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The effects of mannitol and melatonin on MRI findings in an animal model of traumatic brain edema

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Abstract

Objectives : The aim of this study was to compare the effects of mannitol and melatonin on brain edema secondary to trauma using magnetic resonance imaging (MRI).

Methods : A mild traumatic brain injury with the Feeney method was performed upon twelve New Zealand rabbits. Three hours after the trauma was inflicted, MRI images were obtained, then the subjects were divided into two groups : a mannitol group and a melatonin group. The mannitol group (n = 6) was given 2 gr/kg of 20% mannitol IV over 10 minutes and the melatonin group (n = 6) received 100 mg/kg of melatonin IV over 30 minutes. Thirty-three hours after the first MRI, MRI was repeated. The 3-hour and 36-hour post-trauma MRI images in both groups were scored regarding signs of edema and extent of brain tissue protrusion in a blinded fashion by a staff radiologist. Intragroup and intergroup comparisons were made using the Fisher exact test and chi square test. Comparison of brain tissue protrusion measurements was done using the Mann Whitney U test.

Results : Signs of raised intraventricular pressure, contusion and parenchymal edema were more prevelant, and parenchymal protrusion was more prominent on the 36-hour MRI in both mannitol and melatonin groups. No significant difference was found between the melatonin and mannitol groups in any parameter in the MRI images performed 3 and 36 hours after the head trauma.

Conclusions : In this animal model, melatonin and mannitol had similar effects on brain edema, as demonstrated on MRI 3 and 36 hours after head trauma.

Key words : Head trauma ; secondary brain damage ; brain edema ; MRI ; melatonin ; mannitol.

Introduction

Traumatic brain damage is a prominent health problem around the world, with permanent neurologic sequelae in many victims and high medical costs for society (Lee and Newberg, 2005 ; Toyama *et al.*, 2005). Blows to the head cause sudden acceleration, deceleration and/or rotation of the cranial contents. Brain damage after head trauma is classified according to its mechanism and time of development. Primary lesions occuring at the time of impact are commonly intraaxial lesions like acute subdural hematoma, acute epidural hematoma, diffuse axonal damage and intraparenchymal contusion or hemorrhage. Typical secondary lesions are herniation, diffuse brain edema, secondary infarcts and hemorrhages. Because the clinical course is often determined by the magnitude and duration of these secondary lesions, therapy in traumatic brain injury focuses on the prevention and treatment of these problems (Toyama *et al.*, 2005). Expeditious treatment of both primary and secondary problems within the first 48 hours, using close clinical examination and imaging techniques, can minimize the sequelae after brain injury (Lee and Newberg, 2005).

The neuroprotective effects of melatonin in head trauma result from its stimulation of various antioxidative enzymes such as superoxide dysmutase, glutathione peroxides, and glutathione reductase (Maldonado *et al.*, 2007). In previous animal studies, we determined that melatonin decreased the contusion volume in traumatic brain damage (Beni *et al.*, 2004 ; Messenge *et al.*, 1998). This study attempts to compare the effects of mannitol and melatonin-which are widely used to decrease or prevent brain edema- on secondary brain damage by drawing upon the MRI findings.

Methods and materials

The study protocol was approved by the Ethics Committee of our faculty of medicine. Twelve (7 females, 5 males) New Zealand rabbits ranging in size between 2000-2500 gr were divided into two groups : melatonin and mannitol groups. After the subjects were anesthetized with ketamine HCl 50 mg/kg IM and xylasine HCL 15 mg/kg IM, they were subjected to mild traumatic brain injury as described by Feeney *et al.* (Feeney *et al.*, 1981). With the animal in the prone position, the marginal ear veins of rabbits were cannulated under the effect of anesthesia and a craniotomy (1 cm^2) was made in

