Characterization of the expression of Basigin and Monocarboxylate Transporters 1 and 4 in aged mouse brains in response to acute and chronic inflammation

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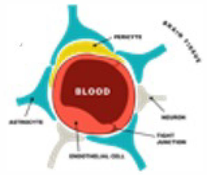
Introduction

The Blood Brain Barrier (BBB) is comprised of blood vessel endothelial cells, astrocytes, and pericytes, which serve to limit the entrance of molecules into the central nervous system (Serlin *et al.*, 2016).



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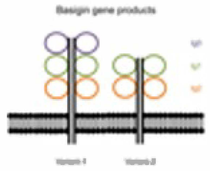
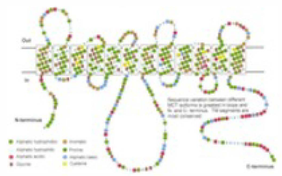


Basigin gene products are Immunoglobulin-like (Ig-like) cell adhesion molecules with extracellular Ig domains, a single-pass transmembrane domain, and a short cytoplasmic tail (Muramatsu, 2016).

Basigin variant-1 is expressed on blood vessel endothelial cells, as well as Müller glial cells of the neural retina and cancerous cells (Muramatsu, 2016). Basigin variant-2 is expressed on photoreceptor neurons of the neural retina (Ochrietor et al., 2003)

Monocarboxylate transporters (MCTs) are a family of membrane-associated transport proteins that move monocarboxylates like pyruvate, lactate, and ketone bodies across the membrane via facilitated diffusion (Halestrap and Price, 1999).



Halestrap and Price, 1999

MCT1 is expressed by blood vessel endothelial cells and microglia in rat brains (Gerhart et al., 1997). MCT4 expression increases on monocytes in response to chronic inflammation (Sun et al., 2020). Both are known to interact with Basigin gene products in the mouse neural retina (Ochrietor and Linser, 2004).

Inflammation, an immune response to harmful stimuli, occurs in two stages. The acute inflammatory response is activated immediately after the harmful stimulus is presented. Chronic inflammation is prolonged activation of the immune system and is a hallmark of many diseases. Specifically, chronic inflammation can affect the integrity of the BBB, which promotes neuroinflammation like that seen in Parkinson's Disease and Multiple Sclerosis (Van der Goes *et al.*, 1998).

Purpose / Hypothesis

The proteins Basigin variant-2 and MCT1 are expressed on blood vessel endothelial cells and therefore may contribute to the integrity of the BBB. The MCT4 protein increases in expression in immune cells in response to chronic inflammation. Therefore, the purpose of the present study was to quantify the expression of the Basigin, MCT1, and MCT4 in brains isolated from aged (6-month-old) mice after 3, 6, 12, and 24 hours of exposure to lipopolysaccharide (LPS). The three hour treatment represents acute inflammation and the 24 hour treatment represents chronic inflammation.

It was hypothesized that the expression of Basigin and MCT1 would decrease and MCT4 would increase in response to chronic inflammation when compared to the saline-treated controls.

Methodology

Animal care and use

All animal procedures were conducted under the approval of the UNF Institutional Animal Care and Use Committee (IACUC). Animals were maintained under standard mouse husbandry conditions with a 12h:12h light/dark cycle and received *ad libitum* water and standard rodent chow. Euthanasia was carried out by asphyxiation using CO_2.

Treatment of tissue

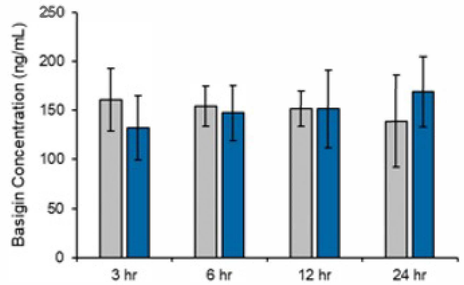
Brains were dissected from mice at postnatal day (PD) 180 (6 months old). The brains were further sectioned into 4 equal parts. Each section was incubated in DMEM with LPS (10 µg/mL, InvivoGen) or a similar volume of Dulbecco's Phosphate Buffered Saline (D-PBS, Gibco) at 37°C with 5% CO_2 for 3, 6, 12, and 24 hours. Three brains were used for each treatment time/condition.

