University at Buffalo The State University of New York | REACHING OTHERS

School of Medicine and Biomedical Sciences, University at Buffalo The State University of New York

CURCA Center for Undergraduate Research and Creative Activities, University at Buffalo The State University of New York

The Effects of ATP on Ethanol-Induced Microglial Phagocytosis

Paul Inzerillo, Melissa Dolan, Zakiya Rhodie and Richard A. Rabin, PhD

Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo

Introduction

- Microglia are the primary innate immune cells of the Central Nervous System (CNS)
- They are responsible for a number of functions including defense against pathogens, cellular debris removal (i.e., phagocytosis) and synaptic pruning
- Microglia are normally found in a resting state, but can be activated by invasion of pathogens or presence of cellular debris[1]
- Activation results in a multitude of phenotypic changes, with our research focusing on phagocytosis[2]
- Previously, our laboratory has found that exposure to ethanol leads to an increase in microglial phagocytosis of unopsonized microspheres.
- This suggests phagocytosis involves scavenger receptors
- The objective of this study was to determine the role of the purinergic scavenger receptor in the ethanol-induced increase of phagocytosis utilizing BV2 mouse microglia as a model system

Hypothesis

Ethanol increases microglia phagocytosis via a purinergic receptor

Methods

- BV2 microglia cells were maintained in DMEM containing 10% fetal bovine serum with 100 units/ml of penicillin, 0.1 mg/ml of streptomycin and 250 mg/ml of amphotericin B (i.e., complete media) at 37° C in an atmosphere of 95% air- 5% CO_2.
- For experiments, cells were plated (0.3 x 10^6 cells/plate) onto 35mm tissue culture plates and allowed to attach overnight.
- Phagocytosis was measured by incubating BV2 cells in DMEM containing 30 x 10^6 Fluoresbrite® YGCarboxylate Microspheres (1.0 µm; Polysciences Inc.,Warrington, PA) in the absence and presence of 100mM alcohol and/or 100µM ATP. Prior to this treatment, the BV2 cells were incubated in DMEM alone for 1 hour at 37° C in an atmosphere of 95% air- 5% CO_2.
- Prior to each experiment, the microspheres were washed with phosphate-buffered saline (PBS) and were incubated in a bath sonicator for 10 minutes in PBS containing 1 mg/ml bovine serum albumin (BSA) to reduce non-specific binding. After centrifugation (13,000 xg for 3.5 minutes) the fluorescent microspheres were incubated in a bath sonicator for 10 minutes in DMEM alone (unopsonized).
- After the BV2 cells were incubated for 1 hour at 37° C, plates were placed on ice and the media was removed. The BV2 cells were rinsed 3X with cold PBS and fixed by a 15 minute incubation in 4% paraformaldehyde (PFA) in PBS. After the removal of PFA, the BV2 cells were rinsed 3X in PBS.
- Flow cytometry was carried out using BD Fortessa SORP Flow Cytometer to determine number of cells with microspheres and results were analyzed using FCS Express 6

Results

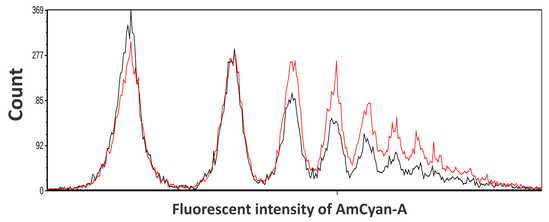

Figure 1. Distribution of fluorescent microsphere uptake

Representative histogram of fluorescent intensity in control (black) and ethanol-treated (red) BV2 cells that have taken up microspheres

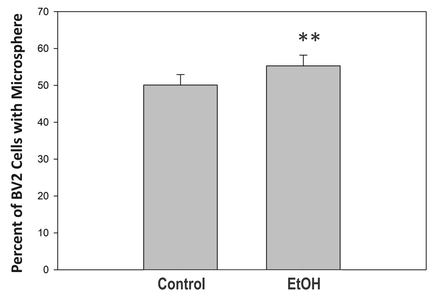

Figure. 2 Ethanol increases phagocytosis of fluorescent microsphere

The presence of 100mM ethanol caused a statistically significant (P=0.004; paired t test; N=8) increase in the percentage of cells that were engaged in phagocytosis

