EFFECT OF CYSTAMINE ON GASTRIC PROPULSIVE FUNCTION AND GAS EXCHANGE IN THE RAT MODEL OF RADIATION-INDUCED MYELOABLATION

Vakunenkova OA[1], Ivnitsky JuJu[1], Danilova OA[2], Schäfer TV[2] ✉, Rejniuk VL[1]

[1] Golikov Research Clinical Center of Toxicology of the Federal Medical Biological Agency, Saint-Petersburg, Russia

[2] State Scientific Research Test Institute of the Military Medicine of Defense Ministry of the Russian Federation, Saint-Petersburg, Russia

Radiation exposure of recipients before hematopoietic stem cell transplantation can cause gastrointestinal (GI) stasis. It is associated with complications of myeloablative radiation therapy: delayed vomiting, excess bacterial growth, endotoxicosis, systemic inflammation, and sepsis. The study was aimed to assess the possibility of GI stasis prevention by intragastric administration of cystamine dihydrochloride when using radiation-induced myeloablation. The severity of GI stasis, levels of enterocyte markers in the small intestinal tissues and the indicator of intestinal endotoxicosis, urinary indican excretion, were assessed in rats 72 h after the single total-body X-ray exposure to the dose of 9.64 Gy (1.1 $LD_{99/30}$); the animals' whole body oxygen consumption was recorded daily. Irradiation caused GI stasis with predominant gastric stasis, the 1.5–4.8-fold decrease in the cholinesterase and alkaline phosphatase activity in the small intestinal tissues, doubled the urinary indican excretion, the whole body oxygen consumption reduction by 17–32%. Cystamine administration generally prevented gastric stasis, but had no significant effect on the characteristics of radiation-induced enterocytopenia and did not prevent accumulation of chyme in the *caecum*, hyperindicanuria, radiation-induced spleen hypotrophy, and decrease in gas exchange rate. Cystamine is promising for testing in large animals as a selective agent for emergency prevention of gastric stasis during myeloablative radiation therapy.

Keywords: rats, radiation myeloablation, cystamine, gastrointestinal stasis, gastric stasis, indican, enterocytopenia, gas exchange

Author contribution: Vakunenkova OA — experimental procedure; Ivnitsky JuJu — rationale, developing the experimental model, data interpretation and discussion; Danilova OA — tissue biochemistry studies; Schäfer TV — experimental procedure, data processing and visualization, developing the experimental model; Rejniuk VL — methodological guidance of gas exchange assessment. All authors contributed to discussion, manuscript writing and editing.

Compliance with the ethical standards: the study was carried out in accordance with the principles of bioethics, approved by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

✉ **Correspondence should be addressed:** Timur V. Schäfer
Lesoparkovaya, 4, Saint-Petersburg, 195043, Russia; schafer@yandex.ru

Received: 29.09.2023 **Accepted:** 20.11.2023 **Published online:** 07.12.2023

DOI: 10.47183/mes.2023.050

ВЛИЯНИЕ ЦИСТАМИНА НА ПРОПУЛЬСИВНУЮ ФУНКЦИЮ ЖЕЛУДКА И ГАЗООБМЕН У КРЫС ПРИ ЛУЧЕВОЙ МИЕЛОАБЛЯЦИИ

О. А. Вакуненкова[1], Ю. Ю. Ивницкий[1], О. А. Данилова[2], Т. В. Шефер[2] ✉, В. Л. Рейнюк[1]

[1] Научно-клинический центр токсикологии имени академика С. Н. Голикова Федерального медико-биологического агентства, Санкт-Петербург, Россия

[2] Государственный научно-исследовательский испытательный институт военной медицины Министерства обороны Российской Федерации, Санкт-Петербург, Россия

Облучение реципиентов перед пересадкой стволовых кроветворных клеток способно вызвать желудочно-кишечный стаз (ЖКС). С ним связаны осложнения лучевой миелоабляционной терапии: поздняя рвота, избыточный бактериальный рост, эндотоксикоз, системное воспаление и сепсис. Целью работы было оценить возможность предупреждения ЖКС при лучевой миелоабляции профилактическим введением в желудок цистамина дигидрохлорида. У крыс определяли выраженность ЖКС, содержание маркеров энтероцитов в тканях тонкой кишки и показатель кишечного эндотоксикоза — экскрецию индикана с мочой — через 72 ч после общего однократного рентгеновского облучения в дозе 9,64 Гр (1,1 $ЛД_{99/30}$); ежедневно регистрировали потребление животными кислорода. Облучение вызывало ЖКС с преобладанием гастростаза, снижало активность холинэстеразы и щелочной фосфатазы в тканях тонкой кишки в 1,5–4,8 раза, вдвое повышало экскрецию индикана с мочой, на 17–32% снижало потребление кислорода организмом. Введение цистамина в основном предупреждало гастростаз, но не оказывало существенного влияния на показатели лучевой энтероцитопении, не предупреждало накопление химуса в слепой кишке, гипериндиканурию, лучевую гипотрофию селезенки и снижение интенсивности газообмена. Цистамин перспективен для апробации на крупных животных в качестве селективного средства экстренной профилактики гастростаза при лучевой миелоабляционной терапии.

Ключевые слова: крысы, лучевая миелоабляция, цистамин, желудочно-кишечный стаз, гастростаз, индикан, энтероцитопения, газообмен

Вклад авторов: О. А. Вакуненкова — выполнение экспериментальной части работы; Ю. Ю. Ивницкий — научный замысел, разработка экспериментальной модели, интерпретация и обсуждение результатов; О. А. Данилова — биохимические исследования тканей; Т. В. Шефер — экспериментальная часть, обработка и визуализация данных, разработка экспериментальной модели; В. Л. Рейнюк — методическое руководство исследованиями газообмена. Все авторы участвовали в обсуждении результатов, подготовке и редактировании рукописи статьи.

Соблюдение этических стандартов: исследование выполняли с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей.

✉ **Для корреспонденции:** Тимур Васильевич Шефер
Лесопарковая ул., д. 4, г. Санкт-Петербург, 195043, Россия; schafer@yandex.ru

Статья получена: 29.09.2023 **Статья принята к печати:** 20.11.2023 **Опубликована онлайн:** 07.12.2023

DOI: 10.47183/mes.2023.050

The term "myeloablation" was proposed in 1952 to define irreversible pancytopenia following the single total body X-ray exposure to a supralethal dose [1]. Radiation-induced myeloablation has found application in clinical practice: it is used to prepare recipients for hematopoietic stem cell transplantation; such preparation is referred to as "conditioning" [2]. Irradiation involving 1–3 fractions with the total doses of 8–12 Gy and transplantation of hematopoietic stem cells after 2–5 days is used for radical treatment of hemoblastoses, some solid tumors, myelodysplastic and autoimmune disorders [3]. In

individuals with acute leukemia, radiation-induced myeloablation is used solo or in combination with chemotherapy. In the latter case, it is considered as a method to combat chemoresistance in cancer cell clones [4]. Radiation-induced myeloablation is the main method to treat T-cell acute lymphoblastic leukemia in children and adults [5, 6]. Irradiation with myeloablative doses can also occur outside the clinic: during the first stage of the nuclear power reactor accident, under exposure to penetrating radiation of a nuclear explosion, when staying in the zones of dangerous or extremely dangerous radioactive contamination of the terrain with the nuclear explosion products [7].

