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MORPHOLOGICAL FEATURES OF THE ACTION OF RONCOLEUKIN ON THE INTERNAL ORGANS OF WHITE MICE

Chronic inflammatory diseases are one of the leading causes of death in the world. A number of pathological conditions are associated with chronic inflammation, such as chronic obstructive pulmonary disease, rheumatoid arthritis, metabolic syndrome, type 2 diabetes mellitus, cardiovascular, chronic kidney disease, cancer, neurodegenerative and autoimmune diseases. To address a number of issues related to this problem, reliable models of chronic inflammation are needed. A number of chemical inductors of this state are known from the literature. One of them is interleukin-2. Roncoleukin is a dosage form of recombinant human interleukin-2. To date, few studies have been presented on changes at the tissue level under the influence of IL-2. In this work, we studied the patterns of morphological changes in the lungs, liver, kidneys, spleen, and thymus of CD-1 mice under the influence of low and high doses of Roncoleukin. We have shown that the drug contributes to an increase in the mass of the spleen by 2 times and a decrease in the mass of the thymus by 1.5 times. Roncoleukin has a stimulating effect on the immunocompetent compartment, leading to cell proliferation of T-dependent zones of the spleen and thymus cortex. In the visceral organs, under its action, a picture of a chronic inflammatory process develops.

Key words: IL-2, planimetric image analysis, chronic inflammation model, Ronkoleukin.

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Ронколейкиннің ақ тышқандардың ішкі мүшелеріне әсер етуінің морфологиялық ерекшеліктерін зерттеу

Созылмалы қабыну аурулары әлем бойынша өлімнің негізгі себептерінің бірі болып табылады. Бірқатар патологиялық ауру түрлері созылмалы қабынумен байланысты, мысалы, созылмалы обструктивті өкпе ауруы, ревматоидты артрит, метаболикалық синдром, 2 типті қант диабеті, жүрек-қан тамырлары, созылмалы бүйрек аурулары, қатерлі ісік, нейродегенеративті және аутоиммундық аурулар. Осы мәселеге қатысты бірқатар мәселелерді шешу үшін созылмалы қабынудың сенімді үлгілері қажет. Бұл күйдің бірқатар химиялық индукторлары әдеби мәліметтерден белгілі. Олардың бірі интерлейкин-2. Ронколейкин – адамның рекомбинантты интерлейкин-2 дәрілік түрінің біріне жатады. Осы уақытқа дейін ИЛ-2 әсерінен ұлпа деңгейіндегі өзгерістер туралы аздаған зерттеулер ұсынылды. Бұл жұмыста біз CD-1 тышқандарының өкпесінде, бауырында, бүйректерінде, көкбауырында, тимусында Ронколейкиннің төмен және жоғары дозалары әсерінен болатын морфологиялық өзгерістерден болатын заңдылықтарды зерттедік. Препарат көкбауырдың массасының 2 есе артуына және тимустың 1,5 есе азаюына ықпал ететінін көрсеттік. Ронколейкин иммунокомпетентті компартменке ынталандырушы ретінде әсер етеді, көкбауырдың және тимус қыртысының Т-тәуелді аймақтарының жасушалық пролиферациясына әкеледі. Висцеральды органдарда оның әсерінен созылмалы қабыну процесі өрши түседі.

Түйін сөздер: ИЛ-2, кескінді планиметриялық талдау, созылмалы қабыну моделі, ронколейкин.

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Морфологические особенности действия Ронколейкина на внутренние органы белых мышей

