Activity of Antioxidant Enzymes in the Skin during Surgical Wounds

E. V. Mikhal'chik, M. V. Anurov, S. M. Titkova, E. A. Miroshnikova*, E. V. Lukasheva*, I. B. Deeva, G. A. Ibragimova, and L. G. Korkina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 12, pp. 622-625, December, 2006
Original article submitted March 29, 2006

Full-thickness skin wounds (460 mm^2) in rats were associated with increased blood chemiluminescence and neutrophil infiltration of the wound tissue and surrounding skin (recorded by myeloperoxidase activity). Activities of glutathione peroxidase and glutathione S-transferase in the skin and wound tissue increased on days 4 and 8. A correlation was revealed between activities of these enzymes and myeloperoxidase activity. Activities of myeloperoxidase and catalase increased in patient's skin excised during plastic surgeries of more than 2.5 h duration.

Key Words: *wounds; skin; antioxidant enzymes; myeloperoxidase*

Reactive oxygen species (ROS) formed in the skin during inflammation play a role of mediators in normal wound healing [9]. However, ROS overproduction serves as an important pathogenetic factor impairing healing (*e.g.*, during radiation exposure) [2]. The imbalance between pro- and antiinflammatory reactions in the skin leads to oxidative damage to cell components (DNA, lipids, and proteins) and skin cell dysfunction, *i.e.* changes in migration, proliferation, and synthesis of extracellular matrix molecules [13]. The discharge from acute and chronic wounds includes products of free radical reactions, carbonylated proteins and malonic dialdehyde (MDA) [8]. Neutrophils serve as a potent source of ROS (superoxide radicals, H_2O_2, and hypochlorite) during the inflammatory phase of wound healing. It was demonstrated that keratinocyte growth factor gene is responsible for the induction of glutathione peroxidase (GP) synthesis, which can protect the proliferating epithelium on wound edges from excess ROS (first of all from H_2O_2 and lipoperoxides) [7].

Russian State Medical University; *Russian University of People's Friendship, Moscow

On the whole, adaptation of various tissues to ROS excess is determined by induction of antioxidant enzymes, which depends on the expression of the corresponding mRNA [14].

Here we measured activities of peroxide-utilizing and lipoperoxide-utilizing antioxidant enzymes GP and glutathione S-transferase (GST) in wound tissue and skin of animals with excision wounds. Catalase activity was measured in human skin after plastic surgeries.

MATERIALS AND METHODS

The skin excised in facelift plastic surgery was used. The skin samples were taken 5-15, 30-150, and 150-250 min after the start of surgery.

Experimental samples were obtained from 16 male Wistar rats (350-400 g) with full-thickness skin excision wounds (459±15 mm^2).

Wound planimetry was performed on days 4 and 8 under general anesthesia. Blood samples from the tail were collected in tubes with heparin. Skin biopsy specimens were taken at a distance of 20 mm from the wound edges. Granulation tissue was sampled. The animals were euthanized.

0007-4888/06/1426-0667

Skin samples were frozen at -18°C and homogenized in a Potter hand glass homogenizer immediately before all study. The samples were taken from the upper layer of human skin (mainly from the epidermis). Homogenates were prepared in a 10-fold volume of 0.1 M potassium phosphate buffer (pH 7.4) and centrifuged at 1000*g* and 10°C for 30 min. The supernatant was separated for biochemical analysis.

Total protein content was measured by the method of Lowry. Myeloperoxidase (MPO) activity was estimated by *o*-dianisidine oxidation [6]. Catalase activity was determined by H_2O_2 degradation [5]. GP activity was measured in the reaction with tert-butyl hydroperoxide in the presence of reduced glutathione. GST activity was measured in the reaction with 1-chloro-2,4-dinitrobenzene [3]. Enzyme activity was expressed in μmol substrate transformed over 1 min. The measurements were performed on a Shimadzu UV-1700 spectrophotometer. The results were standardized for protein content in the sample. The intensity of luminol-dependent chemiluminescence stimulated with phorbol ester was measured in blood samples [10].

RESULTS

The initial area of the wound was 6% of body surface area. Signs of purulent inflammation in the wound were absent throughout the study. On day 4, whole blood chemiluminescence increased from 21.1±3.8 (normal) to 46.3±18.7 mV, which attested to neutrophil activation as a result of trauma. On day 8 this parameter returned to normal (26.2±9.1 mV). The wound area decreased to 253±51 and 150±65 mm^2 on days 4 and 8, respectively.

Variations in MPO activity in the wound tissue reflected the dynamics of local neutrophilic inflammation (Table 1). On days 4 and 8, MPO activity in the skin of treated rats was higher compared to intact animals.

Similar changes were revealed in the adjacent skin. Since activated neutrophils release considerable amounts of ROS under the influence of local stimulatory agents (cytokines, bacteria, and immune complexes), we measured activities of protective antioxidant enzymes in the skin and in the wound.

