

Abstract

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RESISTANCE OF ENAMEL IN SUPRA-OCCLUSION CONTACTS OF INDIVIDUAL TEETH IN RATS OF VARIOUS AGES

Introduction. Supra-occlusion is a position of a group of teeth or individual teeth, the cutting edge or chewing surface of which is located above the occlusal plane. This condition is characterized as primary traumatic occlusion, supra-contact or traumatic nodes, in which a number of structural and functional changes occur in the hard tissues of the teeth and periodontal tissue. Traumatic occlusion of teeth is often caused by occlusal intervention, such as high fillings or prostheses, parafunctional habits. In physiological conditions, the protection of teeth from chemical and mechanical interventions is provided by their structural and functional resistance. However, the influence of supra-occlusion on the resistance of tooth enamel, and especially in different age groups, has not yet been studied. Therefore, we consider this topic relevant.

The **objective** of the work was to study the state of enamel resistance in supra-occlusal contacts of individual teeth in rats of various ages according to the enamel resistance test (ERT-test).

Materials and methods. The study involved 36 white male laboratory rats aged 4 to 22 months and weighing 100 to 210 g. The animals were divided into two groups (control and experimental), each was divided into three subgroups (6 animals each) according to age categories: young, mature, old-aged. In the study group, the state of supra-occlusion was simulated by increasing the height of the lower right second molars via filling 1 mm in height, without preparation of hard tissues of the teeth. Previously, the animals were anesthetized intramuscularly with sodium thiopental solution. After removing the animals from the experiment on the 15th day by decapitation under general anesthesia with sodium thiopental, by intraperitoneal injection, the dental blocks were removed and the structural and functional state of the tooth enamel was determined by the ERT-test. Statistical processing of data was performed using the program AtteStat V.12.5 with the determination of the mean and its error ($M \pm m$). The probable significance of the difference in the obtained data was determined by the Student's t-test ($p \leq 0.05$).

Study results. In the control group, ERT-test values decreased (enamel resistance increased accordingly) in rats, depending on age. In young rats, enamel resistance to acidic dissolution was the smallest, and in mature and old-aged rats it increased. However, the difference between the age groups was not statistically significant ($p > 0.05$). A similar situation was registered in animals of the study group. However, sta-

tistically significant differences were observed only between young and old-aged rats ($p = 0.025$). In the control group, the acid resistance of enamel is higher compared to study group ($p = 0.007$). In young rats from the control group, enamel resistance to acids was 16.7% higher than in the study group ($p = 0.025$). In mature animals of the control group, the resistance of enamel to acid dissolution is 17.7% higher than in the study group ($p = 0.046$), and in old-aged – by 15.2% ($p = 0.2$).

Conclusions. The resistance of enamel of teeth that are subjected to increased stress via supra-occlusal interactions is reduced in young, mature and old-aged rats in comparison with the control group. Reduced enamel resistance can be one of the risk factors in the occurrence of dental caries.

Key words: traumatic occlusion, enamel resistance, ERT-test, rats, supra-occlusion.

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Резюме

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РЕЗИСТЕНТНІСТЬ ЕМАЛІ ПРИ СУПРАОКЛЮЗІЙНИХ КОНТАКТАХ ОКРЕМИХ ЗУБІВ У ЩУРІВ РІЗНОГО ВІКУ

Актуальність. Супраоклюзія – положення групи зубів чи окремих зубів, ріжучий край або жувальна поверхня яких розташована вище оклюзійної площини. Цей стан характеризують як первинну травматичну оклюзію, супраконттакт або травматичні вузли, при якому виникає ряд структурних та функціональних змін в твердих тканинах зубів і пародонта. Травматична оклюзія зубів часто викликається оклюзійним втручанням, таким як високі пломби або протези, парафункціональні звички. У фізіологічних умовах захист зубів від хімічних та механічних втручань забезпечується їх структурно-функціональною резистентністю. Але досі залишаються не вивченими питання впливу підвищеної оклюзії на резистентність емалі зубів, а тим паче в різних вікових групах. Тому цю тему вважаємо актуальною.

Метою роботи було вивчення стану резистентності емалі при супраоклюзійних контактах окремих зубів у щурів різного віку за тестом емалевої резистентності (ТЕР-тест).