The most prevalent and severe complications of myeloablative therapy are represented by the disorders referred to as "oral mucositis" and "gastrointestinal toxicities" in foreign clinical trials [8, 9]. Impaired regeneration of the gastrointestinal tract mucosal epithelium is a common pathogenetic basis of these disorders. Among organs of the gastrointestinal tract, damage to the small intestinal epithelium is the most important. That is why selective radiation shielding of the small intestinal mucosa seems to be a promising approach to prevention of the myeloablative radiation therapy gastrointestinal complications.

One of those is gastrointestinal (GI) stasis, the reversible dose-dependent slowing of the gastrointestinal transit of chyme. There are few reports of such clinical cases, however, the possibility of modeling GI stasis by exposure of rats [10], guinea pigs [11], dogs [12] and monkeys [13] to the doses exceeding 1 Gy suggests that GI stasis comlplicates myeloablative radiation therapy more often, but under the "mask" of other diagnoses. GI stasis occurred in 26% of recipients after the end of primary acute radiation-induced response and manifested itself in the form of nausea, vomiting, bloating and distension of the stomach; it was confirmed by scintigraphy [14].

The GI stasis clinical significance is determined by its influence on the radiation exposure outcome: it hampers the patients' nutrition, makes it pointless to prescribe oral drugs, contributes to damage to the gut-blood barrier with the influx of lipopolysaccharides of Gram-negative bacteria into the blood and the development of sepsis [15]. Its accompanying overgrowth of gastrointestinal microbiota results in realization of the quorum sensing effect, intensification of generation of toxic substances by bacteria, endotoxemia and endotoxicosis [16]. Some of these substances show pulmonary toxicity, and the stomach congested with chyme can limit diaphragmatic excursion. In some recipients, X-ray gastric shadow spreads to large parts of both abdominal and thoracic cavities after the course of myeloablative therapy [17]. That is why abnormal external respiration and gas exchange are potential effects of gastric stasis.

Perhaps, GI stasis is a defensive response to acute radiation-induced mucositis, the main pathogenetic link of which is represented by cytopenia. In this regard, it can be assumed that the drugs preventing cytopenia, radioprotectors, can prevent GI stasis. Of greatest interest are indralin, one of modern standard radioprotective agents [18], and cystamine dihydrochloride that has been earlier used as a radioprotective agent. The latter remained the only sulfur-containing radioprotector registered in our country for a long time; in 1960–2012, it was part of first-aid and sanitation kits for the military unit of the medical service of the Russian Armed forces. There is an experience of using the substance in clinical practice [19]. Despite the fact that this drug is not listed in the State Register of Medicines as at 20 November 2023, it seems reasonable to test the drug as an agent for pathogenetic prevention of radiation-induced GI stasis. The study was aimed to test the hypothesis that cystamine dihydrochloride administered by intragastric route prevented GI stasis, endotoxicosis and gas exchange abnormalities in the rat models of radiation-induced myeloablation.

METHODS

The study involved male Wistar rats (161–190 g), purchased from the Rappolovo laboratory animal nursery. The diet included standard rat food and ad libitum access to water. Animals were randomized into experimental groups. To be deprived of food, rats were placed in the slatter floor cages, preventing coprophagy and consumption of the bedding components, with access to water only.

The time period of myeloablative conditioning was an order of magnitude shorter than the half-time of recovery after radiation exposure (25–45 days in humans). That is why, despite the fact that the myeloablative exposure dose is usually fractionated, the effective value does not differ significantly from the value of the sum of fractions. Therefore, radiation-induced myeloablation was modeled by the single X-ray irradiation in the multifunctional mobile X-ray apparatus (ELTECH-MED; Russia). Rats were placed in the polyethylene terephthalate pencil cases (eight rats per pencil case) positioned radially head to center in the circular polymethyl methacrylate rack. Irradiation parameters: focal range — 564 mm; anode voltage — 60 kV; anode current — 13 mA; filter: polymethyl methacrylate 8 mm + polyethylene terephthalate 0.4 mm; absorbed dose to the geometric center of the body — 9.64 Gy (1.1 $LD_{99/30}$). This dose was identified based on preliminary assessment of the dose dependence of the average life expectancy of exposed rats as a maximum dose, with which life expectancy of all animals was at least 3 days after exposure; in humans this corresponds to the average time period of myeloablative conditioning. The radiation dose rate was 0.27 Gy/min. The minimum to maximum body's exposure dose ratio was 0.9 in the caudo-cranial and 0.5 in the ventro-dorsal direction. Irradiation that lasted for 52 min was applied in three fractions of 12 min with two intervals of 8 min. In preliminary experiments, such exposure resulted in the development of pancytopenia syndrome after 3 days, reduction of relative weight of the spleen by 62 % and femoral bone marrow by 41 %, DNA density in these tissues by 2 and 1.9 times, respectively, as well as in the animals' death after 5.9 ± 1.5 days (M ± *m*, *n* = 16).

Laparotomy and organ harvesting were performed under the mask halothane anaesthesia. The GI stasis severity was assessed based on the relative weight of chyme in the stomach and *caecum* calculated as a difference between the weight of the organ filled with chyme and the weight of the empty organ (*gaster*, *caecum*) in grams relative to body weight in kilograms.

In the first phase of the study we assessed the dynamics of GI stasis following myeloablative conditioning. For that animals were divided into eight groups, among them four were represented by animals deprived of food 2, 24, 48 or 72 h after exposure, while the other (control) ones were represented by animals deprived of food within the same time frame, but non-exposed and provided unlimited access to water. After 72 h severity of GI stasis in animals was assessed.

In the second phase we assessed the cystamine dihydrochloride effects on the GI stasis severity, growth rate of gastrointestinal microbiota and the levels of enterocyte markers in the small intestinal tissues. We used rats having unlimited access to water, but deprived of food between 24 and 72 h after exposure. Animals were divided into three groups, among which the first group was represented by intact animals not receiving cystamine and other groups consisted of exposed animals. Animals of the third group received intragastric injection of 10

mL/kg of the cystamine dihydrochloride (synthesized in the State Scientific Research Test Institute of the Military Medicine of Defense Ministry of the Russian Federation) aqueous solution in a dose of 120 mg/kg 30 min before the beginning of irradiation. This dose, based on the body weight to body surface area ratio of humans, is bioequivalent to the drug dose of 1.2 g prescribed to be taken 30–40 min before irradiation, i.e. to the content of the case contained in individual first-aid kits AI-1, AI-1M and AI-2. Rats were placed in metabolic cages for urine collection 48 h after the exposure. Animals were subjected to laparotomy 72 h after the exposure to assess the GI stasis severity, proximal sections of the *duodenum*, *jejunum* and distal sections of the *ileum* were retrieved. To assess the radioprotector effect selectivity, relative weight of the spleen was determined along with the relative weight of the *gaster* and *caecum* chyme as a measure of myeloprotective effect.

In the third phase we assessed the dynamic changes in the gas exchange and external respiration indicators for 3 days after irradiation of unprotected animals or animals receiving cystamine.

