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Original Article

Multiple different laminar velocity profiles in separate veins in the microvascular network of brain cortex in rats

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Abstract: The orthogonal polarisation spectral (OPS) imaging technique is a method that enables intravital microscopy of the tissue microvasculature particularly including the erythrocytes and leucocytes. As a new finding we here report multi flow, i.e, several different laminar velocity profiles in each and separate veins (diameters < 200 μm) of the microcirculation of the rat brain cortex. The phenomenon was present in all 20 preparations studied and these different laminar velocity profiles were regularly maintained in length beyond 20 times the diameter of parent vessel. In single veins up to 9 different laminar velocity profiles were discernible, each with a different red blood cell velocity. These multi flow profiles may theoretically be anticipated based on what is known in rheological physiology as the Fahreus - Lindqvist effect. It may also be predicted in tissues that have both high and heterogeneous blood flows in conjunction with large local variations in metabolic activity as are present in the cortex of the brain. The new information is that the extent and magnitude of this multi laminar flow phenomenon especially in the venular network of the brain exceeds what has previously been known. The physiological importance of these finding warrants further studies.

Keywords: Dynamic structure of blood flow, Multi-laminar flow profiles, Orthogonal polarization spectral imaging, Rheology

Introduction

The different effects on microvascular flow profiles by alterations in local viscosity and hematocrit were initially described by Fahraeus – Lindqvist [1] in 1931 and have subsequently been firmly established. The concentration of cells in a tube < 0.3 mm in diameter differs from that in the larger feed tube or reservoir because of a difference in the mean velocity of cells and plasma in the smaller vessel that is associated with a non-uniform distribution of the cells. In tubes < 0.3 mm in diameter, the viscosity of the blood decreases with decreasing diameter of the tube, a phenomenon called the Fahraeus – Lindqvist effect [1]. Understanding this effect has been crucial in elucidating the rheological phenomena in the microvascular bed. Recently, with the introduction of orthogonal polarization spectral (OPS) imaging, a new technique has become available that in real time indirectly depicts the red blood cells (RBC), while white blood cells (WBC) are intermittently visible [2]. Using this technique, rheological phenomena are particularly suitable for study in the venous tree (**Figures 1, 2**), because the walls of the veins are thin [3]. The aim of this report is to describe a newly, observed rheological phenomenon in the venular branches and in the direct communications between arteries and arteriolar (a-a shunts) branches in the brain cortex of the rat. This finding in the microvascular network we call the “extended Fahraeus - Linqvist effect”, as it is a consequence of this previously described physiological phenomenon. These findings were made while examining the neuro-vasculature for qualitative and quanti-

tative differences in the microvascular response to inflammation that are known to be prevalent in the brain [4] and that are known to be different to that seen in other organs such as the liver, skeletal muscle, and mesentery [5-8]; differences that have led to a new evolving paradigm for blood cell-endothelial cell interaction in this organ [4].

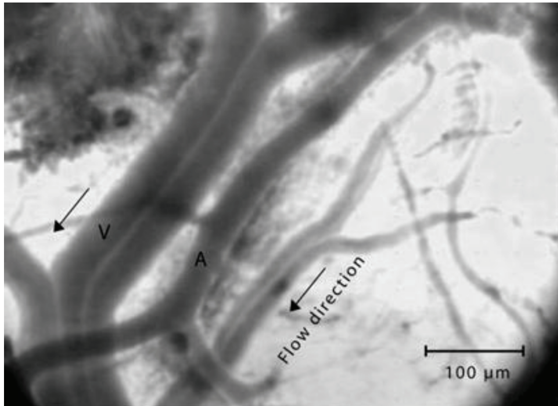

Figure 1. OPS image of the brain microvasculature in the surface tissue after opening of the dura. Central in the image is a larger venule (V) and the artery (A). Arrows indicate flow direction (large (V) and small venule). The Bar corresponds to 100 μm. In the center of the larger venule, there is a white streak indicating the border between the two laminar flow profiles generated by the two veins merging at the top of the image. In the left lower corner yet a third venule is joining and thereby adding a third laminar flow profile.

