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IMPACT OF TRAUMATIC BRAIN INJURY ON SMALL INTESTINAL MORPHOLOGY: AGE-DEPENDENT CHANGES IN LYMPHOID STRUCTURES

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Abstract
This study investigates the morphological changes in the small intestine following traumatic brain injury (TBI) in rats, focusing on the impact of varying injury severity and the role of lymphoid structures within the mucosa. TBI is known to have widespread systemic effects, and this research aims to understand its influence on the gut's structural integrity, particularly in the mucosal immune response. The study examines changes in the small intestine across different age groups (1, 6, and 18 months) of Wistar rats, utilizing histological and morphometric methods to assess the severity of damage and its impact on lymphoid tissue. The findings reveal that TBI leads to significant changes in the small intestine, with varying degrees of inflammation, necrosis, and lymphoid follicle formation depending on injury severity and the age of the rats. Notably, treatment with L-lysine showed promising results in mitigating some of the morphological changes. These findings suggest that TBI-induced changes in the small intestine, particularly in its immune structures, have important implications for digestive and immune system functions, and may inform future therapeutic strategies for TBI patients.

Keywords: Traumatic brain injury, morphological changes, small intestine, lymphoid tissue.

INTRODUCTION

Traumatic brain injury (TBI) remains a significant global health issue, with an estimated 69 million people suffering from TBI each year, leading to a wide range of consequences that can profoundly impact individual health and quality of life. The World Health Organization (WHO) has identified TBI as one of the leading causes of morbidity and mortality, particularly among young adults, and its impact is expected to rise with the increasing prevalence of road traffic accidents, falls, and violence [1]. As TBI rates continue to rise due to road traffic accidents, falls, and violence, there is a growing interest in understanding the multisystem consequences of brain injuries, particularly those extending beyond the central nervous system. While the neurological consequences of TBI have been well studied, the effects on other organs, especially the gastrointestinal system, have received less attention, despite their significant impact on patient outcomes [2], [3].

The small intestine, a crucial organ for digestion, nutrient absorption, and immune function, is particularly susceptible to changes following TBI. Morphological alterations in the small intestine mucosa, such as villous atrophy and disruptions in barrier function, can significantly impair

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digestive processes and overall health. These alterations are thought to result from a combination of factors, including systemic inflammation, ischemia, and neuroendocrine dysregulation following brain injury [4]. Although research into the gastrointestinal effects of TBI is limited, studies indicate that changes in the small intestine can have long-lasting consequences for digestion, metabolism, and immune response, potentially leading to complications such as dysmotility, malabsorption, and increased susceptibility to infections [5].

In Central Asia, TBI is a pressing concern, with increasing rates of road traffic accidents, industrial injuries, and violence contributing to a growing burden of brain injury. Despite the rising incidence, the study of the systemic effects of TBI, including its impact on the GI system, remains underexplored in this region. In many Central Asian countries, the lack of sufficient research, healthcare infrastructure, and awareness of the broader consequences of TBI contributes to an inadequate understanding of the full extent of its impact on health outcomes.

The pathogenesis of these morphological changes is believed to depend on the severity of the TBI as well as the age of the individual. Both the age of the organism and the severity of the injury appear to influence the extent of damage to intestinal structures and function [2], [4]. However, existing data on the impact of TBI on the small intestine are inconsistent, with conflicting results in terms of the severity and type of changes observed. This highlights the need for further investigation into the underlying mechanisms and the extent of gastrointestinal damage in TBI patients.

In particular, the lymphoid structures of the small intestine mucosa are of interest, as they play a vital role in immune responses and can be significantly affected by TBI. Studying these structures in animal models of TBI is crucial for understanding the pathophysiological mechanisms of injury and for developing targeted treatments for TBI-related complications. The aim of this study is to investigate the morphological changes in the small intestine wall in rats with varying degrees of TBI severity, focusing on age-related differences and alterations in lymphoid structures. By elucidating these changes, we aim to improve our understanding of the systemic effects of TBI and to contribute to the development of therapeutic strategies for TBI patients, ultimately leading to better clinical outcomes [1], [3].

