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ORIGINAL ARTICLES

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A STUDY OF THE INFLUENCE OF JUVENILE ADJUVANT ARTHRITIS ON DENTAL HARD TISSUES CONDITION IN EXPERIMENTAL ANIMALS

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ABSTRACT

The aim: To study the intensity and depth of carious tooth lesions in rats with experimental juvenile adjuvant-induced arthritis.

Materials and methods: An experimental study on a model of juvenile adjuvant arthritis (JAA) in 10 one-month-old rats induced by method of A.M. Bendele was carried out. 10 rats of the same age were intact. Injection of adjutant in rats of experimental group led to the development of acute local reaction and then caused generalized joint reaction of autoimmune origin. The performed basic therapy of JAA promoted transition of acute autoimmune process to chronic. Rats were withdrawn from the experiment in 58 days and the dental-jaw blocks were made, in which the intensity and depth of carious lesions of the masticatory group of teeth were determined.

Results: The course of JAA was accompanied by the development of dental caries in 100% of experimental animals. It was found that the intensity of carious teeth lesions in terms of the number of carious teeth and cavities is probably higher than in intact rats (respectively 4.3 ± 0.3 vs. 2.2 ± 0.6 and 4.5 ± 0.3 vs. 2.3 ± 0.7, $p < 0.001$). In rats with JAA, mostly middle and deep carious cavities were revealed, at the same time in intact rats – superficial and middle carious cavities were observed.

Conclusions: It has been established that adjuvant arthritis is accompanied by 100% prevalence of dental caries, high intensity of carious process, presence of middle and deep carious cavities, that confirm the negative influence of autoimmune disease on the condition of the hard tooth tissues.

KEY WORDS: dental caries, experimental study, rats, juvenile adjuvant arthritis

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INTRODUCTION

Despite particular achievements in medicine, adequate treatment of dental caries in children with somatic pathology is important in pediatric dentistry [1, 2]. It is well known that the growth and development of all systems of the child's body, including the dentognathic system, occurs under the influence of many factors of internal and external environment. There is a high susceptibility of teeth to cariogenic factors in children in the presence of autoimmune diseases, including juvenile rheumatoid arthritis (JRA) [3]. Increasing of prevalence and activity of dental caries, the development of multiple foci of enamel demineralization in children with JRA have been clinically proven [4]. These changes, according to the authors [5], occur due to the complex pathogenesis of JRA, metabolic disorders, in particular phosphorus-calcium metabolism, owing to the use of hormonal drugs of corticosteroid class. However, the obtained results by scientists about development of dental caries in this group of sick children were not confirmed in experimental studies.

Currently, there are various models of experimental rheumatoid arthritis, but the closest to human autoimmune disease are the models of adjuvant arthritis for adults [6] and juvenile adjuvant arthritis (JAA) for children [7]. Therefore, for a deep understanding of the development of carious tooth lesions in children with JRA, there is the need to study these issues in experimental animal studies.

THE AIM

The aim of this work was to study the intensity and depth of carious tooth lesions in rats when modelling experimental juvenile adjuvant arthritis.

