A STUDY ON THE DISTRIBUTION OF ^{85}Sr AND ^{134}Cs RADIONUCLIDES AFTER TOTAL BODY IRRADIATION

by

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The kinetics of strontium, ^{85}Sr, and cesium, ^{134}Cs, were evaluated in a mouse experimental model to determine the impact of these radionuclides on a living organism concerning total body irradiation. Our study demonstrates that the elimination rate of ^{134}Cs from the skeleton and teeth is influenced by total body irradiation and the presence of ^{85}Sr. Higher accumulation and faster ^{134}Cs elimination rates were observed in the skeleton and teeth of mice administrated with a mixture of ^{134}Cs + ^{85}Sr radionuclides. Regarding ^{85}Sr, only a minimal effect was observed on its accumulation rate in skeleton, teeth, and muscle in total body irradiation mice. The effect of the ^{85}Sr + ^{134}Cs radionuclide mix on the accumulation of ^{85}Sr was more apparent in teeth, showing a higher retention rate after 10-24 days of administration in non-irradiated mice. The evaluation of the kinetics of these radionuclides provided much-needed insight on their effects during the first two months after exposure, demonstrating that the accumulation rate of ^{85}Sr is greater than that of ^{134}Cs. Further, the elimination rate of the former is slower in comparison to the latter. Interestingly, total body irradiation has a greater effect on the hematological parameters of the mice blood than the radionuclides alone.

Key words: ^{85}Sr, ^{134}Cs, mouse model, internal contamination, total body irradiation

INTRODUCTION

The study of ionizing radiation and radioprotective agents caught special emphasis during the Cold War when the threat of a massive nuclear event was a real possibility. In the present day, the relatively recent disaster at the Fukushima Daiichi nuclear power plant (NPP) and the release of ^{106}Ru in Russia's Southern Urals has directed the attention of the ruling institutions towards the potential fallout of an industrial accident instead. Further, the concentration of radionuclides in the environment is currently increasing despite the implementation of strict safety standards [1, 2] potentially threatening with serious or adverse effects the organisms exposed to this radionuclide contamination. It must be noted that their long-term environmental burden is predominantly defined by long-lived isotopes such as ^{137}Cs and ^{90}Sr [3]. As an example in this regard, the 1957 disaster of the Soviet Union's Mayak plutonium production plant had a broad impact on both the environment and the local population [4, 5]. The major risk put forth by ^{137}Cs and ^{90}Sr release into the environment is given by the overall contamination of the ecosystem and the absorption of these radionuclides into the food chain [6].

Regretfully, the exact toxicity mechanisms of cesium and strontium in humans have not been determined yet. In general, the known effects of cesium and strontium on the human body are based upon the available documented accidents and biokinetic models [4, 7]. Regardless, the effort to summarize a physiologically realistic and time-independent description of the behavior of absorbed radionuclides has yielded a biological model for cesium and strontium poisoning. The basic biokinetic model is based on the gastrointestinal tract model [8] and a refined human alimentary tract model, which already covers all areas of the digestive tract ICRP 100 [9]. If cesium should be injected, then the kinetics model can be found in the ICRP 134 and 137. A study summary on the biokinetics of cesium and strontium can be seen for example in Leggett *et al*., [10].

The correct and full analysis of biological samples treated with radionuclides used to be a complex and demanding process. However, current microwave- and ultrasound-based methods are markedly better, safer, and faster regarding the preparation of samples

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and data acquisition [11-13]. Due to its good solubility, cesium can be easily absorbed into the bloodstream, thus spreading systematically. Once in the organism, cesium has similar kinetics to Potassium and can enter the cells and participate in the electrical charge balance [10]. Cesium is removed from the body soon after absorption and released through urine. In adults, 10-15 % of absorbed ^{137}Cs will be eliminated after 2-3 days, with the rest being eliminated during the following 90 days [14]. Trace amounts of cesium can also be excreted in the feces. The ICRP 54 study shows an 80:20 % elimination ratio of urine to feces. The ^{137}Cs can also be excreted through sweat as well. Strontium has similar kinetics to calcium in the mammalian organism. In adults, strontium is mainly accumulated on the surface of bones (97 %) and teeth (3 %), thus irradiating the bone marrow and bone stem cells in the superficial and soft tissue surrounding the bone [6].

Our study sought to characterize the influence of total body irradiation (TBI) on ^{134}Cs and ^{85}Sr and internal contamination in a mouse model evaluated through a hematopoietic analysis focused on the migration of precursor cells into the thymus and thymocyte development.

MATERIALS AND METHODS

Animals

The C57Bl/6 mice were used in this study (Velaz a.s.; Prague, CZ). The animals were two-month-old at the time of the experiment and had an average weight of 21 g 2 g. The animals were kept in a controlled environment with a 12 hours day/night cycle, a temperature of 22 2°C, and 50 % 10 % humidity. Tap water and standard chow ST-1 diet (CZ) were provided *ad libitum*. The animals were divided into eight experimental groups, each containing 24 animals. The evaluation was carried out in groups of six animals at days 4, 10, 24, and 46 after treatment. The weight and health status of the animals was monitored daily. The protocol was reviewed and approved by The Ethical Committee of the Faculty of Military Health Sciences in Hradec Kralove and by The Ethical Committee of the Ministry of Defence of the Czech Republic.

Total body irradiation and radionuclide administration

A group of mice was set as a control reference (non-irradiated and no radionuclide treatment). The second group was exposed to TBI (4 Gy) from a ^{60}Co gamma source (Chisotron; Chirana, CZ) at a dose rate of 1.3 Gy per minute without anesthesia in vertical cylindrical plexiglass boxes. The radionuclides ^{85}Sr (third group) and ^{134}Cs (fourth group), or their combination (^{85}Sr + ^{134}Cs) (fifth group), were administered via intraperitoneal injection. The sixth (^{134}Cs + 4 Gy), seventh (^{85}Sr + 4 Gy), and eighth groups (^{134}Cs + ^{85}Sr + 4 Gy) received a 4 Gy TBI dose one hour before the application of radionuclides. The radionuclides were administered in liquid solution (^{85}Sr – 20 mg $SrCl_2$ per liter + 3 g HCl per liter 8 %; ^{134}Cs – 20 mg CsCl per liter + 3 g HCl per liter 8 %, Eurostandard CZ s.r.o.).