Хронические воспалительные заболевания являются одной из основных причин смертности в мире. С хроническим воспалением ассоциирован ряд патологических состояний таких, как хроническая обструктивная болезнь легких, ревматоидный артрит, метаболический синдром, сахарный диабет 2 типа, сердечно-сосудистые, хронические заболевания почек, онкологические, нейродегенеративные и аутоиммунные заболевания. Для решения ряда вопросов, связанных с данной проблемой необходимы достоверные модели хронического воспаления. Из литературы известно о ряде химических индукторов данного состояния. Одним из них является интерлейкин-2. Ронколейкин – лекарственная форма рекомбинантного интерлейкина-2 человека. На сегодняшний день представлено мало исследований, посвященных изменениям на тканевом уровне под действием ИЛ-2. В данной работе были исследованы закономерности морфологических изменений в легких, печени, почках, селезенке и тимусе мышей линии CD-1 под действием низких и высоких доз ронколейкина. Нами было показано, что препарат способствует увеличению массы селезенки в 2 раза и уменьшению массы тимуса в 1,5 раза. Ронколейкин оказывает стимулирующее действие на иммунокомпетентный компартмент, приводя к пролиферации клеток Т-зависимых зон селезенки и кортекса тимуса. В висцеральных органах под его действием развивается картина хронического воспалительного процесса.

Ключевые слова: ИЛ-2, планиметрический анализ изображений, модель хронического воспаления, ронколейкин.

Introduction

Chronic inflammatory diseases are one of the leading causes of mortality. Inflammation is a normal response of an organism to injury and pathogen invasion. In some cases, it can take on a chronic character and persist in an organism for a long time, causing a number of pathological changes and leading to a comorbid condition [1]. Conditions such as low grade inflammation and inflammation are known to be associated with metabolic syndrome and aging [2–5]. Conditions associated with chronic inflammation include chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease, chronic kidney disease, cancer, neurodegenerative and autoimmune diseases [6–8]. To solve a number of issues related to this problem, both fundamental and applied, chronic inflammation models are needed. A number of chemical inducers of chronic inflammation are known from the literature, including formaldehyde, dextran, acetic and arachidonic acids, oxazolone, and others [9]. It is also known that high doses of interleukin-2 and its recombinant analogues can lead to systemic inflammation and multiple organ failure [10].

Interleukin-2 (IL-2) is an α-helical cytokine with a molecular weight of 15.5–16 kDa, formerly known as T-cell Growth Factor (TCGF). This cytokine is produced predominantly by $CD4^+$ T cells and to a lesser extent by $CD8^+$ T cells, dendritic cells (DCs), natural killer (NK) cells, and NKT cells in response to antigenic and mitogenic stimulation [11,12]. Recombinant IL-2 was obtained in 1983. Soon, its role in stimulating T cell proliferation and generation of effector and memory T cells was established in mice and humans. Later it was found that another important function of IL-2 is the control of immune responses and the maintenance of self-tolerance, and its absence leads to autoimmunity [13–15]. IL-2 can promote Activation Induced Cell Death (AICD) of T cells and is therefore involved in suppression of antigen-specific T cells after the clonal expansion phase of the immune response. IL-2 may limit IL-17 production and exhibit immunosuppressive properties by stimulating the production and homeostasis of regulatory T cells [16].

According to current concepts, low doses of IL-2 ($1.5 * 10^6$-$3 * 10^6$ international units (IU) once a day for humans or $1.5 * 10^4$-$3 * 10^4$ IU once a day for mice) preferentially stimulates regulatory T cells. The use of high doses is limited by toxicity manifesting as a 'cytokine storm' and

capillary leak syndrome [17]. The greatest limitation of IL-2 treatment has been the associated side effects including hypotension as well as cardiac, gastrointestinal, renal, cerebral, pulmonary, and hepatic toxicity [10,18]. The literature suggests that IL-2 toxicity is due to a cytokine-induced 'systemic autophagic syndrome', related to cytokine-induced autophagy and temporal dysfunction [19].

High doses of IL-2 can lead to inflammation. Thus, signs of inflammation in the respiratory tract are infiltrates in the submucosa of the bronchi of large and small caliber, bronchioles, as well as hyperplasia and metaplasia of the respiratory epithelium [20]. In the liver during inflammation, portal and centrilobular focal or multifocal infiltrates can be observed, containing mainly lymphocytes and macrophages, phenomena of fibrosis of the hepatic capsule, hyperplasia of bile duct cells can also be observed [21]. Signs of inflammation in the kidneys are interstitial infiltrates in the cortex and / or medulla, containing predominantly lymphocytes and macrophages, a small number of neutrophils, as well as degeneration of the epithelium of the tubules of the cortical substance and renal bodies [22].

