[31]. Our study showed a decrease in the number of activated microglia by UCBC administration in the early phase of neonatal HI (fig. 1e, 2e). Pimentel-Coelho et al. [13] also reported that intraperitoneal administration of UCBCs 3 h after insult decreased apoptosis and microglial activation, and improved primitive reflexes in a neonatal HI rat model. Therefore, to suppress the elevation of such proinflammatory cytokines, which lead to decreased apoptosis, UCBC administration in the early phase may be a reasonable therapeutic approach, as demonstrated in the present study.

Oxidative stress plays an important role in HI brain damage [32]. Here, we demonstrated a decrease in 4-HNE- and nitrotyrosine-positive cells in the dentate gyrus following UCBC administration (fig. 1c, d, 2c, d). To our knowledge, this study is the first to report antioxidative effects of UCBCs in a neonatal HI rat model. Arien-Zakay et al. [33] reported antioxidative effects of UCBC-derived neural progenitor cells on insulted PC12 cell lines. Further, suppression of oxidative stress after adult transient focal ischemia was observed in an interleukin-1 knockout murine model [34]. As described above, UCBC administration can decrease the elevated expression of proinflammatory cytokines including interleukin-1 [31]. Moreover, the decreased expression of ED1 in the present study (fig. 1e, 2e) indicates that UCBC administration decreased HI-induced inflammation. Thus, the antioxidative effect of UCBC administration might be exerted directly and/or via suppression of inflammation.

Calculations of the immunohistochemically stained cells were focused on the dentate gyrus of the hippocampus, which is one of the most vulnerable areas to hypoxic ischemic insult. Although we calculated the immunohistochemically stained cells with density counts, which is less sensitive than stereological counts, the results revealed that UCBC administration suppressed apoptosis, as indicated by the decrease in the number of cells positive for active caspase-3 and AIF (fig. 1a, b, 2a, b). In the present study, we performed high-resolution analyses of walking patterns using the CatWalk system; however, they were not sensitive enough to detect motor impairment after HI injury. In contrast to human neonates, rat pups after HI injury did not show obvious locomotor abnormalities, as in other studies using the same model [35]; this may have been because of the higher degree of plasticity of the immature rat brain [36]. We also evaluated the learning memory after HI with the shuttle avoidance test and found only a mild tendency to improve the learning memory in the UCBC group; the difference was not significant. This may be a type 2 error, and further studies are required to clarify the potential effects of UCBC therapy on motor impairment and cognitive deficits after HI. In addition, we found no differences between the vehicle and UCBC groups in absolute tissue loss or the number of neurons in the cortex, corpus callosum or hippocampus (fig. 5). Similarly, some former studies failed to show histological improvement following UCBC therapy [12, 16], whereas others did [14, 15]. We have summarized the experimental protocols and results [37]. In many previous reports, 1×10^7 mononuclear cells were administered intraperitoneally 24 h after the insult. We administered the same dose of cells at an earlier time point. Pimentel-Coelho et al. [13] administered UCBCs even earlier (3 h after the insult) using a lower dose (2×10^6) of cells, and showed improvement in morphology and behavior. It is still unclear how the differences between protocols can affect the results. Other possible reasons for the different outcomes can be the severity of the insult and other experimental settings. Considering the fact that the present study failed to show any effect on morphological changes in the chronic phase or improvement of behavioral impairments, despite the fact that several acute injury markers were suppressed, a modified protocol (e.g. repeated administration, combination with some other treatments) should be tried with an aim to achieve sustained neuroprotection.

In the present study, cryopreserved mononuclear cells were used. Even frozen-thawed UCBCs are known to produce various cytokines and chemokines [38], and exert a neuroprotective effect in various animal models [13, 37, 39]. Moreover, from the viewpoint of clinical applications, cryopreservation is essential in the case of allogeneic transplantation, which may be applicable for patients without access to their own cord blood cells.

We used human UCBCs in a rodent injury model. It might have been more suitable to use rat UCBCs. However, it was very difficult to get sufficient numbers of cells from the umbilical cord of rats without expansion in culture. Because the purpose of the present study was to evaluate the treatment effect of mononuclear cells from the umbilical cord without using culture procedures, we used human cells, as in previous publications [13–18].

Another possible limitation in the present study is that we could not monitor/control body temperature in each pup. We placed the pups on/into the temperature-controlled plate/chamber during the HI insult, and returned them to the dam in a temperature-controlled room after the insult. There might be some variation in brain temperature, leading to variation in the degree of brain damage [40].

The choice of injection site is an important issue when using cell infusion for the treatment of brain injury. We administered UCBCs intraperitoneally, as in most previous studies. According to our recent unpublished results, only a small number of cells either injected intraperitoneally or intravenously could be found in the brain, and cells injected intraperitoneally were less seen in the liver, lung or spleen than cells injected intravenously, indicating that many intraperitoneally injected cells might have stayed in the peritoneal cavity. The treatment effect might be through trophic factors secreted by the cells [41]. The extent of brain damage can be influenced by the peripheral inflammatory response [42]. Modulating peripheral inflammation can be a therapeutic target. In a traumatic brain injury model, multipotent adult progenitor cells exerted a neuroprotective effect through interaction with resident splenocytes [43].

In summary, these results indicate that a single intraperitoneal injection of UCBC-derived mononuclear cells 6 h after HI reduced caspase-3, AIF, microglial activations and oxidative stress, but it did not induce morphological or functional protection. Repeated administration or a combination treatment may be required to achieve sustained protection.

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Disclosure Statement

None of the authors have any conflicts of interest associated with this study.

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