BDE-209 Decreases the Efficacy of Dacarbazine Treatment for Melanoma in C57BL/6 Mice

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Abstract

Humans are widely exposed to environmental chemical toxicants potentially related to disease susceptibility. Polybrominated diphenyl ethers (PBDEs) present a proven risk associated with cancer, but no studies are related with the tumor progression. The decabromodiphenyl ether (BDE-209) is a flame-retardant detected in human plasma, breast milk and umbilical cord. Melanoma is a skin cancer with high metastatic potential and poor response to therapies. The use of alkylating agents such as dacarbazine is still a common protocol for the treatment of melanoma, mainly in Brazilian Public Health Care System. Recently, we reported the role of BDE-209 on the incidence of melanoma metastasis in different organs of mice after inoculation of B16-F10 cells. In the current study, we describe the effects of BDE-209 on dacarbazine treatment for melanoma. Adult male and female C57BL6 mice were exposed to BDE-209 for 45 days, inoculated with B16-F10 cells and treated with dacarbazine for 21 days (five doses of 40 mg.kg⁻¹). At 66th day, the animals were euthanized, and the blood, lung, liver, kidney and brain were sampled for hematological, biochemical and metastasis counting analyses. The results showed a decrease of lung metastases in animals treated with dacarbazine and a significant increase in mice previously exposed to BDE-209. Foremost, BDE-209 impaired dacarbazine treatment. These findings demonstrate the effect of BDE-209 and the decreased efficacy of dacarbazine treatment, favoring cancer progression and affecting the disease prognosis.

1) Introduction

The exposure to environmental toxicants has been a worldwide issue but it is still challenging to extrapolate data to human population. According to Humphrey et al. (2019), the main limitations of this extrapolation are the variability of exposure routes to environmental chemicals, the complexity of the mixtures, biotransformation, metabolic processing, and human lifestyle. In this sense, the relationship between many toxicants and cancer progression remains poorly studied.

Polybrominated diphenyl ethers (PBDEs) are a class of synthetic brominated chemicals introduced in the 1970s as flame retardants to a large variety of plastic products (EPA, 2017). According to Stapleton et al. (2009), a large amount of PBDEs have been produced and applied over the past few decades, resulting in widespread contamination of the environment and accumulation in food webs. Decabromodiphenyl ether (BDE-209) represents more than 90% of the total daily intake of PBDEs in humans (Ji et al., 2017). According to Sjödin et al. (2008), this compound can leach from the plastic products to atmosphere, soil and domestic dust. It can also accumulate in the food chain due to its high lipophilicity and environmental persistency (de Wit et al., 2010). Studies in Africa showed that BDE-209 is present in eggs, fish, meat, milk, nuts and other foods, increasing the human exposure by food (Jing et al., 2019) and domestic dust inhalation (La Guardia et al., 2007). According to Besis et al. (2014), Giordano et al. (2008) and Li et al. (2012), this compound can be detected in the blood, semen, adipose tissue, breast milk, umbilical cord blood and placental tissues.

BDE-209 induces hepatotoxicity (Wang et al., 2018; Zhu et al., 2019), thyroid disturbs (Sun et al., 2018), cardiovascular dysfunctions (Jing et al., 2019), bronchial epithelial cells damage (Montalbano et al., 2020) and progesterone secretion attenuation (Han et al., 2019). In addition, the carcinogenic potential of PBDEs has been suggested by Costa and Giordano (2011), Aschebrook-Kilfoy et al. (2015) and He et al. (2018), although these compounds have not been evaluated by the International Agency for Research on Cancer, IARC (Hurley et al., 2019). Despite of that, Hurley et al. (2019) detected 2.6 to 14.1 ng/g of PBDEs in the blood lipids of a women population in California with breast cancer historical.

Melanoma is the most aggressive and lethal type of skin cancer (Domingues et al., 2018, Kozar et al., 2019, Mishra et al., 2018). According to Kozar et al. (2019), there are about 140,000 new cases of melanoma diagnosed annually and approximately 50,000 deaths per year. There are many options for melanoma treatment, such as surgical excision, chemotherapy, immunotherapy, and targeted therapy (Domingues et al., 2018, Kozar et al., 2019, Mohammadpour et al., 2019). Despite of that, metastatic melanoma is associated with poor prognosis (Pavri et al., 2016) with a survival median of 6 to 9 months, with only 15% of patients reach to 5 years (Hartman and Lin, 2019). For chemotherapy treatment of metastatic melanoma, the most used drug is dacarbazine, a synthetic antitumor agent (Domingues et al., 2018, Mishra et al., 2018), approved in 1975 by Food and Drug Administration (FDA) agency (Pourahmad et al., 2009). Dacarbazine is an alkylating agent that inhibits DNA synthesis through methylation of DNA bases and induction of cell death via apoptosis (Christmann et al., 2011).