The ^{134}Cs has a half-life of 2.0648 years; it has beta decay (β^-) and produces stable ^{134}Ba and ^{134}Xe isotopes. It emits 2.23 gamma-ray photons on average with a mean energy of 0.698 MeV. The half-life of ^{85}Sr is of 64.84 days; it decays to ^{85}Rb via electron capture and emits a strong gamma line of 513.99 keV. The total volume of radionuclides mixed with physiological saline solution applied per animal did not exceed 0.5 mL, tab. 1.

The activity of the ^{85}Sr and ^{134}Cs radionuclides in the mixed sample corresponds to one-half of the activity of the individual radionuclides, thus its total activity is comparable to the individual radionuclides. Only male mice were included in the experiment due to their higher resistance to ionizing radiation. The TBI dose of 4 Gy was selected because it can induce a hematopoietic subsyndrome of acute radiation syndrome (ARS) without being lethal. The ^{85}Sr and ^{134}Cs radionuclides were chosen due to their known effects and ease of detection.

Tissue collection

The animals were euthanized by anesthetic overdose. Peripheral blood, bone, thymus, incisor teeth, and muscle (*m. tensor fasciae latae*) were collected for biokinetics analysis and basic hematological and immunological profile. Except for muscle, these samples were collected on days 4, 10, 24, and 46 after treatment. Muscle samples were collected on days 10, 24, and 46. Approximately 99 % of Strontium is absorbed in the skeleton and teeth. Therefore, we determined the absorbed ratio between them. The muscle tissue was analyzed due to the known kinetics of cesium.

Radioactivity analysis

The samples were evaluated in a gamma spectrometric system consisting of a model 659 bias supply (Ortec; TN, USA), AFT research amplifier (Canberra

Table 1. Radionuclide activity (kBq per 0.5 mL) (applied to individual groups ^{134}Cs, ^{85}Sr, ^{134}Cs + ^{85}Sr, ^{134}Cs + 4 Gy, ^{85}Sr + 4Gy, ^{134}Cs + ^{85}Sr + 4Gy, on 3th February 2017)

Radionuclide		A_0^*	A_1^*	A_2^*	A_3^*	A_4^*
^{85}Sr		182	175	164	141	113
^{134}Cs		159	159	158	156	153
Mixed	^{85}Sr	91	87	82	76	56
^{85}Sr + ^{134}Cs	^{134}Cs	80	79	79	78	76

* A_0 Initial activity at time $t = 0$. * $A_{1,2,3,4}$ activity at time $t = 4$, $t = 10$, $t = 24$, and $t = 46$. Does not consider the biological half-life of ^{85}Sr and ^{134}Cs

Industries), Multiport II (Canberra Industries), and HPGe detector GC3020 (Canberra Industries). The Genie 2000 v. 3.4 software was used in the analysis. Energy calibration was performed using a standard radionuclide mixture (^{241}Am, ^{109}Cd, ^{139}Ce, ^{57}Co, ^{60}Co, ^{137}Cs, ^{113}Sn, ^{85}Sr, ^{88}Y, ^{133}Ba, ^{210}Pb, ^{22}Na, and ^{54}Mn). The efficiency calibration was computed using an ^{85}Sr or ^{134}Cs solution of known activity

$$\varepsilon \quad \frac{NA}{A\,t\,Y} \tag{1}$$

where NA is the net area of the selected peak, A [Bq] – the activity of the sample, t – the live time of measurement, and Y – the yield of the selected peak (branching ratio). The data was corrected using true coincidence summing corrections. The activity of the sample was calculated using the same equation only expressing activity A when using the calculated efficiency.

A_0 (Bq) represents the initial activity of the injected radionuclide at time $t = 0$, A_m represents the average activity per weight unit in skeleton, teeth, and muscles. The A_m/A_0 ratio expresses distribution over time in the treated animals. The average weight of the skeleton (3.572 g), teeth (0.03 g), and muscles (3.247 g) was also determined.

Am can be calculated using the following equation

$$A_{\mathrm{m}} \quad \frac{A\ m_{\mathrm{tot}}}{m_{\mathrm{sample}}} \tag{2}$$

where A is the measured activity of the radionuclide in the sample mass (m_{sample}), and m_{tot} is the weight of the collected tissues as a whole (skeleton, teeth, and muscle).

Tissue processing

The tissue samples were digested as follows: 100 20 mg of bone, 40 20 mg of muscle, or 1.8 0.3 mg of teeth were transferred into an HVT-50 digestion vessel and submerged in 75 % HCl, tab. 2. The amount of sample was chosen according to the parameters of the microwave digestion system. The samples were processed in a Multiwave ECO device (Anton Paar GmbH) to liquify the biological material.

Absolute blood cell count and immunophenotyping

A peripheral blood sample was obtained from a cardiac puncture after euthanasia and collected into a 1.0 mL BD Microtainer MAP K2EDTA (Becton Dickinson, Sarstedt Ltd; UK). An absolute blood cell count was performed in an automated hematological analyzer Pentra 60 ABX (Horiba, Japan). The lymphocytes were isolated by mixing 100 μL of the blood sample with EasyLyse solution (Dako, Glostrup, DK), following the manufacturer's directions. The lymphocytes were resuspended at a cell density of 5 10^6 cells/mL and kept at 4 °C until immunophenotyping. A monoclonal antibody mix (anti-CD3 PECY 7, -CD19 BV 421, -NK-1.1 APC; BD Biosciences) was incubated with the isolated lymphocytes for 30 min/4 °C in the dark. The cells were centrifuged and washed twice in ice-cold staining buffer (PBS, 0.2 % gelatin, 0.1 % sodium azide; Sigma), and evaluated in a CyAn ADP flow cytometer (Beckman Coulter; CA, US).

Table 2. Microwave digestion

Step	T [°C]	Ramp time [min]	Digestion time [min]	Power [W]
1	100	10	10	30
3	130	1	5	65
4	160	1	20	75
5	50	1	20	0

Statistical analysis

The obtained data are shown as the mean value (mean) standard error of the mean (SEM). To determine any statistical significance a two-sided ANOVA with a post-test was used (labeled * for $p < 0.5$, ** for $p < 0.01$, and *** for $p < 0.001$). A Tukey-Kramer Multiple Comparisons Test was also used. If the value of q is greater than 4.616 then the p-value is less than 0.05.

