Reduced Capillary Density in the Prefrontal Cortex in Schizophrenia

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Abstract Neuroimaging studies have shown that the core symptoms of schizophrenia are associated with local changes of cerebral blood flow, particularly in the frontal cortex. Previously we reported ultrastructural damage of capillaries in the upper layers of the prefrontal cortex, Brodmann's area (BA) 10 and in the visual cortex, BA 17 in schizophrenia. An electron microscopic morphometric study was performed to estimate capillary area density (N cap/mm²) in two upper layers of the prefrontal and visual cortices in 26 cases of schizophrenia and 26 normal controls. Capillary area density was lower in the prefrontal cortex in the schizophrenia group (-24%, $p < 0.001$) and in the subgroup of schizophrenia with predominantly negative symptoms ($n=12$, -35%, $p < 0.001$) as compared to controls. Group and subgroup differences were absent in the visual cortex. Dysfunction of neocortical microvasculature in schizophrenia is related to region-specific capillary deficiency in the prefrontal cortex. These changes might contribute to the lowered blood flow, reduced glucose metabolic rates, resting hypofrontality and hypoactivation reported in the prefrontal cortex of patients with schizophrenia.

Keywords: schizophrenia, capillary, electron microscopy, morphometry, prefrontal cortex, visual cortex

1. Introduction

Neuroimaging studies have consistently shown that diverse symptoms of schizophrenia are associated with local changes of cerebral blood flow, particularly in the frontal cortex. Frontal regional blood flow was not increased while performing cognitive tests in patients with schizophrenia as compared to normal controls [1,2,3,4]. Decreased blood flow [5] and glucose metabolic rates [6,7], as well as resting hypofrontality [8] and hypoperfusion [9,10] have been reported in the prefrontal cortex in patients with schizophrenia. Reduced frontal blood flow is associated with negative symptoms of schizophrenia [11,12,13,14]. Hanson and Gottesman [15] suggested that abnormalities of blood flow might lead to altered neuronal-glial relationships resulting in neuronal dysfunction and psychopathology. However, it has remained uncertain whether these changes are related to the abnormalities of microcirculation.

Brain microcircuitry might be abnormal in schizophrenia. Though mean capillary length density was not changed in the prefrontal cortex (BA9) [16] or subcortical brain structures [17] in schizophrenia, decreased numbers of capillary loops were detected by biomicroscopy of the bulbar conjunctiva and capillaroscopy in schizophrenia patients [18]. Ultrastructural alterations of capillaries in human embryos from schizophrenia mothers have been reported previously [19]. Also, an atypical simplified angioarchitecture and abnormal arborization of the brain vessels [20], decreased GFAP labeling of astrocytes adjacent to blood vessels [21]

and lowered number of pericapillary oligodendrocytes [22] have been described in postmortem prefrontal cortex in schizophrenia. Analysis of endothelial cells showed differences in gene expression between schizophrenics and controls [23]. Decreased VEGF mRNA expression has been recently reported in the dorsolateral prefrontal cortex of schizophrenia subjects [24].

Previously we reported ultrastructural damage of capillaries in postmortem prefrontal and visual cortices in subjects diagnosed with schizophrenia [25]. However, it has remained unclear whether capillary density is affected in schizophrenia. We hypothesized that subjects with schizophrenia would show a capillary deficiency in the neocortex.

The aim of the current study was to estimate capillary area density in the upper layers of the prefrontal and visual cortices in schizophrenia and normal controls using quantitative electron microscopy.

2. Materials and Methods

2.1. Subjects and Tissue

Brains were collected from the Mental Health Research Center (MHRC, Moscow, Russian Federation) within a short postmortem delay (4-8 hours). Ethical considerations in obtaining and using human autopsy material were in accordance with the rules of the Ethic Committee of the MHRC, Russian Academy of Medical Sciences. Twenty-six (26) subjects with schizophrenia and 26 normal control subjects matched by age and postmortem delay were used.

Demographic and clinical data are provided in Table 1.