To date, there are quite a few studies on morphological changes under the influence of IL-2. Based on the information presented, as well as our experiments, we suggest that high doses of Roncoleukin can be used to model the inflammatory process *in vivo*.

Materials and methods

Roncoleukin-1000000 IU/ml (LLC Biotech, Russia) was administered intraperitoneally to young CD-1 mice at the following doses: $1*10^4$ IU/kg and $1*10^5$ IU/kg. Animals received unlimited access to water and food, and were kept in vivarium conditions with lighting mode 12/12. 3 groups of 8 animals were formed: control; I group – $1 * 10^4$ IU / kg; II group – $1 * 10^5$ IU / kg.

Slaughter was carried out on the 2nd and 7th day after the administration of the drug. At necropsy, the masses of animals with and without organs were recorded, as well as the masses of each extracted organ.

During slaughter, blood smears were obtained, which were stained according to May-Grunwald (Sigma Aldrich, USA). A manual count of the leukocyte formula per 200 cells was performed for each smear. After necropsy, the organs were fixed in 10% neutral buffered formalin for 24 hours, after which they were processed using standard histological techniques, stained with Carazzi's hematoxylin and eosin [23]. Planimetric analysis of micrographs of lymphoid organs was performed using Levenhuk ToupView 9.3 software and an Optix 600 attachment camera on an MX300T microscope (MicroOptix, China) at x40, x100, x200, x400 magnifications.

To assess the morphophysiological state of the spleen and thymus, morphometric coefficients were used – the stromal-parenchymal ratio (SPR) [24] and the cortico-medullary index (CMI) [25], which were calculated according to the formulas:

$$SPR = \frac{S_{ST}}{S_{WP}} * 100$$

where S_{ST} – area of stroma, S_{WP} – area of white pulp of the spleen.

$$KMI = \frac{S_C}{S_M}$$

where S_C – area of cortex, S_M – area of medulla of the thymus.

The results of quantitative studies were evaluated using One-Way ANOVA analysis followed by statistical analysis using the GraphPad Prism 9.3.1 software package. The significance level p=0.001-0.05 was acceptable.

Results and discussion

To assess the morphological changes under the influence of roncoleukin, an experiment was conducted in which animals were injected with different amounts of the drug (group I – $1*10^4$ IU/kg; group II – $1*10^5$ IU/kg). Animals treated with an equal volume of saline served as the control group. Animals were taken out of the experiment on days 2 and 7 after drug administration.

Histological studies showed that on the 2nd day in group I, small focal perivascular lymphoid infiltrates with impaired blood rheology appeared in the lungs, which were expressed as plasmastases, in the pulmonary arteries (Fig. 1 B); changes in

group II were of a similar nature; erythrostasis were noted in the blood vessels (Fig. 1 C). Focal centrilobular lymphoid infiltrates were observed in the liver of mice treated with both low and high doses of the drug, and lymphocytes were noted in the lumen of the blood vessels of the liver (Fig. 1 E, F). In the kidneys of mice of group I, destructive hemorrhages were noted in the interstitium of the cortical substance (Fig. 1 H); the histostructure of the kidneys in the high-dose group was preserved, and foci of diapedetic hemorrhages were observed in the interstitium of the cortex (Fig. 1 I). In all groups, the area of infiltrates did not exceed 10% of sections of the lungs, liver, and kidneys.

