Acute Alcohol Intoxication Aggravates Brain Injury Caused by Intracerebral Hemorrhage in Rats

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Objective: Alcohol intoxication is associated with worse intracerebral hemorrhage (ICH) outcome, indicating the important role of alcohol in ICH pathogenesis. We intended to investigate the effects of ethanol pretreatment on the severity of ICH-induced brain injury in rats. *Methods:* At 1 hour after intraperitoneal injection of ethanol (3 g/kg), 0.2 U bacterial collagenase was infused into the striatum of male Sprague-Dawley rats to induce ICH. Accumulative mortality rate, body weight changes, and motorsensory and neurological abnormalities were evaluated. The hemorrhagic volume, hematoma expansion, and water content were measured by Drabkin's method, morphometric assay, and dry/wet method, respectively. Blood–brain barrier disruption was assessed using Evans blue assay. Oxidative stress was evaluated by the enzymatic activity of glutathione peroxidase, oxidation of hydroethidine, and the production of malondialdehyde. Cerebral blood flow perfusion volume and hypo-/hyperperfusion neuroimaging were examined by magnetic resonance imaging. *Results:* Ethanol pretreatment aggravates the hematoma hemolysis, hemorrhagic volume, hematoma expansion, brain edema, blood–brain barrier disruption, microglial activation, elevated oxidative stress, and neuroinflammation in the hemorrhagic striatum. The summation effect of these consequences is the major cause of marked neurological impairment and higher mortality rate (64%) in ethanol-pretreated rats with ICH. *Conclusion:* This is a novel model to evaluate the effects of high-dose alcohol administration on experimental ICH rats. *Implications:* The present study may provide clues for making novel strategies in the management of patients with ICH who overconsume alcoholic drinks before the attack. **Key Words:** Alcohol intoxication—blood–brain barrier disruption—brain edema—hematoma hemolysis—neuroinflammation.

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Introduction

Spontaneous intracerebral hemorrhage (ICH), manifesting as bleeding in the brain parenchyma, accounts for approximately 10%-15% of all cerebral strokes[1] and is associated with high morbidity and mortality.[2] Heavy alcohol drinkers have higher risk of ICH strokes.[3,4] Excessive drinking in less than 24 hours increases the risk of both ischemic and hemorrhagic strokes.[5,6] However, the relation between alcohol consumption and ischemic stroke shows a curvilinear pattern, suggesting protective effects of low to moderate alcohol consumption.[7] Acute administration of low-dosage ethanol (1.5 g/kg) exhibited neuroprotective effects in rats with transient cerebral ischemia.[8]

After ICH, hematoma causes mechanical destruction of brain tissues. The hematoma and perihematomal injury also contribute to brain edema, blood–brain barrier (BBB) disruption,[9-12] and neuronal death.[13,14] Hemoglobin and its metabolites also cause brain edema,[15] and BBB can be disrupted via elevated oxidative stress-induced matrix metalloproteinases (MMPs).[16] During neuroinflammatory response after ICH, microglia are activated and release various molecules including proinflammatory cytokines and reactive oxygen species (ROS) to cause neuronal damages.[17,18]

Metabolism of ethanol in neurons can lead to the production of ROS and nitric oxide.[19,20] Ethanol causes osmotic hemolysis of erythrocytes and the release of hemoglobin in vitro.[21] Hemoglobin and its degradation products have been reported to elicit oxidative- and neuroinflammatory damages to the brain.[15,16]

Because of the above pieces of evidence, we hypothesized high serum ethanol concentration should aggravate brain injury after ICH. In the present study, we examined the acute effect of ethanol administration on experimental striatal ICH injury in rats.

Materials and Methods

Animals and Grouping

All experimental protocols were approved by the Animal Care and Use Committee of the Tzu Chi University (Approval no. 101-34), Taiwan, in accordance with guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed under a 12-hour light/dark cycle with free access to food and water.

Totally 129 male Sprague-Dawley rats were randomly assigned into 7 groups: (1) saline pretreatment with sham ICH (saline + sham ICH, n = 6); (2) ethanol pretreatment with sham ICH, 1 day (ethanol + sham ICH, n = 6); (3) ethanol pretreatment with sham ICH, 3 days (ethanol + sham ICH, n = 6); (4) saline pretreatment with ICH, 1 day (saline + ICH, n = 38); (5) saline pretreatment with ICH, 3 days (saline + ICH, n = 10); (6) ethanol pretreatment with ICH, 1 day (ethanol + ICH, n = 39); and (7) ethanol pretreatment with ICH, 3 days (ethanol + ICH, n = 24). Because of the higher mortality of the ethanol + ICH-treated rats, the number of rats used in groups 6 and 7 varied in some experiments. For instance, as shown in Figure 1, A, although 14 rats were used initially, only 5 rats survived in the ethanol + ICH group. As the effect of ethanol pretreatment on ICH outcome was so drastic, we did not make up to 6 animals to obtain other data in the following experiments (i.e., Fig 1, B-D) using this group.

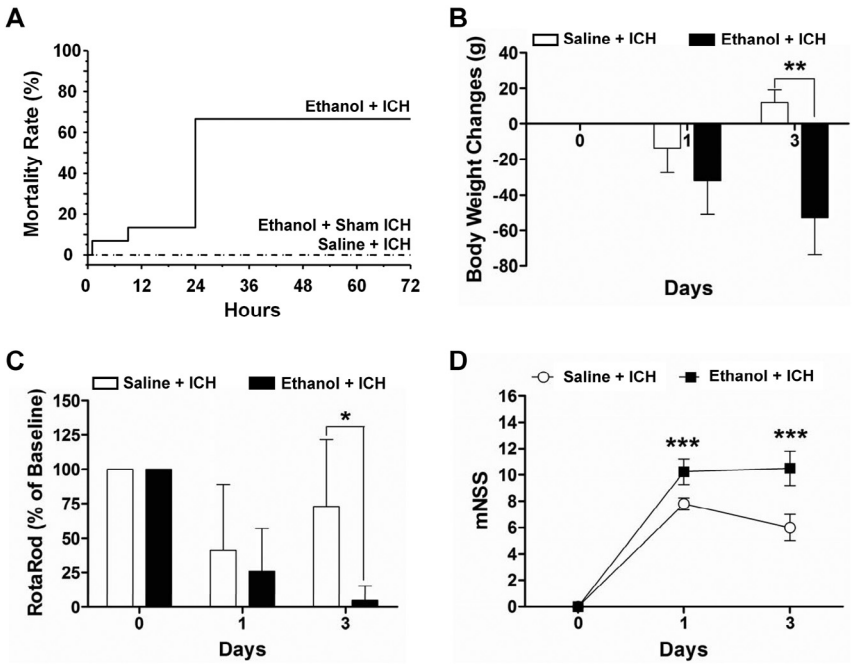

Figure 1. *Effects of ethanol pretreatment on ICH outcomes: (A) accumulated mortality rates after the striatal ICH, (B) body weight changes, (C) motor functions evaluated by RotaRod (Technical & Scientific Equipment GmbH, Bad Homburg, Germany), and (D) neurological functions evaluated by mNSS. Data are presented as mean ± SD. *P < .05, **P < .01, and ***P < .001 versus saline + ICH (n = 6 for both saline + ICH and ethanol + sham ICH groups, n = 14 for the ethanol + ICH group). Abbreviations: ICH, intracerebral hemorrhage; mNSS, modified Neurological Severity Score; SD, standard deviation.*