Morphological Changes in Rat Tracheal Wall in Experimental Hemorrhagic Stroke

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Structure of the tracheal wall of male Wistar rats was studied by histological methods on days 1, 3, and 7 after experimental hemorrhagic stroke. Signs of impaired microcirculation and lymphatic drainage were revealed at all stages of the experiment. In the acute phase of stroke (day 1), these disturbances lead to destruction of epithelial cells and their extensive desquamation, edema of the submucosa, and hemorrhages in the tracheal wall. On days 3 and 7 of the experiment, destructive changes in the mucosa membrane were still present, but swelling of mucosa lamina propria and submucosa of the tracheal wall greatly decreased, probably due to evacuation of tissue fluid excess into the lumen. We can assume that changes in the structure of the tracheal wall in experimental hemorrhagic stroke impair the barrier function of the wall, which can contribute to the development of pneumonia, a serious inflammatory complications of stroke.

Key Words: *hemorrhagic stroke; mucous membrane; trachea*

Currently, hemorrhagic stroke (HS) is a serious medical and social problem because of high levels of mortality and disability [2]. Complications accompanying stroke cause death more frequently than the severity of HS. Early complication of HS is pneumonia [7,9], which develops in 20-25% cases [4]. The morphology of the wall of respiratory organs in experimental HS was not studied. However, the structure of the gastric mucosa undergoes significant changes under similar experimental conditions [1].

Here we evaluate the effect of experimental HS on the structure of rat tracheal wall and, in particular, on the morphology of its mucosa.

MATERIALS AND METHODS

In 25 Wistar male rats weighing 250-300 g, the lower part of the trachea was examined. Stress resistance was evaluated by open-field behavior [3]. Stress-susceptible animals were included in the experiment. The rats were divided into 3 groups: intact (n=5), control (n=5), and experimental (n=15). In animals of the experimental group, HS in the left caudate nucleus was modeled using a modification of the method with 2-fold injection of the blood [10]. In anesthetized animals (hloral hydrate solution, 400 mg/kg body weight intraperitoneally), autologous blood was injected in a volume of 60 μl without heparin. Injections were given by stereotactic coordinates (A=0.7 mm, L=3 mm, H=6 mm), through a hole in the skull with a diameter of 0.5 mm using a gauge 22 needle with rounded tip. After 5 min, the cannula was slowly removed, the hole in the skull was sealed with dental cement, and the skin was sutured. Localization of HS corresponded to hemorrhages from lenticulostriate artery rupture in humans.

The animals of control group were subjected to similar manipulations except blood injection. Intact and control animals were decapitated on day 1 and experimental rats on days 1, 3, and 7 after surgery. Experiments were carried out in accordance with the Order of the Ministry of Higher Education of the USSR "On Regulations of Studies on Experimental

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Animals" No. 742, November 13, 1984. The isolated material was fixed in 10% neutral formalin, 4-5-μ paraffin sections were stained with azure II and eosin, hematoxylin and eosin, and according Brachet and van Gieson. Lymphoid cells were counted on a standard area of histological section (880 μ^2) in 10 fields of view. The results were processed statistically by assessing changes in the cell number in the lymphoid structures using SPSS software (*T* test for paired variables and ANOVA).

RESULTS

In the acute period of HS (day 1), significant changes in the structure of the rat tracheal wall were revealed in all cases. Destruction of the epithelial layer along its entire length was observed. Not only the cilia, but also the apical parts of ciliated cells were destroyed. Against the background of epithelium disintegration, thinning of the basal part of epithelial cells and their complete separation from the basement membrane were observed resulting in the formation of areas without epithelial lining (erosion). The epithelium was populated with lymphocytes. The number of small lymphocytes among the epithelial cells markedly increased compared to the control group of animals (to 1.10±0.31 *vs.* 0.50±0.16 in the control). Lymphocyte and erythrocyte release into the lumen of the organ was noted.

The lamina propria of the mucosa and submucosa of the tracheal wall were considerably loosened and edematous. Solitary lymphocytes were seen in the gland lumens. Lymphoid nodes were surrounded by dilated lymphatic vessels filled with coagulated lymph (sign of disturbance of lymphatic drainage). Moreover, pronounced morphological signs of impaired microcirculation of the tracheal wall were seen. Blood vessels were plethoric, their walls were thickened and edematous. Erythrocyte aggregation in blood vessels and hemorrhages in parenchymal lymphoid aggregates and in the adventitia of the organ were noted.