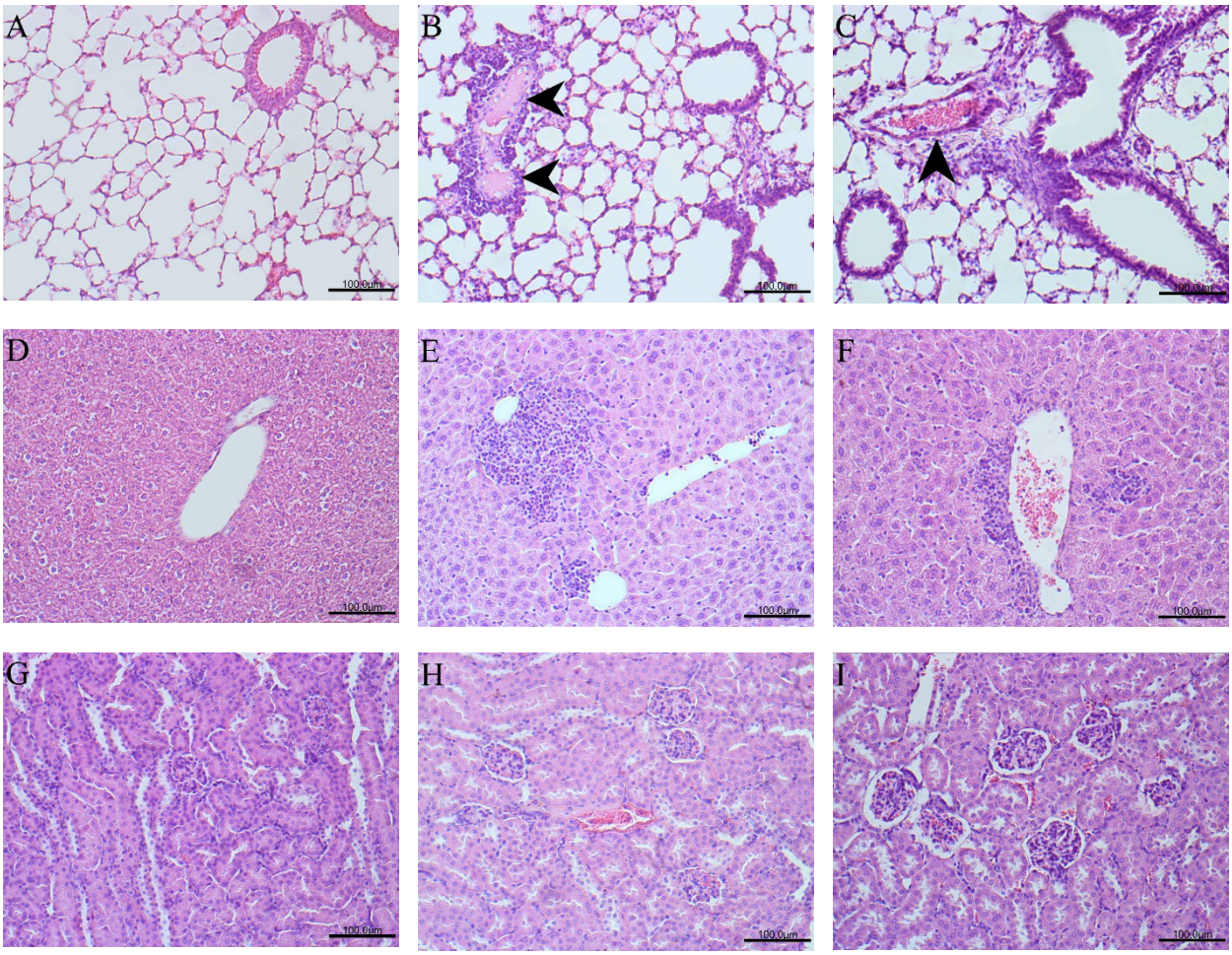

Figure 1 – Changes in the lungs, liver and kidneys on the 2nd day of the study.
A – lungs, control; C – lungs, group I, arterial plasmostasis marked with a black tip; C – lungs, group II, arterial erythrostasis marked with a black tip; D – liver, control; E – liver, group I; F – liver, group II; G – kidneys, control; H – kidneys, group I; I – kidneys, group II. H&E, magnification A-I – x200. Scale – 100 μm.

Using the planimetric analysis of spleen and thymus preparations, we found that on the 2nd day there was a significant increase in the mass of the spleen (Fig. 2 A) and its lymphoid compartment relative to the stroma (Fig. 2 B). From this it follows that under the action of roncoleukin, cells of the lymphoid follicles of the spleen proliferate, due to which an increase in the mass and lymphoid component of the organ is observed. At this time, in the thymus of mice of group II, there is a significant increase in CMI (Fig. 2C), which also indicates the proliferation of cortex lymphocytes under the action of a high dose of the drug. Changes in the thymus mass on day 2 were not significant (Fig. 2D).

