

Yings and Yangs of Acute Ethanol Intoxication in Experimental Traumatic Brain Injury

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Abstract: Although the deleterious effects of acute alcohol intoxication on traumatic brain injury (TBI) are well known, neuroprotective features of lower doses of ethanol (EtOH) before head trauma have been reported during recent years. Inhibition of N-methyl-D-aspartate receptor (NMDA)-mediated excitotoxicity by lower doses of EtOH has been believed to be responsible for this protection. The aim of this study was to show the neuroprotective effects of low and moderate doses of EtOH and to compare their efficacy in each group. Acute EtOH intoxication at low and moderate doses was induced 40 minutes before trauma. Severe TBI was administered in Sprague-Dawley rats using an impact acceleration model. At 24 hours after trauma, all the rats were decapitated and hippocampi were evaluated under light microscopy. According to our results, red neuron formation and vacuolar degeneration in the CA1 and CA3 sectors of the hippocampi were less prominent in the low-dose and moderate-dose EtOH plus trauma groups than in the trauma only group. In addition, edema formation was less prominent in the EtOH plus trauma group. When comparing the low-dose EtOH plus trauma and moderate-dose EtOH plus trauma groups, an almost normal appearance of the hippocampus was noted in the moderate-dose EtOH plus trauma group. EtOH may have a neuroprotective effect when administered at a lower dose, particularly a moderate dose, and this protection may be a result of the inhibition of NMDA receptor-mediated excitotoxicity.

Key Words: ethanol, head injury, traumatic brain injury

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Detrimental effects of acute ethanol (EtOH) intoxication after traumatic brain injury (TBI) have been extensively studied and reviewed by some authors.^{1–10} It has been stated that acute EtOH intoxication is a contributing factor in 30% to 50% of head injuries.¹¹ Diminished motor skills, reaction times, and judgment are the main factors that increase the risk of brain injury.¹² Acute EtOH intoxication at higher doses blunts hemodynamic and respiratory function, which leads to cerebral ischemia.⁴ It increases the permeability of the blood–brain

barrier, thereby exacerbating the formation of cerebral edema.¹³ Furthermore, acute intoxication impairs the function of hemostatic and fibrinolytic cascades, which may contribute to posttraumatic coagulopathy and progressive hemorrhagic intracranial injury.^{14–16}

Some recent in vitro studies have demonstrated the paradoxical effects (“yings and yangs”) of acute EtOH intoxication in TBI, however, and low or moderate doses of EtOH before injury showed neuroprotection.^{17,18} We have previously demonstrated the protective role of moderate doses of EtOH by means of synaptophysin immunoreactivity in hippocampal neurons.¹⁸ This neuroprotective effect of EtOH at lower doses may be a result of the inhibition of N-methyl-D-aspartate (NMDA)-mediated excitotoxicity as stated in the literature.^{2,5,19}

In this study, our aim was to assess the neuroprotective effects of low and moderate doses of EtOH and to compare each in an experimental model of severe head injury. We selected the hippocampus as a classic prediction site for ischemic injury of the selective vulnerability type for histopathologic studies.

MATERIALS AND METHODS

All the procedures were performed according to the accepted standards of the Guide for Care and Use of Laboratory Animals. Thirty-two adult male Sprague-Dawley rats weighing between 260 g and 300 g were used. The rats were randomly divided into 6 groups: a trauma only group (n = 4), a low-dose EtOH (1 mg/kg) only group (n = 5), a low-dose EtOH plus trauma group (n = 7), a moderate-dose EtOH (2.5 mg/kg) only group (n = 7), a moderate-dose EtOH plus trauma group (n = 4), and a sham-operated group (n = 5). The EtOH doses were administered via the intraperitoneal route 40 minutes before trauma.

All surgical procedures were performed under general anesthesia induced by intraperitoneal administration of sodium pentothal (30 mg/kg), with maintenance doses as needed. Rats were endotracheally intubated and mechanically ventilated with room air (Harvard rodent ventilator model 683; Harvard Apparatus, South Natick, MA). To monitor blood pressure and arterial blood gas samples, the tail artery was exposed and cannulated (PE50). In animals belonging to the trauma only, low-dose EtOH plus trauma, and moderate-dose EtOH plus trauma groups, severe TBI was then applied using the impact acceleration model of Marmarou et al,²⁰ the details of which are explained elsewhere.²¹ Briefly, after general anesthesia and endotracheal intubation, the scalp of the animal was shaved,