In the current study, the effects of BDE-209 were investigated in mice exposed to BDE-209, inoculated with B16-F10 murine melanoma cells and then treated with dacarbazine, to address whether BDE-209 can interfere with melanoma treatment and lead to a worse prognosis. The study was based on a recent report that BDE-209 exposure can increase the incidence of melanoma metastases in different organs of mice previously exposed to BDE-209 (Brito et. al., 2020). Despite the global distribution of these current-use flame retardants, there are little and inconclusive data on the effects of polybrominated compounds on the modulation of the dacarbazine chemotherapy for melanoma.

2) Material And Methods

2.1) Experimental Design

The current experimental design was approved by the Ethics Committee on Animal Experimentation at Federal University of Parana (certificate no. #1040/2017). Seven-week-old C57BL/6 mice (50 males and 50 females, no ovariectomized or orchiectomized) were maintained in cages (5 animals per cage). Mice of the same sex were randomly distributed in 3 groups: animals maintained without exposure (no gavage was performed); animals exposed only to corn oil by gavage (vehicle); and animals exposed to BDE-209 ($0.8 \mu\text{g} \cdot \text{kg}^{-1}$ in corn oil by gavage every 5 days; Fig. 1). After 45 days (nine doses), the animals were injected with B16-F10 cells (2.5×10^5 cells in 100 μL of PBS) via caudal vein. In a second phase, five groups (10 animals per group) were organized as follow: control (without gavage), vehicle (gavage with corn oil), dacarbazine (only treated with dacarbazine), BDE-209 (only gavage with BDE-209) and BDE-209

+ dacarbazine (gavage with BDE-209 and treatment with dacarbazine; Fig. 1). The chemotherapy treatment was performed with 40 mg/kg of dacarbazine every 3 days (six doses by intraperitoneal injections) after the 49th day of experiment. Finally, on the 66th day, the mice were anesthetized and euthanized (10 mg.kg⁻¹ of xylazine, 100 mg.kg⁻¹ of ketamine), and the blood, lung, liver, kidney and brain were weighed and sampled for the analyses (Fig. 1).

B16-F10 mouse melanoma cells (American Type Culture Collection - Manassas, USA) were cultured in DMEM culture medium (Lonza Bio Whittaker - Basel, Switzerland), supplement with 0.1 M of HEPES (Lonza Bio Whittaker) and gentamicin (40 mg/L) and fetal bovine serum (10%). Mycoplasma contamination was routinely tested.

2.2) Blood Analysis

The blood was sampled via intracardiac route and a complete blood cell counting (CBC) was performed (total leukocytes, red blood cells, hemoglobin, hematocrit, platelets, segmented, stems, eosinophil, and lymphocytes) at the Veterinary Clinical Pathology Laboratory at Federal University of Parana, Brazil. The plasma was centrifuged (3,000 *g* for 10 min) and utilized for biochemical profile determination (alanine aminotransferase-ALT, aspartate aminotransferase-AST, alkaline phosphatase, cholesterol, triglycerides, creatinine, and urea) using a Mindray BS-200 Automated Benchtop Biochemistry Analyzer.

2.3) Biochemical biomarkers analysis

The lung, liver and kidney were sampled and stored at -80°C. Then, 0.2 g of each organ were homogenized in 2 mL of Tris-buffer (20 mM HCl, 0.1 M EDTA, 1 mM PMSF, pH 7.4) and centrifuged at 12,000 *g* (4°C) for 30 min. The supernatant was recovered, and the total protein concentration was determined according to Bradford (1976).

Non-protein thiols (NPT) concentration was measured in supernatants after protein precipitation with 10% trichloroacetic acid and centrifugation (1,000 *g* for 15 min and 4°C). Supernatant (50 µL) and 230 µL of Tris (0.4 M, pH 8.9) were placed in a 96-well microplate, followed by addition of 20 µL of 2.5 mM DTNB [5, 5'-dithiobis (2-nitrobenzoic acid) in 25% methanol. Absorbances were determined at 415 nm and NPT concentration was calculated by comparison with the standard curve for GSH (Sedlak and Lindsay, 1968).

