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THE QUANTITATIVE CHARACTERISTICS OF HISTOPATHOLOGICAL CHANGES IN LUNGS IN ACUTE BACLOFEN POISONING

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ABSTRACT

The aim of the study was to quantify the changes in the lungs in acute baclofen poisoning in an animal experiment.

Materials and Methods: The study was performed on 20 male Wistar rats weighing 290–350 g and aged 20 weeks. The animals were divided into 4 groups. The reference group of controls included 5 intact rats. The animals of three experimental groups included 5 rats each were treated with baclofen at a dose of 85 mg/kg. The tested rats were euthanized by displacement of cervical vertebrae at 3, 4,5 and 24 hours, respectively. Lung tissue samples were examined by light microscopy using a video system at ×400 magnification.

Results: The area of the intraalveolar septi 4,5 hours after baclofen administration was significantly higher than in the reference group of controls. 3 and 4,5 hours after baclofen administration an increase in the area of alveloli was observed, but the increase was insignificant and the area of alveoli returned to the initial size by the 24th hour. 3 and 4,5 hours after baclofen administration a slight decrease in the area of pulmonary parenchima was observed, which also returned to its initial size in 24 hours. The total area of pulmonary microvessels 4,5 hours after baclofen administration was significantly higher than in the control group and 3 hours after baclofen administration. The area of pulmonary microvessels 24 hours after baclofen administration was significantly higher than in the group of controls, 3 or 4,5 hours after baclofen administration. The area of edema increased and was significantly higher 24 hours after baclofen administration. In baclofen poisoning, a certain complex of general pathological processes in lungs develops. There is a circulatory disorder, increased vascular permeability, infiltration of intraaveolar septis.

Conclusion: The quantitative assessment of the changes can be used to evaluate severity of general pathological process in acute baclofen poisonings and to determine the exact time of the poisonings.

Keywords: Baclofen, poisoning, lungs, lung histopathology, quantitative assessment

INTRODUCTION

Baclofen (Lioresal) is a muscle relaxant. [1-3]. The drug is delivered orally or intrathecally. [1]. Baclofen is used to treat muscle spasticity, clonus, and concomitant pain, sequelae of spinal cord lesions, and multiple sclerosis. It also has several off-label uses. [4]. The exact mechanism of baclofen action still remains unclear. The drug is an agonist at the beta subunit of gamma-aminobutyric acid (GABA) receptors expressed on pre- and post-synaptic neurons. Upon binding to GABAB receptors, baclofen causes an influx of potassium ions into the neuron, leading to hyperpolarization of the neuronal membrane and decreased influx of calcium ions at presynaptic nerve terminals. This results in a decreased rate of action potential threshold being reached by presynaptic neurons and reduced action potential of postsynaptic motor neurons that innervate the muscle spindles. Baclofen inhibits the transmission of both mono-and polysynaptic reflexes at the spinal cord, relaxing spasticity. Baclofen is said to act on some voltage-gated calcium but the clinical significance of this is unclear [1]

Baclofen has a significant psychotropic effect and is often subject to abuse, especially among young people [5,6]. One of target organs in acute baclofen poisoning is lung.

The **aim of the study** was to quantify the changes in the lungs in acute baclofen poisoning in an animal experiment.

MATERIALS AND METHODS

The study was performed on 20 male Wistar rats weighing 200-215 g and aged 20 weeks.

Keeping animals and working with them were performed in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes. The animals were divided into 4 groups.

The reference group included 5 intact rats, three experimental groups included 5 rats each, treated with baclofen at a dose of 85 mg/kg. The animals of the study groups were euthanized by displacement of cervical vertebrae 3, 4,5 and 24 hours, respectively.

The lungs of the rats were fixated in 10% neutral formalin, the samples were embedded in paraffin. We prepared histological sections of 5 μm thickness, placed them on slides and stained with hematoxylin and eosin. We examined the sections by light microscopy using a Nikon Eclipse E-400 microscope with a video system based on a Watec 221S camera (Japan) at ×400 magnification. A morphometric analysis was performed using a grid suggested by G. G. Avtandilov.

