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The effect of UV-filters on the viability of neuroblastoma (SH-SY5Y) cell line

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ABSTRACT

Topical application of cosmetic products, containing ultraviolet filters (UV filters) are recommended as a protection against sunburns and in order to reduce the risk of skin cancer. However, some UV filters can be absorbed through skin and by consuming contaminated food. Among the chemical UV filters, benzophenone-3 (BP-3), 3-(4-methylbenzylidene)camphor (4-MBC) and 2-ethylhexyl-4-methoxycinnamate (OMC) are absorbed through the skin to the greatest extent. So far, these lipophilic compounds were demonstrated to influence the gonadal and thyroid hormone function, but their effect on central nervous system cells has not been investigated, yet.

In the present study, we investigated the effect of some UV filters on cell viability and caspase-3 activity in SH-SY5Y cells. It has been found that benzophenone-2 (BP-2), BP-3, 4-methylbenzophenone (4-MBP) and OMC present in the culture medium for 72 h in high concentration (10^{-5} and 10^{-4} M) and 4-MBC only 10^{-4} M produced a significant cytotoxic effect, as determined both by the MTT reduction test and LDH release assay. In contrast to necrotic changes, all tested UV filters increased caspase-3 activity in much lower concentrations (from 10^{-8} to 10^{-7} M). Proapoptotic properties of the test compounds were positively verified by Hoechst staining.

The obtained results indicated that UV filters adversely affected the viability of nerve cells, most likely by enhancing the process of apoptosis. The most potent effect was exerted by BP-3 and 4-MBC and at concentrations that may be reached *in vivo*. Since human exposure to UV filters is significant these compound should be taken into consideration as one of the possible factors involved in pathogenesis of neurodegenerative diseases.

1. Introduction

Ultraviolet (UV) chemical filters are used in sun protection products and other cosmetics: perfumes, lipsticks, body lotions, shampoos, conditioners and hair sprays. They are also added to the production of food packaging to protect from the damaging effects of sunlight. Because UV light, by induction of DNA damage and inhibition of skin immune system activity, increases the threat of skin cancers, including melanoma, therefore, the appropriate protection is required. However, some compounds used as UV filters can be absorbed through skin and exert systemic effects. The most common group of chemical filters contains benzophenones derivatives: benzophenone-3 (BP-3), benzophenone-2 (BP-2), and 4-methylbenzophenone (4-MBP). They are often used in cosmetic products in combination with two or more sunscreens such as: 3-(4-methylbenzylidene) camphor (4-MBC), and 2-ethylhexyl-4-methoxycinnamate (OMC). In cosmetic products, sunscreens are in a concentration of up to 10% (Schreurs et al., 2002). They are highly lipophilic and can therefore bioaccumulate in humans and in the environment (Fent et al., 2010).

A wide range of *in vitro* and *in vivo* studies have identified sunscreens as an endocrine-disrupting chemicals (EDCs). The most examined so far action of UV filters is related to their effect on the estrogen receptors (ER). *In vitro* studies showed estrogenic activity of some UV filters, binding to estrogen receptors α (ERα) and estrogen receptor β (ERβ) and influence on the ER levels (Schlumpf et al., 2001). However, though there are individual differences in the interaction with ERα and ERβ, they preferentially bind to ERβ.

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In many of ecotoxicological studies, 4-MBC and OMC decreased the level of vitellogenin, which is a phenotypic endpoint for the estrogenic activity in fish. In *in vivo* acute models, estrogenic activity of BP-3, 4-MBC and OMC has been confirmed by demonstrating an increase in uterine weight in immature female rats (Schlumpf et al., 2001, 2004a,b) and oophorectomized rats (Klammer et al., 2005). In addition to estrogenic activity, these compounds *in vitro* exhibit also antiandrogenic and antiprogestagenic action, but such activity *in vivo* was confirmed only for OMC. Another adverse effect of 4-MBC, BP-3 and OMC is related to the interaction with the hypothalamic–pituitary–thyroid axis (HPT) and as a result they can cause thyroid dysfunction (Schmutzler et al., 2007).

Experimental *in vivo* studies showed that BP-3, 4-MBC and OMC could rapidly pass through the skin to systemic circulation. They can be detected in plasma 1–2 h following application (Janjua et al., 2008). In the same experimental study, concentrations of UV filters in male plasma and urine were higher than in female samples, which indicated a gender-related differences in metabolism, distribution and in the accumulation of these compounds (Janjua et al., 2004). Importantly, dermal application is the main route of human exposure of these compounds, thus they enter the systemic circulation without being metabolized by the liver, posing a potential risk to all human tissues. The exposure of human body is significant and still growing. For example, in the U.S. population, the presence of benzophenone-3 and its metabolites was revealed in 98% of urine samples (Calafat et al., 2008). Human exposure to UV filters can occur via dermal absorption, but also through food chain, for example, by consumption of contaminated fish (Weisbrod et al., 2007; Fent et al., 2010). The presence of benzophenone derivatives was also shown in the adipose tissue, breast milk (Ye et al., 2008; Schlumpf et al., 2010) and semen (Schlumpf et al., 2008; Leon et al., 2010) where they can cause adverse effects. Breastfed babies are exposed to the action of UV filters, which are present in 85% samples of human milk (Schlumpf et al., 2010). BP-3 can also penetrate from blood to placenta and its high concentration in mothers' urine were associated with increased birth weight in boys and decreased birth weight in girls (Wolff et al., 2008).