Матеріали і методи. Дослідження було проведене на 36 білих лабораторних щурах-самцях віком від 4 до 22 місяців і вагою від 100 до 210 г. Тварини були розподілені на дві групи (контрольна і дослідна), в кожній з яких виділяли три підгрупи (по 6 тварин) відповідно до вікових категорій: молоді, зрілі, старечі. В дослідній групі моделювали стан супраоклюзії зубів шляхом підвищенням висоти нижніх правих других молярів за рахунок постановки пломби висотою 1 мм без препарування твердих тканин зубів.. Попередньо тварин анестезували внутрішньом'язево розчином тіопентал натрію. Після виведення тварин з експерименту на 15 добу шляхом декапітації під загальною анестезією тіопентал натрію, шляхом внутрішньочеревної ін'єкції вилучали зубошелепні блоки та проводили визначення структурно-функціонального стану емалі зубів за ТЕР – тестом. Статистичну обробку даних проводили за допомогою програми AtteStat v.12,5 з визначенням середньої та її похибки ($M \pm m$). Вірогідну значущість різниці отриманих даних проводили за параметричним критерієм Ст'юдента (при $p \leq 0,05$).

Результати дослідження. В контрольній групі показники ТЕР-тесту зменшувались (резистентність емалі відповідно підвищувалась) у щурів залежно від віку. У молодих резистентність емалі до кислотного розчинення була найменшою, а у щурів зрілого і старечого віку підвищувалась. Однак різниця між віковими групами не мала статистичної вірогідності ($p > 0,05$). У тварин дослідної групи реєструвалась аналогічна ситуація. Проте статистично значимі відмінності відмічали лише між щурами молодого і старечого віку ($p = 0,025$). В контрольній групі кислотостійкість емалі вища за дослідну ($p = 0,007$). У щурів молодого віку з контрольної групи резистентність емалі до дії кислот була на 16,7% вища за тварин дослідної групи ($p = 0,025$). У зрілих тварин контрольної групи стійкість емалі до кислотного розчинення на 17,7% вища за дослідну ($p = 0,046$), а старечого – на 15,2% ($p = 0,2$).

Висновки. Резистентність емалі зубів, які піддаються підвищеному навантаженню через супраоклюзійні взаємовідносини, знижується у щурів молодого, зрілого і старечого віку в порівнянні з контрольною групою. Зниження резистентності емалі може виступати одним з факторів ризику у виникненні карієсу зубів.

Ключові слова: травматична оклюзія, резистентність емалі, ТЕР – тест, щури, супраоклюзія.

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Introduction

Supra-occlusion is a position of a group of teeth or individual teeth, the cutting edge or chewing surface of which is located above the occlusal plane. In this situation, the teeth experience an increased chewing load. This condition is characterized as primary traumatic occlusion, supra-contact or traumatic nodes, in which a number of structural and functional changes occur in the hard tissues of the teeth and periodontal tissue. Traumatic occlusion of teeth is often caused by occlusal intervention, such as high fillings or prostheses, parafunctional habits [1].

Cracks in the enamel may occur at the site of supra-occlusion due to chronic microtrauma [2]. Chronic occlusal trauma due to an increase in the concentration of chewing pressure on the teeth is considered the main local etiological factor that gradually leads to tooth wear and increased attrition of hard tissues [3, 4].

Occlusal trauma of the teeth may be caused not only by their supra-occlusive contacts, but also parafunction in patients. Parafunctions can cause stress and tension in hard tissues, which leads to the appearance of cracks or fractures of teeth [5, 6].

Excessive occlusal load on individual teeth also causes stresses in the enamel, which lead to

the appearance of cervical lesions of the teeth in the form of abfraction defects [7, 8, 9, 10].

In physiological conditions, the protection of teeth from chemical and mechanical interventions is provided by their structural and functional resistance. However, the influence of supra-occlusion on the resistance of tooth enamel, and especially in different age groups, has not yet been studied. Therefore, we consider this topic relevant.

The **objective** of the work was to study the state of enamel resistance in supra-occlusal contacts of individual teeth in rats of various ages according to the enamel resistance test (ERT-test).

Materials and study methods

The study involved 36 white male laboratory rats aged 4 to 22 months and weighing 100 to 210 g. The animals were divided into two groups (control and experimental), each was divided into three subgroups (6 animals each) according to age categories: young, mature, old-aged. The age of the animals was determined by two conditions: number of months from birth and body weight. Young animals included rats at the age of 3–4 months with a weight of 90–100 g; mature animals – at the age of 6–8 months with a weight of 150–170 g; old-aged rats 20–22 months. and a mass of 200–220 g [11].

