Neurodevelopmental effect of endocrine disrupting Bisphenol A compounds

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Introduction

- Numerous studies have documented the toxicity of the endocrine disrupting chemical and potent neurotoxin, Bisphenol A (BPA).
- BPA is a major component of polycarbonate plastics. It is present in various household items such as thermal paper, dental fillings and DVDs (Dekant and Volkel, 2008).
- However, leading concerns for human health have been from oral exposure related to its use within canned food, water bottles and baby bottles (Mercea, 2009).
- The use of related polymer Bisphenol AF (BPAF) for similar products including dishwasher interiors is increasing, yet its toxicity is still relatively unknown.

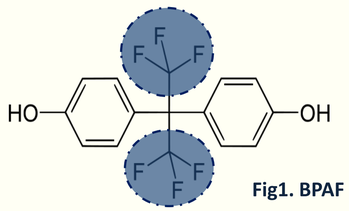

Fig1. BPAF

- The perfluorination of BPA to make BPAF enhances its mechanical and thermal properties but this dense electronegative region could also increase its toxicity.

Aims

- Compare the neurotoxic effects of BPA with BPAF.
- Examine proteomic changes which occur due to exposure to BPA.

Methods

Toxicity testing

- Human Neuronal Precursor Stem Cells (hNPSCs) were seeded onto cell culture plates, and exposed to BPA or BPAF in two separate experiments, for 2 weeks as stem cells, and for 2 weeks whilst the cells differentiated into mature neuronal cell types. Cell viability was examined after 24hrs, 7 days and 14 days exposure by the Alamar Blue reduction assay.

Fluorescence Microscopy

- To examine changes in morphology, size and number of cells due to low dose exposure of BPA or BPAF, hNSCs were seeded onto 4 well chamber slides, and exposed to nanomolar concentrations of BPA or BPAF for 1 week as stem cells. The cells were then differentiated into mature neuronal cell types without further exposure. The slides were then analysed for total cell number by DAPI staining, and for changes in size and morphology using directly labelled fluorescent antibodies to sox2 and nestin. These are markers of pluripotency and a change between DMSO and BPA/BPAF treated cells would indicate that pluripotency is affected.

Western Blotting

- Cells previously exposed to BPA as stem cells for 1 week and then differentiated without the drug present were lysed and electrophoresed then analysed for proteomic changes by western blotting.

Acknowledgments

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Results

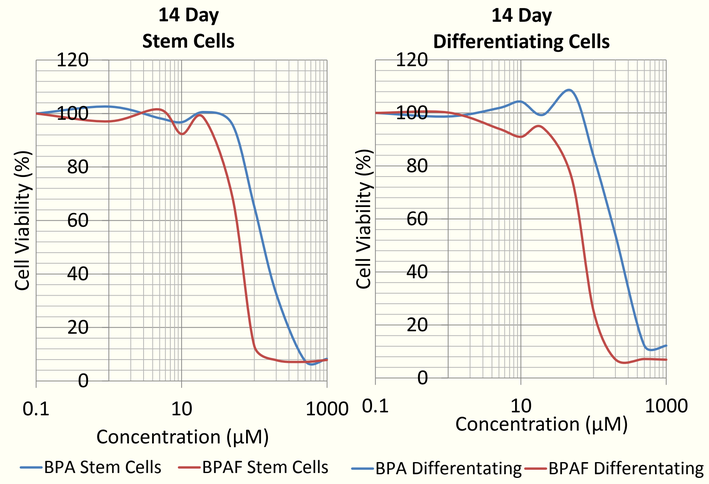

Fig2. 14 Day Stem Cells

Fig3. 14 Day Differentiating Cells

- The highest level of cell death occurred with BPAF treated stem cells. ANOVA tests showed statistically significant cell death at only 50 µM.

	Day 1	Day 7	Day 14
BPAF Stem Cells	150	85	65
BPAF Differentiating	275	90	70
BPA Stem Cells	375	290	130
BPA Differentiating	600	310	230

Fig4. LC50 Values

- LC50 values for 48-Well plates show BPAF to have a 2-3 times greater toxicity to human neuronal cells than BPA.

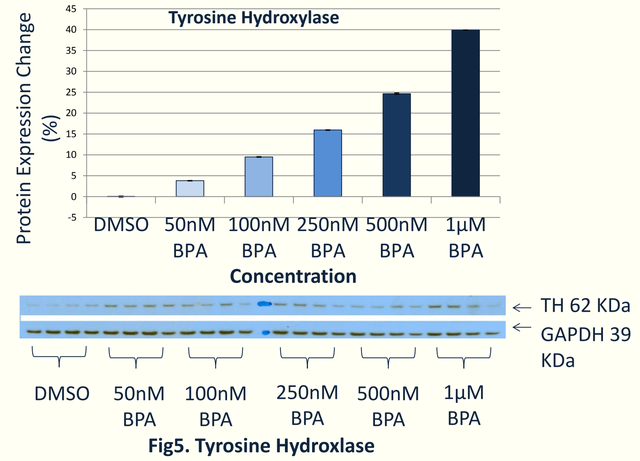

Fig5. Tyrosine Hydroxlase

- The western blot showed increased levels of tyrosine hydroxylase, which is involved in dopaminergic system. Tyrosine hydroxylase catalyses the conversion of L-tyrosine to L-DOPA. L-DOPA formation is the rate-determining step in the synthesis of dopamine.

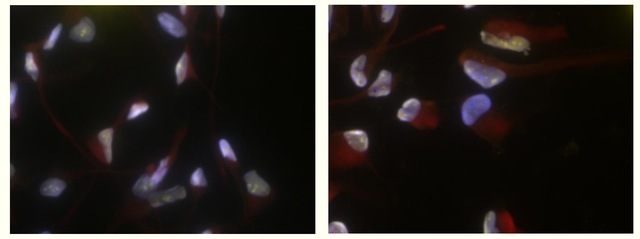

Fig6. Sox2 and Nesin Fluorescence Microscopy

- DMSO (left) and 500nM BPAF (right) slides showed expression of both pluripotency markers sox2 (yellow) and nestin (red) at all DAPI stained nuclei (blue) meaning that pluripotency was not affected. This was the same with BPA.

Discussion

- BPAF is being increasingly used for its favourable properties but is far more toxic to human cells than BPA. The greatest toxicity was to undifferentiated stem cells (akin to early embryonic tissue). Considering the recent ban of BPA use in baby bottles by the FDA, our data strongly indicates that BPAF is a hazard to human health. More research needs to be done to investigate the involvement of dopamine in BPA compound toxicity.

References

Dekant, W. and Volkel, W. (2008) 'Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures', Toxicol Appl Pharmacol, 228(1), pp. 114-34.
Mercea, P. (2009) 'Physicochemical processes involved in migration of bisphenol A from polycarbonate', *Journal of Applied Polymer Science*, 112(2), pp. 579-593.

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