The gastrointestinal microbiota growth rate was assessed based on the urinary indican excretion. The levels of indican in the urine collected within 24 h were determined by the quantitative colorimetric method [20]; indican excretion was expressed in micrograms per kilogram of body weight per hour.

Enterocytopenia was quantified based on the enterocyte membrane marker activity in the tissues of the *duodenum*, *jejunum* and *ileum*: cholinesterase (ChE) and alkaline phosphatase (ALP). The small intestinal segments with the length of 4 cm were weighed, homogenized in the 15-fold larger volume of the Tris-HCl buffer solution (50 mmol/L, pH 7.4), frozen at –20°C, thawed 15 h later at 4°C and centrifuged for 10 min at 2000 g. The supernatant protein content was determined using the Bradford assay. The ChE activity was determined by the Ellman's method in the ChemWell 2910 biochemical analyzer (Awareness Tech.; USA) using acetylthiocholine iodide as a substrate. The ALP activity was measured by the kinetic method using the reagent kit (Olvex Diagnosticum LLC; Russia) at 37°C in the ChemWell 2910 biochemical analyzer (Awareness Tech.; USA).

The whole body oxygen consumption was determined in the apparatus constructed by Miropolsky. Animals were habituated to the respirometry chamber for two days before the beginning of the study. The following equation was used to calculate the whole body oxygen consumption (Q_{O2}, mL/(kg · min)):

$$Q_{O2} = V \cdot F/(m \cdot \Delta t),$$

where V was the volume of manometric fluid in the burette, mL; F was a coefficient used to adjust the oxygen volume to standard conditions; m was the aminal's body weight, kg; Δt was the length of the rat's stay in the sealed chamber, min.

The duration of measurement was 3 min, its absolute error was 0.1 mL (≤ 2 % of V value), and the respirometry chamber volume was 0.9 L. Animals were not secured, they could move freely in the respirometry chamber and looked dazed. During this time the animals' respiratory rate (RR, min^{-1}) was determined, which was considered as a measure of external respiration. The whole body average oxygen consumption per respiratory cycle (mL/kg) calculated as a ratio of Q_{O2} to RR was used as a measure of the external respiration efficiency. The Q_{O2}, RR and Q_{O2}/RR values calculated after irradiation were expressed as a percentage of baseline level taken as 100%.

The results were presented as mean and error of the mean (M ± *m*). The effects of radioprotector on the studied quantitative characteristics were assessed using analysis of variance. When the differences obtained were significant, the intergroup comparison of mean values was performed using the Tukey's honest significance test. The correlations between characteristics were represented as the Spearman's rank correlation coefficients (r_s). The α-value of 0.05 was considered to be a critical significance level.

RESULTS

In rats deprived of food within 48 h before laparotomy, the stomach that was dilated and filled with chyme occupied most of the abdominal cavity 72 h after irradiation; it looked empty in intact animals. The volume of the caecum increased to the lesser extent after the exposure (Fig. 1). Food consumed after irradiation accumulated in the stomach throughout the time of observation, which resulted in the progressive increase in

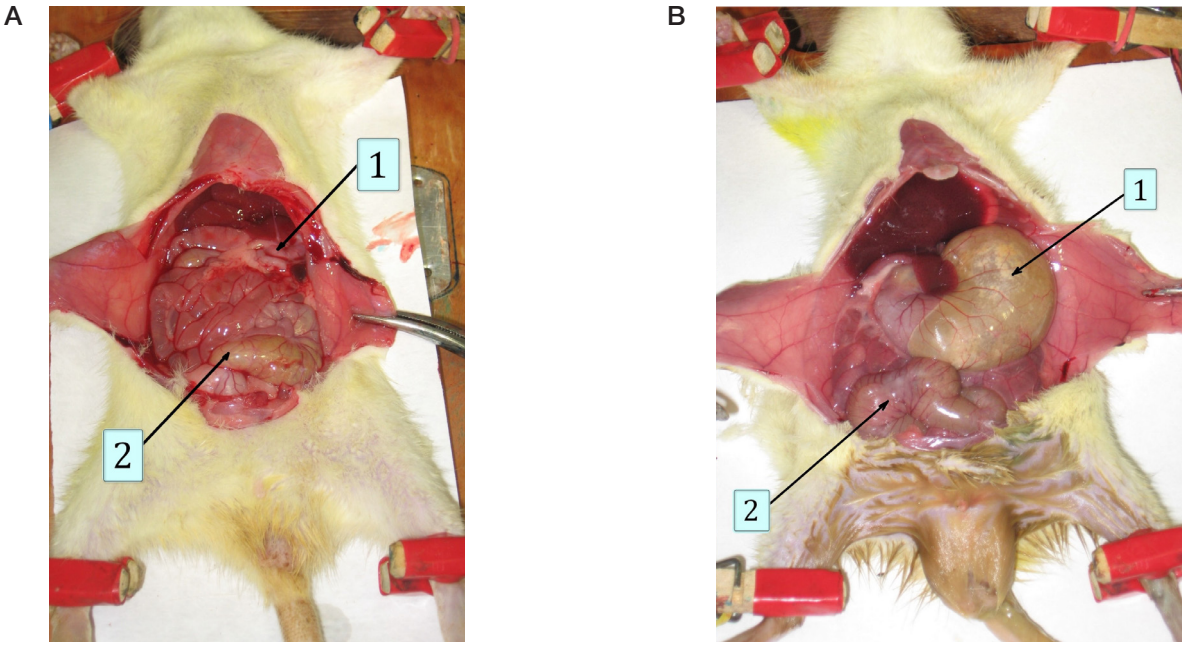

Fig. 1. Abdominal organs of rats deprived of food 48 h before laparotomy. **A**. Intact. **B**. 72 h after single total body X-ray exposure at a dose of 9.64 Gy. Arrows: 1 — stomach; 2 — *caecum*