We evaluated the following signs (volume percent, vol. %): the area of the alveoli, the area of interalveolar septum, the area of pulmonary vessels, the area of WBCs, the area of edema. We also evaluated the WBC count estimated by their area in interalveolar septum tissue.

RESULTS AND DISCUSSION

The results of alveoli, parenchyma, WBCs, edema, vessels, interalveolar septum, edema (vol. %), WBC count estimated by their area in interalveolar septum tissue evaluation are presented in Table 1.

Table 1. Morphometric analysis of histological slides

Parameters (vol. %)	**Reference group**	**Group 1 (Bacl., 3 h.)**	**Group 2 (Bacl.- 4.5 h.)**	**Group 3 (Bacl.- 24 h.)**
Alveoli	36,50 (32,25; 43,0)	41,50 (36,00; 48,5)	39,00 (31,25; 45,75)	36,50 (31,00; 46,00)
Interalveolar septum	44,00 (39,25; 50,0)	37,00 (31,0; 42,75)	32,50* (26,00; 38,50)	33,00 (26,00; 41,00)
WBCs	9,00 (7,00; 11,00)	8,00 (7,00; 10,00)	12,00 (10,00; 15,00)	8,00 (5,25; 11,75)

Edema	6,00 (4,00; 8,00)	8,00 (5,25; 9,75)	9,00 (7,00; 13,00)	11,00* (8,00; 13,75)
Pulmonary microvessels	3,0 (2,00; 3,00)	4,0 (3,00; 5,00)	6,0* (5,00; 7,00)	7,50*^ (6,00; 9,00)
WBC count estimated by their area in interalveolar septum tissue	0,14 (0,11; 0,18)	0,16 (0,12; 0,18)	0,21 (0,16; 0,25)	0,15 (0,08; 0,20)
Total area of parenchima	63,50 (57,00; 67,75)	58,50 (51,50; 64,00)	61,00 (54,25; 68,75)	63,50 (54,00; 69,00)

*Note: * – The parameters that significantly differ from the reference group at p<0,01; ^ – The parameters that significantly differ from group 1 at p<0,01*

The area of the interalveolar septum 4,5 hours after baclofen administration was significantly higher than in the reference group. 3 hours and 4,5 after baclofen administration an increase in the area of alveoli was observed, but it was not significant and the area of the alveoli returned to its initial size in 24 hours. 3 hours and 4,5 after baclofen administration a slight decrease in the area of parenchyma was observed and returned to its initial size 24 hours later The total area of pulmonary microvessels 4,5 hours after baclofen administration was significantly higher than in the group of controls and 3 hours after baclofen administration. The area of pulmonary microvessels 24 hours after baclofen administration was significantly higher than in the reference group, 3 or 4,5 hours after baclofen administration. The area of edema increased and was significantly higher 24 hours after baclofen administration than in the group of controls.

Baclofen is known not to have any direct toxic effect on the bronchi and lungs. However, the drug increases presynaptic blockade of nerve impulses, which originate in the spinal cord, which, in turn, inhibits their transmission. The tone of muscles, including the intercostal ones, decreases. The excessive relaxation of these muscles can cause breathing difficulties followed by hypoxia. The effects of GABA receptor stimulation on bronchial smooth muscles and lungs are worth special attention. GABA A receptor agonists are known to cause contraction of bronchial and bronchiolar smooth muscles ac- companied by spasm and breathing difficulties [7,8]. Baclofen is a selective agonist of GABA B receptors, but it is also capable of stimulating GABA A receptors at high doses [9].

A slight increase in the area of alveoli can be explained by the compensatory reaction to developing hypoxia. Developing hypoxia causes intensification of microcirculation, which we observed in our study. The area of pulmonary microvessels was significantly higher 4,5 hours after baclofen administration. The increase of the area of edema, which we observed 24 hours after baclofen administration, may occur due to increased permeability of microcirculatory vessels caused by hypoxia.

CONCLUSION

In baclofen poisoning, a certain complex of general pathological processes in lungs develops. There are a circulatory disorder, increased vascular permeability, infiltration of interalveolar septum. The quantitative assessment of the changes can be used for valuation of severity of general pathological process in acute baclofen poisonings and determination of the exact time of the poisonings.

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