

BRAIN CAPILLARIES: HETEROGENEITY IS THE WORD OF ORDER

Maria SUCIU^{1*}, Aurel ARDELEAN¹, Anca HERMENEAN¹

¹Institute of Life Sciences, Western University "Vasile Goldis" of Arad, Arad, Romania

ABSTRACT. Capillaries are the smallest vessels of the circulatory tree, having similar functions for the entire body, but also functions that are specific for a certain type of organ or tissue. Capillaries are composed of endothelial cells, pericytes that assist them and a basal membrane that envelopes them. Through this review we wanted to emphasize the fact that capillaries are a generic name for a very malleable structure that is found everywhere in the body. We focused on differences that appear generally between capillaries in the body and specifically in the brain, and we went further to search if there are differences within a single capillary network that connects the arterial and venous sides. There are morphological variations of microvessels within the brain and molecular differences within the same capillary tube, differences that appertain to their arterial or venous characteristics.

Keywords: capillary, brain, difference, blood-brain barrier, histology.

INTRODUCTION.

It is crucial to know in detail the molecular machinery of an organ, especially when it comes to treating certain pathologies. The central nervous system is a very fragile and specialized type of organ that controls all life's aspects of an individual. The neural parenchyma is under constant attack of various agents, and most battles are held at the blood-brain gates: the capillaries' endothelial cells.

When one finds himself in the position to comprehend the microvessel characteristics of the central nervous system, it usually needs to take a step back and search the big picture: the capillaries in general and at other species.

METHODS.

Recent research articles, reviews and books were studied, regarding capillary differences. We used the following search engines: Google, Google Scholar, Google books, Pubmed, ScienceDirect, SpringerLink, and our institute library. The key-words used were "capillary", "arterial", "venous", "differences", "diseases" and similar others in variable combinations.

CAPILLARIES: STRUCTURE AND ULTRASTRUCTURE.

Capillaries are tubular structures within the tissues, formed from the bodies of a single layer of endothelial cells, a basal membrane and the pericytes surrounding them. Capillaries are the smallest vessels connecting the arterial branch and venous branch of the circulatory system. Veins and arteries have a vessel wall: muscular layers, tunica media and tunica adventitia. Compared to the larger vessels, capillaries only have 5-10 µm in diameter and do not have nerve endings and muscular layers, and they are also called microvessels (Eichmann et al., 2005).

They can be found everywhere in the body, being responsible for the transport of nutrients and waste to/from the tissues. They are characterized by a flat-

like endothelial cell wall, increased plasticity, a tendency to form caveolae, clathrin coated vesicles (Fig. 1 c), d), e).), or tubules that transverse the cytoplasm and Weibel-Palade bodies. They can connect to each other, in a tight or loose manner, through tight junctions and adherens junctions, are in close association with pericytes or smooth muscle cells and form a basal membrane that envelopes them and sometimes the pericytes too (Pavelka and Roth, 2010).

Endothelial cells form an apical and basal membrane through the presence of some specific membrane transporters, markers and signal molecules on luminal or abluminal side of the cell. This way they control the traffic of molecules from the blood to the tissue and backwards, and can have a barrier function (Pavelka and Roth, 2010).

CENTRAL NERVOUS SYSTEM CAPILLARIES.

A special type of endothelium can be found in the central nervous system. It has the tightest tight junctions, limited trans-cellular transport and a strict array of transporters and pumps to protect and nurture the brain and spine. Due to its enhanced barrier functions it is called the **blood-brain barrier**, and **blood-spinal chord barrier**, respectively. It is composed of further specialized endothelial cells, pericytes (and the basal membrane in which they are embedded) and they also have another active layer made up from astrocytes' endfeet, a feature restricted to the central nervous system only (Bechmann et al., 2007; Abbot et al., 2006; Hawkins and Davis, 2005).

There are more barriers that protect the nervous system, such as: blood-cerebrospinal fluid barrier and arahnoid membrane, tanyctic barrier in the circumventricular organs, blood-ocular barrier and blood-retinal barrier (Saunders et al., 2012; Bartanusz et al., 2011; Runkle and Antonetti, 2011; Weerasuriya and Mizisin, 2011; Engelhardt, 2008).