During the experiment, the vivarium room was kept at a high temperature, and the rats were

provided with proper care. During the entire period of research, the animals were fed dry granulated food, which was characterized by a balanced content of ingredients necessary for a full life.

In the study group, supra-occlusion of teeth was simulated. Preliminary, the animals were anesthetized intramuscularly with a sodium thiopental solution (2 mg/kg, Thiopental, Kyivmedpreparat PJSC, Kyiv, Ukraine). Excessive mechanical load on the teeth was simulated by increasing the height of the lower right second molars via filling 1 mm in height, without preparation of hard tissues of the teeth. 37% orthophosphoric etching gel for enamel and dentin (Latus, Ukraine), microapplicator (Latus, Ukraine), adhesive system of the fifth generation Prime&Bond NT (Dentsply Sirona, Germany), universal micro-hybrid light-solid composite material Latelux (Latus, Ukraine) and LED.D photopolymerizer (WOODPECKER, China) were used for the filling in accordance with the manufacturer's instructions.

After removing the animals from the experiment on the 15th day by decapitation under general anesthesia with sodium thiopental, by

intraperitoneal injection, the dental blocks were removed and the structural and functional state of the tooth enamel was determined by the ERT-test. [12].

To determine enamel resistance, the dental crowns were treated with distilled water and dried with a cotton swab. A drop of 1% hydrochloric acid solution was applied to the buccal surface of the lower right second molar. After 5 seconds, the acid was washed off with distilled water and the crown of the tooth was dried with a cotton swab. Next, a tampon moistened with 1% aqueous solution of methylene blue was applied to the crown of the tooth. After that, the dye is removed from the enamel surface with a cotton swab with one movement.

Color intensity of the etched enamel area was estimated in points on a ten-field color scale (Fig. 1). The scale is a line of different intensity of blue color areas that correspond to the color of the tooth enamel after acid etching and subsequent staining with a methylene blue dye. The arrangement of shades goes from lighter to darker and corresponds to a score from 1 (the lightest) to 10 (the darkest).

