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The Effects of Ethanol on Microglia

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Introduction

Traumatic Brain Injury (TBI) is characterized by damage to the brain as a result of an impact or blow to the head. Annually, an estimated 1.7 million people sustain a TBI [1]. Consumption of ethanol is associated with an increased risk of accidents and trauma. For example, in 2011 ethanol was involved in 36% of fatal motor vehicle accidents (US Dept of Transportation). Ethanol is also a contributing factor in TBIs as 35-81% of patients with TBIs were intoxicated and 42% of these TBI patients were heavy drinkers before injury [2]. Although ethanol consumption increases the occurrence of TBIs, the effects of ethanol on the severity and clinical outcome are unclear. While serum alcohol levels in humans were reported to correlate with extend of injury [2], others have reported elevated blood ethanol levels were associated with higher survival in patients with severe TBIs [3][4]. Similarly, animal studies have reported both neuroprotective as well as neurotoxic effects of ethanol in TBIs [5].

Activation of microglia, which are the primary innate immune cells of the brain, is a critical component in the response of the brain to TBIs. Extracellular ATP, which is one of the mediators released upon cellular damage, plays a critical role in regulating microglial cell function. The objective of this study was to determine the effects of ethanol and ATP on microglia viability using the BV2 microglia cell line as a model system.

Methods

Cell Culture and Treatments

BV2 microglia cells were maintained at 37ºC in DMEM containing 10% fetal bovine serum, 100Upenicillin, 100µg/ml streptomycin, and 250 ng/ml amphotericin. For experiments, cell were plated onto 24-well tissue culture plates and the next day treated with phenol-red free DMEM containing 2% fetal bovine serum and either 100 µM ATP, 100 mM ethanol, or a combination of ethanol + ATP. Ethanol exposure was carried out by including ethanol in the media and incubating the tissue culture plates at 37ºC in plastic desiccators containing an atmosphere of 95% air and 5% CO_2 that was saturated with the appropriate concentration of ethanol. Initial studies showed that cell viability was comparable when cells were maintained with 10% or 2% fetal bovine serum.

Tetrazolium Dye (MTT) Assays for BV2 Cells

BV2 cell viability was measured using the colorimetric MTT assay. Viable cells convert the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltretrazolium bromide) to a purple formazan. Briefly, cells were rinsed with phosphate-buffered saline (PBS) and incubated in serum-free, phenol red-free DMEM containing 0.5 mg/ml MTT for 2 hr at 37ºC. At end of the incubation the supernatant was removed and the formazan solubilized in isopropanol/ 0.04N HCl. Absorbance of this solution was measured at 540nm.

Trypan Blue Assay

BV2 cell death was determined by spectrophotometrically measuring uptake of the dye trypan blue which is excluded from viable cells. Briefly, cells were rinsed with PBS and incubated with PBS containing 0.05% trypan blue for 15 min at 37ºC.

Trypan Blue Assay(cont.)

The supernatant was then removed and the cells rinsed twice with ice-cold PBS. Cells were lysed with 1% sodium dodecyl sulfate, and absorbance measured at 590nm. Protein content was measured using the colorimetric Bio-Rad protein dye binding procedure with bovine serum albumin (fraction V) as a standard; absorbance was measured at 590 nm

Statistics

Data were analyze by one-way repeated-measures ANOVA and the Student-Newman-Kuels method. An alpha value of 0.05 was used as the criterion for statistical significance.

Results

Fig1. Concentration Response Relationship of ATP-dependent Decrease in Cell Viability

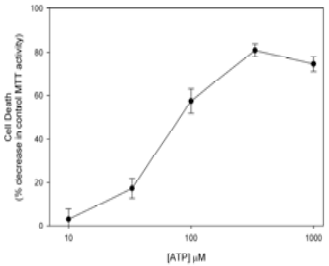

Figure 1. Effects of various concentrations of ATP on BV2 cell viability.
Cells were treated with various ATP concentrations for 24 hours, and cell viability was assessed by measuring MTT activity. A larger % decrease in control MTT activity indicates greater cell death in the samples. Results are expressed as mean ± S.E.M. (N=6). One way RM-ANOVA indicated significant ATP-induced cell death (p<.001) compared to control.