Protein Expression

Proteins were extracted from mouse brains using the TRI Reagent protocol (MRC., Inc.) following the instructions of the manufacturer. The concentration of proteins in each sample was determined using the Bradford Coomassie protocol (Pierce/ThermoScientific) following the instructions of the manufacturer. Expression of Basigin, MCT1, and MCT4 was determined via ELISA analyses using antibodies specific for Basigin (Ochrietor et al., 2003), MCT1 (EMD Millipore) and MCT4 (EMD Millipore), respectively. Total protein (100 µg/mL) isolated from mouse tissue was analyzed in triplicate (Basigin) or duplicate (MCT1/MCT4). An alkaline phosphatase detection system was used, and the absorbance was measured at 405 nm using a spectrophotometer. For Basigin expression, a standard curve was used to determine Basigin protein concentration. For MCT1 and MCT4, the data were plotted as absorbance, which directly correlates to the respective protein expression. The error bars represent the standard error.

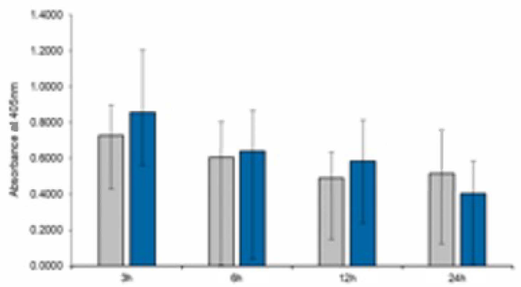
Results

No significant difference in Basigin expression was observed in aged mouse brains exposed to LPS



ELISA analyses using total isolated protein from mouse brains at PD 180 were performed for Basigin. A standard curve was generated to determine Basigin concentration in each sample. The average of the LPS-treated (blue) and D-PBS-treated (gray) tissues at treatment times of 3 hr, 6 hr, 12 hr, and 24 hr were plotted. The error bars represent the standard error. No significant difference in Basigin expression was observed over time in the D-PBS treated samples or when comparing the LPS-treated samples to the D-PBS-treated samples at any treatment time.

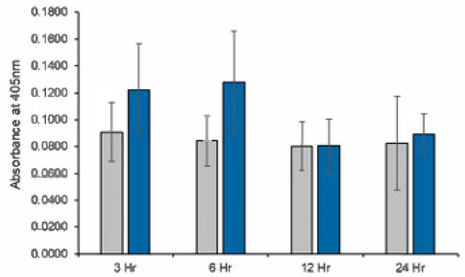
No significant difference in MCT1 expression was observed in aged mouse brains exposed to LPS



ELISA analyses using total isolated protein from mouse brains at PD 180 were performed for MCT1. The average of the LPS-treated (blue) and D-PBS-treated (gray) tissues at treatment times of 3 hr, 6 hr, 12 hr, and 24 hr were plotted. The error bars represent the standard error. No significant difference in MCT1 expression was observed over time in the D-PBS treated samples or when comparing the LPS-treated samples to the D-PBS-treated samples at any treatment time.

Results

No significant difference in MCT4 expression was observed in aged mouse brains exposed to LPS



ELISA analyses using total isolated protein from mouse brains at PD 180 were performed for MCT4. The average of the LPS-treated (blue) and D-PBS-treated (gray) tissues at treatment times of 3 hr, 6 hr, 12 hr, and 24 hr were plotted. The error bars represent the standard error. No significant difference in MCT4 expression was observed over time in the D-PBS treated samples or when comparing the LPS-treated samples to the D-PBS-treated samples at any treatment time.

Conclusions and Comments

The hypothesis was not supported. Regulation of Basigin, MCT1 and MCT4 in aged mouse brains does not significantly change when exposed to LPS for any of the treatment times. These results suggest that Basigin, MCT1, and MCT4 do not play roles in neuroinflammation that occurs through chronic inflammation.

Expression of each target protein appears to remain stable when incubated for as long as 24 hours in culture.

An increase in the expression of MCT4 was observed at 3 hours and 6 hours of treatment with LPS, when compared to the saline-treated controls, but it was not a significant difference. This observation was surprising because an increase was hypothesized to happen in response to the longer treatment times,

Acknowledgements

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