Material and methods

Animal experiment

Twenty female rats (Sprague-Dawley, B&K Universal, Sollentuna, Sweden) weighing from 250 to 270g were used. The rats were housed two to a cage at a constant room temperature (21°C) for 3 days before operation, with free access to water and standard rat chow, and with 12-hour light/dark cycles.

Anaesthesia was induced with 4 % isoflurane (Forene ® 250ml, Abbott, Scandinavia AB, Kista, Sweden) in a mixture (30%:70%) of oxygen:nitrous oxide in a specially designed box. A soft endotracheal tube was used for controlled ventilation (Zoovent, CWC600AP), ULV, Newport, UK) using 1% isoflurane in a mixture (30%:70%) of oxygen:nitrous oxide. The tidal volume and ventilation frequency were carefully regulated using on-site monitoring of blood gases and acid/base status (AVL, OPTI 1 Medical Nordic AB, Stockholm, Sweden). A long-acting non-steroidal anti-inflammatory, carpofen (Rimadyl®, Pfizer, Dundee, Scotland) 0.1ml/kg was given at the beginning of the operation [9].

Microvascular preparation of the brain

A bone flap (0.5 × 0.5 cm²) created in the bregma region of rat's temporal bone along the sagittal suture was removed and the dura opened. A small volume was isolated on the

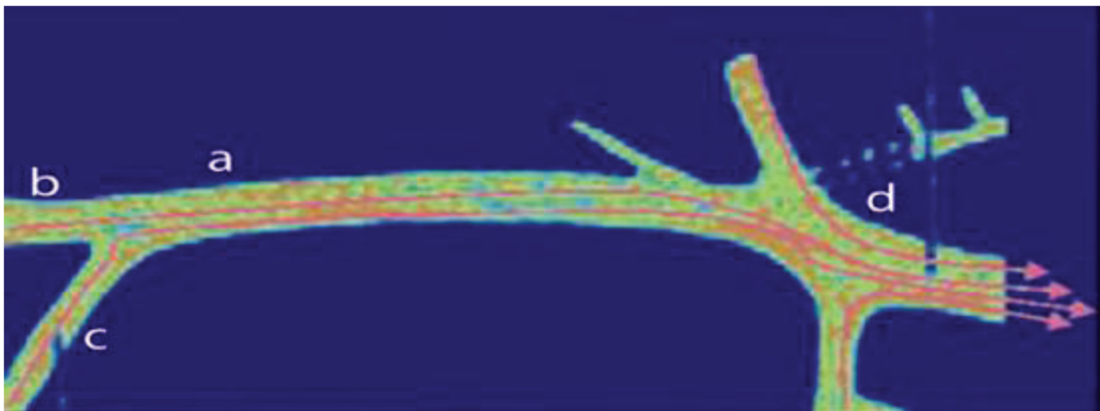

Figure 2. Schematic drawing of the multi laminar flow profiles, generated by the add-on of each and more venular vessels in the microvascular bed of the brain. In the illustration, the blood corpuscles from the branch vessels (c) and (b) do not mix/combine after flowing into a larger vein (a), and continued to travel separately even after reaching the second main branching vessel (d). In this vessel (d), four separate laminar flows with different flow velocities are discernible.

brain cortex with a ring of silicone grease on to which a thin quadratic glass piece had been applied after the volume had been filled by warm Krebs' solution. The microvascular bed was assessed through the glass with the OPS imaging technique as described below. We searched for a visual field that contained at least two venular networks, each of which contained more than six separate areas in which venules were depicted clearly. All measurements were recorded continuously three times, each for five minutes. We examined microvascular networks that contained vessels with the following diameters: *arterioles* ranging from 20 to 60 µm, and *venules* ranging from 20 to 150 µm. The studying of complete microvascular networks also provides information that cannot be obtained from analysis of randomly selected vessels [10].

Ortogonal Polarisation Spectral Imaging (OPS)

The orthogonal polarisation spectral (OPS) imaging technique is a new way of imaging the microcirculation using reflected light that allows imaging of the microcirculation non-invasively through mucous membranes and on the surface of solid organs [11]. It even enables non-invasive visualization of the microvascular bed in humans. As it specifically depicts the separate blood corpuscles it may be used to assess the local vessel hematocrit and blood corpuscular velocity and so provide indirect information on local rheological phenomena.