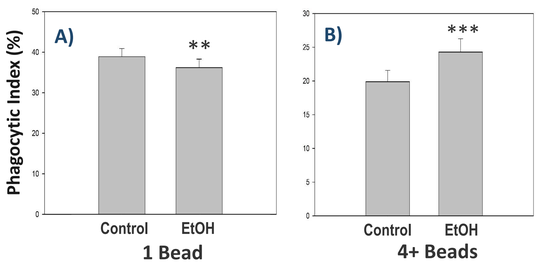

Figure 3. Distribution of Microsphere Uptake In The Presence of Ethanol

Data are expressed as phagocytotic index, which is the percentage of BV2 cells engaged in phagocytosis that have engulfed either 1 (A) or 4+ (B) microspheres, and are plotted as mean ± SEM (N=8) The presence of ethanol caused a statistically significant (P=0.001; paired t test) decrease in the percentage of cells that took up 1 microsphere, but significantly increased the percentage of cells that took up 4 or more microspheres (P<0.001; paired t test)

Results (Cont.)

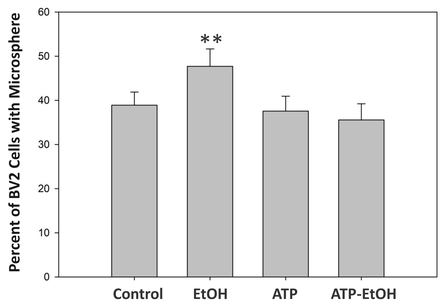

Figure 4. ATP blocks the ethanol-stimulated increase in phagocytosis of fluorescent microsphere

Data are plotted as mean ± SEM (N=11). Repeated measures ANOVA was statistically significant (P=0.004). Post-hoc analysis using Student-Newman-Keuls indicated addition of ethanol alone significantly increased the percentage of BV2 cells engaged in phagocytosis vs. control (P=0.01) and vs. 100mM ethanol + 100µM ATP (P=0.004). Neither ATP nor ethanol + ATP were statistically significant different from control.

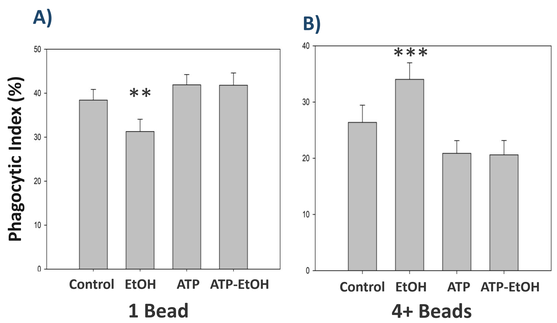

Figure 5. Effects of ethanol and ATP on the distribution of microsphere uptake

5a. Data are plotted as mean ± SEM (N= 11) Repeated measures ANOVA was statistically significant (P=0.001). Post-hoc analysis using Student-Newman-Keuls indicated addition of ethanol alone significantly increased the percentage of BV2 cells that took up 1 microsphere compared to control (P=0.01) or ethanol + ATP (P=0.001). Neither ATP nor ethanol + ATP were statistically significant different from control. **5b.** Data are plotted as mean ± SEM (N= 11) Repeated measures ANOVA was statistically significant (P<0.001). Post-hoc analysis using Student-Newman-Keuls indicated addition of ethanol alone significantly increased the percentage of BV2 cells that took up 4 or more microspheres compared to control (P=0.02) or ethanol + ATP (P<0.001). Neither ATP nor ethanol + ATP were statistically significant different from control.

Conclusions

- Ethanol increased the percentage of BV2 microglial cells engaged in phagocytosis
- Ethanol also altered the uptake process with fewer cells taking up only 1 microsphere and a greater number of cells engaged in uptake of multiple microspheres
- Inclusion of ATP blocked the effects of ethanol on phagocytosis, suggesting the involvement of a purinergic scavenger receptor
- Accordingly, future studies will focus on the role of the purinergic scavenger receptor in the observed effects of ethanol

Acknowledgements

- Center for Undergraduate Research and Creative Activities (CURCA) Funded Project
- Dr. James Olson, PhD, Faculty Member
- Dr. Fraiser Sim, PhD, Faculty Member

References

1. Aloisi, F. (2001). Immune function of microglia. *Glia, 36*(2), 165-179. doi:10.1002/glia.1106
2. Nayak, D., Roth, T. L., & McGavern, D. B. (2014). Microglia Development and function. *Annual Review of Immunology*, *32*, 367–402. http://doi.org/10.1146/annurev-immunol-032713-120240