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EXPERIMENTAL EVALUATION OF BIOCHEMICAL MARKERS OF RAT BONE TISSUE AGAINST THE BACKGROUND OF MODELING CHRONIC EPILEPTIC ACTIVITY AND DENTAL CARIES

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This research is devoted to the modeling of chronic epileptic activity and dental caries with the aim of experimentally evaluating biochemical markers of bone tissues in rats. In the experiment, dental caries was reproduced by transferring the animals to Stephan's cariesogenic diet, while epileptic activity was induced using the kindling and post-kindling models. The rats were divided into groups of six animals each. The results revealed a significant increase in acid phosphatase activity in the dental pulp of rats subjected to the cariesogenic diet and epileptic activity, indicative of tissue resorption processes in the teeth. The decrease in alkaline phosphatase activity signifies impaired mineralization processes in the hard dental tissues and suppression of odontoblast function. The combined influence of these factors exacerbated the pathological changes in the condition of the hard dental tissues, underscoring the need for specialized preventive measures in patients with epilepsy.

Key words: epilepsy, biochemical markers, bone tissue, rats, experiment.

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ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА БІОХІМІЧНИХ МАРКЕРІВ КІСТКОВОЇ ТКАНИНИ ЩУРІВ НА ТЛІ МОДЕЛЮВАННЯ ХРОНІЧНОЇ ЕПІЛЕПТИЧНОЇ АКТИВНОСТІ І КАРІЄСУ ЗУБІВ

Це дослідження присвячено моделюванню хронічної епілептичної активності і карієсу зубів з метою експериментально оцінити біохімічні маркери кісткових тканин щурів. У експерименті карієс зубів відтворювали шляхом переведення тварин на карієсогенний раціон за Стефаном, епілептичну активність – за допомогою моделей кіндлінг та посткіндлінг. Щури були розділені на групи по 6 штук в кожній. Результати виявили значне збільшення активності кислої фосфатази в пульпі зубів щурів, що піддавалися карієсогенному раціону та епілептичній активності. Це свідчить про процеси резорбції тканин зубів. Зниження активності лужної фосфатази свідчить про порушення мінералізаційних процесів у твердих тканинах зубів та пригнічення функцій одонтобластів. Комбінований вплив цих факторів поглибив патологічні зміни у стані твердих тканин зубів, підкреслюючи потребу у спеціалізованих профілактичних заходах для пацієнтів з епілепсією.

Ключові слова: епілепсія, біохімічні маркери, кісткова тканина, щури, експеримент.

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The impact of epilepsy on various body systems, including the condition of the hard dental tissues, remains insufficiently studied, highlighting the need for further research in this area [1]. Given the high prevalence of epilepsy and the considerable risk of developing dental complications in patients with this disease, the importance of conducting comprehensive studies in this field is indisputable [6, 8, 9]. The incidence of epilepsy in different countries ranges from 50 to 70 cases per 100,000 population, with a prevalence of 5–10 cases per 1,000 people (approximately 1 %) [11]. In Ukraine, there are approximately 500,000 patients with epilepsy, of whom about 140,000 are children [2]. The significance of this study is further enhanced by the fact that an integrated approach to investigating this issue may facilitate the development of novel strategies for the prevention and treatment of dental diseases, particularly in patients with epilepsy. Examining the biochemical changes in the hard dental tissues not only provides insights into the effect of epileptic activity on oral health, but also reveals potential biochemical markers that could serve as early indicators of complication development.

The findings of this work will deepen our understanding of the relationship between epileptic activity and the state of the hard dental tissues and will contribute to the development of effective diagnostic, preventive, and treatment methods tailored to the needs of patients with epilepsy.

The purpose of the study was to perform an experimental evaluation of biochemical markers in the jaw and dental pulp of rats under conditions of chronic epileptic activity and dental caries modeling.

Materials and methods. The objects of this experimental study were 30 Wistar rats from the breeding colony of the vivarium at the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"), which were maintained on the vivarium's standard diet. The rats used in the experiments were healthy and had free access to water and food. All experiments on rats were conducted according to standard operating procedures approved by SE "ISMFS NAMS", developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [3].

The animals were divided into the following groups of 6 rats each:

1. Intact group
2. Dental caries model, cariesogenic diet (CD)
3. Kindling model (KM)
4. Post-kindling model (PKM)
5. Dental caries model + post-kindling model (CD+PKM)

The intact group received a standard vivarium diet.

