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WOUND HEALING EFFECT TIOTRIZOLINE OINTMENT FOR ACTION ON SKIN IONIZING RADIATION IN RATS

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Abstract. *The wound healing effect "Unguentum Thiotriazolini 2%" (JSC "chemical-pharmaceutical plant" Red Star ", Ukraine)was studied in rats with local radiation skin lesions. The rats were divided into four groups, each comprised of six rats: intact (group 1), radiation injuries of the skin (group 2), radiation injuries of the skin + ointment: methyluracil (group 3) and Thiotriazoline (group 4). Rats of 2, 3 and 4 groups were exposed to X-rays of the hip (80 Gy). In Group 3 and 4 the ointments were applied on the skin 1 hour before exposure and for 10 days after it once a day. On the 35th day of experiment immunomorphological study of focus of exposure (macrophages, cells are producers of interleukins IL-1 and IL-10) was performed. The results showed that the ointment Thiotriazoline (group 4) caused a more pronounced wound healing effect, manifested increased macrophage reaction, restoration of cytokine balance, compared with group 2 and 3.*

Key words*: Thiotriazoline ointment, wound healing effect, ionizing irradiation of the skin*

Ionizing radiation is a widely used in the treatment of various types of cancer and radiation skin injury is a significant problem. This injury occurs in about 95% of patients receiving radiation therapy for cancer [1]. For many years, radiation burns have been treated like thermal burns, in spite of differences in their pathogenesis [2]. Radiation burns have a dose-dependent clinical manifestation, which includes dry desquamation at 12–20 Gy, moist desquamation at 20 Gy, and necrosis at >35 Gy [3].

Radiation burns associate with violation of skin immunity. Ionizing radiation causes degranulation of mast cells in the dermis. Mast cell–derived histamine, serotonin, tumor necrosis factor-α, and tryptase significantly alter the release of chemokins by dermal fibroblasts [4]. The features of radiation injuries to the skin are suppression of

repair processes, expressed chronic inflammation and low efficacy of therapeutic interventions, in large part due to a violation of the immunological mechanisms [4, 5]. Management of skin radiation injury includes mainly local conservative treatment with ointments, gels, liniments, creams, containing corticosteroids and nonsteroidal agents [6], lanolin-free, water-based moisturizing cream [7], local bone marrow-derived stem cells [2] and other medications. The application of ointments that have a complex multi-directional effect on the wound process is the most promising. In this connection, our attention was attracted by drug of polytropic actions, thiotriazoline ointment "" Unguentum Thiotriazolini 2%" (JSC "chemical-pharmaceutical plant" Red Star " – “Красная Звезда”, Ukraine) with the membrane stabilizing, antioxidant, and anti-inflammatory properties. This determines the expediency of study of this drug efficacy in the skin damage caused by ionizing radiation.

Purpose: to study the wound healing effect "Unguentum Thiotriazolini 2%" in a single local ionizing irradiation of rat skin.

Materials and methods. The study was conducted on 4 groups of WAG rats: Group 1 - intact (n=6), Group 2 - radiation damage to the skin - control (n=6), group 3 (comparator drug) - radiation damage to the skin+methyluracil ointment (n=6), group 4 (main) - radiation damage to the skin+thiotriazoline ointment (n=6). In rats of the 2nd, 3rd,' and 4th groups local radiation damage to the skin was caused by a single local action of X-rays radiation at the thigh area of animals in the exposure dose of 80 Gy (irradiator Tur-60, 5 mA, 50 kV, filter 0 3 mm Al, dose 80.2 Gy / min, irradiation area 20mm^2) [8]. In Group 3 and 4 one hour before irradiation and after it for 10 days once a day the ointment of thiotriazoline and methyluracil respectively were applied on the surface of skin. The severity of skin reactions to radiation exposure was evaluated by clinical manifestations, duration and timing of the healing of radiation damage. All groups of animals were taken out of the experiment at 35 days after irradiation in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals. For objectification of healing morphological and immmunohistochemichal (quantification of macrophages, cells-producer of

interleukin IL-1 and IL-10) studies of the skin in the area of exposure were carried out [9,10]. The data obtained were analyzed by standard method of variation statistics.