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a midline incision was performed, and the periosteum covering the vertex was reflected. The metallic disk was fixed to the central portion of the skull vault of the rat between the coronal and lambdoid sutures. The animals were then placed in a prone position on a foam bed with a known spring constant. The injury was then delivered by dropping a weight (450 g) from a predetermined height of 2 m. Rebound impact was prevented by sliding the foam bed from the tube immediately. The animals were mechanically ventilated during the procedure. The levels of pH, pO₂, pCO₂, and HCO₃⁻ in the blood were determined before impact and over a 2-hour period after trauma. Rectal body temperature was maintained at 37°C ± 0.5°C using hot pads. After termination of the procedure, the incision was sutured. The animals were then extubated, return to their normal environment, and allowed access to food and water. Animals were decapitated at 24 hours after trauma after fixation with transcardiac perfusion of 4% phosphate buffer paraformaldehyde under the general anesthesia. The rats in the control group were also decapitated in the same way. The rat whole brains were removed and fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline for 1 week. After fixation, the brains were embedded in paraffin, and microtome subserial sections of 3 μm to 4 μm thickness passing through the dorsal hippocampus were obtained. The dewaxed sections were stained with hematoxylin and eosin for light microscopic examination.

Histopathologic changes on each slide were graded as 0 (no change), 1 (mild changes), 2 (moderate changes), or 3 (severe changes).

RESULTS

According to our results under light microscopy, a normal structural architecture of the hippocampus was seen in animals belonging to the sham group. In the trauma only group, marked vacuolar degeneration (Fig. 1B) and significant edema (see Fig. 1A) were noted. In the CA1/CA3 sector of the hippocampus and dentate gyrus of the low-dose EtOH only group, there were mild changes in terms of vacuolar degeneration and granular cell vacuolar degeneration, respectively. The same alterations were less severe in the rats belonging to the moderate-dose EtOH only group, however (Table 1). In the low-dose EtOH plus trauma group, mild edema was noted in the CA1/CA3 sectors and dentate gyrus of the hippocampus (Fig. 2). Furthermore, mild vacuolar degeneration and eosinophilic infiltration were present. The histopathologic changes were less severe in the low-dose EtOH plus trauma groups than in the trauma only group. The most intriguing finding in our study was that a normal appearance of the hippocampus was noted in the moderate-dose plus EtOH group (Fig. 3) compared with the trauma only group and the low-dose EtOH plus trauma group.

DISCUSSION

The impact of higher doses of alcohol on the brain after TBI, particularly if the blood concentration is greater than 200 mg %, has been previously demonstrated.¹ The body systems affected by EtOH mainly include the hemodynamic and respiratory brainstem control centers⁴; homeostatic and

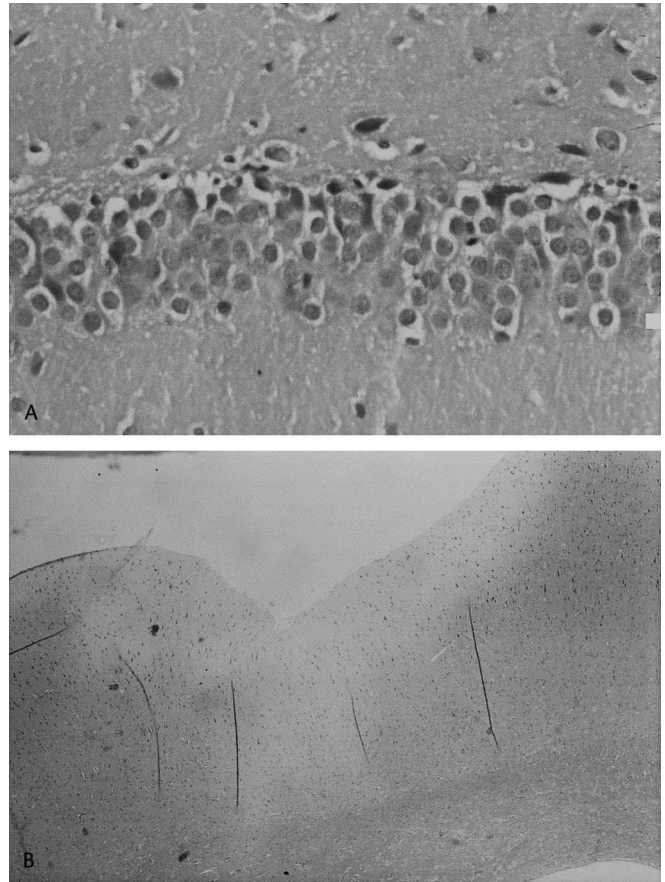


FIGURE 1. Severe vacuolar degeneration (A, original magnification ×400) and prominent edema formation (B, original magnification ×40) are observed in the hippocampi of the trauma only group.

