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Research Report

Regional differences of microglial accumulation within 72 hours of hypoxia-ischemia and the effect of acetylcholine receptor agonist on brain damage and microglial activation in newborn rats

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ABSTRACT

Objective: We examined regional specificity of microglial activation in the developing rat brain for 72 hours after hypoxia-ischemia (HI) and the effect of acetylcholine receptor (AChR) agonist on microglial activation.

Study design: Seven-day-old Wistar rats were divided into two groups: one receiving a single dose of AChR agonist just before hypoxia (carbachol; 0.1 mg/kg) to investigate the reducing effect on brain damage with decreasing activation of microglia and the other group receiving saline as a control. Rats were subjected to left carotid artery ligation followed by 8% hypoxia. Brains were analyzed immunohistochemically at 24, 48, and 72 hours after HI. TNFα production was measured at respective times after HI.

Results: Activation of microglia on the hippocampus of the control group was strong for the first 48 hours and then weakened. In contrast, activation of microglia on white matter and the cortex was weak at 24 hours and then became stronger. A single dose of carbachol significantly reduced brain damage with a marked reduction of microglial activation on the hippocampus, whereas it was less effective regarding microglial activation on white matter and the cortex. TNFα production was low in both groups.

Conclusion: Regional specificity was observed for both microglial activation and susceptibility to carbachol for the first 72 hours after HI. Our data suggested that timely intervention along with region-specific microglial activation, apart from TNFα production, may be critical for the prevention of further brain damage after HI in the newborn.

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1. Introduction

Perinatal hypoxic-ischemic brain damage including periventricular leukomalacia is one of the most important medical problems to overcome (Doi et al., 2012). Multiple pathways of oxidant stress, inflammation, and excitotoxicity affect brain damage at different times and regions of the brain. Among these pathways, it has been proposed that the inflammatory response after hypoxia-ischemia (HI) can induce a cascade of immune responses, in both term and preterm infants, which involves the pathogenesis of early brain injury (Berger et al., 2012).

In the central nervous system, microglia are activated by HI (Wang et al., 2003), which is related to inflammatory processes through the effect of AChR on microglia (Gehrmann et al., 1992; Shytle et al., 2004). In the human developing brain, microglia already exist at 4 weeks of gestation and then populate various sites at different gestational periods (Verney et al., 2010). Activated microglia have been recorded as a marked feature of white matter in all cases involving periventricular leukomalacia in the human preterm fetus (Hirayama et al., 2001). We recently demonstrated that stimulation or inhibition of AChR in the developing rat modifies hypoxic-ischemic brain damage 7 days after HI, as well as regional differences of susceptibility to AChR antagonists or agonists (Furukawa et al., 2011; Furukawa et al., 2013). Additionally, AChR agonist reduced activation of microglia in the hippocampal region 24 hours after HI in the developing rat (Furukawa et al., 2013). Thus, a region-specific AChR-responsive pathway plays an important role in modulating perinatal brain damage, in which early microglial activation after HI may be involved. However, we have not clarified whether the phase of microglial activation differs from the phase of obvious tissue damage after HI, and if the phase differs between brain regions. These issues are very important for the treatment of perinatal brain injury because different processes of inflammation require different treatment strategies for prevention.

Therefore, we hypothesized that regional susceptibility to HI plays an important role in the development of perinatal brain damage. We examined the time course of microglial activation in three different regions comprising the hippocampus, cerebral white matter (WM), and the cortex during the first 72 hours (early phase) after HI in a 7-day newborn rat model, and investigated the effect of AChR agonist on brain damage, microglial activation, and cytokine production.

2. Results

2.1. Brain damage

All the pups survived and were included in the histological and TNFα-production analysis. Fig. 1 shows the relative difference for hemisphere areas (panel B) and hippocampal areas (panel C) at 24, 48, and 72 hours after HI. In the control groups, the relative difference for hemisphere area (panel B) was reduced significantly after 48 hours ($p<0.05$). In the carbachol groups, the relative difference for hemisphere area (panel B) was reduced significantly at 72 hours compared to 24 hours ($p<0.05$). A 0.1 mg/kg dose of carbachol significantly inhibited reducing of the relative difference for hemisphere area to that of controls at 48 and 72 hours ($p<0.05$ and $p<0.05$, respectively). The relative difference for hippocampal area in control groups (panel C) was reduced significantly at 48 and 72 hours compared to 24 hours ($p<0.05$ and $p<0.05$, respectively). No significant difference of the relative difference for hippocampal area was observed throughout study period in carbachol groups. Carbachol significantly inhibited reducing of the relative difference for hippocampal area to that of controls at 48 hours ($p<0.05$).

2.2. Microglial activation

Fig. 2 shows microglial activation at 24, 48, and 72 hours on the cortex (panel C), WM (panel D), and hippocampus (panel E) after HI. Microglial activations on the cortex of controls and carbachols (panel C) were noticeable at 48 hours and then increased and lasted until 72 hours ($p<0.05$). Administration of carbachol did not minimize microglial activation relative to that observed for controls at 24, 48 and 72 hours ($p=0.45$, $p=0.12$ and $p=0.12$, respectively). Microglial activations on the WM of controls and carbachols (panel D) were noticeable at 48 hours and then increased and lasted until 72 hours ($p<0.05$). Administration of carbachol did not minimize microglial activation relative to that observed for controls at 24, 48 and 72 hours ($p=0.77$, $p=0.08$ and $p=0.20$, respectively). Microglial activations on the hippocampus of controls were strong at 24 and 48 hours after HI (panel E). There is no significant difference at 48 hours compared to 24 hours, only significant at 72 hours ($p<0.05$). In the carbachol groups, there is no significant difference throughout study period. A 0.1 mg/kg dose of carbachol resulted in a significant minimizing effect on microglial activation relative to that observed for controls at 24 and 48 hours ($p<0.05$ and $p<0.05$, respectively).

2.3. TNFα production

TNFα production of the ligated hemisphere side at 24, 48 and 72 hours after HI are shown in Fig. 3. TNFα productions in the group of control and carbachol were quite low during the course of the investigation. The TNFα production of both groups ranged from 0 to 2.5 pg/ml. There were no differences of TNFα production at 24, 48 and 72 hours ($p=0.25$, $p=0.89$ and $p=0.90$, respectively) between groups.

3. Discussion

We previously demonstrated that stimulation of AChR reduces brain damage and activation of microglia on the hippocampal region 24 hours after HI in the developing rat (Furukawa et al., 2011, 2013). These findings suggested that AChR stimulation reduces the inflammatory response after HI and is followed by a minimizing effect on damage to the developing brain. However, we have not clarified whether the phase of inflammation differs relative to brain region and time after HI. This is a critical issue because different processes of inflammation require different treatment strategies for prevention. In this