The effects of sunscreens on the central nervous system (CNS) have not be studied, yet. Sunscreens as lipophilic compounds are likely to pass through the blood–brain barrier and, therefore, toxic effects on the central nervous system cells cannot be ruled out. Moreover, other compounds of similar structure and belonging, as UV filters, to the group of endocrine-disrupting chemicals are known to alter neuronal transmission, synaptic plasticity and induce apoptotic or neurotoxic changes (Kajta and Wojtowicz, 2013; Frye et al., 2012). In the present study, we investigated the effect of some UV filters on cell viability (LDH release and MTT reduction) and apoptotic process (caspase-3 activity and Hoechst staining) in human neuroblastoma cell line (SH-SY5Y cells). Considering human exposure to UV filters and their blood concentration, we have selected the following compounds: benzophenone derivatives: BP-3, BP-2, 4-MBP and other substances: 4-MBC and OMC. SH-SY5Y cell line represents a well-established experimental model to study neurotoxic liability of chemical compounds *in vitro*.

2. Materials and methods

2.1. Chemicals

Benzophenone-3 (2-hydroxy-4-methoxybenzophenone), benzophenone-2 (2,2′,4,4′tetrahydroxybenzophenone), 4-methylbenzophenone, 2-ethylhexyl-4-methoxycinnamate, 3-(4-methylbenzylidene) camphor were obtained from Sigma–Aldrich Ltd.

3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT), methyl sulfoxide (DMSO, dimethyl sulfoxide) were obtained from Sigma–Aldrich Ltd.

2.2. Cell cultures

The SH-SY5Y neuroblastoma cell line was obtained from the American Type Culture Corporation (ATCC). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL, Germany) supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, Germany), 100 units/ml of penicillin and 100 μg/ml of streptomycin (Sigma–Aldrich Ltd.), and kept in humidified atmosphere of 5% CO_2/95% O_2 at 37 °C.

2.3. Treatment of cells

One day before the experiment, cells were seeded in 96-well plates in the medium with a reduced amount of serum (1% FBS). For Hoechst staining, cells were cultured on Millicell EZ slide (Millipore). The SH-SY5Y cells were treated with benzophenone-2, benzophenone-3, 4-methylbenzophenone, 3-(4-methylbenzylidene) camphor, 2-ethylhexyl-4-methoxycinnamate at concentrations from 10^{-8} to 10^{-4} M for 24 or 72 h. All investigated chemical compounds (BP-2, BP-3, 4-MBP, OMC, 4-MBC) were dissolved in the smallest possible volume of ethanol and then diluted with water and were added to culture medium in the volume of 10 μl. The control cultures were supplemented with the same amount of an appropriate vehicle in which the final concentration of ethanol was 0.8% for BP-2; 1.5% for 4-MBC and OMC; 1.9% for BP-3 and 2.3% for 4-MBP. To evaluate the effect of solvent on cell viability, the culture without solvent was also added to each assay. As a positive control of cell death and apoptosis respectively 1% Triton and 1 μM staurosporine were used.

2.4. Measurement of lactate dehydrogenase (LDH) release

Toxicity of chemical substances was measured by release of lactate dehydrogenase (LDH) into the culture media at 24 h or 72 h post treatment. LDH activity was determined in medium using a colorimetric method (Cytotoxicity Detection Kit, Roche Diagnostic GmbH, Germany), according to which in the reaction of pyruvic acid with 2,4-dinitrophenylhydrazine amount of colored hydrazone was formed, which is proportional to the LDH activity in the sample. It could be quantified by measuring the absorbance at 490 nm. The results were expressed as a percentage of control cells cultured only with the solvent (mixtures of water and ethanol). In this assay Triton, a compound that damages all cells was used as a positive control.

2.5. MTT reduction assay

Cell viability was measured at 24 h or 72 h post treatment, by determining the cellular reducing capacity. It was estimated from the extent of MTT reduction to the insoluble intracellular formazan, which depends on the activity of intracellular dehydrogenases and is independent of changes in the integrity of the plasma membrane. Briefly, culture medium was replaced and SH-SY5Y cells were incubated with MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyl tetrazolium bromide for 3 h at 37 °C. MTT was prepared in PBS and added at a final concentration of 0.15 mg/ml. Then, the crystals of formazan were dissolved in DMSO and the absorbance of each sample was measured at 570 nm in a Multiscan plate reader (Labsystem, USA). The results were expressed as a percentage of control cells incubated only with solvent.