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Laboratory Investigation

Intracranial Pressure and Experimental Model of Diffuse Brain Injury in Rats

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Objective : In this study, we present a simple closed head injury model as a two-stage experiment. The height of the weight drop enables gradation of head trauma severity.
Methods : The head injury device consists of three parts and there are three adjustable parameters-weight (100-600 g), height of fall (5-100 cm) and elasticity of the springs. Thirty male Wistar rats underwent monitoring of intracranial pressure with and without induction of the head injury.
Results : The weight drop from 45 to 100 cm led to immediate seizure activity and early death of the experimental animals. Severe head injury was induced from 40 cm weight drop. There was 50% mortality and all surviving rats had behavioral deterioration. Intracranial pressure was 9.3 ± 3.76 mmHg. Moderate head injury was induced from 35 cm, mortality decreased to 20-40%, only half of the animals showed behavioral pathology and intracranial pressure was 7.6 ± 3.54 mmHg. Weight drop from 30 cm caused mild head injury without mortality and neurological deterioration. Intracranial pressure was slightly higher compared to sham group- 5.5 ± 0.74 mmHg and 2.9 ± 0.81 mmHg respectively.
Conclusion : This model is an eligible tool to create graded brain injury with stepwise intracranial pressure elevation.

KEY WORDS : Traumatic brain injury · Experimental model · Rat · Intracranial pressure.

INTRODUCTION

Traumatic brain injury (TBI) is a significant cause of death and disability, especially in young males[6]. In the Czech Republic, head injury affects 149.6 persons per annum / 100.000 inhabitans[1]. Over the past few decades there has been an active research into the diagnosis and therapy of TBI. Most of the basic knowledge is acquired through the use of different animal models for head trauma[7]. An experimental model of diffuse brain injury in rats was developed by Marmarou and colleagues[9] and modified by Engelborghs and colleagues[4]. This closed head injury model allows variation in the balance between impact and acceleration forces and mimics clinical features of traumatic injury. This report describes our modification of a rat closed head injury model producing a graded brain injury. Our first objective was to simplify the model as a two-phase experiment. The brain injury should be created within the first part of experiment without a need for surgery, intubation, or ventilation. The anesthesia for the first phase should be very short with rapid recovery. The second part of experiment can be done hours or days later to evaluate the neurochemical, physiological, behavioral, or histological sequela of brain injury. The second objective of this study was to generate graded brain injury with stepwise intracranial pressure elevation.

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MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Ethics Committee (2nd Medical School, Charles University, Prague) - European Union Animal Welfare No. 86/609. Thirty male Wistar rats were used for this experi-ment. They were allowed free access to food and

water and kept under 12/12-h day/night cycle.

Weight drop head injury device

The head injury device consists of three parts - Fig. 1. The first part is a 120 cm tubular plexiglass column with an inner diameter of 24 mm. The column is secured by two snap holders. Segmented weights may fall from designated heights through the plexiglass column. Stainless steel weights assemble from individual cylinders interlocked with a nylon string. Device allows stepwise variations from 100 g to 600 g (step 50 g). The first cylinder is longer, with soft conical impact termination and weights 100 g. The rest of cylinders scale 50 g each. The plexiglass column is marked every 5 cm.

The second part of the trauma device is a rigid platform with four feet (diameter of 17 mm and a length of 55 mm). The third part is a massive horizontal platform with four hollow cylinders. They have an inner diameter of 20 mm and a length of 85 mm. Springs of known elasticity (k = 110 N.m-1) are located inside these cylinders. There are three adjustable parameters - weight (100-600 g), height of fall (5-100 cm) and elasticity of the springs.