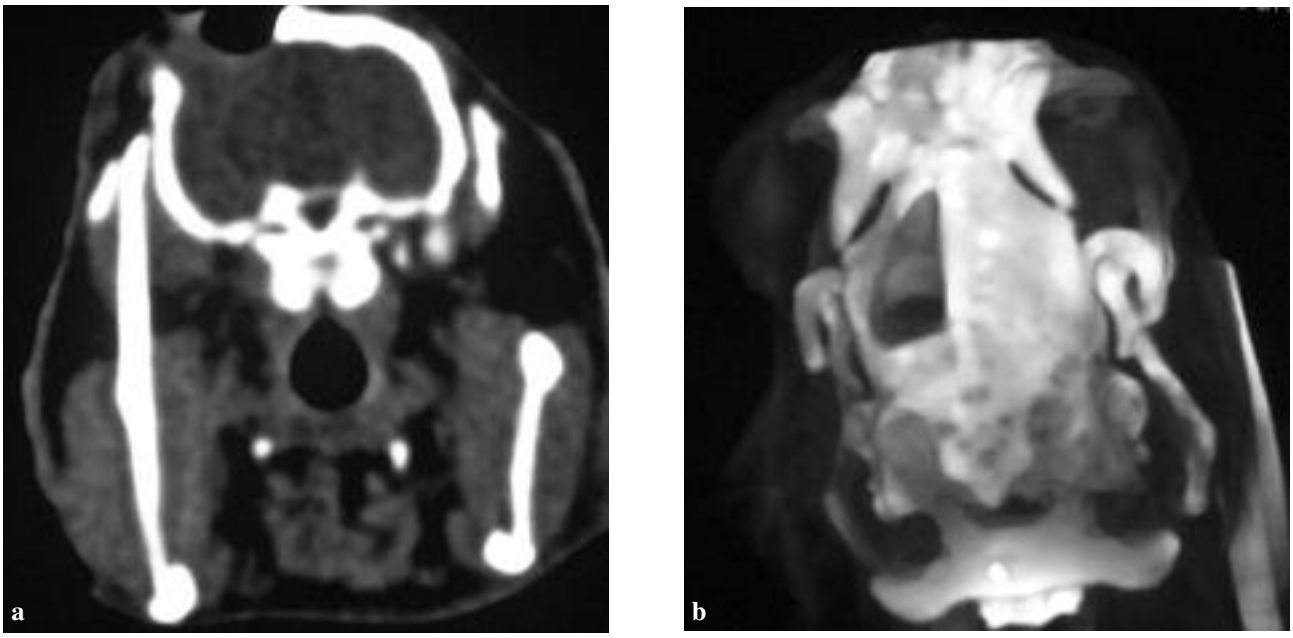

FIG. 1. — Three-dimensional CT image after head trauma was inflicted

left parietal region of the skull, leaving the dura mater intact. Then, a 20 gr steel marble was dropped upon the craniotomy site through a 10 cm long fiberglass tube. After this standardized trauma, the craniotomy area was closed with scalp sutures (Figs. 1 and 2).

Three hours after the trauma, the subject was placed in the prone position and MRI imaging was performed with a Tesla Siemens Symphony® MRI machine (Siemens AG, Erlangen, Germany). After a sagittal pilot image was taken, T2-weighted (4320/105 [TR/TE]), T2-weighted (719/26 [TR/TE]) and FLAIR (8000/113 [TR/TE]) images were obtained in the axial plane (thickness of 2 mm, 8-12 FoV values, and 256 × 512 matrixes).

After the MRI was performed, rabbits in the melatonin group (n = 6) were given a single IV dose of melatonin (dissolved in ethanol then diluted in isotonic NaCl) 100 mg/kg, and the rabbits in the mannitol group (n = 6) were given IV of 20% mannitol 2 gr/kg. Thirty-six hours after the trauma, MRI was performed again using the same sedation and MRI sequences as in the 3-hour post-trauma MRI.

The three- and thirty-six hour post-trauma T2-weighted and FLAIR images were examined for signs of increased intracranial pressure, parenchymal edema, parenchymal contusion and signs of protrusion at the craniotomy site by a staff radiologist who was blinded to the treatment received. On the T2 weighted images, signs of hemorrhage in the parenchyma, subarachnoid space, subdural space, epidural space and ventricles were sought. Signs of ventricular pressure (shrinkage of lateral ventricles, protrusion of brain tissue into the lateral ventriciles, and midline shift), parenchymal edema and protrusion were classified as mild, moderate and severe.