fibrinolytic cascades²²; blood–brain barrier²³; and neuronal membrane receptors, including NMDA, gamma-aminobutyric acid-A (GABA_A), and voltage-dependent calcium channels.² Motor vehicle accident–related injury secondary to acute alcohol intoxication is an endemic problem in many countries and accounts for almost 30% to 50% of TBI.¹¹ Overall, investigations have suggested that alcohol might have a neuroprotective effect when consumed at lower doses, whereas it exerts deleterious effects at higher doses. TBI in combination with higher doses of EtOH results in significant hemodynamic and respiratory depression. Using uninjured anesthetized dogs, McQueen and Posey²⁴ showed that blood alcohol levels 200% above normal resulted in no significant change in intracranial pressure with controlled ventilation but a significant rise occurred with uncontrolled ventilation secondary to decreased ventilatory-derived carbon dioxide retention. In a porcine fluid percussion model of TBI, Zinc et al²⁵ and Zinc and Feustel⁴ showed that intoxicated brain-injured animals had lower cerebral perfusion pressure for up to 3 hours after injury compared with brain-injured control animals and had a longer period of posttraumatic apnea. Marked disruption of the blood–brain barrier, as evidenced by Evans blue extravasation, in acute intoxication at higher doses

TABLE 1. Histopathologic Changes Graded Under Light Microscopy

Groups	Hip.	Hip.	Hip.	Hip.	Dentate
	CA1 VD	CA1 EN	CA3 VD	CA3 EN	Gyrus Gcv
Trauma only	2	2	2	2	1
Trauma only	1	1	2	2	1
Trauma only	2	1	2	1	3
Trauma only	2	1	2	1	3
LDE only	1	1	1	2	2
LDE only	1	0	1	0	1
LDE only	1	2	1	2	1
LDE only	1	1	1	1	1
LDE only	1	1	1	0	1
LDE + trauma	1	2	1	2	2
LDE + trauma	1	2	1	1	1
LDE + trauma	0	2	0	2	2
LDE + trauma	1	1	1	1	1
LDE + trauma	1	2	1	1	1
LDE + trauma	1	1	1	1	1
LDE + trauma	1	1	1	1	1
MDE + trauma	1	1	1	1	0
MDE + trauma	0	1	0	1	0
MDE + trauma	0	1	0	1	0
MDE + trauma	0	0	0	0	0
MDE only	0	1	0	2	1
MDE only	0	0	0	1	1
MDE only	0	2	0	3	1
MDE only	0	1	0	2	1
MDE only	0	1	0	1	1
MDE only	0	2	0	2	1
MDE only	0	2	0	2	1
Sham (n = 5)	0	0	0	0	0

EN indicates eosinophilic neurons; Gcv, granular cell vacuolization; Hip. CA1, Hippocampus CA1 sector; Hip. CA3, hippocampus CA3 sector; LDE, low-dose EtOH; MDE, moderate-dose EtOH; VD, vacuolar degeneration.

in rats and cats receiving a weight-drop cerebral injury has been shown in a number of experimental studies.^{13,26,27} Albin and Bunegin,²³ using a pressure-induced focal ischemia model in dogs, and Shapira et al,⁵ using a weight-drop models in rats, demonstrated a significantly larger lesion size in intoxicated animals compared with nonintoxicated animals. Impaired hemostasis may be one of the most potentially significant risk factors associated with a high mortality rate in the setting of TBI. Higher doses of EtOH cause impairment of platelet aggregation, thereby prolonging bleeding time.²⁸ Changes in the fibrinolytic system secondary to alcohol intoxication, including altered prostacyclin synthesis and thromboxane release, may also hinder hemostasis after TBI.^{20,22,29} Nevertheless, the studies cited previously clearly demonstrate that higher doses leading to acute EtOH intoxication, in combination with TBI, increase mortality, neurologic deficits, lesion size, and brain edema.

Some in vitro studies conducted during the last decade have demonstrated the neuroprotective effects of EtOH at lower doses before TBI.^{6,18,19,30,31} This may largely be a result of the potentiation of GABA_A receptors with simultaneous inhibition

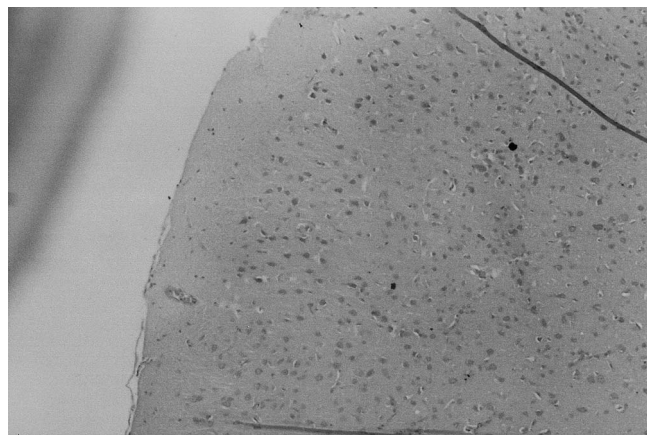


FIGURE 2. Low-dose ethanol plus trauma group: mild edema formation (original magnification ×200).

