MORPHOLOGY AND PATHOMORPHOLOGY

Effects of New Wound Dressings on Healing of Thermal Burns of the Skin in Acute Radiation Disease

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Effects of new wound dressing bacterial cellulose impregnated with SOD and poviargol (Procel-Super and Procel-PA) and Inerpan hydrogel dressing on the reparative processes in deep dermal burns (IIIa-IIIb degree) in rats exposed to total even irradiation in a dose of 4 Gy were studied. Inerpan and Procel-Super dressings proved to be the most effective under these conditions: they accelerated healing of burn wounds by 17.0 and 5.5%, respectively.

Key Words: *thermal burn; combined radiation injuries; wound dressing; reparative regeneration*

Ionizing radiation aggravates the course of reparative regeneration, suppressing the demarcation inflammatory and proliferative regeneration phase of the wound process [11]. Disorders in wound reparation after total irradiation manifest in delayed cleansing from necrotic tissues, suppression or complete cessation of connective tissue growth and epithelialization [10]. Complicated wounds can be locally treated using wound dressings characterized by numerous effects: protecting the burn wound from mechanical injury, creating optimal microenvironment for healing, preventing penetration of microorganisms adsorbing the wound exudation, and providing selective permeability for oxygen and carbon dioxide. Addition of drugs in the wound dressings creates extra potentialities for accelerated wound healing and normalization of the wound process [10]. We studied the effects of new wound dressings Procel-Super, Procel-PA, and Inerpan on the time course of healing of thermal burns complicated by exposure to ionizing irradiation.

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MATERIALS AND METHODS

Experiments were carried out on 60 random-bred albino male rats (190-200 g). In series I total even γ-irradiation in a dose of 4 Gy was carried out on an IGUR-1 device (dose power 1.3 Gy/min); 15 min after the exposure the animals were intramuscularly injected with a mixture of calypsol (70 mg/kg) with droperidol (1 mg/kg) and thermal burns were inflicted by applying tubes with hot water (100°C) to depilated skin on the back (duration of exposure 10 sec, area of burns 5% body surface). Dressings manufactured at Institute of High-Molecular Compounds (St. Petersburg), made from bacterial cellulose impregnated with SOD (Procel-Super) or poviargol (Procel-PA) and Inerpan dressing (a hydrogel film obtained by polymerization of leucin amino acids and methylglutamate; Delalande) were applied. Levomecol ointment [4] served as the reference preparation. Control animals received no treatment. In series II burns alone were modeled (no irradiation) and the animals were divided into groups depending on the treatment protocol similarly as in series I. The time of the scab detachment

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and wound healing were recorded. Hematological studies of the peripheral blood were carried out during healing. Wound biopsy specimens were collected on days 3, 7, and 11 and examined under an optic microscope. The samples were fixed in 10% neutral formalin, treated in ascending alcohols, and embedded into paraffin. Paraffin sections were stained with hematoxylin and eosin and by Hail's method. The preparations were photographed in a microscope with an Opton headpiece. The significance of differences was evaluated using Student's t test.

RESULTS

All studied wound dressings stimulated healing of burn wounds if thermal injury was not aggravated by exposure to ionizing radiation. Procel-Super and Inerpan were more effective and reduced the duration of wound healing by almost 15%; levomecol ointment and Procel-PA wound dressing were less effective and accelerating wound healing by 8%. Differences in the clinical course of the wound process in animals treated by the most effective wound dressings and untreated controls were observed starting from day 3 of the experiment: after application of Procel-Super and Inerpan the scabs on the burn wounds were fine, whitish-grey colored, tightly adhering to the wound, not soldered to the adjacent tissues. Wound discharge was serous. In the control (similarly as in the groups treated with Procel-PA and levomecol) the scabs were dark-brown, thick, with uneven edges, numerous defects and crusts. Discharge from the wounds was mostly purulent, particularly in the control (wound dressing with poviargol suppressed the development of bacterial inflammation in the burn wound).

In control animals and in rats treated with levomecol ointment and Procel-PA wound dressing the scabs detached on days 20-22, while Procel-Super and Inerpan promoted scab detachment by 20% (Table 1).