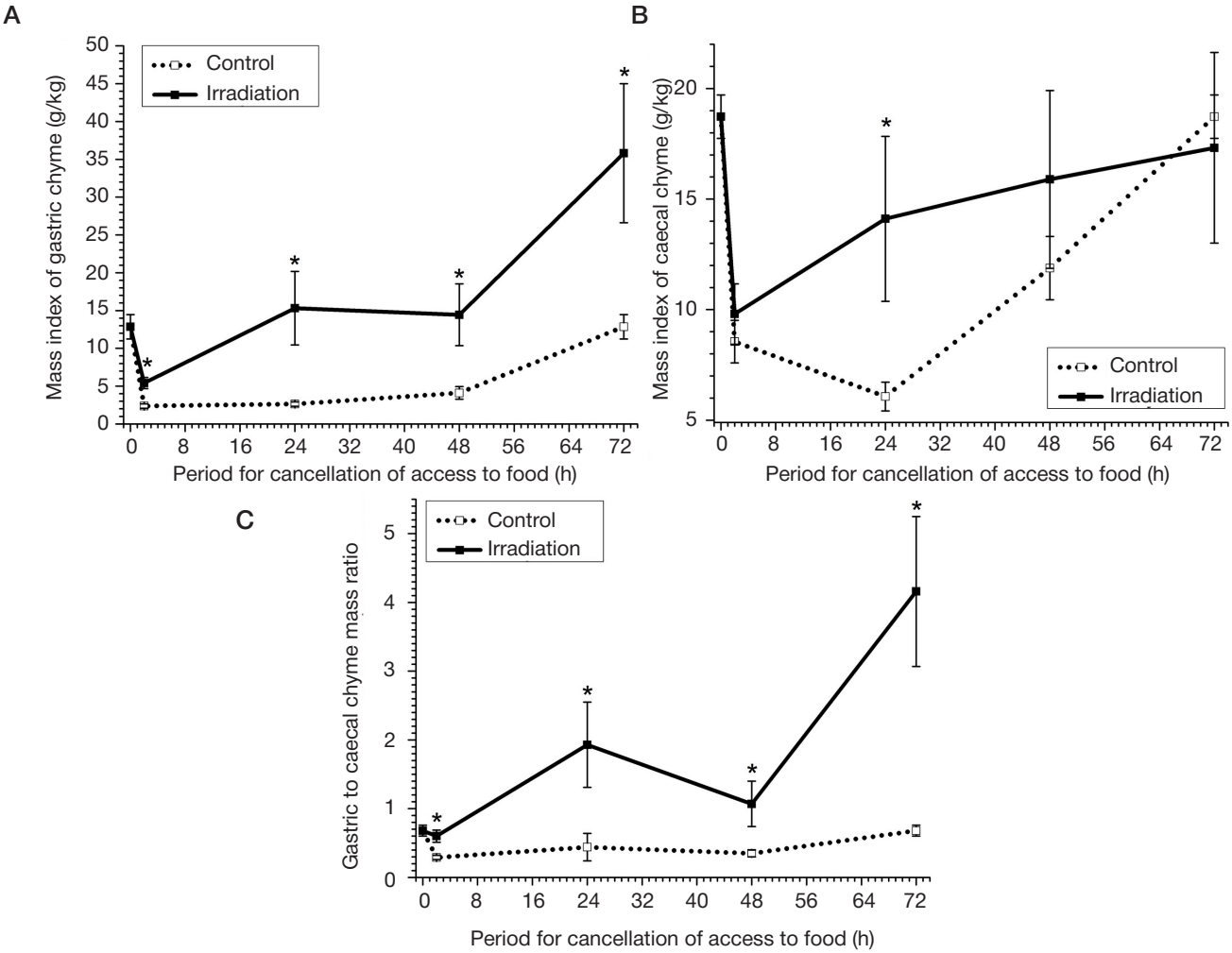

Fig. 2. Mass indexes of gastric chyme (**A**), caecal chyme (**B**) and their ratio (**C**) in rats 72 h after the single total body X-ray exposure at a dose of 9.64 Gy, M ± *m*, *n* = 8, depending on the duration of access to food since the time of irradiation. Control — non-exposed animals. Values of the group of non-exposed rats which had the unlimited access to food are at the zero mark of horizontal axis. * — significant difference from control, $p < 0.05$

the gastric chyme relative weight. Accumulation of chyme in the *caecum* was slower, which increased the gastic to caecal chyme weight ratio by 2–6 times depending on the duration of access to food relative to corresponding values of non-exposed animals (Fig. 2).

In exposed rats not receiving cystamine and deprived of food 24 h after irradiation, body weight was 78.9 ± 1.1% of the baseline value 72 h after exposure. In non-exposed animals deprived of food within the same time frame, it was 84.6 ± 0.7% of the baseline value ($p < 0.05$). Furthermore, the relative weight of gastric and caecal chyme in exposed rats was 5.9 and 2.3 times higher than that of intact rats, respectively. Cystamine administration before irradiation partially prevented gastric stasis: the relative weight of gastic chyme was on average three times lower than that of unprotected animals. The use of cystamine returned the gastic to caecal chyme weight ratio of 1.1 ± 0.2 in unprotected animals to the value of 0.4 ± 0.2 typical for intact rats, with equal duration of access to food ($p < 0.05$). Cystamine had no significant effect on the relative weight of caecal chyme in exposed rats. Cystamine administration also had little effect on the radiation-induced hypotrophy of the spleen (Fig. 3A). Urinary indican excretion measured 72 h after irradiation was on average twice higher than that of intact rats; cystamine had no significant effect on indicanuria (Fig. 3B). Indican excretion by the exposed rats receiving no radioprotector negatively correlated with the relative weight of gastric chyme, $r_s = -0.77$, and positively correlated with the relative weight of caecal chyme, $r_s = 0.68$ ($p < 0.05$); weak correlation was observed against the background of cystamine administration. Irradiation decreased the activity of enterocyte markers (ChE and ALP) in the small intestinal tissues. The most significant decrease (4.8-fold) in the ChE activity was observed in the *ileum*. The values of ChE activity in all parts of the small intestine and ALP activity in the duodenum and ileum tended to moderately exceed these values of unprotected rats against the background of using cystamine. This most prominent increase (2.5-fold) was reported for ChE in the ileum, however, it was represented in the form of the trend only (Fig. 3C and D).

The whole body oxygen consumption was lower than that of intact animals throughout the period after irradiation. On day three, this trend was significant when calculating per both time unit and respiratory cycle; the trend was stronger in the latter case. Intergroup differences in RR were insignificant. Cystamine administration had little effect on the gas exchange and external respiration characteristics (Fig. 4).

DISCUSSION

Modelling myeloablative radiation therapy in rats was associated with deep inhibition of the stomach propulsive function developing in the first hours after irradiation. The time of the chyme gastric transit exceeded two days, while the normal values of healthy humans do not exceed 48 min [21]. Extrapolation of these data to humans shows that gastric stasis

