Initial Stage of Vascular Bed Development in Telencephalon of Human Embryo

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It was found that intracerebral blood vessels in human embryo telencephalon first appear on the 7th week of prenatal development as capillaries with poorly differentiated walls and signs of functional immaturity. The formation of the basal capillary membrane consisting of laminin and type IV collagen starts immediately after the formation of primary capillary network.

Key Words: *human; embryogenesis; blood vessels; encephalon; electron microscopy*

One of the key events in the prenatal development of the brain is the formation of primary vascular network in nonvascularized neuroepithelial plates. Animal studies show the absence of the blood-brain barrier (BBB) during this period [4-6]. The formation of BBB is accompanied by structural rearrangements in the vascular wall [6]. Detailed analysis of BBB ontogeny in humans is hampered by insufficient information about the initial stage of intracerebral angiogenesis [3].

This study was aimed at investigation of the structural and cytochemical organization of blood vessels (BV) in the developing telencephalon in 6-9-week human embryos.

MATERIALS AND METHODS

The study included 15 human embryos (6 to 9 weeks gestation) obtained after induced abortion under specialized hospital conditions. Material for light microscopy was fixed with 80% ethanol containing 0.5% paraformaldehyde and embedded in paraffin. A number of markers of differentiation and vascular wall functional activity (smooth muscle cell actin, α-actin-1), vimentin, endothelial antigen CD31, laminin, type IV collagen, Willebrand's factor were determined immunochytochemically with 1A4, Vim3B4, JC/70A, LAM-89, and CIV22 monoclonal antibodies and polyclonal rabbit antibodies, respectively, using an LSAB2 kit (secondary biotinylated antibodies and a streptavidin-peroxidase conjugate) and a 3,3'-diaminobenzidine-based chromagen. All antibodies and reagents were purchased from DAKO except monoclonal antibodies to laminin (Sigma). After immunocytochemical reactions some sections were stained with hematoxylin. Material for electron microscopy was fixed in 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.4) containing sucrose, postfixed in 2% OsO_4 in a 0.05 M cacodylate buffer, dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and analyzed under a JEM 100B electron microscope (Jeol).

RESULTS

The first BV in developing human telencephalon were revealed in the striatal plate (ganglionic tuber, Fig. 1) early in week 7 and later in the neocortical plate (lateral wall of the lateral ventricle). Their appearance in the neocortical plate preceded massive migration of neuroblasts from the proliferative (ventricular) zone to the cortical plate. Angiogenesis in different parts of the neocortical plate occurred not synchronously but consequently, starting in the lateral part bordering with the ganglionic tuber and spreading dorsally.

BV growing into the neuroepithelial plate propagated from dense capillary network of nondifferentiated meninx, their walls consisted of endotheliocytes and cells with multiple processes (presumably pericytes or their precursors). These cells could not be

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differentiated from meningocytes located near meningeal BV because of the absence of definite basal membrane and typical cytological markers.

Similarly to endotheliocytes of meningeal BV, endotheliocytes of primary telencephalic vessels contained considerable amounts of vimentin intermediate filaments in the cytoplasm and expressed endothelial CD31 antigen. They differed from meningeal endotheliocytes by the synthesis of Willebrand's factor. No positive reaction with the corresponding endothelial antigen was revealed in primary neocortical BV, although more mature striatal BV showed a weak reaction and large meningeal BV — a well-pronounced reaction. It should be noted that during the examined period the intracerebral vascular endothelium contained no typical secretory granules (Veibel—Palad corps) containing Willebrand's factor [2], while these structures were present in the meningeal vascular endothelium.

Intracerebral vascular endotheliocytes were not fenestrated and contained few transport vesicles. Fenestration was not characteristic of the endothelium of meningeal vessels during the period preceding their growth into the neocortical plate. In laboratory animals at the same stage of ontogeny, meningeal vascular endothelium is clearly fenestrated [7]. As seen on cross-sections, endotheliocytes of primary intracerebral vessels formed only point dense contacts, however, these contacts expanded at later stages (weeks 8-9). Initially, vascular endothelium contacted directly with neuroepithelial cells, but later (weeks 8-9) pericytes and basal membrane material presented by

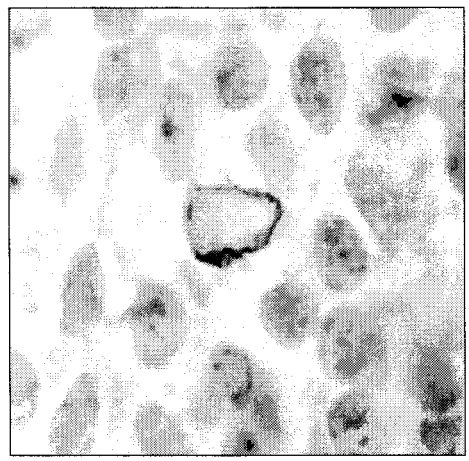

Fig. 1. Blood vessel in the ganglionic tuber of human embryo telencephalon, 7-week embryo, ×1000. Immunocytochemical visualization of type IV collagen, poststaining with hematoxylin.

fused amorphous masses of fine laminin and type IV collagen fibrillae isolated most of the capillary perimeter from surrounding tissues (Fig. 2).