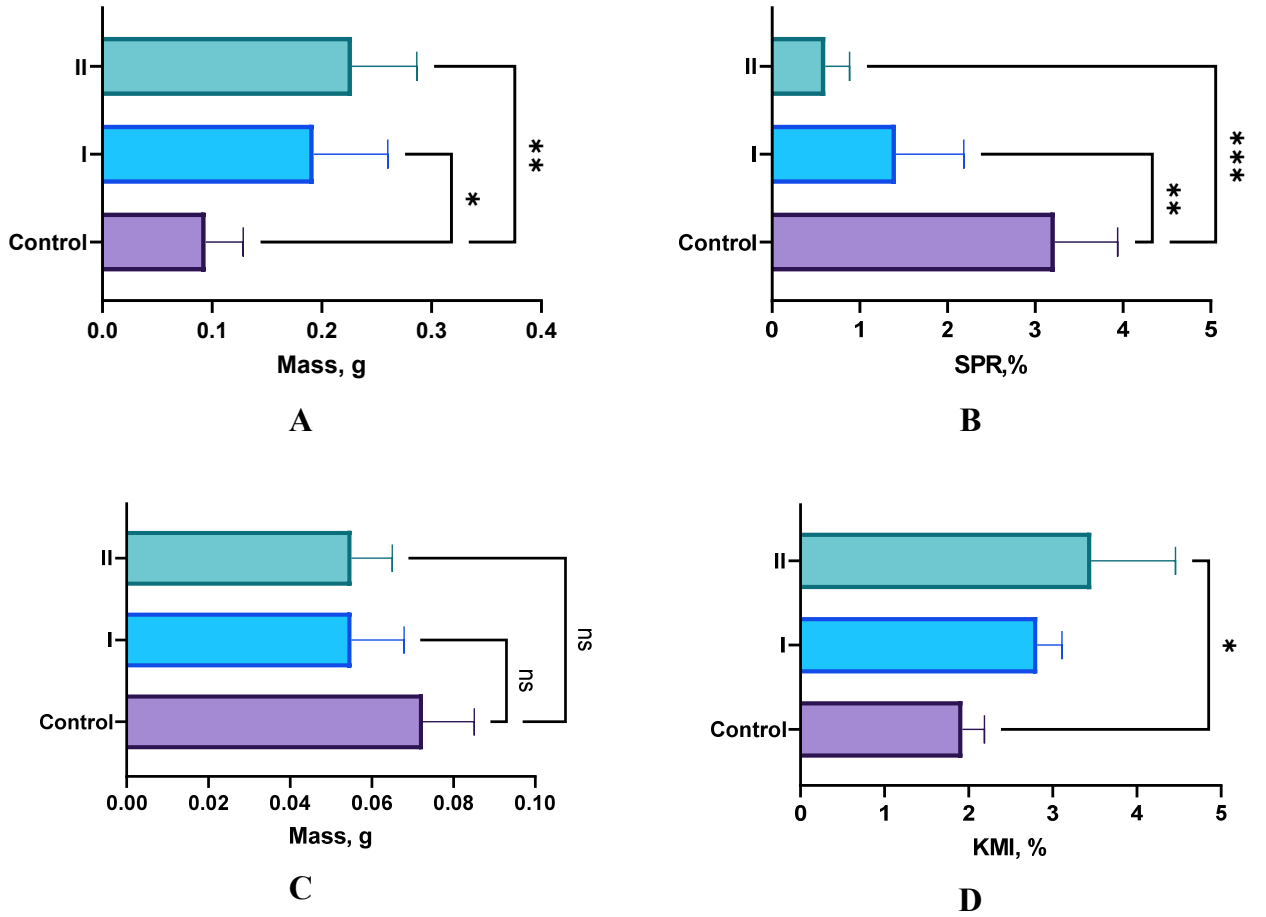

Figure 2 – Morphometric parameters of lymphoid organs on the 2nd day of the study. A – mass of the spleen; B – stromal-parenchymal ratio in the spleen; C – thymus mass; D – cortico-medullary index in the thymus.