Head injury

Male Wistar rats were anesthetized in the induction chamber over 2 minutes with 8% sevoflurane in a mixture of 60% O_2 and 40% N_2O. Subsequently, rats were positioned on the horizontal platform and placed directly under the plexiglass column. The impact site was chosen one centimeter frontal from the interauricular line and in the sagital midline plane. The weight was dropped from a designated height and rats were transferred to an empty plastic cage. Apnea, seizures, bleeding and time to recovery of motor functions were observed. Surviving animals 60 minutes after induction of head trauma were returned to their home cages and allowed simplified access to food and water. The second stage of the experiment was done 12 to 18 hours later. Rats were neurologically tested by experienced animal behavior scientist and rated either normal or pathological. Animals were afterwards anesthetized by intraperitoneal injections of ketamin (100 mg/kg) and intramuscular xylazin (16 mg/kg). Respiration and heart rate were monitored using three subcutaneous electrodes (OmniCare CMS 24, Hewlett Packard). The midline incision over vertex was performed, periosteum retracted, and small twist hole was made behind the coronal suture, 4 mm from

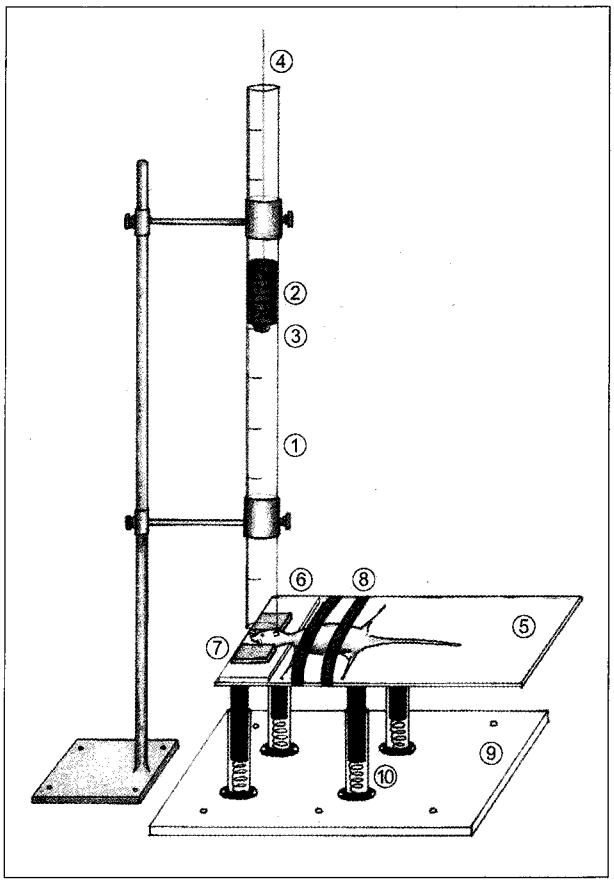

Fig. 1. Weight drop head injury device. ① : hollow plexiglass column; ② : segmented weights; ③ : soft conical termination; ④ : nylon string; ⑤ : floating platform with four feet; ⑥ : platform thickening; ⑦ : head holders; ⑧ : strips for body fixation; ⑨ : a massive horizontal platform with four hollow cylinders; ⑩ : springs.

Table 1. Results : sham group (n = 9)

n = 9	
Weight (g)	360 ± 132
ICP (mmHg)	2.9 ± 0.81
Heart rate / min	242 ± 42
Systolic blood pressure (mmHg)	110 ± 15
Diastolic blood pressure (mmHg)	84 ± 12
Respiration rate/min	51 ± 10

ICP : intracranial pressure

Table 2. Results : head trauma group (n = 21)

Injury	Height (cm)	No. of rats	ICP (mmHg) ± SD	Mortality (%)	Behavioral deterioration (%)	Convulsion (%)
Lethal	45 - 100	7		100		100
Severe	40	6	9.3 ± 3.76	50	100	67
Moderate	35	5	7.6 ± 3.54	20 - 40	50	20
Mild	30	3	5.5 ± 0.74	0	0	33

ICP : intracranial pressure, SD : standard deviation

the midline. The dura was punctured and micro sensor (Codman Microsensor, ICP Express) for intracranial pressure monitoring (ICP) was inserted intraparechymally. Rats in the sham group were cannulated in the carotid artery for blood pressure measurement.

