LABORATORY INVESTIGATION

Effect on Varying the Impact Velocity in the Controlled Cortical Impact Injury Model : Injury Severity and Impact Velocity

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Objective : A study of the histopathologic and neurobehavioral correlates of cortical impact injury produced by increasing impact velocity using the controlled cortical impact(CCI) injury model is studied.
Methods : Twenty-four Sprague-Dawley rats (200~250g) were given CCI injury using a pneumatically driven piston. Effect of impact velocity on a 3mm deformation was assessed at 2.5m/sec (n=6), 3.0m/sec (n=6), 3.5m/sec (n=6), and no injury (n=6). After postoperative 24hours the rats were evaluated using several neurobehavioral tests including the rotarod test, beam-balance performance, and postural reflex test. Contusion volume and histopathologic findings were evaluated for each of the impact velocities.
Results : On the rotarod test, all the injured rats exhibited a significant difference compared to the sham-operated rats and increased velocity correlated with increased deficit $(p<0.001)$. Contusion volume increased with increasing impact velocity. For the 2.5, 3.0, and 3.5m/sec groups, injured volumes were $18.8 \pm 2.3mm^3$, $26.8 \pm 3.1mm^3$, and $32.5 \pm 3.5mm^3$, respectively. In addition, neuronal loss in the hippocampal sub-region increased with increasing impact velocity. In the . TUNEL staining, all the injured groups exhibited definitely positive cells at pericontusional area. However, there were no significant differences in the number of positive cells among the injured groups.
Conclusion : Cortical impact velocity is a critical parameter in producing cortical contusion. Severity of cortical injury is proportional to increasing impact velocity of cortical injury.

KEY WORDS : Controlled cortical impact injury · Impact velocity · Pneumatic pressure · Severity · TUNEL staining.

Introduction

Traumatic brain injury(TBI) is a serious problem that leads to high mortality rates and long-term neurobehavioral and socioeconomic consequences[3,6,7,11]. Unfortunately, however, the patho-mechanism of such insult is complex and many aspects are still not clear[24]. The response of any solid structure to specified loading depends critically on its composition, geometry and in particular, on its deformation characteristics[25]. Different types of stress and corresponding strain occur depending on how the load is applied[19]. Various modes include: 1) elongation, 2) compression, 3) bending, 4) shear, and 5) torsion (twisting)[19]. In TBI, the severity of the trauma is related to these variable factors of the force[10].

Investigation of the complex brain injury mechanism req-uires experimental models that exhibit posttraumatic anatomic and functional responses that mimic those described clinically[13,14]. Ideally, trauma models should produce a graded reproducible injury with quantifiable endpoints[10]. Models currently in use include inertial loading without impact, fluid percussion, and direct impact[1,4,9,10,14]. Inertial loading or acceleration injury produces diffuse axonal injury and, at high impulse levels, can lead to subdural hematoma, hippocampal neuronal loss, and parenchymal distribution but generally fails to produce cerebral contusions[10]. The cortical impact model using pneumatic piston is the most recently introduced animal model of TBI[10].

We examined the pathological features of the controlled cortical impact(CCI) injury model in the rat over a range of impact velocities.

Materials and Methods

Animals, surgery, and controlled cortical injury

Male Sprague-Dawley rats weighing 200~250g were used for this study and were allowed free access to food and water during the pre-operative period, with constant temperature and humidity conditions. All procedures were performed in accordance with the guidelines for care and use of laboratory animals approved by the Chung-Ang University Institutional Animal Care and Use Committee.

Surgical procedures

The rats were anesthetized initially with ketamine hydrochloride (15mg/kg, i.m.) and the head was fixed in a stereotaxic device (Small animal stereotaxic instrument, David Korf instrument, Tujunga, CA, USA). Anesthesia was maintained at 2% halothane mixed with oxygen and compressed air. After performing a 1.5cm midline skin incision and scalp retraction, a 5mm diameter craniectomy was made over the right parietal cortex with an electrical drill. The medial margin of the bone flap was 1.5mm lateral to the sagittal suture and the anterior margin was 1.5mm posterior to the bregma. Great care was taken to avoid damaging the underlying dura matter during the drilling and removal of the bone flap.

Experimental controlled cortical injury

After removal of the bone flap, experimental brain injury was produced using the CCI device (CAUH-1) method as described previously[4]. The device consists of a 4mm metal impact tip that is pneumatically driven at a predetermined depth (3.0mm) and duration (0.2sec) of brain deformation.