RESULTS

The initial effect of radiation and/or radionuclides was evaluated through the overall health and weight of the treated animals. A temporarily lower physical activity and reduced appetite were observed in the irradiated mice during the first days after treatment. The mice exposed to TBI also showed hair loss and lower weight, otherwise, the mice treated with radionuclides or TBI were not different from the control group, tab. 3.

The accumulation of ^{134}Cs in the experimental mice did not exceed 10 % of the initial radionuclide activity throughout the evaluation period, reaching a higher accumulation rate in bone and muscle, fig. 1. Within the study period, ^{134}Cs was gradually eliminated from the muscles and skeleton of non-irradiated mice from day 4 figs. 1(a) and 1(e).

The skeleton and teeth of TBI-treated mice displayed a higher radionuclide deposition rate between days 10 and 24 after treatment with ^{134}Cs when compared to ^{134}Cs + ^{85}Sr-treated mice, suggesting that the mice treated with ^{134}Cs show a slower elimination rate from skeleton and teeth in comparison with ^{134}Cs + + ^{85}Sr-treated mice. At the end of the 24-46 day period when compared to the ^{134}Cs contaminated group. However, this effect could not be observed in the muscle figs. 1(e) and 1(f). Interestingly, the effect of the radionuclide mix in the muscles was only apparent in the higher accumulation of ^{134}Cs at day 10 after treatment fig. 1(e). A significantly higher ^{134}Cs accumula-

Table 3. Average mice weight at 4, 10, 24, and 46 days after treatment with ^{85}Sr, ^{134}Cs, ^{85}Sr + ^{134}Cs, and 4 Gy TBI

Radionuclide/irradiation		Weight [g]			
		day 4	day 10	day 24	day 46
Negative control (NC)	Mean	28	28.4	28.4	28.6
	SEM	2	2	2	2.1
4 Gy	Mean	23.3	25.2	24.5	27.1
	SEM	3.6	1	1.2	1.4
^{85}Sr	Mean	25.1	26.5	24.3	26.8
	SEM	0.6	2.5	1.4	0.6
^{85}Sr + ^{134}Cs	Mean	25.6	25.1	26.6	27.8
	SEM	1.7	0.9	1.5	0.7
^{134}Cs	Mean	25.1	25.6	25.2	26.1
	SEM	3	1.5	2.6	0.9
^{85}Sr + 4 Gy	Mean	25.3	24.7	25.6	26.9
	SEM	2.5	1.6	1.1	0.8
^{85}Sr + 1^{34}Cs + 4 Gy	Mean	24.4	24.4	26.8	27.6
	SEM	0.9	1.2	1.1	0.8
^{134}Cs + 4 Gy	Mean	24.4	24.6	25.5	26.5
	SEM	0.3	1.2	0.3	0.4

tion rate was evident in the teeth during the first days after treatment with ^{134}Cs when compared to ^{134}Cs + ^{85}Sr contaminated mice. In general, the teeth displayed up to two times higher ^{134}Cs accumulation whereas ^{134}Cs + ^{85}Sr-treated mice showed lower ^{134}Cs accumulation in bone.

In comparison to ^{134}Cs-treated mice, TBI led to an order of magnitude higher accumulation of ^{134}Cs in both skeleton and teeth following ^{134}Cs + ^{85}Sr treatment figs. 1(b) and 1(d), however, this effect could not be observed in muscle tissue. The overall decreased activity in muscle and teeth did not exceed 1 % of the initial activity at day 46 in either the ^{134}Cs- or ^{134}Cs + ^{85}Sr-treated groups. During the 4-46 day period, ^{134}Cs accumulation in teeth did not exceed 0.01 % of the initial activity. It must be highlighted that ^{134}Cs was unevenly eliminated in TBI mice throughout the evaluation period, fig. 1. Further, TBI did not influence the ^{134}Cs accumulation value in muscle. Regardless, a slight increment in ^{134}Cs accumulation was evident in bone and teeth due to TBI, thus demonstrating its strong influence in ^{134}Cs kinetics. This higher ^{134}Cs accumulation in TBI mice could be in response to ARS, where blood cell differentiation is disrupted.

Concerning ^{85}Sr, most of the applied radionuclide was also deposited in the skeleton and teeth. Interestingly, its elimination rate was also affected by TBI in bone, teeth, and muscle tissue, fig. 2. The effects of strontium were strongly influenced by calcium kinetics. Seemingly, the quantity of ^{85}Sr and ^{85}Sr + ^{134}Cs accumulated in the contaminated mice was an order of magnitude greater than that of ^{134}Cs, figs. 1 and 2. In general, it can be stated that a majority (as much as 99 %) of the accumulated strontium was built into the bone structure. With one exception, the activity values detected in teeth and muscle did not exceed 1 % of the initial activity fig. 2(d).

Notably, ^{85}Sr elimination from bones was slower than that of ^{134}Cs, fig. 2. Regardless, activity values greater than 10 % of the applied dose were measured in the bone at day 46 after treatment, thus validating that ^{85}Sr has a stronger affinity for this tissue than ^{134}Cs. Further, TBI had only a minimal effect on the elimination rate of ^{85}Sr and ^{85}Sr + ^{134}Cs from bones. In comparison with the ^{85}Sr-treated group, a higher ^{85}Sr accumulation was observed in the teeth of ^{85}Sr + ^{134}Cs-treated animals at days 10-24. Further, TBI-treated mice showed a higher ^{85}Sr accumulation on day 46 after treated animals. In contrast, TBI had only an ephemeral effect on the accumulation of ^{85}Sr in muscle, which was observed during the first days of the experiment. Interestingly, strontium was accumulated at a higher rate in muscle in the ^{85}Sr-treated mice when compared to the ^{85}Sr + ^{134}Cs-treated group on day 10. On day 46, ^{85}Sr accumulation values in muscle low, accounting for only 0.01 % of the initial dose.

