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PAPER

PATHOLOGY/BIOLOGY

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Alcohol Intoxication May Exacerbate the Effects of Blunt Cranial Trauma Through Changes in Brain Free Magnesium Levels

ABSTRACT: Moderate to high levels of alcohol decrease brain intracellular free magnesium concentration, a factor known to be critical in brain injury. Phosphorus magnetic resonance spectroscopy was used to examine changes to brain free magnesium concentration after blunt cranial trauma in alcohol-intoxicated rats. Rats exposed acutely or chronically to alcohol sufficient to increase blood alcohol levels to between 150 and 350 mg/dL demonstrated a brain free magnesium level that was 20–50% less than in nonintoxicated animals ($p < 0.01$). After injury, brain free magnesium levels declined more rapidly and to a greater extent in alcohol-affected animals than in nonintoxicated control animals ($p < 0.001$). As both preinjury depletion of magnesium and degree of magnesium decline after brain injury have been associated with poor recovery, these findings suggest that moderate to severe alcohol intoxication may predispose the brain to a worse outcome by reducing brain free magnesium levels, both before and after injury.

KEYWORDS: forensic science, alcohol, blunt cerebral trauma, traumatic brain injury, magnesium, intoxication, death

Our understanding of the pathogenesis and manifestations of many aspects of blunt cranial trauma is incomplete. Debate occurs around a wide range of topics including the speed with which brain swelling may develop after impact, the possible significance of the so-called "second impact syndrome," and the relationship of hypoxic brain damage to the development of subdural hemorrhage in infants (1–3). Another issue that arises in forensic practice concerns the effect of high levels of alcohol on the response to cerebral trauma. The effects of alcohol intoxication on outcome after blunt cranial trauma are unclear, with some reports suggesting that a low blood alcohol concentration may be beneficial through attenuation of posttraumatic injury factors (4), while others have suggested that higher alcohol levels may result in a more severe clinical outcome or even death (5–7). The significance of the latter hypothesis is clear; that is, if high levels of alcohol act synergistically with blunt trauma, then a far worse outcome may follow less severe impact. Proposed mechanisms have centered around interference with brainstem cardiorespiratory centers with depressed respiratory drive, impaired hemodynamic response with reduced cerebral perfusion, and elevation of brain and cerebral venous blood lactate levels (5,6,8–11); however, little work has been carried out on possible underlying biochemical changes.

A reduction in free magnesium levels within the brain and a depressed bioenergetic state have been associated with the development of neurological deficits following blunt cranial trauma in animals (12–14). In addition, preinjury depletion of magnesium has been shown to exacerbate injury (15), with attenuation of these electrolyte changes being neuroprotective in terms of motor and cognitive outcome (16). Recent studies have demonstrated that acute doses of alcohol administered to rats will also cause a decrease in brain magnesium and a depressed bioenergetic state (17,18). The similarity of these responses raises the possibility that there may be a relationship between the decline in magnesium following brain trauma and that following alcohol exposure, thus providing a potential mechanism for an exacerbation of injury.

In the following paper, a death owing to blunt cranial trauma is reported to illustrate features of a typical case where high levels of alcohol were considered to have played a role in the lethal outcome. In addition, to further investigate possible underlying mechanisms, a study was undertaken to examine the effects of acute and chronic alcohol exposure on brain magnesium following closed cranial trauma in an animal model. Phosphorus magnetic resonance spectroscopy (MRS) was used to noninvasively measure brain intracellular magnesium levels prior to, and following, brain trauma, with prior acute or chronic exposure to alcohol.

Materials and Methods

A case of lethal head trauma associated with an elevated blood alcohol level was taken from the files of Forensic Science SA, Adelaide, Australia.

Animal studies were conducted according to the National Health and Medical Research Council (Australia) guidelines for the use of laboratory animals in experimental research following approval by the local animal ethics committee.

Experimental Design

Adult male Sprague Dawley rats ($n = 18$; 300–350 g) were fed and watered ad libitum throughout. Animals were randomized to receive either chronic alcohol exposure, acute alcohol exposure, or

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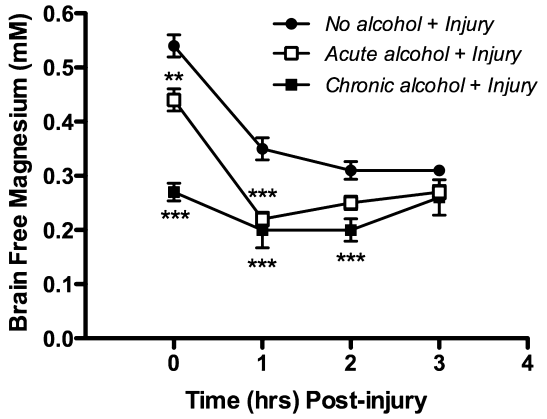

FIG. 1—*Changes in brain free magnesium concentration in the rat brain following acute or chronic (30 day) alcohol exposure and subsequent induction of moderate blunt cranial trauma (2.6–2.8 atmospheres by lateral fluid percussion injury). Data are mean ± SEM.* **p < *0.01 versus no alcohol controls;* ***p < *0.001 versus no alcohol controls.*

no treatment, and then subject to closed cranial trauma using the lateral fluid percussion injury method (19,20). Following injury, animals were monitored for changes in magnesium using phosphorus MRS over a 3-h posttraumatic interval.

