Photoprotective Activity of Melanin Preparations in Human Skin Exposed to UV Irradiation: Dependence on Previous Photoexposure

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Photoprotective effects of three melanin preparations (from black yeast fungi and *Sepia sp.*) were studied. These preparations in aqueous solutions (5 μg/ml, dark exposure for 7 days) demonstrated high photomodification capacity upon exposure to visible light in doses of up to 1.8 kJ/m^2. Preliminary exposure of these solutions to visible light in a dose of 360 kJ/m^2 notably decreased the photoprotective effect of melanins during UV exposure of the skin treated with these solutions (at UV dose of 3.4 kJ/m^2). This necessitates empirical selection of the dose and storage condition of melanin preparations for attaining the optimal photoprotective effect.

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

Production of melanins increases in response to exposure to some extreme factors [6], which suggests protective (and "rejuvenating") effects of these substances. Now melanins are widely used in dermatology and cosmetology. Biological effects of melanins can be explained by their antioxidant and antiradical activities [4,6], as well as by prooxidant effects of melanins of animal origin [5]. Different scientists demonstrated opposite effects of the same melanin preparation [4,7]. Analysis of these papers showed considerable differences in experimental conditions, agent concentrations, and illumination doses used by these authorities (see also [5]).

It was previously shown that the effect of melanin (after preliminary photoexposure) on skin exposed to a fixed dose of UV depended on the dose and varied from photoprotective to photosensitizing (even to photoburn) [3]. (In paper [3] UVA dose was 17 kJ/m^2 (283 mW/cm^2, 600 sec.) The specified dose 1050 kJ/m^2 is an error). Here we studied the effect of preliminary (before application to the skin) photoexposure on the efficiency of photoprotective concentration of different melanin preparations. The degree of photomodification (changes in absorption spectrum) after changes in background illumination should be proportional to the donor-acceptor activity of the substance [1]. Therefore we selected melanin preparations characterized by high photomodication capacity at doses of scattered solar light not exceeding 10% of the dose absorbed by the skin over 30 min at radiation density of 1 mW/cm^2 (open air under cloudy sky), *i.e.* not exceeding 1.8 kJ/m^2.

MATERIALS AND METHODS

The source of UV light was a DRSh-100 mercury-quartz lamp (80% UV radiation in the 320-380 nm band with the maximum at 365 nm). Photometric sensor was FDK-227 photodiode with sensitivity of 7.2 μA/W (integral, 400-740 nm) and 1.54 μA/W (monochromatic, 365 nm) calibrated with a standard set of optic filters with known optical density. The absorp-

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tion spectra of the studied melanin solutions (20 samples, including synthetic DOPA melanin, ICN) were recorded on a SF-46 spectrophotometer at 310-460 nm at 1-nm band width.

Preparations M1 and M2 were obtained from biomass of black yeast fungi *Aureobasidium pullulans* var. *pullulans* (SPCFA 2275) and *Nadsoniella nigra* var. *hesuelika* (All-Russian Collection of Industrial Microorganisms F-2137), respectively; M3 was a preparation of animal origin (from *Sepia sp.*, ICN). Yeast cultures were grown for 14 days at 25±1°C in shaking flasks (200 ml, 220 rpm) in the Capek-Dehoux liquid synthetic nutrient medium, after which melanin was isolated from the biomass (0.5 n. NaOH, 121°C, 1 h) and purified as described previously [2]. Aqueous solutions of melanins (5 μg/ml) were kept in the dark for 7 days at 4°C in sealed vials, and thus the initial ("unexposed") preparations were obtained. Photomodification of the preparations during exposure to visible light in doses of up to 1.8 kJ/m^2 is shown in Fig. 1. Before application to the skin the preparations were exposed to bright sunlight (20 mW/cm^2, 30 min) on air.

Two healthy volunteers took part in the study. One of them was not tanned, and during irradiation he felt warm, the sensation being nearly painful by the end of the procedure. The other was sun-tanned (the intensity of tan approached the maximum for this individual) and during irradiation he felt no pain. Seven skin sites (3-cm^2) on the inner side of the forearm were irradiated with UV-A (320-380 nm, 1.13 mW/cm^2, 300 sec) through a cardboard mask; one site served as the control and others were treated with exposed and unexposed M1-M3 preparations.

