OBJECTIVE: Neonatal hypoxic-ischemic encephalopathy (HIE) is the important etiology that leads to disability of children in our country. Mesenchymal stem cells (MSCs) could secrete a series of growth factors and neurotrophic factors and have the potential ability to differentiate to the neural cells in vitro and in vivo. We expect to transplant and directly induce MSCs to differentiate into neurons in order to cure HIE. The aim of this study was test the hypothesis that MSCs could enter the brain of newborn Wistar rats through their blood-brain barrier (BBB), and observe the characteristics of the distribution and differentiation of MSCs in brain tissues, then explore the effects of hypoxic-ischemic brain damage to the penetration and differentiation of MSCs.

METHODS: Isolation and purification of MSCs were established from the whole bone marrow of juvenile Wistar rats by removing the nonadherent cells in primary and passage cultures. For cellular identification, MSCs of 3-5 passages were continuously prelabeled with 5-bromo-2-deoxyuridine (BrdU) for 72 hours before transplantation. Animal models of HIE were built in 7d-postnatal Wistar rats according to Rice method. Two hours after hypoxia-ischemia, rats with HIE group (n=8) were intraperitoneally infused MSCs (4×10^6,0.5 ml) . In control group (n=8), 7d-postnatal normal Wistar rats were intraperitoneally infused with the same amount of MSCs. In 14d after transplantation all rats were sacrificed and their cerebra were sectioned by cryomicrotome. Immunohistochemical staining with chromogen diaminobenzidine (DAB) was used to detect and measure the cells derived from MSCs, and study the characters of distribution. To determine the differentiation of the BrdU positive cells entering the brains, Immunofluorescence double labeling for BrdU and neural cells specific antigens was performed.

RESULTS: MSCs were distributed throughout the cerebra in both groups at the 14th day after transplantation. The number of MSCs detected was 2415±226 in the control group, and 3626±461 in HIE group,t=6.68;P<0.05. More BrdU reactive cells were observed in the right ischemic hemisphere& than in the contralateral hemisphere&;t=1904±267;P<0.05. No significant difference was proved while comparing both cerebral hemispheres of the control group,t=0.31, P>0.05. In the HIE group, MSCs distributed more extensively, and some focus aggregations of MSCs were found. A few MSCs expressed Nestin—protein marker of neural progenitor cells, and almost none of the MSCs expressed proteins characteristic of neuron (e.g. NSE) and astrocyte (e.g. GFAP) was detected at the 14th day after transplantation.
CONCLUSIONS: 1. MSCs can enter the cerebral parenchyma through BBB and migrate throughout the brain by intraperitoneal infusion. 2. Hypoxic-ischemic brain damage could improve MSCs entrance and migration. 3. Transplanted MSCs could not differentiate to adult neural cells without other interventions during 14 days after transplantation.