RESULTS

Sham group

Nine male Wistar rats underwent monitoring of intracranial pressure, blood pressure, heart rate and respirations without induction of the head injury. Their weight was 360 ± 132 g. There was a temporary increase of intracranial pressure after insertion of ICP probe (3 to 10 minutes) so we recorded all the data at least 15 minutes after the insertion of ICP micro sensor. The results of our study are summarized in Table 1. Mean intracranial pressure was 2.9 ± 0.81 mmHg. The systolic and diastolic pressures were 110 ± 15 mmHg and 84 ± 12 mmHg respectively. Heart rate reached 242 ± 42 beats per minute and respiration rate was 51 ± 10 per minute.

Head trauma group

The collective results of 21 Wistar rats are summarized in Table 2 and in Fig. 2. The trauma was induced with 400 g weight dropped from 30 to 100 cm. Using designated height 45 to 100 cm; there was always immediate seizure activity and early death of the experimental animals. The majority of the animals also showed bleeding from the nose. There was a bleeding from both ears following weight drop from 100 cm. Weight drops from lesser heights (45 to 60 cm) resulted in surviving for a few minutes-however animals remained comatose (3-60 minutes). Six rats underwent induction of trauma from 40 cm. Four animals (67%) suffered immediately seizures, five rats (83%) bled from the nose. Three of the animals (50%) perished 3, 55, and 70 minutes after impact (there was a partial recovery from comatose state in two rats). Three animals survived the weight drop from 40 cm. Their neurological performance was pathological 12 hours later (protracted motion, shivering, lack of escape reaction, ataxia). The average intracranial pressure was 9.3 ± 3.76 mmHg in this group.

Five Wistar rats were impacted from the 35 cm. Only one animal had posttraumatic convulsions (20%), four of them (80%) bled from the nose. The rat with seizures expired 22 minutes later, four animals survived first 12 hours after injury. Additional animal died during the second stage of the procedure, 840 minutes after trauma. This rat neurologically deteriorated already before the second anesthesia with gasping and shivering. Three animals completely went through the second stage. Two of these rats were neurol-

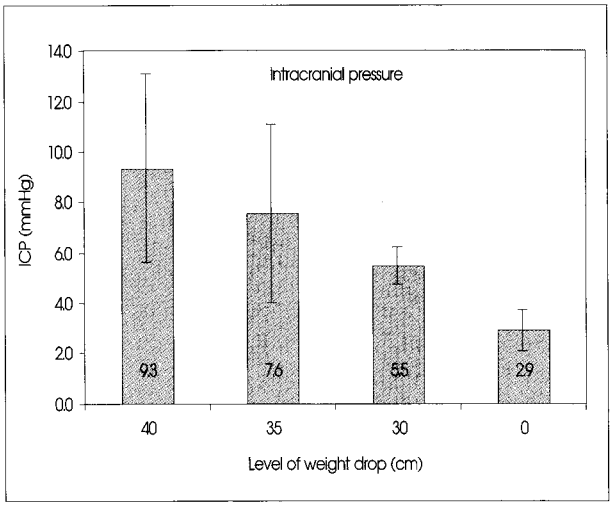

Fig. 2. Results : intracranial pressure and the level of the weight drop injury.

