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The effects of experimentally irradiated pituitary gland on the growth of rats' tibia, skull, maxilla and mandible

Uticaj eksperimentalno zračene hipofize na rast potkolenice, lobanje, gornje i donje vilice pacova

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Abstract

Background/Aim. The reaction of an organism to radiation depends on the level of irradiation and the sensitivity of the affected tissue cells. The biological effects on the cells and tissues are proportional to the absorbed radiation energy. The aim of our study was to examine the effects of hypofunction of the pituitary gland, previously irradiated with x-rays, on the growth of rat's craniofacial and stomatognathic system as well as rat's tibia. **Methods.** A total dose of x-rays of 27.92 Gy was applied in eight sessions in the period from 8 to 63 days of animal age on their heads and the effects of irradiation-induced hypofunction of the pituitary gland on the growth of rat's craniofacial and stomatognathic system and tibia were examined. In order to differentiate the effects of the irradiated pituitary gland from the direct effects of x-rays on the head, the experiment was set as a double study. One group of animals had the pituitary gland protected with a lead plate set beneath the projection of the gland, while the second group was irradiated with the same dose, but without the protection. The control group of animals were non-irradiated. **Results.** Growth measurements of numerous parameters of the craniofacial and stomatognathic system as well as tibia in rats with the entire locally irradiated head showed a statistically significant delay, compared to both the non-irradiated control group and the group with the protected pituitary gland. **Conclusion.** The damage of the stomatognathic and craniofacial system was greater in animals with irradiated head without pituitary gland protection compared to those with the gland protection whereby the growth of the craniofacial system was most affected. Irraduition-induced pituitary gland hypofunction and irradiation itself showed a stronger effect on the lower jaw growth rate impairment compared to the upper jaw. Similarly, the delay in tibia growth was more pronounced in animals without pituitary gland protection during irradiation of their heads compared to non-protected ones.

Key words:
pituitary gland; x-rays; rats; growth; tibia; skull; maxilla, mandible.

Apstrakt

Uvod/Cilj. Reakcija organizma na zračenje zavisi od doze zračenja i osetljivosti ćelija tretiranog tkiva. Biološki efekti zračenja na ćelije i tkiva proporcionalni su apsorbovanoj radijacionoj energiji. Cilj istraživanja je bio da se ispitaju efekti hipofunkcije prethodno x-zracima ozračene hipofize na rast kraniofacijalnog i stomatognatnog sistema pacova. **Metode.** Primenjena doza x-zračenja od 27,92 Gy aplicirana je na dve grupe pacova u 8 seansi tokom perioda od 8 do 63 dana njihove starosti, uz praćenje efekata zračenja na rast kraniofacijalnog i stomatognatog sistema. U nameri da u merenju brojnih parametara pomenutih sistema razdvojimo efekte ozračene hipofize od direktnih efekata x zraka koje smo primenili na glavu, postavljen je eksperiment sa ozračivanjem glave dvema grupama životinja istim dozama x zraka, ali je jednoj zaštićena hipofiza olovnom pločicom postavljenom iznad hipofiza. **Rezultati.** Zaostajanje u rastu stomatognatog sistema kod pacova kojima je lokalno ozračena cela glava u odnosu na kontrolne životinje predstavlja zbirni efekat delovanja zračenjem izazvane hipofunkcije hipofize i direktnog delovanja x zračenja, dok zaostajanje u rastu stomatognatog sistema kod pacova sa ozračenom glavom i hipofizom zaštićenom od zračenja predstavlja isključivo efekat x zraka. **Zaključak.** Oštećenja stomatognatog i kraniofacijalnog sistema bila su veća kod životinja kojima hipofiza tokom zračenja glave nije bila zaštićena u odnosu na one sa zaštitom žlezde prilikom zračenja, pri čemu je rast kraniofacijalnog sistema u sagitalnom pravcu bio najviše pogođen. Veći zaostatak u rastu usled kombinovanoig dejstva hipofunkcije hipofize izazvane zračenjem i samog zračanje ustanovljen je na donjoj vilici u odnosu na gornju vilicu. Slično ovome, zao

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stajanje u rastu tibije bilo je izraženije kod životinja kojima tokom zračenja cele glave hipofiza nije bila zaštićena u odnosu na neštićene životinje.

