Photoprotective Activity of Melanin Preparations from Black Yeast-Like Fungus during UV Irradiation of Human Skin: Dependence on the Concentration

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The effect of melanin solutions on the skin exposed to UV irradiation (1050 kJ/m^2) depended on its dose and varied from photoprotection (0.005 mg/ml) to photosensitization and phototoxicity (burn, 0.1 mg/ml). These results suggest that doses of melanin preparations should be empirically selected to achieve optimum photoprotective effect.

Key Words: *melanin; photoprotection; black yeast fungi*

The biological role of melanins attracted much recent attention. These pigments are synthesized in organisms belonging to various taxonomic groups (from unicellular organisms to humans) under extreme conditions [5], which probably attests to their protective (or even rejuvenating) properties. In view of this, melanin preparations are widely used in dermatology and cosmetology. Melanins also possess antioxidant and antiradical activities [3]. However, eumelanins (dihydroxyphenylalanine preparations) produce a prooxidant effect [4]. Detailed studies showed that treatment with melanin preparations in various concentrations and under different lighting regimens is followed by various changes. Therefore, the effects of melanin preparations depend of these factors [6]. The concentration of molecular oxygen also modulates the effects of melanins. It is known that substances acting as antioxidants produce prooxidant effects at oxygen concentrations of about 10^{-6} M [1].

Here we studied the dependence of photoprotective activity of 1,8-dihydroxynaphthalene melanin (F-48) on its concentration during UV-A irradiation.

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MATERIALS AND METHODS

Black yeast-like fungus of the ascomycete affinity *Aureobasidium pullulans* (var. *aubasidani*, All-Russia Collection of Industrial Microorganisms, F-448) were used as the source of melanin. The culture was grown for 14 days in 200-ml shaker flasks with synthetic liquid nutrient Czapek—Dox medium (220 rpm and 25±1°C). Melanin was extracted from the biomass with 0.5 N NaOH (121°C, 1 h) and purified as described elsewhere [2].

Two healthy volunteers were examined. Melanin was dissolved in a water-glycerol mixture to a concentrations of 0.1, 0.05, 0.025, and 0.005 mg/ml and applied to the skin in the lower abdominal region. Treated zones were irradiated in the UV-A range (320-380 nm, 1050 kJ/m^2) through a 3-cm^2 cardboard shield. Before application to the skin, melanin preparations were exposed to scattered sunlight (1 mW/cm^2) for 30 min.

The irradiated skin was examined for 10 days. Skin biopsy samples taken on days 1, 3, and 7 after irradiation were fixed in 2.5% glutaraldehyde in phosphate buffer at 4°C for 2 h, washed, fixed in 2% OsO_4 at 4°C for 12 h, dehydrated in increasing ethanol concentrations (40-100%), and embedded into Epon-Araldite mixture. Ultrathin sections were obtained on a LKB ultratome and contrasted with lead salts by the

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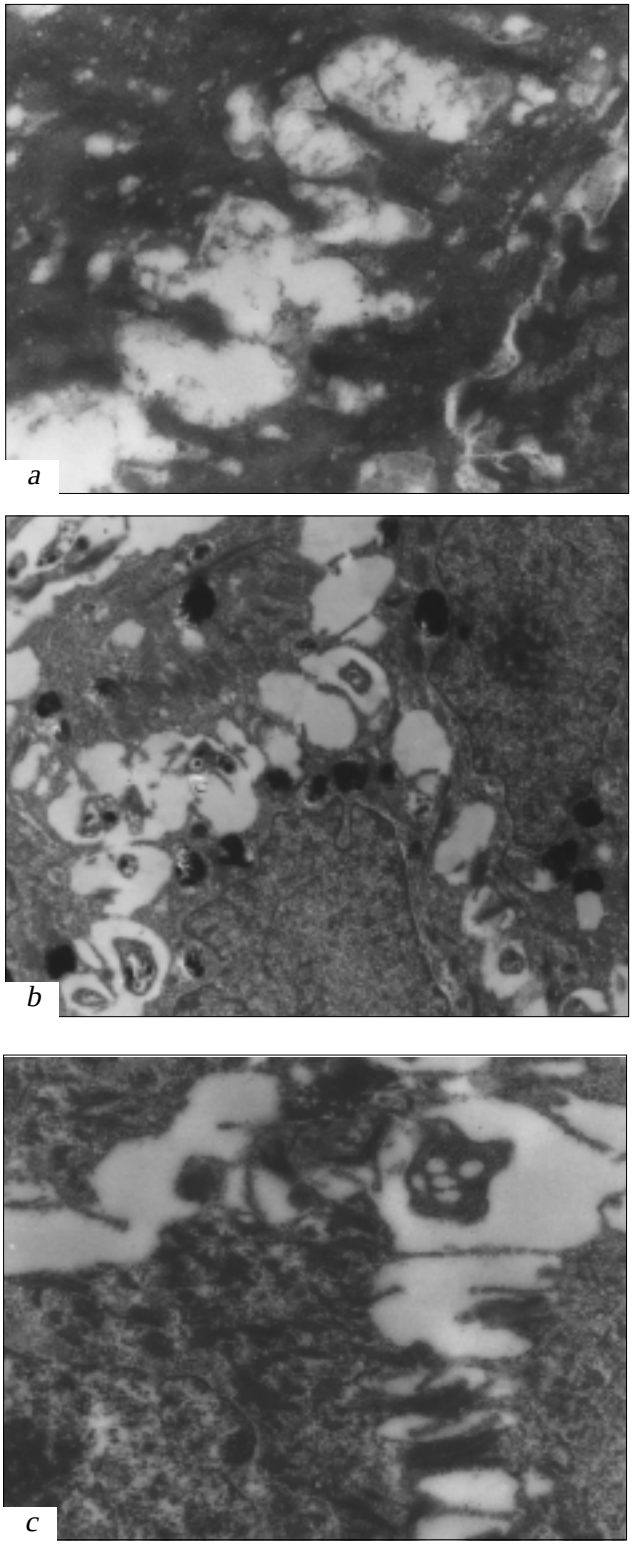