Irradiation was associated with the development of the typical pancytopenic syndrome: on day 10 after radiation exposure the erythrocyte count decreased by

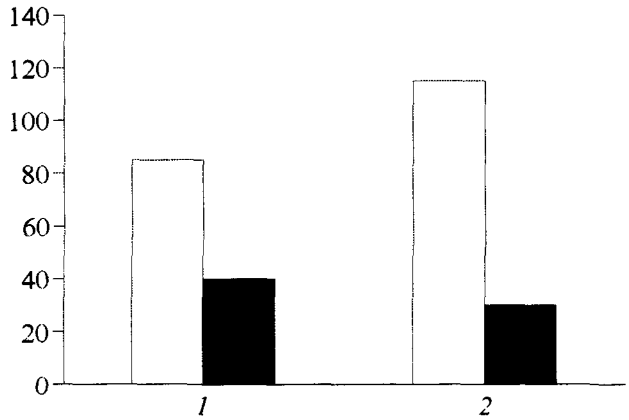

Fig. 1. Erythrocyte (*1*) and leukocyte (*2*) counts in the blood of animals on day 10 after burn (light bars) and combined radiation and burn injury (CRI; dark bars).

60% and leukocyte count by 70% in rats with combined radiation and burn injuries (CRI) in comparison with the control (Fig. 1).

The course of the wound process was also different. The initial signs of scab detachment, appearance of foci of granulation tissue and marginal epithelialization of the burn wound in irradiated animals were observed 6 days later than in rats with thermal injury, and wound healing was over only by day 50 (*vs.* day 41-42 in control rats with burns; Table 1). Discharge (mainly serous and purulent) from the wounds was also more intense in animals with CRI.

Only the use of Procel-Super and Inerpan brought about a significant effect in CRI. Wound healing was 6 and 17% sooner, respectively, and the time of the scab detachment was 14-15% more rapid *vs.* the control. Use of Procel-PA wound dressing also accelerated scab detachment, but application of this dressing did not significantly improve wound healing in irradiated animals.

Wound-healing effect of Procel-Super and Inerpan wound dressings was confirmed by the results of histological studies of wound biopsy specimens. Dry scab formed as soon as on day 7 after burns after

TABLE 1. Effects of Wound-Healing Preparations on the Terms of Burn Healing in Animals with Thermal Injuries and CRI

Preparation	Day of healing		Day of scab detachment	
	in burns	in CRI	in burns	in CRI
Control (no treatment)	41.3±0.6	49.5±0.6$^+$	21.0±0.8	26.7±0.9
Levomecol	38.6±0.5*	49.3±0.5$^+$	19.7±0.8	24.4±0.6
Procel-Super	35.4±0.6*	46.7±0.4*$^+$	17.8±0.6*	19.4±0.7*
Procel-PA	38.4±0.5*	48.2±0.6*	19.6±0.7	22.8±0.8*
Inerpan	35.4±0.5*	41.5±0.6*$^+$	17.2±0.8*	18.2±0.8*

Note. $p<0.05$ compared to *control, $^+$the parameter in animals with burns.

application of these dressings; necrotic tissues were delineated by a well-formed leukocytic roll. By the beginning of the peak of acute radiation disease (ARD) zones of detachment were formed along the lower border and on the lateral walls of the leukocytic roll (Fig. 2, *a*). The development of young granulation tissue was seen under the demarcation zone; this tissue consisted of layers of horizontal fibroblasts and numerous blood vessels (Fig. 2, *b*; 3). Granulation growth was paralleled by intense accumulation of acid glycosaminoglycanes in the tissue. Skin appendages (hairy follicle epithelium) serving as the substrates for regeneration retained viability in the depth of the derma for a long time (within day 11).

By the peak of ARD (days 11-12) keratinocyte mitoses appeared in these zones and fine blood vessels grew from sites of viable derma at the interface with subcutaneous fat. Histochemical study showed numerous mast cells in dead tissues forming the burn scab (Fig. 2, *c*). Accumulation of these cells can be explained by long duration of capillary functioning in the paranecrotic zone under the wound dressing. Moreover, application of wound dressing accelerated wound epithelialization: a multilayer "wedge" of new keratinocytes on an underlayer of formed granulation tissue was formed at the interface between necrotic epidermal plast and the underlying derma on day 3 of CRI (Fig. 2, *d*).

Thermal burns of IIIa-IIIb degree occupying up to 5% of skin surface are not shockogenic traumas and are considered as slight injuries [11]. In our experiments neither isolated burns, nor CRI caused death of experimental animals, but radiation exposure aggravated the course of burn disease, which was seen from longer healing of burns in animals with ARD in comparison with non-irradiated controls. Healing was more rapid in animals with CRI treated with Procel-Super and Inerpan wound dressings, but still the duration of healing did not approximate that in non-irradiated animals in any of the groups (Table 1).

