Dr Michael Antoniou and Dr Robin Mesnage, King’s College London, in collaboration with Dr Elisabete Silva, from Brunel University, were awarded a grant of £45,000 to examine the cancer-causing potential of bisphenols. Work began in April 2018 and was completed in December 2021.

Brief Summary of Results

The research examined the effects of bisphenol mixtures on human breast cells grown in cell culture and monitored changes in gene expression. The findings showed that low concentrations of bisphenol mixtures can impact numerous pathways including the ubiquitin-proteasome pathway, which has implications for breast cancer development and growth.

Study background

Bisphenol A (BPA) used in the manufacture of certain clear plastics, including those used for food and drink packaging, has been shown to be a potent mimic of oestrogen. This has led to fears that exposure to BPA can stimulate the growth of many breast cancers. As a result, plastics manufacturers have been replacing BPA with other types of bisphenols, claiming that these BPA alternatives are less oestrogenic and thus safer.

Project aims

This research investigated the breast cancer-causing and growth stimulating properties of a mixture of BPA and 6 substitutes, using cultures of human breast epithelial cells, including those that can form 3-dimensional breast-like cellular structures known as “mammospheres”. These are more representative of breast architecture than standard breast cell culture systems, as they can reproduce features of malignant changes which can be
observed microscopically and monitored using molecular biology methods such as transcriptomics (gene expression analysis) (1).

Little is known about the molecular events resulting from bisphenol exposure that cause a normal breast cell to enter a cancerous state. Gene expression analysis enables a better understanding of the biochemical pathways associated with breast cancer development.

The work is a continuation of an earlier BCUK-funded project which found bisphenols used as alternatives to bisphenol A in “BPA-free” products were equally, or in some cases more, oestrogenic than BPA and so able to stimulate growth of breast cancer cells in vitro. (2).

Mixtures of endocrine disrupting chemicals, including those that can mimic oestrogen, can have potent biological effects at concentrations at which they are inactive when tested individually (3). Different concentrations of bisphenol mixtures, including bisphenol A, bisphenol S, bisphenol F, bisphenol AP, bisphenol AF, bisphenol Z and bisphenol B were tested for oestrogenic effects using human breast cancer cells and normal breast cells grown in cell culture and using a 3D cell culture systems.

**Summary of Results**

A 3-dimensional breast cell culture system was established using human mammary epithelial cells (cell line MCF-12A) and cells were grown in the presence and absence of bisphenols and bisphenol mixtures. However, this model system was unable to reveal differences resulting in exposure to mixtures of bisphenols, either based on altered morphology or differences in gene expression analysis (using transcriptomics). Although gene expression differences were seen, when experimental groups were compared, most differences were attributed to the cell culture batch. This meant it was not possible to determine the effects of experimental treatments. This could be explained by the differences in size and morphology observed in the 3D cell culture system. To further understand whether the instability was due to the cell line or the 3D model system and understand effects of bisphenol
mixtures on breast cells, the remaining experiments used cells grown in traditional (2D) cell culture.

Cell cultures of three different mammary epithelial cell lines (MCF-7, MCF-10A and MCF-12A) were exposed to high and low concentrations of bisphenol mixtures and assessed for alterations in their transcriptome (gene expression profiles). These were further analysed to identify differentially expressed genes (DEGs) and affected pathways. MCF-10A was the most affected with 2,205 DEGs, while MCF-7 had 71 DEGs and MCF-12A had 35 DEGs. Numerous pathways were affected in MCF-10A cells, including a perturbation in the ubiquitin-proteasome pathway (UPP). Alterations in the UPP have been linked with various cancerous states, including breast cancer. Findings showing that bisphenols can impact this pathway may have implications for breast cancer development and progression. A follow-up project (beginning in February 2022) will enable further understanding of biochemical pathways affected by bisphenol exposures.

References

