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MORPHOMETRIC CHANGES OF ODONTOGENESIS CAUSED BY LOW-DOSE IONIZING RADIATION IN THE EXPERIMENT

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Morphometric methods are increasingly being used to evaluate clinical, pathological and experimental data. A systemic morphometric analysis of the main pathological processes and the most common diseases has been developed. However, these data regarding dental pathology are missing.

The aim of the study. *A morphometric investigation of the effects of low doses of ionizing radiation on odontogenesis in rats.*

Objects and methods of the study. *The experiments were carried out on albino rats of mongrel gregarious breeding. The irradiation of the rats and their offspring was carried out at the Institute of Radiobiology of the National Academy of Sciences of Belarus on the Gammarid-192/120 installation with an exposure dose rate of 110 mR/h since the 1st day of pregnancy before sampling on the 16th, 18th and the 20th day. The irradiation of the offspring was continued on the 1st and 3rd days after birth. The absorbed dose in all groups did not exceed 0,5 Gy. Histological, histochemical and morphometric methods of investigations were applied.*

Results of the study and their discussion. *Low doses of ionizing radiation at the early stages of odontogenesis (the 16th and 18th days of antenatal life) caused the greatest decrease in almost all morphometric indexes of tooth germs in experimental animals. Morphometric indexes in the control animals significantly exceeded those in the experimental animals. By the 20th day, almost a complete repair of these changes had occurred. However, in 3-day-old rats some differences were still present, for example in the odontoblast layer thickness in the control animals.*

Conclusion. *Low doses of ionizing radiation during all periods of odontogenesis affected the structure and development of tooth germs, although they did not disrupt the genetically programmed course of odontogenesis. In our opinion, it can be considered as one of the manifestations of the non-threshold effect of ionizing radiation.*

Key words: *morphometry, odontogenesis, ionizing radiation, low doses.*

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МОРФОМЕТРИЧЕСКИЕ ИЗМЕНЕНИЯ ОДОНТОГЕНЕЗА ПОД ДЕЙСТВИЕМ МАЛЫХ ДОЗ ИОНИЗИРУЮЩЕЙ РАДИАЦИИ В ЭКСПЕРИМЕНТЕ

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Морфометрические методы все чаще применяются для оценки клинических, патологоанатомических и экспериментальных данных. Разработан системный морфометрический анализ основных патологических процессов и наиболее распространенных заболеваний. Однако эти данные в отношении патологии зубов отсутствуют.

Цель исследования. Морфометрическое изучение воздействия малых доз ионизирующей радиации на одонтогенез у крыс.

Объекты и методы исследования. Исследования проводили на белых крысах стадного разведения. Облучение крыс, а затем и приплода проводили в Институте радиобиологии НАН РБ на установке «Гаммарид-192/120» с мощностью экспозиционной дозы 110 мР/ч с 1-х суток беременности до забора материала на 16, 18-е и 20-е сутки. Облучение приплода продолжали в 1-е и 3-и сутки после рождения. Поглощенная доза во всех группах не превышала 0,5 гр. Применялись гистологические, гистохимические и морфометрические методы исследования.

Результаты исследования и обсуждение. *Малые дозы ионизирующей радиации на ранних этапах одонтогенеза (16-е и 18-е сутки антенатальной жизни) вызывали наибольшее снижение величины почти всех морфометрических показателей зачатков зубов у подопытных животных. Морфометрические показатели у контрольных животных значительно превышали таковые у подопытных. К 20-м суткам*

наступала почти полная репарация этих изменений. Однако и у 3-суточных крысят имелись некоторые отличия от контрольных животных, например, в толщине слоя одонтобластов.

Заключение. *Малые дозы ионизирующей радиации во все изученные нами сроки одонтогенеза оказывали влияние на структуру и развитие зачатков зубов, хотя и не нарушали генетически запрограммированного хода одонтогенеза. По нашему мнению, это можно рассматривать как одно из проявлений беспорогового действия ионизирующей радиации.*

Ключевые слова: *морфометрия, одонтогенез, ионизирующая радиация, малые дозы.*

Morphometric methods are increasingly being used to evaluate clinical, pathological and experimental data. A systemic morphometric analysis of the main pathological processes and the most common diseases has been developed. However, these data regarding dental pathology are missing [1]. We have found the only research [6], the authors of which studied the size of odonto- and enameloblasts, daily deposition of dentine and enamel in the first molar in rats in the postnatal period.

The aim of the study

A morphometric investigation of the effects of low doses of ionizing radiation on odontogenesis in rats.