Experimental groups

The effect of altering impact velocity at a 3mm deformation depth was studied in detail. The rats were randomly assigned to the following groups : sham impact (n=6), 2.5m/sec impact (n=6), 3.0m/sec impact (n=6), and 3.5m/sec impact (n=6). Following the impact, the craniectomy and incision were repaired, and anesthesia was discontinued.

Neurobehavioral tests

Twenty-four hours following the procedure, the subjects were evaluated neurologically as previously described[5]. The neurobehavioral battery of tests consisted of a rotarod test, beam-balance performance, and posture reflex test. The followings are short descriptions of each test.

Rotarod test

Locomotor behavior was assessed on a turning rod. The rats was allowed to remain stationary for 10sec at 0rpm. The speed was slowly increased to 3rpm for 10sec and was steadily increased by 3rpm in 10-sec intervals until the maximum of 30rpm was reached. Trials were halted if the subject fell off the rotarod or hung on (not walking) for two consecutive turns. Each subject performed the rotarod test three times for a maximum of 60sec at 30rpm. The duration of each test was recorded and the mean time was determined for each rat.

Beam-balance performance

Vestibular function was evaluated on the balance beam. The rats were placed on the beam with its head away from the wall and allowed to remain for 60sec. Each rat was given three trials, and a mean score was determined.

Posture reflex test

The posture reflex test evaluates movement asymmetry of the forelimbs as the rat is suspended by the base of its tail. Normal posture is determined as the symmetrical reaching of both forelimbs toward the ground and was given a score of 3. Moderately abnormal posture, which is the flexion of the contralateral forelimb toward the body, was given score of 2. Severely impaired posture, characterized by flexion of the forelimb and rotation of the shoulder and/or body, was given a score of 1.

Extraction of the brain

After neurological evaluation, the rats were again anesthetized with halothane and decapitated. The brain was removed from the calvarium and weighed. There was an identifiable focal cortical injury at the right cortical area.

The brain was cut into 2mm-thick slices with a rat brain matrix (Harvard Bioscience, Holliston, MA, USA), and stained with 2% TTC (2,3,5-triphenyl tetrazolium chloride, Sigma, Inc., St. Louis, MO, USA) at 37°C for 10 min. After staining, the tissue slices were fixed with 4% buffered paraformaldehyde.

Contusion volume

The stained brain slices were image-recorded with a digital camera (FinePix S2pro, Fuji Photo Film Co., Ltd, Minato-ku, Tokyo, Japan) and the injury volume was calculated with an image analyzer program (OPTIMAS 6.5, Optimas, Inc., Bothell, WA, USA).

Pathologic evaluations

Evaluation of neuronal loss

The brain slices, which were fixed with 4% buffered paraformaldehyde, were paraffin-embedded. The prepared brain

sections were cut into 5μm-thick slices for hematoxylin and eosin(H-E) staining. Neuronal cells were counted in the hippocampal regions of CA1, CA2, and CA3 by an evaluator blinded to the study groups. For each rat, mean counts of neurons were obtained by examining at least three serial coronal sections in the hippocampus underlying the area of the contusion. In each section, the neurons were counted in three nonoverlapping fields at a magnification of 400x in each subregion (CA1, CA2, and CA3).

Only complete neuronal cells with a clearly defined cell body and nucleus were counted.

Apoptosis staining

TUNEL (TdT-mediated dUTP-biotin nick end-labeling) staining was performed using previously described methods[18,23]. Staining was carried out using the TACS2 TDT-DAB kit (Trevigen, Inc, Gaithersburg, MD, USA). The number of positive cells was determined by taking a series of nonoverlapping pictures at a magnification of 400x.

Statistical methods

Neurological scores, contusion volume, and counts of neuronal cells are presented as the mean ± SD of six rats per group. Data were compared between groups using one-way analysis of variance(ANOVA) followed by the Tukey-Kramer multiple-comparisons method. P values of < 0.05 were considered statistically significant.

Results

Impact velocity

As described previously, the impact velocity could be adjusted between 2.5 and 3.8m/sec by controlling the pneumatic pressure (a range from 60psi to 125psi)[4]. The pressures of 60, 70, and 110psi to the impactor resulted in impact velocities of 2.5, 3.0, and 3.5m/sec, respectively.