Ključne reči:
hipofiza; x zraci; pacovi; rast; tibija; lobanja; gornja vilica; donja vilica.

Introduction

The reaction of an organism to radiation depends on the level of irradiation and the sensitivity of the affected tissue cells. The biological effects on the cells and tissues are proportional to the absorbed radiation energy. A marked damage, with the same dose, is evident in tissues made up of cells with a high replication index, or it occurs at an undifferentiated embryonic level. This is one of the milestones of radiobiology, set up by Tribondeau and Reaminer [1] in 1905.

One of the most marked negative effects of the irradiation of the neck and head region is pituitary hypofunction. There is a number of opposed opinions regarding the sensitivity of the pituitary gland to radiation [2]. In some studies, it was considered that the pituitary gland of adults is resistant to radiation [3] and that high doses are needed in order to induce hormonal changes. Nowadays, it is thought that the hypothalamic pituitary unit is a particularly radiosensitive region, but due to a high hormonal reserve, the negative effects become evident after a longer latent period. It is established that the growth hormone secreting acidophilic pituicytes are sensitive at a single dose greater than 30 Gy [4, 5], and this is often associated with the impact on growth, sexual function and physical and psychological health [6]. Consequently, hypopituitarism develops after radiotherapy for sellar neoplasms, brain tumors and head and neck tumors [7].

Postradiation therapy hypopituitarism shows more damage in younger patients [8]. Growth failure develops after the radiotherapy tumor doses of 46 Gy [9]. Growth hormone insufficiency of 50–100% is usually the only abnormality after irradiation of hypothalamic-pituitary axis with the doses < 30 Gy [10]. Children irradiated for the head and neck tumors have significant alterations in some skeletal measurements, such as asymmetry, potential deformity [11] and deviations in craniofacial structures [12]. The mandible is more sensitive to radiation compared to the maxilla, and especially the condylar region as the center of growth. Doses of 30 Gy to 40 Gy and above are particularly harmful regarding facial bone growth [13, 14].

The majority of bones of rat's neurocranium grow by apposition along the sutures, and except for the frontonasal bone, this ends by the age of 34 days. The scull must be observed as a whole, as each part affects the development not only of adjoining structures, but also of distant structures. Differential growth along the sutures gives the final shape to the scull [15].

Not many authors have researched the scull and jaw growth in rats [16–18], although in examining the adverse effects of various *noxae* on rat's head and jaw growth many authors also recorded values from control, untreated groups [19–21].

The pituitary gland in rat is located ventral to the diencephalon and caudal to the *chiasma optici* in a shallow groove at the base of the sphenoid bone [22]. The adenohypophysis is a heart-shaped disk joined to the neurohypophysis at the medial line. The *pars intermedia* separate these two parts. The horizontal cleft of the hypophyseal space is located between the neurohypophysis and the *pars intermedia* and it persists throughout the lifespan [23].

Since the 1980s, extensive studies have been carried out on the effects of irradiation on the development and growth of rats. Some researchers irradiated whole animals, and some only certain parts of their bodies. The studies on the influence of limb irradiation on the growth of long bones showed a significant, but reversible delay in the growth of femur or tibia as early as at doses of 5 to 7 Gy [24, 25]. At a dose of 17.5 Gy, a greater delay in tibial growth in width than in length was observed [26]. Severe damage in femur osteogenesis was caused by a dose of 20 Gy, while three doses of 10 Gy each caused mild, but significant changes [27].

Different doses were also used to irradiate rat's head in examining the effect on the skull and jaw growth. Neurological and cognitive impairments have also been reported [28, 29], as well as a decline in immunity [30].

Sedlecki et al. [31] and Demajo [32] have studied the effects of irradiation on the growth of the scull and teeth in rats. Their results indicate that doses above 4.8 Gy (maximal dose 9.6 Gy) result in measurable effects on the decrease in jaw parameters. A dose of 20 Gy caused a significant delay in the growth of the lower jaw [33].

