

IMMUNOCHEMICAL ANALYSIS OF BCL-2 AND BAX EXPRESSION IN BRONCHIAL EPITHELIUM IN RATS IN ACUTE BACLOFEN POISONING

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ABSTRACT

Baclofen (also sold under trade names Lioresal, Baclosan) belongs to a group of drugs called antispasmodics. This drug is available in oral and intrathecal forms. Baclofen has a pronounced psychotropic effect and is often a subject to abuse, especially among young people.

The aim of the study was to detect apoptosis in the bronchial epithelium in rats.

The study was performed on 20 male Wistar rats (weight: 290-350 g., age: 20 weeks). The animals were divided into 4 groups. The group of controls included 5 intact rats, each of three experimental groups included 5 rats treated with baclofen at a dose of 85 mg/kg and euthanized by the displacement of the cervical vertebrae after 3, 4,5, and 24 hours, respectively.

In the bronchial epithelium of the controls bax was weakly expressed, which shows a low level of apoptosis. 3 and 4,5 hours after baclofen administration bax was strongly expressed and 24 hours after baclofen administration bax was moderately expressed.

In the bronchial epithelium of the controls bcl-2 was weakly expressed; 3 hours after baclofen administration bcl-2 was moderately expressed; 4,5 hours and 24 hours after baclofen administration bcl-2 was strongly expressed.

The results from the present study suggest the involvement of apoptosis in the development in the lesion of bronchial epithelium.

Furthermore, bax and bcl-2 expression in the regenerating epithelial cells may play a role in the recovery of this lesion.

Keywords: baclofen, poisoning, immunohistochemistry (IHC), apoptosis, bcl-2, bax.

INTRODUCTION

Baclofen (also sold under trade names Lioresal, Baclosan) belongs to a group of drugs called antispasmodics [1].

This drug is available in oral and intrathecal forms. This is a prescription drug, which is used to treat muscle

spasms, multiple sclerosis, and injuries of the spinal cord [2-4]. Baclofen also has a pronounced analgesic effect [1]. This drug has a psychotropic effect [1,5] and is often a subject to abuse especially among young people [5].

The exact mechanism of baclofen action still remains unclear, the drug is considered to be an agonist at the beta subunit of gamma-aminobutyric acid (GABA) receptors, which are expressed on pre- and post-synaptic neurons. After binding to GABAB receptors, baclofen causes an influx of potassium ions into the neuron, which causes hyperpolarization of the neuronal membrane and decreased influx of calcium ions at presynaptic nerve terminals. This causes 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 also acts on some voltage-gated calcium but the clinical significance of this is unclear [2].

Baclofen has a pronounced psychotropic effect and is common among drug addicts, mainly young people. They can consume up to 6-14 tablets at once [6]. Drug intoxication occurs in about 30-40 minutes. Its main symptoms are as follows: nausea and vomiting, visual and auditory hallucinations, tremor of the hands, drowsiness, apathy, mydriasis, slurred speech, dizziness [7-9].

In suspicious baclofen poisonings differential diagnosis with other poisoning is necessary to take optimal rehabilitation measures. Understanding the processes that occur in the body at different stages of this poisoning allows us to provide timely assistance to the category of patients. In case of a lethal outcome, it is necessary to reveal the immediate cause of death [9].

Baclofen has an indirect effect on respiratory system [1], which contributes into the pathogenesis of baclofen poisoning.

The aim of the study was to detect apoptosis in the bronchial epithelium in rats under baclofen intoxication.

MATERIALS AND METHODS

The study was performed on 20 male Wistar rats (weight 290-350 g., age: 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 group of controls included 5 intact rats, three experimental groups included 5 rats treated with baclofen at a dose of 85 mg/kg and euthanized by the displacement of the cervical vertebrae after 3, 4, 5, and 24 hours, respectively. Their lungs were fixated in 10% neutral formalin and the samples were embedded in paraffin. Histological sections of 5 µm thickness were prepared.

Immunohistochemical staining was performed using rabbit polyclonal antibodies against rat bcl-2 (AF 6139) and bax (AF 0120).

We also used ab64261 - Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (Abcam).

We deparaffinized and rehydrated formalin-fixed paraffin-embedded tissue section, added enough drops of Hydrogen Peroxide Block to cover the sections. The samples were incubated for 10 minutes and washed 2 times in buffer.

Then we applied Protein Block and incubated for 10 minutes at room temperature to block nonspecific background staining.

We washed the samples 1 time in buffer, applied primary rabbit polyclonal antibodies against rat bcl-2 (AF 6139) and bax (AF 0120) incubated for 10 minutes, then washed 4 times in buffer, applied enough Biotinylated Goat Anti-Polyvalent to cover the tissue sections and incubate for 10 minutes at room temperature, washed 4 times in buffer.

After that we applied Streptavidin Peroxidase and incubated for 10 minutes at room temperature, rinsed 4 times in buffer, added 30 µl (1 drop) DAB Chromogen to 1.5 ml (50 drops) of DAB Substrate, mixed by swirling and applied to tissue. Incubation for 5 minutes, rinsed 4 times in buffer.

Then we applied counterstain – Mayer's hematoxylin dehydrated and coverslipped.

We assessed the expression of bax and bcl-2 as negative (-), weak positive (+), moderately positive (++) and strongly positive (+++).

RESULTS AND DISCUSSION

The results of the study are presented below.

Table 1. Comparative data on bax and bcl-2 expression in bronchial epithelium in rats after baclofen ingestion

Antigen	the controls	3 hours	4,5 hours	24 hours
bax	+	+++	+++	++
bcl-2	+	++	+++	+++
Note – + -weak expression, ++ – moderate expression, +++ – strong expression				

In the bronchial epithelium of the controls bax was weakly expressed, which shows a low level of apoptosis. 3 hours after baclofen administration bax was moderately expressed, 4,5 and 24 hours after baclofen administration bax was strongly expressed.

In the bronchial epithelium of the controls bcl-2 was weakly expressed; 3 hours after baclofen administration bcl-2 was moderately expressed. 4,5 and 24 hours after baclofen administration bcl-2 was strongly expressed.

It is now known that the bcl-2 gene family regulates apoptosis [6].The bax and bcl 2 genes encode homologous proteins that have opposite effects on cell activity. bcl-2 prolongs cell survival, bax accelerates apoptosis [Reed et al., 1994]. The ratio of bcl-2 and bax proteins might present the main determinant of cellular apoptosis ability.

The present study confirmed that the cells of bronchial epithelium in the group of controls express bcl-2 (weak expression) and bax (weak expression), suggesting that bcl-2 and bax expression might regulate apoptosis in the physiological state.

3 hours after baclofen poisoning the expression of bax was strong and the expression of bcl-2 was moderate. These data suggest that the increased expression of bax may contribute to the apoptosis in the lesion. 4,5 hours after baclofen ingestion the levels of bax and bcl-2 was strong. 24 hours after baclofen administration the expression of bax was moderate and the expression of bcl-2 was strong, which reflects the process of healing.

CONCLUSION

In conclusion, the results from the present study suggest the involvement of apoptosis in the development in the lesion of bronchial epithelium.

Furthermore, bax and bcl-2 expression in the regenerating epithelial cells may play a role in the recovery of this lesion.

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