On the 7th day of the study, the lungs of animals treated with both low and high doses showed focal moderate lymphoid infiltrates located peribronchial (Fig. 3 B, C). Large multifocal centrilobular lymphoid infiltrates with division of blood into plasma and formed elements in the central veins were noted in the liver of group I mice; lymphocytes were also observed in the lumen of blood vessels (Fig. 3 E). With a high dose drug administration, multifocal portal pulverized obesity of hepatocytes without inflammation was recorded (Fig. 3 F). In groups I and II, multifocal interstitial lymphoid infiltrations accompanied by destructive hemorrhage were observed in the kidneys (Fig. 3H, I). In both experimental groups, the area of lymphoid infiltrates in the lungs, liver and kidneys reached 30% of the section of each organ – the severity of chronic inflammation was higher than on the 2nd day of the study.

The mass of the spleen on the 7th day remained significantly increased (Fig. 4 A), while the SPR value did not significantly differ from the control group (Fig. 4 B), which suggests that the mass of the organ was increased equally due to lymphoid , and stromal compartments. In the thymus, on the other hand, a significant decrease in the mass of the organ was noted both in groups I and II (Fig. 4 C), while the CMI coefficient was significantly higher only in group I (Fig. 4 D).

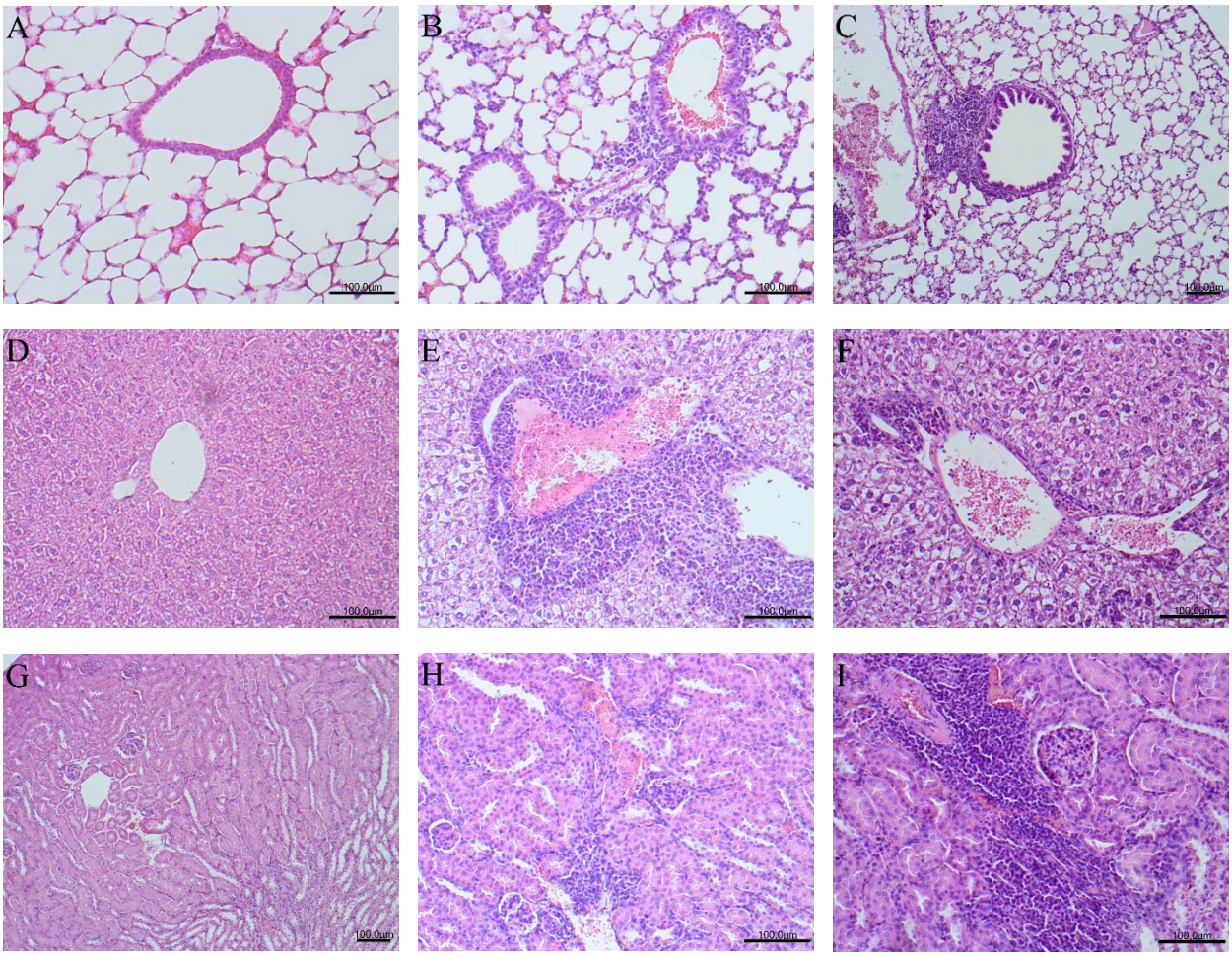

Figure 3 – Changes in the lungs, liver and kidneys on the 7th day of the study.
A – lungs, control; B – lungs, group I; C – lungs, group II; D – liver, control; E – liver, group I; F – liver, group II; G – kidneys, control; H – kidneys, group I; I – kidneys, group II. H&E, magnification C, G – x100; A, B, D-F, H, I – x200. Scale – 100 µm.

In the peripheral blood of mice of group I on the 2nd day of the study, an increase in the number of neutrophils and monocytes was observed, while in group II – only monocytes, which indicates the initiation of an inflammatory reaction. On the 7th day, a further increase in the number of neutrophils was observed in both experimental groups, while the number of monocytes decreased (Table 1).

Based on the histological description, it was found that in the lungs, liver and kidneys there is an increase in inflammatory infiltrates from 2 to 7 days after the administration of the drug. The area of lesions increased from 10% on day 2 to 30% by day 7, with the highest degree of inflammation recorded in the kidneys. The data of planimetric analysis indicated that on the 2nd day there was an increase in mass in the spleen, however, changes in the thymus occurred only under the influence of a high dose. Thymus mass on day 7 in all experimental groups significantly decreased. The functional activity of the spleen on the 7th day in all experimental groups decreased, while it increased in the thymus at a low dose.

