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The effect of dermal benzophenone-2 administration on immune system activity, hypothalamic-pituitary-thyroid axis activity and hematological parameters in male Wistar rats

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ABSTRACT

Benzophenones used as UV filters, in addition to the effects on the skin, can be absorbed into the blood and affect the function of certain organs. So far, their effects on the sex hormone receptors and gonadal function have been studied, but not much is known about their potential action on other systems. The aim of the present study was to determine the effect of benzophenone-2 (BP-2) on immune system activity, hypothalamic-pituitary-thyroid (HPT) axis activity and hematological parameters.

BP-2 was administered dermally, twice daily at a dose of 100 mg/kg for 4-weeks to male Wistar rats. Immunological and hematological parameters and HPT axis activity were assayed 24 h after the last administration.

It was found that BP-2 did not change relative weights of the thymus and spleen and did not exert toxic effect on tymocytes and splenocytes. However, this compound increased proliferative activity of splenocytes, enhanced metabolic activity of splenocytes and thymocytes and nitric oxide production of these cells. In animals exposed to BP-2, the HPT axis activity was increased, as evidenced by reduction in the thyroid stimulating hormone (TRH) level and increase in free fraction of triiodothyronine (fT3) and thyroxin (fT4) in blood. BP-2 had no effect on leukocyte, erythrocyte and platelet counts or on morphology and hemoglobin content in erythrocytes.

The conducted research showed that dermal, sub-chronic BP-2 administration evoked hyperthyroidism, increased activity or function of the immune cells but did not affect hematological parameters. We suggest that topical administration of BP-2 leading to a prolonged elevated BP-2 level in blood causes hyperthyroidism, which in turn may be responsible for the increased immune cell activity or function. However, only future research can explain the mechanism and functional importance of the changes in thyroid hormones and immunological parameters observed after exposure to BP-2.

1. Introduction

Benzophenone derivatives are the compounds most often used as UV filters. They are components of many cosmetics and are also added to the production of food packaging to protect the content from the damaging effects of sunlight (Gonzalez et al., 2006). Recently, however, it has been noted that these compounds may adversely affect the function of the organism, because as lipophilic compounds they are absorbed through the skin from the applied preparations and additionally also from the digestive tract after consumption of contaminated food products. Benzophenones belong to the group of endocrine-disrupting chemicals (EDCs) and most of the current data concern their impact on the sex hormone receptors and in consequence, impairment of gonadal function. Other side effects of benzophenones, such as the effects on thyroid hormone synthesis, on immune system function, hematopoietic activity and viability of brain cells are very poorly identified at the moment.

In the benzophenone group, a majority of data on absorption, concentration and effects concern benzophenone-3, the most commonly used compound. It has been shown that benzophenone-3 is absorbed through the skin in humans and its blood concentration reaches up to 250 ng/ml (Janjua et al., 2008; Tarazona et al., 2013). In experimental

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animals, this compound accumulates in organs (Okereke et al., 1994). In contrast to BP-3, there is no data on BP-2 concentrations in human blood and on its levels in the tissues of experimental animals while some BP-2 effects in experimental animals, for example, stimulation of rat uterine weight, are stronger than those of BP-3 (Schlecht et al., 2004).

In our previous studies (unpublished data), we have shown that BP-2 is absorbed through the skin in rats and 24 h after the last administration its blood concentration is ca. 300 ng/ml. Even higher levels of BP-2 in rat blood were shown after oral treatment, however, after administration via this route, BP-2 was quickly eliminated from the body (Schlecht et al., 2008). Estrogen-like benzophenone-2 activity and adverse effects on gonadal function have been relatively well studied in the experimental animals (Jarry et al., 2004; Schlecht et al., 2004, 2006). However, acting as an agonist on estrogen receptors and as antagonist on androgen receptors this compound can disturb not only gonadal function but may affect many organs, because these receptors are present in almost all cells of the body. Moreover, beside action via sex hormone receptors, some data indicate that also other mechanisms, for example via the aromatic hydrocarbon receptor (AhR) or the impact on gene transcription are involved in the action of benzophenones (Hofmann et al., 2009; Schlecht et al., 2004).

An important problem in the context of identifying potentially adverse BP-2 activities is related to its impact on the immune function. UV radiation, the target of protective action of chemical UV filters, exerts immunosuppressive effects on the immune system of the skin and thus increases the risk of skin cancers, including melanoma, development. Data available so far, coming primarily from in vitro experiments, suggest that benzophenones also exhibit immunosuppressive effects and shift the balance between Th1 and Th2 lymphocytes toward Th2 response (Kato et al., 2006). For example, immunosuppressive action of benzophenone-4 on dendritic cells and lymphocytes T has been documented (Frikeche et al., 2015). Also BP-2 at 10^{-5} M concentrations, in an in vitro model decreased IFN-γ production and increased IL-10 level and changed the Th1/Th2 lymphocyte population ratio (Rachoń et al., 2006). Moreover, since 17β-estradiol shows also similar effect to that exerted by BP-2, it is suggested that BP-2 action on Th lymphocytes may be mediated by stimulation of estrogen receptors. To date, however, the effects of benzophenones on the immune system function have been determined only in vitro, on individual cell populations and are considered primarily to be an effect on the immune system of the skin. Because BP-2 is absorbed into the blood, thus, it may affect not only the immune system of the skin but may influence the function of the whole immune system. Therefore, the main aim of the present study was to determine the effect of BP-2 on immunity after sub-chronic, dermal administration of this compound. The relative weight of thymus and spleen, viability of thymocytes and splenocytes, proliferative activity of splenocytes and their ability to produce nitric oxide (NO) were determined. The parameters that characterize the immune system were determined in male Wistar rats which received BP-2 dermally, for 4 weeks. In this model, we previously measured the levels of this compound in blood, peripheral tissues and in brain structures (our unpublished data).