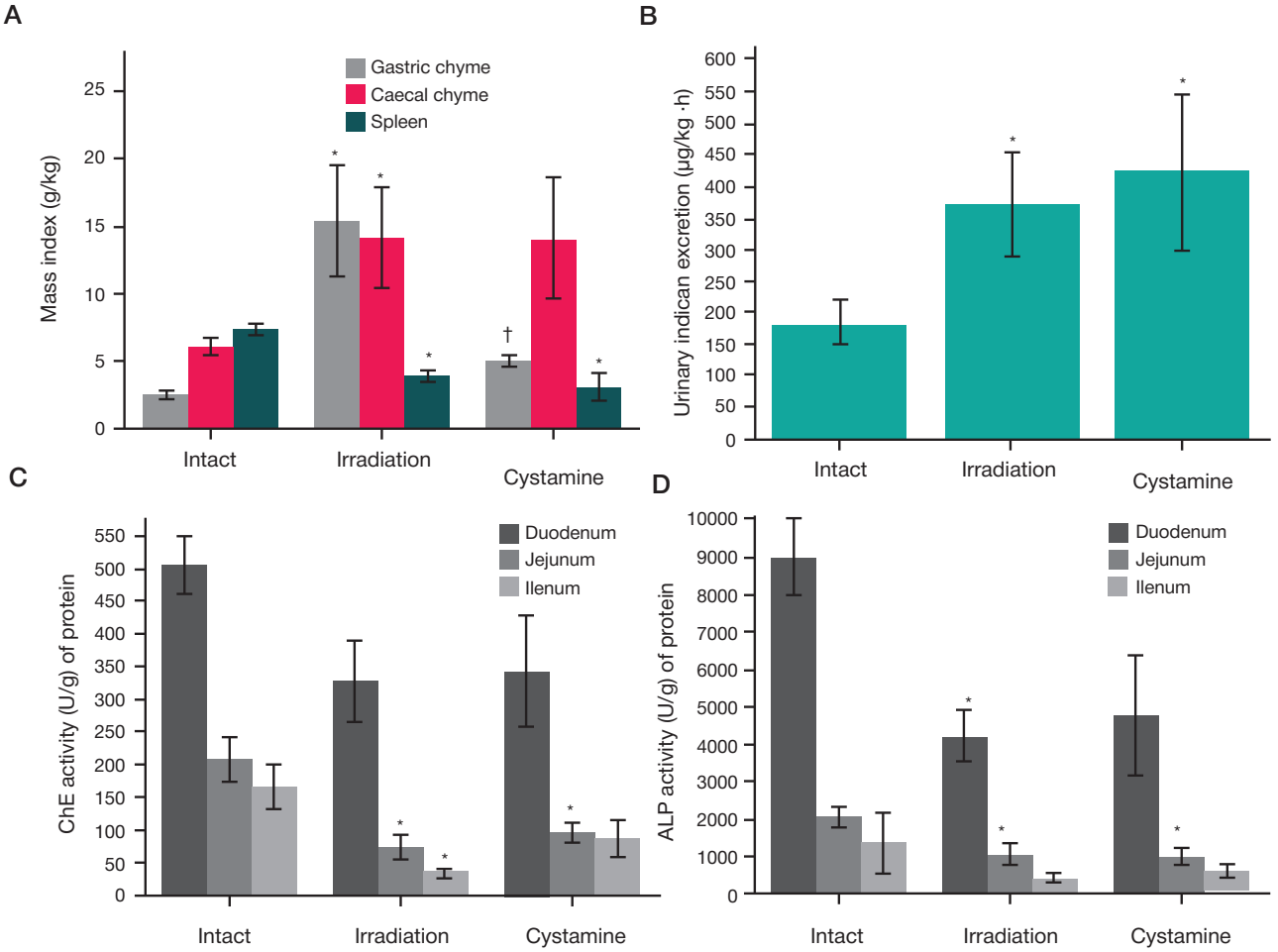

Fig. 3. Mass indexes of gastric chyme, caecal chyme and the spleen (**A**), urinary indican excretion (**B**), cholinesterase (**C**) and alkaline phosphatase (**D**) activity in the intestinal tissues of rats 72 h after the single total body X-ray exposure to the dose of 9.64 Gy, M ± *m*, *n* = 8, depending on the duration of access to food since the time of irradiation. "Intact" — non-exposed rats which obtained no medication. "Irradiation" — rats exposed without administration of radioprotector. "Cystamine" — intragastric administration of cystamine dihydrochloride in a dose of 120 mg/kg 30 min before the beginning of irradiation. All animals were deprived of food 24 h after irradiation. Significant difference, $p < 0.05$: * — from intact group; † — from "Irradiation" group

persists for most of the myeloablative conditioning course. It can be associated with the complaints typical for such patients: loss of appetite, nausea, vomiting, pain, epigastric heaviness and bloating. Rodents lack emesis relieving the stomach; that is why the stomach overfilling could be more prominent in rats, than in humans exposed to equal doses.

Despite inhibition of the chyme release into the caecum resulting from gastric stasis, the relative weight of caecal chyme measured 3 days after irradiation was 2.3 times higher than that of non-exposed animals, which reflected the decrease in the colonic propulsive function. The total relative weight of gastric and caecal chyme increased by 3.4 times in exposed rats: on average to 29.5 vs. 8.8 g/kg in controls. Despite accumulation of chyme, body weight after irradiation was 7% lower than in non-exposed animals fasting during the same time period, which indicates possible involvement of GI stasis in deterioration of body's general condition. Intestinal endotoxicosis, indicated by the two-fold increase in the urinary indican (indoxyl sulfate) excretion, could be one of the mechanisms underlying such an effect. Indoxyl sulfate is the end product of indole oxidation to indoxyl and its sulfonation in the liver. The reaction catalyzed by the gut microbiota-derived tryptophanase is the only source of indole in the body. Toxicity is exhibited by both indoxyl sulfate concentration two order of magnitude exceeding physiological levels [22] and indole [23]. Hyperindicanuria is indicative of more intense production of ammonia, the other toxic product of the tryptophanase reaction, in the gastrointestinal tract, along with indole. Endotoxicosis may involve other intestinal toxic substances and products of their biotransformation: bacterial endotoxin, *p*-cresol, *p*-cresyl sulfate, trimethylamine, trimethylamine N-oxide, influx of which into blood is increased when there is GI stasis [22].

The content of bacteria in the colonic chyme, 10^{11} mL^{-1}, is eight orders of magnitude higher than that in the gastric lumen, ≤ 10^{3} mL^{-1} [24]. That is why accumulation of chyme in the caecum played a major role in the development of intestinal endotoxicosis. Under these circumstances, gastric stasis that slowed chyme entry into the *caecum* could limit intestinal endotoxicosis. This is indicated by negative correlation between the relative weight of gastric chyme and the urinary indican excretion, as well as by positive correlation between the latter and the relative weight of caecal chyme in exposed rats not receiving radioprotector.

The gastric stasis protective role could come not only from its inhibiting effect on production of toxic substances in the intestine, but also from prevention of further damage to the small intestinal epithelium by chyme released from the stomach under conditions of emerging enterocytopenia. It was indicated by the decrease in the enterocyte marker (ChE and ALP) activity in the small intestinal tissues after irradiation.

A more than three-fold decrease in the relative weight of gastric chyme associated with intragastric administration of cystamine resulted from its local radioprotective effect on the gastric mucosa (Fig. 3A). This was evident from the lack of