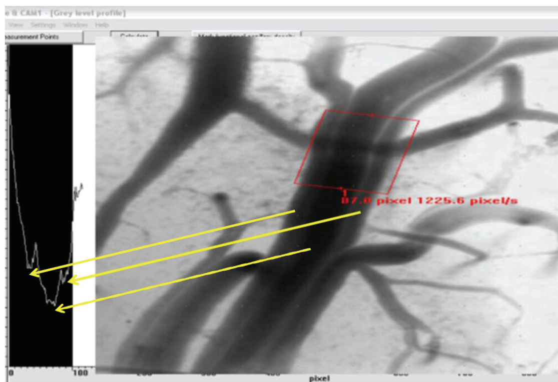

Figure 3. Screen picture (OPS image) of the brain microvascular bed (3 Venules). Relative velocity estimates are provided from the area (red box) positioned on the large venule in the center of the image. The corresponding relative blood cell velocity is pictured to the left in the image (black rectangle with a white line). In the velocity profile of this box three separate flow maximums are depicted and marked with the corresponding part of the vessel in the microvascular image (3 arrows). The relative flow profile was computed using KK-Technology software.

The OPS imaging device used was a Cytoscan Model E-II (Cytometrics Inc., Philadelphia, Pa., USA), a newly developed instrument that functions as an intravital microscope and is small and easily portable. By the use of orthogonal polarization spectral (OPS) imaging, the Cytoscan Model E-II delivers images of the microcirculation that are comparable to those achieved with intravital fluorescence video microscopy (IFM), but without the use of fluorescent dyes [12]. Arterioles, venules, and capillaries can be indirectly seen, and the movement of individual red blood cells can be observed through them [13].

Data analysis

The images recorded of the microvascular beds were stored on DVD discs. The dimensions of the microvascular bed were assessed by specially designed software (KK-Technology, Sweeting House, EX14 1LJ, England). Using this software the red cell velocity profile of a given vessel may also be depicted (Figure 3).

Results

Venular network

The alterations in hematocrit seen for separate veins – that is a central concentration and plasma skimming at the vascular wall effect was observed in all preparations (n=20), of which 15 such venular preparations are shown (Figure 4). Depending on the number of venular branches that joined to form a larger vessel, a corresponding number of different laminar flow profiles were seen. The multi laminar flow effect of any given vein was maintained for an extended length beyond the point where new vessels had joined and new laminar flow profiles were added. These profiles were maintained from 2 to 9 venular vessels, each in themselves adding a new laminar flow profile (Figure 5). These separate flow profiles were also kept well beyond a distance longer than that of 20 diameters of the parent vessel (Figure 6). When the dimension of a vessel was changing, this also affected the width of the laminar flow profile in that vessel, which changed accordingly. The profile varied over time in parallel to pulsations