From week 8 of embryogenesis radial glial cells formed enlarged perivascular processes containing the deposits of basal membrane material near the vascular wall. High-resolution examination of these sites revealed accumulation of amorphous material in both the basal part of endotheliocyte and in gliocyte podia (Fig. 3) suggesting the involvement of both endothelium and radial glia in the formation of basal membrane.

At this stage of prenatal ontogeny, intracerebral vessels did not differentiate into arteries and veins and

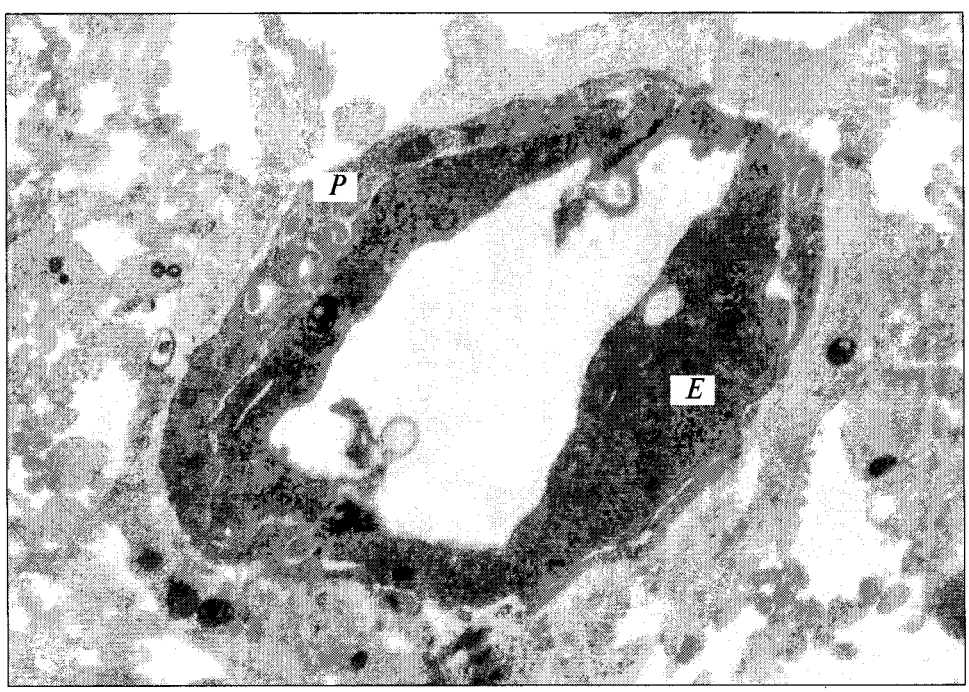

Fig. 2. Blood vessel in the neocortical plate in 9-week human embryo, ×8000. *E*: endotheliocyte cytoplasm; *P*: pericyte cytoplasm.

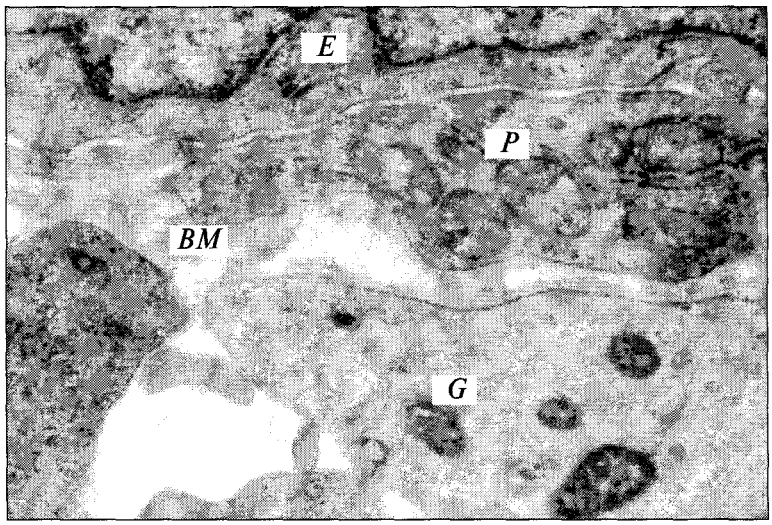

Fig. 3. Vascular wall fragment in the neocortical plate of 8-week human embryo, ×14,000. *E*: endotheliocyte; *P*: pericyte process; *G*: perivascular process of radial gliocyte; *BM*: basal membrane material.

retained their capillary structure. Immunocytochemical detection of α-actin-1 revealed no smooth muscle cells in telencephalic BV. Therefore, high activity of NO synthase in vessels of the developing brain [1] was not associated with myorelaxation.

Thus, the formation of intracerebral vascular network in humans starts in the ganglionic tuber (striatal plate). Primary BV are presented by capillaries with low-differentiated walls and signs of functional immaturity. The formation of capillary basal membrane from laminin and type IV collagen begins immediately after the formation of primary capillary network.

These findings provide the basis for comparative morphological analysis of structural and cytochemical changes in BV of human brain and the formation of the blood-brain barrier during prenatal ontogeny.

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