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The microvascular alterations in frontal cortex during treatment with antipsychotics: a post-mortem study

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Abstract

Introduction: Schizophrenia is the most severe psychiatric illness, with a biological support in the brain. There is evidence that the adequate dopamine balance in the frontal cortex is associated with a better outcome of the disorder, while the alteration of dopamine mechanism at this level may affect the vascular system leading to secondary neuronal alterations. Our study was conducted post-mortem and its objective was to identify the alterations in the neuronal architecture, in the integrity of the microvascular unit in the frontal cortex of patients treated with potent and excessive D2-blocking antipsychotics. *Materials and Methods*: We studied post-mortem sections of the frontal cortex of three patients (two women and one man) diagnosed with schizophrenia or schizophrenia-spectrum disorders and treated with antipsychotics for the last 24 months. The slides were prepared according to the classical histopathological protocols. *Results*: Various alterations were found at the neural and vascular levels in the frontal cortex. The most significant was the neural loss as the result of severe changes in the microvessels (diameter reduction, hyaline and collagen deposits, edema, pinocytosis and vacuolization). *Discussion*: The evidences shown in our study highlight the fact that antipsychotics with potent antagonist action on D2 receptors may affect the neurovascular unit and small vessels in frontal cortex by altering the balance vasoconstriction-vasodilatation, thus reducing the blood flow and metabolism and generating structural microvascular changes proportional with the level of apoptosis at this level. The functional integrity of the dopaminergic system in frontal cortex depends on the vascular support and the capabilities of the neurovascular unit and any dysfunction increases the neuronal loss with clinically significant changes. *Conclusions*: The pathological data of our study raises the hypothesis for the pathogenic stages at the level of microvessels in the frontal cortex of the patients with schizophrenia or schizophrenia-spectrum disorders treated with D2-blocking antipsychotics: a stage with functional, reversible alterations that may be correlated with the impairments of working memory and presence of extrapyramidal symptoms and a lesional, irreversible stage with significant deterioration of cognition and global functioning. Further studies are needed to verify this hypothesis.

Keywords: cerebral small vessels, frontal cortex, cognitive deficit, neurovascular unit, antipsychotics.

⊟ Introduction

Schizophrenia is the most severe psychiatric illness, with a biological support in the brain. The cognitive dysfunction has been mentioned as early as the introduction of the concept of schizophrenia, both by Kraepelin (dementia praecox) and Bleuler, who considered that this cognitive disability may be the fundament of the schizophrenic process.

The identification of brain mediators and, most important, the correlation of dopamine function with acute psychotic phenomena [1] were the cornerstones of the psychopharmacological research that lead to the first generation of antipsychotic agents with a proven therapeutic efficacy on the positive symptoms that are delusions and hallucinations. The initial enthusiasm was soon tempered by the adverse effects of the excessive blocking of D2 receptors, leading to extrapyramidal symptoms and decrease of dopamine levels in frontal cortex with hypo-dopaminergic syndrome. This syndrome, known as the hypofrontality syndrome may be induced on animal model by D1 antagonists that determine long-term depression [2].

The importance of the frontal cortex and particularly of the dorso-lateral prefrontal area is mentioned in many studies [3] showing that the physiologic level of dopamine in frontal cortex is maintained by a biphasic release of dopamine that is indirectly regulated by the synergic action of the balance between D2 and D1 receptors at this level. The correct dopamine integration in the frontal cortex facilitated the informational gating from the prefrontal area to the median subcortical dopaminergic area. We may argue that, in the frontal cortex, dopamine is a real pacemaker for the regulation and control of the balance long-term depression – long-term potentiation [4].

The role of the frontal cortex functioning in schizophrenia depends on the neuronal dopaminergic transmission, but also on the influence of dopamine blockade at the level of small cerebral vessels, with the alteration of vascular perfusion and metabolic support.

Our study was conducted post-mortem and its objective was to identify the alterations in the neuronal architecture, in the integrity of the microvascular unit in the frontal cortex of patients treated with potent and excessive D2-blocking antipsychotics.

⌕ Patients, Materials and Methods

The study was conducted on three patients, two women and one man, with ages at the time of death of 76, 54 and 55 years, respectively, registered in psychiatric settings of Galati County (Romania), with schizophrenia and schizophrenia-spectrum disorders that were treated with antipsychotics for the last 24 months (Table 1).

