is also caused by the fact that vessels provide migration of neuronatal of precursors to a number of zones of brain [1]. At the same time the growth of vessels is bound with the vascular endothelial factor of growth (VEGF) which is expressed by astrocytes [8]. Being the key regulator of cellular growth, migration and differentiation the transforming growth factor β (TGF-β) has neuroprotective effect promoting synthesis of fibronectin of basal membranes, stimulates expression of VEGF and therefore participates in neoangiogenesis [9, 10]. So TGF-β is proved to be an antiinflammatory cytokine [11].

Aim of the Research

To study of pathogenetic mechanisms of perinatal affection of CNS in human fetuses under prenatal encephalitis.

Materials and Methods

We investigated the brain of 47 fetuses which have divided into 2 groups. The first group included 37 fetuses at the term of a gestation of 26–27 weeks in which were revealed the symptoms of congenital encephalitis and encephalomeningitis. Termination of pregnancy had spontaneous character in 54.1% of cases. In the second (control) group there were 10 fetuses at the term of a gestation of 26–27 weeks obtained after induced abortion for maternal medical reasons (young primipara, psychoneurological disorders, etc.). For light microscopy, an immunohistological and chemical analysis the brain fragments and its thalamic area were fixed in 10% solution of neutral formalin. It was carried out by a standard technique. We prepared serial paraffin cuts of 5 micron in thick. Then we painted them with hematoxylin and eosin (according to Nissl) with following light microscopy (Axiostar, Zeiss, Germany). By means of monoclonal antibodies (Novocastra Laboratories, UK) with use of a peroxydase label by the method of immunohistological and chemical typing on paraffin cuts in cells we determined glial fibrillary acidic protein (GFAP), endothelial tissue of vessels (CD34), proliferative activity of cells of PVZ (Ki-67) and also expression in them of the vascular endothelial factor growth (VEGF), the tumor necrose factor α (TNF-α), the transforming growth factor β. In thalamic area we counted the numerical density (N_{ai}) of all cells of PVZ, glial cells and neurons in the test area of 25 μm^2. In the PVZ we counted the number (N_{ai}) of vessels and PVZ cells expressing GFAP, the vascular endothelial factor of growth, the tumor necrose factor α, the transforming growth factor β, Ki-67 in the test area 25 μm^2. Taking into account normal distribution of signs we applied the Student's t-criterion to/for assessment of significant difference of average sizes at $p < 0.05$.

Results and Discussion

PVZ in fetuses of the second (control) group of PVZ (control) it was morphologically presented by a uniform distribution of the germinal cells. PVZ in fetuses of 1 (prenatal encephalitis) group was presented by non-uniform accumulation of germinal cells with foci of their accumulation and depression, sanguine vessels, the foci of the hemorrhages limited by ependymocytes (Figure 1). In fetuses of the first (prenatal encephalitis) group the pathomorphological changes in a brain of fetuses were characterized by focal (perivascular) productive and productive and necrotic inflammation (Figure 2) in case of prenatal encephalitis. Also this prosses was accompanied by formation of granulomas with involvement of me-

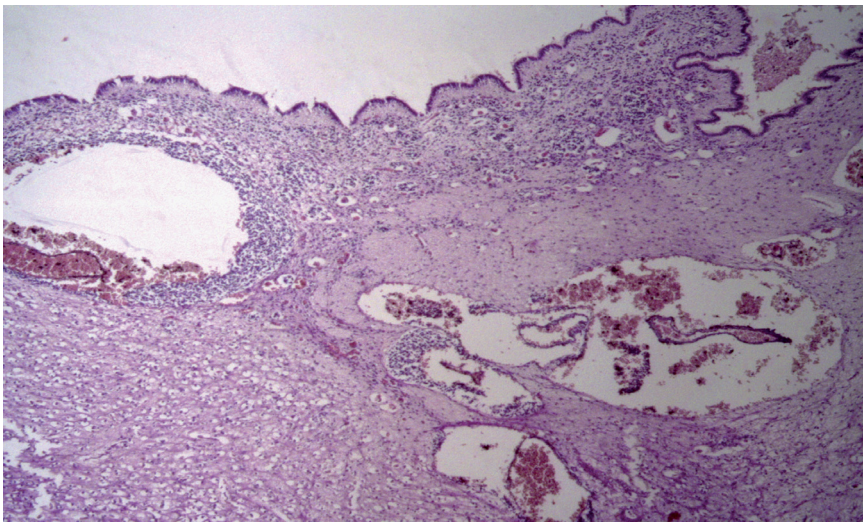

Figure 1. The paraventricular zone at prenatal encephalitis. Stained with hematoxylin and eozin. Magnification 100

