Endothelial Dysfunction of Cerebral and Major Arteries during Chronic Obstructive Disease

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Vasomotor activity of the major and cerebral arteries was studied in mice with chronic obstructive pulmonary disease. Regional differences were revealed in the endothelium-dependent response of arteries. The development of chronic obstructive pulmonary disease was associated with a paradoxical response of the dilatational component of vasoregulation against the background of increased constrictive influences of the vascular endothelium in the major and cerebral vessels.

Key Words: *mouse; blood flow in major and cerebral vessels; magnetic resonance tomography; endothelial vasomotor function*

According to principles of evidence-based medicine, chronic obstructive pulmonary disease (COPD) with various clinical manifestations belongs to a group of systemic disorders [12]. Little is known about the pathogenetic role of progressive decrease in cerebral metabolism leading to mental disturbances [2,14]. The cascade of thanatogenesis is induced by chronic hypoxemia and by hypercapnia (in severe disease). Brain dysfunction in COPD contributes to impairment of respiratory and vasomotor reactivity to hypercapnia due to a decrease in the sensitivity of central chemoreceptors (up to 80% chemosensitivity to CO_2) [15]. Change in central vasomotor regulation results in inadequate blood supply to various areas, including the brain [15]. The vascular endothelium is an important regulator of hemoperfusion in the brain [5,10,15]. Previous studies revealed specific endothelium-dependent reactions in the cerebral arteries [11]. Endothelial dysfunction during COPD plays an important role in various stages of pathogenesis (from the maintenance of systemic inflammation to the formation of chronic pulmonary heart) [3]. It is interesting to evaluate the role of the vascular endothelium in dysfunction of cerebral circulation during COPD.

Here studied vasomotor activity of the endothelium in the major and cerebral arteries of mice with COPD.

MATERIALS AND METHODS

Experiments were performed on male C57Bl mice (n=40) aging 6-8 weeks and weighing 24.2±1.5 g. The animals were maintained in a vivarium under standard conditions and natural light/dark regimen and had free access to water and food. The animals were randomized into 2 groups (treatment group, n=30; control group, n=10). COPD was induced by the tobacco smoking protocol [13]. The animals were subjected to tobacco smoke from 1 cigarette 3 times a day (2-h smokeless period between breakfasts). This treatment was performed 5 days a week for 5 months in a special smoking chamber. Smoke from Prima cigarettes (tar 13 mg, nicotine 0.7 mg) was produced by a smoking device.

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The development of COPD in treated mice was verified histologically (emphysema and remodeling of the respiratory pathway).

The animals were subjected to magnetic resonance scanning. The mice were fixed with rometar in a dose of 5.5 mg/kg (Xylazinum, Spora) and immobilized with relanium (37 mg/kg intraperitoneally). Magnetic resonance tomography (MRT) was performed on a PharmaScan US 70/16 tomograph for experimental studies (Bruker; magnetic field strength 7.0 T, frequency 300 MHz, BGA 09P coil). Anatomical and topographic characteristics of the great and cerebral blood vessels were evaluated. Frontal, sagittal, and horizontal sections were used to get T2 tomograms and proton density-weighted tomograms (RARE_8, MSME, and 3DTOF pulse sequence). Occipital 3D construction of the vascular bed was obtained using ParaVision 3.0.2 software (Bruker).

Vasomotor responses were studied in the following pharmacological tests: endothelium-dependent vasodilation (VD_{ED}, acetylcholine test); endothelium-independent vasodilation (VD_{EI}, nitroglycerine test); endothelium-dependent vasoconstriction (VC_{ED}, N-monomethyl-L-arginine (L-NMMA) test); and endothelium-independent vasoconstriction (VC_{EI}, norepinephrine test) [4]. The diameters of the middle cerebral artery and carotid artery were measured before and after the test. Two points were set by the cursor of a magnetic resonance tomograph (adventitia/media boundary in the lateral arterial wall and media/adventitia boundary in the medial arterial wall). The vasomotor response was estimated from changes in the diameter of the artery during the test and expressed in percents of the rest value. The index of regional correspondence was calculated as the great/cerebral artery vasomotor response ratio multiplied by 100 [5].

The results were analyzed by Excel 2003 software. The significance of intergroup differences was estimated by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Magnetic resonance scanning showed that the initial diameter of vessels in COPD mice tended to increase compared to control animals (Tables 1 and 2).

Endothelium-dependent stimulation was followed by expected VD_{ED} in control animals. Equivalent stimulation caused paradoxical VC of the cerebral and carotid arteries in COPD mice. It should be emphasized that VC of the carotid arteries was more pronounced compared to the cerebral arteries ($p<0.01$). VD_{EI} of the middle cerebral artery and carotid artery in mice with COPD was much lower compared to the control (by 4.5 and 3.25 times, respectively, $p<0.001$). COPD was accompanied by a decrease in VD_{ED} and VD_{EI}. These changes were primarily mediated by the endothelium-dependent mechanism, which sometimes appeared as constrictive distortion (Tables 1 and 2).

