Wound-Healing Effect of Papaya-Based Preparation in Experimental Thermal Trauma

E. V. Mikhal'chik, A. V. Ivanova, M. V. Anurov*, S. M. Titkova*, L. Yu. Pen'kov, Z. F. Kharaeva***, and L. G. Korkina**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 6, pp. 638-640, June, 2004
Original article submitted December 17, 2003

Treatment with the phytopreparation from papaya accelerated wound healing and reduced the severity of local inflammation in rats with burn wounds. The effect of this phytopreparation can be related to an increase in the effectiveness of intracellular bacterial killing by tissue phagocytes due to the inhibition of bacterial catalase. Antioxidant activity of the preparation decreases the risk of oxidative damage to tissues.

Key Words: *burns; papaya; antioxidants; bacterial catalase*

Papaya fruits and leaves are traditionally used in South-East Asia and Africa for wound healing and treatment of inflammation (*e.g.*, during burns) [8]. Papaya fruits contain considerable amounts of proteolytic enzymes that promote wound sanation from necrotized tissue. Preparations from fermented papaya fruits can modulate the content of biologically important free radicals in the organism [7]. The release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from leukocytes in the zone of injury during burn trauma accompanies phagocytosis, is initiated by bacterial toxins and, therefore, depends on the severity of infection. Local treatment with antioxidants reduces the severity of tissue damage in the wound, but increases the risk of wound infection due to suppression of ROS generation. Wound microorganisms escape phagocytosis due to the presence of catalase, which degrades hydrogen peroxide. In the presence of myeloperoxidase, hydrogen peroxide and chlorine ions are transformed into hypochlorite with high bactericidal activity.

Antioxidant and antibacterial activity of the preparation from fermented papaya was studied in model systems. We studied the antiinflammatory and wound-healing effects of this preparation in rats with burn trauma.

MATERIALS AND METHODS

Antioxidant activity of the preparation from fermented papaya (BioRex, 003117.I.392.07.2001) was evaluated by means of chemiluminescence assay. Generation of hydroxyl and superoxide radicals was studied in H_2O_2—$FeSO_4$ and xanthine—xanthine oxidase model systems, respectively. The total production of RNS and ROS by leukocytes was measured in blood samples stimulated with phorbol ester [2].

Peripheral blood neutrophils were isolated in a double density gradient of Ficoll and Verografin to study phagocytic reactions.

Cultures of *Staphylococcus aureus* with catalase activity were isolated from the blood of patients with generalized infection. Bacterial cultures not possessing catalase activity were isolated routinely from tonsil smear.

Cultures of *Staphylococcus aureus* were treated with BioRex. The solution of BioRex in 0.9% NaCl (1 ml, 100 mg/ml) was added to the cell suspension (1 ml, 1.5×10^9 cells/ml) and incubated at 37°C for 1 h. Bacteria were washed with 0.9% NaCl to estimate the effectiveness of intracellular bacterial killing by human neutrophils [6] and bacterial catalase activity [1].

Department of Molecular Biology, *Department of Physiology, Russian State Medical University; **Department of Surgery, Institute of Pediatrics and Child Surgery, Russian Ministry of Health, Child Municipal Clinical Hospital No. 9, Moscow; ***Kabardino-Balkarian State University, Nalchik. ***Address for correspondence:*** lemik@zmail.ru. Mikhal'chik E. V.

0007-4888/04/1376-0560

Burn trauma was produced in 12 male Wistar rats weighing 400-450 g. An aluminum cylinder filled with water (85°C) was applied to the skin for 10 sec under constant pressure [3]. This treatment resulted in the appearance of burns IIIA-B (20% of skin area). Eschar was formed on day 4 and removed by tangential excision. The gel of BioRex and Vaseline meshed tulle *Lomatuell* were applied to the wound in rats of the main group. Control animals were treated only with *Lomatuell*. Necrectomy and treatment with the preparation were repeated on day 8. The animals were examined for 12 days. The wound area was measured planimetrically on days 4, 8, and 12. The intensity of ROS generation in the blood of animals was estimated by luminol-dependent chemiluminescence stimulated with phorbol myristate acetate (PMA). Activities of myeloperoxidase, glutathione peroxidase, catalase [4], and superoxide dismutase (SOD) [5] in the homogenate of granulation tissue were measured on day 12.

