**KARAGANDA MEDICAL UNIVERSITY**

ANNOTATION

dissertations for the academic degree Doctor of Philosophy PhD

 Baigulakov Azamat Turashevich

on the topic:

**"Pathomorphological substantiation of a non-invasive method of treatment of dental caries (experimental study)".**

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| Speciality:Direction of doctoral studies: Duration of doctoral studies:  |  6D110100 MEDICINEScientific and pedagogical3 years |
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| Scientific consultant: | Tuleutaeva Svetlana Toleuovna, Candidate of Medical Sciences, Professor of the School of Dentistry, NC JSC “KMU” |
| Scientific consultant:Foreign scientific consultant: | Tusupbekova Maida Maskhapovna, Doctor of Medical Sciences, Professor, Department of Pathology, NC JSC “KMU”Doctor of Medical Sciences, associate professor, head of the Department of Therapeutic Dentistry, Kuban State Medical University, Ministry of Health Care of Russia |

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**Actuality of the topic**

Due to the high prevalence of initial caries the search for new means to reverse the process of demineralization of enamel is still going on. This possibility is provided by preservation of protein matrix of enamel. According to the world literature it is known that amelogenin protein is able to stop and reverse caries formation by stimulating biomimetic remineralization. In addition, it stimulates the construction of the correct crystal lattice of hydroxyapatite from human saliva minerals. On the basis of this lattice there is a process of restoration of enamel prisms. The use of a synthetic analogue of the human protein amelogenin (SAHPA), developed by a Kazakh researcher (B.A. Aituov, 2015), may also be able to lead to the restoration of enamel destroyed by caries by noninvasive and painless application in the form of drops.

However, this technique has no experimental studies on animals and has insufficient clinical data in comparison with other non-invasive methods of prevention and treatment of focal demineralization of teeth enamel and has no morphological substantiation of enamel regeneration processes. In this regard, the study of morphological manifestations of the structural state of enamel when using SAHPA is relevant and will allow a more detailed study of the processes occurring in tooth enamel under the influence of this drug.

**Purpose of the study**

Pathomorphological justification of non-invasive method of prevention and treatment of dental caries in experiment.

**Research objectives**

1) To carry out a complex evaluation of carious process prevalence in children undergoing orthodontic treatment;

2) To carry out the morphological evaluation of the dental enamel condition in laboratory rats before and after the white stain stage caries treatment by non-invasive methods in the experiment;

3) Carry out morphological evaluation of enamel condition of extracted human teeth before and after treatment of caries in the stage of white spot by noninvasive methods

4) To evaluate the effectiveness of non-invasive method of dental caries treatment in the experiment.

**Scientific novelty:**

1. For the first time in the experiment the morphological changes of laboratory white rats teeth enamel were studied when using the preparation containing synthetic analogue of human protein amelogenin (SAHPA) and morphological substantiation of the effectiveness of non-invasive method of treatment was given;

2. Morphological characterization of enamel condition on extracted human teeth before and after treatment of focal demineralization by noninvasive methods and remineralizing potential with the use of the preparation containing synthetic analog of human protein amelogenin has been given for the first time.

 **The main statements made for the defense:**

1. When the enamel of teeth with initial caries in laboratory rats is exposed to the preparation containing synthetic analogue of human protein amelogenin, the formation of organic matrix within 1 month followed by restoration of mineral structure occurs.

2. When a preparation containing SAHPA is used on the enamel of extracted human teeth after acid demineralization, the formation of an organic matrix occurs, which contributes to the restoration of the mineral structure of the enamel.

3. Remineralizing ability of synthetic analogue of human protein amelogenin in demineralization of enamel continues for 3 months after application of the preparation.

 **Practical significance of the work:** the results of the study will substantiate the introduction into clinical dental practice of new data on the possibility of using the preparation containing SAHPA in the initial stages of the carious process and may serve as the basis for the development of measures to prevent and early non-invasive treatment of focal demineralization of teeth enamel.