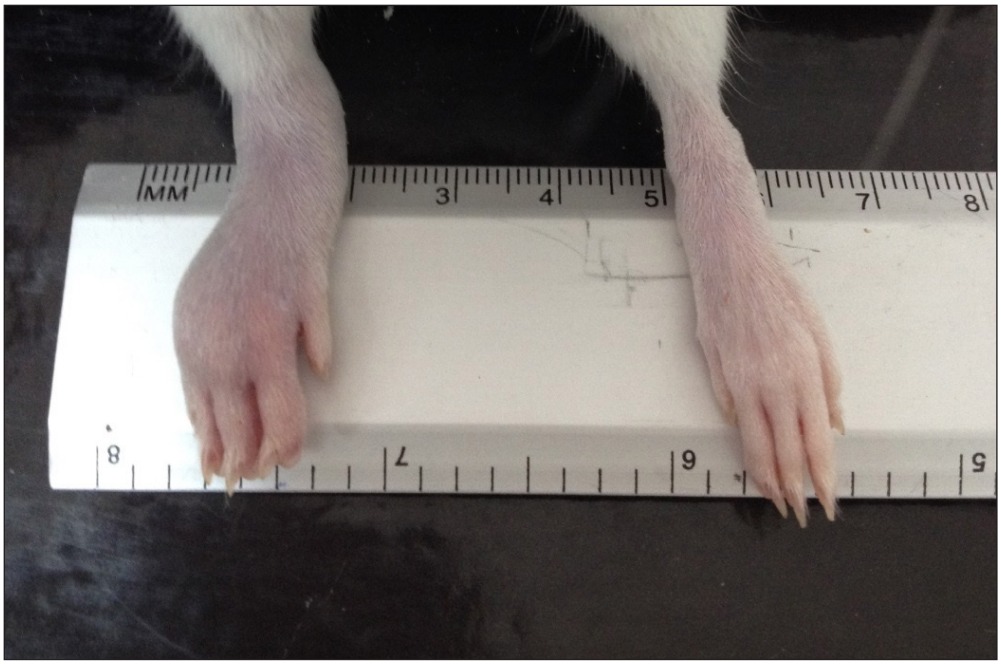

Fig. 1. Picture of a rat number 3 (group II), 3rd day of experimental research. Inflammatory reaction of rat's right hind paw after injection of Freund's adjuvant: limb enlargement, edema of the ventral part of the paw, skin redness of the damaged hindlimb

Fig. 2. Picture of rat number 4 (group II), peak of the inflammatory process (14th day of the experimental study): generalized reaction and progression of JAA, the spread of the inflammatory reaction to the intact left rat's paw, skin redness and swelling in the area of the joints of both extremities

MATERIALS AND METHODS

To achieve this goal, 20 white laboratory rats of both sexes of 1-month-mature with an average weight of 49.0 ± 0.916 g were used. Two groups of rats with 10 animals in each were formed: the control (group I) and the experimental (group II). All rats of the control (intact) and experimental (main) groups were kept in the standard conditions of the vivarium: air temperature, humidity, diet and water intake.

In rats of group II the JAA was induced by the method of A.M. Bendele [8]: 0.1 ml of Freund's adjuvant was injected once subcutaneously in rat's right hind paw (subplantarly). Composition of Freund's adjuvant is antigen inactivated and dried mycobacteria (Bacillus Calmette-Guérin, BCG) in oil emulsion. According to the author [8], this agent is the most adequate for reproducing JAA and extrapolating the results to humans.

The experiment lasted 58 days with daily visual examination and assessment of the clinical condition of rats upon reaching the peak of the inflammatory process (14th day), the formation of chronic immune inflammation (28th day) and the end of the experiment

Fig. 3. Dental-jaw blocks of the upper and lower jaws of rat number 7 (group II), 58th day of experiment, Dental-Missing-Filled-Teeth (DMFT) scores = 9

(58th day). The appearance of animals, changes in behavior, reaction to external stimuli, the condition of the joints of rats, motor activity were evaluated. Local (swelling in the right hindlimb) and generalized reaction (swelling in the left hindlimb) to Freund's adjuvant were observed. Hematological parameters were used as criteria of the development of chronic autoimmune inflammation, which were determined on the 28th day of the experimental study. Treatment of JAA in rats of the experimental group was performed for 30 days (from 14th day of the experiment) according to the protocols of basic therapy of autoimmune disease by administering drugs intragastrically in doses according to the recommendations of Yu. R. Rybolovlev and R. S. Rybolovlev [1979]. Basic therapy included the use of disease-modifying antirheumatic drugs, glucocorticoids, nonsteroidal anti-inflammatory and antirheumatic drugs, calcium supplements, vitamins in accordance with the Order of the Ministry of Health of Ukraine dated April 11, 2014 No. 263 "Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care and medical rehabilitation". In 58 days, all animals were withdrawn from the experiment by total bloodletting under propofol anesthesia (60 mg / kg).

To compare the condition of the hard tooth tissues of intact and experimental animals, dental-jaw blocks were made, in which the intensity and depth of carious lesions of the masticatory group of teeth were determined using a binocular magnifying glass. The intensity of the carious process was studied by counting the number of carious teeth and cavities, on average, per rat. The depth of carious lesion was determined in scores: superficial – carious cavity within the enamel (1 score), middle – carious cavity within the upper layers of dentin (1.5 scores), deep – a large carious cavity reaching the pulp chamber (2 scores).

Experimental studies were carried out in accordance with the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Declaration of Helsinki (2000), according to the policies of general ethical principles of experiments on animals (Kyiv, 2001). The study protocol was approved by the Biomedical Ethics Committee of National Pirogov Memorial Medical University, Vinnytsya. Ethics and morality were not violated during the study. Rats were housed in the vivarium room with a constant temperature, humidity and 12-hour dark-light cycle.