Objects and methods of the study

The experiments were carried out on albino rats of mongrel gregarious breeding. These animals were chosen because the development and structure of their molars are largely similar to those of humans [7, 8]. There were 11 pregnant females in the control group and 16 in the experimental group. The irradiation of the rats and their offspring was carried out at the Institute of Radiobiology of the National Academy of Sciences of Belarus on the Gammarid-192/120 installation with an exposure dose rate of 110 mR/h since the 1st day of pregnancy before sampling on the 16th, 18th (3 experiments each) and the 20th day (4 experiments). The irradiation of the offspring was continued on the 1st and 3rd days after birth (3 experiments each). The absorbed dose in all groups did not exceed 0,5 Gy.

The material was fixed in a 10 % solution of neutral formalin. The heads of the newborn rats were additionally decalcified in a 5 % solution of nitric acid.

The objects were embedded in paraffin according to generally accepted methods. Sagittal “serial-selective” sections [5] were stained with haematoxylin and eosin, Van Gieson’s picrofuchsin; nucleic acids were detected with gallocyanin using Einarson’s method; elastic fibers were detected with Hart’s stain, argyrophilic fibers were detected according to Bilshovsky method modified by G. A. Berlov [2]. The degree of teeth calcification for 1- and 3-day-old rats was detected using Alizarin red S according to O. N. Koroleva’s recommendation [4]. The first three methods were used in morphometry; and for light-optical study, all the above-listed methods were used.

In 16-, 18-, and 20-day-old rats, contouring measurement of the area of the enamel organ pulp (1), the enamel organ (2), dental papilla (3), and the thickness of the outer (4) and inner enamel epithelium (5), as well as dental papilla (6) was performed using the «Bioscan-AT» image analysis system.

In 1- and 3-day-old rats, the thickness of odontoblast (7), dentine (8) and enameloblast layers (9) was measured. The above structures were measured at least ten times in each case of the control and the experiment.

A total of 2206 histological specimens were studied and 3623 measurements were performed. The data were processed by the method of variation statistics using Student’s t-test.

Results of the study and their discussion

The area of the enamel organ pulp was greater in the control animals than in the experimental animals (table 1). The 16-day-old fetuses showed the greatest difference: 1,35 ± 0,20* and 0,66 ± 0,08 μm^2 ($P < 0,01$), a 2,05-fold increase. The area of this structure had increased by the 20th day of the antenatal period both in the control and in the experiment. Compared to that of 18-day-old fetuses, by this time it had increased by 5,01 and 5,08 times.

The area of the enamel organ in the control animals was greater than in the experimental animals. The greatest differences were revealed in 16-day-old fetuses. In the control, the area was 2,79 times greater than that in the experiment: 4,02 ± 0,66 and 1,44 ± 0,10 μm^2 ($P < 0,001$). This area had increased by 3,17 and 3,43 times in 20-day-old fetuses compared to 18-day-old ones.

Note: * all the values of area in the text and in Table 1 should be multiplied by 10^4

Примечание: * все показатели площади в тексте и табл. 1 следует умножить на 10^4

The area of the dental papilla at the 16th and 18th days was greater in the control group fetuses and at the 20th day, in the experimental group. The greatest differences – 1.91 times – between the control and the experiment were observed in 16-day-old fetuses. From the 16th to the 18th days, the growth of dental papilla area was somewhat more intense than from the 18th to the 20th day of the antenatal period. By the 18th day compared to the 16th day, the increase was 2,14 and 2,92 times in the control and the experiment, respectively (table 1).