The exposed skin was observed for 2 months. Skin sites were dissected under 1% procaine anesthesia on day 6 after irradiation, fixed in 2.5% glutaraldehyde in Hanks' solution (2 h, 4°C), washed, postfixed in 2% OsO_4 (12 h, 4°C), dehydrated in ascending (from 40 to 100%) alcohols, and embedded in Epon-812-Araldite mixture. Ultrathin sections were made on an LKB ultratome, contrasted with uranyl acetate and lead salts

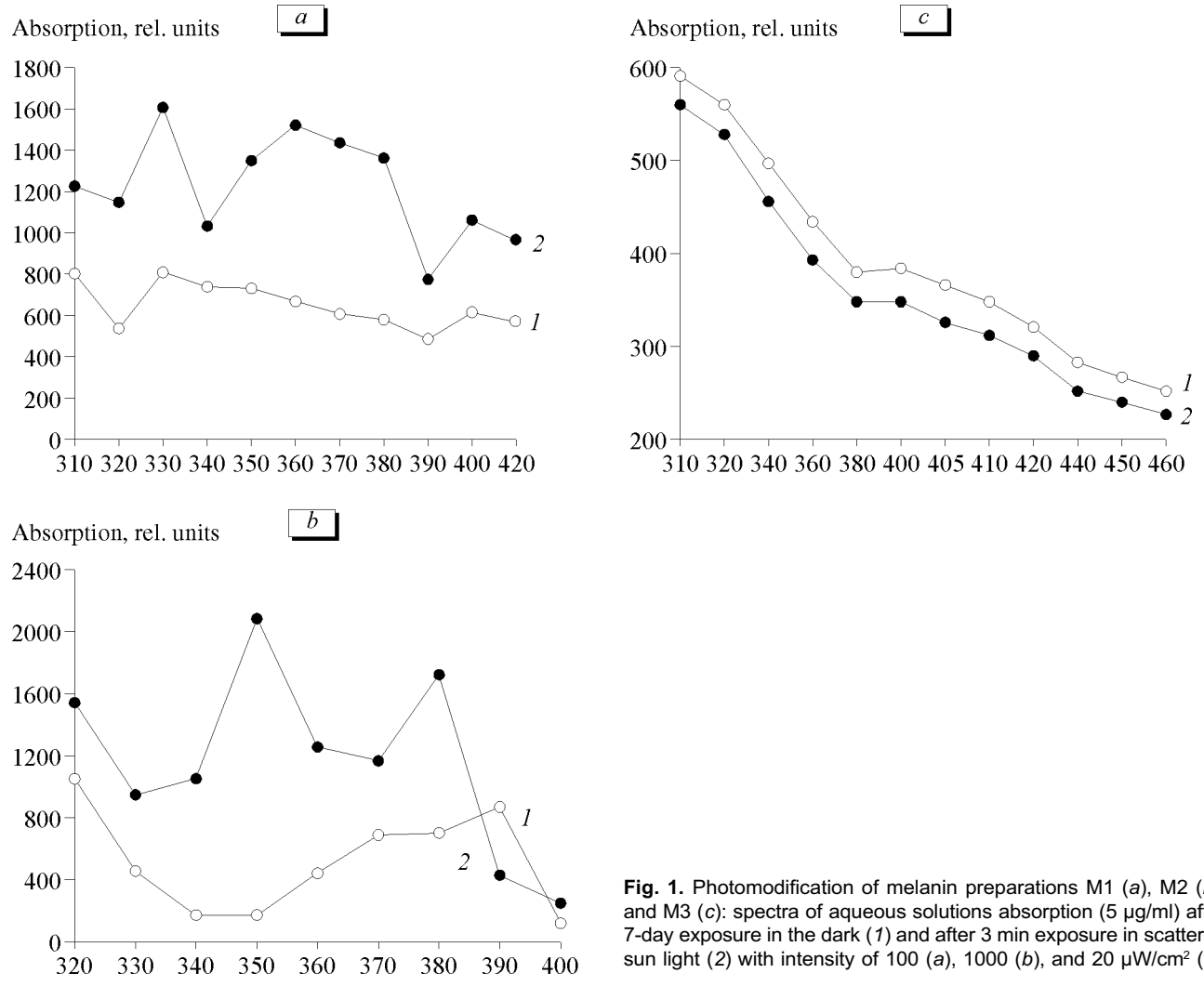

Fig. 1. Photomodification of melanin preparations M1 (*a*), M2 (*b*), and M3 (*c*): spectra of aqueous solutions absorption (5 μg/ml) after 7-day exposure in the dark (*1*) and after 3 min exposure in scattered sun light (*2*) with intensity of 100 (*a*), 1000 (*b*), and 20 μW/cm^2 (*c*).

after Reynolds, and examined under a JEM-100c electron transmission microscope.

RESULTS

During 24 h after the exposure all patients developed hyperemia and edema, which were most severe in control sites and less pronounced in sites treated with M1 and M3 melanins (changes in volunteer No. 1 were more pronounced than in volunteer No. 2). Hyperpigmentation (tan) developed during the next day and persisted throughout the observation period. It was more pronounced at sites treated with unexposed melanins and at sites treated with melanins M1 and M3 (also more pronounced in volunteer No. 1). After 5-7 days volunteer No. 1 developed hyperkeratosis, most severe on the control site and less pronounced at sites treated with exposed M1 and M3 melanins; no hyperkeratosis was observed at sites treated with unexposed M1-M3.

Microscopic examination showed pronounced activation of melaninogenesis in the control skin site of volunteer No. 1: the number of melanin granules increased in melanocyte cytoplasm and basal keratinocytes, as well as in melanocyte processes (increased melanin transport). Signs of intraepidermal edema were seen, extracellular spaces were extended (especially around melanocytes), there were some small intraepidermal vesicles. Minor ultrastructural changes were seen (desmosomes were partially melted). The horny layer thickened (to 35-40 layers of scales). Plethoric vessels, edema, and mast cell degranulation were observed in the derma.