 **Putting into practice**

 The obtained data have been implemented and are used in educational process of school of stomatology of NC JSC “KMU” on disciplines "Pediatric Stomatology", "Therapeutic stomatology", "Pathological anatomy" and "Clinical pathomorphology". (Acts of implementation in the appendix).

**The results of the study**

The results of the study were presented at the 18th International Dental Congress "18th Debrecen Dental Days" (Debrecen, Hungary, 30 March-1 April 2017); at the 8th International Conference "Social Science and Humanity" (London, UK, 23-29 March 2018); at the 8th International Dental Congress "8th Scientific Congress of Bulgarian Dental Association, Sofia Region Body" (Sofia, Bulgaria, 17-18 November 2018); at the Republican Scientific and Practical Conference with international participation dedicated to the 40th anniversary of the Faculty of Dentistry "Dentistry: yesterday, today, tomorrow" (Karaganda, 22-23 November 2018). ); on Republican scientific-practical conference with international participation, devoted to 40th anniversary of Stomatology Department "Stomatology: yesterday, today, tomorrow" (Karaganda, 22-23rd of November 2018); on Republican scientific-practical conference with international participation "Actual dentistry problems" (Bukhara, Uzbekistan, 29-30 of March 2019); on World Dental Congress "ADA FDI 2019 World Dental Congress" (San Francisco, California, USA, 4-8 of September 2019).

**Publications**

On the materials of the dissertation work 11 scientific works were published, including: 2 certificates on the object of intellectual property, 4 articles in editions recommended by the Committee for Control in the sphere of Education and Science of MES RK; 1 article in the journal "Public Health", indexed in the database WoS Q2; 1 article in the collection of materials of the 8th International Conference "Social Science and Humanity"; 3 theses in the proceedings of international scientific conferences and congresses, including 1 thesis published in the journal "International Dental Journal", indexed in the database WoS Q2, 1 thesis in the journal "Pediatric Dentistry and Prevention".

**Structure and scope of the dissertation**

The thesis is presented on 96 pages of the computer set of the text editor Microsoft Word, consists of the introduction, the main part, the conclusion and the list of the used sources. The dissertation has four appendices. The work contains 25 tables and 40 figures. The list of references contains 135 sources in Russian and English.

 **Materials and methods of study.** Design of the study. Inclusion and exclusion criteria. In a basis of work the epidemiological stomatological examination of children of the city of Karaganda, experimental research of the removed teeth of the person and laboratory animals was put. Selection of the material included in research, was made according to criteria of inclusion and the criteria of an exception presented in the tables 1, 2, 3.

Table 1 - Inclusion and exclusion criteria of epidemiological dental examination of children of the city of Karaganda.

|  |  |
| --- | --- |
| Inclusion criteria | Exclusion criteria |
| * Children of both sexes aged 12 and 15 years without concomitant somatic pathology;
* Children of both sexes aged 12 and 15 years undergoing orthodontic treatment;
* Children whose parents agreed to participate in the study.
 | * Children whose parents refused to participate in the study;
* Children who have chronic illnesses that require inhaled medications;
* Children who have general somatic abnormalities.
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Table 2 - Inclusion and exclusion criteria for the study of laboratory rats

|  |  |
| --- | --- |
| Inclusion criteria | Exclusion criteria |
| * laboratory rats of both sexes;
* laboratory rats by day 20-25 of life;
 | * Lab rats over 25 days old
* Lab rats younger than 20 days old
 |

Table 3 - Inclusion and exclusion criteria for the study of extracted human teeth.

|  |  |
| --- | --- |
| Inclusion criteria | Exclusion criteria |
| * Removed teeth of men and women;
* Removed permanent teeth;
* Intact teeth removed for orthodontic reasons;
* Intact teeth removed for periodontal disease;
 | * Removed teeth of deciduous bite;
* Removed teeth with carious lesions;
* Removed teeth with a filling;
* Removed teeth with enamel hypoplasia;
* Removed teeth with fluorosis;
* Removed teeth with a wedge-shaped defect;
* Extracted teeth with enamel erosion;
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In the first part of the study, 2 groups with 2 subgroups were formed.