Acute Alcohol Exposure

Acute effects of alcohol were examined after administration of three equal volume intraperitoneal injections of 50% ethanol/water aliquots totaling 3 g/kg over 20 min ($n = 6$). Previous studies have demonstrated that this dosage results in a blood alcohol level of approximately 300 g/100 mL (21). In the present study, blood alcohol concentrations in a subgroup of animals ($n = 3$) were measured at 350 ± 16 mg/dL within 15 min of the final injection.

Chronic Alcohol Exposure

Chronic alcohol exposure was induced by the vapor inhalation method (22) as used Previously in our laboratory (18). Briefly, rats ($n = 6$) were housed in clear Plexiglas cages connected by tubing to a conical flask containing an alcohol/water mixture. Air was bubbled through the mixture to the cages over a period of 30 days, with the alcohol concentration in the mixture increasing from 50% for the first 7 days, to 75% for the next 14 days, and finally to 90% for the remaining 9 days. Typically, blood alcohol concentration in these animals was between 150 and 200 mg/dL at the end of the 30-day exposure. Throughout the exposure period, rats were supplied with food and water ad libitum.

Closed Cranial Trauma

Closed cranial trauma was induced using the lateral fluid percussion injury model as described in detail elsewhere (19,20). Briefly, animals were anesthetized with 60 mg/kg intraperitoneal sodium pentobarbital and a femoral venous catheter inserted for continuous infusion of anesthetic (sodium pentobarbital; 8 mg/kg/h). The animals' core temperatures were monitored and maintained at 37.5 ± 0.5°C using a thermostatically heating pad. A sagittal incision (2 cm) was made on the dorsal scalp of the head, and the temporal muscles were reflected before a 5 mm in diameter craniectomy was trephined into the skull centered 3 mm right of the sagittal suture and midway between the bregma and lambda. The dura was kept intact at the opening and a female Leur-lock connection (BD, Sydney NSW, Australia) was secured into the craniectomy with cyanoacrylate adhesive. Once fixed, the animals were placed in a prone position onto a foam block and the Leur-lock connection was filled with isotonic saline. The animals were then attached to the fluid percussion injury device (19) via the Leur-lock connection and moderate trauma induced with a force of 2.6–2.8 atmospheres. After injury, animals were disconnected from the device and manually resuscitated if required until stable respiration had resumed (<5 min). At that time, the Leur-lock connection was removed from the skull.

Phosphorus MRS

All animals ($n = 18$) were monitored by phosphorus MRS prior to and for 3 h following induction of closed cranial trauma. A 9 × 5 mm single tuned surface coil was placed centrally over the craniectomy site and phosphorus MRS spectra were then acquired using an Oxford Instruments 7.0 tesla horizontal bore magnet (Abingdon Oxon, UK) interfaced with a Varian spectrometer console (Agilent Technologies, Santa Clara CA) as previously described (23,24). Acquisition parameters were such that the 90° pulse was centered at a cortical depth of 2 mm, spectral width was 6000 Hz, and repetition rate was 0.7 sec. Peak chemical shifts and integrals were determined using the Varian computer software after applying a routine convolution difference (20/500 Hz) procedure to each acquired spectrum.

Free magnesium concentration was determined from the chemical shift difference between the α and β peaks of ATP as previously described (14,23) using the equation:

$$[\mathrm{Mg}_f] = \mathrm{K}_d \frac{(10.82 - \delta_{\alpha-\beta})}{(\delta_{\alpha-\beta} - 8.35)}$$

where $\delta_{\alpha-\beta}$ is the chemical shift difference between the α and β peaks of ATP. The K_d for MgATP was initially assumed to be 50 μM at pH 7.2 and 0.15 M ionic.

Data Analysis

All data are expressed as mean ± standard error. Significance was determined using repeated measures analysis of variance (ANOVA) followed by Bonferroni correction. A p-value of <0.05 was considered significant.

Results

Illustrative Case

A 39-year-old man was observed by a number of individuals to be intoxicated at a party. He was involved in a fight during which he was observed to have his head hit against furniture and a tiled verandah. His assailant also had grabbed him around the neck during the struggle. He was subsequently noted to be not breathing and attempted resuscitation by ambulance personnel was unsuccessful. At autopsy, there was a 35-mm laceration of the posterior occiput with no underlying skull fractures. Multiple minor bruises and abrasions elsewhere were compatible with the history but were not medically significant. Layer dissection of the neck do not reveal any injuries and there were no facial or conjunctival petechiae to implicate strangulation. Neuropathological evaluation showed minor patchy subarachnoid hemorrhage with small cerebral contusions that were considered by the neuropathologist to be insufficient to

account for death. There were no underlying organic diseases present that could have caused or contributed to death. Toxicological studies showed a blood alcohol level of 0.2%, with therapeutic levels of diazepam and nordiazepam and low levels of tetrahydrocannabinol. Death was, therefore, attributed to blunt cranial trauma associated with an elevated level of blood alcohol. It is recognized that the other drugs that were present may also have played a role in central nervous system depression.

