BIOLOGICAL EFFECT OF SHOCK WAVES: BRAIN DAMAGE BY SHOCK WAVES IN RATS – PRESSURE DEPENDENCE

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Abstract: To introduce shock waves (SW) as treatment modality in the vicinity of brain, it is necessary to understand the threshold of brain damage. In this study, we have evaluated pressure dependent SW induced brain damage.

Brain of Sprague-Dawley rat (8week old) was exposed to single shot of SW produced by AgN3, and were divided into 7 groups according to the exposed overpressure of shock wave, and time of sacrifice. Group A served as control. Estimated overpressure of applied SW was over 10 MPa in group B, C, D, and nearly 1 MPa in group E, F, G. The rat was sacrificed at 4, 24, 72 hours, respectively. The specimen were evaluated morphologically (hematoxykin and eosin stain) and biochemically (heat shock protein 72 and TUNEL stain). Subcortical hematoma and contusion were common findings in group B, C, D. In goup C and D, marked necrosis, and TUNEL positive cells could be observed. Heat shock protein 72 was positive in the cells around these necrotic area. In group D, E, and F, fusiform changes in nucleaus were observed in some specimen.

Present results show that two type of neuronal death, i.e. necrosis and apoptosis, can be induced by SW beyond 1 MPa.

Introduction

Since the clinical application of extracorporeal shock wave lithotripsy, shock waves have been recognized as safe and potential treatmnet modality, especially for the fragmentation of renal stones and for stones at other location. Shock wave has also been used as new treatment modality for the nonunion at fracture of long bone and pain management in calcified tendonitis in orthopedic field [2], and revasculization in severe heart failure patients in cardiovascular medicine [8]. We have been performing research work to introduce this unique property of shock wave to develop minimally invasive treatment for revasculization in cerebral embolism [3, 4], cranioplasty [7], tumor chemotherapy [5]. On the other hands, shock wave is known to cause complications, such as morphological changes in necleus, and complications mostly related to vessel rupture. Overpressure, wavelength, impulse, dose, and other various factors are considered to be involved in the occurence of these tissue damage. Among them, overpressure plays an important role in the occurence of vessel rupture, leading to tissue damage [12]. The threshold of shock waves is reported to be over 3-10 MPa for mammalian kidneys and lungs [2].

To introduce shock waves as safe treatment modality in the vicinity of central nervous systems and skull, it is mandatory to understand the threshold. In this study, we have developed reliable experimental settings to produce shock wave induced brain damage in rats, and performed preliminary study concerning pressure dependent damage.

Materials and Methods

Animal Experiments

Twenty 8-week-old male Sprague-Dawley rats weighing 250 to 270 g were initially anesthetized with halothane, and maintained using a mixture of 70% nitric oxide, 30% oxygen, and 1.0% halothane. The rats were then placed on a heating pad to control the rectal temperature at 38°C during the surgery. Rat's head was shaved and fixed with a head holder. The left parietal bone was exposed after making a 4-cm skin and galeal incision at the midline. A bone defect (5 ×7 mm) was made bilaterally in the convexity using surgical drill. The edge of the bone defect was made precisely vertical to the dura, which was kept intact. A shock wave generator was placed above the left side. The shock wave was focused at 3mm lateral from midline and 4mm caudal of bregma. Water bath, made by acrylic cylinder and skin flap, was placed between brain and shock wave generator to avoid attenuation of shock wave. Water bath was filled with physiological saline and kept at 38°C.

The rats were divided into 7 groups according to the given pressure of shock wave, and acquired time of the specimen after shock wave exposure. Each group was included 4 rats. Group A, served as control, and did not receive any shock wave exposure after craniotomy. Group B, C, D received single exposure of shock waves over 10 MPa. The rats were sacrificed at 4, 24, and 72 hours after shock wave exposure, respectively. Group E,

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F, G received single exposure of shock wave around 1 MPa. The rats were sacrificed at 4, 24, and 72 hours after shock wave exposure, respectively. All animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University.

Shock waves generator

In the ellipsoidal shape shock wave generator, set of AgN3 in Nd YAG laser. And shock waves focus to one point.