Table 1 – *Clinical and socio-demographic characteristics of the patients*

Age [years]	Gender	Diagnosis (year of diagnosis)	Daily treatment regimen (last 24 months)	Year of death
76	F	1. Brief psychotic disorder (2008); 2. Delusional disorder (2010).	Haloperidol 10 mg	2011
54	F	Schizoaffective disorder (2010)	Olanzapine 15 mg Valproate 500 mg Clonazepam 2 mg	2013
55	M	Paranoid schizophrenia (2007)	Risperidone LAI 25 mg Levomepromazine 25 mg Valproate 500 mg	2010

F: Female; M: Male; LAI: Long-acting injectable.

Because the patients died during hospitalization, autopsies were performed in order to determine the cause of death. During the procedure, brain sections from the frontal area were prelevated and fixated in 10% neutral formalin solution and embedded in paraffin, according to the classic pathological protocol. The biological material embedded in paraffin was processed at the Research Center for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova, Romania. The cuts of the paraffin blocks were performed with the Microm HM350 rotary microtome equipped with a system of transferring the sections on water bath (STS, microM). Thus, we obtained 3–4 μm sections. For the classic histopathological study, we used the Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome stainings. The objective of the immunohistochemical study was to evaluate the capillary density and vascular alterations in the frontal lobe of the cerebral hemispheres. From the same material, we cut 3 μm sections that were collected on slides covered with poly-L-lysine, dried in a thermostat at 37^0C for 24 hours. After that, the paraffin was removed and the sections were hydrated. For revealing the specific antigen, the slides were boiled in a solution of sodium citrate (pH 6) in a microwave oven for 21 minutes (seven cycles of three minutes). After the slides cooled, they were washed in distilled water for 15 minutes. For the inactivation of endogenous peroxidase, we incubated the sections in 3% hydrogen peroxide for 30 minutes, at room temperature, followed by washing in distilled water for 10 minutes and then in 1% phosphate-buffered saline (PBS) for five minutes. Finally, the non-specific sites were blocked using 2% skim milk for 30 minutes.

The prepared sections were incubated with the primary antibody for 18 hours (overnight) in a refrigerator, at 4^0C. Next day, we applied the secondary biotinylated antibody for 30 minutes, at room temperature, then we washed the slides in 1% PBS (three cycles of five minutes), then we applied Streptavidin–HRP (horseradish peroxidase) for 30 minutes, at room temperature, then we washed the slides in 1% PBS (three cycles of five minutes). The signal was detected using 3,3'-diaminobenzidine (DAB, Dako) and the reaction was stopped in 1% PBS. The next procedures were contrasting with Mayer's Hematoxylin, dehydrating in alcohol, clarifying in xilene and mounting the slides with DPX (Fluka). For highlighting the capillaries (endothelial cells), we used the anti-CD34 antibody (clone EP373Y/ab81289 Abcam, 1:100 dilution).

⌕ Results

As documented in the patients' files, the onset of the disorder was psychotic with acute, polymorphic delusions and hallucinations and disruptive behavior (aggressivity and violence, significant impairment of social functioning with severe conflictual behavior). All patients have lacked treatment compliance and adherence that were maintained by the cognitive deficit (Mini Mental State Examination – MMSE = 24+1), particularly regarding the working memory, and a chronic background of extrapyramidal symptoms.

The histopathological assessment showed multiple alterations of the cerebral parenchyma and vessels. In the cerebral cortex, we found a reduction in the number of neurons, especially in the upper layers (the molecular and the external granular layers) (Figure 1). In these areas, the neurons had cytoplasmatic and nuclear condensations with cellular pinocytosis. On some samples, we found large vacuoles because of neuronal death (Figure 2). In the profound layers, the significant alterations were cellular pyknosis with perineuronal edema, capillary collapse and pericapillary edema (Figure 3).

The large cerebral vessels (arterioles and venules) had a thickened wall due to hyalinization and fibrosis of tunica media and externa that resulted in the increase of vascular rigidity, thus showing a reduction of the capability in regulating the cerebral blood flow (Figures 4 and 5). Also, the vessels in the parenchyma had a significant reduction of their diameter due to the same phenomena of cerebral arteriosclerosis, either concentric or eccentric (Figure 6). The metarterioles had large deposits of collagen and hyaline material in their walls with a severe reduction of smooth muscular fibers in the tunica media with consecutive abolition of the blood flow regulating mechanisms in the brain (Figure 7).