Of interest to our discussion might also be the endothelial component of the **choroid plexus**, which

has normal fenestrated endothelia. At this level the barrier function is mostly located at the epithelial component; **The circumventricular organ** has the leakiest type of endothelium component, a capillary network that permits the rapid transfer of neurohormones from the neuronal side into the blood. At this location, the barrier properties are found at the epithelial component as well (Saunders et al., 2012; Engelhardt, 2008).

All the other barriers above mentioned are of epithelial or ependymal origin, not endothelial, and they do not make a subject of this review.

CAPILLARIES ROLES AND FUNCTIONS.

Capillaries have: 1) a transport role, made trough blood flow, 2) a nutritive role, assured through trans-endothelial transport, for both digestion-derived nutrients transport and for oxygen transport point of view, 3) a clearance role, trough tissue-blood transport of catabolic products and renal filtration, 4) they have a barrier role against harmful agents, trough tight junction formation and membrane defense mechanisms, and a role in 5) pathologic conditions, inflammation and local tissue remodeling through molecule signaling (Lin et al., 2000).

CAPILLARY DIFFERENCES.

There are differences between endothelia of different species, races, age, types of vessels, and

organs (Farrall and Wardlaw, 2009; Kalinowski et al., 2004). There are differences in the way endothelial cells react and respond to stimuli even within the same organs (Cines et al., 1998).

Histological differences.

Capillaries can have a continuous, fenestrated or discontinuous wall. Sinusoids from liver, spleen and bone marrow have discontinuities and are very permeable. Capillaries of the retina, dermis, bone tissue, skeletal muscle, myocardium, testes and ovaries are continuous. But those in the endocrine glands and kidney are fenestrated. Fenestration is dependent on vascular endothelial growth factor A (VEGFA) and endocrine gland derived vascular endothelial growth factor (EG-VEGF) secretion (Ribatti and Crivellato, 2012; Roberts and Palade, 1997; 1995). In the brain we can find continuous, fenestrated and discontinuous capillaries (Saunders et al., 2012). A comparison between continuous brain capillary and liver sinusoidal capillary can be seen in Fig. 1 a) and b).

One of the major morphological differences in capillaries noted in recent literature is in the origin of the capillary: that is venous or arterial. The single most important characteristic of capillaries, which gave the venous or arterial characteristic, was until recently the direction of the blood flow – from the arterial towards the venous end of vascular branches (Casley-Smith, 1971).

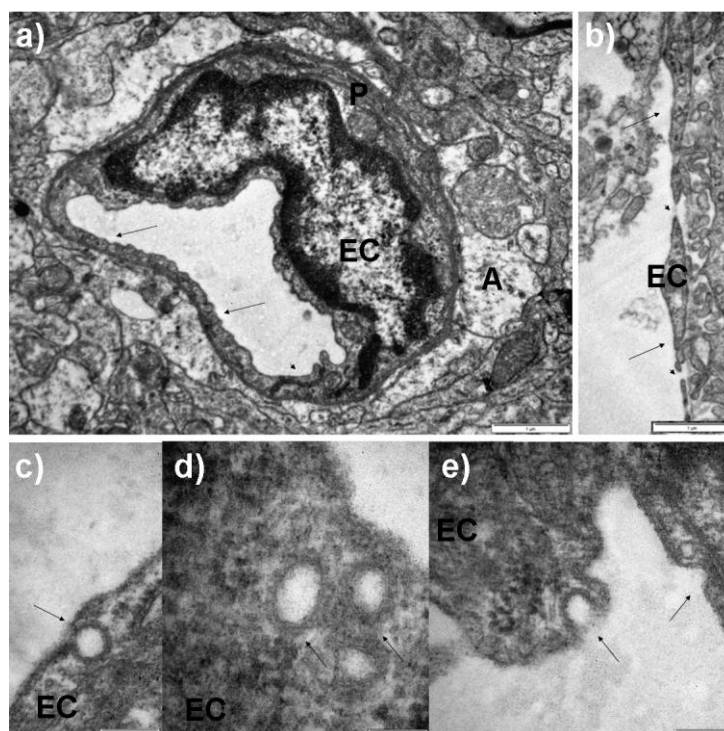


Figure 1. Examples of capillaries. a) Brain endothelial cell (EC) with continuous vessel wall (arrows) and tight junctions (arrow head); P – pericyte, A – astrocyte endfeet; b) Hepatic endothelial cell (EC) with a thin vessel wall (arrows) and fenestrae (arrow heads); c-e) clatrin coated vesicles budding, intracellular and fusing (arrows) in brain endothelial cells (EC).