Superoxide Dismutase (SOD) activity was measured in supernatants (1.0 mg protein/mL) after addition of ethanol (1:5) and new centrifugation (9,000 *g*, 4°C for 30 min). Supernatant (20 µL) and 35 µL of 572 µM NBT chloride in 0.1 mM EDTA were mixed in a 96-well microplate. The reaction was initiated through the addition of 145 µL of 51 mM hydroxylamine chloride in 0.5 M sodium carbonate (pH 10.2). The reduction of NBT by O₂⁻ to blue formazan was measured spectrophotometrically as a constant increase of absorbance at 560 nm for 30 min. The rate of NBT reduction in the absence of samples was used as the reference rate. One unit of SOD was defined as the enzymatic activity able to inhibit the reduction of NBT to 50% of the reference rate (Crouch et al., 1978).

Catalase (CAT) activity was measured in supernatants (1.0 mg protein/mL). Supernatant (10 μ L) was mixed with reaction medium (290 μ L, 20 mM H_2O_2 , 50 mM Tris-base, 0.25 mM EDTA, pH 8.0, 25°C) in UV-star™ 96-well microplates (Greiner Bio-One) and absorbance decrease was immediately measured at 240 nm for 1 min. Molar extinction coefficient for H_2O_2 of $40 \text{ M}^{-1}.\text{cm}^{-1}$ was used to calculate catalase activity (Aebi, 1974).

Glutathione S-transferase (GST) was measured in supernatants (1.0 mg protein/mL). Supernatant (50 μ L) was placed in 96-well microplate, immediately followed by reaction medium (100 μ L, 1.5 mM GSH (glutathione), 2.0 mM CDNB (1-chloro-2,4-dinitrobenzene), 0.1 M potassium phosphate buffer, pH 6.5). Absorbance increase was measured at 340 nm for 2 min and the molar extinction coefficient for CDNB of $9.6 \text{ mM}^{-1}.\text{cm}^{-1}$ was used to calculate the activity (Keen et al., 1976).

Lipid peroxidation (LPO) was measured by Ferrous Oxidation-Xylenol (FOX) assay (Jiang et al., 1992). From the cell homogenates, 50 μ L were separated for protein quantification and to the remaining volume (450 μ L), an equal volume of methanol was added. After centrifugation (1,000 g for 10 min at 4°C), 30 μ L of supernatant and 270 μ L reaction solution (0.1 mM xylenol orange, 25 mM H_2SO_4 , 4.0 mM BHT (butylated hydroxytoluene) and 0.25 mM $\text{FeSO}_4.\text{NH}_4$ (ammonium ferrous sulfate) in 90% methanol) were mixed in a 96-well microplate. Samples were incubated at room temperature for 30 min, centrifuged (1,000 g, room temperature for 5 min), 250 μ L of supernatant were added to 96-well microplates and absorbance was measured at 570 nm. To determine the hydroperoxides concentration, the apparent molar extinction coefficient for H_2O_2 and cumene hydroperoxide of $4.3 \times 10^4 \text{ M}^{-1}.\text{cm}^{-1}$ was used.

2.4) Histological analysis

The lung was chemically fixed in paraformaldehyde solution (4%, pH 7.2–7.4 for 4 h), washed in ethanol (70%), dehydrated in ethanol graded series, diaphanized in xylol and embedded in Paraplast Plus resin (Merck® - Darmstadt, Germany). Lung samples (n = 5/group) were cut in microtome (5 μ m thickness) and stained with hematoxylin-eosin. The images were obtained using motorized Axio Imager Z2 epifluorescence microscope (Carl Zeiss, Jena, DE), equipped with an automated scanning Metafer 4/VSlide (Metasystems, Altlussheim, DE) and a conventional light microscope for metastasis analysis.

2.5) Integrated response of biochemical biomarkers (IBR)

The data from biomarkers corresponding to the tested doses and the control group were first transformed to logarithm to reduce the variance. These data (Y_i), the mean (μ) and the standard deviation (s) were calculated from all analyzed samples. The formula $Z_i = (Y_i - \mu)/s$ was applied for each treatment, and the difference between the treated and control group was used to obtain the value of A (corresponding to the integrated result for each biomarker) (Beliaeff and Burgeot, 2002; modified by Sanchez et al., 2013). Then, these results were plotted on a radar-type graph, where values above and below zero (control) indicate the stimulus and inhibition of a given biomarker, respectively. Finally, to calculate the IBR index from each experimental group, the values of A were converted in absolute numbers (S) and summed.