The Aim of the Study

The aim of the study is to examine the morphological changes in the wall of the small intestine in rats with varying degrees of traumatic brain injury, with a focus on changes in the lymphoid structures of the mucosa.

MATERIALS AND METHODS

In this experimental study, 180 outbred white male rats aged 1, 6, and 18 months were used. Prior to the study, all animals were quarantined for one week, during which they were carefully monitored to exclude the presence of infectious diseases. Afterward, they were transferred to standard vivarium conditions, where the temperature was maintained at 22-24°C, humidity was 50-60%, and the lighting cycle consisted of 12-hour light/dark periods. Throughout the experiment, the rats had unlimited access to food and water. The study protocol included the following stages: modeling of traumatic brain injury (TBI) and morphological study. Four groups

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(n=12) of animals were distinguished: 1) intact; 2) TBI with a load lifting height of 0.6 m, weight of 145 g; 3) TBI with a load lifting height of 0.6 m, weight of 155 g; 4) TBI with a load lifting height of 0.6 m, weight of 170 g. The control group animals were fixed in the setup, but no injury was inflicted. In the control groups, on the 3rd and 6th days after TBI modeling, in compliance with the principles of humane animal treatment, some animals were removed from the experiment by euthanasia under chloroform anesthesia, via puncture of the left ventricle until complete exsanguination. The obtained biomaterial (intestine) was fixed in a 10% formalin solution.

For the modeling of TBI, a controlled skull impact method was used with a special device that allowed precise regulation of the impact force. Depending on the impact force, TBI was classified into mild, moderate, and severe degrees. Before the impact, the animals were anesthetized (ketamine and xylazine) to minimize pain and stress. The control group underwent similar procedures, including anesthesia, but no impact was applied. All rats were divided into control and experimental groups. In each age category (1, 6, and 18 months), a group of animals that did not undergo TBI was included, as well as groups with mild, moderate, and severe damage. The study included 10 animals in each control group and 20 animals in each of the TBI groups. To evaluate the dynamics of morphological changes in the small intestine, tissues were collected from animals on days 1, 6, and 18 after injury, and on each of these days, 6-7 animals from each experimental group were euthanized. The main focus of the study was to investigate the morphological changes in the small intestine mucosa after TBI. The analysis included assessment of mucosal thickness, the area of inflammatory infiltrates, and the degree of necrosis. Histomorphometric and microscopic methods were used to obtain quantitative data.

Statistical data processing was carried out using Microsoft Excel 7.0 and GraphPad Prism software. The analysis included the calculation of mean values, standard deviations, median values, as well as the application of Mann-Whitney, Student's t-test, and Fisher’s exact test. The levels of statistical significance varied: high ($P<0.001$), moderate ($P<0.010$), low ($P<0.050$), and insignificant ($P>0.050$). Correlation analysis allowed identification of relationships between various parameters.

RESULTS AND DISCUSSION

Microscopic analysis revealed that the mucosa of the small intestine in 1-month-old Wistar rats had the same histotopographic structure, although the thickness and length of the villi varied. The surface of the mucosa was covered by a single layer of cylindrical epithelium. Lymphoid follicles were found in small numbers in the lamina propria. The connective tissue of the lamina propria was in the stage of formation, consisting of an infiltrate of young lymphohistiocytic cells and thin-walled blood vessels. In most areas of the small intestine wall, the lymphoid tissue was underdeveloped. Lymphocytes and plasma cells were scattered throughout the lamina propria of the mucosa. In some areas of the mucosa, lymphoid cells formed clusters that developed into primary lymphoid follicles. These clusters consisted of loosely arranged small and medium-sized lymphocytes, with a small number of reticular cells in their stroma, arranged haphazardly. Enlarged post-capillary venules were located at the periphery of these clusters, through which lymphocyte migration was observed. In areas of the mucosa where lymphoid follicles were forming, villi were absent, and the covering epithelium showed uniform thickening. In 1-month-