Dental caries was induced in rats of Groups 1 and 5 starting at one month of age by transferring the animals to Stephan's cariesogenic diet [5], which they received for 30 days.

Chronic epileptic activity (EpA) was modeled using two approaches: kindling and post-kindling. Pharmacological kindling was induced in rats of group 3 starting at one month of age by daily intraperitoneal (i.p.) injections of picrotoxin (PCT, "Sigma-Aldrich," Germany) at subthreshold doses (ranging from 0.9 mg/kg to 1.1 mg/kg, individually selected based on the intensity of seizures to prevent fatality) over a 24-day period.

In rats of group 4, post-kindling was modeled by allowing a 14-day period with no convulsant administration following the last PCT injection [9].

In group 5, the animals were exposed to both factors: they were switched to the cariesogenic diet at one month of age and subsequently underwent post-kindling for 14 days without convulsant administration, following 24 days of daily PCT injections.

The animals were euthanized under thiopental anesthesia (40 mg/kg). The lower jaws and dental pulp were collected for analysis.

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Student's test. The difference was considered statistically significant at $p<0.01$ [4].

Results of the study and their discussion. Table 1 presents the findings on the condition of the dentoalveolar system in rats under various experimental influences (cariesogenic diet, kindling, post-kindling).

Table 1

Condition of the dentoalveolar system in rats under experimental pathologies, M±m

No.	Animal groups	Degree of alveolar process atrophy, %	Number of carious cavities per rat	Depth of carious lesions (points)
1	Intact, n=6	15.8±0.7	2.3±0.2	3.0±0.2
2	Cariesogenic diet (CD), n=6	20.7±0.8 p<0.001	3.1±0.3 p<0.001	3.4±0.3 p>0.1
3	Kindling model (KM), n=6	24.4±1.3 p<0.001	3.4±0.3 p<0.001	4.0±0.4 p<0.001
4	Post-kindling model (PKM), n=6	25.2±0.9 p<0.001	3.7±0.2 p<0.001	4.3±0.3 p<0.001
5	CD+PKM, n=6	29.2±1.9 p<0.001	4.0±0.4 p<0.001	4.8±0.4 p<0.001

Note: p – significance of differences compared to the intact group.

One month of a cariesogenic diet led to a marked 31.0 % increase in alveolar process atrophy compared to the control group, indicating alterations in bone structure due to a diet high in sugars. Further modeling of epilepsy using the kindling and post-kindling approaches resulted in additional increases of 54.4 % and 59.5 %, respectively, highlighting the negative effect of neuropathological conditions on bone tissue. The greatest atrophy (84.8 %) was noted in the rats exposed to the combined influence of both factors, indicating a synergistic effect of these conditions on bone resorption processes.

Maintaining rats on a cariesogenic diet for 30 days also significantly increased the mean number of carious cavities per rat by 34.7 %, while the depth of carious lesions (in points) grew insignificantly (by 9.6 %). Modeling epileptic activity in Groups 3 and 4 led to further increases in the number of carious

cavities by 47.8 % and 60.8 %, respectively, underscoring the relationship between neuropathological changes and oral health. The most pronounced changes were found in the combined-exposure group, where the number of carious cavities rose by 73.9 % and the depth of lesions by 54.8 %, demonstrating the substantial impact of the cariesogenic diet and epilepsy on the deterioration of the dentoalveolar system. These findings emphasize the need for targeted strategies in preventing and treating dental diseases in individuals with neurological disorders.

The mineralization capacity of the dental pulp is reflected in the activity levels of acid and alkaline phosphatases. The results of these measurements in the rat dental pulp are presented in Table 2.

Table 2

Phosphatase activity in rat dental pulp, M±m

No.	Animal groups	Activity	
		acid phosphatase (nkat/g)	alkaline phosphatase (μkat/g)
1	Intact, n=6	65.6±5.1	2.17±0.15
2	Cariesogenic diet (CD), n=6	107.3±9.8 p<0.001	1.52±0.09 p<0.001
3	Kindling model (KM), n=6	103.2±8.6 p<0.001	1.40±0.14 p<0.001
4	Post-kindling model (PKM), n=6	110.6±9.3 p<0.001	1.24±0.08 p<0.001
5	CD+PKM, n=6	142.4±13.8 p<0.001	0.75±0.04 p<0.001

Note: p – significance of differences compared to the intact group.