2.6) Bioinformatic analyses - molecular docking and metabolism prediction

Dacarbazine and BDE-209 are metabolized mainly in the liver, primarily by cytochrome P450 isoform CYP1A2 (Letcher et al., 2014; Pourahmad et al., 2009). In this sense, molecular docking analysis was performed to assess the potential binding of BDE-209 and Dacarbazine at the CYP1A2 binding site as an *in-silico* alternative to show a possible model of how BDE-209 can interfere with dacarbazine metabolism. The 3D structure of BDE-209 was obtained from PubChem and the crystallographic structure of CYP1A2 from the Protein Data Bank (ID: 2HI4; Resolution: 1.95 Å). The files were prepared for the docking procedure in the AutoDock Tools software using a specific site docking strategy (Coordinate Grid - x: 30; y: 30; z: 30) and the LGA (Lamarckian Genetic Algorithm) algorithm to search the best ligand conformation, following the exact protocol proposed by Forli et al. (2016). Each ligand/protein pair was docked in triplicate to calculate the average affinity score (Kcal/mol) of protein-ligand complex. Finally, the protein-ligand complex was analyzed in the program Chimera 1.14 (Petersen et al., 2004) and Discovery Studio Visualizer (Biovia) in order to obtain the 3D and 2D model of the protein-ligand interaction, respectively. In addition, the prediction of pharmacokinetic metabolism of dacarbazine and BDE-209 were evaluated by the pkCSM server, which uses graph-based signatures to develop predictive models of central ADMET properties based on SMLIES chemical sequence (<http://structure.bioc.cam.ac.uk/pkcsm>) (Pires et al., 2015). Metabolism parameters were evaluated for inhibition of the P450 complex (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) and P-glycoprotein inhibitor/substrate.

2.7) Statistical procedures

The control and vehicle groups were compared using the t-test revealing no statistical differences, and so only the vehicle group is shown in the results. For all comparisons, two-way ANOVA was performed, considering sex and treatment, followed by Tukey's post-tests. Between sexes, to compare all treatments related to the control/vehicle group, one-way ANOVA was performed with Tukey's as posteriori test. To answer the effects of dacarbazine the Dunns's posterior test was performed comparing the groups dacarbazine, BDE-209 and BDE-209 + dacarbazine.

3) Results

3.1) Animal health conditions

According to biometric parameters, the total body and liver weight were higher in male individuals than in females, but the opposite occurred for the lung weight. The exposure to BDE-209, with and without treatment with dacarbazine, did not present effects on body weight, but led to increases of lung weight in female mice. Conversely, exposure to BDE-209 followed by treatment with dacarbazine led to decreased liver weight in both male and female mice (Fig. 2).

For the blood analyses, sex difference was observed with higher number of white blood cells, lymphocytes, monocytes, and granulocytes in female mice and platelets in males, without effects in

individuals exposed to BDE-209. However, an increase of lymphocytes was found in female animals exposed to BDE-209 and after treatment with dacarbazine (13.12 ± 7.12 and 15.02 ± 0.042 , respectively ($\times 10^3 \text{uL}^{-1}$); $p < 0.05$). The biochemical profile of the blood showed increases of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, urea, cholesterol and VLDL-cholesterol levels in the female individuals comparatively to males. For the treatments, ALT activity was higher in female individuals exposed to BDE-209 and after treatment with dacarbazine ($107.7 \pm 50.41 \text{ U.L}^{-1}$, $p < 0.003$).

3.2) Biochemical biomarkers

The biochemical parameters of lung, liver and kidney are presented in the Fig. 3. In the lung of mice, the exposure to BDE-209 and dacarbazine presented no effects on superoxide and catalase activities, and lipid peroxidation. However, higher concentration of non-protein thiols was observed in female mice exposed to BDE-209 and treated with dacarbazine ($p < 0.05$). Likewise, glutathione *S*-transferase activity was higher in female mice exposed to BDE-209 than in those of the vehicle. Male and female mice had differences for all biochemical parameters, except for lipid peroxidation (Fig. 3A). For the liver, dacarbazine and BDE-209 exposure followed by dacarbazine treatment led to decreased lipid peroxidation in male mice, whereas no effects were observed for non-protein thiols, superoxide dismutase, catalase and glutathione *S*-transferase activities (Fig. 3B). In the kidney, the exposure to BDE-209 followed by treatment with dacarbazine led to increased superoxide dismutase activity in both male and female mice, whereas no effects were observed for non-protein thiols, lipid peroxidation, catalase and glutathione *S*-transferase activities (Fig. 3C).

