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The Effects of Alcohol on Microglial Phagocytosis

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Abstract

- Microglia, which are the innate immune cells of the central nervous system (CNS), are involved in normal brain function and have been linked to CNS disorders such as multiple sclerosis and traumatic brain injuries (TBI).
- As a key player in the inflammatory response, microglia remove cellular debris by phagocytosis (i.e., uptake of cellular debris).
- Alcohol is one of the most used and abused drugs in the United States. It affects all organ systems in the body and especially alters functioning of the central nervous system.
- Given the high prevalence of alcohol use and the prominent role of microglia in normal and pathological brain function, the objective of this study was to determine the effects of alcohol on microglial phagocytosis
- BV2 cells, a model microglial cell line, were treated with alcohol and fluorescent microscopy used to measure the uptake of fluorescent beads.
- Alcohol increased phagocytosis of unopsonized microspheres, but did not alter uptake of opsonized microspheres
- The results suggest an effect of alcohol on phagocytosis mediated by scavenger or integrin receptors, but not the complement or Fc receptors
- These studies will help further our understanding of microglial activation and involvement in neurodegenerative disorders and TBIs.

Introduction

- In the U.S., alcohol (ethanol) is one of the most used and abused drugs causing drastic health and societal problems with an annual economic burden of $223.5 billion:

Time Period	Percent of Adults Consuming Alcohol
Lifetime	86.7
Previous Year	70.7
Previous Month	56.4

Table 1. Prevalence of Alcohol in the U.S. In 2013, the National Institute of Alcohol Abuse and Alcoholism (NIAAA) reported the use of alcohol among individuals aged 18 or older.

- The consumption of alcohol also is linked to a higher susceptibility of contracting infection.
- alcohol has been reported to alter microglia function and microglia have been suggested to play an important role in alcohol-induced brain injury and cognitive deficits.[5]
- The innate immune system is the body's first line of defense and can be rapidly activated by pathogen invasion or the presence of cellular debris.
- Microglia are the primary innate immune cells of the Central Nervous System (CNS).
- Normally, microglia exist in a “ramified/resting” state. Upon a pathogen invasion or the presence of neuronal damage, microglia become activated.
- Entering this state of activation allows the microglial cells to perform a variety of functions such as phagocytosis, which is the engulfment of pathogens and cellular debris.
- Microglia carry out phagocytosis through the actions of various receptors.

Hypothesis

Microglial phagocytosis is ***inhibited*** in the presence of alcohol (ethanol).

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 poly-lysine coated 35mm tissue culture plates and allowed to attach overnight.
- To measure phagocytosis, BV2 cells were incubated in complete media containing 30 x 10^6 Fluoresbrite® YGCarboxylate Microspheres (1.0 µm; Polysciences Inc.,Warrington, PA) in the absence and presence of 100mM alcohol. 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.
- Before use, the fluorescent microspheres were washed in phosphate-buffer saline (PBS) and incubated in a bath sonicator for 10 minutes in PBS containing 1 mg/ml bovine serum albumin (BSA) to reduce non-specific binding. The fluorescent microspheres were collected by centrifugation (13,000 xg for 5 minutes) and incubated in a bath sonicator for 10 min in either complete media (opsonized) or DMEM alone (unopsonized).
- After the BV2 cells were incubated for 1 hour at 37° C or 4° 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.
- Bright-field and fluorescent images of the cells were captured using an Olympus IX70 inverted fluorescence microscope (20X objective) equipped with a Olympus mercury bulb and a FITC filter plus a Hamamatsu orca-ER CCD camera. The images were analyzed using Image J software from the National Institute of Health (NIH).
- For each experimental condition, 1-2 plates (presence or absence of alcohol) were used and 6-8 randomly selected fields per plate were captured. For the experiments, 229-520 cells were analyzed per plate.

Results

Effects of 100mM Alcohol on Uptake of Fluorescent Microspheres

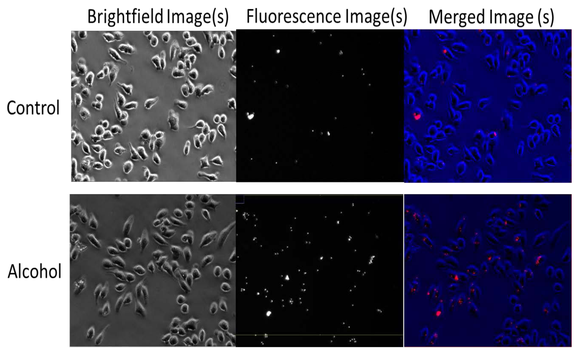

Figure 1 shows representative images of the effects of 100 mM alcohol on uptake of unopsonized fluorescent microspheres by BV2 microglia cells. To enhance contrast, images were pseudo-colored and merged using NIH Image J software.