Allocation to the groups was carried out in compliance with the homogeneity criteria of the sample, taking into account the inclusion and exclusion criteria.

1. Group 1, (Group 1) consisted of 200 children at the age of 12 in Karaganda;

Group 1, subgroup 1 (Group 1.1) - 12-year-old children without orthodontic treatment;

Group 1, subgroup 2 (Group 1.2) - 12-year-old children undergoing active orthodontic treatment;

Group 1, subgroup 3 (Group 1.3) - 12-year-old children who are in the retention period and finished active orthodontic treatment;

2. Group 2, (Group 2) consisted of 200 children at the age of 15 years in Karaganda;

Group 2, subgroup 1 (Group 2.1) - 15-year old children without orthodontic treatment;

Group 2, subgroup 2 (Group 2.2) - children 15 years old undergoing active orthodontic treatment;

Group 2, subgroup 3 (Group 2.3) - children 15 years old who have completed active orthodontic treatment and are in the retention period.

In the second part of the study, the subject was divided into 4 groups

 (Table 4).

Table 4 - Scheme of separation by groups of extracted teeth of laboratory rats.

OBJECT OF STUDY

teeth of laboratory animals (80 teeth in each group)

Group-II (Main)

2 months after the сariesogenic diet 3 months after SAHPA treatment

Group-IV (Control)

period of 2 months teeth without treatment after cariesogenic diet

Group -III (Control)

2 months after cariesogenic diet and dental ICON treatment

Group -I (Main)

2 months after the cariesogenic diet

1 month after SAHPA treatment

The distribution into groups was carried out in accordance with the homogeneity criteria of the sample, taking into account the inclusion and exclusion criteria.

1. 80 teeth of laboratory rats after 2 months of cariesogenic diet 1 month after treatment with a preparation containing synthetic analog of human protein amelogenin;

2. 80 teeth of laboratory rats after 2 months of a cariogenic diet 3 months after treatment with a preparation containing a synthetic analogue of the human protein amelogenin;

3. 80 teeth of laboratory rats after 2 months of cariogenic diet treated with synthetic resin "ICON" according to the conventional method;

4. 80 teeth of laboratory rats after 2 months of cariesogenic diet without remineralizing or infiltrating therapy;

The subject of the third part of the study was divided into 4 groups, of which the first and third groups were divided into 2 subgroups (Table 5).

Table 5 - Scheme of distribution by groups and subgroups of the study of extracted human teeth

**OBJECT OF STUDY**

**Human teeth removed for orthodontic indications ( 100 teeth in each group)**

Subgroup 1.

(Gr.1.1) teeth

after acid demineralization before treatment with SAHPA

Subgroup 2 (Group 1.2) One month after dental treatment SAHPA

Subgroup 1 (Gr.3.1) teeth after acid demineralization before treatment with SAHPA

Subgroup 2 (Group 3.2) teeth 3 months after treatment with SAHPA

Group-III (Main) observation period 3 months

Group-II (Group 2) (Control) Teeth without SAHPA treatment 1 month after acid demineralization

Group-I (Main)

observation period 1 month

Group-IV (Group 4) (Control) without SAHPA treatment 3 months after acid demineralization

Allocation to groups in the third part of the study was also performed in accordance with the homogeneity criteria of the sample, taking into account the inclusion and exclusion criteria.