The integration of biochemical biomarkers (IBR) showed that the lung, liver and kidney of male individuals were more affected by BDE-209 exposure followed by dacarbazine treatment, while the lung and kidney were more affected by dacarbazine treatment and the liver by BDE-209 exposure in female mice (Fig. 4).

3.3) Metastasis nodules counting

The number of pulmonary nodules was higher in the female mice than in male (Fig. 5). As expected, individuals not exposed to BDE-209 and treated with dacarbazine showed a higher efficiency reducing the number of nodules, particularly in female mice (Fig. 5, dacarbazine group). For the group of individuals exposed to BDE-209, the number of nodules was higher when compared with the vehicle group in females, but not for the male group. However, the prior and concomitant exposure to BDE-209 impaired the treatment with dacarbazine, as observed by the higher number of metastasis in BDE-209 + dacarbazine groups when compared to the dacarbazine group (Fig. 5).

Tumor nodules were found in organs considered as no target for the tumor cells inoculation protocol (i.e., through caudal vein), such as the brain, liver and kidney (Fig. 6). However, as observed for the lung, these other organs presented the same effect of dacarbazine, which decreased the incidence of nodules in the vehicle group, but had its efficacy impaired by exposure to BDE-209 (Fig. 6).

3.4) Histological analysis

The histological analysis showed the occurrence of peripheric or internal nodules in the lung of all individuals from the studied groups. The frequency of nodules in male and female mice treated with dacarbazine was lower than in those of the BDE-209 and BDE-209 + Dacarbazine groups (Figs. 5 and 7). In individuals from the vehicle group, the nodules are more developed (Fig. 7A) than in those treated with dacarbazine (Fig. 7B). In the dacarbazine groups, the tumor nodules decreased in number and size (Fig. 7B).

The lungs of animals exposed to BDE-209 during the whole experiment showed a large number of well-developed pulmonary nodules and at distinct stages of development (Fig. 7C). Likewise, the lungs of animals exposed to BDE-209 and treated with dacarbazine showed more developed nodules in the lung than those treated with dacarbazine (Fig. 7D). In this case, there was no chemotherapeutic effect like the one observed in individuals from dacarbazine group (Fig. 7B).

3.5) Bioinformatics analysis

The *in-silico* results showed that dacarbazine and BDE-209 can interact with the same active site of CYP1A2 (Fig. 8A-B), presenting a binding affinity of -5.2 and - 8.13 kcal/mol for dacarbazine and BDE-209, respectively. Comparatively, the possibility of non-covalent interactions with CYP1A2 is lower for dacarbazine (Fig. 8C) than for BDE-209 (Fig. 8D). Furthermore, the metabolic prediction indicates that BDE-209 acts as an inhibitor of CYPs (CYP1A2, CYP2C19 and CYP2C9) and P-glycoprotein (type I) (Fig. 8E).

4) Discussion

It is well described that both the health of patients and environmental conditions can modulate the development of cancer, but few studies reported the role of pollutants in cancer prognosis or its effects on cancer treatment efficacy. Recently, we reported the modulation of BDE-209 increasing the metastatic spread of melanoma in the lung, brain, liver and gonads of C57BL/6 mice (Brito et. al., 2020). These findings raised concerns on the risk of exposure of people with cancer to environmental pollutants. In the current study, we describe that the exposure to low doses of BDE-209 can impair the chemotherapy treatment with dacarbazine, favoring the cancer progression and melanoma metastases in mice previously exposed to the contaminant. Nowadays, a new clinical condition revealed that this issue should be better investigated, mainly because the tested doses of BDE-209 in the current study were at least 10 times lower than the reference doses (700 ng.kg^{-1}) established by U.S. Environmental Protection Agency (2008) for safe exposure.

In the present study, the liver of male individuals showed a higher incidence of metastasis than in females, but the opposite was observed for pulmonary nodules. These data agree with the studies developed by Dobos et al. (2013) and Hirayama et al. (1985), respectively. Furthermore, there is evidence in the literature to support this difference: in malignant skin melanoma, women have a more favorable

prognosis if compared to men (Miller and Mac Neil, 1997; Kemeny et al, 1998), but according to Dobos et al. (2013), this difference was not correlated with hormonal factors. The lung nodules are an estimated parameter to evaluate the level of metastases colonization in the lung after caudal injection of tumor cells.