Table 1. Demographic and clinical data

		N per group	Gender	Age (years)	PMI(hours)	Duration of disease (years)	Age at onset of disease (years)	Neuroleptic medication
				(Mean ± SD)				
Controls		26	5F/21M	52.3±14.8	5.5±0.9			
Schizophrenia		26	15F/11M	53.5±16.2	6.0±2.1	20.6±13.8	33.3±15.5	79.35 ±104.2
Schizophrenia	SPNS	12	5F/7M	52.5 ±15.7	5.6±1.5	21.6±14.9	32.2±15.3	112.4 ±35.0
	SPPS	14	10F/ 4M	54.3 ±17.2	6.3±2.6	19.8±13.3	34.2±16.3	54.6 ±70.2
	Nonparanoid schizophrenia	9	4M/ 5F	44.1 ±16.3	5.4±3.1	13.0±14.0	31.2±16.2	21.4 ±36.5
	Paranoid schizophrenia	17	7M/ 10F	58.4 ±14.3	6.3±1.5	25.2±11.9	34.5±15.6	108.3 ±15.7
Causes of death		Controls	Cardiovascular disease, myocardial infarction, blood vessel rupture, pneumonia					
		Schizophrenia	Cardiovascular disease, myocardial infarction, pulmonary embolism, aspiration asphyxia					

SPNS - schizophrenia with predominantly negative symptoms. SPPS - schizophrenia with predominantly positive symptoms. Neuroleptic medication for one year prior to death is expressed as CPZ-equivalents, mg.

Clinical records were obtained and both ICD-10 and DSM-IV diagnoses were made by MHRC psychiatrists. The Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) were used to rate negative and positive symptoms during the last hospitalization of schizophrenia subjects. Summary scores of negative and positive symptoms were determined on the basis of the ratio of the percentage of negative and positive scores. 12 cases with predominantly negative symptoms, 14 cases with predominantly positive symptoms and 26 normal control cases were studied. The schizophrenia group included 17 cases of chronic paranoid schizophrenia and 9 cases of other types of chronic schizophrenia: undifferentiated schizophrenia (n=4), catatonic schizophrenia (n=2), residual (n=2), unspecified schizophrenia (n=1) (nonparanoid schizophrenia).

Cases were coded, and the researchers were blind to patient diagnosis. For electron microscopy for each case, 10 small serial tissue samples containing grey matter of the central part of the prefrontal cortex (BA10) and of the visual cortex (BA17) from the left hemisphere were obtained in a plane vertical to the pial surface. The tissue samples were fixed in a mixture of 2.5% glutaraldehyde and 4% paraphormaldehyde in 0.1M phosphate buffer for one week. Then, tissue pieces were postfixed in 1% osmium tetroxide for one hour, stained with uranyl acetate for one hour, dehydrated in ethanol series and embedded in Araldit epoxy resin. Three prefrontal cortex tissue blocks and three visual cortex tissue blocks were randomly sampled. Sections were cut using an Reichert ultramicrotome, and 1 μ m thick sections stained with toluidine blue were used for orientation in cortical layers. Small pyramids were trimmed on the two upper layers (containing polymorphous cells and located above layer 3 containing pyramidal neurons), and ultrathin sections were cut at 60nm. We have chosen these layers on the basis of the previous evidence for ultrastructural damage of capillaries in the upper layers of the prefrontal and visual cortices in schizophrenia [25]. Ultrathin sections were taken and mounted onto Formvar/carbon coated slot-type copper grids, counterstained with uranyl acetate and lead citrate and examined in a Philips EM 420 electron microscope.

2.2. Quantitative Data Collection

Three prefrontal cortex tissue blocks and three visual cortex tissue blocks examined per subject. Capillary area density was estimated in two upper layers of the prefrontal and visual cortices in the control and schizophrenia groups. Capillaries were identified according to criteria described by Peters [26]. Capillary density in the prefrontal cortex was estimated within a tissue area of on average 84,700 μ m² per brain for the control group and 84,400 μ m² per brain for the schizophrenia group. The tissue area examined in the visual cortex was on average 88,000 μ m² per brain for the control group and 101,300 μ m² per brain for the schizophrenia group. The number of all capillary profiles < 10 μ m in diameter were counted using 4,000x magnification. Mean number of capillaries (mean $\pm$ SD) per brain calculated in the prefrontal cortex was 20.3 $\pm$ 4.3 for the control group and 15.6 $\pm$ 3.4 for the schizophrenia group. In the visual cortex the mean number of capillaries per brain was 15.5 $\pm$ 4.7 for the control group and 17.9 $\pm$ 5.8 for the schizophrenia group. After adjusting for magnification, the number of capillaries per unit tissue area was estimated by dividing the total number of capillary profiles counted by the total area examined. Then the capillary area density was estimated as the number of capillaries per 1mm².