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old mice, it was found that small lymphocytes made up the majority of the cell composition of lymphoid follicles, accounting for 82.5±5.6%. Reticular cells (2.8±0.2%) and lymphoblasts (1.1±0.2%) constituted the structural basis of the lymphoid follicles, remaining in a functionally passive state and making up only 3.7%. In the germinal zone, large lymphocytes (1.9±0.5%) and medium-sized lymphocytes (5.6±0.7%) were found, though in small quantities. At the periphery of the lymphoid follicles, there were cells undergoing disintegration and death (1.7±0.4%), as well as macrophages phagocytizing them (0.7±0.2%).

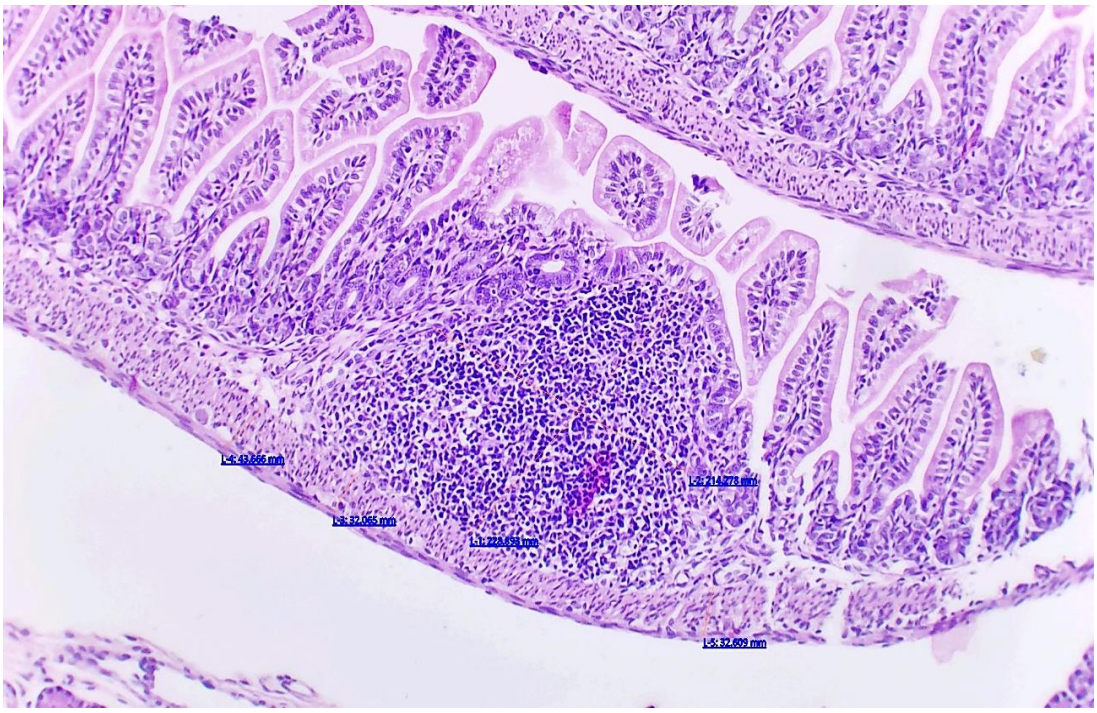

Figure 1. Formation of secondary lymphoid follicles and an increase in the number of palisade-like cells in the crypts of the small intestine in 1-month-old Wistar rats. Staining: hematoxylin-eosin. Magnification: ×10, objective 20.

In cases of mild traumatic brain injury in 1-month-old Wistar rats, morphological changes in the lymphoid structures of the small intestine wall observed on days 3 and 6 were as follows: lymphocytes predominantly accumulated around post-capillary venules, forming primary lymphoid follicles in the maturation stage. However, these structures lacked a clear reticular stroma, zones of cell development, and clusters of small lymphocytes surrounding them. At this stage of the study, other layers of the small intestine wall had also not yet fully developed. The mucosa, being in direct contact with the muscular layer, lacked a well-defined submucosa. The muscle layer consisted of loose and convoluted muscle fibers, with a small number of blood vessels and nerve plexuses between them (**Figure 1**).