Endothelial dysfunction is an essential component in the pathogenesis of COPD and develops at the early stage of the disease [3]. Inhibition of endothelial function with irresponsiveness or inversion of the response to relaxing influences can be observed at the late stage of COPD [3,9]. The effect of acetylcholine depends on functional and anatomical properties of the endothelium. Normally, acetylcholine binds to specific receptors on endotheliocytes and induces the cascade of NO synthesis, which results in VD_{ED}. However, acetylcholine can cause contraction and VC during direct interaction with smooth myocytes [1,8]. It means that administration of acetylcholine to animals with intact endothelium and preserved endothelial function leads to arterial dilation. Endothelial dysfunction/damage is accompanied by insufficient VD or pathological VC. Pathological VC attests to severe damage to the endothelium [6]. The decrease in the sensitivity of vascular smooth muscle cells to nitrovasodilators is induced by hypoxia typical of COPD [7,9]. COPD is also accompanied by remodeling of the arterial wall and increase in vessel rigidity. Hence, the artery cannot respond adequately to various influences [8,9].

Significant intergroup differences were revealed in constrictive vasomotor function of the vascular endothelium. The VC_{ED} test showed that VC of the middle cerebral artery in COPD mice was 2-fold higher than in control animals ($p<0.01$, Table 1). Constriction of the carotid artery in response to administration of L-NMMA was more pronounced in mice with COPD (by 1.7 times compared to the control, $p<0.01$, Table 2). These data suggest that COPD is accompanied by an increase in VC_{EI} and, particularly, in VC_{ED} of cerebral and major arteries. VC_{EI} of the major vessels increased compared to the control. However, only slight changes in VC_{EI} were revealed in cerebral arteries. Our results demonstrate the existence of regional differences in vasomotor dysfunction in various regions of blood flow during COPD.

The increase in VC_{ED} during COPD is associated with systemic inflammatory response, oxidative stress, hypoxemia, and hypercapnia leading to increased production of endothelin-1 and other constricting agents by endotheliocytes and reduced synthesis of endogenous NO and prostacyclin [3,9, 14]. The observed regional differences can result

from high activity of the endothelial NO system due to autoregulation of cerebral vessels [1,8].

For more comprehensive analysis of the observed differences in vasomotor responses of the vascular endothelium in cerebral and carotid arteries vasomotor activity of the vascular endothelium was analyzed by calculating the index of regional correspondence. It was found that the vasomotor response of the major arteries in the majority of control mice was more significant compared to variations in the diameter of the cerebral arteries. Some authors reported that these features are determined by metabolic demands of blood-supplied regions and specific autoregulation of cerebral blood flow [9,11,13]. The mean index of regional correspondence in control mice was 182.4±5.3 arb. units. The vector of changes in the vascular lumen was similar in mice with COPD. The increased index of regional correspondence moderately increased in these animals (237.4±6.2 arb. units), which was mainly associated with changes in vasomotor activity of the carotid arteries ($p<0.05$ compared to control mice). The increase in the index of regional correspondence for the vasomotor response probably reflects inhibition of autonomic reactions in cerebral arteries of COPD animals. Discrete analysis of variations in the index of regional correspondence in COPD mice during each pharmacological test revealed critical strain of the mechanisms for regional coupling of the vasomotor responses. For example, the index of regional correspondence for VD_{ED} in COPD animals increased to 412.8±9.7 arb. units (*vs.* 172.8± 6.1 arb. units in the control group, $p<0.001$). Our results demonstrate exhaustion of relaxation reserves in the cerebral arteries during stimulation of NO expression. The index of regional correspondence for VD_{EI} in COPD mice significantly exceeded that in control animals (201.7±5.9 and 138.0±4.1 arb. units, respectively, $p>0.01$). Hence, the cerebral arteries were much more rigid than the major arteries under conditions of systemic impairment of the total decrease in vascular VD reactivity. The index of regional correspondence for VC_{ED} decreased in both vascular regions of COPD animals. The index of regional correspondence in COPD mice and control animals was

TABLE 1. Vasomotor Function of Cerebral Arteries (*M*±*m*)

Parameter	Control	COPD
Initial arterial diameter, mm	0.19±0.02	0.21±0.01
Arterial diameter in the acetylcholine test, mm	0.20±0.01	0.21±0.02
VD_{ED}, %	4.26±0.88	-1.56±0.91*
Arterial diameter in the nitroglycerine test, mm	0.23±0.01	0.22±0.01
VD_{EI}, %	15.07±2.42	3.20±1.01*
Arterial diameter in the L-NMMA test, mm	0.17±0.01	0.164±0.011
VC_{ED}, %	-11.49±0.93	-23.1±2.9*
Arterial diameter in the norepinephrine test, mm	0.18±0.01	0.191±0.029
VC_{EI}, %	-8.08±0.83	-10.43±1.92

Note. *$p<0.001$ compared to the control.