Measurement of neurologic behavior

The effects of varying impact velocity were evaluated according to the neurobehavioral assessment (Table 1). The sham-operated rats performed well on all three neurological assessments. In comparison, on the rotarod test the injured rats exhibited deficits which increased with increasing velocity (Fig. 1) (p < 0.001). According to the beam-balance performance and posture reflex test, subjects of the 2.5m/sec group were not significantly impaired (p > 0.05). The injured rats of the 3.0 and 3.5m/sec groups exhibited significantly greater deficits compared to the sham-operated rats (p < 0.05). However, there was no significant difference between these two groups (Table 1) (p > 0.05).
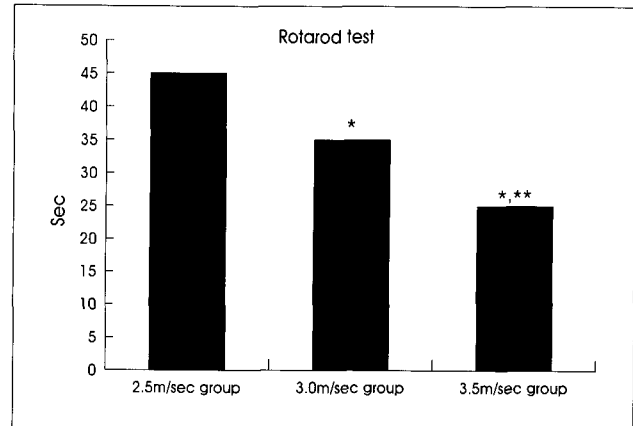

Fig. 1. Rotarod test after controlled cortical impact (CCI) injury. There was significant difference in varying impact velocity (p <0.05). The deficit was increased with increasing impact velocity. ** p < 0.05, compared with 2,5m/sec group *** p<0.05, compared with 3.0m/sec group.

Table 1. Neurologic assessment 24 hours after CCI injury*

	Rotarod (sec)		Beam-balance (sec)		Posture reflex score	
	Pre	Post	Pre	Post	Pre	Post
2.5m/sec group	60	44.3±3.0	60	53.0±4.9	3	2.2±0.4
3.0m/sec group	60	33.5±2.3**	60	46.5±5.0	3	1.8±0.4
3.5m/sec group	60	25.0±4.1**,***	60	42.5±5.1	3	1.7±0.5

* All data are presented as the mean ± standard deviations. ** p < 0.05,compared with 2,5 m/sec group *** p < 0.05, compared with 3.0 m/sec group CCI; controlled cortical impact, Pre; preoperative, Post; postoperative
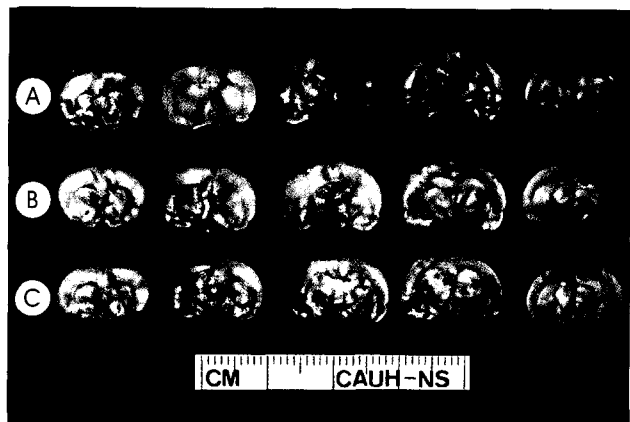

Fig. 2. Brain slices stained with 2,3,5-triphenyltetrazolium chloride (TTC) at 24 hours after controlled cortical impact(CCI) brain injury. The brain slices show margins of injury area surrounding contused brain tissue. A : 2.5m/sec, B : 3.0m/sec, C : 3.5m/sec of impact velocity.

Measurement of injured area

No cortical lesions were detected in the sham-operated rats using TTC staining. Subjects in the injured groups exhibited definite contused and ischemic findings in the pericontusion area (Fig. 2). In the 2.5, 3.0, 3.5m/sec groups, injured volumes were found to be $18.8 \pm 2.3mm^3$, $26.8 \pm 3.1mm^3$, and $32.5 \pm 3.5mm^3$, respectively. The contusion volumes signific-antly increased with increasing impact velocity (Fig. 2) (p<0.05).

Table 2. Number of neuronal cells in sub-region of hippocampus[a]

	CA1 (%)	CA2 (%)	CA3 (%)
Sham operated group	32.5±3.0	31.2±1.5	57±1.1
2.5m/sec group	24.3±3.4*	23.5±3.6*	52.2±5.7*
3.0m/sec group	13.3±2.3*,**	16.2±1.7*,**	47.2±3.3*,**
3.5m/sec group	6.8±2.3*,**,***	5.3±3.5*,**,***	35.8±8.0*,**,***

a All data are presented as the mean ± standard deviations. * p < 0.05, compared with sham operated group ** p < 0.05, compared with 2.5 m/sec group *** p < 0.05, compared with 3.0 m/sec group CA; cornu ammonis

Fig. 3. Photomicrographs of hematoxylin and eosin staining of ipsilateral hippocampal lesion at 24 hours after traumatic brain injury. Loss of neuronal cells is increased with increasing impact velocity. A. Sham–operated rat, B. 2.5 m/sec C. 3.0 m/sec, D. 3.5 m/sec of impact velocity(x 40).

