Characterization of the SIM-A9 cell line as a novel microglia activation model to screen potential therapeutics for Neuropathic pain

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Purpose: Resident microglia of the central nervous system are being increasingly recognised as key players in diseases such as neuropathic pain. Biochemical and behavioural studies in neuropathic pain rodent models provide compelling evidence that ATP-mediated P2X4R- brain-derived neurotrophic factor (BDNF) signalling in activated microglia is a critical signalling pathway in the pathogenesis of neuropathic pain caused by peripheral nerve injury (PNI). Therefore, knockdown of BDNF expression in microglia using molecules like siRNA can be a promising strategy for neuropathic pain therapy. A robust, reliable, and validated in vitro model system simulating in vivo pathogenesis of pain hypersensitivity enables high-throughput efficacy screening of therapeutics for neuropathic pain. Though reliable, in vitro experimentation utilizing primary microglia has challenges associated with time-consuming and expensive isolation methods, inadequate yield, purity, and intra-subject variability. On the other hand, cell lines transformed by viral induction might alter the microglial phenotype and thus possess only short term microglial properties. A novel, spontaneously Immortalized Microglia-A9 (SIM-A9, non-transformed) cell line has shown microglial phenotypes (i.e., Iba1 protein expression) and functional properties (i.e., phagocytosis) similar to cultured primary microglia, but not yet studied for pain-related phenotypes. In order to test whether SIM-A9 is a suitable cell line for screening neuropathic pain therapeutics, we characterized and validated the expression of Iba1 (microglia activation marker), P2X4R, and BDNF proteins in SIM-A9 cell line during their resting (normal) state as well as in the presence of external stimuli.

Methods: The SIM-A9 cell line were cultured with and without external stimuli, adenosine triphosphate (ATP) and/or lipopolysaccharide (LPS) under a variety of experimental conditions i.e., the concentration of stimuli, incubation time, and cell regeneration time. The cytocompatibility of SIM-A9 cells with ATP and LPS were determined using two different assays, ATP assay and MTS assay. Intracellular Iba1, P2X4R, BDNF, and α-tubulin protein expression in the resting and ATP stimulated cells were characterized using western blot and Immunocytochemistry (ICC).

Results: Western blot study for intracellular protein evaluation showed that SIM-A9 cells at resting conditions expressed Iba1, P2X4R, and BDNF (monomer, dimer, and pro-BDNF). LPS at a concentration as low as 2.5ng/mL for 24h showed a significant (p<0.0001, cell viability<80%) reduction in SIM-A9 cell viability. On the other hand, ATP was well tolerated by SIM-A9 cells incubated for 24 h even at a high concentration of 250µM. Western blot and ICC studies consistently showed that cells stimulated with 50µM ATP for 4h showed maximum Iba1, BDNF (14kD) and BDNF dimer (28kD) compared to other tested ATP concentrations and incubation times.

Conclusion: SIM-A9 expressed all microglial phenotypes—i.e. P2X4R, Iba1 and BDNF—involved in the pathogenesis of PNI-induced neuropathic pain. ATP at a safe dose showed a time-dependent increase in Iba1 and BDNF expression without intracellular toxicity. LPS as an external stimulus molecule was found to be toxic and non-specific to SIM-A9 cells. Hence, an ATP activated SIM-A9 cell line model
system can be utilized for screening of neuropathic pain therapeutics, including small molecule as well as macromolecular therapies such as proteins and nucleic acids.

References