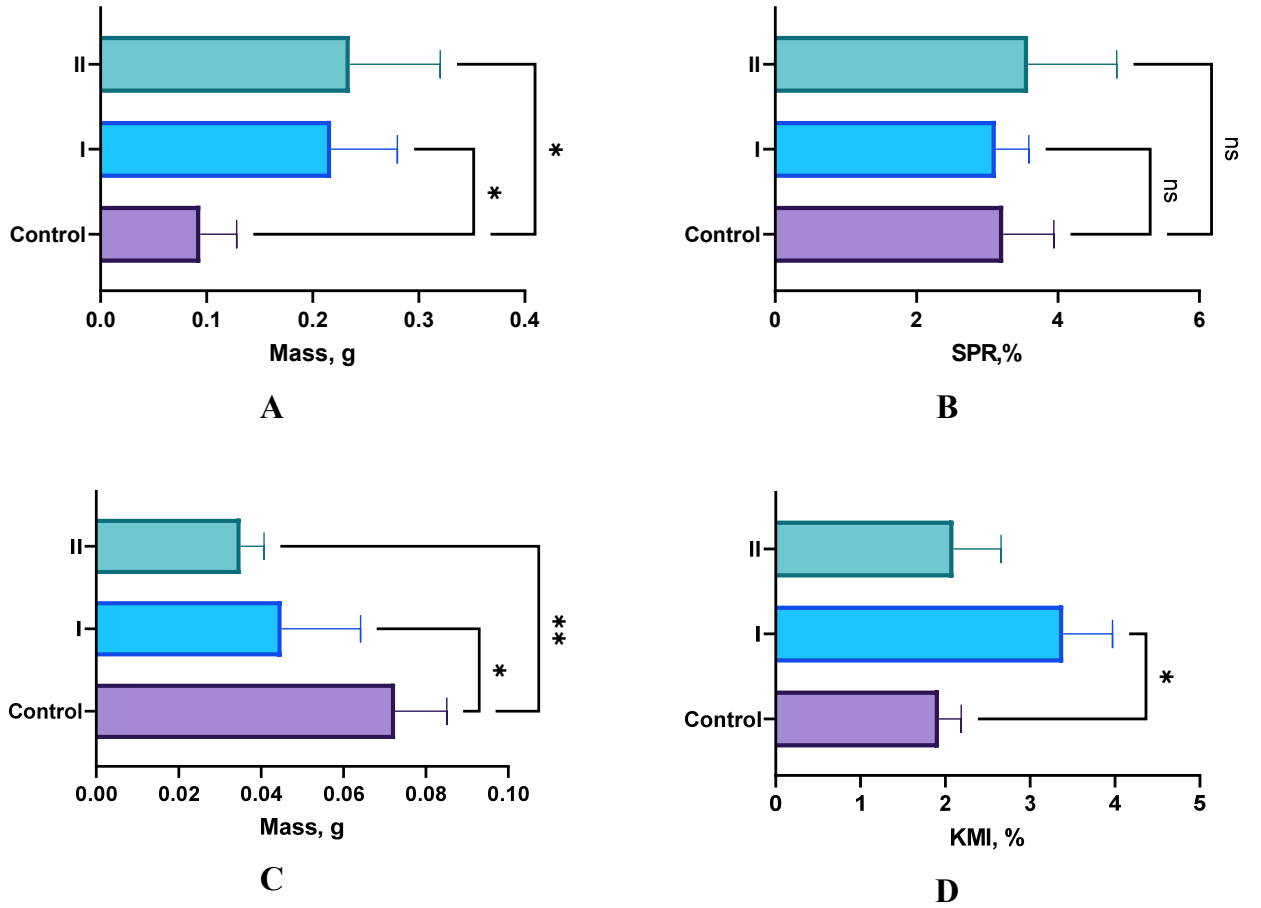

Figure 4 – Morphometric parameters of lymphoid organs on the 7th day of the study. A – mass of the spleen; B – stromal-parenchymal ratio in the spleen; C – thymus mass; D – cortico-medullary index in the thymus.

Table 1 – Leukocyte formula

Group / measure	Control	10^4 IU/kg		10^5 IU/kg	
		Day 2	Day 7	Day 2	Day 7
Lymphocytes	159,6±5,92	133±11,5	106±14,5	150,75±16,25	143,25±5,75
Neutrophils	37,2±6,56	52±11,5	65,75±25,25	38,75±11,25	53,5±6
Monocytes	3±1,2	9,75±2,75	1	5,75±4,25	1,5±0,75
Basophils	0,2±0,32	2	1±0,67	2,25±0,875	0
Eosinophils	0	3,25±1,375	2±0,67	2,5±1	1,75±1,25

Conclusion

Thus, we studied the morphological features of the action of roncoleukin at doses of 1*104 and 1*105 IU/kg on the internal organs of CD-1 mice. Signs of chronic inflammation were noted in all the organs studied, and the severity of the inflammatory process was not dynamic and was weak in the lungs and liver, but the dynamics and moderate severity were detected in the kidneys. There was a significant decrease in the stromal-parenchymal ratio in the spleen and an increase in the cortico-medullary index in the thymus in proportion to the increase in the dose of Roncoleukin. The state of the lymphoid component of the immune organs

indicated an increase in their functional activity. Roncoleukin at a dose of 1 * 10^5 IU / kg leads to greater damage to internal organs, especially the kidneys, compared with a dose of 1 * 10^4 IU / kg.

Under the action of a high dose of Roncoleukin, a chronic inflammation develops in the visceral organs, and the drug can be recommended as an agent for modeling this phenomenon, in particular, to obtain a picture of renal, pulmonary and hepatic insufficiency. There is also evidence in the literature that high doses of IL-2 can cause a "cytokine storm" [17], and further immunological studies are needed to study the patterns of its course at the morphological level.

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