It is easily perceived that ^{134}Cs does not affect ^{85}Sr accumulation in the bones figs. 2(a) and 2(b). Moreover, the total absorbed activity in both muscles and teeth was insignificant in comparison with the skeleton with the measured values not exceeding 1 % of the initial dose on day 46. Considering the measured activity value and the comparatively small tooth mass, a higher ^{85}Sr accumulation could be observed in teeth when compared to bone. Further, ^{85}Sr elimination from the teeth of ^{85}Sr- and ^{85}Sr + ^{134}Cs-treated mice was virtually inexistent as it formed much stronger bonds in this tissue than in bone or muscle, thus resulting in a slower elimination rate, fig. 2.

Lymphocyte number is strongly dependent upon TBI

Non-irradiated mice showed a comparable lymphocyte count to the control group on days 4, 10, 24, and 46 after radionuclide treatment. There was a significant decrement in the absolute lymphocyte count at day 4 in the ^{134}Cs-treated mice in comparison with the ^{134}Cs + ^{85}Sr group. However, the absolute lymphocyte

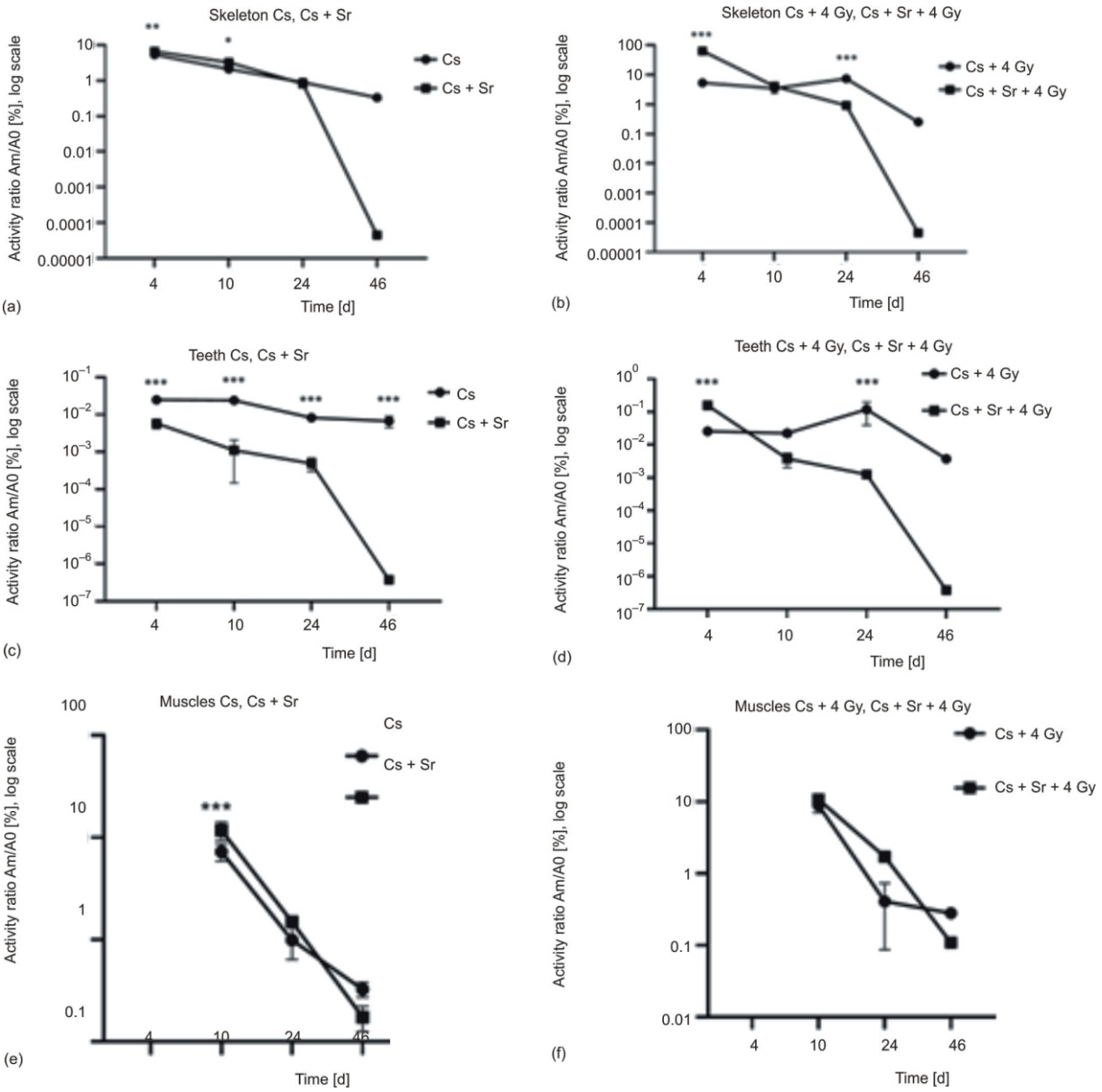


Figure 1. The ^{134}Cs activity at days 4, 10, 24, and 46 after treatment with/out 4 Gy TBI in the skeleton (a), (b), teeth (c), (d), and muscles (e), (f). The data was obtained from six mice and is shown as the mean SEM. The ANOVA with post-test *p < 0.05, **p < 0.01, ***p < 0.001. The data from control mice is not included because its value is zero. Muscle values are not shown because this tissue was not collected on day 4 after treatment. Other missing values were lower than the minimum detectable activity (MDA) of the instrument

count was significantly increased in the ^{134}Cs-treated mice on day 24 in comparison with the other groups fig. 3(a).

The absolute number of lymphocytes in peripheral blood became significantly lower from day 4 through all of the experimental TBI groups fig. 3(b). It must be highlighted that the irradiated mice suffered a dramatic blood cell number decline compromising their health, an effect that was not observed in the animals treated with radionuclides only. Thereafter, gradual increments in lymphocyte number could be observed at days 10, 24, and 46, although it did not reach the value of the NC cell population observed in non-irradiated mice throughout the evaluation period. The administration of radionuclides did not induce an inflammatory response; therefore, the absolute number of neutrophils did not increase during the experiment. Regardless, a significant increment in neutrophil number could be observed at day 24 after the administration of ^{134}Cs into the previously ^{85}Sr-treated mice fig. 3(c). No significant difference in neutrophil number could be observed in comparison with the control group. The TBI and ^{85}Sr, ^{85}Sr + ^{134}Cs-treated mice displayed a significant reduction in the absolute number of neutrophils on day 4 in comparison with the non-irradiated control group (NC). However, the neutrophil population returned to comparable values with the non-irradiated control group at day 10 fig. 3(d). The absolute lymphocyte count remained variable throughout the evaluation period.

