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Research Report

Galectin-1 suppresses methamphetamine induced neuroinflammation in human brain microvascular endothelial cells: Neuroprotective role in maintaining blood brain barrier integrity

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ABSTRACT

Methamphetamine (Meth) abuse can lead to the breakdown of the blood–brain barrier (BBB) integrity leading to compromised CNS function. The role of Galectins in the angiogenesis process in tumor-associated endothelial cells (EC) is well established; however no data are available on the expression of Galectins in normal human brain microvascular endothelial cells and their potential role in maintaining BBB integrity. We evaluated the basal gene/protein expression levels of Galectin-1, -3 and -9 in normal primary human brain microvascular endothelial cells (BMVEC) that constitute the BBB and examined whether Meth altered Galectin expression in these cells, and if Galectin-1 treatment impacted the integrity of an in-vitro BBB. Our results showed that BMVEC expressed significantly higher levels of Galectin-1 as compared to Galectin-3 and -9. Meth treatment increased Galectin-1 expression in BMVEC. Meth induced decrease in TJ proteins ZO-1, Claudin-3 and adhesion molecule ICAM-1 was reversed by Galectin-1. Our data suggests that Galectin-1 is involved in BBB remodeling and can increase levels of TJ proteins ZO-1 and Claudin-3 and adhesion molecule ICAM-1 which helps maintain BBB tightness thus playing a neuroprotective role. Galectin-1 is thus an important regulator of immune balance from neurodegeneration to neuroprotection, which makes it an important therapeutic agent/target in the treatment of drug addiction and other neurological conditions.

1. Introduction

Galectins are a family of β-galactoside-binding lectins that regulate a variety of biological functions and modulate immune response under various pathophysiological conditions. Expression of Galectins is significantly increased under neuroinflammatory conditions and neuroinflammation contributes to the pathogenesis of several neurological diseases. 3 members of the

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Galectin family namely Galectin-1, -3 and -9 in particular are believed to be involved in the neuromodulation via cytokine production contributing to CNS pathology and/or neuropreservation.

Galectin-1 regulates microglial activation that targets the activation of p38MAPK-, CREB-, and NF-κB-dependent signaling pathways and suppresses downstream proinflammatory mediators, such as iNOS, TNF-α, thereby deactivating microglia and restrict brain inflammation and neurodegeneration and therefore has important therapeutic implications in neuroinflammation (Starossom et al., 2012; Nonaka and Fukuda, 2012). Similar, neuroprotective properties of Galectin-1 were demonstrated in astrocytes via the production of brain-derived neurotrophic factor (BDNF) which is known to promote neuronal survival and hence brain function and the inhibition of glutamate toxicity via modulation of NMDA receptor expression (Endo, 2005; Sasaki et al., 2004; Lekishvili et al., 2006).

Galectin-3 expression is significantly enhanced in IFN-γ-stimulated glia and produced high levels of proinflammatory mediators via the activation of the JAK–STAT pathway. Galectin-3 presence in the brain could thus be indicative of neuropathology in the CNS. (Jeon et al., 2010)

Under basal conditions astrocytes did not express Galectin-9 however IL-1β, IFN-γ, and TNF-α via the activation of Galectin-9 modulate the neuroinflammatory processes and contribute to CNS pathology. Galectin-9 functions as an astrocyte-microglia communication signal and promote cytokine production from microglia (Steelman et al., 2013). Galectin-9 is induced in astrocytes by TNF-α via the JNK/c-Jun pathway and astrocyte-derived Galectin-9 functions as an immunoregulatory protein in response to ongoing neuroinflammation (Steelman and Li, 2014). Astrocytes produce Galectin-9 in response to the stimulation with IL-1β, which contributes to neuroinflammation in the CNS (Yoshida et al., 2001).

Previously, we have shown that addictive drugs such as opiates like morphine and Meth increased the expression of Galectin-1 in human monocytes-derived macrophages (MDM) and exogenous Galectin-1 and morphine potentiated HIV-1 infection of MDM (Reynolds et al., 2012a, 2012b). These studies demonstrated that Galectin-1 was an important mediator involved in immune-mediated responses observed in HIV-1 infected drug abusing subjects and that Galectin-1 could be included as a therapeutic target in combination therapy for HIV-1 infected drug abusers. Targeting Galectin-1 could decrease cell adhesion between viral proteins and host macrophages thereby decreasing HIV-1 infectivity of the macrophages in HIV-1 infected drug abusers.