Histological Analysis

In each group, the animals were killed by intraperitoneal injection of pentobarbital (20 mg/kg) and specimen were obtained by perfusion fixation. Heparinized saline were injected through the left ventricle with followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The rats were decapitated and their brains were stored in fixative overnight at 4°C. The fixed brains were embedded in paraffin blocks and cut coronally into5μm-thick sections. Each section was stained with hematoxylin and eosin (H&E) and examined using optical microscopy.

Immunohistochemistry

5μm-thick sections made from paraffin-embedded tissue were used. The sections were incubated with blocking solution and reacted with mouse monoclonal anti-Hsp70 antibody (Stressgen Bioreagent #SPA-810) at a dilution of 1:500. Immunohistochemistry was performed using the avidin-biotin technique, and then the nuclei were counterstained with methyl green.[10]

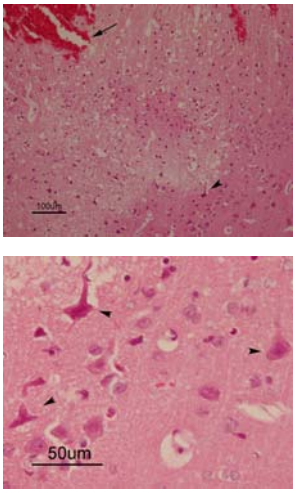
Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end-labeling staining

To clarify the spatial distribution of DNA fragmentation, terminal deoxynucleotidyl transferase-mediated biotinylate dUTP nick end labeling (TUNEL) were performed. The sections fixed by 4% paraformaldehyde were prepared as described above and were incubated with NeuroPore (Trevigen, Gaithersburg, MD #4820-30-01) for 30 min. They were placed in 1x terminal deoxynucleotidyl transferase (TdT) buffer (Invtrogen) for 15 min, followed by reaction with a TdT enzyme (Invtrogen #10533-065) and biotinylated 16-dUTP (Roche Diagnostics #1093070) at 37°C for 120 minutes. The sections were washed two times in saline-sodium citrate (150 mmol/l sodium chloride and 15 mmol/l sodium citrate, pH 7.4) for 15 minutes, followed by washing in PBS two times for 15 minutes. The avidin-biotin technique was applied, and then the nuclei were counterstained with methyl green solution for 20 min. [9]

Results

Morphological changes of rat brain

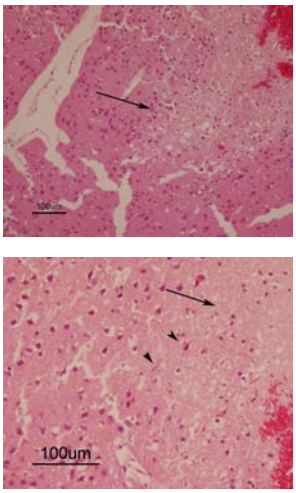
Subcortical hematoma and contusion were commnon findings in group B, C and D. Fragmented neuron, oligodendroglia and astrocyte could be observed around the hematoma. In addition, necrosis was widely distributed in these lesions in group C and D. Although there were no marked morphological changes in group E, F, and G, fusiform shaped nucles were observed in some neuron. However, this change could no be observed in oligodendroglia and astrocyte.



(a)

(b)

Figure 1: In group B, hemorrhage can be observed in the cortex (arrow). Beyond the lesion, spindle-shaped necleus cell were found in some specimen (arrow head). (a) H&E stain x10, (b) x40.



(c)

(d)

Figure 2: In groupC, the necrotic changed area (arrow) was more remarkable than 4hours after exposed shock wave, and spindle-shaped cell were highly transformed (arrow head). (c) H&E stain x10, (d) x20.