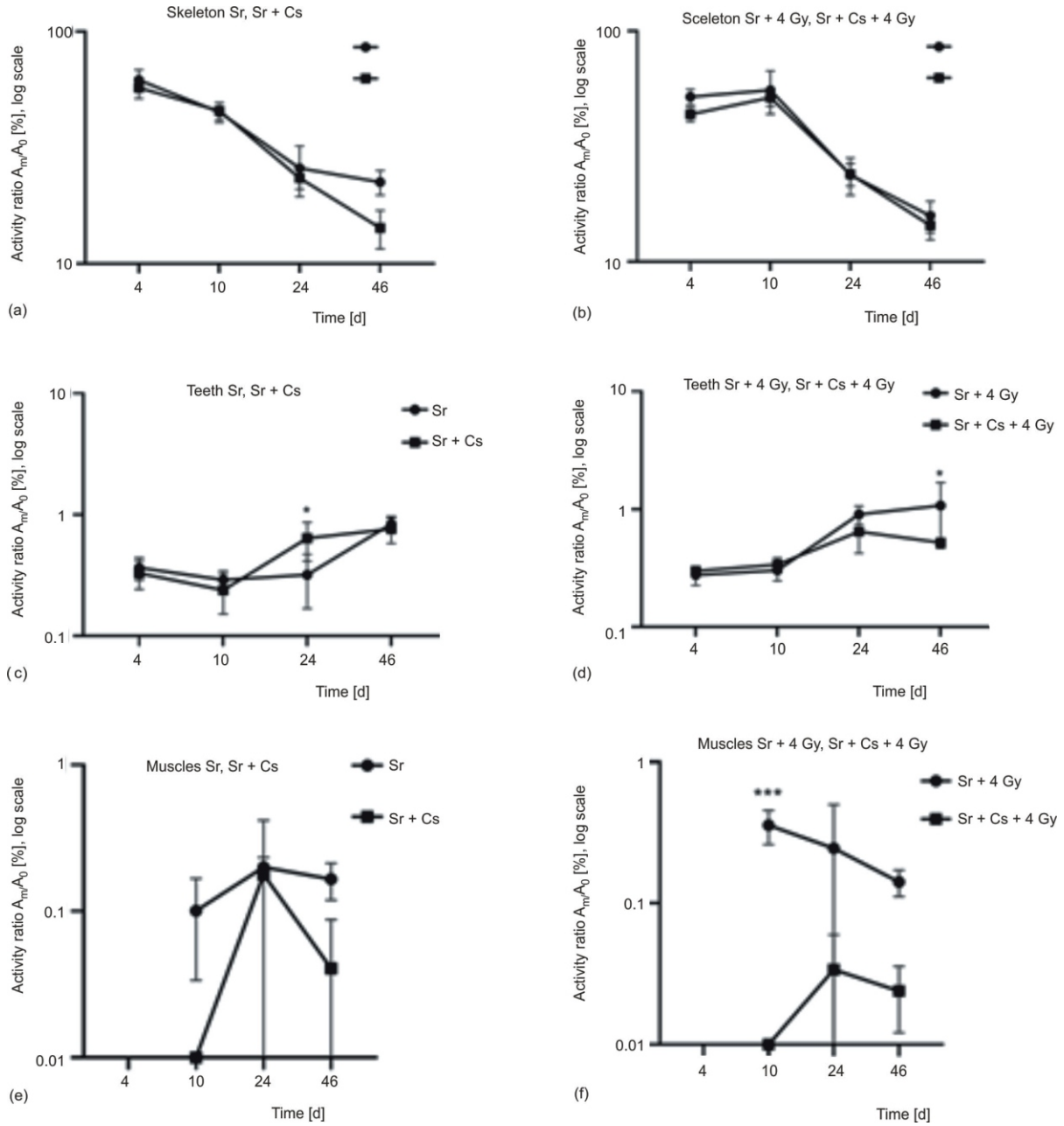
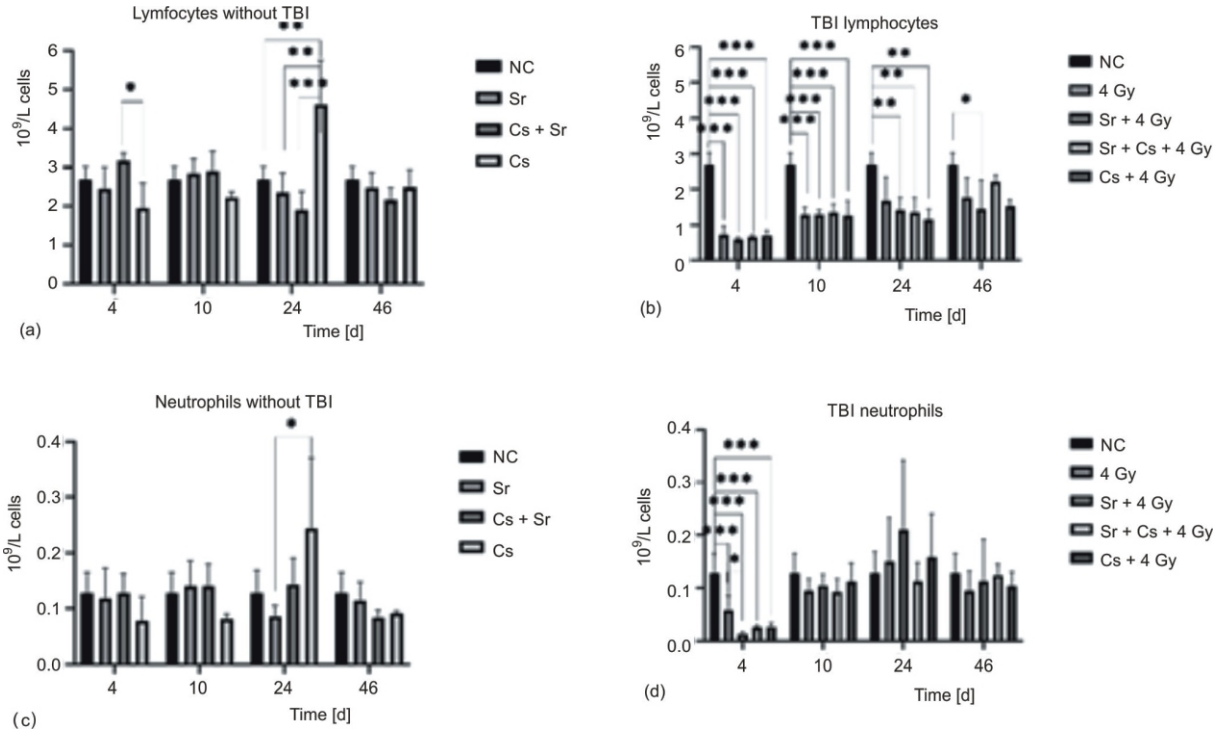