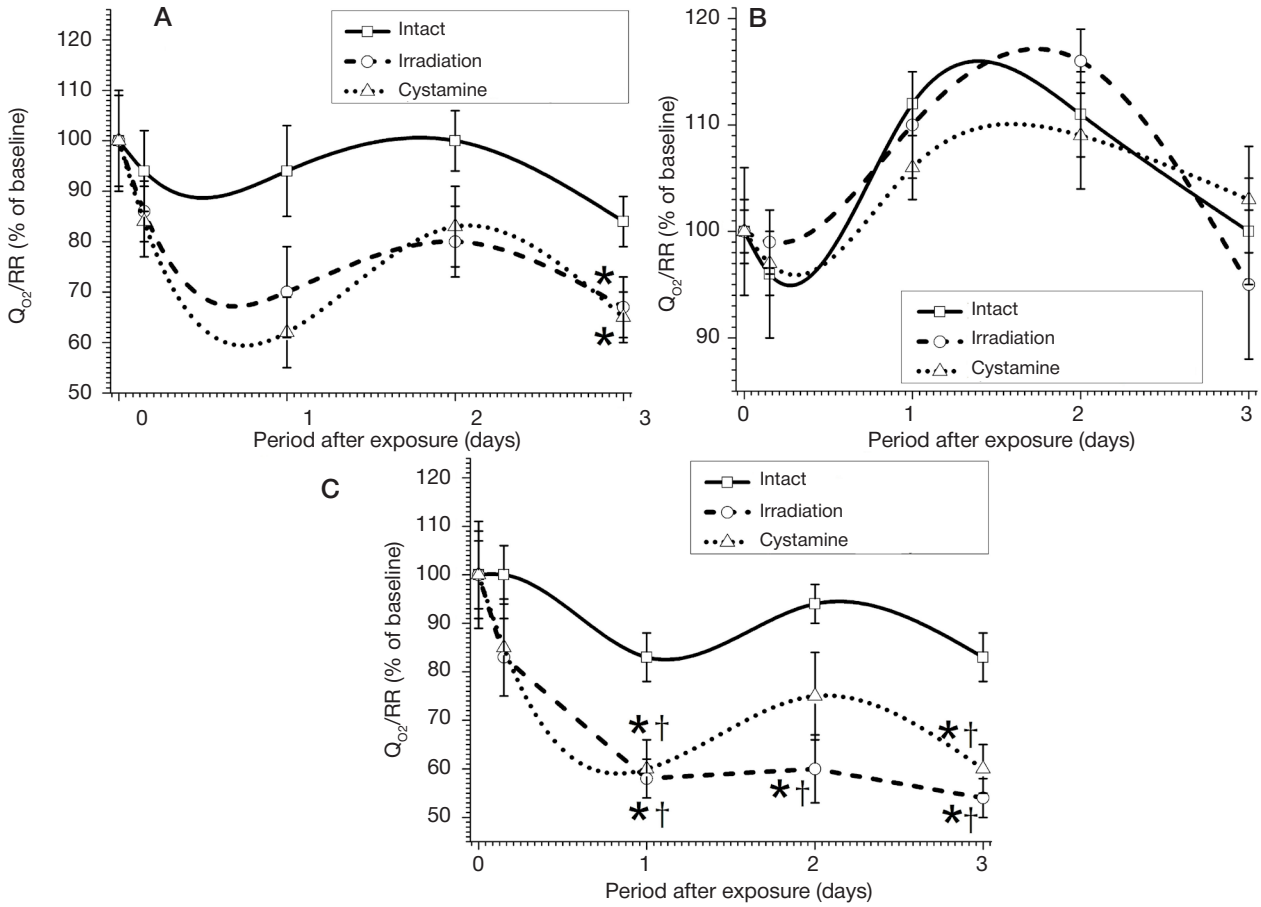

Fig. 4. The whole body oxygen consumption (**A**), respiratory rate (**B**) and oxygen consumption per respiratory cycle (**C**) in rats 72 after the single total body X-ray exposure at a dose of 9.64 Gy, M ± *m*, *n* = 8. Q_{O2} — the whole body oxygen consumption; RR — respiratory rate. 100% is the parameter values determined at 4 h before irradiation. * — significant difference from baseline, $p < 0.05$; † — significant difference from intact group, $p < 0.05$

significant cystamine effect on the radiation-induced spleen hypotrophy, the sensitive indicator of systemic radioprotector effects. Such a result is consistent with impossibility to reproduce the cystamine systemic radioprotective effect by itragastric cystamine administration to rats reported in the literature [19]. Cystamine prevented gastric stasis, despite its thiol form (cysteamine) capability of reversible inhibition of gastric chyme evacuation via enchanced hydrochloric acid secretion by the gastric parietal cells known from the literature [25].

Gastric stasis prevention could not be mediated by the cystamine local radioprotective effect in the small intestinal mucosa: the effect was weak, which was evident from the lack of significant influence on the enterocytopenia characteristics, ChE and ALP activity in the small intestinal tissues (Fig. 3C and D). It can be assumed that the anatomical structure of the rat stomach lead to the fact the radioprotector solution failed to enter the small intestine till the end of irradiation and contacted mostly with the gastric mucosa.

The hypothesis of gastric stasis prevention as a result of cystamine local radioprotective effect on the gastric mucosa is consistent with the emergence of GI stasis in rats after local irradiation of the abdomen reported more than 70 years ago, while irradiation with equal doses without abdominal shielding never causes GI stasis [10]. The findings suggest that the GI stasis triggers are localized in the mucous membranes of appropriate gastrointestinal tract parts and can be “switched off” through local exposure to cystamine.

In case of ingestion of equivalent cystamine dihydrochloride dose (1.2 g) by humans 30–40 min before radiation exposure to the dose causing bone marrow syndrome, the nominal radiation dose change factor is 1.4 [7]. This means that in case of ingestion of the recommended dose of cystamine by humans, gastric stasis prevention would be associated with systemic radioprotective effect that is unwelcome when preparing patients for hematopoietic stem cell transplantation. That is why selectivity of emergency gastric stasis prevention involving cystamine during exposure of rats to myeloablative doses cannot be unconditionally extrapolated to humans. Using cystamine to prepare patients for hematopoietic stem cell transplantation requires determination of the conditions for realization of its capability of preventing gastric stasis without exhibiting myeloprotective activity in large animals.

In this study we assessed gas exchange under conditions that were close to the conditions for determination of basal metabolic rate, that is why the oxygen consumption decrease observed in exposed animals could not result from their dazed state. The finding is consistent with the reduced oxygen consumption in rats earlier followed up for 3 days after the X-ray exposure to the doses of 300–1000 R [26]. Gas exchange inhibition could not result from reduction of respiratory volume due to restriction of diaphragmatic excursion by the dilated stomach: this was evident from the lack of significant irradiation effect on the RR (Fig. 4B). This also could not result from the direct damaging effect of the applied radiation dose on the tissue energy metabolism: there is no information about such effect in the literature. Reduced whole body oxygen consumption could be a manifestation of intestinal endotoxicosis indicated by the increased urinary indican excretion in exposed animals (Fig. 3B). Such gut microbiota products, as bacterial endotoxin and *p*-cresyl sulfate, are characterized by the capability of

causing damage to the blood-gas barrier followed by pulmonary edema [27, 28]. Indoxyl sulfate and bacterial endotoxin damage mitochondria, thereby disturbing oxygen utilization at the cellular level [29, 30]. The hypothesis of the intestinal endotoxicosis involvement in the effect of gas exchange reduction following radiation exposure is supported by no effect of cystamine on the latter; preventive cystamine administration did not prevent hyperindicanuria.

The data obtained show that the pathogenetic approach to prevention of gastric stasis caused by myeloablative radiation exposure involving the use of radioprotectors is promising. This approach cannot be considered as an alternative to using symptomatic drug treatment to relieve primary systemic response to irradiation (particularly, treatment with 5-HT_3 receptor antagonists).

CONCLUSIONS

The single total-body X-ray exposure of rats to the dose of 9.64 Gy corresponding to that used for myeloablative conditioning leads to the decrease in enterocyte counts in the small intestinal mucosa after 3 days, as well as to gastrointestinal stasis with predominant gastric stasis and hyperindicanuria being an indicator of excessive growth of intestinal microbiota producing indole. Intragastric administration of the cystamine dihydrochloride dose equivalent to that recommended as a single dose for humans to rats 30 min before irradiation partially prevents gastric stasis and has no significant influence on the characteristics of enterocytopenia, caecal stasis, as well as on the hyperindicanuria severity.