There was a small number of cerebral capillaries, collapsed, with pericapillary edema (Figure 8). The presence of perivasculary edema shows a significant alteration in the functionality of the blood-brain barrier.

The immunohistochemical investigation of cerebral microcirculation employed the anti-CD34 antibody and revealed the fact that the alteration of cerebral vascular network was uneven. In the areas with severe neuronal alterations, the number of capillaries was significantly more reduced that in the other areas, suggesting that the main factor involved in the neuronal death may be the vascular factor. Chronic ischemia is the cause of the microscopic neural alterations.

The results obtained in the post-mortem histological

analysis of the frontal cortex demonstrate the relation between structural neuronal alterations and the intensity of vascular change and suggest the existence of two stages potentially induced by the vascular alterations secondary to the dopaminergic blockade in the microcapillary: a functional, initial stage and a lesional, irreversible one.
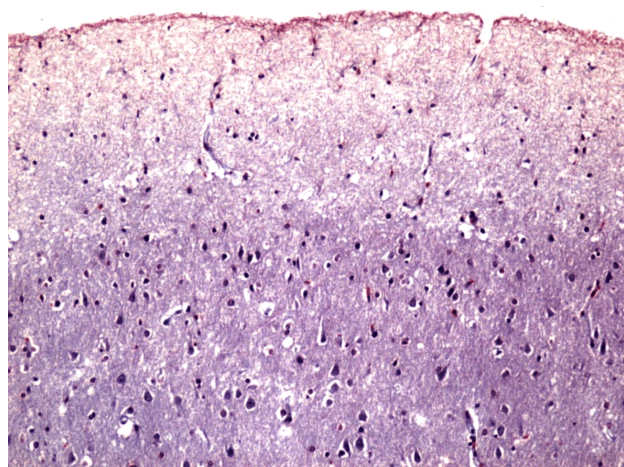

Figure 1 – *Histopathological overall picture of the frontal cortex in assessed patients with neuronal depopulation in the I and II layers. HE staining, ×100.*
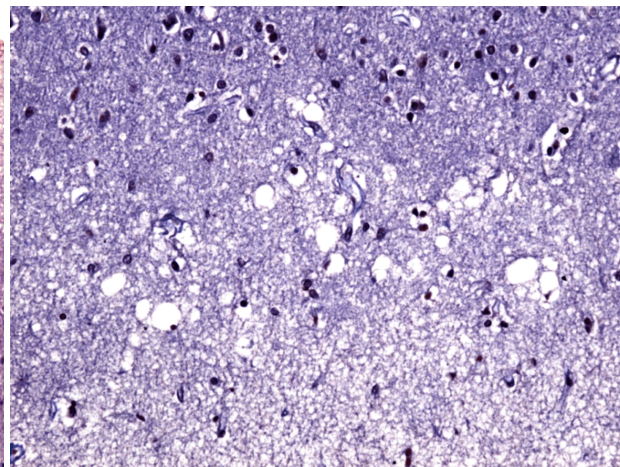
Figure 2 – *Cerebral cortex with neuronal pyknosis and multiple vacuoles as the result of neuronal death. HE staining, ×200.*
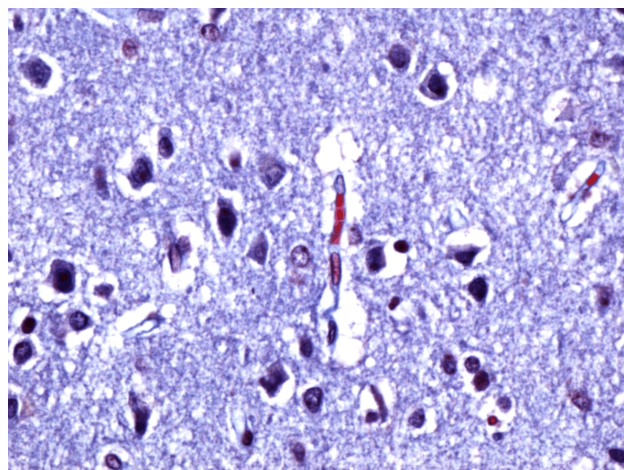
Figure 3 – *Pyramidal, granular and stellar neurons in the profound layers of cerebral cortex, with aspect of moderate pinocytosis, perineuronal edema, capillary collapse and pericapillary edema. GS trichrome staining, ×400.*
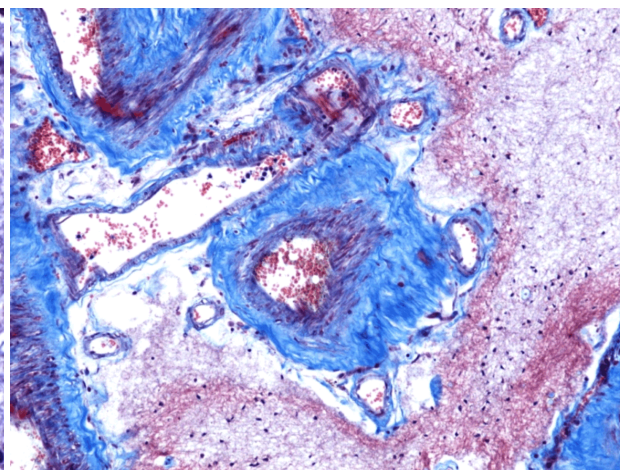