In 6-month-old rats, pronounced changes in the mucosa of the small intestine were observed. The villi were elongated, forming various branched structures. The covering epithelium predominantly consisted of hyperchromatic enterocytes, with a small number of goblet cells. Crypts were

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numerous, varying in shape and size, and were also composed of hyperchromatic enterocytes. At this stage of postnatal ontogenesis, the lamina propria of the mucosa remained underdeveloped, consisting of loose connective tissue with infiltration of lymphohistiocytic cells. Lymphoid cells were scattered, and small primary lymphoid follicles were formed in certain areas. They localized in the submucosal layer, with poorly defined borders and undifferentiated germinal centers. Partial hypoplasia of the mucosa was also observed in this age group. The villi remained short, the crypts were sparse and varied in size. Goblet cells were present in small numbers, some of them undergoing vacuolar dystrophy. Particularly pronounced hypoplasia was noted in the lamina propria of the mucosa, where lymphoid cells were practically absent, the number of blood vessels was reduced, and the connective tissue fibers were sparsely and disorderly arranged. The submucosa was underdeveloped, and the crypts were in direct contact with the smooth muscle layer.

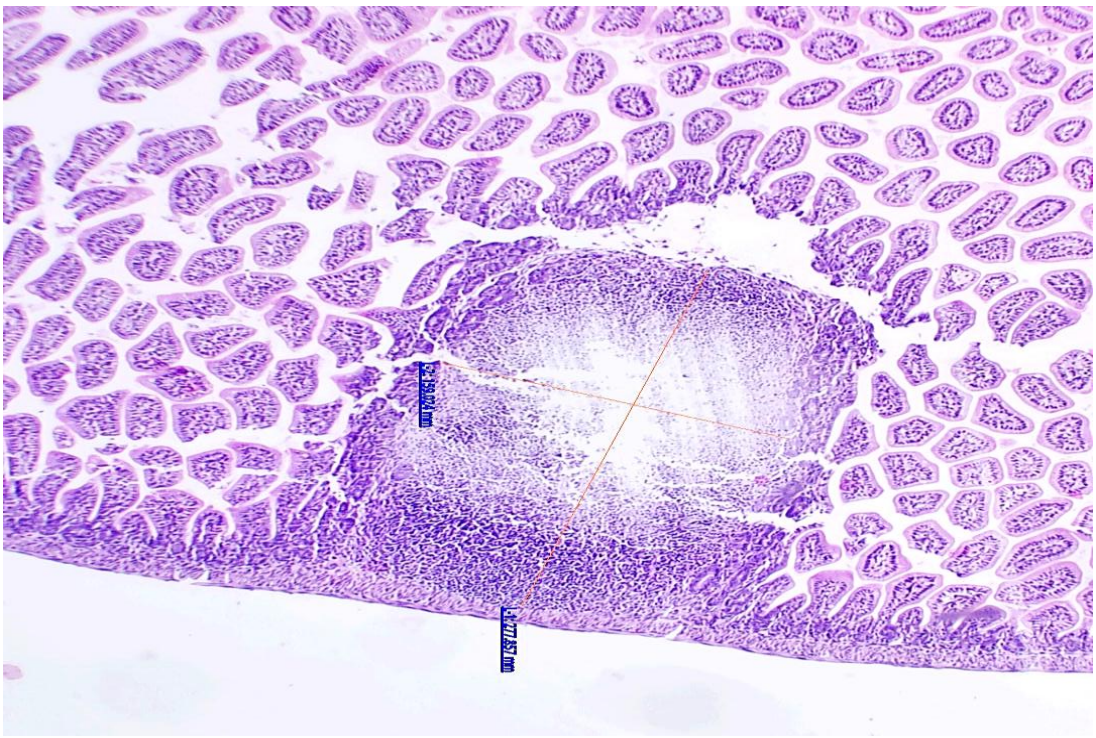

Figure 2. The onset of necrosis in the germinal centers of lymphoid follicles in the small intestine. Staining: hematoxylin-eosin. Magnification: ×10, objective 20.