On day 3 after HS, the structure of rat tracheal wall was not fully restored. Signs of destruction of the epithelial layer were still evident, namely disintegration of the epithelium, destruction of cilia and apical parts of ciliated epithelial cells; many of them were separated from the basement membrane, and erosive areas were seen. However, the number of small lymphocytes among epithelial cells was reduced compared with the previous term of the experiment (from 1.10±0.31 cells on day 1 to 0.40±0.16 cells on day 3). It should be noted that on day 3 after HS, the submucosa of the tracheal wall seemed to be more compact and less edematous than on day 1 of the experiment. Solitary lymphocytes were still present in tracheal gland lumens. Lymphatic vessels in the lamina propria were still enlarged and their lumens contained coagulated lymph. Signs of impaired microcirculation in the tracheal wall were still evident, that is, not only plethora, but also erythrocyte aggregation in the lumens were present. Erythrocytes are seen in the parenchyma of lymphoid clusters and adventitia (result of hemorrhage).

On day 7 after HS, many structural defects of rat tracheal wall detected at earlier periods of the experiment persisted. Signs of epithelial lining destruction were pronounced, the number of small lymphocytes among epithelial cells increased again (up to 1.30±0.21 cells) compared to the previous term. Morphological signs of impaired microcirculation and lymph drainage revealed earlier were still present. It should be noted that on day 7 after HS, the submucosa of the tracheal wall was compact again, edema was not identified.

Thus, the structure of the lower part of rat tracheal wall changed considerably in HS, destruction of epithelial layer and swelling of the lamina propria of the mucosa and submucosa were detected. These changes were a result of impairment of microcirculation and lymph drainage in tissues of the trachea, morphological signs of which were registered at all stages of the experiment. Similar changes in the mucosa were detected during studying of the gastric wall in rats with HS [1]. By the 7th day after experimental exposure, the structure of the tracheal wall did not completely recover. Signs of destruction of the tracheal epithelial lining were still observed. At the same time, the amount of interstitial fluid in the wall of the trachea was significantly reduced (submucosa looked compact). Apparently, edema decreased due to drainage of tissue fluid excess into the lumen of the trachea. Lymphoid cells migrated into the lumen of the organ together with interstitial fluid. According to data obtained by us earlier, this affected cytoarchitectonics of lymphoid formations of the tracheal wall in the lower part of the organ [5]. In the upper part of the trachea, tissue fluid excess was also removed through lining of the tracheal glands, which are particularly abundant in here [6].

Thus, marked morphological changes in the wall of the trachea have occurred as a result of disturbance of microcirculation and lymphatic drainage during HS. Hence, the barrier function of mucosa was violated, which may contribute to pneumonia. By day 7 of the experiment, the structure of the tracheal wall was not fully restored.

REFERENCES

1. Al-Rayashi Salim, E. V. Shvetsov, and E. V. Koplik, *Morfologiya,*, **29**, No. 4, 8-11 (2006).
2. E. I. Gusev, A. J. Konovalov, and G. S. Burd, *Neurology* [in Russian], Moscow (2000), pp. 259-290.

3. E. V. Koplik, *Vestn. Novykh Med. Tekhnol*., **9**, No. 1, 16-18 (2002).
4. Yu. S. Martynov, O. N. Kevdina, N. A. Shuvahina, *et al., Nevrol. Zh.*, No. 3, 18-21 (1998).
5. L. A. Svistukhina, G. G. Aminova, and E. V. Shvetsov, *Morfol. Vedomosti*, Nos. 3-4, 71 (2008).
6. L. A. Svistukhina and E. V. Shvetsov, *Ibid*., Nos. 3-4, 113 (2009).
7. V. I. Skvortsova and V. V. Krylov, *Hemorrhagic Stroke* [in Russian], Moscow (2005).
8. E. V. Shvetsov, Al-Rayashi Salim, G. G. Aminova, and E. V. Koplik, *Morfol. Vedomosti*, No. 2, 32-34 (2007).
9. A. Chamorro, S. Amaro, M. Vargas, *et al.*, *J. Neurol. Neurosurg. Psychiatry*, **77**, No. 11, 1279-1281 (2006).
10. W. Deinsberger, J. Vogel, W. Kuschinsky, *et al., Neurol. Res.*, **18**, No. 5, 475-477 (1996).