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Review

Propentofylline, a CNS glial modulator does not decrease pain in post-herpetic neuralgia patients: In vitro evidence for differential responses in human and rodent microglia and macrophages

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ABSTRACT

There is a growing body of preclinical evidence for the potential involvement of glial cells in neuropathic pain conditions. Several glial-targeted agents are in development for the treatment of pain conditions. Here we report the failure of a glial modulating agent, propentofylline, to decrease pain reported in association with post-herpetic neuralgia. We offer new evidence to help explain why propentofylline failed in patients by describing in vitro functional differences between rodent and human microglia and macrophages. We directly compared the proinflammatory response induced by lipopolysaccharide (LPS) with or without propentofylline using rat postnatal microglia, rat peritoneal macrophages, human fetal microglia, human peripheral macrophages and human immortalized THP-1 cells. We measured tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and nitrite release (as an indicator of nitric oxide (NO)) as downstream indicators. We found that LPS treatment did not induce nitrite in human microglia, macrophages or THP-1 cells; however LPS treatment did induce nitrite release in rat microglia and macrophages. Following LPS exposure, propentofylline blocked TNF-α release in rodent microglia with all the doses tested (1–100 μM), and dose-dependently decreased TNF-α release in rodent macrophages. Propentofylline partially decreased TNF-α (35%) at 100 μM in human microglia, macrophages and THP-1 macrophages. Propentofylline blocked nitrite release from LPS stimulated rat microglia and inhibited nitrite in LPS-stimulated rat macrophages. IL-1β was decreased in LPS-stimulated human microglia following propentofylline at 100 μM. Overall, human microglia were less responsive to LPS stimulation and propentofylline treatment than the other cell types. Our data demonstrate significant functional differences between cell types and species following propentofylline treatment and LPS stimulation. These results may help explain the differential behavioral effects of propentofylline observed between rodent models of pain and the human clinical trial.

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Abbreviations: ATP, adenosine tri-phosphate; BPI-sf, brief pain inventory short form; CNS, central nervous system; GFAP, glial fibrillary acidic protein; GM-CSF, granulocyte macrophage colony stimulating factor; Iba-1, ionized calcium binding adaptor molecule 1; IC50, inhibitory concentration 50; IFN-γ, interferon-gamma; IL-1β, interleukin-one beta; LPS, lipopolysaccharide; M-CSF, monocyte colony stimulating factor; NO, nitric oxide; PBMC, peripheral blood monocyte; PGIC, patient's global impression of change; PHN, post-herpetic neuralgia; PMA, phorbol-12-myristate-13-acetate; S1P1, sphingosine-1-phosphate receptor 1; SF-MPQ, short form of the McGill pain questionnaire; SNRI, serotonin–norepinephrine reuptake inhibitor; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; VAS, visual analog scale.

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Introduction

Over the past decade there has been an exponential increase in research studying the role of glia in many central nervous system (CNS) diseases and syndromes including chronic pain (Fields, 2009; McMahon and Malcangio, 2009). CNS glial cells (microglia, astrocytes, and oligodendrocytes) constitute over 70% of the total cell population in the brain and spinal cord. Once thought of as merely a physical support system for neurons, glia have come under investigation as key neuromodulatory, neurotrophic, and neuroimmune elements in the CNS. Many studies have been directed toward the characterization and elucidation of pathophysiological mechanisms of nerve injury-induced behavioral hypersensitivity in rodent models. The focus of our laboratory has been to identify distinct targets that regulate spinal cord mechanisms underlying neuropathic pain. Since CNS injury can induce hyperalgesia in association with changes in peripheral and CNS glia and immune cells, neuroimmune responses may initiate and maintain chronic pain (DeLeo and Yezierski, 2001; Watkins and Maier, 2003).

Microglia, cells of monocytic origin are the macrophages of the brain and, as such, perform a vast number of immune-related duties and are the first cell type to respond to various CNS injuries (Hickey and Kimura, 1988; Kreutzberg, 1996). Pathological stimuli, such as abnormal signals from nociceptors, provoke a graded transformation of microglia beginning from a highly ramified surveillance state ultimately to a phagocytic macrophage (Austin and Moalem-Taylor, 2010). Microglial "activation" involves a stereotypic pattern of cellular responses, such as proliferation, increased expression of immune molecules, recruitment to the site of injury (migration), and functional changes that include the release of cytotoxic and/or inflammatory mediators (Austin and Moalem-Taylor, 2010). Currently, it is not well understood what is the initial signal of microglial activation, but neuronal depolarization combined with extracellular ion changes after CNS injury may be a contributor (Caggiano, et al., 1996). Alternatively, neuronal signals such as NO, adenosine triphosphate (ATP) or proinflammatory cytokines may provide the stimuli for this activation (Hauser, et al., 1996); (Merrill and Benveniste, 1996).