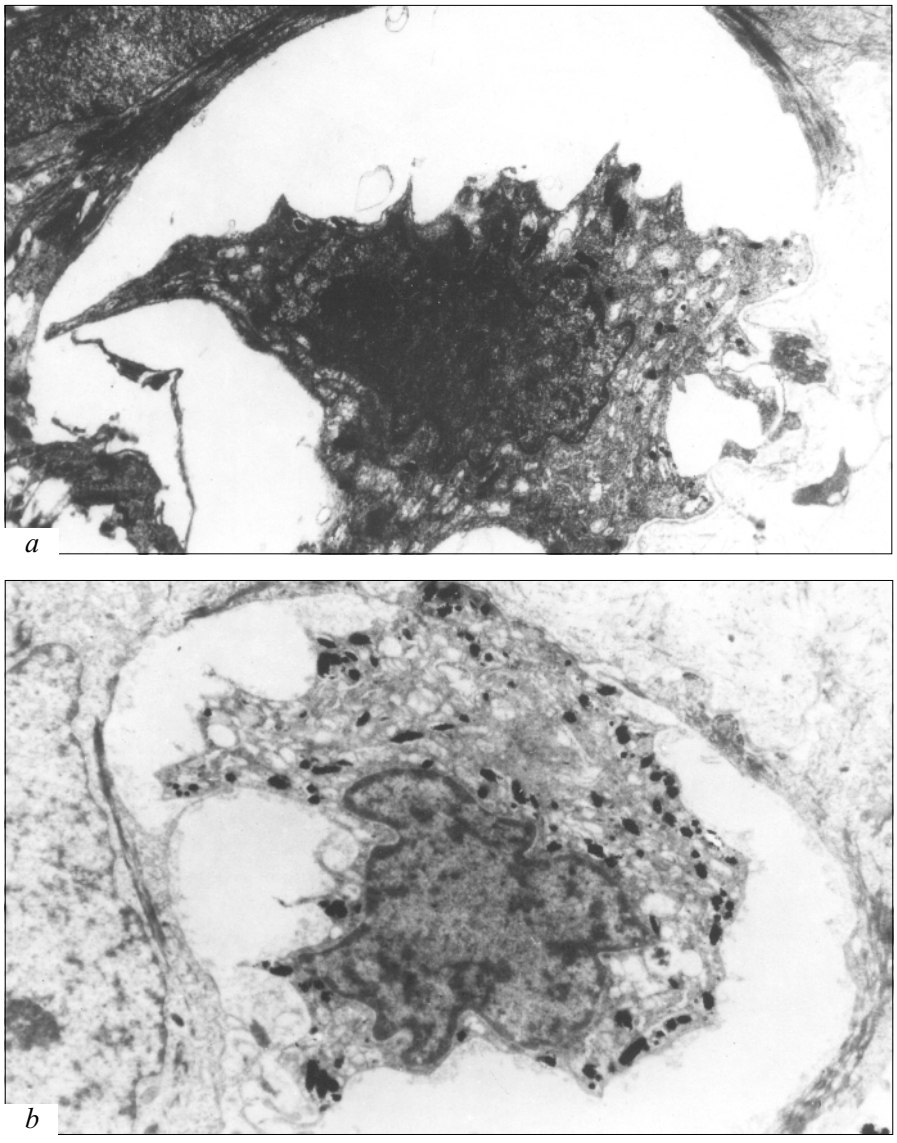

Fig. 2. Melanocytes on day 6 after UV irradiation after treatment with exposed (*a*) and unexposed (*b*) M3 melanin, ×5000. *a*) slight stimulation of melaninogenesis; *b*) active pigment production.

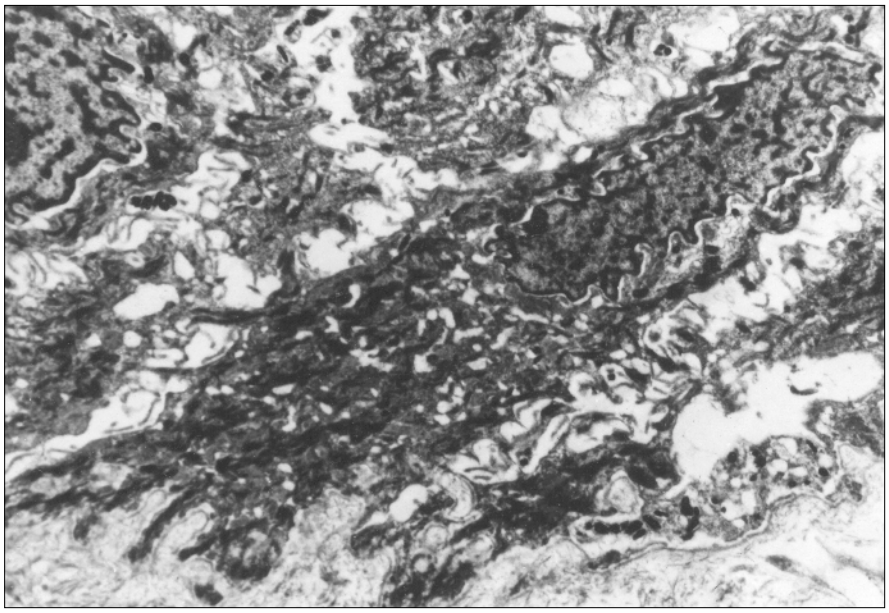

Fig. 3. Epidermal basal layer cells on day 6 after UV irradiation; treatment with exposed M2 melanin, ×4000.

Stimulation of melaninogenesis was more intense at sites treated with unexposed M1 and M3 melanins and less pronounced at sites treated with exposed melanins (Fig. 2). At sites treated with M2 the alterations were more pronounced after treatment with exposed preparation (Fig 3).

Examination of exposed skin in volunteer No. 2 with initially greater skin pigmentation showed less pronounced stimulation of melaninogenesis and less pronounced changes than in volunteer No. 1. In other aspects the changes observed in irradiated skin sites were similar to those in volunteer No. 1.

Hence, these findings indicate that preliminary exposure of melanin preparations significantly reduced their photoprotective effect, which necessitates empirical selection of the dose and storage conditions of melanin preparation for attaining the optimal photoprotective effect.

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