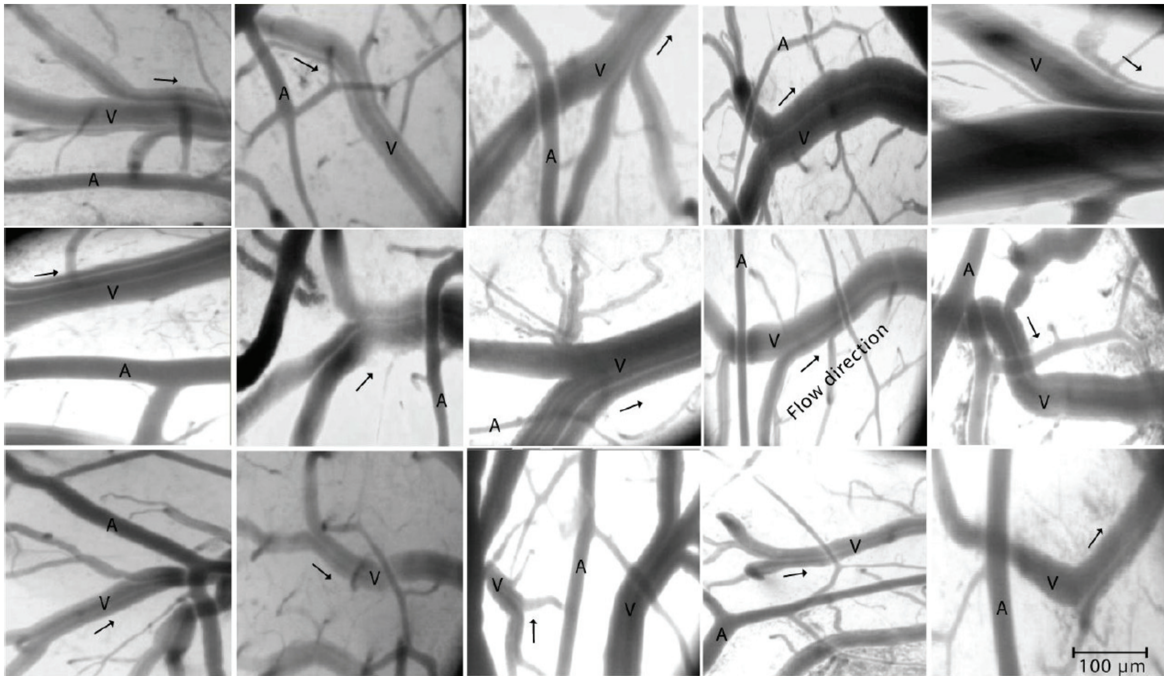

Figure 4. OPS imaging showing the venular network in the microvascular preparation of the brain. The phenomenon with maintained multi laminar flow profile effects was recorded in all 20 animal preparations, here showing 15 of them. Calibration bar is 50 μm; (V) venule; arrows indicate flow directions; Arterioles (A). Visualizations were by the CYTOSCAN E-II device with x 10 lens.

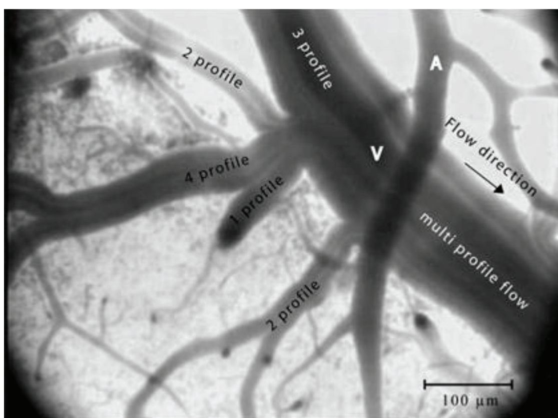

Figure 5. Depending on the number of venules joining to form a larger vessel (center of image), there is a corresponding number of flow profiles. For each vessel the number of laminar blood flow patterns visible is depicted (black text). In this preparation more than 8 separate laminar flow profiles were discernible when the large vessel was formed and that exits the image to the lower right. Bar indicate 100 μm.

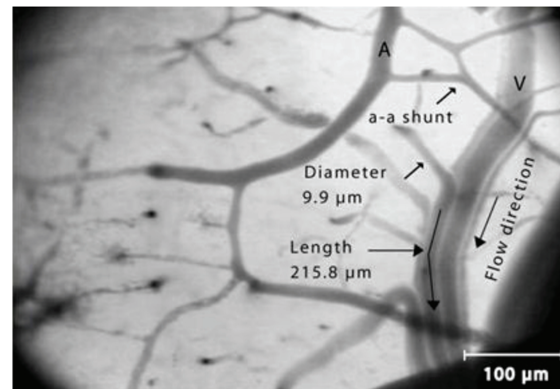

Figure 6. The laminar blood flow profiles generated from the venule marked with an arrow (diameter 9.9 μm) as it is joining the larger venule (with its 4 laminar flows (Flow direction and arrow)) the length of the maintained flow profile is at least 215.8 μm, also marked with an arrow. A-a indicates arterial – arterial anastomoses. Bar corresponds to 100 μm.

or effects of ventilation (Figure 7). When different laminar flow profiles were examined, it was evident that each of these profiles depicted a different blood cell velocity (Figure 3). This was also clearly evident by just examining the smaller dimension vessels in the OPS system.