Crawford (2018) described a decrease of lung weight after chemotherapy treatment, but these results were not confirmed by the current study, since females from BDE-209 and BDE-209 + dacarbazine groups showed an increase of lung weight. These findings may be associated with the higher number of tumor nodules, or the pro-inflammatory condition characterized by the increase of white blood cells and lymphocyte number in individuals exposed to BDE-209. Additionally, Fearon et al. (2006) described the presence of edema normally found after a signaling cascade of pro-inflammatory factors. For the liver, the weight decreased in BDE-209 + dacarbazine group. According to Feng et al. (2015), a decrease of liver weight was found in C57BL/6 mice after exposure to higher dose of BDE-209 (1000x) for two years. This suggests that even low doses BDE-209, such as those tested in here, may be harmful, which was confirmed by the increased ALT activity in the blood.

The redox imbalance may explain many cell effects including a pro-inflammatory state also favoring associated diseases, including cancer (Montalbano et al., 2020). According to Giordano et al. (2008), Tagliaferri et al. (2010) and Vagula et al. (2011), PBDEs exposure is known to generate *in vitro* and *in vivo* oxidative stress and consequent tissue damage due to a pro-oxidant state of cells, as demonstrated by the alteration of biomarkers in the present study. The integration of the biochemical biomarkers related to redox imbalance revealed the susceptibility of different organs of mice exposed to BDE-209 and sex differences. According to IBR analysis, BDE-209 exposure affected mainly the liver of female mice, and the lung and kidney of males. Additionally, the results revealed that the combination BDE-209 + dacarbazine led to harsher effects on male organs. The use of integrative analysis, such as IBR, is appropriate to elucidate some physiological significance of biological disturbs in an organism (Beliaeff and Burgeot, 2002). The development of metastases in the lung was quantifiable externally and each nodule is considered as an independent tumor or metastasis (Ferrari de Andrade et al., 2016). The results showed that BDE-209 increases the spread of metastasis in C57BL/6 mice after injection of B16-F10 cells, confirming the data reported by Brito et al. (2020). Hurley et al. (2019) described PBDEs exposure as a risk factor for breast cancer in the women population of California, highlighting the estrogenic role of brominated compounds.

According to Domingues et al. (2018), the use of single agents or combined therapies in cancer varies because it depends on different conditions, such as patient's health, stage of cancer, and tumor localization. Although the immunotherapies are promising for patients with melanoma, chemotherapy with dacarbazine is the most used therapy in Brazil (Corrêa et al., 2019). As expected, the treatment with dacarbazine decreased the number of tumor nodules in the lung of mice, but it was more effective in females than males. In general, sex differences in the drug toxicokinetics and tumor microenvironment may affect the sensitivity of tumor cells to the treatment (Skvortsova, 2019). Indeed, dacarbazine is a pro-drug that is bioactivated in humans by cytochrome P450 (CYP) isoforms CYP1A1, CYP1A2 and CYP2E1

(Reid et al., 1999; Al-Badr et. al., 2016), but according to Renaud et al. (2011) there are differences in the expression of many CYPs between male and female mice. According to Huang et al. (2007) and Wang and Huang, (2007), many anticancer drugs, including methotrexate, paclitaxel, fluorouracil, capecitabine, gemcitabine and topotecan present sex differences in their clearance. Foremost, BDE-209 showed to favor the metastatic spread of melanoma in the lung of mice (Brito et al., 2020), and impaired the dacarbazine beneficial effects on metastatic melanoma treatment. These findings highlight the role of BDE-209 on cancer prognosis and the high sensitivity of female mice to dacarbazine treatment and polybrominated exposure, revealing that human lifestyle is vital for cancer treatment efficacy.