Fig. 2. Desmosomes in the spinous epidermal layer 7 days after irradiation and treatment with melanin: ×20,000 (*a*, *c*) and ×15,000 (*b*). *a*) degeneration of desmosomes (0.1 mg/ml), *b*) most desmosomes are preserved (0.025 mg/ml, *c*) normal desmosomes and increased content of pigment (0.005 mg/ml).

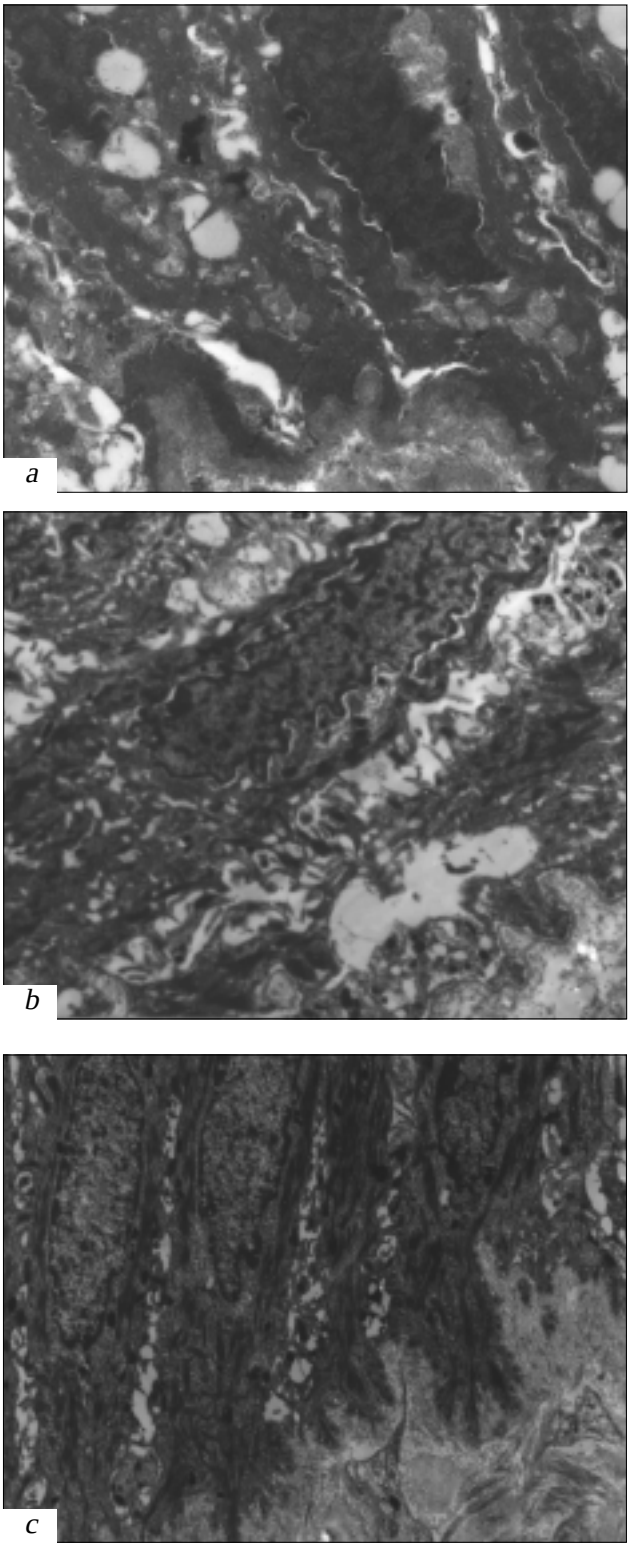

Fig. 1. Keratinocytes in the basal epidermal layer 3 days after irradiation and treatment with melanin: ×10,000 (*a*, *b*) and ×6000 (*c*). *a*) carbonization of keratinocytes (0.1 mg/ml), *b*) pronounced damages (0.025 mg/ml), *c*) minimum changes (0.005 mg/ml).