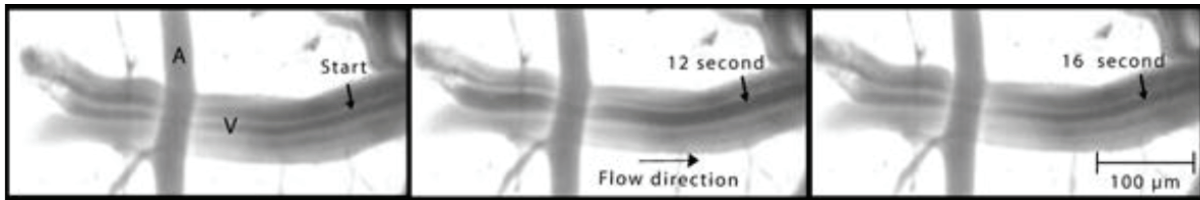

Figure 7. The multi laminar flow profiles are maintained during tissue pulsations due to arterial pulse pressure changes and changes due to respiration. In this image a smaller arteriole (A) is crossing larger venule (V). The width of the laminar profile is increasing from image A (left) to B (center; an image frame 12 seconds later) and again smaller image C (right; an image frame 16 seconds after the first image). Arrow indicates flow direction. Bar corresponds to 100 μm.

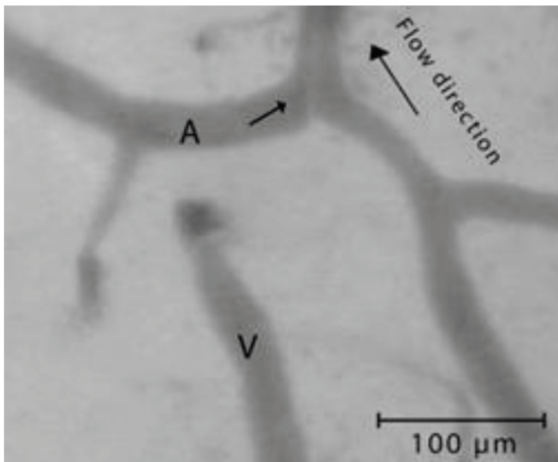

Figure 8. The direct communication observed between two arteriole (a-a shunts) in the rat brain. Arrow indicates flow direction. Also in the a-a conection the laminar flow profiles are maintained. Bar marks 100 μm.

Arteriolar network

We observed arteriolar (a-a shunts) regularly in the preparation of the cortex. In these a-a shunts, double parabolic flow profiles were also seen (**Figure 8**).

Discussion

We describe extensive multi laminar flow profiles in separate veins of the brain microcirculation that lasted more than 20 vessel diameters in the venular microcirculation. To our knowledge, although the phenomenon may be predicted by the effects described by Fahreus-Lindquist [14], the extent of this phenomenon in the present preparation (rat brain) has not, to our knowledge been described previously.

The findings suggest that the consequence of the Fahraeus-Lindqvist effect is more extensive in the brain venular microvascular bed than previously known. The finding, which we have called "multi laminar velocity flow profiles" is that in the microvasculature the blood corpuscles do not mix when two vessels meet, but the concentration and its distribution of red cells that corresponds to the diameter of the parent vessel is maintained, and that the plasma skimming at the vessel border is also kept after several new venular vessels have joined.

The finding was appreciated in all of the preparations of the brain cortex that we examined and was most prevalent for the venular network, though similar changes were also seen on the arterial side. We have been unable to find publications that have described such findings, except in an experimental series done during severe hemodilution, in which a similar but lesser effect was noted [15]. Of importance is that we also noted that the red cell velocity seemed to be highest in the centerline of each laminar flow profile as has previously been described for single vessels [16].

Although we have examined only the microcirculation of the brain cortex it is possible that this phenomenon, albeit less extensive, may also be present in more venular microvascular beds of other organs. The finding may, however, be particularly prevalent in the cortex of the brain for several reasons: first and most importantly the high, and locally varying cell metabolism of the brain leads to local variations in metabolic needs and co-existent microvascular flow; secondly, the high flow in this organ leads to high

blood cell velocity, which if it is different, counteracts mixing of blood corpuscles [17]; thirdly, the high vessel density in this tissue may contribute further to the phenomenon.