Animal Study

Prior to injury, brain magnesium was 0.54 ± 0.2 mM in untreated animals, 0.44 ± 0.02 mM in acute alcohol-treated animals at 90 min postadministration, and 0.27 ± 0.02 mM in chronic alcohol-treated animals. Both alcohol-treated groups had significantly lower magnesium values than untreated controls ($p < 0.01$). These results are consistent with previously published values (18,25,26). After trauma in untreated animals, magnesium gradually declined to 0.31 ± 0.1 mM over the following 3 h. Following acute alcohol treatment, there was a rapid decline of magnesium to a minimum value of 0.22 ± 0.01 mM by 1 h after trauma, which was significantly less than that observed at the same timepoint in untreated controls ($p < 0.001$). There was also no significant change from this value over the ensuing 2 h. In chronic alcohol-treated rats, magnesium decreased to 0.20 ± 0.02 mM in the first hour, and thereafter, did not change significantly from this value. The magnesium levels in the chronic alcohol-treated rats were significantly less than in alcohol-free, injured controls ($p < 0.001$) (Fig. 1).

Discussion

The reported case demonstrates a situation where death occurred following a witnessed closed head injury with relatively unimpressive findings on formal neuropathological examination. Lethal mechanisms were, therefore, considered to have involved the synergistic interaction of elevated alcohol levels with blunt cranial trauma, as studies have suggested that deaths following blunt cranial trauma in individuals affected by moderate to severe alcohol intoxication may be owing to alcohol augmentation of the effects of concussive brain injury (27). Specifically, these authors reported that individuals intoxicated (with blood ethanol levels ranging from 220 to 330 mg/dL) died after blunt trauma to the face, despite the fact that the injuries were predominantly soft tissue in nature with no skull fractures, intracranial bleeding, or detectible injury to the brain. While the underlying process was unclear, they suggested that resultant posttraumatic apnea was the mechanism of death. Certainly, posttraumatic apnea in brain-injured animals has been reported to be significantly longer if animals are intoxicated (28). Moreover, dilation of pial arterioles in response to hypoxia and hypercapnia is significantly reduced in the presence of alcohol (29) and hypoxia and low doses of alcohol when present together, despite having minimal effects on their own, can act synergistically to produce a significant degree of neuronal injury (30).

In the current study, we have demonstrated that alcohol can also have a deleterious effect on ion metabolism in the brain after blunt cranial trauma, specifically that of magnesium homeostasis. Alcohol exposure caused a depletion of magnesium prior to the induction of blunt cranial trauma, while the trauma itself exacerbated the degree and rate of magnesium decline such that it was significantly greater than in animals not previously exposed to alcohol. Previous studies have shown that decline in magnesium levels after brain injury is associated with the development of neurological deficits (26,31,32) and the fall in levels in untreated animals after trauma to 0.31 ± 0.1 mM over the ensuing 3 h would be in keeping with the development of such deficits (12,26). Moreover, magnesium depletion induced prior to brain injury exacerbates neuronal cell death and the development of neurological deficits (15,33,34), with attenuation of the decline reducing neuronal cell death and improving neurological outcome (35–37). We have shown that alcohol not only causes preinjury depletion of magnesium but also exacerbates the rate and degree of the decline in magnesium levels after trauma. This combination could predispose the brain to a worse outcome, given the adverse effect that magnesium decline could have on a number of cellular processes. For example, a reduction in magnesium levels would facilitate the activity of the N-methyl-D-aspartate channels thus upregulating glutamate-induced excitotoxicity after trauma (38). Although it has been postulated that this effect may relate to alcohol dosage (4), a similar effect has been observed with barbiturates (29). The decline would also have adverse effects on all energy producing and consuming reactions given the essential role magnesium plays in these processes. Thus, bioenergetic state and the ability of the cell to recover from injury would be severely compromised (13). Indeed, reductions in bioenergetic state as a result of magnesium depletion have been associated with increased incidence of cerebrospasm and stroke (39) as well as an increased mortality from head injury and stroke (34). The decline in magnesium levels would also inhibit cellular ion homeostasis, especially that of sodium, potassium, and calcium, given that they all require the activity of magnesium-dependent ATPase. A decline in Na^+/K^+ ATPase activity in particular would promote posttraumatic edema formation (40), which in itself has also been associated with repeated alcohol exposure (41). Whether reduced magnesium concentration together with alcohol exposure act synergistically to promote edema formation is currently unknown. Finally, membrane structure itself may be adversely affected by reduced levels of magnesium (42), including a disruption of blood-brain barrier permeability with consequent neuroinflammation (43).

Our findings demonstrate both an alcohol-induced preinjury depletion of magnesium and an enhanced decline in magnesium levels after brain injury with alcohol exposure. Both of these factors could predispose the injured brain toward a worse outcome than might occur with blunt trauma in isolation. It may be that the adverse effects that elevated alcohol levels have on outcome following blunt head trauma are initiated by a more complex cascade of events involving an interplay of both cellular and hemodynamic processes.

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