The irradiation of the rat's head with the doses of 20, 22, and 24 Gy caused a significant decrease in the secretion of the pituitary gland hormone, the most sensitive being the growth hormone [34]. A dose of 10 Gy of γ-radiation applied to the whole body, abdomen and head caused increased hypothalamic-pituitary axis activity and greater hormone secretion in order to protect the body from radiation [35].

The aim of our study was to examine the effects of hypofunction of the pituitary gland, previously irradiated with a total dose of x-rays of 27.92 Gy applied in eight sessions in the period from the 8 to 63 days of animal age, on the rats' growth, as well as on their growth of the craniofacial and stomatognathic system. In order to differentiate the effects of the irradiated pituitary from the direct effects of x-rays on the head, the experiment was set as a double study. One group of rats had the pituitary gland protected with a lead plate set beneath the projection of the gland. The second group was irradiated with the same dose, but without the protection with a lead plate.

Methods

Experimental animals

In the experiment, Wistar rats (n = 45) with a genetic uniformity of 98% were studied, thus ensuring a uniform response to irradiation. The rats were introduced in the trial at the age of eight days. Up to the age of 30 days, they were kept with their mothers in groups of 6–8 animals per cage. They were weaned at the age of 30 days and set in separate individual cages. All animals were kept under a uniform light regime (light from 5.00 AM to 7.00 PM). Room temperature was 22 ± 2 °C, and they were fed with standard food chaw and watered *ad libitum*.

The first group was not irradiated (control group, n = 15). In the second group (n = 15) the rats' heads were irradiated, but their bodies were shielded and thus protected. This group is described as the irradiated group. The rats in the third group (n = 15) had not only their bodies protected, but the region of the pituitary gland as well. This group is described further in the paper as the protected group. During the experiment, a number of rats died, making the final number of animals 30. Thus, 10 animals in each group were finally studied.

Irradiation

The heads were irradiated with a Philips roentgen, usually used for radiotherapy. The conditions were as follows: 240 kV; 7.5 mA; filter 10 mm Al; speed 18.321 Gy/min and time 19 min 2 sec. The field width was 14 cm and the distance of the focus from the skin was 87.5 cm.

The overall irradiation dose of 27.92 Gy was divided into eight single doses of 3.49 Gy each. The animals were treated twice a week (Friday and Tuesday), every alternate week. The rats were treated for the first time at the age of eight days. After that, they were treated on the 12^{th}, 22^{nd}, 26^{th}, 36^{th}, 40^{th}, 50^{th} and 54^{th} day of age. During each session, rats were irradiated and the room temperature in which the irradiation treatment was carried out was 18 °C. Only the heads were irradiated, as a 6 mm thick lead plate of fitting dimensions protected the rest of the bodies. In the protected group, the pituitary gland was also protected with 6 mm thick lead plate.

The rats were set in a specially constructed wooden stand. In order to stop frantic movements, the extremities were fixed by rubber tubing. The head was fixed in such a way that a thin wire was placed on one side behind the incisors and on the other side, in order to immobilize them in such a fashion as not to hurt the soft tissues.

Measurements of the tibia

The right tibia was prepared [36] and its weight was determined on an automatic Mettler scale. The length was measured with a precise measuring instrument with a precision of a tenth of a millimeter.

Measurements of the skull

The heads and the jaws were cleaned of all soft tissues [36]**,** with an orthodontic measuring instrument Dentaurum, with a precision of a tenth of a millimeter, and the following parameters were taken: 1. skull length – from the tip of the nasal bone to the most distal point of the occipital bone (Figure 1); 2. skull width – the distance between the most prominent points of parietal bones (Figure 1); 3. skull front height – from the mid coronary suture between the frontal and parietal bones up to the middle of the suture between the sphenoid bone and the sphenoid base (Figure 2); 4. skull hind height – from the midsuture between the intraparietal and occipital bones down to the midline of the lower Foramen magnum edge (Figure 2); 5. length of maxilla – the distance between anterior and posterior nasal spines (Figure 3); 6. length of mandible – from the posterior edge of the angulus to the apex of the alveolar calyx of the incisor on the vestibular side (Figure 4); 7. height of mandible – from the point between ramus and corpus on the upper edge of mandible to the most convex point of incisura premaseterica on the lower edge of the corpus. (Figure 4); 8. length of ramus – from the tip of the condylar extension to the lower edge of the angulus (Figure 4); 9. width between the condyles – distance between most lateral points of condylar extensions (Figure 5).