Endocrine-disrupting chemicals, including chemical UV filters, in addition to the effects on the sex hormones, most often also disturb the function of the thyroid gland (Hofmann et al., 2009; Schmutzler et al., 2004). Few data yet indicate that also BP-2 inhibits synthesis or release of thyroid hormones (Schmutzler et al., 2007). So far, thyroid hormone levels were determined only in female ovariectomized rats, so in a model in which the effect of sex hormones was eliminated and after oral BP-2 administration that is, in conditions of rapid elimination from blood of the test compound (Jarry et al., 2004; Seidlová-Wuttke et al., 2005; Schmutzler et al., 2007). Since after 4 weeks, dermal BP-2 administration, the blood concentration of this compound in male rats was relatively high, the second objective of the current research was to determine if in this model of exposure, BP-2 induces changes in thyroid function. To determine the activity of the hypothalamic-pituitary-thyroid (HPT) axis the concentrations of free fractions of triiodothyronine (fT3) and thyroxin (fT4) as well as thyroid stimulating hormone (TSH) were determined. Free hormone fractions were assayed, because their blood levels better reflect the thyroid function than total T3 and T4. Moreover, bidirectional communication occurs between thyroid hormones and the immune system and especially a lot of evidences show that disturbance in thyroid hormone levels affects the immune system function. Hyperthyroidism is frequently connected with abnormal antibody production, increased migration of polymorphonuclear leukocytes, lymphocyte proliferation, increased reactive oxygen species (ROS) production by macrophages and reduced levels of pro-inflammatory cytokines, while hypothyroidism usually produces opposite effects (Jara et al., 2017).

To date, there is no evidence on the impact of the BP-2 on the number and morphology of blood cells. Only in the case of one benzophenone derivative from fruit of *Garcinia indica* (garcinol), it has been shown that this compound affects the membrane of human erythrocytes and causes cell shrinkage (Fazio et al., 2015). The next aim of this study was to evaluate the effects of BP-2 on hematological parameters of peripheral blood in rats. We determined red blood cell (RBC) count, mean corpuscular volume (MCV), hematocrit (HCT), hemoglobin concentration (HGB) and mean cell hemoglobin concentration (MCHC). Also the leukocyte count in blood (WBC) was estimated, to determine the effect of BP-2 on the pool of blood immune cells, and platelet count (PLT) was measured because these cells are not only involved in the coagulation process but also are a source of mediators of inflammation.

To analyze the effect of BP-2 on immunological and hematological parameters and on HPT axis activity, we used a model of 4-week, 2 times a day dermal BP-2 administration, because this is the most common route of exposure to this compound in humans.

2. Materials and methods

2.1. Materials

Benzophenone 2 (2,2′,4,4′-tetrahydroxybenzofenon; CAS No 131-55-5; Batch No MKBN6515 V) was purchased from Sigma-Aldrich (St. Louis, MO). The purity of this compound determined by the manufacturer using HPLC is 98.6%.

2.2. Animal treatment

The experiments were performed on male Wistar rats (250–280 g) delivered from the animal house facility of the Jagiellonian University Medical College in Cracow. The animals were kept under natural day-night cycle (lights on at 6:00 a.m.) at 22 ± 2 °C with food and water available at libitum. All procedures were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Local Ethics Committee. The rats were randomly divided into two groups of ten animals in each, the first group received gel with BP-2 and the second group pure gel.

Hair on the back of the neck through half-way towards the tail region was shaved off prior to treatment. Animals were reshaved during the course of treatment as soon as the hair began to reappear. BP-2 was dissolved in small amount of ethanol (1.5 g) and olive oil (3 g) and formulated with 95.5 g Hascobase (Hasco-Lek, Poland) to the ointment. The Hascobase contained: liquid paraffin, white vaseline, glycerol monostearate SE, cetostearyl alcohol, polysorbate 40, triglycerides of saturated fatty acids with medium chain length, propylene glycol, anhydrous colloidal silica, sorbic acid and purified water).This formula was applied at a dose 100 mg/kg twice a day (8:00 and 17:00) for 4 weeks. At the beginning of the experiment when the weight of the animals were between 250 and 280 g, BP-2 was given at dose from 25 to 28 mg on rats (depending on the weight). At the end of experiment when the weight of the animals were between 320 and 350 g BP-2 was