Table 1. **Changes in the size of dental germ structures in rats' fetus**

Таблица 1. **Изменение величин структур зачатков зубов плодов крыс**

Index	16 days		18 days		20 days	
	Control	Experiment	Control	Experiment	Control	Experiment
Area of the enamel organ pulp, μm²	1,35 ± 0,20 *n* = 23 *v* = 71,85	0,66 ± 0,08* *n* = 34 *v* = 72,73	1,54 ± 0,25 *n* = 22 *v* = 74,68	1,37 ± 0,14 *n* = 30 *v* = 54,01	7,72 ± 0,38 *n* = 20 *v* = 21,50	6,92 ± 0,40 *n* = 31 *v* = 39,31
Area of the enamel organ, μm²	4,02 ± 0,66 *n* = 24 *v* = 80,85	1,44 ± 0,10* *n* = 33 *v* = 39,58	5,06 ± 0,51 *n* = 22 *v* = 45,85	3,85 ± 0,46 *n* = 30 *v* = 65,45	16,05 ± 0,70 *n* = 20 *v* = 19,13	13,20 ± 0,62* *n* = 31 *v* = 25,98
Area of the dental papilla, μm²	3,90 ± 0,61 *n* = 22 *v* = 74,10	2,04 ± 0,13* *n* = 33 *v* = 37,75	8,35 ± 0,76 *n* = 22 *v* = 41,80	5,96 ± 0,75* *n* = 30 *v* = 69,13	13,21 ± 1,39 *n* = 20 *v* = 45,80	15,18 ± 1,23 *n* = 31 *v* = 45,26
Thickness of the outer enamel epithelium, μm	46,44 ± 4,80 *n* = 58 *v* = 78,77	25,26 ± 1,56* *n* = 102 *v* = 62,43	38,19 ± 2,25 *n* = 62 *v* = 46,37	19,30 ± 1,62* *n* = 92 *v* = 80,73	31,69 ± 2,04 *n* = 70 *v* = 53,96	34,07 ± 1,61 *n* = 140 *v* = 56,06
Thickness of the inner enamel epithelium, μm	46,17 ± 3,84 *n* = 64 *v* = 66,47	24,99 ± 1,51* *n* = 108 *v* = 62,79	46,76 ± 3,70 *n* = 68 *v* = 65,29	27,71 ± 1,85* *n* = 110 *v* = 70,05	39,75 ± 2,19 *n* = 87 *v* = 51,30	38,32 ± 1,70 *n* = 161 *v* = 56,26
Thickness of the dental papilla, μm	50,24 ± 2,97 *n* = 108 *v* = 61,43	47,32 ± 2,00 *n* = 202 *v* = 60,02	83,91 ± 4,23 *n* = 129 *v* = 57,22	63,39 ± 3,64* *n* = 190 *v* = 79,24	170,26 ± 11,86 *n* = 105 *v* = 71,38	187,4 ± 8,99 *n* = 190 *v* = 66,13

Note: in Table 1 and Table 2, the reliability of differences compared to the control group is marked with *, $P < 0,05–0,001$; *n* – the number of measurements, *v* – the coefficient of variation

П р и м е ч а н и е: здесь и в табл. 2 (звездочкой) * обозначена достоверность различий по сравнению с контрольной группой, $P < 0,05–0,001$; *n* – количество измерений; *v* – коэффициент вариации.

At all times, the area sizes were greater in the control animals, with the exception of the area of the dental papilla in 20-day-old experimental fetuses. The maximum differences in the control and the experiment occurred in the early odontogenesis period, i. e. the 16th day of intrauterine life.

On the 16th and 18th days of the antenatal period, the thickness of the outer enamel epithelium in the control animals exceeded it in the experimental animals by 1,84 and 1,98 times, respectively ($P < 0,001$). In 20-day-old fetuses, this index was 1.08 times higher in the experiment ($P > 0,05$). The thickness of the outer enamel epithelium in the control decreased uniformly by 1,22 and 1,21 times with increasing age of the animals. In the experiment, in 18-day-old fetuses compared to 16-day-old ones, it decreased by 1,31 times. In 20-day-old fetuses, on the contrary, an increase in thickness by 1,77 times was noted compared to 18-day-old experimental fetuses (table 1).

In the fetuses of all three age groups, the thickness of the inner enamel epithelium in the control exceeded that one in the experiment (table 1). The greatest difference in thickness (by 1,85 and 1,69 times) was in 18- and 16-day-old fetuses: 46,17 ± 3,84 and 24,99 ± 1,51 μm, 46,76 ± 3,70 and 27,71 ± 1,85 μm ($P < 0,001$). The thickness of the inner enamel epithelium in the control decreased by 1,18 times in 20-day-old fetuses compared to 18-day-old ones. In the experimental animals, on the contrary, from the 16th to the 18th, and then to the 20th day of the antenatal period, the increase in thickness by 1,11 and 1,38 times respectively was observed.

The thickness of the dental papilla in the control animals was greater than in the experimental animals, with the exception of 20-day-old fetuses (table 1). The greatest differences in thickness occurred on the 18th day: dental papilla in the control animals' fetuses was 1,32 times as thick as the dental papilla in the experimental animals ($P < 0,001$). Intensive growth of the dental papilla thickness occurred from the 18th to the 20th day of the antenatal period. The data for 20-day-old fetuses exceeded similar findings for 18-day-old fetuses in the control and the experiment by 2,03 and 2,96 times.

The thickness of the main structures of tooth germs in the control animals exceeded those in the experimental animals, except the thickness of the outer enamel epithelium and the dental papilla thickness in 20-day-old fetuses.