Fig. 4. Photomicrographs of TUNEL staining in hippocampus at 2.5m/sec (A) and 3.5m/sec (B) of impact velocity. Although there is no significant difference in number of TUNEL–positive cells per HPF between these two groups (p>0.05) increased velocity has much extent TUNEL–positive area. TUNEL; TdT–mediated dUTP–biotin nick end–labeling. A. 2.5m/sec, B. 3.5m/sec of impact velocity (x 400).

Pathologic finding

H & E staining

All the injured rats exhibited definite histopathological findings (Fig. 3). Moderate (3.0mm) deformation injury at 2.5m/sec produced predominantly cortical-thick contusions, whereas 3.0mm deformation injuries at 3.0 and 3.5m/sec spanned some of the contiguous white matter and the hippocampus, as well as the full cortical thickness (Fig. 3). Neuronal cell loss ipsilateral to cortical impact paralleled impact velocity (Table 2) In all subregions of CA1, CA2, and CA3, there was significant difference in number of cell loss (p<0.05).

Apoptosis staining

These TUNEL positive cells were identified throughout the injured cerebral cortex and especially TUNEL positive cells at 3.5m/sec were showed at hippocampal area and some white matter (Fig. 4). However, the number of positive cells per HPF did not appear to be significantly different among the series presented (p>0.05).

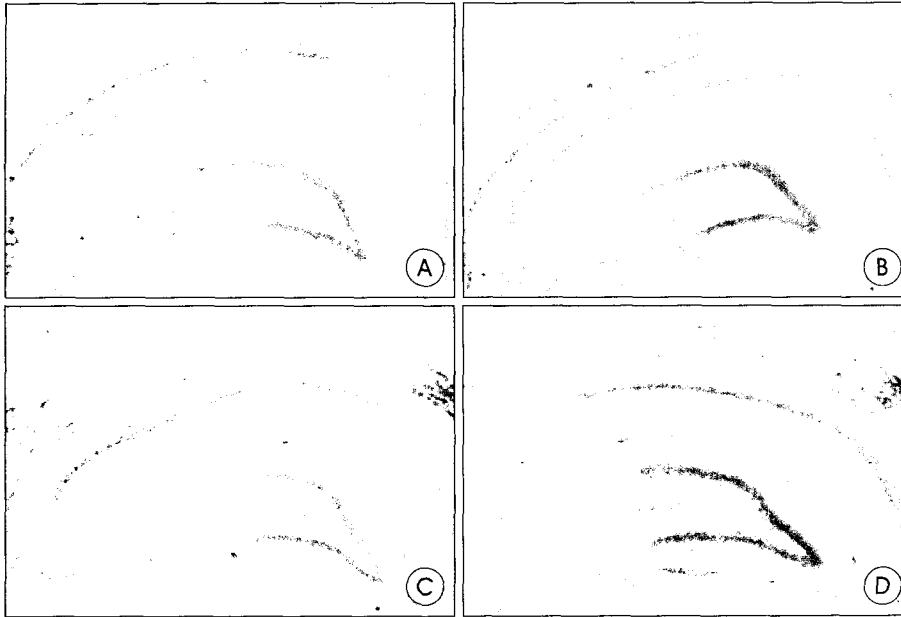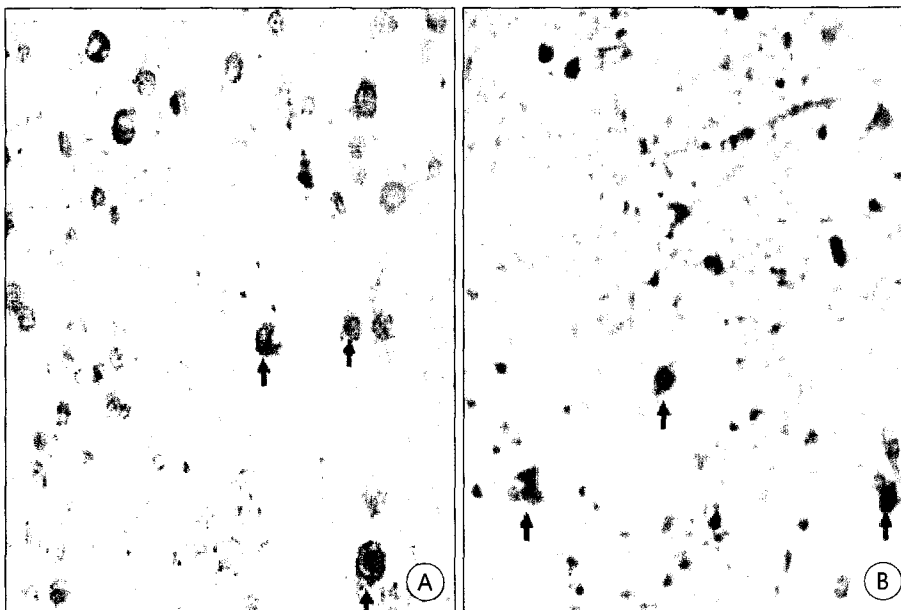## Discussion