Fig2. Effect of Purinergic Ligands on BV2 Cell Viability

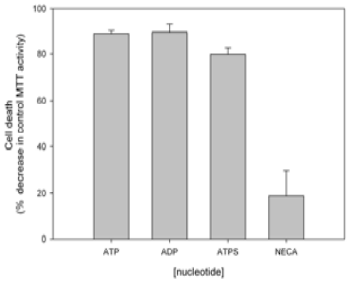

Figure 2. Effects of various purinergic ligands on BV2 cell viability
Cells were treated with 100µM ATP, 100µM ADP, 100µM ATPγS, or 50µM NECA for 24 hours, and cell viability was assessed by measuring MTT activity. Results are expressed as mean ± S.E.M. (N=4-6). ATP, ADP and ATPγS each caused a significant (p<.001) decrease in cell viability.

Fig3. EtOH Does Not Alter ATP-induced decrease in BV2 Cell Viability

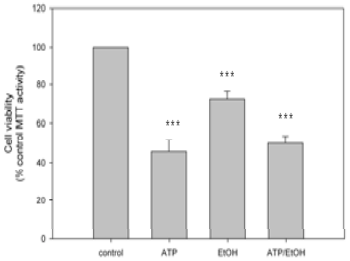

Figure 3. Effects of ethanol on ATP-induced BV2 cell viability.
Cells were treated with 100µM ATP, 100mM ethanol or combination of ATP and ethanol for 24 hours, and cell viability was assessed by measuring MTT activity. Data are expressed as mean ± S.E.M. (N=6). A significant (p<0.001) decrease in cell viability was found in cells exposed to ATP or ethanol. However, inclusion of ethanol did not alter the ATP-induced reduction in BV2 cell viability.

Fig4.Trypan Blue Exclusion Assay Confirms Ethanol Does Not Enhance ATP-Induced Cell Death

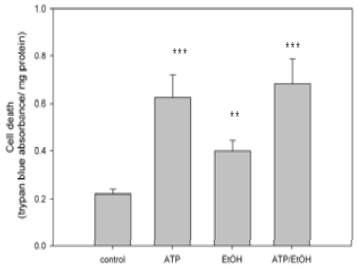

Figure 4. Effects of ethanol on ATP-induced BV2 cell death
Cells were treated with 100µM ATP, 100mM ethanol or combination of ATP and ethanol for 24 hours, and cell death was expressed using the trypan blue exclusion assay. Because viable cells exclude trypan, an increase in dye uptake indicates dead or dying cells. Data are expressed as mean ± S.E.M. (N=8). *** p<0.001, ** p<0.02 compared to control.

Fig5. 6 Hour Exposure to ATP Does Not Alter BV2 Cell Death

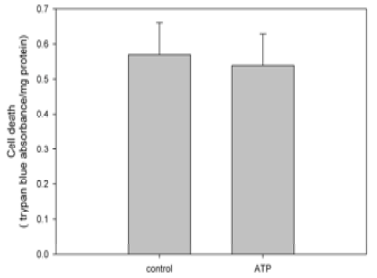

Figure 5. Effects of a 6 hour exposure of ATP on BV2 cells
Cells were treated with 100 µM ATP for 6 hours. Data are expressed as mean ± S.E.M. (N=8). Data revealed that a 6 hour treatment did not significantly alter BV2 cell death.

Discussion

• In BV2 microglia cells ATP caused a concentration-dependent reduction in cell viability. Using two different assays, the present study showed that while ATP or ethanol each increased BV2 cell death, inclusion of ethanol did not significantly alter the lethal effects of ATP.

• A decrease in cell viability was also observed with ADP and ATPγS, but not with the adenosine analogue NECA. This suggests an involvement of the purinergic P2Y receptor, rather than P2X or adenosine receptors. In addition, the need for an exposure of greater than 6 hr for ATP-induced cell death suggests an involvement of apoptosis.

•Future studies will investigate the question of whether ethanol alters other effects in microglia, such as chemotaxis and phagocytosis. From our results, we know that these effects will not be confounded by ethanol's effect on ATP-induced cell death.

References

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