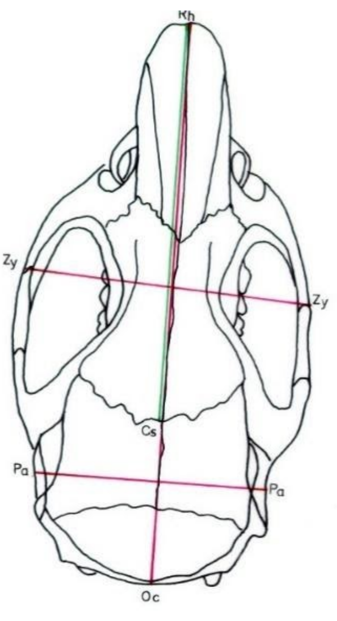

Fig. 1 – Skull length (Rh–Oc), skull width (Pa–Pa).

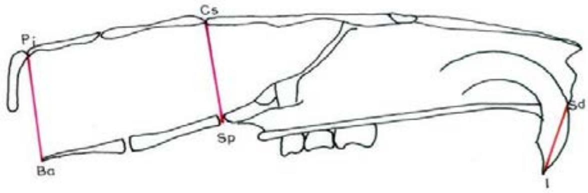

Fig. 2 – Frontal (Cs–Sp) and hind (Pi–Ba) skull height.

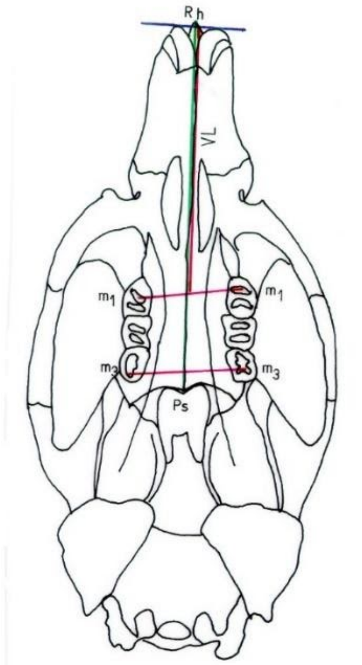

Fig. 3 – Length of maxilla (Rh-Ps).

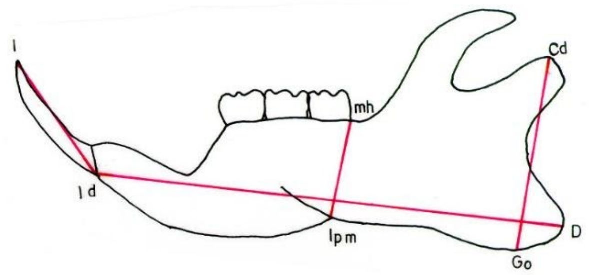

Fig. 4 – Length (Id-D) and height (mh-Ipm) of mandible and length of ramus (Cd-Go).

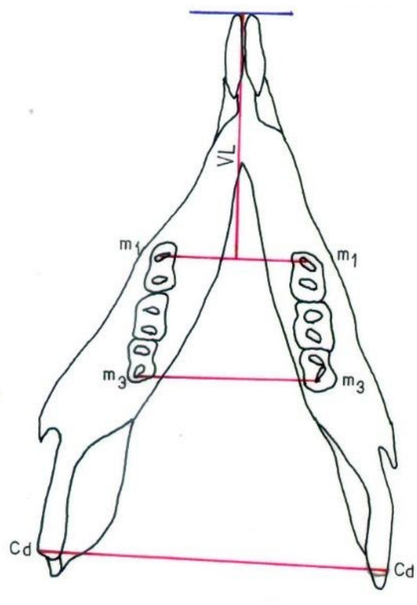

Fig. 5 – Width between the condyles (Cd-Cd).