Our past research evaluated the role of microglia, astrocytes, cytokines, chemokines, innate immunity including toll-like receptor 4 (TLR4) and CD14 and adaptive T-cell immunity in the genesis and maintenance of chronic pain (Cao and DeLeo, 2008; Cao, et al., 2009; Tawfik, et al., 2008). These data together with substantial results from laboratories throughout the world formed the foundation to move forward to clinical trials using the glial modulator, propentofylline. Unexpectedly, this trial demonstrated no efficacy in alleviating chronic pain associated with post-herpetic neuralgia. In light of these findings, the pain scientific community should be cautious about collecting and interpreting data using techniques and animal models that may not translate to patients. It is imperative that we learn from clinical trials and attempt to increase our understanding of the role of glial cells in the context of human pain. In order to enhance the translation from preclinical animal models to patients, we propose to compare rodent and human cellular responses to novel pharmacological approaches as a previous step to clinical trials. However, due to the difficulty of obtaining and/or using human microglial cells, it would be useful to find a cell type that can be easily obtained and used as a surrogate for human microglia. Since microglial cells are defined as the macrophages of the CNS, it is reasonable to hypothesize that peripheral macrophages may be used as a surrogate for microglia.

In part I of this report, we briefly review the design and results of the Solace (SLC022 = propentofylline) clinical trial for the treatment of post-herpetic neuralgia (PHN). In part II, we present in vitro data on the differential responses of human and rodent macrophages and microglia in response to an inflammatory stimulus, LPS in the presence or absence of propentofylline. We chose to measure IL-1β, TNF-α and NO release as potential proalgesic mediators. TNF-α is a proinflammatory cytokine that is associated with nerve injury-induced behavioral hypersensitivity. Application of exogenous TNF-α induces thermal hyperalgesia and mechanical allodynia in rodents, and blocking TNF-α is anti-allodynic (Homma, et al., 2002; Murata, et al., 2006; Sorkin, et al., 1997). The proinflammatory cytokine IL-1β was increased in the CNS following peripheral nerve injury. In rodent models of neuropathy, blockade of IL-1β decreased behavioral hypersensitivity (Kiguchi, et al., 2010; Sweitzer et al., 2001a, 2001b). Increased NO release has been shown to be involved in the development of hypersensitivity during inflammatory and peripheral nerve injury conditions. The inhibition of NO expression alleviated animal hypersensitivity in a rodent model of neuropathic pain (De Alba, et al., 2006; Lam, et al., 1996). These data reported herein provide important, novel information on potential species and cellular differences in response to an inflammatory stimulus in the presence or absence of propentofylline, a glial modulator. In addition, the results from these studies may help direct future glial-pain investigations and improve on potential clinical translation.

Part I

Methods and results for part I clinical trial

The title of the proof-of-concept clinical trial was: "A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study Evaluating the Efficacy and Tolerability of Oral SLC022 300 mg TID, a Glial Cell Modulating Agent, Versus Placebo in the Treatment of Post Herpetic Neuralgia," Protocol SLC022/201, EudraCT number 2008-002108-24, US IND number: 104, 119, registered on ClinicalTrials.gov. Efficacy evaluation was based on: 1) daily pain rating via electronic diary; 2) Visual Analog Scale (VAS) of pain intensity from the short form of the McGill Pain Questionnaire (SF-MPQ); 3) selected questions from the Brief Pain Inventory Short Form (BPI-sf); and 4) Patient's Global Impression of Change (PGIC). Inclusion criteria were the following: male or female age 18 or older; history of cutaneous herpes zoster infection with sustained pain associated with the site of the skin rash for >6 months after onset of the skin rash; pain intensity score of a ≥4 on a 11 point numeric scale for at least 5 of the 7 days immediately prior to randomization; mean pain intensity of ≥4 on a 11-point numeric rating scale during Baseline Phase; completion of at least 5 out of 7 daily pain reports during Baseline Phase. Patients