Figure 4 – *The scissural area with larger cerebral vessels (arterioles and venules) with thicker outer layer due to fibrosis and hyalinization of tunica externa. GS trichrome staining, ×40.*
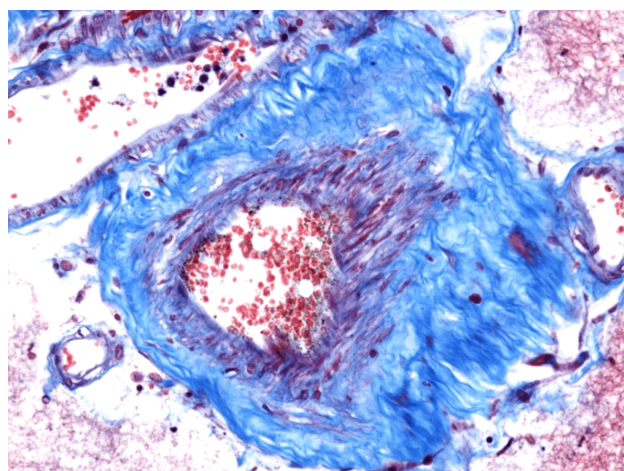

Figure 5 – *Detail of previous figure with excessive fibrosis and hyalinization of the vascular wall. GS trichrome staining, ×200.*
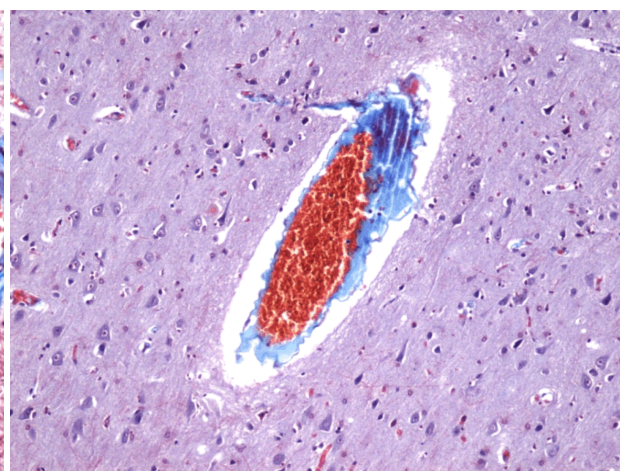

Figure 6 – *Venule in parenchyma with thickened wall after eccentric deposit of collagen and hyaline fibers. The lesions are associated with significant perivascular edema. GS trichrome staining, ×100.*
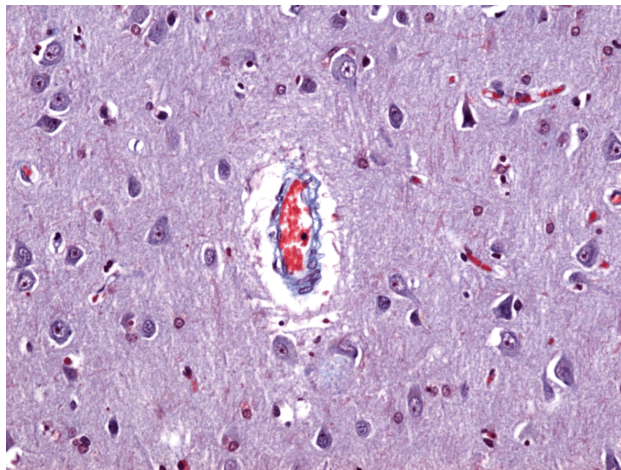
Figure 7 – *Metarteriole with high density of collagen fibers in the wall and the consecutive reduction of smooth muscles fibers. GS trichrome staining, ×400.*