Over 30 days of the cariesogenic diet, there was a substantial increase in acid phosphatase activity in the rat dental pulp, indicating intensified bone resorption processes, particularly through enhanced odontoclast activity. This rise in acid phosphatase activity was observed not only under the influence of the cariesogenic diet but also in the epilepsy models (kindling and post-kindling), with the enzyme activity increasing by 1.57- and 1.68-fold, respectively, whereas the combined effect of these states led to a 2.17-fold increase. These findings emphasize the impact of neuropathological changes on metabolic processes in the teeth.

Parallel to the elevated acid phosphatase activity, there was a noted decrease in alkaline phosphatase activity, suggesting suppression of the mineralizing functions of odontoblasts and a reduced overall mineralizing potential of the pulp. Observations across different study groups showed the greatest decline in alkaline phosphatase activity in the group experiencing both the cariesogenic diet and epileptic activity modeling, which was 2.9 times lower compared to the control group.

Overall, the results demonstrate that destructive processes prevailed over mineralizing processes in rat dental pulp under the influence of the studied factors, leading to a decrease in the overall mineralizing potential of the pulp in pathological conditions. The most pronounced shift toward destruction was noted in group 5, in which the rats were subjected to the combined influence of the pathogenic factors.

Our findings indicate a marked exacerbation of destructive processes in the hard dental tissues of rats under the combined impact of a cariesogenic diet and chronic epileptic activity. These results are in line with previous observations demonstrating the detrimental effects of neuropathological conditions on bone tissue homeostasis [1, 6]. In particular, the synergistic effect we noted – with significantly elevated acid phosphatase activity and reduced alkaline phosphatase activity – corresponds to research illustrating intensified bone resorption under conditions of both chronic epilepsy and metabolic stress [2, 9]. This dual exposure may accelerate pathological mechanisms that weaken mineralized tissues, as also suggested by studies reporting impaired alveolar bone density in individuals with epilepsy [1]. In comparing our data with those obtained from clinical and experimental observations, it is evident that epileptic activity may contribute to microcirculatory disturbances, altered calcium homeostasis, and shifts in enzymatic profiles that favor catabolic pathways in bone [11]. The significant increases in caries indices we observed align with previous work indicating a higher susceptibility to dental lesions in epileptic patients, partly attributable to both direct disease-related factors and secondary influences, such as medication-induced changes in saliva and dietary habits [8, 10]. Other authors have likewise emphasized the need for specialized oral care in this patient population, underscoring the combined influence of caries risk factors and systemic disorders on overall oral health [6]. The sustained decrease in alkaline phosphatase activity found in our experiment points to suppressed odontoblast function, possibly linked to neurogenic regulation deficits tied to epilepsy [2]. These alterations highlight a broader issue of compromised tissue regeneration and mineralization capacity, stressing the importance of preventative and therapeutic interventions. Such

interventions could extend beyond conventional dental treatments to include targeted anticonvulsant strategies that minimize detrimental side effects on oral tissues, an approach advocated by those exploring the neuro-oral axis [9]. Indeed, the development of multi-modal treatment regimens holds promise not only for stabilizing epileptic activity but also for maintaining or restoring the integrity of dental hard tissues. Given the high prevalence of epilepsy and its significant oral health implications, continued research into comprehensive treatment strategies is warranted. Future studies may focus on optimizing the management of caries and periodontal conditions through tailored pharmacological regimens, dietary counseling, and innovative biomaterials designed to enhance remineralization [1, 5, 7]. Further experimental investigations combining epilepsy models with other comorbidities could also deepen our understanding of the complex interactions between systemic neurological disorders and local tissue pathologies. Ultimately, such integrative efforts will facilitate the development of more effective protocols aimed at preventing and mitigating dental complications in patients with epilepsy, thereby improving both their oral and overall health.

Conclusions

1. A significant increase in acid phosphatase activity was found in the dental pulp of rats subjected to a cariesogenic diet and epileptic activity, indicating the activation of tooth tissue resorption processes. At the same time, there was a decrease in alkaline phosphatase activity, signifying impaired mineralization processes in the hard dental tissues and suppression of odontoblast functions.

2. Modeling chronic epileptic activity and dental caries in experimental animals significantly affected the dentoalveolar system, as evidenced by a marked increase in the degree of alveolar process atrophy, a rise in the number of carious cavities per rat, and greater depth of carious lesions.

3. The combined effect of the cariesogenic diet and chronic epileptic activity produced the most pronounced changes in the condition of the hard dental tissues, demonstrating a synergistic action of these factors.

4. These findings highlight the need for developing targeted preventive and therapeutic strategies for patients with epilepsy to reduce the risk of dental diseases.

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