**Bisphenol A-induced mammary gland carcinogenesis in mothers: effects in senility after exposure during pregnancy and lactation**

**Introduction**

Endocrine disruptors are compounds that could trigger tumorigenic processes through endocrine pathways. Among these, xenoestrogens, especially bisphenol A (BPA), have been associated with disorders in the female reproductive system and accessory glands. The mammary gland (MG) is widely studied in the field of carcinogenic agents research, since it presents morphological plasticity linked to hormonal alterations. The present study aimed to analyze the histopathological effects of the gestational-lactational BPA disruption in MG of senile females.

**Methods**

Female Mongolian gerbils (N=20) were allocated with fertile males. The 1st offspring was discarded to determine the start of the 2nd pregnancy. From the 8th gestational day onwards, females were randomly divided into 4 experimental groups (n=5): control (C) – daily water gavage; vehicle (V) – daily corn oil gavage; ↓BPA (50 μg/kg) and ↑BPA (5000 μg/kg) – daily gavage with BPA diluted in 0.1 ml corn oil. Mothers’ exposure happened during pregnancy (24-26 days) and lactation (21 days). After this period, they were allocated in individual insulators until 18 months-age and then euthanized. MG was removed, fixed in 4% paraformaldehyde and processed for histology and immunohistochemistry (IHC) routines. Analyses of epithelial lesions and quantification (percentage) of collagen and elastic fibers areas were performed. IHC analysis (cells/mm2) of phospho-histone H3 (PHH3), active caspase-3 (CASP3), metalloproteases (MMP-2, -3, -9), FAP (activated fibroblasts), and TGF-β were also performed. The procedures were authorized by CEUA from IBILCE/Unesp (Protocol number: 217/2019).

**Results**

Analyses led to observe that in control and vehicle groups, MG showed tissue regression typical of perimenopause, with increased elastin area (C: 15.52% ± 1.31; V: 10.39% ± 1.34; ↓BPA: 3.79 ± 0.64; ↑BPA: 4.53 ± 0.71), and **CASP3** (C: 15.46 ± 1.16; V: 6.41 ± 0.51; ↓BPA: 2.84 ± 0.53; ↑BPA: 2.35 ± 0.39). However, in the BPA groups, development of multifocal carcinomas was supported by an epithelial-mesenchymal transition (EMT) process – confirmed by vimentin staining and absence of cytokeratin. **TGFβ**+ epithelial cells increased in BPA groups (C: 5.91 ± 0.54; V: 8.33 ± 0.91; ↓BPA: 17.4 ± 0.85; ↑BPA: 19.4 ± 0.99) associated with the disruption process and a drastic increase in proliferation (**PHH3**+ - C: 3.49 ± 0.53; V: 16.9 ± 0.95; ↓BPA: 39.47 ± 2.68; ↑BPA: 41.84 ± 1.62). MG stroma from BPA-exposed groups showed an increase in collagen fibers (C: 4.30 ± 0.42; V: 5.75 ± 0.46; ↓BPA: 11.93 ± 0.52; ↑BPA: 14.16 ± 0.63), and in **FAP**+ cells (C: 3.76 ± 0.22; V: 2.82 ± 0.32; ↓BPA: 8.92 ± 1.05; ↑BPA: 13.88 ± 1.28), *i.e.*, cancer associated fibroblasts. Furthermore, the MMPs (**MMP-2** - C: 1.05 ± 0.41; V: 2.14 ± 0.35; ↓BPA: 15.04 ± 1.27; ↑BPA: 14.34 ± 1.1; **MMP-3** - C: 2.29 ± 0.31; V: 1.72 ± 0.32; ↓BPA: 9.97 ± 1.3; ↑BPA: 13.56 ± 1.6; **MMP-9** - C: 0.92 ± 0.32; V: 1.35 ± 0.27; ↓BPA: 7.41 ± 1.17; ↑BPA: 15.06 ± 1.21) increased in both BPA exposed groups, presenting stromal cells staining, supporting a tumor microenvironment, and also positive carcinoma cells, increasing neoplastic invasiveness.

**Conclusion**

In conclusion, the gestational and lactational windows of susceptibility present a period of carcinogenic induction by BPA, evidenced in MG of senile females. Elements to support a tumor microenvironment and a remarkable EMT process were found in MG after the endocrine disruption.

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