Previous studies showed that trauma was not accompanied by catalase induction in intact rat skin [2]. Hence, we studied GP and GST as the protective peroxide-utilizing and lipoperoxide-utilizing enzymes. Activities of both enzymes in the wound tissue and skin significantly increased on days 4 and 8. GST activity in wound tissue progressively increased until day 8 and exceeded that in skin samples (Table 1).

A positive correlation was revealed between activities of MPO and GP in the skin on day 4 (r=0.90, p<0.05; Fig. 1, *a*). On day 8, MPO activity in the wound tissue positively correlated with activities of GP (r=0.96, p<0.05; Fig. 1, *b*) and GST (r=0.89, p<0.05).

Specific MPO activity progressively increased in human skin samples obtained during facelift surgery (Fig. 2). The samples were excised with widening of the surgical field, which corresponded to sampling of the adjacent skin under experimental conditions.

When studying activity of catalase as a peroxide-utilizing enzyme in human skin, we found that specific catalase activity in human skin samples tended to increase 20-150 min after the start of surgery and then 1.7-fold surpassed the basal level (p=0.039, Fig. 2).

Wound healing proceeds through fibroblast-mediated contraction of the wound edges, formation of the granulation tissue, growth of keratinocytes, and remodeling of the epidermis [4]. The inflammatory stage is characterized by neutrophil migration to the wound and takes about 4 days. The increase in MDA concentration reflects activation of free radical reactions in the wound tissue. The concentration of hydroperoxides in aseptic wound tissue of rats with a wound area of 400 mm^2 increased on days 1-3 and 6-12 [1]. The contents of MDA and conjugated dienes increased on day 1 [2]. Our experiments were performed in rats with similar

TABLE 1. Enzyme Activities in Wound Tissue and Adjacent Skin of Rats

Parameter	Normal	Time after surgery, days			
		4		8	
		wound	skin	wound	skin
MPO, μmol/mg	33.9±16.1	95.1±35.8*+	183.2±16.3*	112.4±36.7*	113.2±39.0*
GP, U/mg	0.11±0.02	0.28±0.05*	0.26±0.07*	0.26±0.11*	0.27±0.06*
GST, μmol/g	4.4±0.8	9.4±2.4*	10.6±2.5*	15.1±4.0*+	9.3±1.2*

Note. p<0.05: *compared to normal; +compared to the adjacent skin.

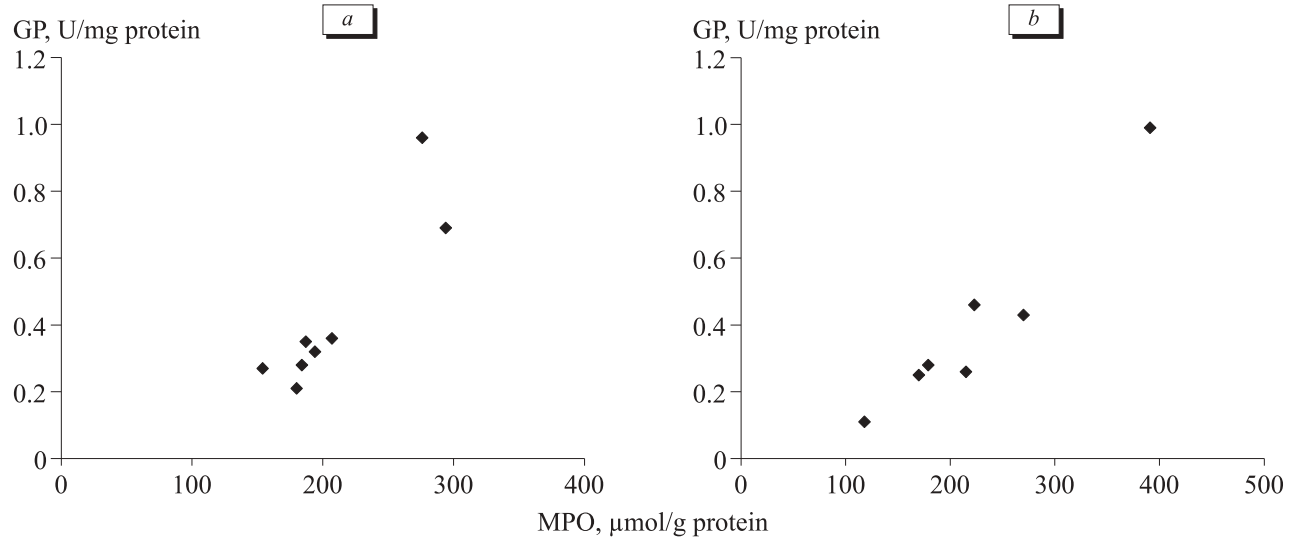

Fig. 1. Relationship between activities of MPO and GP in rat skin (*a*) and wound tissue (*b*) on days 4 and 8 after surgery, respectively.