Results

The irradiation of rats' heads with multiple doses of x-rays results in impaired growth. The protection of the pituitary gland during irradiation decreased the negative effects of radiation on the growth of rats, and on the growth of rats' jaws and teeth. The first few doses in the protected group even showed a stimulative effect on the overall growth.

Body length and mass growth, as well as the pituitary weight in the completely irradiated group, were significantly impaired ($p < 0.001$) as it had been shown earlier [37, 38].

During the experiment, some changes that could be attributed to the effects of radiation were also recorded. However, further studies of these changes were not planned, but we did consider it of interest to make a few comments on them. The epilation of hair on the head started at the age of 25–29 days (after the fourth irradiation treatment) and was evident all the time during the experiment. In the protected group, hair loss was obvious after the fourth treatment only on the exposed parts of the head. The protected areas had normal fur. Some of the irradiated rats by the 60th day of age had inflamed eyes with concurrent bleeding, apathy, weakness (the rats were somnolent and they gathered at the bottom of the cage). Water consumption increased and feed consumption decreased. There were no cases of spontaneous teeth fallout.

At the age of 60 days, the rats reach sexual maturity and secondary sexual characteristics are evident. The most prominent sexual feature is large testicles, which are lacking fur and are easily seen. The irradiated rats at the age of 60 days had testicles, which were atrophic and furry. When decapitated, the bleeding was very scarce and the blood was dark and dense.

Length and weight of the tibia

In our experiment, tibia was measured in order to determine the effect of pituitary gland irradiation on the growth of long bones, which were not in the radiation field.

The average weight of the tibia was the highest in the control group (403.37 mg), slightly lower in the protected group and the lowest in the irradiated group. Analysis of variance and the Student's test showed a statistical significant differences in the weight and length of the tibia of irradiated non-protected rats compared to the control and the protected group ($p < 0.001$). There was no significant difference between the protected and the control group (Table 1).

The results showed that the tibiae were the longest in the control group and the shortest in the irradiated group. The difference was statistically significant between the control and the irradiated group. The length of the tibia was shown to be the most variable within the irradiated group.

Within the control and the protected group, there was no significant correlation between the weight and the length of the tibia. Within the irradiated group of rats, the correlation was negative; however, this was not up to a significant degree. The negative value of correlation in the irradiated group of animals was the result of a more intensive tibia weight loss compared to the length.

Skull length

The longest average length was measured in the control group of animals. This value was significantly lower in the protected group ($p < 0.05$). The lowest results ($p < 0.001$) were recorded within the irradiated group (Table 2).

Skull width

The same as for the skull length, the largest average values of skull width were recorded in the control group. In the protected group, the values were significantly lower ($p < 0.05$). The lowest values for the skull width were in the irradiated non-protected group of rats. The values for the irradiated, non-protected group were significantly lower compared to the control group ($p < 0.001$) (Table 2).

There was no significant correlation between the measured parameters in all observed groups.

A greater delay in the width than in the length of the skull in the protected group compared to the control group is probably due to the protection of the pituitary gland by the lead, which also included the bones of the roof of the skull. The protection did not cover the parietal bones where the width of the skull was measured, so the irradiation had a greater effect on the transverse than on the sagittal dimension.

Frontal and hind skull height

In the control group, the frontal skull average height was significantly higher ($p < 0.05$) compared to the protected group. In the irradiated, non-protected group, this difference was even more impressive ($p < 0.001$). The variations of the measured values were moderate (Table 3).

From the obtained readings for the hind height it can be clearly seen that the average readings were significantly lower in the irradiated, protected group ($p < 0.05$) and the irradiated non-protected group ($p < 0.001$) (Table 3).

In the control and in the protected group, there was no significant correlation between the skull front and hind height. The correlation values were almost identical, as in the irradiated, non-protected group the lack of frontal and hind skull height was comparable. Within the non-protected group, the correlation between the measured parameters was high. Based on this finding, it can be concluded that in the irradiated, non-protected group, the radiation resulted in a significant inhibition of the growth of both the frontal and hind portions of the skull.

