EFFECT OF NICOTINE ON ANTIOXIDANT STATUS IN HEMn-LP MELANOCYTES

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SIGNIFICANCE

Nicotine is one of the tobacco alkaloids and due to its addictive properties is responsible for the high smoking prevalence in the world. Nicotine draws attention because of its presumed neuroprotective and antioxidant features as well as common use in smoking cessation therapies. The evidence for nicotine accumulation in melanin containing tissues was provided. Studies on mice revealed that pigmented tissues can store nicotine up to 30 days after a single injection. It is suggested that nicotine may be accumulated in human tissues containing melanin. This may in turn influence biochemical processes in human cells producing melanin.

Melanin is a polymeric pigment and the major determinant of skin color, of skin, eyes and hair. It is also present in the inner ear, heart, lungs, liver, lymphocytes and brain. Melanin biopolymer is produced, stored and transported in melanosomes which are the specialized membrane-bound organelles of melanocytes. The role of melanin is to protect cells from UV radiation by creating a supranuclear cap in a cell, absorbing energy and working as an antioxidant agent and free radicals scavenger. Melanin is capable of binding many chemical substances, including drugs, like aminoglycoside antibiotics, fluorquinolones, anticancer agents, psychotropic drugs and also nicotine.

AIM OF STUDY

Retention of nicotine in melanin-containing tissues may affect biochemical processes in melanocytes. The purpose of this work was to estimate the effect of nicotine on antioxidant defense system in cultured normal human melanocytes HEMn-LP.

METHODS

Cell culture

The normal human epidermal melanocytes (HEMn-LP, Cascade Biologics) were grown according to the manufacturer’s instruction. The cells were cultured in M-254 medium supplemented with HMGS-2, penicillin (100 IU/ml), neomycin (10 μg/ml) and amphotericin B (0.25 μg/ml) at 37°C in 5% CO2. Cells were exposed to nicotine in concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mM for 24 h.

Hydrogen peroxide assay

Hydrogen peroxide (H2O2) content was measured using an assay kit (Cell Biolabs, Inc., USA) according to manufacturer’s instruction. This method is based on the ability of sorbitol to convert peroxide to a peroxyl radical, which oxidizes Fe3+ into Fe2+. Then Fe2+ reacts with a micromolar amount of xylene orange in the presence of acid to create a purple product that absorbs light at maximal wavelength 595 nm. The antioxidant -- butylated hydroxytoluene (BHT) is provided to prevent further undesirable chain peroxidation. Hydrogen peroxide content in the samples was expressed in μmol/mg protein.

Superoxide dismutase (SOD) assay

Superoxide dismutase (SOD) activity was measured using assay kit (Cayman, MI, USA) according to manufacturer’s instruction. This kit utilizes tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and xanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. SOD activity was expressed in U/mg protein.

Catalase (CAT) assay

Catalase (CAT) activity was measured using assay kit (Cayman, MI, USA) according to manufacturer’s instruction. This kit utilizes tetrazolium salt for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The formaldehyde produced is measured colorimetrically with 4-aminophenylhydroxymethylamine (Purpuril) as the chromogen. One unit of CAT was defined as the amount of enzyme that causes the formation of 1 nmol of formaldehyde per minute at 25°C. CAT activity was expressed in nmol/mg/min protein.

Glutathione peroxidase (GPx) assay

Glutathione peroxidase (GPx) activity was measured using an assay kit (Cayman, MI, USA) according to manufacturer’s instruction. The measurement of GPx activity is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydroperoxide by GPx is recycled to its reduced state by GR in the presence of nicotinamide-adenine dinucleotide phosphate (NADPH). The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25°C. GPx activity was expressed in nmol/min/mg protein.

Statistical analysis

In all experiments, mean values of at least three separate experiments (n=3) performed in triplicate were calculated. The results were analyzed statistically using GraphPad Prism 6.01 Software. A value of p<0.05 (*) or p<0.005 (**) obtained with a Student’s t-test, by comparing the data with those for control (cells without nicotine), was considered statistically significant.

RESULTS

It has been demonstrated that the activity of SOD increases with rising concentration of nicotine (Fig. 1). The treatment of cells with 0.1, 0.5 or 1.0 mM of nicotine significantly increased the SOD activity by 10.7%, 13.1%, or 18.3%, respectively, as compared with the controls. The intracellular CAT activity was also increased by 10.3%, 18.0% or 25.2% for cells treated with nicotine in concentration of 0.1 mM, 0.5 mM or 1.0 mM, respectively (Fig.2). Treatment of melanocytes with 0.1, 0.5 and 1.0 mM of nicotine enhanced the H2O2 content by 13.8%, 25.5% and 37.0%, respectively, as compared with the controls (Fig. 3). Nicotine in the concentration of 0.01 and 0.05 mM had no effect on cellular SOD and CAT activities as well as on H2O2 content. In contrast to SOD and CAT, nicotine had no statistically significant influence on the activity of GPx (Fig. 4).

CONCLUSION

Our results demonstrate that nicotine in non-cytotoxic concentrations causes significant alterations of biochemical processes in melanocytes, like induction of oxidative stress (increase in SOD, CAT activity and H2O2 content).

After treatment of cells with nicotine in lower concentrations (0.01 mM and 0.05 mM), the activities of SOD and CAT, and the H2O2 content were similar to the controls. Simultaneously, in case of all tested concentrations, no significant changes in cellular GPx activity were observed, what indicates that in melanocytes catalase is the main enzyme responsible for inactivation of the proradical hydrogen peroxide.

In case of light pigmented melanocytes, which contain less melanin than dark pigmented cells, the amount of nicotine bound to melanin is relatively low and therefore the antioxidative enzymes response is sufficient in various forms of smoking cessation therapies. The evidence for nicotine accumulation in melanin containing tissues was provided. Studies on mice revealed that pigmented tissues can store nicotine up to 30 days after a single injection. It is suggested that nicotine may be accumulated in human tissues containing melanin. This may in turn influence biochemical processes in human cells producing melanin.

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