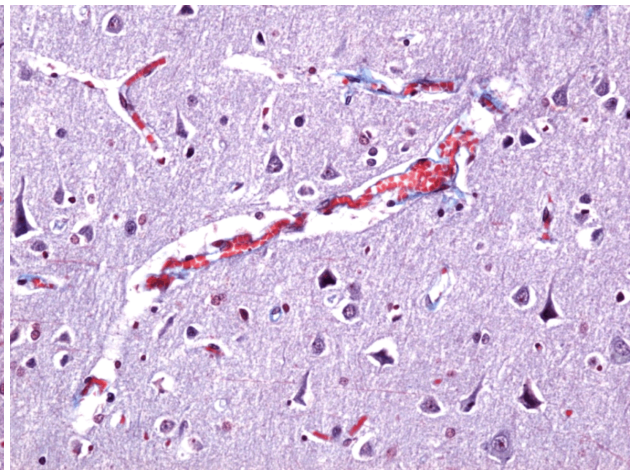**Figure 8 –** *Cerebral capillaries with sinuous pathway, reduces diameter and pericapillary edema. GS trichrome staining, ×200.*
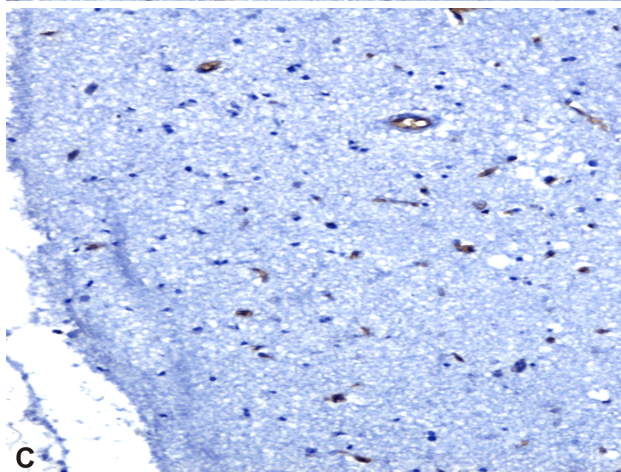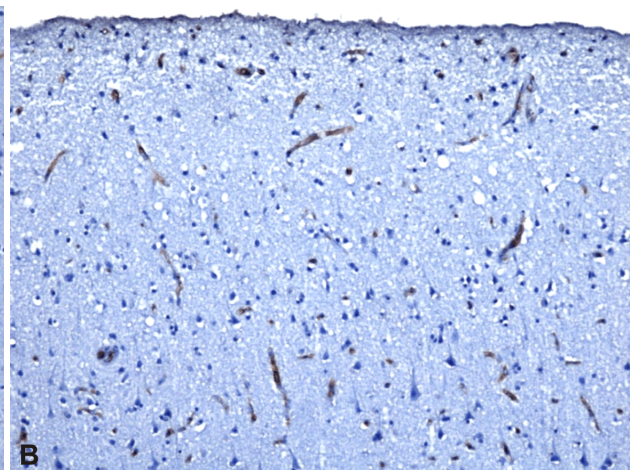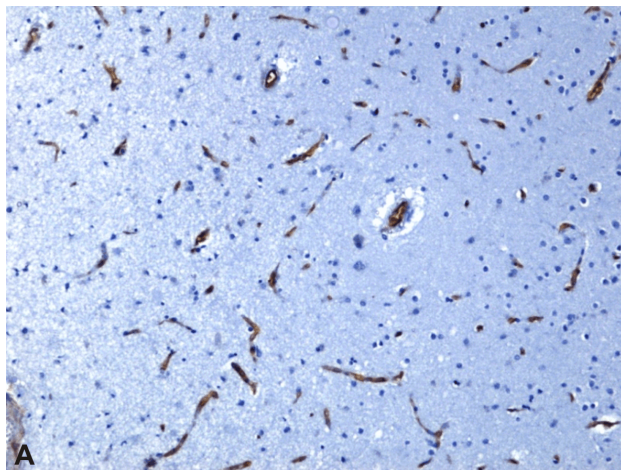

