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| Project Title | Mechanisms of Action of Mesenchymal Stromal Cell Therapy in Newborn Rats from Pregnant Rats with Intrauterine "Augmented Inflammation" | |
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## IMSUT International Joint Usage/Research Center Project <International>

## Joint Research Report (Annual)

**Annual Report**

**Report**

**Title: Mechanisms of action of mesenchymal stromal cell therapy in newborn rats from pregnant rats with intrauterine "augmented inflammation"**

**Background and objectives :**

Low birthweight (LBW, < 2500 g) occurs in approximately 14.6% of infants. LBW infants exhibit a higher risk for neurodevelopmental disorders (NDDs), such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). These disorders are associated with sensory and sensorimotor disturbances, deficits in cognition, memory and learning, emotional disorders including anxiety and psychiatric disorders (Coq JO, et al*, Front Neurol* 9:423,2018). Fundamentally, LBW and prematurity appear to have two main prenatal causes: **hypoxia-ischemia** related to intrauterine hypoperfusion and **infection/inflammation**. Umbilical cord-derived mesenchymal stromal cells (UC-MSCs) have the potency to suppress inflammation and exhibit neuroprotective, and neurorestorative properties.

Our previous reports demonstrated that the behavioral deficits and inflammatory levels were slightly affected by intrauterine hypoperfusion (MIUH) and the beneficial effects of early therapy with UC-MSCs were marginal, although this MSC therapy prevented LBW pups and rats from lower hippocampal neuron density and from hyperexcitability in the spinal cord. Our objectives are to extend the previous study to make LBW rats by hypoxia with MIUH and uterine inflammation with LPS injection.

**Methods:**

Fist, we established a rat model of prenatal “augmented inflammation”, based on the intraperitoneal injection of an endotoxin, the lipopolysaccharides (LPS) into pregnant rats on gestational day 17 to induce prenatal systemic infection/inflammation, with a student (Zhang T. in Nagamura’s laboratory).

In 2025 FY, we created a model of “augmented inflammation” combining hypoxia/ischemia and infection/inflammation during the prenatal period. We first put coils around the 4 uterines arteries at embryonic day 17 (E17) to reduce blood perfusion to the placentas and fetus leading to fetal growth restriction. Then, with the same procedure, we injected LPS into each sac containing a fetus to produce systemic infection/inflammation during the prenatal period at E17. Rat behaviour was asseesed using the following tests: the surface righting reflex test, the negative geotaxis test, the cliff avoidance test, the inter-limb coordination test, the open-field test, the memory object-recognition test and the elevated plus maze test.

**Results and Discussions :**

We obtained 9 MIUH+LPS pups with a birth weight below 6.0 g (LBW) treated with the vehicle. These animals were used to study body weight gain with age, as well as early and adult sensorimotor abilities, anxiety and disinhibition, and short-term memory.

***Weight gain with age***

We first examined body weight gain with age in controls i.e., sham non-operated, MIUH and MIUH+LPS rats from P0 to P60 (2 months old). Compared to the control group, MIUH and MIUH+LPS group showed lower weitght at P0 up to P30, however, the three groups did not differ significantly at P60, likely due to small samples. Our results show that by P60, MIUH and MIUH+LPS rats appeared to catch up the control rats in terms of weight.

***Early and adult sensorimotor abilities***

In the surface righting reflex test, we measured the latency of pups to regain contact with the surface with all four paws after being placed in a supine position from P0 to P16. MIUH+LPS pups showed longer latencies to straighten than controls at P2 and from P9 to P16.

In the negative geotaxis test, we measured the latency to turn through 180° from a head-down position on an inclined 45° plane surface from P0 to P16. There were no statistically differences in latencies between MIUH+LPS and control rats of any age.

In the cliff avoidance test, each pup was placed on the edge of a table with their forepaws and head hanging over the edge. The time required to complete backing and turning away from the cliff was recorded from P0 to P16. Compared to controls, MIUH+LPS pups took longer to withdraw at P9, P14 and P16.

The inter-limbs coordination of young-adult rats at P60 was tested using a rotarod apparatus with an increasing speed ranging from 4 rpm to 45 rpm over a period of 5 minutes. MIUH+LPS rats had a shorter latency to fall down than controls, but this difference was not significant. The maximum speed reached by the MIUH+LPS rats was lower than that of controls, but this was not statistically different.

To sum up, MIUH+LPS pups exhibited lower performances and a delayed emergence of early sensorimotor neuroreflexes while the adult sensorimotor coordination was not significantly different between the two groups, likely dur to the small sample size.

***Anxiety and disinhibition***

Adult rats were exposed to an unknown open-field under high illumination during 5 min to assess the levels of anxiety. The expected behavior in control rats that present tendencies to be scared and anxious in open-field spend more time in the periphery and corners than in the center more exposed of the open-field. Compared to controls, MIUH rats spent more time in the center than in the periphery and corners ; whereas MIU+LPS rats exhibited comparable behaviors as controls. However, MIUH+LPS rats travelled the most in the open-field, compared to control and MIUH rats, suggesting spontaneous exploratory hyperactivity.

In the elevated plus maze under high illumination, adult rats were exposed for 5 min to two arms that are open and anxious while two other arms in a cross shape are closed and less anxious than the open arms. Corresponding to the expected behavior of controls, our control rats spent more time in the closed arms than in the open arms, showing to anxiety and inhibition. Compared to controls, MIUH rats spent significantly more time in the closed arms than in the open arms, roughly like controls. In opposition, MIUH+LPS rats spent more time in the open arms than control and MIUH rats, and about same time in both open and closed, suggesting less anxiety in MIUH+LPS rats, along with exploratory hyperactivity.

***Short-memory abilities***

In the memory object-recognition test, two objects were presented in the already known open-field for 5 min. After 5 min of retention, one object from the two objects was replaced with a new one to assess the reaction to novelty and thus the short-term or working memory abilities. Control and MIUH rats exhibited comparable poor performances with little reaction to novelty, whereas MIUH+LPS rats exhibited exploratory hyperactivity and the highest reaction to novelty. These preliminary results are surprising and counter-intuitive, and needs confirmation before a conclusion. Thus, our preliminary results with the combination of intrauterine hypoxia/ischemia and infection/inflammation in MIUH+LPS rats show greater exploratory hyperactivity than MIUH rats (i.e., only hypoxia/ischemia), longer latencies and delayed emergence of sensorimotor neuroreflexes, less anxiety so more disinhibition in the elevated plus maze.

The preliminary results in open-field and in memory object-recognition are surprising and require more animals before conclusions can be drawn. These preliminary results are interesting and encouraging but require more animal and additional testings in MIUH+LPS animals treated with vehicle or with UC-MSCs, the latter will be tested in FY2026. We have recently obtained an additional (n =13) MIUH+LPS pups, which have been treated with either vehicle or UC-MSCs injection. To analyze the influence of inflammation on the pups, we plan to investigate the expression levels of 27 pro- and anti-inflammatory cytokines.