Neuroinflammatory response in the hippocampal formation of dams induced by gestational diabetes

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AIM

Assess the long-term effects of GDM in terms of cognitive dysfunction and hippocampal neuroinflammation response with microglial activation.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a metabolic disease, characterized by insulin resistance or insulin deficiency and affects up to 6% of all pregnancies. [1] Hyperglycaemia, oxidative stress and neuroinflammation are considered some causes of the physiopathology of GDM that can ultimately lead to cognitive deterioration, such as loss of memory and impaired learning abilities, leaving some questions as to the extent of such deficits. [2,3] Apart from this, little is known about the effects of GDM in the central nervous system of pregnant female rats and its possible implications in cognitive impairment.

METHODS

To reach the pregnant stage, daily vaginal cytologies were made in order to determine the oestrus cycle of the dams and once they found themselves in the pro-estrous stage of the cycle, the mating would occur. Pregnant Female Wistar rats were randomly assigned to three groups:

- Control (C) n=16
- G65: Intraperitoneal injection of Streptozotocin
- G69: Sham Surgery
- STZ n=14
- Intraperitoneal Injection
- STZ+Insulin n=15
- G65: Intraperitoneal injection of Streptozotocin
- G67: Induced Diabetes Confirmation
- G69: Sham Surgery
- G6D: Saline Intraperitoneal Injection

During the pregnancy, the dams were regularly weighted, and their blood glucose levels were measured to assure their well-being and the success of the procedures.

At the end of the weaning period of the pups, the dams were studied to evaluate possible changes in anxiety, locomotor activity, memory and spatial learning using the Morris water maze, elevated plus-maze, open field, as well as the novel object recognition and the forced swim test.

RESULTS

Blood glucose levels were measured to assure the well-being and the success of the procedures.

BEHAVIOURAL STUDIES

To conclude, we describe here behavioural effects of gestational diabetes in dams. Diabetic rats have normal long- and short-term acquisition performances, locomotor activity and display no anxiety-like behaviours compared to both controls and insulin-treated diabetic rats.

FUTURE WORK

- Assess the total number of resting and activated microglia, and GFAP positive cells in all the subfields of the hippocampal formation and measurement of the expression levels of pro-inflammatory and anti-inflammatory cytokines by qRT-PCR.
- Evaluation of the aeral densities of the apoptosis related proteins on the hippocampal formation subfields and of the gene expression of both BAX and BCL-2.

Fig. 1: Representative images of vaginal cytology. (A) Proestrus phase, predominance of nucleated epithelial cells. (B) Estrus phase, characterized by a predominance of numerous cornified squamous cells. (C) Metestrus phase, predominance of leukocytes, cornified and nucleated epithelial cells in the same proportion. (D) Diestrus phase, consists of mainly leukocytes.

Fig. 2: Representative graph of serum glucose concentrations of normal, diabetic and insulin-treated dams. Columns represent means and vertical bars SEM. Tally test: *p < 0.0001 compared to both control and insulin-treated diabetic rats.

Fig. 3: (A–D) Morris water maze test. The graph in A shows the mean ± SEM total distances travelled (cm) to find the hidden platform for each block of the four consecutive trials in the Morris water maze test. There were no significant differences in acquisition performance between groups. The histograms in B show mean ± SEM of the number of platform crossings over the duration of the test (grade trial). No significant differences were noted among groups. (C) Elevated plus-maze test. Graphical representation of the mean ± SEM time spent in the arms and central square of elevated plus-maze. There were no significant differences between controls, diabetic, and insulin-treated diabetic rats. (D) Open-field test. The histograms show the mean ± SEM time spent in outer and inner zones of the open-field. There were no significant differences between groups. (E–F) Novel Object Recognition test. (E) Trial Test, with one novel object. (F) Forced swim test. The histograms show the mean ± SEM percentage of cumulative immobility time over the duration of the test. No differences were found between groups.