Figure 2. The ^{85}Sr activity at 4, 10, 24, and 46 days after treatment with/out 4 Gy TBI in the skeleton (a), (b), teeth (c), (d), and muscles (e), (f). The data was collected from six mice and is shown as the mean SEM. ANOVA with post-test *p < 0.05, **p < 0.01, ***p < 0.001. The data from control mice is not included because its value is zero. Muscle values are not shown because this tissue was not collected on day 4 after treatment. Other missing values were lower than the (MDA) of the instrument

Regarding the T, B, and NC cell populations in peripheral blood, there was a significant increment in the absolute number of T cells throughout the evaluation period in particular for ^{85}Sr, ^{85}Sr + ^{134}Cs-treated groups at day 10 after the administration and ^{134}Cs-treated groups at day 24 after the administration, fig. 4. On day 46, the number of T cells dwindled after the administration of ^{85}Sr. Further, the TBI-treated groups showed a similar trend, although it occurred sooner at day 10. The absolute number of B lymphocytes in the irradiated mice diminished rapidly from day 4 in comparison with the non-irradiated control group (NC). On the other hand, the number of NC cells increased from days 10-24 after radionuclide administration. On day 46, the population of NC cells became depleted throughout the experimental groups. Further, at this stage, there was a clear trend in all lymphocytes population depletion when compared with non-irradiated mice.

DISCUSSION

The toxicity and kinetics of strontium and cesium radionuclides are not fully understood. Previous studies have determined that the negative effect of radionuclides



***NC – Negative control**

Figure 3. Lymphocyte (a), (b) and neutrophil (c), (d) count at days 4, 10, 24, and 46 after treatment and with/out TBI. The data was obtained from six mice and is shown as the mean SEM. The ANOVA with post-test *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Other missing values were lower than the (MDA) of the instrument

is directly connected with their radioactive characteristics rather than with the nature of the element in question [15, 16]. Interestingly, stable cesium and strontium isotopes exhibit very low toxicity [15, 16].

Our study sought to clarify and evaluate the potential problem of combined exposure to external and internal irradiation sources that may occur after a nuclear disaster. It must be noted that a single irradiation dose is extremely unlikely under the premise of a nuclear event.Therefore, an optimistic prognosis for the exposed individuals is not to be expected. The hematopoietic system and the gastrointestinal tract are particularly sensitive to TBI. Regarding the former, the number of peripheral lymphocytes decreases as soon as 24 hours after the exposure event, resulting in a state of induced immunosuppression. Therefore, the risk of subsequent infections represent a major hallmark of ARS. Besides, the hematopoietic stem cell population can also become depleted, thus compromising the process of hematopoiesis.

In our experiments, we observed a diminished number of lymphocytes in all of the TBI-treated groups, an effect that was also present in the non-irradiated, $^{134}Cs + {}^{85}Sr$ -treated group after three weeks. In contrast, individual treatment with either of the evaluated radionuclides did not affect the lymphocyte population during the first six weeks after TBI-treatment, thus suggesting their minimal impact in this regard. In the case of neutrophils, the effect of the radionuclides and TBI could only be observed after 24 days. Chua *et al*. [17] reported a significantly lower neutrophil and lymphocyte count in TBI-treated mice after 20 months, confirming an initial decline in the number of neutrophils and the gradual recuperation of the lymphocyte population in a period of 1.5-5 months after TBI. In general, exposure to ionizing radiation often results in neutropenia, thrombocytopenia, and anemia [18, 19], all of which may become a prevailing condition in mice for more than 30 days.

It must be highlighted that the cells of the immune system do not share the same degree of radiosensitivity and display variable depletion and renewal times. This may affect the balance between the diverse immune cell subpopulations and result in an altered immune function. Lymphocytes are particularly radiosensitive and can be affected in a higher or lesser degree according to their phenotype as follows (in descending order): B cells [20], T regulatory cells, T helper cells, cytotoxic T cells, memory T cells, and NC cells [21]. The capacity to correctly evaluate the amount of absorbed ionizing radiation in an exposed person, either by accident or as part of treatment, merits a whole separate study [22]. One such was conducted by Bertho *et al*. [23], who evaluated the effects of chronic exposure to ^{137}Cs and ^{90}Sr in mice.

Due to its high solubility and similar kinetics of Potassium, radioactive cesium is transferred as a free ion into the peripheral blood after oral administration, thus presenting an immediate systemic distribution [10]. The divergence between K^+ and Cs^+ ions deter-