One physiologically important consequence of the described phenomenon is that it, although we did not specifically examine this in the present paper, may affect the process of extravasation of the WBC and the exchange of molecules including oxygen and carbon dioxide between erythrocytes and the tissues, and thereby also have consequences for the inflammatory process. This is currently of particular importance as there has been an evolving new description of the blood cell-endothelial cell interactions in the cerebral circulation [4]. There is evidence that suggests that there are qualitative and quantitative differences in the microvascular response to inflammation in the brain compared with other organs such as liver, skeletal muscle and mesentery [4]. The cerebrovascular endothelial cells have a number of unique characteristics, including their barrier, transport, metabolic, and cell traffiking properties. These differences may also depend on a different underlying rheology. The multi laminar blood flow profiles that have been described would theoretically lead to a decreased extravasation of WBC´s as they would make trafficking even more difficult. Such effects may for example, emphasize the finding of few WBC in the brain parenchyma, which has led to the claim that the brain is an immunologically privileged organ [4]. The rheological state described, multi laminar flow profiles, may further contribute to difficulties for the WBC to extra-vasate. For example the WBC extravasation in the brain is claimed to be less than 1:20 of that seen in the skeletal muscle [18]. This finding has previously been described and claimed to be the result of several factors such as: low basal expression of endothelial cell adhesion molecules [18]; a high electrostatic charge on the cerebrovascular endothelial cells [19]; and high venular shear rates that tend to oppose adhesion of blood cells [8]. Added to these factors may also be the multi laminar blood flow profile effect described here and this needs to be further examined and confirmed in future studies.

An important contributing factor that has led to the presented findings is the use of a new imaging technique (OPS). We think that the specifics of this technique provide the technical background to picture local variations in hematocrit more easily. OPS is based on that the polarized light is absorbed by the red blood corpuscles and the red blood cells are seen as dark images. The corresponding layers of plasma skimming do not absorb the light and are depicted in the video images as white streaks that enable the observer to distinguish them from the mass of red cells [20]. That combined with the thin layer of the venular wall, facilitates the observation of varying local vessel hematocrit. The lower blood cell velocity in the veins further amplifies the discriminative power of the investigator. Apart from the fact that they are less prevalent, it was more difficult technically to find corresponding phenomena in the arterial tree. This may be partly due to the difficulty of OPS light penetration through the arterial wall, and the higher blood cell velocity in these vessels. Arterial to arterial branching was also less redundant.

The underlying mechanisms for these rheological findings is that the resistance to flow is less in the areas of the vessel border, which leads to a central concentration of the red blood cells, and simultaneously plasma is marginalized at the endothelial end. The underlying rheological physiology also leads to a velocity increase of the red cells at the central core of the vessel. As the blood cells are affected by the increasing speed at the center, forces are liberated that tend to rotate the blood cell and further facilitate the migration of the red blood corpuscle towards the center line [21, 22].

Another important factor that contributes to the findings of the present study is the use of a validated animal model. This model is based on inhalation anesthesia, and special care was taken not to alter the circulatory or ventilatory settings once the preparation was stable [9]. Using a from environmental high oxygen and low carbon dioxide closed microvascular preparation also reduces any effects of the atmospheric gases, particularly vasoconstriction induced by a low carbon dioxide, which are known to affect the microvascular preparations and alter microvascular rheology [23].

In summary we have shown that multi laminar flow profiles are common phenomena in the venous microvasculature of the rat brain. The findings may be explained by the high and heterogeneous metabolic rate and the complex

microvascular architecture of the brain. Contributing factors to these findings are also the specifics of the OPS imaging system, which clearly visualizes the red corpuscles in the microvasculature and so increases the chances of observing rheological phenomena. The physiological relevance of the multi laminar flow profiles is unclear. However, it may be of importance for the extravasation of WBC, and as such have implications for the inflammatory process in the brain, however this needs further investigations

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