The BDE-209 metabolism in the liver is related to the upregulation of CYP1A2 and GSTM1, where the metabolism is primarily through oxidative pathways (Chabot-Giguère et al., 2013). BDE-209 bioaccumulates from dietary exposure and generates a range of lower-brominated compounds (Stapleton et al., 2009). According to Hakka and Letcher (2003), the tissue distribution of BDE-209 is the following: liver (0.9%), muscle (0.7%), skin (0.4%), adipose tissue (0.3%), colon wall (0.25%) and other tissues (< 0.1%). The *in vitro* debromination products of BDE-209 described by Chabot-Giguère et al. (2013) in liver include three hepta-BDEs (BDE-183 > - 170 > - 179), five octa-BDEs (BDE-197 > - 196 ≈ - 201 > - 203 > - 202) and two nona-BDEs (BDE-207 > - 208). The current results demonstrated that the tested low concentration of BDE-209 is enough to induce metastasis spread (Brito et al., 2020) to non-target organs and interfere negatively with the dacarbazine treatment.

As described by Letcher et al. (2014), the estimated concentration of 80% of other BDEs in the tissues and plasma is due to BDE-209 debromination by hepatic CYP1A1. Curiously, dacarbazine N-demethylation also depends on CYP1A1, as well as CYP1A2 and CYP2E1 enzymes in humans, which is necessary for dacarbazine metabolic activation (Al-Badr et. al. 2016). The same CYP isoform (CYP1A1), described as responsible for BDE-209 bioactivation, is also involved in N-demethylation of dacarbazine, but only in extrahepatic metabolism. In these terms, this enzyme does not interfere with dacarbazine activation as this event is mainly hepatic and involves CYP1A2 (Pourahmad et al., 2009). According to Chabot-Giguère et al. (2013), CYP1A2 enzyme acts as the most important site of hepatic BDE metabolism, and the results of molecular docking demonstrated that both molecules present physical proximity with the same enzyme site, but the binding affinity of BDE-209 is 56% higher than of dacarbazine. On this way, the potential binding of BDE-209 and inhibition of the CYPs, especially CYP1A2, can impair dacarbazine activation, as demonstrated by the bioinformatics analysis. These results could explain the low efficacy of dacarbazine treatment after the previous exposure of animals to BDE-209. Also, the inhibition of dacarbazine effects could be related to the low efficacy or inhibition of drug-efflux transporters by PBDEs, such as P-glycoproteins and others ABC transporters (Marchitti et al., 2017; Yu et al., 2017). Based on the pharmacokinetic prediction, BDE-209 has chemical properties similar to a P-gp I inhibitor (first generation), such as verapamil (Palmeira et al., 2012). Thus, it is possible that BDE-209 mechanism of action is also involved with multidrug resistance, promoting the low therapeutic activity of dacarbazine.

The histological analysis revealed that the lungs of animals treated with dacarbazine presented less developed tumor nodules. According to Soengas and Lowe (2003) and Triozzi et. al. (2012), apoptotic

and antiangiogenic effects of chemotherapy may impair tumor growth. The animals exposed to BDE-209 showed larger tumor nodules than those exposed to BDE-209 and treated with dacarbazine. These nodules showed many melanocytic cells and a well-developed blood vessel around them, similarly to detected in the vehicle group, indicating that the brominated pollutant could modulate the development of tumors *in vivo* (Brito et al., 2020). In addition, the animals from BDE-209 and BDE-209 + dacarbazine groups had more tumors than those from the dacarbazine group.

5) Conclusion

In the present study, we showed that exposure to BDE-209 can increase the number of metastasis nodules in the lung of mice and impair the chemotherapy treatment with dacarbazine, with important sex differences between male and female mice. The decrease of dacarbazine efficacy could be explained by the impairment of dacarbazine bioactivation due to interactions of BDE-209 with CYP1A2 and drug efflux transporters, according to the predictions, but it must be confirmed. Finally, the exposure to pollutants such as BDE-209 may represent a threat to humans with melanoma due to its effects on metastasis spread and chemotherapy impairment. Overall, at least three aspects should be investigated to explain the role of BDE-209 in the melanoma treatment with dacarbazine: (i) phenotypic changes of melanoma cells with increased malignancy due to BDE-209 exposure, (ii) toxicological interactions with activation/inactivation of biochemical pathways related to toxicokinetics of dacarbazine by BDE-209, and (iii) immune modulation by BDE-209.

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Figures

Figure 1

Experimental design. Seven-week-old C57BL/6 mice were maintained in cages (5 animals per cage, two cages per group) and exposed to BDE-209 (0.8 µg/kg in corn oil) by gavage every 5 days (phase I). Then, the mice were injected with B16-F10 cells via caudal vein and treated with dacarbazine (40 mg/kg, phase II). Control and vehicle groups were maintained in parallel during the whole experiment.