1. Group 1, subgroup 1 (Group 1.1) consisted of 100 extracted teeth after acid demineralization before treatment with SAHPA and observation for 1 month;

2. Group 1, subgroup 2 (Group 1.2) - 100 extracted teeth after acid demineralization 1 month after SAHPA treatment;

3. Group 2 (Group 2) - 100 extracted teeth without treatment 1 month after acid demineralization;

4. Group 3, subgroup 1 (Group 3.1) - 100 extracted teeth after acid demineralization before SAHPA treatment and follow-up for 3 months;

5. Group 3, subgroup 2 (Group 3.2) - 100 extracted teeth after acid demineralization 3 months after SAHPA treatment;

6. Group 4 (Group 4) 100 extracted teeth without treatment 3 months after acid demineralization.

 **Ethical approval**

Ethical approval of the study was granted by the Bioethics Committee of Karaganda State Medical University, Minutes № 26 of 20.12.2017. Performance of this scientific work involves work with laboratory animals - white rats. The study is also conducted on extracted teeth of patients with focal demineralization of tooth enamel. Epidemiological dental examination of children was carried out with the written informed consent of their parents.

**Research Methods**

 1. Epidemiological dental examination.

 2. Vital staining with caries marker solution;

 3. laser detection of dental caries using "Diagnodent" laser of "Kavo" company;

 4. Morphological methods: collection of material for histological study, the material was fixed in 10% neutral formalin buffer solution, with further processing according to the well-known procedure in a carousel-type tissue processor according to the standardized protocol, followed by making paraffin blocks and 5-6 micron thick sections, staining with hematoxylin and eosin, histological description of the material obtained;

 5. electron microscopy and spectral analysis.

 6. Statistical analysis: computer software packages for nonparametric statistical methods (SPSS 21).

 **Results of the research.**

The prevalence of initial caries in the chalky stain stage was significantly higher in children of both age groups with fixed orthodontic appliances and was 75.5% (95% CI:71-80) in children 12 years old and 72% (95% CI:68-76) in children 15 years old. The prevalence of caries in the chalky stain stage was significantly lower in children without orthodontic treatment and was 28% (95%DI:25-31) and 23% (95%DI:18-26) in children 12 and 15 years old, respectively. During the retention period after the active stage of orthodontic treatment, the prevalence of dental hard tissue disease increased due to the progression of caries, while the prevalence of periodontal disease decreased. The prevalence of caries in the chalky stain stage in children during the retention period decreased markedly and was 33% (95% CI:29-37) at 12 years and 26.5% (95% CI:22-31) at 15 years. This seems to be explained by the progression of caries from the chalky stain stage to the pigmented stain stage, either with the formation of a carious cavity or by the reversal of the carious process with improvement of oral hygiene and remineralization of initial caries in children during the retention period.

 The results of the second phase of the study revealed that 66 teeth of the laboratory rats (82,50%) of the first group were healthy (Code - 0), and 14 teeth (17,50%) had signs of a carious process within the enamel (Code - 1). The second group had the lowest prevalence of carious process: 78 teeth (97.50%) were healthy (Code - 0) and 2 teeth (2.50%) had signs of carious process inside the enamel (Code - 1). In the third control group, 74 teeth (92.50%) had a healthy condition (Code - 0), which is slightly more than in the first group and comparatively less than in the second group. At the same time, 6 teeth in the third group (7.50%) had caries of the superficial layers of enamel (Code - 1). The fourth group had the highest prevalence of caries process: only 11 teeth (13,75%) were healthy (Code - 0), and 69 teeth (86,25%) had pronounced signs of enamel caries (Code - 1).

 The results of the third phase of the study revealed that 81 teeth (81%) of the first group were healthy (Code - 0) and 19 teeth (19%) had signs of focal demineralization inside enamel (Code -1), while only 36 teeth of the second group (36%) had a healthy state (Code - 0) and 64 teeth (64%) had pronounced focal demineralization of surface enamel layers (Code - 1). The third group had the lowest prevalence of focal demineralization: 93 teeth (93%) were healthy (Code - 0) and 7 teeth (7%) had focal demineralization of enamel (Code - 1). The fourth group had the highest prevalence of demineralization: 32 teeth (32%) were healthy (Code - 0), 68 teeth (68%) had pronounced foci of enamel demineralization (Code - 1).