Other morphological differences are in the number of fenestrae, which are up to 12 times more numerous in the venous than the arterial capillaries of the small intestines of mice, and most of the venous fenestrae

have diaphragms (Casley-Smith, 1971). Braverman and Yen demonstrated in the late 70s with the use of an electron microscope that skin capillaries can be differentiated between arterial and venous morphology

by their appearance of the basement membrane: the arterial capillary is homogenous and the venous one is multilayered and inhomogeneous (Braverman and Yen, 1977 (a); Yen and Braverman, 1976).

Endothelial cells can spring from different microvascular beds and have organ specific molecular differences. These depend on genetic and local factors, such as: extracellular components, cytokines, growth factors, neighboring cells and mechanical forces. Apart from having local-specific characteristics, they can also influence local development and features through some signal molecules secreted into the blood and interstitium or by expressing them on the cell surface (Ribatti and Crivelatto, 2012; Pusztaszeri et al., 2006). Some endothelial cells can even be found circulating in the blood, but that is typically a sign of vessel damage and vessel associated disease (Lin et al., 2000).

Physiological differences.

Watzman and colaborators (2000) studied the oxygen saturation in brain capillaries and found an arterial/venous ratio of 16:84 in physiological and pathologic conditions.

Molecular differences.

They reside in the arterial and venous derived characteristics. The **von Willebrand factor** is a marker generally considered to identify endothelial cells. But even so it seems to be expressed in venous rather than arterial capillary type and absent from sinusoids and from the endothelia of the glomeruli, spleen and lung (Pusztaszeri et al., 2006).

Platelet endothelial cell adhesion molecule (CD31 or PECAM1) is an endothelial marker mostly studied in the lung, where it has a homogenously strong expression (Muller et al., 2002).

CD34 stains endothelial cells in general, having the strongest expression in the capillaries, but it is also expressed in larger vessels (Kawanami et al., 2000).

Friend leukemia integration 1 (Fli-1 or ERGB) is a marker that stains all endothelial cells, and is stronger at tumor sites (Hewett et al., 2001).

Ephrins (receptors and ligands) are expressed differently in the arterial and venous capillaries, as well: ephrinB2 in arterial routes including capillaries and ephrinB4 in the venous counterparts Ribatti and Crivelatto, 2012). Their expression seems to be strongly dependent on the blood flow (le Noble et al., 2004). Also it seems that ephrinB2 is expressed in most of smooth muscle cells and pericytes of the arteries but not the veins (Gale et al., 2001; Shin et al., 2001; Wang et al., 1998). Also based on the same morphological and genetic difference it was proven that the neo-vascularisation appears at the arterial side of the capillary and not the venous, as previously believed (Shin et al., 2001), although this is still a matter of debate even at present times (Red-Horse et al., 2010).

Our group has also found differences in the brain capillaries expression of some **tight junction proteins**, within the same type of continuous capillaries (unpublished data). Vascular endothelial cadherin has similar expression in both types of blood vessels (Bianchi et al., 1999).

Enzymatic differences.

There were **enzymatic differences** noted between capillary beds as well: arterial ones express alkaline phosphatase and the venous ones express dipeptidylpeptidase IV (Koyama et al., 1998). **Receptor-type protein-tyrosine phosphatase μ** expression is higher in the arteries than in the venous circulation (Bianchi et al., 1999).

Braverman and Yen (1977, (b)) found a difference between arterial and venous capillaries in psoriasis: they noticed that all capillary loops formed in the psoriasis damaged skin had venous morphology and after treatment they returned to normal arterial appearance.

