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Full Length Research Paper

Optimal incubating time of *in vitro* bromodeoxyuridine labeling of human umbilical cord blood- mononuclear cells and their functional assessment in ICH rats

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Bromodeoxyuridine (BrdU) is a thymidine analog that is ready to be incorporated into DNA and can be used as a marker for *in vitro* pre-labeling of isolated stem cells. The objective of this study was to explore the optimal incubating time of *in vitro* BrdU labeling for human umbilical cord blood - derived mononuclear cells (HUCB-MCs). The mononuclear cells were isolated by the standard density gradient technique and were labeled with 10 µM/L BrdU. Then, the cells were incubated at 37°C for 1, 6, 12, 24, 48 and 72 h, respectively. Immunocytochemistery was performed respectively to calculate the labeling index and the cells' activity for different time. Efficacy of BrdU positive HUCB-MCs was evaluated by *in vivo* study in intra-cerebral hemorrhage (ICH) rat model. The current findings indicated that labeling ratio significantly increased after 24 incubating hours. Cell viability remained high (75%) after 24 h. There was a significant recovery in behavioral performance 2 weeks after HUCB-MCs infusion to ICH rats and BrdU+ cells were localized by immunohistochemistry in the injured area of brain. This study shows that the most appropriate time for BrdU labeling HUCB-MCs is 24 h and these BrdU+ cells have high efficacy and stability in cell therapy.

Key words: Bromodeoxyuridine (BrdU), cell labeling, human umbilical cord blood derived mononuclear cells (HUCB-MCs), optimal incubating time.

INTRODUCTION

Umbilical cord blood (UCB) is a prominent source of non-embryonic multipotent stem cells. Since it's initial clinical use in 1989 UCB, which is a rich source of hematopoietic stem cells (HSCs), has been considered as an exchangeable hematopoietic alternative to bone marrow and peripheral blood HCSs (Sirchia and Rebulla, 1999). It has been shown that UCB stem cells have the ability to regenerate numerous tissue types, and when transplanted into animals and humans, have produced measurable functional enhancement (Harris et al., 2008; Sanchez-Ramos et al., 2001).

HUCB-MCs appear to be unique in their ability to undergo pluripotential differentiation. Other tissue-derived stem cells have limited self-renewing capacity and are unable to regenerate a whole organ system (Hill et al.,2006; Seaberg and van der Kooy, 2002; Seaberg et al., 2004; Toma et al., 2001; Tropepe et al., 2000; Yoon

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