Statistical analysis of the study results was carried out using computer programs Microsoft® Excel 2017 for Mac (corporate license, Product ID: 02984-001-000001; Device Code: 86C36D0C-8F15-59CA-A81E-B1D889205F71) and the licensed package "Statistica 6.1" (serial number BXXR901E246022FA). Statistical data processing was performed by methods of variation statistics with the calculation of average arithmetic values and errors (M ± m), standard deviation (t) and the significance of differences (p-value, the differences were considered statistically significant at $p < 0.05$).

RESULTS

At the beginning of experimental study, rats of both groups were active, the motor activity of the joints were not limited, the general state were not disturbed. In 1-3 days after injection of adjuvant subplantarly in animals of the experimental group the increased aggression, irritability, alertness, increased frequency and depth of respiration and also a significant decrease in motor activity of the left limb and local inflammatory reaction of the injured paw (increased its volume, edema, skin hyperemia, pain sensitivity of the joint) were revealed (Fig. 1).

Table I. WBC count in rats with JAA on the 28th day of the experiment

Types of WBCs	Groups of experimental rats		Types of WBCs	Groups of experimental rats	
	Group I, n = 10	Group II, n = 10		Group I, n = 10	Group II, n = 10
WBCs x 10^9	11.3 ± 0.47	14.27 ± 0.57	Eosinophils, %	1.7 ± 0.30	2.28 ± 0.36
	p <0.001			p >0.05	
Banded neutrophils, %	0.8 ± 0.29	2.4 ± 0.27	Lymphocytes, %	75.3 ± 2.18	63.8 ± 2.23
	p <0.001			p <0.001	
Segmented neutrophils, %	16.6 ± 2.08	25.18 ± 1.94	Monocytes, %	5.60 ± 1.13	5.63 ± 0.59
	p <0.01			p >0.05	
Basophils, %	0.48 ± 0.13	-	Plasma cells,%	0.1 ± 0.05	-

Note: p – the significance of the difference between types of WBCs of the control group and the group of rats with adjuvant arthritis.

Table II. Indicators of caries intensity in experimental rats

Groups of experimental animals	Number of carious teeth	Number of carious cavities
The control group, n = 10	2.2 ± 0.61	2.3 ± 0.65
Experimental group, n=10	4.3 ± 0.30	4.5 ± 0.31
p	p <0.001	p <0.001

Note: p – the significance of the difference between indicators of caries intensity in the control and experimental groups of rats

Table III. Indicators of the depth of tooth carious lesions in experimental animals (in scores)

Groups of experimental animals	Superficial caries	Middle caries	Deep caries
The control group, n = 10	2.1 ± 0.61	0.3 ± 0.20	0
Experimental group, n=10	3.0 ± 0.26	2.1 ± 0.25	0.2 ± 0.20
p	p >0.05	p <0.001	p >0.05

Note: p – the significance of the difference between indicators of the depth in the control and experimental groups of rats

On the 14th day, the progression of JAA and the development of generalized reaction with lesions of both extremities (Fig. 2) were observed: edema, redness and pain in the joints of the intact limb, increased inflammation of the injured rat's paw, ulcers on the skin of its paw, phalanges and knee joint. The peak of the inflammatory process on this day of observation was accompanied by a significant decrease in the overall activity of animals, their mobility and a decrease in food and water intake.

General therapy of adjuvant arthritis from the 14th day of the experiment contributed to the gradual reduction of acute inflammatory phenomena in the joints, which was manifested in the reduction of edema and pain when flexing the inflamed joints. There was a certain tendency to normalize motor activity and emotional state of animals. Treatment of JAA in rats from the 14th to the 28th day of the experiment helped to increase general and motor activity, reduce the severity of the inflammatory process in the hindlimbs: reduce redness, swelling, pain when flexing the joints. There was a certain tendency to normalize motor activity of animals, but was not the full disappearance of inflammatory phenomena.

Due to the frequent transition of acute autoimmune inflammation to chronic, the study of the most obvious indicators of homeostasis – white blood cell (WBC) count in experimental animals were evaluated, the results of which are shown in Table I.

When analyzing the results, it was found that the average statistical parameters of WBC count in intact rats were corresponded to normal values, while in the experimental group – to the changes, that characterized chronic autoimmune process. This was indicated by a significant increase in WBCs, the percentage of banded and segmented neutrophils with a moderate shift to the left and a similar decrease of lymphocytes with a statistically significant difference compare to the control group of rats (p <0.05), as well as a significant increase of eosinophils and the appearance of basophils in the peripheral blood.