Differences between larger vessels, such as arteries and veins include: the expression of fibulin-5 (also known as EVEC or DANCE), Hairy-related transcription factor 1-3 (HRT1-3), and latent tumor growth factor β -binding protein-2 are higher in arterial smooth muscle cells at developmental stages (Kowal et al., 1999; Nakamura et al., 1999; Nakagawa et al., 1999; Fang et al., 1997). Fully differentiated arteries can be identified with delta-like protein 4 (Dll4), hairy/enhancer-of-split related with YRPW motif protein 1 (Hey1), neurogenic locus notch homolog proteins 3 and 4 (Notch3, Notch4), decidual protein induced by progesterone (Depp), Sox-13, endothelial PAS domain-containing protein 1 (EPAS-1) neuropilin-1 and gridlock protein (Red-Horse et al., 2010; Eichmann et al., 2005; Ema et al., 1997; Flamme et al., 1997). Veins express neuropilin-2 in early development, and later apolipoprotein A-I regulatory protein 1 (NR2F2 protein or Coup-TF2), vascular endothelial growth factor receptor 3 (VEGFR3), nitrophorin 2 (Np2) and apelin receptor (Aplnr) (Red-Horse et al., 2010; Eichmann et al., 2005). But these markers were not shown to be expressed at capillary level as well (Shin et al., 2001) and until now there are no markers found that work reliably in all species, tissues, vascular beds, and in all physiological and pathologic conditions (Baluk and McDonald, 2008). And finding one is a very challenging task because vessels are very labile structures: capillaries can have arterial characteristics at one point, then change to venous, develop to be arteries or veins and then regress to be capillaries again, as shown at the beginnings of *in vitro* studies by Clark and Clark (1932).

Table 1. Differences noted in literature between capillaries from morphological and molecular points of view.

Element Of Difference	Arterial Capillary	Venous Capillary	Organs/Tissues	Developmental Stages	Adulthood	References
fenestrae	low numbers	up to 12x as many	microvilli of small intestine	N/A	yes	Casley-Smith, 1971
von Willebrand	low expression	high expression	bronchial and biliary epithelia and	N/A	yes	Bianchi et al., 1999

factor			cardiocytes but not the endocardium			
CD31	high expression		lung, and other	N/A	yes	Muller et al., 2002
CD34	high expression		all endothelia	N/A	yes	Kawanami et al., 2000
Fli-1	equal expression		all endothelia	N/A	yes	Hewett et al., 2001
ephrinB2 receptor	high expression including smooth muscle cells and pericytes	no expression	kidney, heart, liver, spleen, intestinal fat, leg muscle, brain	yes	yes	Gale et al., 2001; Shin et al., 2001; Wang et al., 1998
ephrinB4 ligand	low expression	high expression	kidney, heart, liver, spleen, intestinal fat, leg muscle, brain	yes	yes	Gale et al., 2001; Shin et al., 2001; Wang et al., 1998
VE-cadherin	equal expression		bronchial and biliary epithelia and cardiocytes but not the endocardium	N/A	yes	Bianchi et al., 1999
RPTP μ	high expression	low expression	bronchial and biliary epithelia and cardiocytes but not the endocardium	N/A	yes	Bianchi et al., 1999

*N/A – not available

CONCLUSION.

Although large blood vessels can be easily recognized morphologically and identified through specific molecular markers to be veins or arteries, this has been a problem when it came to identifying the smallest of vessels: the capillaries. Capillaries are very variable in morphology and functions depending on their localization. In the brain, capillaries are paradoxically the tightest (at the blood-brain barrier sites) and the leakiest (at the site of the circumventricular organ, in the third ventricle).

Still, few markers were found to stain microvessels: von Willebrand factor, CD31, CD34,

CD105, VEGFR2 Fli-1, but endothelial cells are very heterogeneous even in the expression of these markers throughout the body, and not all these markers are specific to capillary endothelia. Also, these markers change their expression and distribution in pathologies. Making sense through all these bits and parts of information regarding capillaries characteristics and identification is still a matter of ongoing debate. And last but not least, to make it even more complicated, it seems that endothelial cells bare markers of their venous or arterial origin, giving the capillaries another factor of distinction.

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***Correspondence:** 80-86, Liviu Rebreanu Str.,
 Institute of Life Sciences, Western University
 "Vasile Goldis" of Arad, Arad, Romania