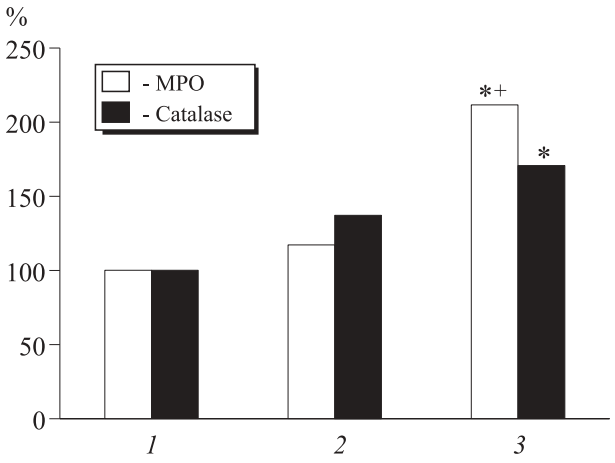

Fig. 2. Intraoperative changes in activities of MPO and catalase in patient's skin during plastic surgeries: 5-15 (*1*), 20-150 (*2*), and 150 min or later after the start of surgery (*3*). $p<0.05$: *compared to the parameter observed after 5-15 min; +compared to the parameter observed after 20-150 min.

wound areas (459±15 mm^2). We found that MDA content in skin samples from the wound edges increases on day 4 (0.86±0.19 *vs*. 0.47±0.07 μmol/g protein in intact skin, $p<0.05$).

Activation of antioxidant enzymes in the adjacent skin is a normal adaptation reaction to inflammation (*e.g.*, ROS generation). GST activity differed in skin samples and wound tissue on day 8. Probably, the wound tissue accumulates a greater amount of lipoperoxides and degradation products that serve as the substrate for GST.

Our results contradict published data that rat skin cannot adapt to oxidative stress [10]. Previous studies showed that antioxidant enzymes are not induced in subjects with a wound area of 800 mm^2 [10]. It probably results from hyperactivation of leukocytes and direct inactivation of antioxidant enzymes with wound radicals. We conclude that wound tissue and adjacent skin in rats may respond to the inflammatory reaction by activation of peroxide-utilizing enzymes. Our results are consistent with published data that expression of mRNA for GP and other enzymes in mice increases in the skin of the wound edges [11]. Rapid migration of neutrophils to the perioperative area of human skin is followed by catalase activation.

REFERENCES

1. V. V. Zakharov, L. A. Mamedov, S. A. Meshcheryakova, *et al*., *Byull. Eksp. Biol. Med.*, **107**, No. 6, 690-692 (1989).
2. T. V. Zvyagintseva, *Teor. Eksper. Med*., No. 1, 44-47 (2001).
3. *Medical Laboratory Technologies* [in Russian], Ed. A. I. Karpishchenko, St. Petersburg (1999), Vol. 2, pp. 20-26.
4. *Methodical Manual on Wound Healing* [in Russian], Ed. P. Khartmann, Moscow (2000), pp. 24-35.
5. H. Aebi, *Methods of Enzymatic Analysis*, New York, London (1984), Vol. 2, pp. 673-684.
6. P. C. Andrews and N. I. Krinsky, *CRC Handbook of Methods for Oxygen Radical Research,* Ed. R. A. Greenwald, Florida (1985), pp. 297-302.
7. S. Frank, B. Munz, and S. Werner, *Oncogene*, **14**, No. 8, 915-921 (1997).
8. R. Moseley, J. R. Hilton, R. J. Waddington, *et al*., *Wound Repair Regen*., **12**, No. 4, 412-419 (2004).
9. M. Rojkind, J. A. Dominguez-Rosales, N. Nieto, and P. Greenwel, *Cell. Mol. Life Sci*., **59**, No. 11, 1872-1891 (2002).
10. A. Shukla, A. M. Rasik, and G. K. Patnaik, *Free Radic. Res*., **26**, No. 2, 93-101 (1997).
11. H. Steiling, B. Munz, S. Werner, and Brauchle, *Exp. Cell. Res*., **247**, No. 2, 484-494 (1999).
12. F. Takayama, T. T. Egashira, and Y. Yamanaka, *Nippon Yakurigaku Zasshi*, **111**, No. 3, 177-186 (1998).
13. P. T. Thang, S. Patrick, L. S. Teik, and C. S. Yung, *Burns*, **27**, No. 4, 319-327 (2001).
14. M. Teidge, S. Lortz, J. Drinkgern, and S. Lenzen, *Diabetes*, **46**, No. 11, 1733-1742 (1997).