Tetrabromobisphenol A (TBBPA), as the active endocrine disruptor that has a negative impact on the placenta functions

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ABSTRACT

Halogenated compounds are persistent organic pollutants that are widely distributed in the environment. This class includes brominated flame retardants (BFRs) that are applied to inhibit or resist the spread of fire. Tetrabromobisphenol A (TBBPA) is the most widely used compound among BFRs in industrial and consumer products. This chemical is applied as a flame retardant in a wide variety of commercial and household products, from which is released and circulates as dust throughout homes, offices and the interior of passenger cars. TBBPA is a highly lipophilic compound, and thus has high potential to cross cell membranes. Moreover, this chemical is easily transported through the placental barrier, as it has been detected in umbilical cord tissue. The potential for TBBPA to adversely affect early life stages should be a matter of greater concern. That widespread use leading to chronic human exposure to TBBPA is supported by numerous epidemiological studies, which have noted its presence in human body fluids, such as blood and breast milk. The endocrine-related biological activity has been described for TBBPA both in *in vitro* and *in vivo* tests, although limited information is available for this chemical with regard to physiologically important tissue, the human placenta. The presence of TBBPA in the placenta tissue poses a serious threat to the proper functions of the organ acting as an endocrine gland, which in consequence, generates a risk for pregnancy health and proper development of the fetus. Thus, the aim of the present study was to investigate the effect of TBBPA on JEG-3 human placental choriocarcinoma cells *in vitro*. This cell line is a reliable model in studies of placental function: it possesses many biological and biochemical characteristics of syncytiotrophoblasts and expresses enzymes involved in steroidogenesis, therefore, it is used to examine the hormonal function of trophoblasts and intracellular receptor mechanisms.

JEG-3 cells were cultured in DMEM without phenol red, supplemented with 5 % charcoal-stripped FBS in the presence of increasing concentrations of TBBPA (1, 10, 50, 100 [nM] oraz 1, 10, 50, 100 [μM]) for 24, 48 and 72 h. After an appropriate culture time, the medium was collected to determine the level of estradiol, progesterone and human chorionic

gonadotrophin (hCG) secretion by an enzyme-linked immunosorbent assay (ELISA) using commercial kits (DRG, Germany). The cytotoxic potential of TBBPA was determined by the cytotoxicity detection kit (Roche). The assay is based on the detection of lactate dehydrogenase (LDH) released from dead cells as a result of cytotoxicity. JEG-3 cell line proliferation was assessed by the alamarBlue® assay. Caspase-3 activity was used as a marker for cell apoptosis and was measured with the AC-DEVD-pNA colorimetric assay. The apoptotic action of TBBPA was also confirmed by Hoechst 33342 staining. The aromatase activity *in vitro* was measured using fluorogenic substrate dibenzylfluorescein (DBF). Intracellular concentrations of cAMP were determined using a commercial cAMP immunoassay (Cell Biolabs, Inc.). Aromatase and PPARγ protein expression was examined by Western Blot analysis.

The present study demonstrated that TBBPA did not remain indifferent to placental cells. This chemical impaired secretion of three major hormones in normal pregnancy: estradiol, progesterone and hCG by JEG-3 cells *in vitro*. TBBPA, at a wide range of concentrations (1×10^{-9}–1×10^{-4} M), induced estradiol and progesterone secretion and decreased hCG secretion by these cells. At this range of concentrations, TBBPA did not significantly affect JEG-3 cell proliferation or viability. Only the highest concentrations of this compound (50 and 100 μM) showed a strong cytotoxic effect. This study also showed that TBBPA has an adverse effect on aromatase, the key enzyme that regulates the synthesis of estrogens. This compound caused a trend towards the induction of aromatase enzymatic activity at all time points. This activity increased in a concentration-dependent manner. Moreover, it was confirmed that TBBPA-induced changes in aromatase enzymatic activity were associated with up-regulation of aromatase protein expression. Aromatase activity is controlled by cAMP-dependent intracellular signal pathways in different cell types, including placental cells. To investigate the mechanism of aromatase induction in JEG-3 cells, the ability of TBBPA to increase intracellular levels of cAMP was examined. The data showed that TBBPA exerted a marked stimulatory effect on the level of cAMP in JEG-3 cells. Time-response curves for cAMP induction and concentration-response curves for aromatase induction by TBBPA were similar. The impact of TBBPA on cAMP-dependent signal transduction pathway might play a crucial role in regulating the activity of aromatase, and thus the level of estradiol in JEG-3 cells. Endocrine disrupting chemicals have been shown to affect the normal function of human placenta by interfering with receptor signaling or activating other signaling pathways. It has recently been proved that peroxisome proliferator-activated receptor gamma (PPARγ) is shown to be target for TBBPA action. However, there

is no information available about the mechanism of PPARγ activation by TBBPA in the placental cells and potential consequences of the process. PPARγ is essential for the maturation of a functional placenta. It has been postulated that PPARγ stimulates hCG synthesis and trophoblast differentiation. Based on the results, which showed an inhibitory effect of TBBPA on hCG secretion, the aim of the present study was to investigate the involvement of PPARγ in the mechanism of TBBPA action in JEG-3 cells. It was found that, the expression of PPARγ was modulated by TBBPA and was concentration-dependent. The lower concentration of TBBPA (10 nM) increased, while the higher (10 μM) decreased PPARγ protein expression compared to control cells. PPARγ protein expression also increased after treatment with agonist GW1929 of this receptor. In the presence of PPARγ antagonist GW9662, TBBPA at 10 nM had no effect on PPARγ protein expression. In order to clarify the relationship between TBBPA-mediated decrease in hCG secretion and PPARγ expression, the level of hCG secretion was determined in the presence of agonist and antagonist of PPARγ receptor. Combined treatment with GW1929 and 10 nM or 10 μM TBBPA, enhanced TBBPA-mediated decrease in hCG secretion. GW1929 alone had the same effect. Combined treatment with GW9662 and 10 nM or 10 μM TBBPA did not restored TBBPA-mediated decreased in hCG secretion but potentiated its effect. Similarly effect was observed after GW9662 alone. The results indicated that impaired hCG secretion by TBBPA is not related only with activation of PPARγ. Clearly there are alternate mechanism involved in its biological actions, in particular in a PPARγ independent manner. In addition to the hormonal homeostasis that is necessarily for proper placental development, apoptosis has been described as important for the normal placental development and for placenta related alterations. Numerous studies revealed that abnormal apoptosis is related to the pathogenesis of miscarriages, preeclampsia or intrauterine growth restriction. This study revealed that TBBPA induced a prominent increase in caspase-3 activity and formation of apoptotic bodies indicating that this chemical has a proapoptotic activity.

In summary, the results presented herein showed that TBBPA altered placental hormone secretion and impaired apoptosis and thus may interfere with normal placental development during early pregnancy. TBBPA exerts its endocrine-disrupting effects through complex interactions at the molecular level. Therefore, further studies of the mechanism of TBBPA action in human placenta are needed. Noteworthy in this respect, a potential risk for fetal-maternal health after TBBPA exposure appears plausible.