Results. All rats in group 2 at 24 hours after irradiation developed primary erythema. On the third day true bright erythema appeared and remaineded until the 7th day. Reaction of wet and dry desquamation developed sequentially from the 7th day after irradiation. On 7-9 day dry epitheliitis, that is, the appearance of dry yellow crusts and marked desquamation, were observed in all rats. Dry desquamation lasts for 8 days. In some animals during this period already the integrity of the skin was violated, and epithelium exfoliation occured. Moist desquamation developed on day 15 after exposure and its duration was on average 17 days. Thus the large areas of weeping surface appeared followed by the formation of brown crusts and ulcer. Ulcers with purulent necrotic manifestations that was persisting to the end of the observation period, developed in 83% of animals. According to immunohistological studies this group developed severe ulcerative destructive changes with signs of chronic radiation ulcers, degenerative changes in fibrous stroma accompanied by macrophages deficiency, 2.8-fold increase in cells-producers of proinflammatory interleukin IL-1 and 1.8 times decrease in cells-producers of anti-inflammatory interleukin IL-10 as compared with intact rats (Table 1).

Reduced the number of macrophages that regulate the proliferation of fibroblasts and provide a link between the inflammatory and reparative response, confirms the absence of repair processes in animals of this group. In addition, lack of anti-inflammatory cytokines contributes to the development of immune deficiency and the formation of chronic inflammation [11].

In the third group of animals (radiation skin damage methyluracil ointment+) radiation reactions proceeded more easily than in the controls. Pronounced erythema developed in 83% of the exposed rats lasted for 6 days. Dry epidermitis, characterized by the appearance of yellow crusts on the background of hyperemia with further development expressed peeling, lasted 7 days. Moist desquamation, which was observed for 15 days, was characterized by the emergence of sites weeping surface followed by the formation of brown crusts with skin cracks.

Table 1

The relative amount of macrophages and cells-producers of interleukins in the skin of rats on 35 day after radiation

Groups	Macrophages	Cells-producers of interleukin	
		IL-1	IL-10
Group 1 - intact	8,33±0,55	2,50±0,42	1,33±0,21
Group 2 - radiation damage to the skin	8,83±0,31	7,00±0,36$^{###}$	0,75±0,11$^{\#}$
Group 3 - radiation damage to the skin + methyluracil ointment	8,83±0,31	3,17±0,31***	1,42±0,37*
Group 4 - radiation damage to the skin +thiotriazoline ointment	11,17±0,31**	4,67±0,42***	3,33±0,33***

Notes:

1.Significance of differences comparing to group 1(# – p≤0,05; ## – p≤0,01; ### – p≤0,001)

2.Significance of differences comparing to group 2 (* – p≤0,05; ** – p≤0,01; *** – p≤0,001)

In 67% of the rats irradiation led to the formation of a surface covered with sores. The disappearance of the radial change began on 28 day after exposure. At the stage of healing the irradiated area of the skin was a glossy surface with traces of peeled crusts. Immunomofrologic data confirmed the improved course of healing in comparison with the control, but severe destructive processes with reduced regenerative activity, as well as local immune processes (2.2-fold reduction in cells producing IL-1 and increase in cells producing IL-10 up to 1 9 times) reminded (Table 1). The amount of macrophages did not change.

Radiation damage in group 4 (radiation skin damage + thiotriazoline ointment) were less pronounced compared to the control group and group 3 (radiation skin damage + methyluracil ointment), and faster died down. So, erythema was observed for 5 days in 66% of rats. Dry desquamation accompanied by slight peeling lasted not more than 6 days. The duration of course of moist dermatitis was reduces to 11 days; its severity also was reduced. Ulcerative defect occurred only in 33% of the exposed rats. Restoration of the epithelium integrity and the healing was noted from 22nd day that is 6 days earlier than in the third group. In all rats the area of irradiation was epithelized completely, providing a surface that is completely healed. Thiotriazoline ointment showed more pronounced effect in contrast to the previous groups:

epithelialization of skin defects was associated with preservation vessels of microvascular circulation and appendices of the skin, increase in the number of macrophages by 26.5%, decrease in cells-producers IL-1 by 1.5 times with greater increase in cells producing IL-10 (4.4-fold) (Table 1). Apparently, marked macrophage reaction promoted rapid wound cleansing of cellular debris and, due to macrophage-fibroblast interaction it accelerated fibroblast proliferation, synthesis and secretion of collagen. Activation of cell-producers proinflammatory cytokine IL-1 promoted the development of an adequate inflammatory response, and growth of cells that produce anti-inflammatory cytokine IL-10 stimulated the production of macrophages, fibroblast growth factor, which is one of the links in the activation of healing processes that have been found in our histological studies.

Thus, thiotriazoline ointment reduces the intensity of development and decreases the time course of acute radiation damage to the skin of rats in the irradiation, as evidenced immunomorphologic data: increased macrophage reaction, restoring the cytokine balance.

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