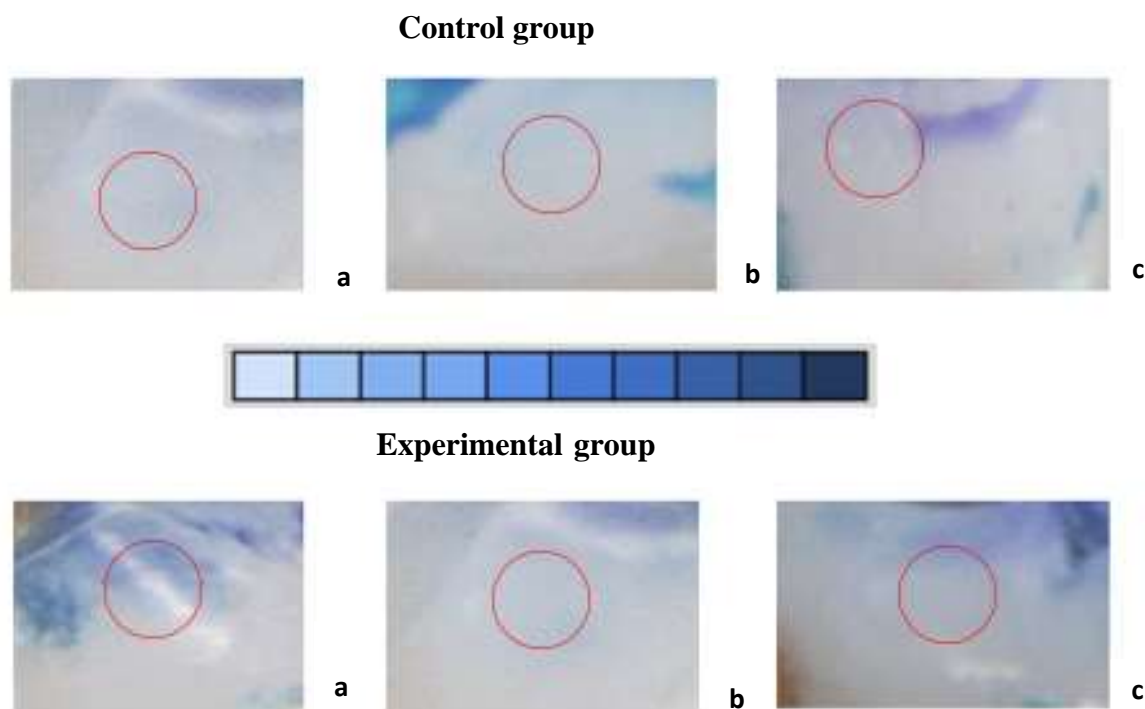


Figure 1 – ERT-test in rats by age: a – young, b – mature, c – old-aged

Experimental rats were cared for in accordance with the provisions of the European Convention on the protection of vertebrates, used in experiments and other scientific purposes (Strasbourg, 1986)

and the General ethical principles of animal experiments, approved by the First National Congress on Bioethics (Kyiv, 2001).

Statistical processing of data was performed using the program AtteStat V.12.5 with the determination of the mean and its error ($M \pm m$). The probable significance of the difference in the obtained data was determined by the Student's t-test ($p \leq 0.05$).

Study results and discussion

The study of enamel resistance indicators in rats showed (Table 1) that the value of the ERT-test differs both between groups of study animals and within groups, depending on age.

Table 1 –ERT-test results in rats of various age, points

| Rat age | Group of rats | | | | P |
|----------|---------------|-----------------|-------|-----------------|-------|
| | Control | | Study | | |
| | n | $M \pm m$ | n | $M \pm m$ | |
| Young | 6 | 2.5 ± 0.22 | 6 | 3.0 ± 0.0 | 0.025 |
| Mature | 6 | 2.33 ± 0.21 | 6 | 2.83 ± 0.17 | 0.046 |
| Old-aged | 6 | 2.17 ± 0.31 | 6 | 2.5 ± 0.22 | 0.2 |
| Total | 18 | 2.33 ± 0.14 | 18 | 2.78 ± 0.10 | 0.007 |

In the control group, ERT-test values decreased (enamel resistance increased accordingly) in rats, depending on age. In young rats, enamel resistance to acidic dissolution was the smallest, and in mature and old-aged rats it increased. However, the difference between the age groups was not statistically significant ($p > 0.05$). A similar situation was registered in animals of the study group. However, statistically significant differences were observed only between young and old-aged rats ($p = 0.025$).

The decrease in enamel resistance in young animals is due to its insufficient mineralization after teething, the peculiarities of the enamel structure, and the specific structure (permeability, microhardness stability in an acidic environment) [13]. It is proved that the tooth enamel at the early stages of physiological "maturation" due to an insufficiently formed prismatic structure, lack of protective layer, significant permeability, large number of retention points has morphological characteristics of hypomineralization and low caries resistance and acid resistance [14]. This is confirmed by the data of clinical and laboratory studies, which indicate the crucial importance of the enamel mineralization processes in maintaining the state of caries resistance [15].

Conclusions

The resistance of enamel of teeth that are subjected to increased stress via supra-occlusal interactions is reduced in young, mature and old-aged rats in comparison with the control group. Reduced enamel resistance can be one of the risk factors in the occurrence of dental caries.

Analyzing the state of enamel resistance between the studied groups of animals, we also registered a significant difference in the resistance of tooth enamel. In the control group, the acid resistance of enamel is higher compared to study group ($p = 0.007$). This trend is typical for animals of all age groups, but the ERT-test data varies depending on the age of the rats and the experimental conditions.

In young rats from the control group, enamel resistance to acids was 16.7% higher than in the study group ($p = 0.025$). With age, the resistance of tooth enamel increases, but the general trend in the difference of the ERT-test values remains. Thus, in mature animals of the control group, the resistance of enamel to acid dissolution is 17.7% higher than in the study group ($p = 0.046$), and in old-aged – by 15.2% ($p = 0.2$).

That is, the results of the experiment indicate a decrease in the resistance of the enamel of teeth that are subjected to excessive stress. If we assume that the teeth are subjected to a similar load in young people engaged in physical exercises with weights, as well as in orthodontic patients whose teeth are forcibly moved, our data coincide with the results of other researchers [16].

Prospects for future research

The obtained data on the functional state of the enamel of teeth that are subjected to mechanical overload give grounds to investigate structural changes in the tissues of the teeth and periodontium in the supra-occlusal interactions of individual teeth in the age aspect.

Conflict of interest

The authors declare no conflict of interest.

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