Figure 9 – *Immunohistochemistry image of cerebral microvascular network in assessed patients. Immunostaining with anti-CD34 antibody, ×200: (A) Virtually no microvascular alteration in the frontal cortex; (B) Minimal microvascular alterations; (C) Significant microvascular alterations in the frontal cortex.*

⊟ Discussion

The functional integrity of the dopaminergic system in frontal cortex depends on the vascular support and the capabilities of the neurovascular unit that provides the physiological cerebral blood flow in small arteries, arterioles, venules and capillaries. Any dysfunction of this system leads to the so-called small vessel disease with the increase of dopaminergic neuronal losses and excessive release of glutamate from the astroglia structure with consecutive hyperintensities in the white matter (Figure 10).

The cerebral blood flow in small vessels is regulated by the systemic dopamine levels and the actions on dopamine receptors:

▪ D1 receptors stimulated with agonist agents generated vasodilatation in the brain, as reported by studies on animal model [5] and human *in vivo* research [6].

▪ D2 receptors in small cerebral vessels, particularly in frontal cortex, reduce the blood flow through multiple vasoconstriction mechanisms, with a significant reduction of cAMP (cyclic adenosine monophosphate) [7, 8].

The microvascular circulation in frontal cortex of the

patients with schizophrenia is altered [9] and depends in our view on the effect and individual efficacy of dopamine on D1 or D2 receptors. Moreover, we estimate that the dopaminergic antagonism of haloperidol on D2 receptors in frontal cortex induces functional deafferentation [10], with an excessive release of glutamate.
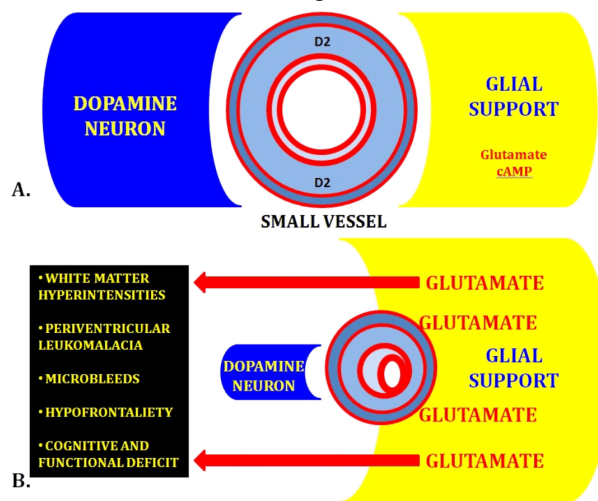

Figure 10 – (A) The functional model of the neuro-vascular unit in the frontal cortex, mediated by D2 receptors in the small cerebral vessels; (B) Dysfunctional and lesional consequences on the excessive blockade of D2 receptors in small cerebral vessels.

Assuming the existence of a dopaminergic system of D2 and D1 receptors in small cerebral vessels of the frontal cortex, we consider that the action of any antipsychotic with potent D2 antagonism may induce a functional deafferentation with a potential reversibility by agonism on D1 receptors (Figure 11).
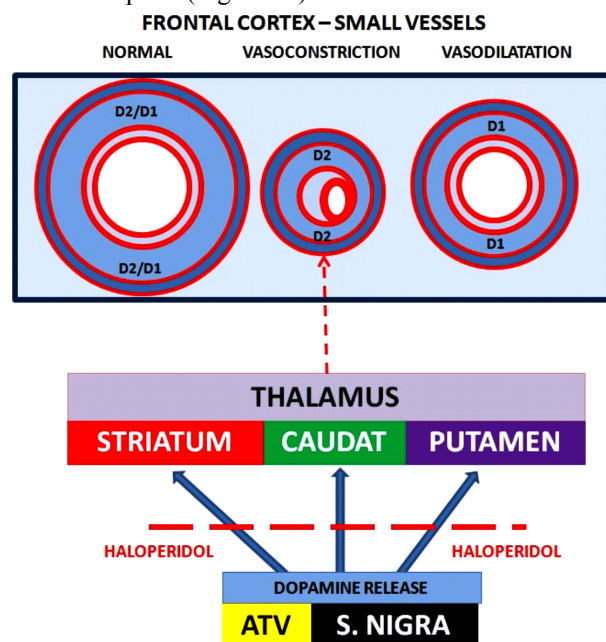

Figure 11 – The model of D2 blockade secondary to the administration of haloperidol and the effect of functional deafferentation in the small vessels of the frontal cortex.

