Philippa Gould – SRF Vacation Scholarship report 2015

The role of maternal low protein diet on neural stem cells and neurogenesis in the offspring brain

Background

Malnutrition, especially protein undernutrition, is the greatest threat to global public health according to the World Health Organization, affecting some 13% (1 in 7), (1, 2) and causing 54% of child mortality (3). Nutrition directly affects cell metabolism and growth. Maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. It is important therefore for women during pregnancy and lactation to ingest sufficient nutrients for themselves and their child to prevent the risk for abnormal development and disease in offspring as conceptualised in the Barker hypothesis (4).

Animal models of protein undernutrition produce effects similar to human health problems found in underdeveloped countries (5, 6). However, the importance of nutrition during embryogenesis for early brain development is unknown. Previous project data has shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice(first 3.5 days of pregnancy) is sufficient to induce cardiometabolic and locomotory behavioural abnormalities in adult offspring. The Emb-LPD and sustained LPD reduce neural stem cell (NSC) and progenitor cell numbers through suppressed proliferation rates in both ganglionic eminences and cortex of the early (E14.5) fetal brain. Suggesting, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels. Demonstrating poor maternal nutrition around conception, already associated with adult behavioural deficit, adversely affects early brain development.

Aims: My aim was to see if the maternal LPD during gestation and the periconceptional period (Emb-LPD), effects we have seen at E14.5 are also present at E12.5.

Methods

Flow cytometry analysis of markers

Cortex and Ganglionic eminiences were dissected from each foetal brain and were mechanically dissociated and washed 3 times with FACS buffer. Cells were incubated in Live/Dead Violet Viability stain (Invitrogen, Paisley, United Kingdom) for 30 minutes at 4ºC. Samples were treated with BD Cytofix/Cytoperm kit (554714). Cells were washed 3 times in FACS buffer and incubated with mouse anti-nestin IgG conjugated to phycoerythin (PE) (IC2736P, R&D systems) and mouse anti- Beta-III-tubulin conjugated to allophycocyanin (APC) (Biolegend) or isotype control antibodies for 30 at 4ºC degrees. Cells were then washed again three times in FACS buffer and re-suspended in 250 µl for FACS analysis. Phenotypic characterization of GE and cortex (n=3 foetus per litter) was performed with a 9-color FACSAria cell sorter and FACSDiva Software (version 5.0.3; BD Biosciences, Oxford, United Kingdom). Dead cells were excluded from analysis and 10,000 live cells per sample were counted.

FA*CS gating*

Dissociated cells from the foetal brain tissue that had been prepared for FACS were run on a 9-color FACSAria cell sorter using FACSDiva Software (version 5.0.3; BD Biosciences, Oxford, United Kingdom). IgG controls are also run. Based on cell density on the flow cytometry plots early progenitors PE^{hi} APC^{lo} and late progenitors populations PE^{lo} APC^{hi} can be picked out as shown in fig 1. Dead cells were excluded from analysis. To detect the different cell-type populations, FACS gates were calibrated so as to only detect specific wave lengths of light, produced by the two markers upon excitation. Q1 only detected the presence of nestin, Q4 detected the presence of Beta-III-tubulin, Q2 detected neural progenitors which are both positive for nestin and Beta-III-tubulin and Q3 detected unstained cells. Percentage populations of PE+ APC- , PE- APC+, PE^{hi} APC^{lo} and PE^{lo} APC^{hi} cells were calculated for statistical analysis.

Statistical analysis

The FACS staining data were analyzed using a multilevel random-effects regression model using PASW for Windows program version 21 (SPSS UK, Woking, Surrey, United Kingdom). This showed that differences identified between treatment groups are independent of maternal origin of litter and litter size (7).

Results

Flow cytometry on E12. 5 cortex and ganglionic eminences cells were stained for Nestin and Beta-III-tubulin and sorted. Nestin only positive cells represented only a small percentage of the whole population and showed a decrease in Emb-LPD in cortex, with a trend to decrease in cortex LPD and ganglionic eminences Emb-LPD showed a significant decrease with a trend to decrease in the LPD compared to NPD. Beta-III-tubulin only positive cells represent young neurons and showed a trend to increase in Emb-LPD and to decrease in LPD, compared to NPD, in the cortex, ganglionic eminiences had a significant increase in these cells in the LPD.

Two different populations can be detected in the double positive cell population. These were separated as follows: $Nestin^{hi}$ $Beta\text{-}III\text{-}tubulin^{lo}$ (representing early neuronal progenitors), and $Nestin^{lo}$ $Beta\text{-}III\text{-}tubulin^{hi}$ (representing late neuronal progenitors). When analysed separately, these two neuronal progenitor populations showed very different

results in both ganglionic eminences and cortex: the early progenitors decreased significantly in both Emb-LPD and LPD compared to NPD whereas the late progenitors increased significantly in both Emb-LPD and LPD compared to NPD.

Conclusion

These results indicate that maternal diet affects the availability of neural stem cells but also their pattern of differentiation towards a neuronal fate. Therefore, in Emb-LPD there is a decrease in the neural stem cells and early neural progenitor cells and an increase in late neuronal progenitors and young neurons. In contrast, LPD induced a decrease in stem/progenitor cells, an increase in late neuronal progenitors and an increase in young neurons in just the ganglionic eminiences.
This suggests that a maternal LPD affects neural stem cells and neural progenitor cells even during the early period (Emb-LPD) at E12.5 and further supporting the information the group know at E14.5.

Acknowledgements

I would like to thank Joanna Gould for helping me in the lab and Professor Tom Fleming for supervising me and helping me throughout the project giving me the opportunity to carry out this project. I would also like to thank the Society for Reproduction and Fertility for kindly granting me this vacation scholarship and funding this work.

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