The significantly low values for the skull length and width in the irradiated, non-protected group are the result of the joined effects of radiation and pituitary hypofunction on the growth of the skull.

Table 1

Tibia length and weight in irradiated (IRR) rats

Group	Tibia length (mm)		Tibia weight (g)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	31.74 ± 0.25	0.7886	403.37 ± 2.89	0.7164
IRR-protected	31.52 ± 0.15	0.4914	400.78 ± 0.89	0.2225
IRR	26.74 ± 1.22*†	4.5535	366.12 ± 8.10*†	2.2133

***p* < 0.001 v.s. Control; †*p* < 0.001 v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Table 2

Skull length and width in irradiated (IRR) rats

Group	Skull width (mm)		Skull length (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	15.48 ± 0.10	0.6673	39.81 ± 0.98	2.4710
IRR- protected	15.34 ± 0.12*	0.7653	38.88 ± 0.29*	0.7454
IRR	14.15 ± 0.39**†	2.7922	33.78 ± 1.63**†	4.8197

***p* < 0.05, ***p* < 0.001 v.s. Control; †*p* < 0.001 v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Table 3

Frontal and hind skull height in irradiated (IRR) rats

Group	Frontal skull height (mm)		Hind skull height (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	10.96 ± 0.37	3.3923	9.79 ± 0.30	3.0245
IRR-protected	10.60 ± 0.19*	1.8340	9.53 ± 0.12*	1.2172
IRR	9.03 ± 0.33**†	3.6932	8.32 ± 0.37**†	3.6971

***p* < 0.05, ***p* < 0.001 v.s. Control; †*p* < 0.001 v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

In the protected group, these parameters were lower compared to the control group, but significantly higher compared to the irradiated, non-protected group. Based upon statistical analysis, the difference between the protected group and the control group was smaller than between the irradiated, non-protected group and the protected group. These data highlight the fact that the greater negative effect on the sagittal and transversal growth of the skull was due to the irradiated pituitary gland hypofunction, compared to the direct effects of radiation. The decreased growth of the skull width, compared to skull length in the protected group and compared to the control group, was probably due to the protection of the pituitary by a lead plate that covered the bones of the skull. However, the plate did not cover the parietal bones. Thus, the radiation affected more the transversal dimension than the sagittal one.

In the irradiated, non-protected group, the sagittal dimension was more affected as more bones and sutures are present in this direction compared to the transversal. Hence, it is more probable that the lack of the growth hormone affects more the sagittal dimension. In the protected group, the height of the skull was smaller compared to the control group, but significantly higher compared to the irradiated, non-protected group. This difference was the direct consequence of the effects of radiation. Within the irradiated, non-protected group, there was a simultaneous effect of radiation on the bones and insufficient growth hormone synthesis due to the radiation damage of the pituitary gland. The results in the irradiated, protected group could have been affected by the lead protection as the plates covered also the hind portions of the skull. Thus, the differences in frontal skull height were a more reliable indicator for the studied groups.

Maxillary length

Maxillary length in the control group was significantly longer than in both irradiated the protected and non-protected group, but the level of significance was different. In regard to the protected group, it amounted $p < 0.05$ and in regard to the non-protected one, it was $p < 0.001$. Such a large backlog of the irradiated, non-protected group compared to the control group is a result of the summarized effect of the radiation and pituitary gland hypofunction on the growth of the upper jaw. In the protected group, only the effect of the radiation was manifested, which is why it is smaller than that in the control group, but also significantly longer than in the irradiated, non-protected group. Based on these observations, it can be said that the maxillary growth into the sagittal direction is more influenced by the hypofunction of the irradiated pituitary gland than by the radiation itself (Table 4).

Mandibular length

The control group of animals had longer mandibles than the other two groups. The level of difference in length compared to the control group in the irradiated, protected group was at the significance level $p < 0.05$ and in the non-protected group the level was $p < 0.001$. In the protected group, the length of the lower jaw was longer than in the non-protected one (the significance of the difference was at the $p < 0.001$ level). All this suggests that pituitary hypofunction, conjoined with the consequent growth hormone deficiency, caused a greater delay in the sagittal growth of the mandible than direct radiation exposure. A similar finding was ascertained in the analysis of sagittal parameters of the maxilla, so that it can be said that the irradiation of jaws causes a smaller growth delay of the sagittal dimension of the head and jaws compared to the lack of the growth hormone (Table 4).