Modelling radiation-induced myeloablation in rats is associated with reduction of the animals' oxygen consumption not resulting from the influence of gastric stasis on the diaphragmatic excursion within 3 days after exposure. Intragastric administration of cystamine prior to irradiation does not prevent this effect. In rats, local radioprotective effect of cystamine dihydrochloride injected in the stomach is not associated with the emergence of the signs of systemic radioprotective effect, which, when repoduced in large laboratory animals, makes this drug a promising agent for prevention of gastric stasis during myeloablative radiation therapy.

References

1. Lorenz E, Congdon C, Uphoff D. Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology. 1952; 58 (6): 863–77.
2. Savchenko VG, redaktor. Protocoly transplantacii allogennyh gemopoeticheschih stvolovyh cletok. M.: Praktika, 2020; 320 s.
3. Pop VP, Rukavicyn OA. Allogeneic hematopoietic stem cell transplantation: prospects and alternatives, own experience. Ros zhurn detsk gematol onkol. 2017; 4 (2): 46–69. Russian.
4. Keit E, Liveringhouse C, Figura N, Weigand J, Sandoval M, Garcia G, et al. Feasibility and toxicity of full-body volumetric modulated arc-therapy technique for high-dose total body irradiation. Technol Cancer Res Treat. 2023; 22: 15330338231180779.
5. Battipaglia G, Labopin M, Mielke S, Ruggeri A, Zubeyde Nur Ozkurt, Bourhis J, et al. Thiotepa-based regimens are valid alternatives to total-body irradiation-based reduced-intensity conditioning regimens in patients with acute lymphoblastic leukemia: a retrospective study on behalf of the acute leukemia working party of the European society for blood and marrow transplantation. Transplant Cell Ther. 2023; Oct 8: S2666-6367(23)01582-8. Online ahead of print.
6. Cahu X, Labopin M, Giebel S, Aljurf M, Kyrcz-Krzemien S, Socié G, et al. Impact of conditioning with TBI in adult patients with T-cell ALL who receive a myeloablative allogenic stem cell transplantation: a report from the acute leukemia working party of EBMT. Bone marrow Transplantation. 2016; 51 (3): 351–7.
7. Drachyov IS, Zacepin VV, Ivanchenko AV, Ivnitsky JuJu, Kryukov EV, Seleznyov AB. Acute lesions resulting from external irradiation of the human body. In book: Sofronov GA, Kryukov EV, ed. Military toxicology, radiology and medical protection. Saint Petersburg: VMedA, 2023; p. 507–39. Russian.
8. Konishi T, Ogawa H, Najima Y, Hashimoto S, Wada A, Adachi H, et al. Safety of total body irradiation using intensity-modulated radiation therapy by helical tomotherapy in allogenic hematopoietic stem cell transplantation: a prospective pilot study. J Radiat Res. 2020; 61 (6): 969–76.
9. Nakagaki M, Kennedy G, Gavin N, Clavarito A, Whitfield K. The incidence of severe oral mucositis in patients undergoing different conditioning regimens in haematopoietic stem cell transplantation. Support Care Cancer. 2022; 30 (11): 9141–9.
10. Conard RA. Effect of X-irradiation on intestinal motility of the rat. Am J Physiol. 1951; 165 (2): 375–85.
11. Krantis A, Rana K, Harding R. The effects of γ-radiation on intestinal motor activity and faecal pellet expulsion in the guinea pig. Dig Dis Sci. 1996; 41 (12): 2307–16.
12. Erickson BA, Otterson MF, Moulder JE, Sarna SK. Altered motility causes the early gastrointestinal toxicity of irradiation. Int J Radiat Oncol. Biol. Phys. 1994; 28 (4): 905–12.
13. Dorval ED, Mueller GP, Eng RR, Durakovic A, Conclin JJ, Dubois A. Effect of ionizing radiation on gastric secretion and gastric motility in monkeys. Gastroenterology. 1985; 89 (2): 374–80.
14. Eagle D, Gian V, Lauwers G, Manivel J, Moreb J, Wingard J. Post-transplant complications. Gastroparesis following bone marrow transplantation. Bone Marrow Transplantation. 2001; 28: 59–62.
15. Chapman MJ, Nguyen NQ, Deane AM. Gastrointestinal dysmotility: clinical consequences and management of the critically ill patient. Gastroenterol Clin North Am. 2011; 40 (4): 725–39.
16. Patel R, Soni M, Soyantar B, Shivangi S, Satarija S, Saraf M, et al. A clash of quorum sensing vs quorum sensing inhibitors: an overview and risk of resistance. Arch Microbiol. 2023. 205 (4): 107.
17. Annede P, Prieux-Klotz C, Dubergé T, Chargari C, Gisserot O, de Jaureguiberry J-P. Radiation induced gastroparesis – case report and literature review. J Gastrointest Oncol. 2017; 8 (4): E52–5.
18. Vasin MV. The drug B-190 (indralin) in the light of the history of the formation of ideas about the mechanism of action of radioprotectors. Rad Biol Radioecol. 2020; 60 (4): 378–95. Russian.
19. Kuna P. Chemical radioprotection: Transl. from Czech. M.: Medicina, 1989; p. 192. Russian.
20. Balahovskij SD, Balahovskij IS. Methods of chemical analysis of blood. 3th ed. M.: Medgiz, 1953; p. 746. Russian.
21. O'Grady J, Murphy CL, Burry L, Shanahan F, Buckly M. Defining gastrointestinal transit time using video capsule endoscopy: a study of healthy subjects. Endosc Int Open. 2020; 8 (3): E396–E400.
22. Ivnitsky JuJu, Schäfer TV, Rejniuk VL, Golovko AI. Endogenous humoral determinants of vascular endothelial dysfunction as triggers of acute poisoning complications. J Appl Toxicol. 2023; 43 (1): 47–65.
23. Martynova NA, Gorohova LG. Toxicological evaluation of indole. Bjul. VSNC SO RAMN. 2006; 65 (1): 248–51. Russian.
24. Sender R, Fuchs S. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016; 14 (8): e1002533.
25. Tanaka H, Takeuchi K, Okabe S. Role of accumulated gastric content in the pathogenesis of cysteamine- and mepirizole-induced duodenal ulcers in the rat. J Intern Med Suppl. 1990; 732: 69–75.
26. Mole RH. The effect of X-irradiation on the basal oxygen consumption of the rat. Q J Exp Physiol Cogn Med. Sci. 1953; 38 (2): 69–74.
27. Russ M, Boerger E, von Platen P, Francis R, Taher M, Boemke W, et al. Surfactant depletioncombined with injurious ventilation results

in a reproducible model of the acute respiratory distress syndrome (ARDS). J Vis Exp. 2021; 170: e62327.
28. Chang J, Liang S, Thanasekaran P, Chang H, Wen L-L, Chen C, et al. Translational medicine in pulmonary-renal crosstalk: therapeutic targeting of p-Cresyl sulfate triggered nonspecific ROS and chemoattractants in dyspneic patients with uremic lung injury. J Clin Med. 2018; 7 (9): 266.
29. Thome T, Salyers Z, Kumar R, Hang D, Berru F, Ferreira L, et al. Uremic metabolits impair skeletal muscle mitochondrial energetics through disruption of the electron transport system and matrix dehydrogenase activity. Am J Physiol Cell Physiol. 2019; 317 (4): C701–13.
30. Kim Y-S, Lee H, Lee M, Park Ye, Sehwan M, et al. The effect of mitochondrial transplantation on sepsis depend on the type of cell from which they are isolated. Int J Mol Sci. 2023; 24 (12): 10113.