A comparative assessment of the content of trace elements in human teeth in the groups by spectral analysis revealed significant differences in the state of enamel in the removed human teeth when treated with the preparation containing the synthetic analog of amelogenin protein in comparison with the control groups. The average calcium level after 1 month after SAHPA application (Gr.1) was 6.5%, which was 1.85 times higher than in the group after 1 month without SACHPA treatment (3.5%) (Gr.2) and more than 6 times higher than in the group after 3 months without treatment with SAHPA (1.0%), (Gr.4). Phosphorus levels were also higher in group 1 (7.5%), (Group 1) a mean of more than 5 times that of the second control group (1.5%), (Group 2) and 15 times that of the fourth control group (0.5%), (Group 4). The levels of calcium and phosphorus continued to increase with time and at 3 months after the treatment with SAHPA (Group 3), the average calcium level was 37.5%, which is more than 5 times higher than the average normal calcium level in the group one month after treatment with SAHPA (6.5%), (Group 1). Phosphorus level in the third group was 23.0% (Gr.3), which is more than 3 times higher than in the first group (7.5%) and corresponds to the norm.

The results of comparative evaluation of the picture of electronograms and ultrastructural changes in the removed human teeth showed that there is a partial restoration of enamel in 1 month after treatment with SAHPA preparation, and in 3 months after treatment with this preparation there is a complete restoration of the surface layer of demineralized enamel with the formation of a homogenous structure and enamel prisms, located evenly in parallel rows.

 At the same time, in the control groups without treatment, pronounced foci of demineralization with abnormalities in the surface layers of enamel were observed. The high percentage of healthy enamel in the first and third groups of teeth studied compared to the control groups can be explained by the high remineralizing ability of the synthetic analog of the human protein amelogenin by restoring the organic enamel matrix, which contributes to the incorporation of minerals from the artificial saliva solution.

**Conclusions:**

1. In the key WHO age groups, the prevalence of initial caries in the chalky stain stage during orthodontic treatment at age 12 years was 75.5% (95% CI:71-80); at age 15 years corresponded to 72% (95%DI:68-76).

2. Statistically significant differences were found in the state of dental enamel in laboratory rats 1 month after application of the preparation containing SACHPA (group I) in comparison with the control group (group IV) in the absence of treatment (χ2=75,73; p=0,00000), with healthy rat enamel obtained in 82,50% cases; it was found that more effective enamel restoration occurred 3 months after application of the drug (group II) compared to the control group (χ2=113.66;p=0.0000), which was observed in 97.50% of cases and statistically significantly different from group I (χ2=10.00; p=0.00157);

 3. In human enamel on extracted teeth complete enamel restoration was obtained after 1 month after application of the drug in 81% of cases, which statistically significantly differed from the control group (χ2 =41.70; p=0.00000); after 3 months complete enamel restoration was observed in 93% of cases, which also statistically significantly differed from the control group (χ2 =79.38; p=0.00000);

 4. In spectral analysis, there are significant differences in the content of calcium and phosphorus in the enamel of extracted human teeth when treated with SAHPA-containing preparation compared to the control groups. The average calcium level at 1 month after SAHPA application (Tr.1) was 6.5%, which was 1.85 times higher than in the group at 1 month without SAHPA treatment (3.5%) (Tr.2) and more than 6 times higher than in the group after 3 months without SAHPA treatment (1.0%) (Tr.4). Phosphorus levels were also higher in group 1 (7.5%) (Group 1), on average more than 5 times higher than in group 2 (1.5%) (Group 2) and 15 times higher than in group 4 (0.5%) (Group 4). The levels of calcium and phosphorus continued to increase and at 3 months after the treatment with SAHPA (Group 3), the calcium level was 37.5%, which was 5 times higher than the calcium level in the group one month after treatment with SAHPA (6.5%) (Group 1). The level of phosphorus in group 3 was 23,0% (Gr.3), which is more than 3 times higher than in group 1 (7,5%).