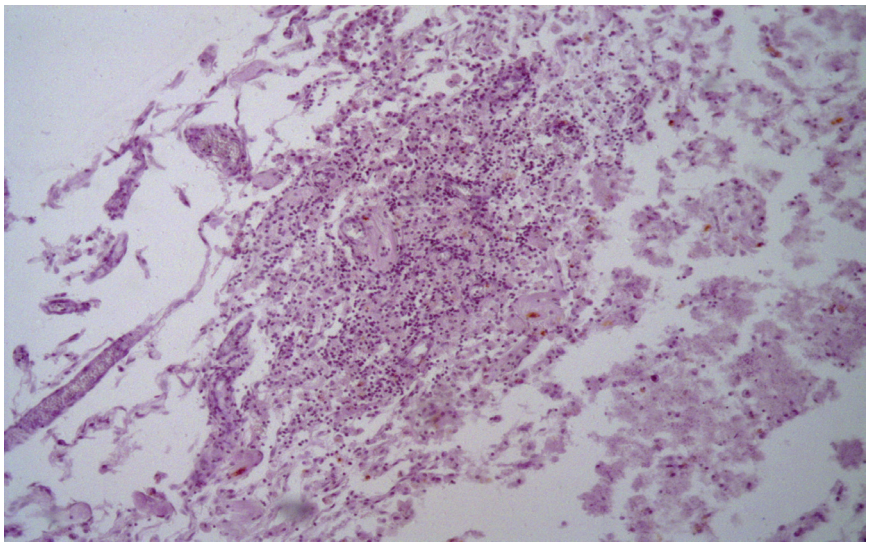

Figure 2. A focal perivascular infiltration from lymphocytes and macrophages in brain substance at prenatal encephalitis. Stained with hematoxylin and eozin. Magnification 200

ninx vasculosas into inflammatory process. Earlier in fetuses of the first group (prenatal encephalitis) in one observation Kozyaev M.A. et al. (2011) revealed expression of a virus of simple herpes of the second type, and in another one observation they revealed expression of a virus of cytomegalovirus [12]. Perinatal CNS lesion was represented in fetuses of the first subependymal group by subependymal hemorrhages (20.8 %) and intra ventricular hemorrhages (16.7%) [13].

Fetuses in the first group had a total quantity of cells of PVZ of thalamic area of a brain (Table 1) less then in the second (control) group by 33%. And their proliferative activity was increased: expression of Ki-67-positive cells was 1.86 times more in fetuses of the first group then in fetuses of the second (control) group.

Results of a morphometric studies of cells in thalamic area (Table 2) have shown that in fetuses of the first group (prenatal encephalitis) the amount of neurons and glial cells is increased. Their numerical densities (N_{ai}) were 1.2 and 2.5 times higher then in the second (control) group respectively. The numerical density of PVZ cells (expressing GFAB) in fetuses of the first group was 37% higher then in the second (control) group.

In fetuses of the first group we revealed reduction of numerical density of vessels of PVZ 3.4 times (Table 3). Taking into account that the growth of vessels is connected with VEGF, we assume that its reduced expression in PVZ cells (1.5 times) has caused abnormality of angiogenesis in PVZ. In fetuses of the first group with prenatal encephalitis we revealed the 1.4 times increase of numerical density of cells expressing TNF-α in comparison with the same indicator in fetuses of the second control group. And the number of cells expressing TGF-β was smaller by 26%.

Taking into account close structural-functional relationship of neurons, a glial reticulum and vasculature of a brain in the prosses of normal functioning

Table 1

The numerical density (N_{ai}) of PVZ cells in brain of fetuses and their proliferative activity at congenital encephalitis (M ± m)

The studied groups	The general cellularity of the paraventricular zone	Ki-67
The first (prenatal encephalitis)	31.92 ± 0.55*	3.62 ± 0.45*
The second (control)	42.52 ± 0.68	1.95 ± 0.19

* Significant difference of average sizes in comparison with the same indicators in fetuses of the second (control) group ($p < 0.05$).

Table 2

The numerical density (N_{ai}) of PVZ cells in thalamus of a brain of human fetuses at prenatal encephalitis (M ± m)

The studied groups	Expression of GFAB in PVZ cells	Neurons	Glial cells
The first (prenatal encephalitis)	15.60 ± 0.56*	15.36 ± 0.45*	49.37 ± 1.49*
The second (control)	11.33 ± 0.57	13.05 ± 0.52	20.40 ± 0.75

* Significant difference of average sizes in comparison with sizes of the same indicators in fetuses of the second (control) group ($p < 0.05$).

