No.	K22-1059	
Project Title	Mesenchymal Stromal Cell Therapy to Prevent Neurodevelopmental Disorders related to Low-Birth-Weight	
Principal Investigator	Jacques-Olivier COQ (Centre National de la Recherche Scientifique・Professor)	
Project Member(s)	IMSUT Host Researcher	Tokiko Nagamura-Inoue (The Institute of Medical Science, The University of Tokyo・Associate professor)
	Project Member (s)	Coq, Jacques-Olivier (Centre National de la Recherche Scientifique・Professor/Senior Reasearcher)
	Project Member (s)	Nagamura-Inoue, Tokiko (Department of Cell Processing and Transfusion・Professor)
	Project Member (s)	Tsuji, Masahiro (Kyoto Women's University/Department of Food and Nutrition・Associate Professor)
	Project Member (s)	Mukai, Takeo (Department of Cell Processing and Transfusion・Associate Professor)
	Project Member (s)	Sei, Kenshi (Department of Cell Processing and Transfusion・PhD student)
	Project Member (s)	Vianefe, Maele (Aix Marseille University・Master 2 student)
	Project Member (s)	Satoshi Uematsu (Division of Metagenome Medicine・Project Professor)
	Project Member (s)	Kosuke Fujimoto (Division of Metagenome Medicine・Project Assistant)

(Form 2-2)

Principal Investigator
Jacques-Olivier Coq

IMSUT International Joint Usage/Research Center Project <International>
Joint Research Report (Annual)

Annual Report

Report: Mesenchymal Stromal Cell Therapy to Prevent Neurodevelopmental Disorders related to Low-Birth-Weight

<Background> Low birth weight (LBW) increases the risk of neurodevelopmental disorders (NDDs) such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), as well as cerebral palsy, for which no prophylactic measure exists. We have established the rat model of prematurity and LBW based on intrauterine hypoperfusion. Neuroinflammation in fetuses and neonates plays a major pathogenic role in NDDs (Delcour et al., 2011, 2012ab; Ohshima et al., 2016; Coq et al., 2018, 2019; Tsuji et al., 2018). Meanwhile, umbilical cord-derived mesenchymal stromal cells (UC-MSCs) exhibit immunomodulatory properties. Therefore, we hypothesized that systemic administration of UC-MSCs in the early postnatal period may attenuate neuroinflammation and thereby prevent the emergence of NDDs. The aim of this project is to investigate the effects of an early therapy based on MSCs with specific anti-inflammatory properties on the organization and functions of the brain in LBW rats, as well as some mechanisms of actions of these MSCs.

<Methods> Human UC-MSCs, was approved by the Ethics Committee of the Institute of Medical Science, the University of Tokyo (IMSUT), and Kyoto Women's University, Institut de Neurosciences de la Timone, Centre National de la Recherche Scientifque, France. UC-MSCs were provided by cord blood and umbilical cord bank from the research hospital, IMSUT (IMSUT CORD), Japan (Ethical committee of IMSUT number 33-2). We used 85 LBW rats subjected to MIUH and 56 sham-operated rats of either sex; all rats were operated on at E17. Thirty-nine LBW rats and 26 sham pups received intravenous injection of MSCs on P1, and 46 LBW and 30 sham rats were intravenously injected with a vehicle solution on P1. Ten rats from all four experimental groups (sham-vehicle, sham-MSC, LBW-vehicle and LBW-MSC) underwent the same assessments of body weight, behavioral performance including negative geotaxis (P6 to P8), brain weight, and neuronal counts (7 weeks of age). As behaviors may differ between males and females at puberty and later life stages, behavioral tests performed from 3 to 7 weeks of age (i.e., the open-field test and three-chamber sociability test, as well as body weight and brain weight tests) were separately analyzed in males and females. Males and females in the sham MSC groups were excluded from these behavioral test analyses due to the low number of males in this group (n=3). We evaluated cytokine expression (P2), electrophysiological post-activation depression (P5 to P6), and KCC2 expression in the lumbar spinal cord (P8) using Western blotting in different sets of experimental rats for the four groups.

<Results> The LBW pups born to dams subjected to mild intrauterine hypoperfusion exhibited a significantly lesser decrease in the monosynaptic response with increased frequency of stimulation to the spinal cord preparation from postnatal day 4 (P4) to P6, suggesting hyperexcitability, which was improved by intravenous administration of human UC-MSCs (1×10^5cells) on P1. Three-chamber sociability tests at adolescence revealed that only LBW males exhibited disturbed sociability, which tended to be ameliorated by UC-MSC treatment. Other parameters, including those determined via open-field tests, were not significantly improved by UC-MSC treatment. Serum or cerebrospinal fluid levels of pro-inflammatory cytokines were not elevated in the LBW pups, and UC-MSC treatment did not decrease these levels.

<Discussion and Conclusion> To our knowledge, this is the first study to assess the impact of early administration of human UC-MSCs on physiology and behaviors associated with NDDs in a rat model of LBW based on intrauterine hypoperfusion. Our results indicate a positive effect of UC-MSC administration in reducing hyperexcitability in the lumbar spinal cord and a marginal positive effect on social interactions in males in the three-chamber test. Thus, UCMSC treatment may improve different aspects of neurological problems in LBW children. We observed no significant effects of UC-MSC treatment in LBW rats on the (1) physical development of pups (body weight gain), (2) delay in acquiring a physiological reflex (negative geotaxis), (3) spontaneous hyperactivity and less anxiety-like behavior in the open-field test, and (4) reduced neuronal counts in the hippocampus, as observed in the LBW-vehicle rats. Considering the limited preventive measures for NDDs and cerebral palsy in LBW children, we believe that the beneficial effects of UC-MSCs are worth exploring for possible clinical translation. In conclusion, UC-MSC treatment may have a potential to prevent the emergence of NDDs. (Tsuji et al, Sci Rep. Sci Rep. 2023 Mar 7;13(1):3841.).