In our study, when analyzing the morphological changes in the lymphoid structures in the wall of the small intestine in 6-month-old Wistar rats with mild brain injuries on day 3, the following changes were observed: in the germinal centers of the lymphoid follicles, signs of necrosis were clearly seen in the center against the background of hyperplasia, and around the germinal center, the number of cells and their clusters increased (**see Figure 2**). The onset of necrosis indicates that in these zones, cells die, forming bright areas. Lymphoid follicles, grouped in specific regions, were found only in the distal part of the small intestine in small numbers. Although these follicles have a solitary structure, they form centers of cellular division and develop into secondary follicles, suggesting that the mucosa of the small intestine was exposed to exogenous antigens multiple

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times, leading to the development of secondary immunity. It was also found that in some rats of this age, lymphoid follicles appeared not only in the wall of the small intestine but also in the wall of the cecum, including the inner layers of the muscular layer. On the surface of the mucosa, lymphocytes penetrate between the cells of the covering epithelium, establishing a symbiotic interaction with the epithelium.

In 18-month-old rats, age-related changes in the structure of the small intestine begin to manifest: the villi become shorter and thicker, which may indicate the onset of degenerative processes. The density of epithelial cells decreases, and the border of the brush membrane may become less distinct. The depth of the crypts may decrease, and the number of Paneth cells and basal cells decreases, slowing down regeneration processes. Thickening of the mucosal layer and basal membrane is observed, associated with progressive age-related changes. Small areas of subepithelial inflammation or fibrosis may appear. The muscle layer begins to gradually thin, which could lead to decreased motility in the small intestine.

Between 1 and 18 months, in rats with brain injuries, no significant changes were found in the cellular composition of the lymphoid nodes of the small intestine within 3 days. The number of small lymphocytes slightly increased by 2.3%, while reticular cells made up 2.6±0.3%, blast lymphocytes 1.7±0.4%, large lymphocytes 1.4±0.3%, and medium lymphocytes 4.4±0.8%. In rats of this age, it was found that the number of degenerating and dying cells, as well as macrophages on the periphery of the lymphoid follicles in the large intestine, increased twofold.

In our study, when analyzing the morphological parameters of the small intestine wall in the controlled groups of rats, we observed the following changes. Measurements were taken from the surface epithelium to the submucosal layer. In one-year-old rats, the villus thickness was 150 ± 10 μm, villus height was 350 ± 25 μm, the number of cells per square millimeter was 2500 ± 200, and the muscle layer thickness was 100 ± 8 μm. In six-month-old rats, the villus thickness increased to 170 ± 12 μm, villus height was 300 ± 22 μm, the number of cells per square millimeter was 2600 ± 205, and the muscle layer thickness was 120 ± 9 μm. In eighteen-month-old rats, the villus thickness was 160 ± 10 μm, villus height was 330 ± 20 μm, the number of cells per square millimeter was 2500 ± 200, and the muscle layer thickness was 120 ± 9 μm (**table 1**).

When calculating the morphometric parameters of the cells constituting the structure of the lymphoid follicles in the mucosa of the small intestine, it was found that the percentage of cells differed significantly at different stages of the rats' life. In three-month-old rats, small lymphocytes (82.5 ± 5.6%) made up the majority of the cells in the lymphoid follicles, while reticular cells (2.8 ± 0.2%) and lymphoblasts (1.1 ± 0.2%) comprised only 3.7%. Small (1.9 ± 0.5%) and medium-sized (5.6 ± 0.7%) lymphocytes were also found in the germinal area. In the peripheral regions of the lymphoid follicles, destroyed cells (1.7 ± 0.4%) and macrophages phagocytosing them (0.7 ± 0.2%) were observed. These data on the cell composition of lymphoid follicles in rats at rest, without antigenic exposure, indicate their functional inactivity.