Mandibular height and ramus length

Mandibular height in the protected group was significantly lower than in the control group, but also significantly higher compared to the irradiated, non-protected group. Based on the level of significance, it can be seen that there was a greater backlog of the non-pretected group compared to the protected one than of the protected group compared to the control group (Table 5).

Table 4

Length of maxilla and mandible in irradiated (IRR) rats

Group	Maxillary length (mm)		Mandibular length (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	21.73 ± 0.75	3.4712	20.68 ± 0.63	3.3965
IRR-protected	20.71 ± 0.42*	2.0159	19.95 ± 0.51*	2.5805
IRR	18.43 ± 0.62**†	3.3646	17.96 ± 0.61**†	3.3736

***$p < 0.05$, **$p < 0.001$ v.s. Control; †$p < 0.001$ v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Table 5

Height of mandible and ramus in irradiated (IRR) rats

Group	Mandibular height (mm)		Ramus height (mm)	
	mean ± SD	CV(%)	mean ± SD	CV(%)
Control (non-IRR)	6.18 ± 0.16	2.6197	10.02 ± 0.53	5.2555
IRR-protected	5.88 ± 0.18*	3.0850	9.48 ± 0.08*	0.8323
IRR	5.47 ± 0.24**†	4.3126	8.55 ± 0.22**†	2.5415

***$p < 0.05$, **$p < 0.001$ v.s. Control; †$p < 0.001$ v.s. IRR-protected (*t*-test).**
CV – coefficient of variation; SD – standard deviation.

Ramus length in the protected group was significantly lower than in the control group, but also significantly higher compared to the irradiated, non-protected group. Based on the level of significance, it can be noticed that there was a greater backlog of the non-protected group when compared to the protected one than of the protected group compared to the control group. This ratio was conditioned by the greater influence of the lack of the growth hormone induced by irradiation on the growth of the mandible in the vertical direction than the negative effect exerted by radiation itself. Since the same happens with the length of the mandible, it can be said that the total growth of the body of the mandible was more damaged by the irradiation-induced pituitary gland hypofunction than by radiation. The mandible growth center is in the condyle, so any adverse effects on the wrist have an impact on the entire jaw. The x-ray direction was such that they passed through the skull before the condyle, so they probably lost their strength and could not fully affect the joint (Table 5).

Compared to the control group, a significant delay in the ramus length of the 57th day of age [32] was found in the group of the rats whose heads were irradiated with 9.6 Gy on the 8th day of age [32], which is consistent with our findings.

The growth hormone deficiency had a somewhat greater effect on ramus length compared to the effect of x-rays, but it can be summarized that, compared to the control group, there was an equal delay in the growth of the vertical dimension of the mandible in both experimental groups.

Bicondylar distance

As with the other parameters of the mandible, the values of the bicondylar distance were the lowest in the irradiated, non-protected group. The protected group was lagging behind the control group. However, there was a greater backlog of the non-protected group compared to the protected one. Irradiation had less effect on the transverse growth of the mandible than the hypofunction of the irradiated pituitary gland (Table 6).

Table 6

Bicondylar distance in irradiated (IRR) rats

Group	Bicondylar distance (mm)	
	mean ± SD	CV(%)
Control (non-IRR)	19.68 ± 0.31	1.5845
IRR-protected	19.09 ± 0.41*	2.1592
IRR	15.40 ± 0.24**†	1.5903

***p* < 0.05, ***p* < 0.001 v.s. Control;**
†*p* < 0.001 v.s. IRR-protected (*t*-test).
CV – coefficient of variation; SD – standard deviation.

