Effect of β-estradiol on myelination of neurons by oligodendrocytes in co-culture: a new long term in vitro model of central myelination

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INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), the cause of MS is not clear, but its pathology consists of immune infiltration into the CNS, inflammation, demyelination and final axonal degeneration. The development of therapies to promote remyelination in MS is a key research aim, to both restore and maintain electrical impulse conduction in nerves and provide neuroprotection, reducing disability in patients. Currently, most researchers use in vitro models of developmental myelination and in vivo models of remyelination. In vitro systems culturing oligodendrocyte precursor cells (OPCs) with CNS or peripheral nervous system neurons are relatively simple, not relevant to physiology and not adapted to high-throughput screening, and testing a remyelination therapy in the many and various in vivo models of MS is expensive in terms of time, animals and money. Thus, a relevant and robust system mimicking the physiological aspect of the central myelination is needed to find new potential treatments. Myelination in the CNS involves sequential developmental processes in which OPCs migrate, proliferate, and differentiate into newly formed oligodendrocytes, after which those oligodendrocytes (OL) selected by target-dependent neuronal mechanisms wrap myelin membrane around the axons to form the sheaths. Each oligodendrocyte can myelinate many axons, with the number of wraps proportional to the axon segment and regulated tightly by reciprocal signaling between oligodendrocyte and axons (see figure below).

To be relevant, a model of CNS myelination has to reproduce chronologically different steps of the process. Here, we developed a new and reproducible in vitro myelination model based on primary cocultures of central neurons and OL cultured in 96-well plate and then adapted to high throughput screening. We also have developed an automated system of quantifying myelination, in order to use this model as a fast and objective screen.

MATERIALS & METHODS

CENTRAL NEURONS (CN) / OLIGODENDROCYTES (OL) CO-CULTURE: Oligodendrocytes were cultured as previously described by Charles et al., 2000. Forebrain were removed from 17-day-old wild rat isolutes (Jaxier Lab), dissociated by trypsinization (trypsin EDTA, 0.05%) for 20 min at 37°C. The reaction was stopped by addition of DMEM containing 10% of fetal bovine serum (FBS) in the presence of DNase I (20 μg/ml). The suspension was trituated with a 10 μl pipette. Cells are then centrifuged at 180 g for 10 min at 4°C on a layer of BSA (3%) in 1.5 ml tubes. The supernatant is discarded and the cells of pellet are resuspended in neurobasal (Gibco) containing 2% of B27 (Gibco), 2% of penicillin-streptomycin (Pan), 1% of L-glutamine, 1% of FBS and 10 ng/ml PDGF-AA (Pan). Viable cells were counted in a Neubauer cytophotometer using the trypan blue exclusion test (Sigma) and seeded on the basis of 3,000 cells per well in 96 well plates (Greiner-traslated laminin (Sigma). The plates were maintained at 37°C in an humidified incubator, in an atmosphere of air (95%)CO2 (5%). The cultures were maintained in standard neurobasal medium for 7 days. On day 7, the cultures were fed with standard neuronal medium with 50 mM β-estradiol (Sigma).

RESULTS

Kinetic curves of myelination process: A decrease of natural precursors (NG2 staining) was observed in the 6 first days of the culture corresponding to their differentiation into OPC and glial cells. A large proliferation of OPC (A2B5 and O4 markers) was observed (between day 10 to 14) and their migration to neurons enhancing their differentiation into OL (MAG) and myelinating OL (MBP). Kinetics of myelin formation was assessed showing the compact myelin formation (MBP staining) around neuron axons (see picture). Immature and mature neuron of integrity was studied using respectively microtubule-associated-protein 2 (MAP-2) and neurofilament (NF) staining. The overlapping between MAG and MAP-2 staining was observed on the mature myelinating OL endrehealing around the axons.

CONCLUSIONS

The goals of the research reported here were multiple, first to establish a robust and relevant myelination system allowing chronologically following the process of myelination of central neurons and then better understanding it (in term of protein expressions and timing of expression). Second to test in this model, the effect of β-estradiol known to be active on OPC proliferation and sheet formation (Martino-Husstege et al., 2004) in order to validate our model.

REFERENCES