Several studies report the expression of Galectins in tumor-associated endothelial cells (EC) and have implicated Galectins in angiogenesis process; however no data are available on the expression of Galectins in normal human brain microvascular endothelial cells. (D'Haene et al., 2014). Based on previous reports Galectin-1, -3 and -9 play an important role in the CNS, however their expression levels, cellular localization and role in human central nervous system tissue is not well defined. Galectins may modulate cell adhesion by inhibiting or enhancing adhesive potential between cells or between cells and the extracellular matrix (Wada and Makino, 2001). The brain microvascular endothelial cells and the astrocytes are the two major cells that constitute the blood brain barrier. Given the important role of Galectins on cell adhesion, migration, polarity, and chemotaxis, it is likely that modulation of Galectin levels in the brain microvascular endothelial cells that constitute the blood brain barrier (BBB) could affect the integrity of the blood brain barrier and consequently contribute to neuroinflammation. Further, we have shown that the Galectin expression levels may be modulated by viral infections and inflammatory agents such as drugs of abuse but whether they directly or indirectly alter BBB permeability is not known (Reynolds et al., 2012a, 2012b).

We have previously shown that Meth treatment alter BBB permeability via the release of pro-inflammatory cytokines (Mahajan et al., 2008; Reynolds et al., 2011), however a detailed investigation of the effect of Meth on Galectin expression and its role in modulating BBB permeability has not been done. In the current study we first evaluated the basal gene expression levels of Galectin-1, -3 and -9 in normal human brain microvascular endothelial cells (BMVEC). Our results showed that Galectin-1 was highly expressed in BMVEC as compared to Galectin-3 and -9, therefore subsequent studies examined if Meth treatment altered Galectin-1 expression in these cells. A previous study using vascular endothelial cells demonstrated that increased Galectin expression contributed to increased leukocyte traffic through the vascular endothelium (Ishikawa et al., 2004; Imaizumi et al., 2002). Therefore, we examined whether treatment of an in-vitro BBB with Galectins impacted the integrity of the BBB and evaluated if Galectin-1 treatment modulated the expression of tight junction proteins ZO-1 and Claudin-5. Since Galectins play a major role in cell–cell and cell-ECM adhesion, we further examined if Galectin-1 treatment modulated levels of intercellular adhesion molecule-1 (ICAM-1) that could enhance the responsiveness of endothelium thereby

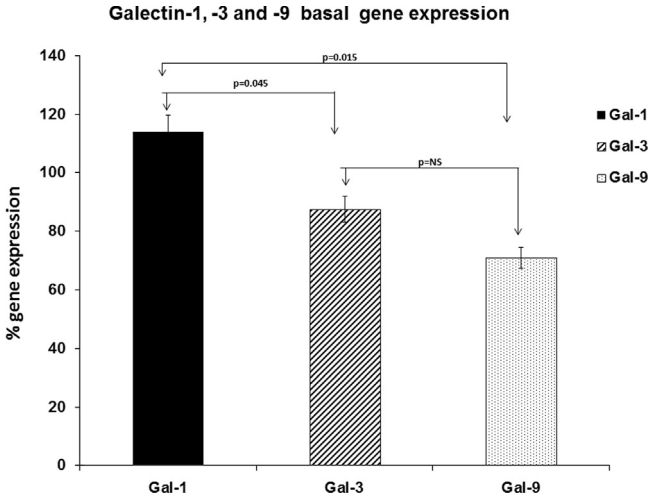

Fig. 1 – Basal galectin expression in BMVEC. Figure shows the basal Galectin-1, 3 and 9 gene expression levels The basal gene expression levels were calculated using the delta delta CT method where Δ Reference Gene vs. Δ Target Gene expression was computed using the formula ΔΔCT = (CT(ref, untreated) − CT(ref,treated)) − CT(target,untreated) − CT (target,treated). Results are expressed as the mean ± SD from n = 3 separate experiments. A p Value of < 0.05 is considered a statistically significant difference.