Based on these premises, our post-mortem study on the frontal cortex of patients treated with potent D2-blocking antipsychotics (haloperidol, risperidone, olanzapine) focused on the identification of histopathological stages of microvascular alterations in the frontal cortex.

The disabilities of the social domain of cognition have a significant severity in psychiatric disorders such as schizophrenia and psychotic disorders, bipolar disorder of major depression with severe impact on major areas of these cognitive abilities (identification and recognition, social perception and decision-making in social life). Also, the theory of mind is affected, which is the mechanism that helps the individual to understand and predict behaviors based on reason and belief, thus leading to adequate reactions to perceived intentions by attributing to them states of mind or intentions [11, 12]. The cognitive process based on the same theory of mind (mentalizing) includes primary elements such as decoding facial expression and understanding movements and actions, but also specialized processes such as reasoning on the mental state of others based on representations [13].

These two cognitive processes have a well-defined neurobiological support (medial prefrontal cortex, temporo-parietal junction, temporal poles, superior temporal sulcus, cerebral amygdala and orbito-frontal cortex) that offer the individual the capability of making rapid and precise inferences related to the state of mind of another person. This has been demonstrated on imaging studies that proved the correspondence between specific social cognitive processes and processing areas in the brain [14] with a higher rate of metabolism in the areas involved in social cognition compared with the areas involved in non-social cognition during a continuous adjustment in processing social information [15, 16].

Out of these cerebral area, the medial prefrontal cortex in involved in mentalizing processes related to planning [17], working memory [18], memory for serial order and temporal information [19], aspects of language [20], attention [21] and social information processing [22], as well as the more general abilities associated with executive function [22], affect [23] and olfaction [24]. Differences in memorization were found in the favor of tasks involved in image formation based on personal impression rather than on description.

This cerebral area is activated only during the perceptive and impression-forming processes and is also involved in action monitoring and error correction, while the orbito-frontal cortex is automatically activated when social norms are deliberately or involuntary broken or in the presence of aversive reactions such as rage.

Damage to orbital prefrontal cortex has been shown to lead to impulsive aggressive behavior and variety of social behavioral problems [25], as well as impairments of social cognition and deficits in social information processing [26] and risk judgment [27].

In the case of schizophrenia and psychotic disorders, the social behavior of an individual is directly affected, including social cognition and theory of mind. These patients lack the ability of conducting daily and leisure activities, independent life, normal interpersonal and professional relations. They also show disabilities in neurocognitive domains (attention, verbal memory, executive function and cognitive flexibility) and neurosocial domains (perception and recognition, generation and selection of the response, verbal and/or non-verbal response).

Conclusions

The functional and structural integrity of the frontal cortex is crucial for a good outcome of schizophrenia or schizophrenia-spectrum disorders with significant improvement of symptoms, cognitive preservation and functional recovery. The evidences shown in our study highlight the fact that antipsychotics with potent antagonist action on D2 receptors may affect the neurovascular unit and small vessels in frontal cortex by altering the balance vasoconstriction–vasodilatation, reducing the blood flow and metabolism and generating structural microvascular changes proportional with the level of apoptosis at this stage. The histopathological data of our study raises the hypothesis for the pathogenic stages at the level of microvessels in the frontal cortex of the patients with schizophrenia or schizophrenia-spectrum disorders treated with D2-blocking antipsychotics: a stage with functional, reversible alterations that may be correlated with the impairments of working memory and presence of extra-pyramidal symptoms and a lesional, irreversible stage with significant deterioration of cognition and global functioning. Further, more ample studies are need in order to verify these observations that may shape preventive interventions for an improvement of schizophrenia outcome and functional recovery by preserving vascular perfusion in the frontal cortex.

Conflict of interests

The authors declare that they have no conflict of interests.

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