Литература

1. Lorenz E, Congdon C, Uphoff D. Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology. 1952; 58 (6): 863–77.
2. Савченко В. Г., редактор. Протоколы трансплантации аллогенных гемопоэтических стволовых клеток. М.: Практика, 2020; 320 с.
3. Поп В. П., Рукавицын О. А. Аллогенная трансплантация гемопоэтических стволовых клеток: перспективы и альтернативы, собственный опыт. Рос. журн. детск. гематол. онкол. 2017; 4 (2): 46–69.
4. Keit E, Liveringhouse C, Figura N, Weigand J, Sandoval M, Garcia G, et al. Feasibility and toxicity of full-body volumetric modulated arc-therapy technique for high-dose total body irradiation. Technol Cancer Res Treat. 2023; 22: 15330338231180779.
5. Battipaglia G, Labopin M, Mielke S, Ruggeri A, Zubeyde Nur Ozkurt, Bourhis J, et al. Thiotepa-based regimens are valid alternatives to total-body irradiation-based reduced-intensity conditioning regimens in patients with acute lymphoblastic leukemia: a retrospective study on behalf of the acute leukemia working party of the European society for blood and marrow transplantation. Transplant Cell Ther. 2023; Oct 8: S2666-6367(23)01582-8. Online ahead of print.
6. Cahu X, Labopin M, Giebel S, Aljurf M, Kyrcz-Krzemien S, Socié G, et al. Impact of conditioning with TBI in adult patients with T-cell ALL who receive a myeloablative allogenic stem cell transplantation: a report from the acute leukemia working party of EBMT. Bone marrow Transplantation. 2016; 51 (3): 351–7.
7. Драчёв И. С., Зацепин В. В., Иванченко А. В., Ивницкий Ю. Ю., Крюков Е. В., Селезнёв А. Б. Острые поражения, возникающие в результате внешнего облучения организма человека. В книге: Софронов Г. А., Крюков Е. В., редакторы. Военная токсикология, радиология и медицинская защита. СПб.: ВМедА, 2023; с. 507–39.
8. Konishi T, Ogawa H, Najima Y, Hashimoto S, Wada A, Adachi H, et al. Safety of total body irradiation using intensity-modulated radiation therapy by helical tomotherapy in allogenic hematopoietic stem cell transplantation: a prospective pilot study. J Radiat Res. 2020; 61 (6): 969–76.
9. Nakagaki M, Kennedy G, Gavin N, Clavarito A, Whitfield K. The incidence of severe oral mucositis in patients undergoing different conditioning regimens in haematopoietic stem cell transplantation. Support Care Cancer. 2022; 30 (11): 9141–9.
10. Conard RA. Effect of X-irradiation on intestinal motility of the rat. Am J Physiol. 1951; 165 (2): 375–85.
11. Krantis A, Rana K, Harding R. The effects of γ-radiation on intestinal motor activity and faecal pellet expulsion in the guinea pig. Dig Dis Sci. 1996; 41 (12): 2307–16.
12. Erickson BA, Otterson MF, Moulder JE, Sarna SK. Altered motility causes the early gastrointestinal toxicity of irradiation. Int J Radiat Oncol Biol Phys. 1994; 28 (4): 905–12.
13. Dorval ED, Mueller GP, Eng RR, Durakovic A, Conclin JJ, Dubois A. Effect of ionizing radiation on gastric secretion and gastric motility in monkeys. Gastroenterology. 1985; 89 (2): 374–80.
14. Eagle D, Gian V, Lauwers G, Manivel J, Moreb J, Wingard J. Post-transplant complications. Gastroparesis following bone marrow transplantation. Bone Marrow Transplantation. 2001; 28: 59–62.
15. Chapman MJ, Nguyen NQ, Deane AM. Gastrointestinal dysmotility: clinical consequences and management of the critically ill patient. Gastroenterol Clin North Am. 2011; 40 (4): 725–39.
16. Patel R, Soni M, Soyantar B, Shivangi S, Satarija S, Saraf M, et al. A clash of quorum sensing vs quorum sensing inhibitors: an overview and risk of resistance. Arch Microbiol. 2023. 205 (4): 107.
17. Annede P, Prieux-Klotz C, Dubergé T, Chargari C, Gisserot O, de Jaureguiberry J-P. Radiation induced gastroparesis – case report and literature review. J Gastrointest Oncol. 2017; 8 (4): E52–5.
18. Васин М. В. Препарат Б-190 (индралин) в свете истории формирования представлений о механизме действия радиопротекторов. Рад Биол Радиоэкол. 2020; 60 (4): 378–95.
19. Куна П. Химическая радиозащита: пер. с чешск. М.: Медицина, 1989; 192 с.
20. Балаховский С. Д., Балаховский И. С. Методы химического анализа крови. 3-е изд. М.: Медгиз, 1953; 746 с.
21. O'Grady J, Murphy CL, Burry L, Shanahan F, Buckly M. Defining gastrointestinal transit time using video capsule endoscopy: a study of healthy subjects. Endosc Int Open. 2020; 8 (3): E396–E400.
22. Ivnitsky JuJu, Schäfer TV, Rejniuk VL, Golovko AI. Endogenous humoral determinants of vascular endothelial dysfunction as triggers of acute poisoning complications. J Appl Toxicol. 2023; 43 (1): 47–65.
23. Мартынова Н. А., Горохова Л. Г. Токсикологическая оценка индола. Бюл. ВСНЦ СО РАМН. 2006; 65 (1): 248–51.
24. Sender R, Fuchs S. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016; 14 (8): e1002533.
25. Tanaka H, Takeuchi K, Okabe S. Role of accumulated gastric content in the pathogenesis of cysteamine- and mepirizole-induced duodenal ulcers in the rat. J Intern Med Suppl. 1990; 732: 69–75.
26. Mole RH. The effect of X-irradiation on the basal oxygen consumption of the rat. Q J Exp Physiol Cogn Med Sci. 1953; 38 (2): 69–74.
27. Russ M, Boerger E, von Platen P, Francis R, Taher M, Boemke W, et al. Surfactant depletioncombined with injurious ventilation results in a reproducible model of the acute respiratory distress syndrome (ARDS). J Vis Exp. 2021; 170: e62327.
28. Chang J, Liang S, Thanasekaran P, Chang H, Wen L-L, Chen C, et al. Translational medicine in pulmonary-renal crosstalk: therapeutic targeting of p-Cresyl sulfate triggered nonspecific ROS and chemoattractants in dyspneic patients with uremic lung injury. J Clin Med. 2018; 7 (9): 266.
29. Thome T, Salyers Z, Kumar R, Hang D, Berru F, Ferreira L, et al. Uremic metabolits impair skeletal muscle mitochondrial energetics through disruption of the electron transport system and matrix dehydrogenase activity. Am J Physiol Cell Physiol. 2019; 317 (4): C701–13.
30. Kim Y-S, Lee H, Lee M, Park Ye, Sehwan M, et al. The effect of mitochondrial transplantation on sepsis depend on the type of cell from which they are isolated. Int J Mol Sci. 2023; 24 (12): 10113.