

Alcohol Induced Oxidative Stress leads to Blood Brain Barrier Dysfunction in an iPSC-derived Model

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Background: The NIH states that 6.2% of adults in the U.S. are diagnosed with an alcohol use disorder. Previous animal studies have indicated that ethanol can lead to dysfunction in the blood brain barrier (BBB). The BBB is critical in maintaining homeostasis between the vasculature and the brain parenchyma. The mechanisms underlying alcohol-induced barrier breakdown are relatively unclear. Utilizing a human derived BBB model we investigated the cellular effects of ethanol on several critical barrier properties. We hypothesized that human induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells (BMECs) produced excessive reactive oxygen species (ROS) due to increased ethanol metabolism and ultimately contributes to a leaky BBB.

Designs/Methods: iPSC-derived BMECs were exposed to ethanol (10, 50, 100 mM) for one hour and barrier integrity was monitored with trans-endothelial electrical resistance (TEER) and sodium fluorescein permeability assays. A DCFDA assay kit monitored ROS. To determine the role of ethanol metabolism, BMECs were treated with 4-methylpyrazole (4-MP), an ethanol metabolism inhibitor, and Trolox, a ROS scavenger. Finally, we determined the role of alcohol-induced ROS on barrier integrity in the presence of 4-MP and/or Trolox.

Results: iPSC-derived BMECs exposed to ethanol (50 and 100 mM) had a significant reduction in TEER indicative of an impaired BBB. BMECs exposed to ethanol showed an increase in DCFDA fluorescence indicating an increase in ROS production. When BMECs were treated with 4-MP or Trolox, alcohol-induced ROS production was decreased.

Potential Impact: Recent studies indicate that dysregulation of the BBB could be associated with the pathogenesis of neurological and psychiatric disorders. With such a high prevalence of alcohol consumption in the U.S. a significant portion of the population could be at a higher risk of developing a neurodegenerative disease. Determination of the underlying cellular mechanisms responsible for alcohol-induced barrier damage could potentially contribute to therapeutic interventions.