The thickness of the odontoblast layer in 1- and 3-day-old rats of the control group exceeded the similar finding in the experimental rats by 1,61 and 1,2 times (table 2). It increased in 3-day-old animals in the control by 1,52 times, and in the experiment by 2,03 times.

The dentine thickness in the control animals was greater than in the experimental animals: 14,11 ± 1,43 and 11,00 ± 1,05 μm respectively, and 25,00 ± 1,79 and 21,95 ± 1,35 μm ($P > 0,05$). The thickness of both dentine and odontoblasts increased by 1,77 times

Table 2. **Changes in the thickness of rats' tooth germs structures after birth**

Таблица 2. **Изменение толщины структур зубных зачатков крыс после рождения**

Index	1st day		3rd day	
	Control	Experiment	Control	Experiment
Odontoblasts layer thickness, μm	34,61 ± 2,94 *n* = 71 *v* = 71,68	21,50 ± 1,27* *n* = 37 *v* = 35,86	52,54 ± 2,60 *n* = 65 *v* = 39,97	43,68 ± 2,31* *n* = 86 *v* = 48,99
Dentine layer thickness, μm	14,11 ± 1,43 *n* = 111 *v* = 107,02	11,00 ± 1,05 *n* = 49 *v* = 66,64	25,00 ± 1,79 *n* = 107 *v* = 74,20	21,95 ± 1,35 *n* = 131 *v* = 70,52
Enameloblasts layer thickness, μm	25,55 ± 1,44 *n* = 125 *v* = 62,94	33,00 ± 2,81* *n* = 53 *v* = 62,0	20,96 ± 0,77 *n* = 106 *v* = 37,69	20,70 ± 0,81 *n* = 158 *v* = 49,08

in the control and 2 times in the experiment by the 3rd day compared to that on the 1st day of the postnatal period.

The thickness of the enameloblast layer in experimental 1-day-old rats turned out to be 1,29 times greater than in the control rats: 33,00 ± 2,81 and 25,55 ± 1,44 μm, respectively ($P < 0,05$). In 3-day-old animals, on the contrary, the findings in the control animals slightly exceeded those in the experiment: 20,96 ± 0,77 and 20,70 ± 0,81 μm ($P > 0,05$).

The thickness of the enameloblast layer decreased from 25,55 ± 1,44 μm in 1-day-old rats to 20,96 ± 0,77 μm in 3-day-old rats in the control and from 33,00 ± 2,81 to 20,70 ± 0,81 μm in the experiment: the decrease by 1,22 and 1,59 times.

So, the thickness of the structures of tooth germs after birth in the control animals was higher than similar indexes in the experimental animals, with the exception of the thickness of the enameloblast layer in 1-day-old rats.

The thickness of the odontoblast layer and the thickness of dentine increased with the age of the animals, and the thickness of the enameloblast layer decreased both in the control and in the experiment.

Changes in area and thickness (their increase or decrease) were more intense in the irradiated animals.

Low doses of ionizing radiation during all periods of odontogenesis affected the structure and development of tooth germs, although they did not disrupt the genetically programmed course of odontogenesis. The greatest differences between the control and the experiment were noted in 16- and 18-day-old rat fetuses. The indexes in the control animals were significantly higher than those in the experimental animals. By the 20th day of the antenatal period, the differences in the size of the structures of the tooth germs in the experimental group compared to the control group were, as a rule, unreliable. In the control animals, by this time the area of the pulp, the enamel organ, and the thickness of the inner enamel epithelium had become greater; the area and thickness of the dental papilla and the outer enamel epithelium, the dental papilla turned out to be lesser. In the postnatal period in 1- and 3-day-old rats, the thickness of the odontoblasts and dentine layers were greater in the control animals. By the 1st day, the thickness of the enameloblast layer turned out to be significantly greater in the experimental rats, and by the 3rd day there were practically no differences in the enameloblast layer thickness. It can be considered as one of the manifestations of the conditionally non-threshold effect of ionizing radiation [3], since ionizing radiation even in low doses demonstrated its negative effect on tooth germs.

Conclusion

Thus, low doses of ionizing radiation at the early stages of odontogenesis (the 16th and 18th days of antenatal life) caused the greatest decrease of almost all morphometric parameters of tooth germs in the experimental animals. By the 20th day, almost a complete repair of these changes had occurred. However, in 3-day-old rats some differences were still present, for example in the odontoblast layer thickness in the control animals. These deviations in odontogenesis can play a negative role in the further formation and morphofunctional state of the teeth in adult animals.

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