of NMDA receptors and voltage-activated calcium channels. As measured by lactate dehydrogenase release in embryonic rats by Chandler et al,¹⁹ whole-brain culture showed marked attenuation of NMDA receptor-mediated excitotoxicity by EtOH. The degree of inhibition was concentration dependent, with a 38% reduction seen at EtOH doses of 25 mM and a 96% inhibition recorded at EtOH doses of 200 mM. This reduction seems to be related to a diminished calcium influx through the NMDA receptor-mediated excitotoxicity after TBI.^{30,31} In a cortical contusion model in rats, Kelly et al¹⁷ demonstrated that injured animals receiving low and moderate doses of EtOH had significantly less severe beam-walking impairment compared with no EtOH and high-dose EtOH groups. Additionally, the mean lesion volumes were markedly smaller in animals receiving low and moderate doses of EtOH than in the no EtOH and high-dose EtOH groups. According to the authors, the neuroprotection from a low or moderate dose of EtOH was a result of the inhibition of NMDA receptor-mediated excitotoxicity, whereas the loss of protection and increased mortality observed with high-dose EtOH might be related to EtOH-induced hemodynamic and respiratory depression. In another study, we

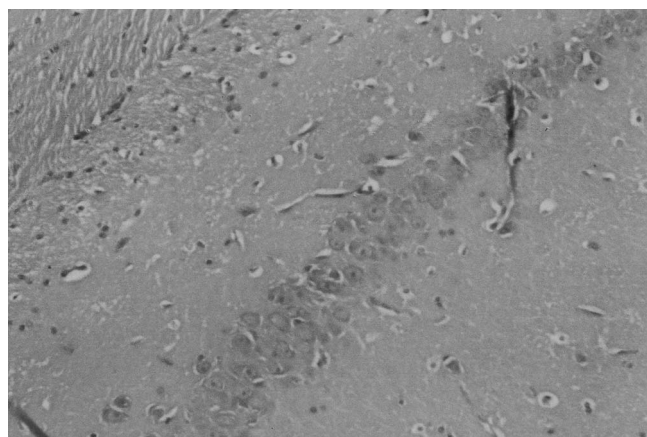


FIGURE 3. Near-normal appearance of the hippocampi in the moderate-dose ethanol plus trauma group (original magnification ×400).

demonstrated the neuroprotective effect of moderate doses of EtOH in a severe traumatic injury model in rats.¹⁸ We used synaptophysin immunostaining to investigate the regenerative process associated with synaptic remodeling. Injured animals receiving a moderate dose of EtOH showed marked synaptophysin immunoreactivity in the stratum oriens and stratum radiatum of the CA1 sector of the hippocampus compared with the no EtOH group. Furthermore, vacuolar degeneration and red neuron formation were less prominent in the pyramidal cell layer of the CA1 and CA3 sectors of the hippocampus in the trauma plus EtOH group than in controls.

It has also been demonstrated that preinjury or early postinjury administration of NMDA-receptor antagonists significantly reduced intracellular calcium accumulation, hyperglycolysis, and lesion size and improved functional recovery in animal models of TBI.^{32–34}

Consistent with the previous studies, our results showed a neuroprotective effect of lower doses of EtOH in a setting of severe TBI. At a moderate dose, EtOH inhibits NMDA receptor-mediated neurotoxicity more effectively than at low-dose EtOH. Formation of eosinophilic neurons and vacuolar degeneration in the hippocampi in the trauma only group were pathologic findings. When these findings are compared with those of the low- or moderate-dose EtOH plus trauma group, fewer prominent pathologic changes are noted. In addition, such pathologic changes were almost absent in the moderate-dose EtOH plus trauma group. Formation of edema and vacuolar degeneration are also significantly decreased when EtOH is administered before TBI.

Overall, studies, including ours, showing the neuroprotective effects of low or moderate doses of EtOH in severe head injury suggest EtOH-induced blockade of NMDA receptor-mediated excitotoxicity. The release of excitatory amino acids, particularly glutamate and aspartate, has been well documented after focal or diffuse TBI in animals.^{12,35,36} These excitatory amino acids may contribute to most of the secondary brain damage after TBI by means of a massive efflux of potassium and influx of calcium by NMDA receptors.^{37,38} Many authors think that the neuroprotection of EtOH at lower doses is a result of inhibition of NMDA receptors, preventing electrolyte derangements at this particular point in time.^{2,5,19} Our results as well as results reported elsewhere demonstrate the potency of EtOH as an NMDA receptor antagonist, and any therapeutic trial of EtOH in TBI would clearly have to take into account these deleterious dose-related sequelae.

CONCLUSION

In conclusion, we suggest a neuroprotective effect of EtOH when it is administered at lower, especially moderate, doses before TBI. This effect of EtOH may primarily be a result of inhibition of NMDA receptors.

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