Observation of experimental group animals before the end of the basic therapy of adjuvant arthritis (58th day) revealed some improvement in the general condition and local inflammatory reaction in rats, but not their complete normalization, which confirmed the development of chronic autoimmune process, despite the performed treatment course. It should be noted that the death of rats both the control group and with simulated JAA throughout the experiment was not observed.

At the next stage of the experimental study, when comparing the condition of the hard tooth tissues of in-

tact and experimental animals, the carious lesions were revealed in 60% of dental-jaw blocks of rats in group I and in 100% – in group II.

When analyzing the nature of the carious lesion of the teeth (Table II) a low level of caries intensity and almost the same average statistical indicators of the number of carious teeth and cavities in rats of the control group were revealed. At the same time, in animals with adjuvant arthritis, these values were higher in twice then in the control group with a high level of significance of the difference in values ($p < 0.001$). We give an example of a high level of intensity of tooth damage by the carious process in rat number 7 on the 58th day of the experiment after subplantar injection of Freud's adjuvant (Fig. 3).

To assess the dental caries' findings of lesions in experimental animals, it is important to determine the depth of the carious process, the results of which are shown in Table III.

It can be seen from the data given above that in rats with JAA superficial caries was diagnosed by 1.5 times more often than in the control group ($p > 0.05$), and middle caries by 7 times ($p < 0.001$). Deepening of the carious process in rats with adjuvant arthritis was indicated by revealed carious cavities reaching the pulp chamber, while in intact animals there were no deep carious lesions of the teeth.

DISCUSSION

Taking into account the influence of JRA on the clinical course of dental carious lesions, established by the authors in single works [9], the study of this issue was carried out on an experimental model of the disease described by A.M. Bendele [8]. The model, induced by Freund's adjutant according to scientists [10] is the closest to JRA in children.

Inducing of the experimental model of JAA in rats by a single subplantar injection of adjutant into the right hind paw contributed to the development of autoimmune disease: acute local and subsequent generalized joint inflammation with a peak of inflammatory autoimmune process on the 14th day, which confirmed the adequacy of experimental JRA model [11]. The performed basic therapy of JAA in rats did not lead to the elimination of pathological immune inflammation, on the contrary, contributed to the transition from acute to chronic autoimmune process, that agrees with the data literature about clinical manifestations of JRA in childhood [12]. This was evidenced by a significant decrease of protective factors level in the blood of experimental animals: neutrophilic leukocytosis with a moderate shift to the left, lymphocytopenia, a tendency to increase eosinophils and the presence of basophils in the WBC count.

Experimental confirmations of the negative influence of autoimmune disease on the course of dental caries in children, have been described in clinical studies of the authors [13], were: 100% damage of the teeth by carious process of animals with JAA; significantly higher indicators of the number of affected teeth and their cavities compared to intact rats ($p < 0.001$); detection of carious cavities mainly of medium depth and the presence of single deep cavities in experimental animals in contrast to control rats, in which only superficial and sometimes middle caries were diagnosed.

CONCLUSIONS

Thus, a single injection of Freund's adjuvant subcutaneously in right hind paw of experimental rats leads to the manifestation of acute autoimmune inflammation of the joints (juvenile adjuvant arthritis) and the development of local and generalized reaction of animals' organism.

In the process of basic treatment of induced JAA the changes in peripheral blood count (neutrophilic leukocytosis with a shift to the left, the decrease in the percentage of lymphocytes and increase in eosinophils and the presence of basophils), that confirm the transition from acute to chronic autoimmune inflammatory process in experimental animals.

In rats with experimental JAA model, 100% of carious tooth lesions were revealed. Peculiarities of carious tooth lesions in rats with JAA were: significantly higher indicators of caries intensity (number of affected carious teeth and cavities) than in intact animals ($p < 0.001$); the increase in the number of carious cavities of medium depth ($p < 0.001$) and the appearance of deep caries cavities. Thus, in intact rats mainly superficial and middle carious lesions were revealed, but in animals with JAA – middle and deep carious lesions. The negative influence of chronic adjuvant arthritis in experimental animals on the condition of dental hard tissues was confirmed.

The obtained results of the study allow to recommend the use of JAA model in experimental rats for testing of prevention methods of carious tooth lesions in children with JRA.

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The Authors declare no conflict of interest.

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