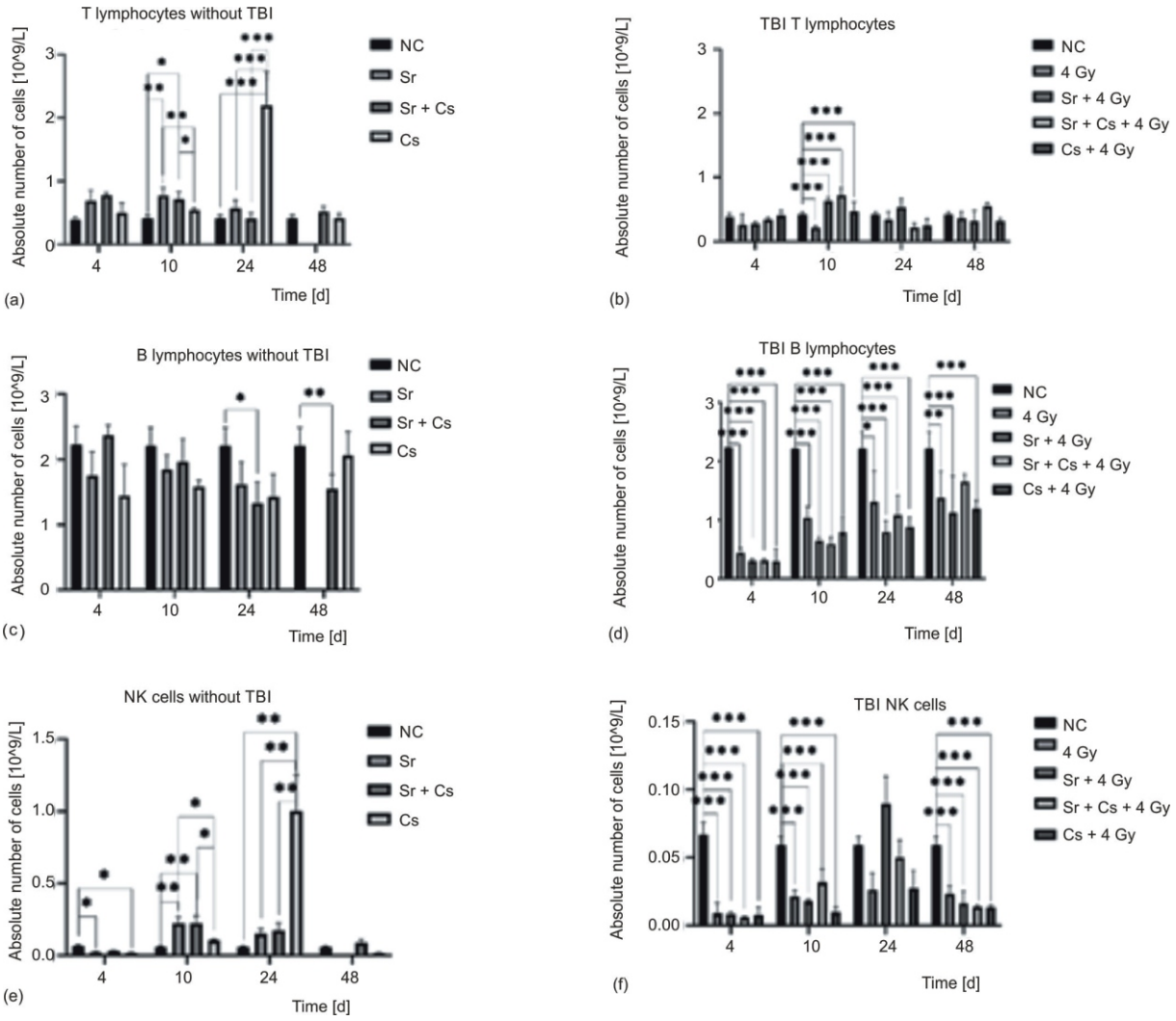


Figure 4. The NC, T, and B cell population analysis in non-irradiated and TBI-treated mice. The T lymphocytes (a), (b); B lymphocytes (c), (d); NC cells (e), (f). The data was obtained from six mice and is shown as the mean SEM. ANOVA and Tukey-Kramer test $p < 0.5$*, $p < 0.01$ $p < 0.001$***. Other missing values were lower than the MDA of the instrument**

mines their function within the organism; on the one hand, cesium is unable to completely replace the function of Potassium thus compromising normal cell function, especially in muscle and neural cells [10, 15]. Additional studies have shown a similar distribution of cesium in rats, mostly accumulating in muscles, skin, gastrointestinal tract, and liver and with only low concentrations found in the peripheral blood and brain shortly after oral or intraperitoneal administration [24]. In our study, we found a higher accumulation of ^{134}Cs in muscle and skeleton, with only minimal accumulation in teeth.

An autoradiographic study evaluated the distribution of cesium in rats after intraperitoneal administration of 1.5 Bq cesium over a period of 5 minutes to 3 months finding a higher concentration of ^{137}Cs in the abdomen and sinuses after 1 hour [25]. Nelson *et al*. reported that the accumulation of ^{137}Cs in teeth and bones could not be detected within the first hour after administration, further, the highest activity recorded was found in cartilage [25]. However, an increasing amount of ^{137}Cs could be observed in muscle after one day of administration, increasing during the following 3 days. The ^{137}Cs activity decreased significantly after one month in both muscle and bones [25]. In our study, ^{134}Cs activity decline in bones and muscle could be observed from days 4 to 46 after treatment. Contrary to the report by Nelson *et al*., [25] the activity of ^{134}Cs was not increased, probably due to the different sampling period.

Our experiments demonstrated that TBI in the presence of ^{85}Sr and ^{134}Cs had a significant effect on the accumulation and elimination rates of ^{134}Cs in both the skeleton and teeth and partially in muscles. On day 10 after ^{134}Cs administration, approximately 10 % of the initial activity could be measured in the muscles, at day 46. However, the measured activity values were two orders of magnitude lower. This suggests the relatively strong ability of an organism to eliminate cesium ions, very likely through urine. In this regard, a previous study demonstrated the high concentration of ^{137}Cs in the kidneys and urinary bladder of mice after

one day of treatment, thus supporting the hypothesis that the administered radionuclide is excreted through urine during the first few days [25].

In contrast, TBI-treated mice displayed a higher rate of cesium resorption. Carsten *et al*. [26] reported that, after whole-body irradiation, the number of bone marrow cells in the treated mice was drastically diminished showing a gradual recuperation over time; however, the hematopoietic stem cell population did not recover.

The exact location for ^{85}Sr accumulation in the gastrointestinal tract remains unclear. A previous study performed on hamsters suggests that ^{85}Sr could be accumulated in the stomach and small intestine [27]. Chemically soluble strontium gradually permeates into the bloodstream becoming systemically distributed in the form of protein complexes [27]. The excretion mechanism of strontium has been closely related to its initial form of entry, *e.g.* after an intravenous application of ^{85}Sr approximately 35 % is excreted through urine. Strontium can mimic the kinetics of calcium in the organism; therefore, it is accumulated in the surface of bones and the teeth [16], where approximately 99 % of the absorbed strontium becomes strongly bound.