TABLE 2. Vasomotor Function of Carotid Arteries (*M*±*m*)

Parameter	Control	COPD
Initial arterial diameter, mm	0.34±0.01	0.352±0.011
Arterial diameter in the acetylcholine test, mm	0.36±0.01	0.329±0.013
VD_{ED}, %	7.33±0.64	-6.44±0.81***
Arterial diameter in the nitroglycerine test, mm	0.41±0.02	0.375±0.019*
VD_{EI}, %	20.86±1.88	6.41±0.62***
Arterial diameter in the L-NMMA test, mm	0.27±0.01	0.23±0.02
VC_{ED}, %	-20.65±1.04	-34.11±4.71***
Arterial diameter in the norepinephrine test, mm	0.25±0.01	0.28±0.04
VC_{EI}, %	-25.51±1.31	-19.41±1.62**

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

147.8±4.5 and 179.1±4.7 arb. units, respectively ($p<0.001$). Administration of norepinephrine to COPD mice was accompanied by a decrease in the index of regional correspondence to 186.5±4.1 arb. units (*vs*. 211.9±8.3 arb. units in control animals, $p<0.01$). Therefore, changes in the index of regional correspondence were most pronounced for the dilatational vasomotor response. Regional inconsistency was maximum during endothelium-dependent stimulation. These changes occurred against the background of decreased physiological regional differences in the constrictive response of the cerebral and major vessels in COPD mice.

Our results indicate that COPD is accompanied by endothelial dysfunction of the cerebral and major arteries, which results in a significant increase and prevalence of arterial constriction. The development of COPD is associated with paradoxical vasomotor response of the vascular endothelium in the acetylcholine test. COPD in mice manifested in the appearance of regional regulatory differences in circulatory homeostasis. The excessive decrease in dilation reserves of regional autonomy in the cerebral arteries was accompanied by asymmetric increase in carotid constriction.

These data provide new insights into dysfunction of the cerebral arteries and specific regional regulation of blood flow in COPD.

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REFERENCES

1. V. N. Anan'ev, B. N. Manukhin, and O. V. Anan'eva, *Adrenoreactivity and Cholinoreactivity of Systemic and Regional Blood Supply during Cold Adaptation* [in Russian], Moscow (2002).
2. V. I. Bobrova, *Nervous System and Chronic Obstructive Pulmonary Diseases with Systemic Arterial Hypertension* [in Russian], Kiev (2001).
3. T. A. Brodskaya, V. A. Nevzorova, B. I. Geltser, and E. V. Motkina, *Ter. Arkhiv*, No. 3, 76-84 (2007).
4. B. I. Geltser, V. N. Kotelnikov, I. G. Agafonova, and P. A. Luk'yanov, *Byull. Eksp. Biol. Med*., **140**, No. 11, 587-591 (2005).
5. B. I. Geltser, S. V. Savchenko, V. N. Kotelnikov, and I. V. Plotnikova, *Kardiologiya*, No. 4, 24-28 (2004).
6. T. Yu. Demidova, A. S. Ametov, and L. V. Smagina, *Klin. Med*., No. 10, 25-30 (2005).
7. N. A. Manak, I. A. Kozich, and I. S. Karpova, *Kardiologiya SNG*, No. 5, 26-29 (2003).
8. P. A. Motavkin, *Regulation of Cerebral Blood Flow* [in Russian], Vladivostok (1992).
9. E. V. Motkina and V. A. Nevzorova, *Tikhook. Med. Zh*., No. 2, 34-37 (2005).
10. N. N. Fedosova, V. N. Tsyuryupa, and I. V. Vlasova, *Ul'trazvuk Funkts. Diagnost*., No. 3, 72-77 (2005).
11. O. V. Filatova, V. D. Kiselev, O. V. Trebukhov, and L. D. Kozlova, *Ros. Fiziol. Zh.*, No. 12, 1503-1511 (1999).
12. A. Agusti and T. A. Neff, *Proceedings of the ATS*, **3**, No. 6, 478-481 (2006).
13. T. S. Shim, J. H. Lee, S. Y. Kim, *et al*., *Chest*, **120**, No. 5, 1506-1513 (2001).
14. M. J. Van de Ven, W. N. Colier, M. C. Van der Sluijs, *et al*., *Eur. Respir. J*., **18**, No. 1, 61-68 (2001).