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Table 1. Morphological Parameters of the Small Intestinal Wall in Rats with Traumatic Brain Injury.

Parameters	Units of Measurement	1 Month	6 Months	18 Months
Papilla Thickness	µm	142 ± 10	164 ± 10	152 ± 9
Papilla Height	µm	287 ± 19	328 ± 18	320 ± 19
Cell Count (per unit area)	Cells/mm²	2350 ± 200	2550 ± 205	2450 ± 195
Cell Density	%	40 ± 3	45 ± 4	44 ± 3
Muscle Layer Thickness	µm	95 ± 8	112 ± 7	105 ± 6
Submucosal Layer Structure	-	Edema, tissue destruction	Inflammatory infiltration	Breakdown of connective tissue and fibrosis

After treatment with L-lysine in rats with mild traumatic brain injury (TBI), aimed at determining the morphological changes in the lymphoid structures of the small intestine over the course of a month, we added an L-lysine solution to an isotonic solution and administered it to the rats in a dosage corresponding to their body weight. We observed a positive impact on the morphological changes occurring in the small intestine.

When studying the potential effects of L-lysine in the context of traumatic brain injury (TBI), it was found that this amino acid plays a crucial role in protein synthesis and has the potential to modulate inflammatory processes. It aids in tissue recovery and reduces changes, which is of significant importance in TBI. Specifically, the effect of L-lysine on the mucosa helps reduce inflammation and alleviate edematous processes (**table 2**).

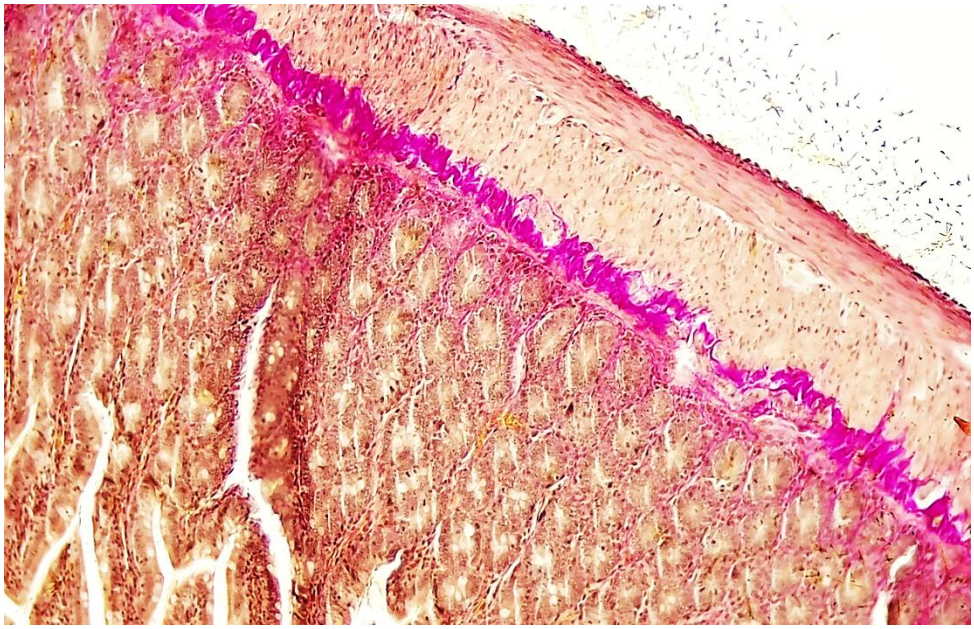

Figure 3. After treatment with medications for mild traumatic brain injury in 18-month-old white mice, partial recovery, preservation of necrotic changes, and restoration of microcirculation are observed in the wall of the small intestine. Staining: Van-Gieson. Magnification: approximately 10, objective 20.