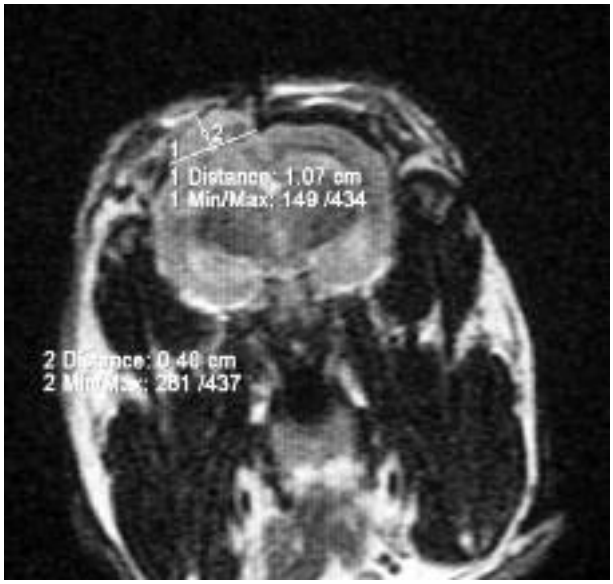

FIG. 2. — Measuring protrusion of brain tissue from the craniotomy site on the MR image on the perpendicular line extending from the tangential line drawn across the craniotomy site.

Brain tissue protrusion was measured from the most prominent point of brain tissue outside a tangential line connecting the two inner tables at the craniotomy site (Fig. 3).

Three- and thirty-six-hour MRI findings were recorded and compared with Fisher Exact test and chi-square testing (SPSS for Windows® version 15, SPSS, Inc., Chicago, USA). Mann Whitney U test was used for comparison of the protrusion measurements. A P value of less than 0.05 was considered statistically significant.

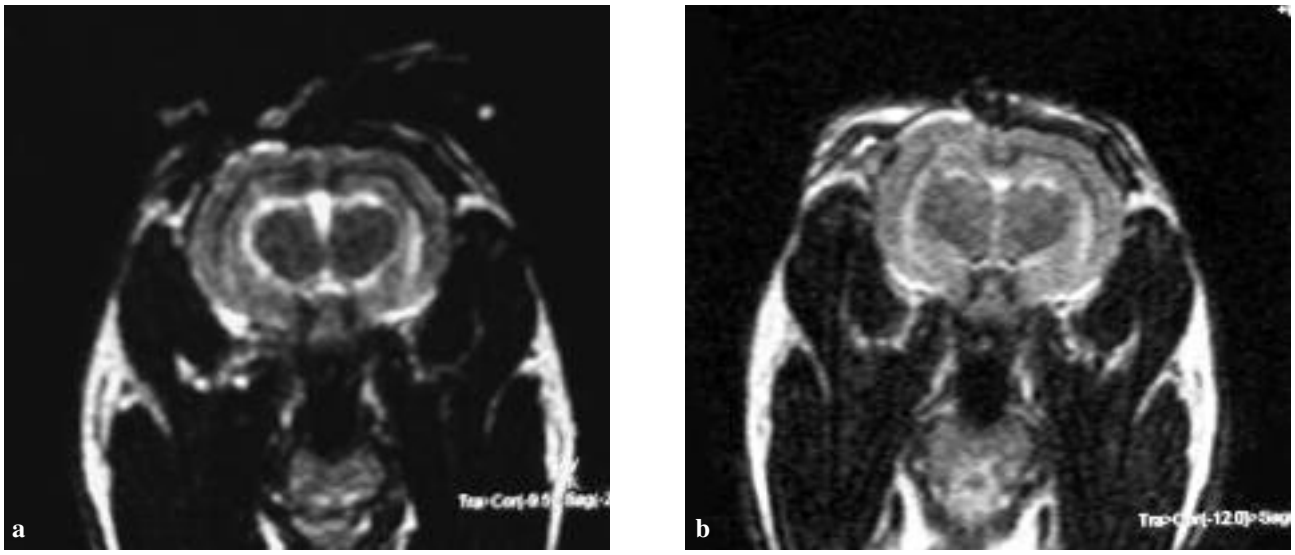

FIG. 3. — MRI images of a subject in the mannitol group (a) 3 hours after trauma, and (b) 36 hours after trauma. In (a), a mild parenchymal contusion is evident in the right occipito-parietal region. In (b), mild brain tissue protrusion is present.