Upon *i.v.* entry, Strontium rapidly finds its way into a critical organ, leading to a higher accumulation rate (55 %) and a lower elimination rate when compared to oral intake (11 %) [19]. The report by Nelson *et al*. [23] indicates that 5 minutes after intraperitoneal application, ^{90}Sr is mostly accumulated in the bones. However, the unabsorbed radionuclide remains within the peritoneal cavity. Four hours after administration, the incorporation of ^{90}Sr from soft tissues and peripheral blood into the bones is complete. Four days after administration, ^{90}Sr retention in the pineal and dentinal epiphyseal segments is now apparent, and no remaining radionuclide can be detected in soft tissues [25]. Buldakov and Moskalev [28] demonstrated that 42.6 % and 17.8 % of the administered ^{90}Sr are accumulated in the skeleton after 4 to 6 days. In apparent contradiction, our study revealed that ^{85}Sr activity could still be measured in muscles after 24 days. However, this may be due to the more sensitive method used in our protocol. Nelson *et al*. [25] also reported that the maximum activity values of ^{90}Sr in teeth were reached 1 hour after administration and remained constant for 16 days, quickly dropping afterward. In our study, the accumulation of ^{85}Sr had a growing tendency throughout the evaluation period. Previous studies demonstrated that the administration of higher ^{90}Sr doses (higher 8.0 0.3 MBq *vs*. low dose 200 0.3 kBq) had a direct effect on the ability of the organism to excrete the radionuclide in as much as a 6:4 ratio (low vs. high dose), moreover, a higher dose also influences the accumulation rate in the body [6].

The toxicity of strontium was previously evaluated during a study focused on the treatment of osteoporosis using strontium-ranelate, an anti-osteoporotic agent [29], proving that traces of strontium become heterogeneously incorporated into the skeleton [27]. Another study conducted on rats reported that strontium is highly accumulated in the hip joint (iliac crest), incisors, the lower mandible, and the skullcap (*calvaria*) [30]. A similar study performed on monkeys showed that a high strontium intake leads to its inclusion into the mineral structure of both compact and porous bone [31], being especially predominant in new bone [32]. The mechanisms for the inclusion and bonding strength of strontium into tooth and bone structures differ slightly. In teeth, strontium is often accumulated in dentine (*dentium*), enamel (*enamelum*), and cementum [33]. The mineral component of teeth plays an important role in its composition and its mostly represented by hydroxyapatite in various modifications. Under an excessive amount of strontium in the organism, approximately 1 out of 10 Ca^{2+} ions are replaced with the Sr^{2+} cations [30, 34], binding with the tooth's apatite crystal lattice instead of calcium.

It is common knowledge that an altered calcium balance leads to bone decalcification and that the exchange between extracellular fluid and bone depends strongly upon the level of physical activity. In general, 500 mg of calcium on average are eliminated from the bones, largely through stool [35]. These facts provide a clearer explanation for the results of our experiments. The radioactive strontium isotopes were gradually eliminated from the skeleton throughout the evaluation period. However, a gradual increment was detected in the teeth throughout the same. This increased accumulation could be explained by the higher elimination rate of calcium and strontium from the bones, besides, part of the strontium released into the bloodstream could be re-incorporated into the teeth. The structure of the enamel is also influenced by several factors, including diet. In our case, the mice were provided with untainted food and water and the bedding was changed daily. Therefore, these can be ruled out as potential strontium sources, assuming instead that the resorption of circulating ^{85}Sr into the muscles was insignificant.

Comparing our results in strontium and cesium biokinetics, it was apparent that the former was more strongly accumulated in the organism thus extending its elimination time in comparison with cesium, an observation that is consistent with another study [6]. Cesium is also characterized by a faster penetration rate into all tissues of the body and thus by a more even distribution resulting in a constant irradiation rate for most cells; in change, strontium mainly led to the irradiation of soft tissue cells in bone and adjacent tissues. The synergistic effect of strontium and cesium was probably due to the radioactive effect of ^{85}Sr incorporation in the bone. Interestingly, the excretion of cesium was influenced in part by TBI and by the presence of 85Sr within the organism.

A higher incidence of breakage was recorded when collecting the bone samples (bone fragility)

from the treated mice, thus impeding their extraction. Bone strength is dependent upon its mass and microarchitecture, further, mineralization influences the mechanical resistance and density of bones. Therefore, a higher propensity to mechanical damage after 24 to 46 days after the administration of strontium should be expected.

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AUTHORS´ CONTRIBUTIONS

The idea for the study and tissue collection was put forward by M. Nemcova. The radionuclide analysis, tissue processing, data evaluation, and interpretation were carried out by M. Nemcova and J. Janda. L. Andrejsova, and Z. Šinkorova were responsible for adjusting and measuring using a flow cytometer. A. Lierova and M. Jeličova were responsible for absolute blood cell count and immunophenotyping.

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СТУДИЈА О РАСПОДЕЛИ РАДИОНУКЛИДА ^{85}Sr и ^{134}Cs НАКОН ТОТАЛНОГ ОЗРАЧЕЊА ТЕЛА

Кинетика стронцијума, ^{85}Sr, и цезијум, ^{134}Cs, процењена је на експерименталном моделу миша како би се утврдио утицај ових радионуклида на живи организам у погледу тоталног озрачења тела. Наша студија показује да на стопу елиминације ^{134}Cs из костура и зуба утиче тотално озрачење тела и присуство ^{85}Sr. Већа акумулација и брже елиминисање ^{134}Cs забележене су у скелету и зубима тотално озрачених мишева којима се даје смеша радионуклида од ^{134}Cs + ^{85}Sr. Што се тиче ^{85}Sr, забележен је само минималан ефекат на његову акумулацију у скелетима, зубима и мишићима код тотално озрачених мишева. Ефекат мешавине радионуклида ^{85}Sr + ^{134}Cs на акумулацију ^{85}Sr био је очигледнији у зубима, показујући већу стопу задржавања након 10-24 дана примене код неозрачених мишева. Процена кинетике ових радионуклида пружила је преко потребан увид у њихове ефекте током прва два месеца након излагања, показујући да је брзина акумулације ^{85}Sr већа од стопе ^{134}Cs. Даље, стопа елиминације ^{85}Sr је спорија у поређењу са ^{134}Cs. Уочљиво је да тотално озрачење тела има већи ефекат на хематолошке параметре крви мишева од самих радионуклида.

Кључне речи: ^{85}Sr, ^{134}Cs, модел миша, унутрашња контаминација, тотално озрачење тела