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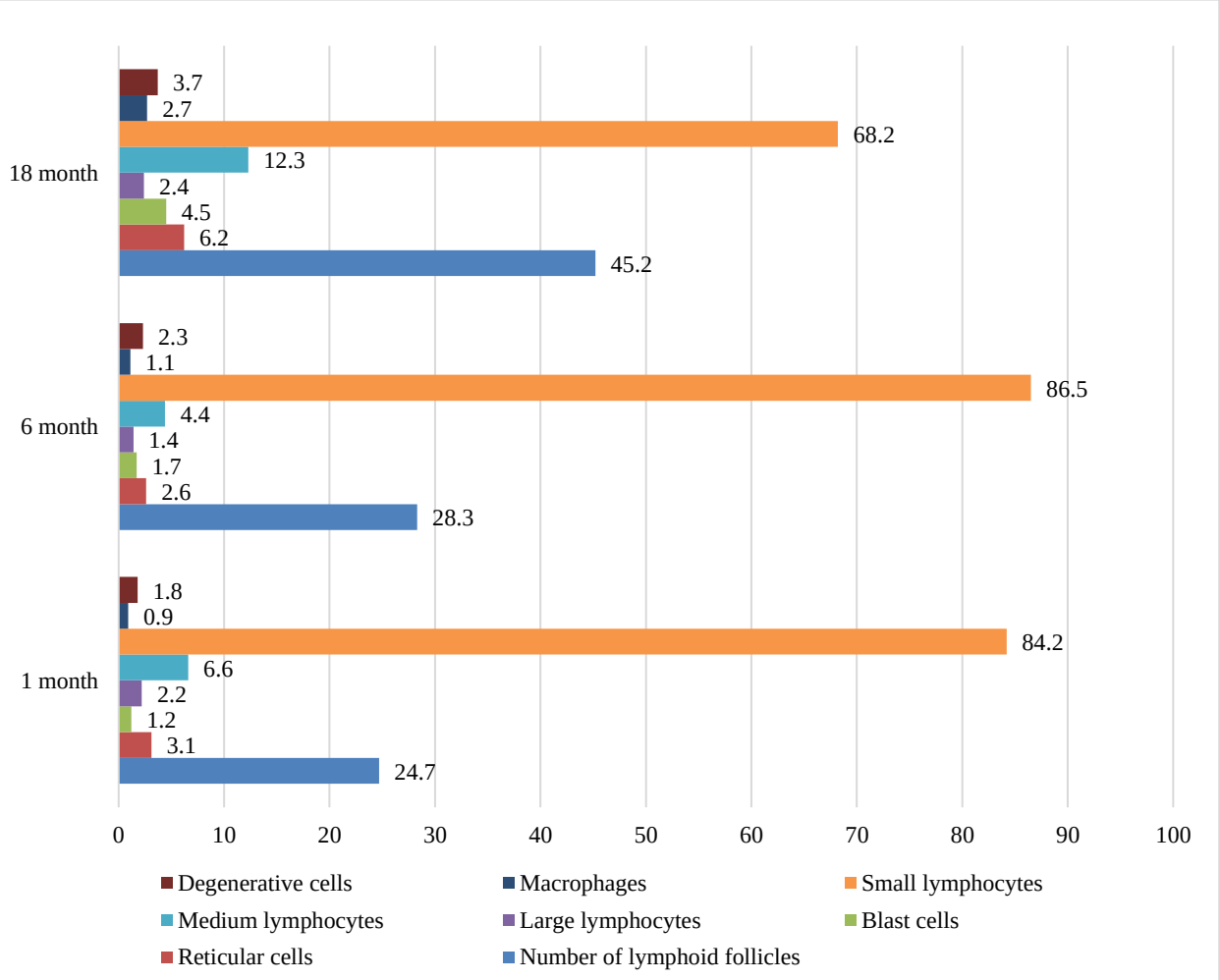
In our study, the morphological parameters of the small intestine in groups of mice with brain injuries that were exposed to L-lysine were analyzed across different age groups, from the surface epithelium to the submucosal layer. In 1-month-old mice, the villus thickness was 142 ± 10 μm, villus height was 287 ± 19 μm, cell density per square millimeter was 2350 ± 200, and the muscular layer thickness was 95 ± 8 μm. In 6-month-old mice, the villus thickness increased to 164 ± 10 μm, villus height was 328 ± 18 μm, cell density per square millimeter was 2600 ± 205, and the muscular layer thickness was 120 ± 9 μm. In 18-month-old mice, the villus thickness was 152 ± 9 μm, villus height was 320 ± 19 μm, cell density per square millimeter was 2450 ± 200, and the muscular layer thickness was 105 ± 6 μm.