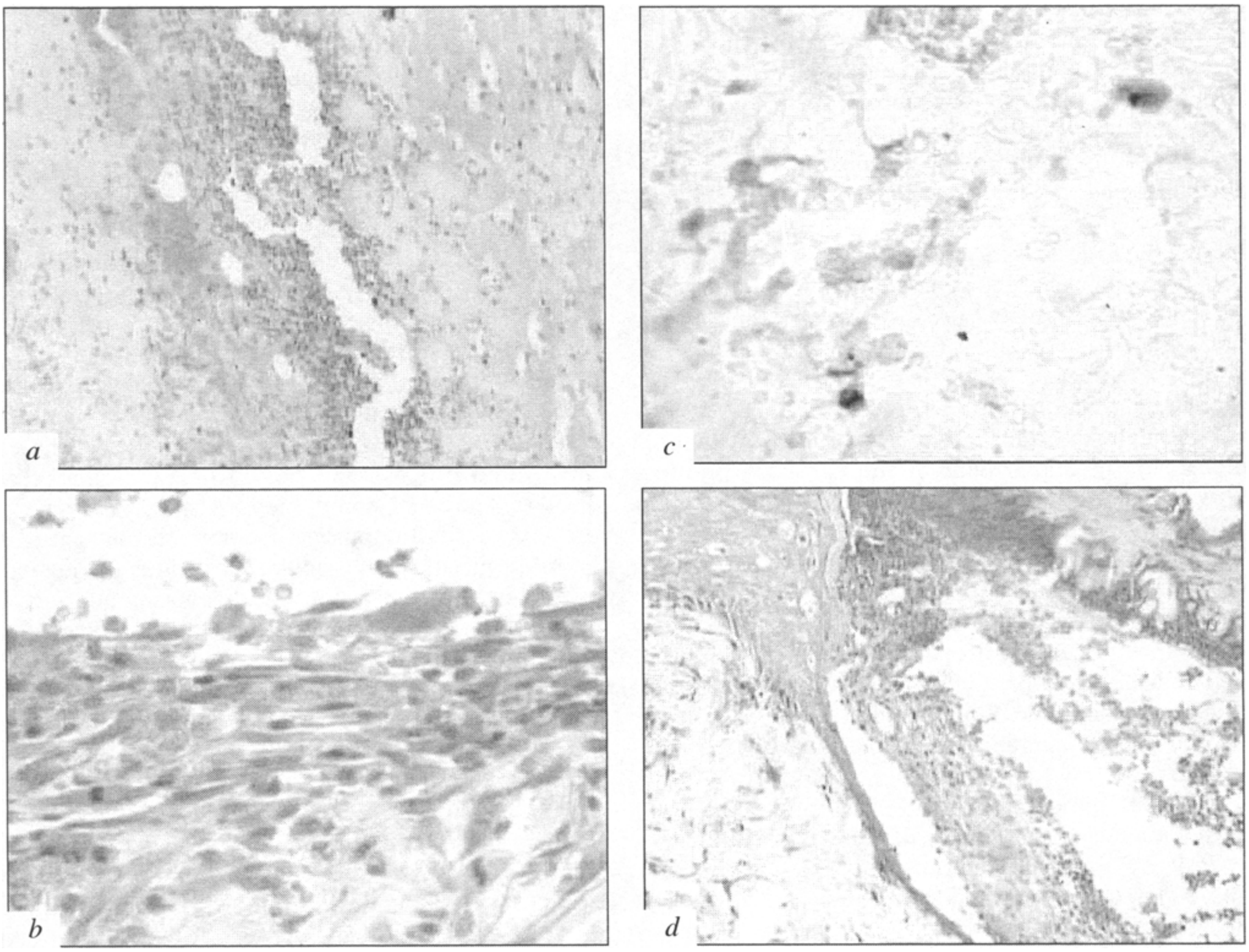

Fig. 2. Application of Inerpan wound dressing in CRI (day 12). *a*) demarcation and detachment of burn scab, ×21; *b*) intense growth and development of granulation tissue under the scab, ×70; *c*) the remainder of mast cells in necrotic tissues, ×70; *d*) growth of a young multilayer plast of keratinocytes under detaching burn scab, ×21. *a-d*: *hematoxylin and eosin staining.*

Inerpan is permeable for water vapor, water, salines and antiseptic solutions and impermeable for microorganisms. The hydrogel dressing creates the optimum microenvironment for reparative processes, prevents infection, stimulates proliferation and protein synthesis in the skin [9]. Presumably, the efficiency of Inerpan dressing in animals exposed to ionizing radiation can be explained by the radioprotective effects of the amino acids in its composition [3].