Figure 2

Biometric analysis in C57BL/6 mice exposed to BDE-209, after injection with B16-F10 cells and treatment with dacarbazine. Two-way ANOVA (sex and treatment), ANOVA pos hoc test Tukey's and Dunn's (treatments for sex), $p < 0.05$. The asterisk (*) indicates differences between the sexes, while the different letters indicate differences between treatments. Two independent experiments were performed, $n = 10$.

Figure 3

Biochemical biomarkers in the lung (A), liver (B) and kidney (C) of male and female C57BL/6 mice exposed to BDE-209, injected with B16-F10 cells and treated with dacarbazine. Two-way ANOVA (sex and treatment), ANOVA pos hoc test Tukey's and Dunn's (treatments for sex), $p < 0.05$. The asterisk (*) indicates differences between the sexes, while the different letters indicate differences between treatments. Two independent experiments were performed, $n = 10$.

Figure 4

Integrated data (IBR) of biochemical biomarkers in the lung, liver and kidney of C57BL/6 mice exposed to BDE-209, injected with B16-F10 cells and treated with dacarbazine. The highest values are highlighted in bold.

Figure 5

Metastasis nodules in the lung of C57BL/6 mice exposed to BDE-209, injected with B16-F10 cells and treated or non with dacarbazine. Metastasis formed by melanoma cells (black spots) in the lung of mice and quantification. Two-way ANOVA (sex and treatment), ANOVA pos hoc test Tukey's and Dunn's (treatments for sex), $p < 0.05$. The asterisk (*) indicates differences between the sexes, while the letters indicate differences between treatments. Data are representative of two independent experiments with $n = 10$ for each one.

Figure 6

Metastasis distribution in different organs of C57BL/6 mice exposed to BDE-209, injected with B16-F10 cells and treated with dacarbazine, A) Brain, B) Liver, C) Kidney. Two-way ANOVA (sex and treatment), ANOVA pos hoc test Tukey's and Dunn's (treatments for sex), $p < 0.05$. The asterisk (*) indicates differences between the sexes, while the letters indicate differences between treatments. Two independent experiments were performed, with $n=10$ for each one.

Figure 7

Lung histological sections of mice inoculated with B16-F10 cells. A. Panoramic image of the lung section in individuals from the vehicle group. Note the external (black arrows) and internal (white arrow) nodules. Images a1, a2, a3 and a4 show details of the nodules in different development stages. Observe the presence of large nodules with melanin-containing cells (large black arrows), cell death (small black arrows) and the presence of vessels (white arrows). Scale bars = 600 μ m (A), 200 μ m (a1) and 50 μ m (a2, a3 and a4). B. Panoramic image of the lung section in individuals from the dacarbazine group. Note the absence of external nodules and the presence of smaller internal nodules (white arrow). The small black arrow indicates a vessel, while the white arrow points to dead cells (b1, b2, b3 and b4). Scale bars = 600 μ m (A), 200 μ m (b1) and 50 μ m (b2, b3 and b4). In C, the panoramic image shows external (black arrow) and internal (white arrow) nodules in the lung of individuals exposed to BDE-209, while in c1 and c2 the black arrows indicate nodules in different stages of development. Observe the presence of cell

death area (large arrow) and a well-developed nodule (small arrow) in c3. Observe a nodule detail with melanin-containing cells (arrow) in c4. Scale bars = 600µm (C), 200µm (c1) and 50µm (c2, c3 and c4). The figure in D shows the presence of external and internal nodules (black and white arrows respectively) in individuals exposed to BDE-209 and treated with dacarbazine. In figures d1 – d4 the arrows indicate nodules in different stages of development, while in d3 observe a nodule detail with melanin-containing cells (arrow). Scale bars = 600µm (D), 200µm (d1 and d2) and 50µm (d3 and d4). Hematoxylin and eosin stain.

Figure 8

Molecular Docking analysis of dacarbazine and BDE-209 physical interactions with CYP1A2. (A) Overview of CYP1A2 showing the interaction site of enzyme. (B) Spatial conformation of dacarbazine (gray arrow) and BDE-209 (dark arrow) interactions with the enzyme. (C) and (D) 2D representation of binding interactions between dacarbazine and BDE-209 with CYP1A2 amino acids, respectively. (E)

Pharmacokinetic metabolism predicted by pkCSM server. P-gp: glycoprotein P; P-gp I: inhibitor type I; P-gp II: inhibitor type II.

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