Results

All twelve rabbits were able to complete the study as designed. The only findings on the three-hour post-traumatic MRI images were mild parenchymal contusion in two rabbits, and mild parenchyma edema in three rabbits (all were those which later received mannitol). None of the subjects had brain tissue protrusion, subdural, epidural, or parenchymal hemorrhage.

In the mannitol group (n = 6) 36 hours post-trauma, MRI images showed mild signs of elevated ventricular pressure and parenchyma edema in three subjects. Each of these three subjects in the mannitol group also had a small parenchymal hemorrhage, but no brain tissue protrusion, parenchymal contusion, subdural or epidural hemorrhage. The other three subjects in the mannitol group had mild parenchyma edema with mild brain protrusion, but without signs of elevated intracranial pressure, intraparenchymal, subdural or epidural hemorrhage. One of these three had severe, and the other two had mild brain tissue protrusion. One had a parenchymal contusion (Fig. 4). No significant differences were found in the three-hour and 36-hour post-trauma MRI images in the mannitol group rabbits.

MRI images of the melatonin group taken 36 hours after head trauma showed mild parenchymal edema, mild brain tissue protrusion, and mild signs of raised intracerebral pressure in two subjects. The other four subjects had parenchymal edema, two of which had mild protrusion of brain tissue. None of the melatonin subjects experienced subdural or epidural hemorrhage (Fig. 5). No significant differences were found in the three-hour and 36-hour post-trauma MRI images in the melatonin group rabbits.

MRI findings of signs of ventricular pressure, parenchymal edema, parenchymal hemorrhage and contusion were not significantly different between the mannitol and melatonin groups. The amount of brain protrusion measured 3 and 36 hours later were compared between groups. In the mannitol group, brain protrusion (mean ± SD) was 2.0 ± 0.8 cm three hours after trauma and 3.4 ± 1.7 cm 36 hours after trauma. In the melatonin group, brain protrusion (mean ± SD) was 2.2 ± 0.5 cm three hours after trauma and 2.8 ± 0.6 cm 36 hours after trauma (Fig. 1). No significant differences were found between melatonin and mannitol groups in point of brain protrusion measurements in the three-hour and 36-hour post-trauma ($p = 0.095$, $p = 0.07$) .

Discussion

Traumatic secondary brain damage is the most common cause of mortality in head trauma patients (Marshall and Gautille, 1990). Secondary brain damage occurs as a result of ischemia and decreased cerebral perfusion which are caused by an increase in intracranial pressure together with brain edema (Graham *et al.*, 1989). Hours after head trauma, vasogenic fluid accumulates, which leads to brain edema, raised intracranial pressure (ICP), and eventually to systemic hypotension, all of which result in cerebral ischemia. Free radical scavengers such as N-methyl-D-aspartate and calcium blockers, are being tested in an attempt to prevent traumatic secondary brain damage (Bullock *et al.*, 1999 ; De With *et al.*, 1995).