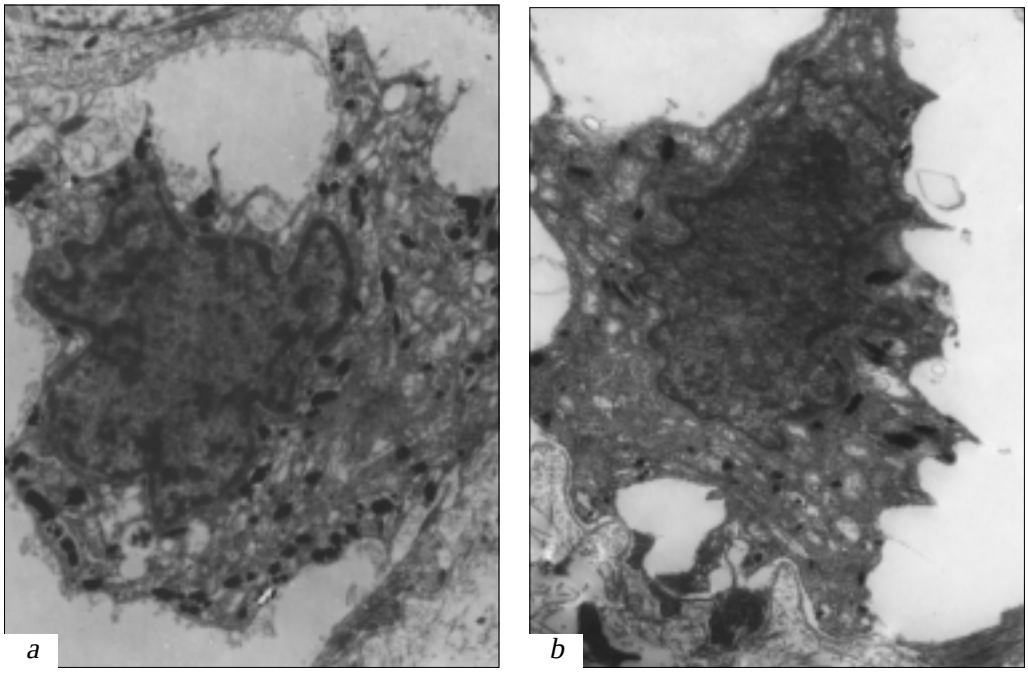

Fig. 3. Melanocytes on day 7 after irradiation and treatment with melanin in concentrations of 0.005 (*a*) and 0.025 mg/ml (×20,000, *b*). *a*) intensive production of pigment, *b*) less pronounced stimulation of melanogenesis.

method of Reynolds. The preparations were examined under a JEM-100c microscope.

RESULTS

Hyperpigmentation of irradiated skin immediately after irradiation was more pronounced at higher melanin concentrations and persisted throughout the observation periods. Edema and hyperemia of the surrounding skin were observed over 2-3 days after irradiation. On days 1 and 3 after irradiation the derma was characterized by inflammatory signs, including edema, mast cell degranulation, and plethora. These signs disappeared on day 7 after exposure.

Morphological examination revealed different reactions of the epidermis to UV irradiation after application of melanin in various concentrations. Most pronounced damages to the skin were found after application of 0.1 mg/ml melanin. Absorption of light energy by melanin molecules contributed to carbonization of all epidermal layers (including the basal layer, Fig. 1, *a*). Empty spaces, destruction of organelles and cytoskeleton, degeneration of desmosomes (Fig. 2, a), and condensation were seen in skin cells. Damaged fibroblasts and collagen fibers associated with the derma were found. Ultramicroscopic signs corresponded to thermal burns of the skin (photoburn).

Ultrastructural changes were less pronounced after application of melanin in other concentrations. After application of 0.05 mg/ml melanin, epidermal cells were also destructed, but nucleus matrix was more homogenous, perinuclear empties were not found and condensation was less pronounced. After application of melanin in a concentration of 0.025 mg/ml skin damages were mosaic. Some keratinocytes retained viability, while other cells died (Fig. 1, *b*). Most desmosomes were preserved (Fig. 2, *b*). The most pronounced changes were found in cells enriched with melanin granules. After treatment with 0.005 mg/ml melanin damages to the skin were also mosaic, but without keratinocyte death (Fig. 1, *c*) and desmosome degeneration (Fig. 2, *c*).

Melanin in low concentrations, particularly in a dose of 0.005 mg/ml, stimulated melanogenesis (Fig. 3, *a*). The effect was less pronounced after application of 0.025 mg/ml melanin (Fig. 3, *b*).

Our results show that application of melanin to the skin produced dose-dependent changes, which included photoprotection, photosensitization, and photoburn. Therefore, the doses of melanin preparations should be empirically selected to achieve the optimum photoprotective effect.

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