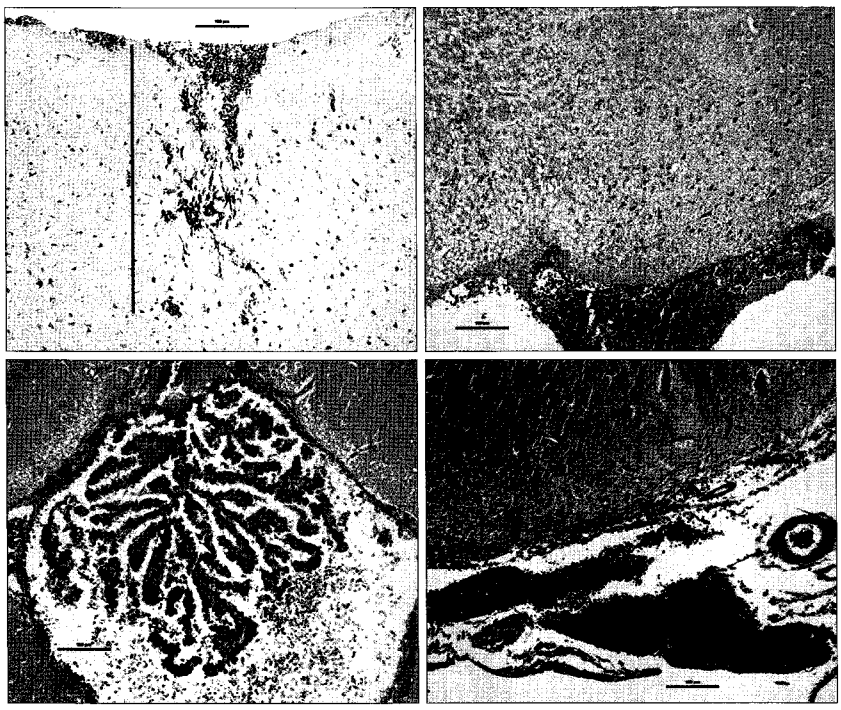

Fig. 3. Histopatological study : diffuse brain injury (hematoxylin and eosin staining, magnification 400×, scale 100 μm) : bottom left - hemorrhagic foci within the choroid plexus; bottom right - bleeding within interpeduncular fossa; top left - contusion from micro sensor insertion; top right - bleeding and edema of the external capsule.

ogically intact, the third one showed pathological behavior. The average ICP was 7.6 ± 3.54 mmHg.

Weight drop from 30 cm was performed in three rats. There was no bleeding after the impact; one animal had short term convulsions. The respiration after the injury was regular and recovery to full vigil state was very short (2 to 3 minutes). Neurological examination twelve hours post injury showed appropriate performance status. The average recorded intracranial pressure was 5.5 ± 0.74 mmHg.

DISCUSSION

Different experimental models of head trauma have been used over the past years[2,3,5,8,10-13]. The fluid percussion model[3], rotational injury model[11], the cryogenic injury model[5,12], the cortical impact model[8] and weight drop injury model[4,9,10] were created to represent heterogeneous pathophysiological changes associated with traumatic brain injury[2].

In this study, we present a simple closed head injury model. This model is an eligible tool to create graded brain injury with stepwise intracranial pressure elevation. Histopatological investigation eighteen hours after severe trauma showed diffuse brain injury. Macroscopic observation exposed subarachnoidal hemorrhage and a thin layer of subdural hematoma. Light microscopy studies demonstrated subdural, subarachnoidal and intraventricular bleeding. There were focuses of edema and punctuate hemorrhages in the brain parenchyma (Fig. 3).

There is no need for intubations and ventilation in the experimental animals before and during the induction of head trauma. There are also no surgical wounds in the course of the first step, so during the recovery period, the questions of pain control or animal suffering are not pressing. There are three adjustable parameters of the model-weight (100-600 g), height of fall (5-100 cm) and elasticity of the springs. In our adjustment, the elasticity k = 110 N.m-1 and 400 g weight were used. Weight drop from 45 cm and above was lethal. The injury was accompanied with generalized convulsions, skull base and facial fractures and respiratory depression. Weight drop from 40 cm created severe brain injury. Mortality rate was 50% and all survivors had behavioral deterioration. There was also marked increase in the intracranial pressure. Using a height of 35 cm induced moderate brain injury. Survival probability was 80%, behavioral deterioration was reciprocal and convulsions were rare. There was also a smaller increase in intracranial pressure compared to severe brain injury group. Weight drop from 30 cm generated mild head injury. There was no post-impact bleeding or behavioral deterioration after the recovery.

CONCLUSION

This simple model in rats produces diffuse reproducible brain injury. The height of the weight drop enables gradation of head trauma severity. This experimental model may be a valuable tool in the basic research to bring new insights into neurochemical, physiological, behavioral, or histological sequela of brain injury.

• Acknowledgements

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