Local treatment of CRI is as a rule focused on prevention and treatment of infectious complications with the various antibacterial means [11]. A new silver-containing antiseptic poviargol attracts special interest among modern antibacterial agents; this drug is highly active towards gram-positive and gram-negative microflora, including that resistant to traditional antiseptics and antibiotics. In addition to the antiseptic effect, silver stimulates skin cell proliferation, directly stimulates cellular and humoral immunity, and indirectly stimulates nonspecific resistance [7,12]. The drug is effective in the treatment of thermal burns and mechanical wounds. In our study the wound dressing based on this drug was more or less effective in the treatment of isolated burns and ineffective in CRI. It is obvious that prevention of wound suppuration under these conditions is not sufficient for stimulating their reparation.

The use of agents with antioxidant effects is a principally new trend in local therapy of wounds and burns. Use of these agents is also pathogenetically justified in CRI. Tissue damage in thermal burns is associated with excessive generation of AOF and drastic intensification of LPO processes in the pancreonecrotic zone and in adjacent tissues [2]. Increased production of radical forms of oxygen and their derivatives is the cause of endotheliocyte damage to the microcirculatory bed [1] and their paralytic dilatation, which leads to impairment of the capillary permeability. Oxygen radicals and their derivatives destroy many components of the extracellular matrix (hyaluronic acid, proteoglycanes, collagen) and lead to cytolysis of connective tissue cells (fibroblasts) [1,13,14]. All these disorders result in the formation of secondary necrotic zones and deeper involvement of tissues in burns. Irradiation, paralleled by water radiolysis and formation of free radicals augmenting damage to burnt tissues is a factor aggravating the mechanism of tissue damage.

Dibunol liniment containing ionol [6,7] is the best known antioxidant used for local therapy. Wound dressing Procel-Super tested in our study is based on enzyme isolated from human erythrocytes or biotechnologically from recombinant *Saccharomyces cerevisiae* strain.

Local use of SOD is effective in the treatment of thermal and solar burns. This enzyme characterized by pronounced antiinflammatory effect shortens wound process phases, promotes connective tissue proliferation and wound epithelialization, and in some cases makes the involvement less deep [5,9]. Wound dressing with SOD used in our study was effective in thermal burns and CRI, though in the latter case its effect was less pronounced than that of Inerpan; it is, however, obvious that local use of antioxidants in CRI is pathogenetically justified.

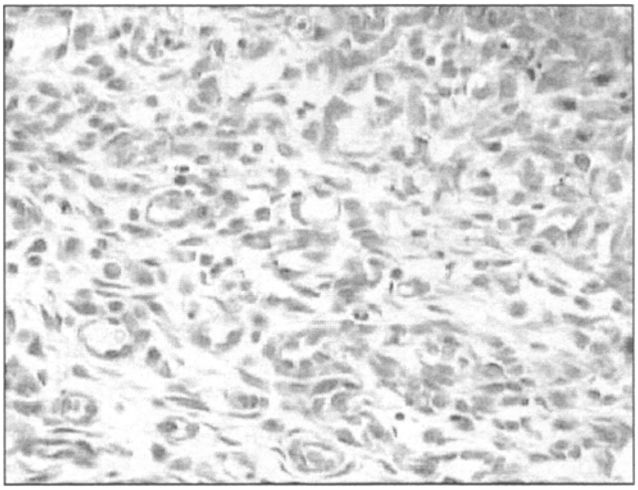

Fig. 3. Application of Procel-Super wound dressing in CRI (day 12). New capillaries in the fascia propria of the skin. Hematoxylin and eosin staining; ×70.

The majority of local therapeutic means do not ensure comprehensive effects on the wound process [6,7].

Wound-healing means are often little effective in CRI not only because of inhibitory effects of ionizing radiation on the reparative potential, but also because of impaired reactivity of irradiated organism to drugs [11]. Adequate local therapy of CRI requires careful selection of drugs best corresponding to the phase of the wound process and period of ARD. Therefore, good prospects of comprehensive approach based on the use of multicomponent compositions with universal properties and modulating various pathogenetic mechanisms of reparation simultaneously are obvious. Our studies indicate that the development of complex wound dressing including antibacterial, antiinflammatory, antioxidant, and radioprotective compounds, is an important task.

Hence, wound dressings Procel-Super and Inerpan stimulate healing of thermal burns of the skin in irradiated animals. The use of these synthetic wound dressings promotes wound healing process in CRI. These preparations decrease the duration of separate phases of the wound process and accelerate healing.

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