Osmotic therapy is still the most efficient method for decreasing brain edema, and is still used to prevent and treat secondary brain damage (Davis and Lucatorto, 1994). Mannitol is the most commonly used osmotic agent ; it decreases intracel-

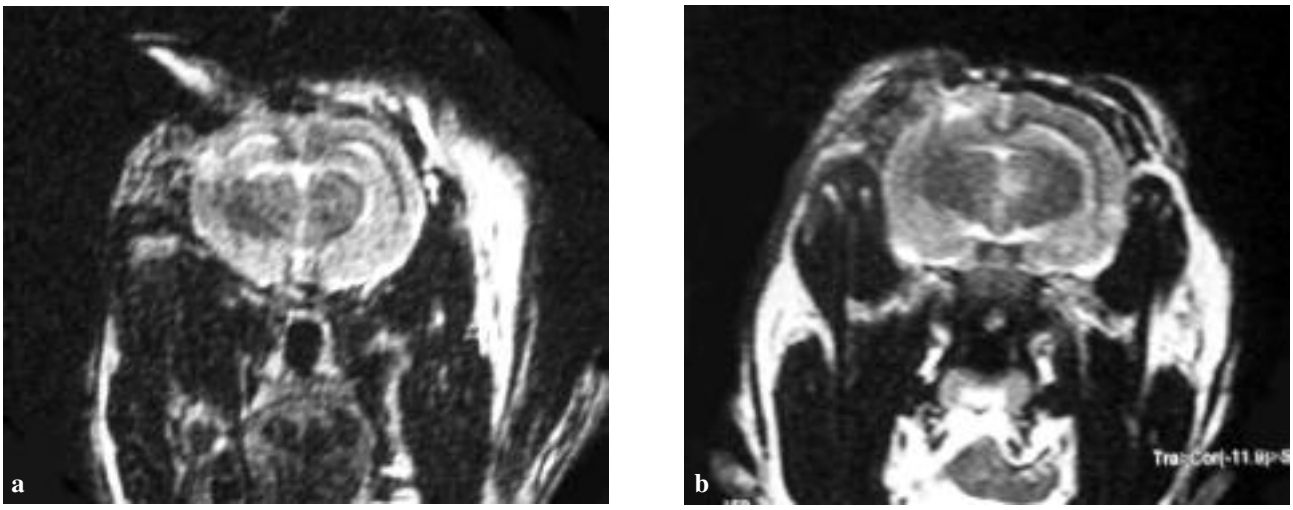

FIG. 4. — Melatonin group. (a) MRI image 3 hours after head trauma. Mild parenchymal contusion and edema are present in the occipitoparietal region on the right. (b) MRI image taken 36 hours after trauma. The parenchymal contusion has grown and the pressure on the ventricles has partially increased.

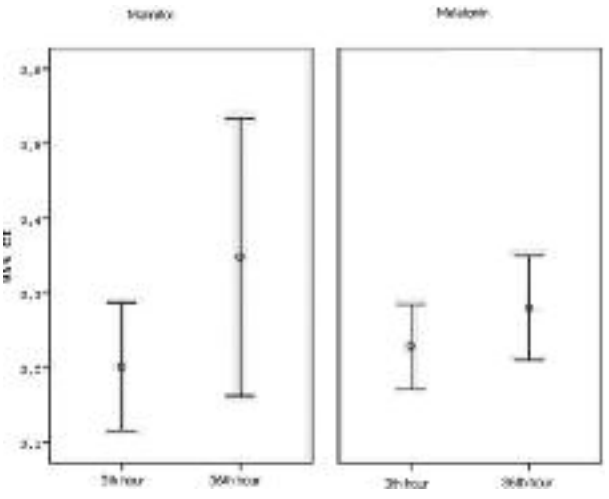

FIG. 5. — Brain tissue protrusion measurements taken from MRI images obtained three and 36 hours after head trauma in both mannitol (n = 6) and melatonin (n = 6) groups (mean ± SD with 95% confidence intervals displayed).

lular and CSF fluid, decreases blood viscosity, and decreases ICP. The decrease in blood viscosity also increases the perfusion of the brain tissue (Davis and Lucatorto, 1994). Mannitol has also been shown to have protective effects against biochemical damage (Davis and Lucatorto, 1994 ; Lt. Col. Jha, 2003).