Discussion

In the available literature on this topic, we have not encountered any works that have separately examined the effects of irradiated pituitary gland and the corollaries of direct effect of x-rays on the growth of head bones. Therefore, some of the results obtained could not be compared or discussed with other authors. These results can be considered to be our original contribution to the study of the effects of pituitary hypofunction on the growth of rats' head bones. At the same time, it represents a baseline study in research of the effects of radiation-induced pituitary hypofunction on the growth of facial and jaw bones in patients irradiated in this region during the adolescence.

Length and weight of the tibia

Measuring tibial length as an indicator of systemic effects of head irradiation was performed by Demajo [32]. In the control group, the values agree with our finding, while the values of tibia length of the animals irradiated with 9.6 Gy were greater than the values of the irradiated animals from our study. Regardless of fractionation, the dose of 27.92 Gy is much higher than that used by Demajo [32], so the values are lower.

Frontal and hind skull height

The anterior skull heights were measured at the same points by Haralabakis and Dagalakis [18]. In the 60-day-old control group, the value is about 11 mm, which is slightly higher than in the animals in our control group.

Maxillary length

The animals from the same farm where we conducted the research were measured by Demajo [31, 32]. In the control group, the maxilla length was 21.82 ± 0.31 mm, which is entirely in accordance with our results in the control group. In the group where the heads of the rats aged eight days were irradiated with 9.6 Gy, the length of the maxilla on the 57th day was lower than the value recorded in our irradiated group. Although the rats in our study had received almost three times higher dose, their maxillae were less delayed in the sagittal growth. Total dose fractionation and the time between irradiation applications enabled the activation of reparative processes, which prevented more drastic growth retardation.

Mandibular length

In animals whose heads were irradiated with 9.6 Gy when they were eight days old, Sedlecki et al. [31] recorded a significantly shorter mandible length on day 57 compared to the control group. In the 57-day-old animals, Demajo [32] measured higher values than ours (26.03 ± 0.21 mm), and in the group where the heads were irradiated with 9.6 Gy when the animals were 8 days old, the values of the sagittal dimension of the mandible were also significantly higher than in our irradiated group (23.87 ± 0.38 mm).

Duterloo and Vilmann [17] measured a mandible length of 18.02 mm in the 30-day-old rats and found that the mandible had grown 2.17 mm from the 14th day. In our control group, the rats are twice older and the difference in length was slightly more than 2 mm. As the growth rate is twice faster in the first month than in the second month of life, our results are in line with the findings of these authors.

Mandibular height and ramus length

Sedlecki et al. [31] measured the height of the mandible of the first molars, unlike our measurement of the third molars; they also found that the irradiation of the head of 8-day-old rats caused a significant retardation in growth, recorded on day 57.

In the 57-day-old rats, Demajo [32] measured a mandible height that was lower than in animals from our control group. In animals whose head was irradiated with 9.6 Gy on day 8, the height of the mandible on day 57 was also lower than the value in our irradiated group.

Bicondylar distance

In the 30-day-old irradiated males rats [17], the measured bicondylar width was slightly lower than the values measured in the twice older animals in our irradiated group, so it can be said that the x-radiation with pituitary gland hypofunction caused the growth of the mandible in the transverse direction that is twice slower.

Conclusion

In the animals with locally irradiated heads, the measurements of the craniofacial system showed statistically significantly lower values compared to the measurements of the animals in the control group, as well as in the group where their pituitary glands were protected, which is a consequence of the combined effect of the x-ray radiation and irradiation-induced pituitary gland hypofunction.

The growth of the craniofacial system in rats in the sagittal direction was most sensitive to radiation and radiation-induced growth hormone deficiency.

The measurements of the growth of numerous parameters of the stomatognathic system in rats with the entire locally irradiated heads showed statistically significant delays compared to both non-irradiated control group and the group where the pituitary gland was protected.

In the group where the pituitary gland during the irradiation of the head was protected, there was a statistically significant delay in the growth of the stomatognathic system compared to the (non-irradiated) control group.

Damage to the stomatognathic system was greater due to irradiation-induced pituitary gland hypofunction than due to the direct exposure to x-rays itself.

Radiation-induced pituitary hypofunction and direct irradiation caused greater growth delay on the lower than on the upper jaw.

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