RESULTS

BioRex in a concentration of 1-5 mg/ml *in vitro* suppressed generation of superoxide (C_{50}=5 mg/ml) and hydroxyl radicals (C_{50}=1.1 mg/ml) and total production of radicals in human blood cells (C_{50}=2 mg/ml) by 50%.

Pretreatment of *S. aureus* with BioRex decreased bacterial catalase activity by 35%. These changes were accompanied by a 500-fold increase in the effectiveness of intracellular killing of staphylococci. The number of survived bacteria in control and treated rats was 5×10^6 and 10^4 cells/ml, respectively.

The preparation had a dose-dependent effect on intracellular death of bacteria possessing catalase activity. BioRex produced no direct bacteriostatic or bactericidal effects.

Experiments with model systems revealed the antioxidant and indirect antibacterial effect of BioRex. Therefore, this phytopreparation holds much promise as a local wound-healing drug.

The acute inflammatory response developed in rats with experimental burn trauma. The intensity of radical generation in the blood increased by 2.7-3 times on day 4 after trauma. Chemiluminescence of the whole blood from control rats surpassed normal in various periods of observations. However, in animals treated with BioRex this index decreased by the 12th day after trauma (Table 1).

A planimetric study revealed considerable differences in the rate of wound healing in BioRex-treated and control rats on day 8 of the experiment. On day 12 after trauma, the wound area in BioRex-treated rats was 2-fold lower than in control animals (Table 2).

Myeloperoxidase serves as a marker of local inflammatory reaction. Enzyme activity in BioRex-treated rats was slightly lower than in control animals (328.1±37.8 and 414.0±66.3 μmol/g protein, respectively). No intergroup differences were observed in SOD and glutathione peroxidase activities in the granulation tissue. Catalase activity significantly differed in samples of granulation tissue from BioRex-treated and control rats (15.6±5.5 and 70.5±30.2 U/mg protein, respectively, $p<0.05$).

Our results suggest that local treatment with the preparation from papaya accelerates wound healing in rats with burn trauma. This preparation decreased production of free radicals in the whole blood of animals, which reflects changes in the general inflammatory reaction. Myeloperoxidase activity in the wound tended to decrease. Local antiinflammatory activity of this phytopreparation was related to the indirect antibacterial effect and antioxidant properties.

Catalase activity decreased, while activities of other antioxidant enzymes in the granulation tissue remained unchanged. These data suggest that BioRex directly inhibits catalase in both the macroorganism and wound bacteria.

TABLE 1. Entire Production of Radicals in Blood Samples from Animals (PMA-Stimulated Luminol-Dependent Chemiluminescence, arb. units)

Period after the incidence of burn, days	Group	
	control	treatment
Before burn	9.3±4.0	10.9±4.5
1	20.9±11.9	18.4±6.7
6	24.6±9.4	24.3±8.9
12	33.5±17.6	17.5±0.1

TABLE 2. Planimetry of Burn Wound (Area, mm², *M*±*m*)

Period after the incidence of burn, days	Group	
	control	treatment
4	4858±452	5104±316
8	4085±319	3123±540*
12	2092±196	884±531*

Note. *$p<0.05$ compared to the control.

REFERENCES

1. O. V. Bukharin, S. V. Cherkasov, A. V. Sgibnev, *et al*., *Byull. Eksp. Biol. Med.*, **130**, No. 7, 80-82 (2000).
2. E. V. Mikhal'chik, L. G. Korkina, S. D. Shiyan, and N. G. Bovin, *Biol. Membrany*, **11**, 581-587 (1994).
3. N. A. Busch, E. M. Zanzot, P. M. Loiselle, *et al*., *Infect. Immun*., **68**, No. 6, 3349-3351 (2000).
4. R. A. Greenwald, *Handbook of Methods for Oxygen Radical Research*, Florida (1986), pp. 291-302.

5. H. P. Misra and I. Fridovich, *J. Biol. Chem*., **247**, 3170-3175 (1972).
6. S. L. Nielsen, F. T Black, M. Storgaard, and N. Obel, *J. Biochem*., **118**, 271-277 (1995).
7. J. A. Osato, I. B. Afanas'ev, Z. P. Cheremisina, *et al*., *Phys. Chem. Biol. Med*., **2**, 87-95 (1995).
8. I. F. Starley, P. Mohammed, G. Schneider, and S. W. Bickler, *Burns*, **25**, No. 7, 636-639 (1999).