Table 2. Average number and cellular composition (in percentages) of lymphoid follicles in the intestines of 1-month-old, 6-month-old, and 18-month-old rats with brain injury.



In the small intestines of rats, early histological changes were observed on the first day after brain injury, increasing in severity depending on the extent of the injury. In the mild form of brain injury, mild degeneration of the epithelium was noted. These changes indicate the initial damage of tissues that have not yet reached a definitive stage. In moderate severity, degeneration of the epithelial surface and microcirculatory disturbances were observed, indicating more serious structural damage to the tissues. In severe cases, epithelial necrosis and particularly noticeable microvascular changes were evident, indicating tissue damage.

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When analyzing the histological changes in the small intestinal wall at various stages of brain injury, we observed significant alterations across all three groups of rats, depending on the severity of the injury. Our findings indicated that the degree of damage to the brain had a direct impact on the structural changes observed in the small intestine. In the control group, no significant changes were observed in the small intestine. The structure of the small intestine remained intact, with no apparent histological alterations.

In day 1 post-TBI mild inflammation and epithelial dystrophy are observed. These changes suggest that the tissues have sustained initial damage, but the alterations are not yet severe. In cases of moderate severity, inflammation and disturbances in microcirculation become evident. These changes indicate that the injury has caused structural disruptions in the tissues, reflecting a more pronounced impact on the small intestine. In severe cases, there is significant inflammation, desquamation of the epithelium, and noticeable microcirculatory changes. These histological alterations indicate substantial tissue damage in response to the severe injury.

In day 3 post-TB for mild injuries, the inflammation begins to show minimal recovery. The tissue structures may return to a near-normal state, with only slight residual changes. **TBI w**ith moderate severity, partial inflammation and necrosis are present, along with the restoration of dystrophic processes. This suggests that the tissue is in the process of repair but has not fully recovered. Severe injury leads to necrosis and noticeable inflammation. These changes are accompanied by significant microcirculatory damage, highlighting the severity of the injury.

By the sixth day, mild injuries show almost complete recovery, with minimal residual changes. The tissue structure is largely restored, with few lasting effects. Moderate injuries exhibit moderate residual inflammatory changes and regenerative necrosis. These alterations reflect ongoing healing processes, but the tissues are still in a state of recovery. In severe cases, partial recovery is observed, with necrotic changes still present and microcirculatory function beginning to return to normal. This suggests that while some repair is occurring, significant damage persists in the tissues.

In cases of severe brain injuries and treatment in 18-month-old white mice, morphological changes in the wall of the small intestine vary depending on the severity of the condition. In mild injuries, almost complete recovery is observed with minimal residual changes, in moderate injuries — reparative regeneration, and in severe cases — partial recovery of tissue and microcirculation, with necrotic changes persisting.

CONCLUSIONS

The study showed that traumatic brain injury (TBI) leads to significant changes in the morphology of the small intestinal mucosa, which depend on the severity of the injury and the age of the animals. In 1-month-old rats, the changes were minimal, with a small number of inflammatory infiltrates. In 6-month-old animals, more pronounced changes were observed, including villous elongation and hyperplasia of lymphoid follicles. In 18-month-old rats, the changes included signs of aging and degeneration of the mucosal lining. This study highlights the importance of considering age-related changes when analyzing the impact of TBI on the intestine and opens up opportunities for further research in the fields of gastroenterology and neurology.

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