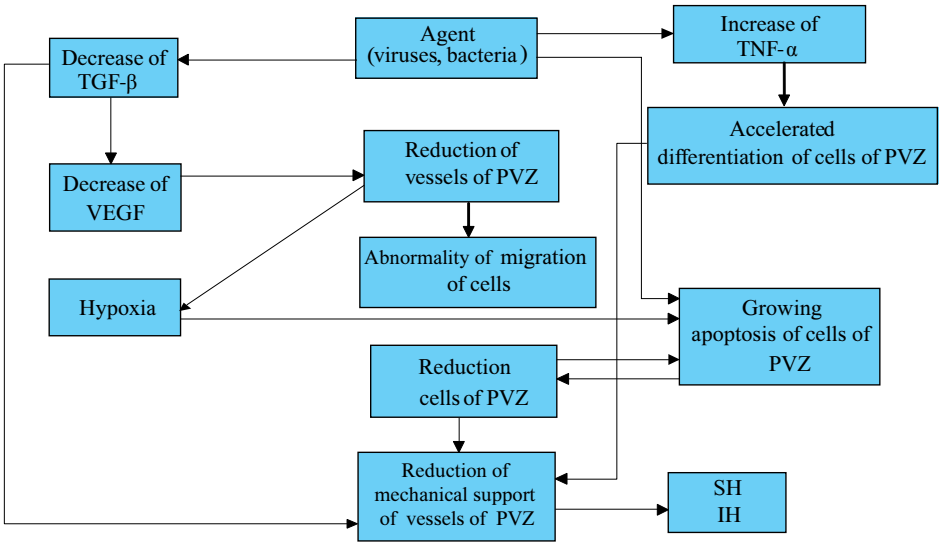

Figure 3. The scheme of pathogenesis of perinatal CNS lesion in case of prenatal encephalitis (transforming growth factor β (TGF-β); tumor necrose factor α (TNF-α); the vascular endothelial factor of growth (VEGF); paraventricular zone (PVZ); subepedymal hemorrhages (SH); intraventricular hemorrhages (IH))

and pathological influence, the brain is considered as the uniform glial vascular system. At prenatal encephalitis the quantity of neuroepithelial cells in PVZ is decreased in spite of their increased proliferative activity, while the amount of neurons and glial cells in thalamic area is increased. And also the quantity of cells expressing GFAP is increased, that can be considered as the accelerated maturation of a neurepithelium into neurons and glial cells and their migration from PVZ [14] (Figure 3).

Liu X. et al. (2006) shown that the accelerated differentiation of cells can be connected with secretion by inflammatory cells of the cytokines exerting the regulating action on the processes of differentiation and migration of cells in PVZ, such as IFN-γ, TNF-α, IL-1β, IL-6 [14]. In fetuses of the first group with prenatal encephalitis we also revealed the increase in numerical density of PVZ cells expressing TNF-α. This can be probably connected with virus etiology of encephalitis. Besides, considering proapoptopic activity of viruses one of the mechanisms of disappearance of cells from PVZ can be their apoptosis induced by viruses. Decrease of quantity of cells expressing of VEGF is caused by essential decrease in number of vessels in PVZ. It could be followed by disturbance in processes of the cellular migration ongoing along the vessels, and development of a pool of cells in other region of brain [15]. Eventually it could lead to change of neurogenesis in adults [7].

In fetuses of the first group with prenatal encephalitis the reduced quantity of the cells expressing VEGF could be bound with decreased expression of TGF-β by PVZ cells with stimulating effect on VEGF. Being an antiinflammatory cytokine the decrease of expression TGF-β is probably caused by the marked expression of pro-inflammatory TNF-α. Change of migration of cells from PVZ and their differentiation into neurones and glial cells (including astrocytes, expressing VEGF); decrease of expression of TGF-β taking part in formation of the gliosis; depression of quantity of pericytes of capillaries and their secretion of a fibronectin of basal membranes could promote saving/conservation of unripe vessels of PVZ and in the conditions of a prenatal hypoxia or a childbearing could promote their burst and hemorrhages.

Table 3

The numerical density (N_{ai}) of vessels and cells expressing VEGF, TNF-α, TGF-β in PVZ of brain of human fetuses at prenatal encephalitis (M ± m)

The studied groups	Expression by cells of paraventricular zone of cytokines			Vessels of paraventricular zone (CD34)
	TNF-α	VEGF	TGF-β	
The first (prenatal encephalitis)	10.61 ± 0.48*	44.24 ± 0.47*	17.92 ± 1.46	24.15 ± 1.36*
The second (control)	7.56 ± 0.87	64.41 ± 1.75	22.73 ± 1.84*	81.95 ± 3.79

* Significant difference of mean values in comparison with those in fetuses of the second (control) group ($p < 0.05$).

Conclusion

At a prenatal encephalitis decrease of the general population of cells and increase of proliferative activity of cells in PVZ were followed by growth of quantity of neurones and glial cells in thalamic area, and integration of PVZ by cellular elements with saving/conservation of unripe capillaries.

At a prenatal encephalitis in PVZ the found decrease of number of vessels was probably caused by decrease of expression of VEGF and TGF-β having an effect on the processes of cellular migration and differentiation of the neuronatal of precursors with saving/conservation of structural immature of vessels of PVZ causing a perinatal CNS lesion. At a prenatal encephalitis in PVZ we detected the increase by its cells of an expression of pro-inflammatory cytokine TNF-α influencing expression of cytokines TGF-β and VEGF.

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