Results (Cont.)

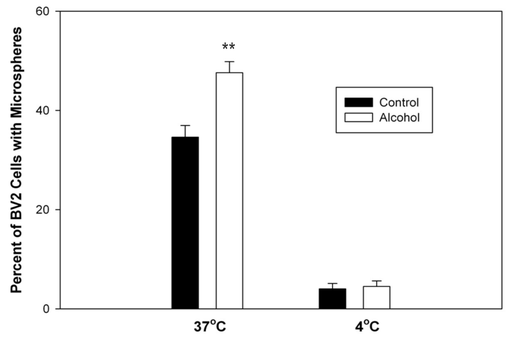

Figure 2. Fluorescence Microspheres Associated with BV2 Cells in the Absence and Presence of 100 mM alcohol.

BV2 cells were incubated with unopsonized microspheres at 4°C and 37°C in the presence and absence of 100 mM alcohol. Because phagocytosis does not occur at 4°C, microspheres associated with the cells at this temperature was due to non-specific binding. Data are expressed as mean ± SEM (N=7-8). **: P = 0.003 (Student’s t test) Alcohol caused a statistically significance increase the percentage of BV2 cell that were engaged in phagocytosis of the unopsonized microspheres. Alcohol did not alter non-specific binding of the microspheres.

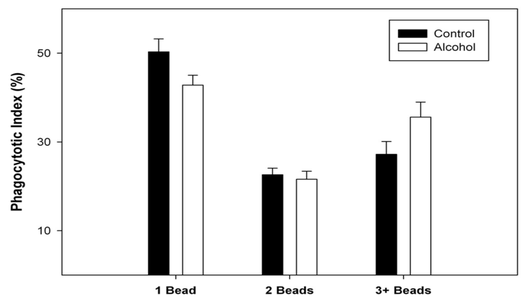

Figure 3. Distribution of Microsphere Uptake

The number of unopsonized microspheres per BV2 cell was determined at 37oC in the absence and presence of 100 mM alcohol. Data are expressed as phagocytotic index which is the percentage of BV2 cells engaged in phagocytosis that have engulfed 1, 2, or 3+ microspheres. Data are plotted as mean ± SEM (N=7-8).
Analysis of the data by 2-way repeated measure ANOVA indicated no statistically significant difference in the phagocytotic index between the control and alcohol-treated cells.

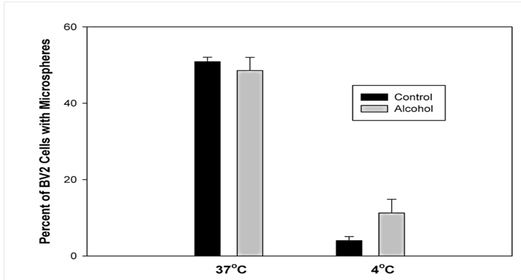

Figure 4. Fluorescence Opsonized Microspheres associated with BV2 Cells Measured at 37°C and 4°C in the Presence and Absence of Alcohol

The data are plotted as mean + SEM (N=4).
There was no statistically significance difference between the percent uptake in the control and alcohol-treated cells. Similarly, alcohol had no statistically significance on the non-specific binding of the opsonized microspheres at 4°C.

There was no statistically significant difference in the phagocytotic index of opsonized microspheres between the control and alcohol-treated cells. (Data not shown)

Conclusions

- The present data demonstrates that alcohol stimulates microglial phagocytosis of unopsonized, but not opsonized microspheres. The results suggest an effect of alcohol on phagocytosis mediated by scavenger or integrin receptors, but not the complement or Fc receptors. Interestingly, a previous report using radiolabeled bacteria (E.coli) rather than fluorescent microspheres found that alcohol inhibited phagocytosis[1]. As phagocytosis may involve a variety of different receptors, it appears that alcohol exerts a receptor-specific action.
- As microglia are involved in numerous processes in the brain (e.g., fighting infection, neurodegeneration, traumatic brain injury, brain development and plasticity), alcohol might exert different effects on each of these processes due to its differential effects on the various phagocytic receptors.
- In the future, we plan to examine the specific mechanism(s) by which alcohol alters the pathways and receptors involved in microglial phagocytosis.

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