Experimental models of traumatic brain injury(TBI) that can be biomechanically characterized are required for physical and analytical modeling of tissue deformation[13]. These modeling efforts are useful for the correlation of experimental results and injury response with human injury initiation and response[14].

Direct mechanical injury related to impact is primarily necrotic, as is cell lysis related to osmotic shifts and intracellular swelling; however, the proportion of necrotic to apoptotic death are dependent upon the extent of injury, brain region, and other secondary factors[12,15].

Experimental models of TBI (e.g., fluid percussion, controlled

cortical impact, and weight drop) have revealed that the cortex, hippocampus, and thalamus are selectively vulnerable to injury, whereas other regions of the brain are usually spared[22].

The direct cortical impact model results in hemodynamic cerebral blood flow, intracranial pressure, and neurochemical alterations, which directly parallel the magnitude of cortical deformation and impact velocity[3]. In this study, impairment in the rotarod test (as a neurobehavioral assessment) increased with increasing impact velocity and was a more sensitive and efficient index for assessing motor impairment produced by brain injury, compared to the beam balance performance and posture reflex tests. In agreement with the results of this study, Hamm et al. demonstrated that the rotarod test was indeed a sensitive index of injuryinduced motor dysfunction following even mild fluid percussion injury[11].

Previous authors used TTC staining for quantification of infarction volume after focal cerebral ischemia and TTC staining has also been utilized to assess the effectiveness of potential neuroprotective agents[1]. CCI brain injury induces an increased percentage of water content, an indicator of brain edema, on days 1-3, with a peak increase on day 1[1]. Moreover, the time course of brain edema was found to correlate well with the injury volume quantified by TTC staining. However, cortical injury after TBI usually has a complex three-dimensional shape, sometimes within distinct borders, resulting in volumes that are difficult to define and accurately measure[1]. For the well-stained contusion zone, we made 2-mm brain slices and then the TTC staining was performed to the slices. We found that the volume of contusion increased with increasing impact velocity.

Specific sub-regions within the hippocampus are also selectively sensitive to traumatic injury[8]. In this experiment, loss of neuronal cells was definitely confirmed in sub-regions of the hippocampus. Previous reports showed that the hilus and CA3 regions are preferentially injured, whereas the CA1 region and dentate gyrus are uniquely spared[8].

Previous studies have suggested that the severity of insult is a major determinant of the progression of either apoptosis or necrosis[21]. Neuronal apoptosis has been identified as a significant component of the pathology of neurodegenerative diseases, including head trauma[2,16,17]. After TBI, acute programmed cell death (6~48h) is seen in cortical neurons, white matter, and the hippocampus[20]; we found similar results. In this experiment, the positive cells of TUNEL staining were identified mainly at the pericontusional area. Although there was not a significant difference in number of TUNEL positive cells per HPF among the various impact velocities (p>0.05), increased velocity was increasing area of TUNEL positive cells. TUNEL positive cells at 2.5m/sec were showed at cortical area, whereas at 3.5m/sec, they were showed at hippoc-

ampal area. However, we did not estimate the volume of TUNEL positive area in detail, further study should be needed.

This study demonstrates that the volume of cortical contusion and the extent of hippocampal neuronal loss produced in the rat by cortical impact can be varied by altering the impact velocity. Within a given deformation of cortical injury, the impact velocity directly correlates with the extent of injury.

Conclusion

Cortical impact velocity is a critical parameter in producing cortical contusion. Severity of cortical injury increased with increasing impact velocity in the CCI model.

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