2.3. Statistical Analysis

Statistical analysis was performed using the STATISTICA software package for Windows (StatSoft. Inc, Tulsa, OK, USA) to test the hypothesis that schizophrenia subjects have deficient capillaries in the neocortex. Capillary area density between the groups was analyzed. In addition, the control group was compared with two sets of clinical subgroups: cases with predominantly positive or negative symptoms and cases of paranoid or nonparanoid schizophrenia. Group comparisons were made using one-way ANOVA. Differences between the control group and different clinical subgroups were examined using one-way ANOVA with the control group and each of two sets of clinical subgroups, followed by post hoc test (Duncan's test, $\alpha < 0.05$) using the Bonferroni correction. Correlational analysis was performed for the control and

schizophrenia group to examine the effects of postmortem delay, age, age at onset of disease, duration of disease, medication (expressed as chlorpromazine equivalents according to Davis [27]). A normal distribution of the data by Kolmogorov–Smirnov tests was obtained, so the Pearson correlation coefficient was used. Gender effects were analyzed using a two-way ANOVA.

3. Results

Figure 1 illustrates the electron microscopic appearance of blood capillaries in the prefrontal cortex from control and schizophrenia brains. Characteristic thickening of capillary basement membrane and swelling of

pericapillary astrocytic end-feet were observed in the schizophrenia subjects (Figure 1B) in contrast to the control subjects (Figure 1A). There was no correlation between capillary area density and either age or postmortem interval. Means $\pm$ SEM of capillary area density for control group, schizophrenia group and schizophrenia subgroups are given in Table 2. Differences between the control group and schizophrenia group and subgroups are given in Figure 2. One-way ANOVA demonstrated a reduction in the capillary area density by 24% in the schizophrenia group as compared to the control group in the prefrontal cortex: ($F = 12.53$, $df = 1,50$, $p = .0009$) (Figure 2).

Figure 1. Electron micrographs of capillaries in layer 2 of the prefrontal cortex from control (A) and schizophrenia (B) subjects. Cap – capillary, AP – astrocytic process, arrows – capillary basal lamina. Scale bars = 2 μ m

Table 2. Capillary area density (Ncap/mm²) in the prefrontal and visual cortices in the control group, schizophrenia group and in different schizophrenia subgroups (mean $\pm$ SEM)

Brain structure	Control group	Schizophrenia group	SPPS	SPNS	Nonparanoid schizophrenia	Paranoid schizophrenia
Prefrontal cortex	252.6 $\pm$ 13.4	191.5 $\pm$ 10.8	214.7 $\pm$ 13.7	164.3 $\pm$ 14.1	182.1 $\pm$ 18.7	196.4 $\pm$ 13.6
Visual cortex	186.44 $\pm$ 12.6	182.8 $\pm$ 14.5	192.4 $\pm$ 21.6	171.7 $\pm$ 19.4	192.0 $\pm$ 27.1	177.9 $\pm$ 17.5

Figure 2. Capillary area density (means $\pm$ SEM) in the prefrontal cortex and in the visual cortex in control group, schizophrenia group, in the subgroup with predominantly positive symptoms (SPPS), in the subgroup with predominantly negative symptoms (SPNS) and in the subgroups of nonparanoid and paranoid schizophrenia

When the control group and clinical subgroups were compared, the subgroups of schizophrenia subjects with predominantly negative or positive symptoms differed significantly from the control group in the prefrontal cortex ($F = 8.99$, $df = 2,49$, $p = 0.0005$). Post hoc analysis demonstrated a significantly decreased capillary area density in the subgroup with predominantly negative symptoms ($n = 12$, -35% , $p < 0.0003$) as compared to the control group (Figure 2). Capillary density tended to decrease in the prefrontal cortex in the schizophrenia subgroup with predominantly positive symptoms as compared to control group, however the decrease did not reach significance ($n=14$, -15% , $p = 0.08$). Comparison of the control group with the subgroups of paranoid and nonparanoid schizophrenia demonstrated that capillary area density differed significantly in the prefrontal cortex ($F = 6.33$, $df = 2,49$, $p = 0.003$). Post hoc analysis revealed that the nonparanoid subgroup had significantly lower capillary density as compared to the control group ($n = 9$, -28% , $p = 0.005$) (Figure 2). No significant differences in capillary area density were found between groups or between schizophrenia subgroups and the control group in the visual cortex ($p > 0.7$) (Figure 2).