Melatonin decreases morbidity and mortality in patients with head trauma, possibly due to its antioxidant and free radical scavenging effects in the central nervous system and subcellular organelles (Maldonado *et al.*, 2007 ; Kerman *et al.*, 2005 ; Reiter, 1998 ; Reiter *et al.*, 1998). Melatonin removes hydroxyl and superoxide anion radicals and peroxylnitride anions. In addition, it induces endogenous antioxidant enzymes like super-oxide dismutase, glutathion reductase, glutathion peroxidases and glucose-6-phosphate dehydrogenease (Reiter, 1998 ; Reiter *et al.*, 2001). Melatonin can also lessen neuroimmunological disorders which develop after head trauma (Maldonado *et al.*, 2007). It acts as an immune-modulator, stimulates the release of pro-inflammatory cytokines, and plays an important role in the regulation of circadian rhythm (Carillo-Vico *et al.*, 2005 ; Carillo-Vico *et al.*, 2003).

Based upon preliminary positive studies, we attempted to determine the effects of melatonin on post-traumatic brain edema. We thought that it might prevent not only brain edema, but also increased intracerebral pressure, contusion and intraparenchymal hemorrhage ; all of which are important indicators of secondary brain damage. To clarify the effects of melatonin, we imaged the brain of our subjects three and 36 hours post-trauma, when signs of secondary brain damage become evident.

Melatonin may have a lower toxicity than conventional medicines used in head trauma, and can even be given to newborn infants without signs of toxicity (Maldonado *et al.*, 2007). When given IV, its half-life is one hour. Even at high doses (100 mg/kg), cardiovascular depression did not occur. Kerman, *et al.* found positive effects on free radicals which play an important role in secondary brain damage (Kerman *et al.*, 2005). Melatonin also has a protective effect against ischemic damage in immature brain tissue (Carillo- Vico *et al.*, 2003 ; Hatton, 2001 ; Wakatsuki *et al.*, 2001 ; Okatani *et al.*, 2000 ; Wada *et al.*, 1999).

Melatonin, in a dose of 100 mg/kg IV, given immediately after head trauma prevented acute hippocampal neuron loss and long-term cognitive dysfunction (Ozdemir *et al.*, 2005). To date, the

effects of mannitol and melatonin on traumatic secondary brain damage had not been compared. Some studies have examined the effects of different dosages of melatonin on brain damage though (Ozdemir *et al.*, 2005 ; Sarrafzadeh *et al.*, 2000). In one, with a cortical impact model, animal subjects were given high dose (100 mg/kg) melatonin : 20 minutes before trauma, just after the trauma, an hour after the trauma, and two hours after the trauma (Reiter *et al.*, 2001). Twenty-four hours after trauma, brain edema, brain liquid content, cerebral perfusion pressure and ICP were measured. No difference in the melatonin and placebo-treated animals was noted except in the ones given melatonin before head trauma was inflicted : brain contusion volume was significantly less in the melatonin group (Reiter *et al.*, 2001). In this study, worsening of MRI findings between the three- and 36-hour images was seen in both groups of animals. However, the severity of MRI findings in melatonin and mannitol-treated animals were not significantly different from each other in the 3rd hour or 36th hour post-trauma. The fact that protrusion measurements in the 3rd hour post trauma were not significant indicates that traumatic effects occurred equally in both groups as neither groups had received any treatment yet. The fact that protrusion measurements in the 36th hour post trauma were not significant in both groups is an important sign that both agents have equal effects on brain edema since the measurements were obtained after the groups received mannitol and melatonin treatment.

The protrusion measurement obtained in the 36th hour post trauma was considerably higher in one subject of the mannitol group than the others. And this led the measurements of the mannitol group in the 36th hour to increase standard deviation significantly.

Major limitation of this study is that it lacks placebo control group. Subjects were only given melatonin, and MRI findings were compared with those of a mannitol group ; no placebo group was used. A study planned with placebo control group in future can be very useful for mannitol and melatonin efficiency to compare.

Conclusion

Protrusion of brain tissue and signs of brain edema were similar in animals receiving melatonin and mannitol, as determined on MRI images taken in the 3rd and 36th hours after mild traumatic brain injury. Melatonin should be tested in a variety of head injury models to determine if these results are robust, eventually leading to studies in humans.

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