Overall, no effects of postmortem delay, age, age at disease onset, duration of schizophrenia or chlorpromazine equivalents on the parameter measured were found. In addition, no effect of gender on capillary area density was revealed.

4. Discussion

To our knowledge, this study is the first electron microscopic morphometric study of blood capillary density in schizophrenia. We used electron microscopic morphometric method because previously we reported significant ultrastructural alterations of capillaries in the same brain structures and in the same brain samples in schizophrenia as compared to controls [25]. The present study provides evidence for region-specific capillary deficit (by 24%) in the upper layers of the prefrontal cortex, but not in the visual cortex in schizophrenia as compared to controls. Previously we reported that capillary diameter was not changed in schizophrenia patients [25], and the data is in agreement with the results of stereological study of Sinka et al. [28], who showed that capillary diameter was not changed in the anterior cingulate cortex in schizophrenia patients. So capillary size could not influence the estimation of capillary area density. Reduced capillary density cannot be explained by the effects of aging since the control and schizophrenia groups were matched by age and postmortem delay, and these variables did not correlate with capillary area density in either group. No effects of chlorpromazine equivalents on capillary area density were found. These findings are in line with the results of in vivo studies demonstrating that while the atypical neuroleptic clozapine can improve negative symptoms and cognitive dysfunctions, it cannot improve reduced blood flow in the frontal lobes [29]. Both antipsychotics and antidepressants are known to increase proliferation of neuronal and endothelial cells in the hippocampus [30]. Together these data suggest that reduced capillary density found in the prefrontal cortex in

schizophrenia is not attributable to the effects of neuroleptic medication.

Kreszmanski and colleagues [16] found no differences in mean capillary length densities in the prefrontal BA 9 in schizophrenia subjects as compared to normal controls in a light microscopic morphometric stereological study. The absence of differences in mean capillary length densities in the prefrontal BA 9 in schizophrenia does not directly contradict the regional and layer-specific reduction of capillary area density found in the upper layers of the prefrontal BA 10 in the present study. Our data is consistent with other abnormalities reported in schizophrenia patients: decreased number of capillary loops by biomicroscopy of the bulbar conjunctiva and capillaroscopy [18], atypical simplified angioarchitecture in orbitofrontal cortex in paranoid hallucinatory schizophrenia patients, and abnormal arborization of the brain vessels [20].

When the subgroups of cases with predominantly positive and negative symptoms were analyzed at separately, capillary area density was significantly 35% lower in the subgroup with predominantly negative symptoms in the prefrontal cortex as compared to controls and nonsignificantly (15%) lower in the subgroup with predominantly positive symptoms. The data are in good agreement with the results of our previous studies demonstrated a significant 22% deficit of pericapillary oligodendrocytes in the prefrontal BA 10 in the schizophrenia group, including a 20% deficit of pericapillary oligodendrocytes in the subgroup of subjects with predominantly negative symptoms as compared to normal controls [22]. Importantly, our data are also in accordance with a significantly (by 24%) lower number of neurons in layer 2 of the prefrontal cortex [31], as well as decreased expression of the GAD67 protein, reelin mRNA, and density of reelin-immunopositive neurons in layer 1 of the prefrontal cortex [32] reported in schizophrenia. In addition, nonparanoid subgroups showed significantly lower capillary density as compared to the control group.

We found no significant changes in the capillary area density in the visual cortex in schizophrenia as compared to controls. Previously we reported a decreased synaptic size in layers 1 and 2 in the prefrontal cortex but not in the visual cortex [33] and a significant decrease in numerical density of axospinous synapses in layer 1 of the prefrontal cortex [31]. Moreover, reduced synaptophysin immunoreactivity has been reported in the prefrontal cortex but not in the visual cortex in schizophrenia [34]. Together these data provide evidence that the regionally specific reduction in capillary area density in the prefrontal cortex found in schizophrenia is related to the disease.

It is of interest to note that the aging-related loss of synapses in the prefrontal BA 46 but not in the visual BA17 showed a significant correlation with behavioral measures of memory function in monkeys [35,36]. Since the prefrontal cortex has a greater role in cognition than the primary visual cortex, these data suggest that the deficit of synapses in layer 1 and decreased synaptic size in the upper layers of the prefrontal cortex might be associated with cognitive disturbances and microvascular abnormalities found in schizophrenia. Deficits in capillary density might reduce cerebral blood flow and metabolic support to neurons, and these abnormalities might be

associated with the capillary damage in subjects with schizophrenia.

The present findings are also in accordance with imaging data suggesting that negative symptom profile in schizophrenia patients displays cognitive deficits and lower cerebral blood flow in the frontal lobes [11,12,14], as well as with the reported negative correlation of negative symptoms with regional cerebral blood flow in the left frontal lobe [13]. Taken together with our results, these data support the notion that the abnormal capillaries revealed in the prefrontal cortex in schizophrenia might reduce cerebral blood flow and consequently result in deficit of metabolic support to neurons. In addition, these abnormalities might also contribute to reduced blood flow and thus might be involved in the production of negative symptoms and cognitive disturbances in schizophrenia.

In vivo studies demonstrated that regional cerebral blood flow abnormalities in patients with chronic schizophrenia do not directly correlate with the illness duration or the effects of medication. Lower blood flow in frontal cortex is already present at the early stage of schizophrenia, suggesting that a similar neural dysfunction occurs in both first-episode and chronic schizophrenia [37,38,39]. While microvascular density in each brain region could be genetically programmed, the developmental mechanisms controlling vasculature growth should be dynamic, because changes in neural activity during development are known to result in changes in microvascular density [40]. Capillary density appears to develop in accordance with local functional demands [41]. Increase in metabolic demand produces significant growth of new capillaries, while decreased activity reduces vascular growth [42,43,44,45]. Decreased VEGF mRNA expression has been recently reported in the dorsolateral prefrontal cortex of schizophrenia subjects [24]. The development of cortical vascularization is mediated by VEGF [45]. VEGF significantly influence angiogenesis and microvascular plasticity by stimulating neovascularization and increasing the number of blood vessels. VEGF influences neurogenesis, it is involved in the regulation of blood flow and has both neurotrophic and neuroprotective properties [46]. These data suggest that reduced capillary density might be of neurodevelopmental origin.

Significant ultrastructural alterations of capillaries in the prefrontal and visual cortices were found in schizophrenia compared to normal controls in our previous study of the same brain samples [25]. Abnormalities of capillaries and of their microenvironment in schizophrenia brain included thickening, deformation, vacuolation of basal lamina, prominent swelling and vacuolation of astrocytic end-feet, alterations of pericapillary oligodendrocytes and signs of activation of microglial cells. Alterations in microvessel ultrastructure could potentially contribute to changes in both blood flow and transport of materials across the capillary wall. Reduced frontal blood flow during task performance and incapacity to increase blood flow velocity during the time course of cognitive activation compared to control subjects have been reported [47,48,49]. These data suggest that the ultrastructural capillary abnormalities and deficiency in the prefrontal cortex in schizophrenia might contribute to hypofrontality, reduced blood flow reported in the prefrontal cortex in schizophrenia.

Altered hemodynamics may lead to hypoactivation [5,9,10,13,37], altered metabolic rates [7] and atrophy of neurons and synapses [33,50,51,52] reported in the prefrontal cortex in schizophrenia. Capillary loss has been reported in the white matter in vascular dementia in leukoaraiosis [53], decreased vascular density has been found in Alzheimer's disease, and cerebrovascular dysfunction precedes and accompanies cognitive dysfunction and neurodegeneration [54]. These data suggest that capillary abnormalities in the prefrontal cortex in schizophrenia detected in the present study might contribute to cognitive impairments in schizophrenia patients.

The limitations of the study are that we did not use stereological methods, and the groups were not gender matched. Future studies of blood capillaries in different brain regions in schizophrenia are required, taking into account the importance of region-specific reduction in the capillary density in schizophrenia reported in the present study.

5. Conclusions

Reduced capillary area density found in the prefrontal cortex in schizophrenia could be related to the dysfunction of neocortical microvasculature in schizophrenia. Decreased capillary area density in the prefrontal cortex but not in the visual cortex was most prominent in subjects with predominantly negative symptoms, which is in line with the results of neuroimaging studies. Capillary deficiencies in the prefrontal cortex might contribute to lower blood flow, reduced glucose metabolic rates, resting hypofrontality and hypoactivation detected in the prefrontal cortex of patients with schizophrenia.

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Statement of Competing Interests

The authors have no competing interests.

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