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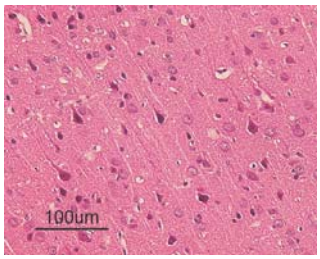
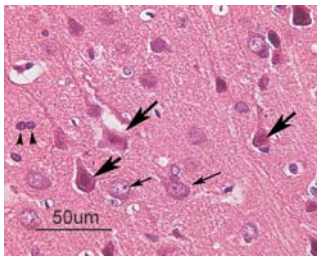


(e)

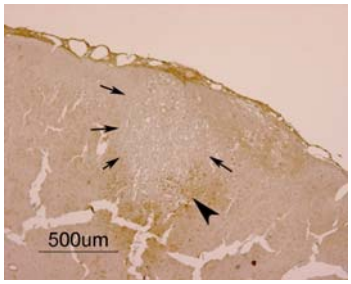


(f)

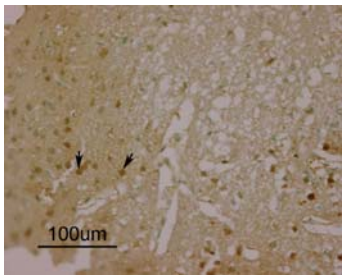
Figure 3: In group F, oligodendroglia (arrow head) and astrocyte (small arrow) did not shape shift, but neuron morph into fusiform-shaped (big arrow). (e) H&E stain x20, (f) x40.

Immunohistochemical detection of heat shock protein 72

In group C and D, heat shock protein 72 positive cell was detected periphery brain damaged area. A number of positive cell in group C was higher than group D.



(g)

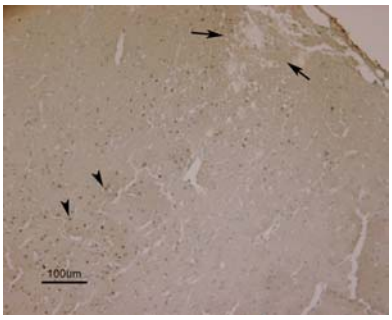


(h)

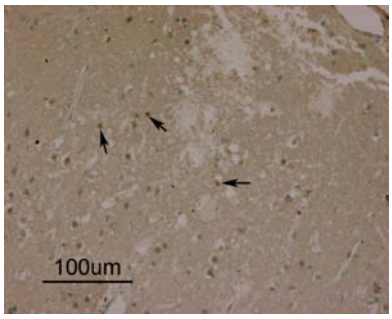
Figure 4: Immunohistochemical detection of heat shock protein 72 in the groupC rat brain. Heat shock protein 72 positive cells were showed at the periphery of necrosis. (g)x4, (h)x20.

TUNEL positive cell

In goup C and D, marked TUNEL positive cells could be observed around brain damaged area. In contrast, cleary TUNEL positive cell could not be obserbed in group E, F, G (nearly 1 MPa shock wave exposed rat).



(i)



(j)

Figure 5: TUNEL positive cells (arrow) could be observed. in goupC rat brain . apoptosis cells were scattered in tissure brain damaged area. (i)x10, (j)x20.

Conclusions

In the past, extensive studies have been conducted on tissue damage induced by shock waves in the kidney, lung, liver, and gallbladder. Histologically, the main features of damage are the rupture of blood vessels leading to hematoma, hemolysis leading to thromboembolism, and laceration of tissues when the exposed overpressure exceeds the threshold of the target tissue. In the present experiment, subcortical hemorrhage and contusion were common findings after application of shock wave over 10 MPa. In addtion, fragmentation of neuron and glial cell could be observed from 4 hours after application of shock wave. It is noteworthy that spindle shape deformity could sometimes be observed after application of 1 MPa. Kodama, et al reported this spindle shape change after application of shock waves using microexplosive in rabitt liver at MPa. These result may indicate the vulnerability of brain tissue to pressure dependent shock wave insult. This vulnerability might be due to existence of fine vessel in the brain [12]. It is noteworthy that necrosis prominently appeared from 24 hours after shock wave expossure around the hemorrhage lesion. Becase prominent appearance of necrosis usually occure after 24 hours, it might be suspected that shock wave induced brain insult is different in both mechanism and show time course compared to ischemic and traumatic induced brain

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injury. In the future work, the difference between shock wave induced brain insult and ischemic / traumatic insult should be clarified including long term effect.

Heat shock protein 72, when induced in the cellular response to cytotoxic stress, prevents cell death triggered by a variety of apoptotic stimuli, including heat shock, and ischemia.[1,11]Heat shock protein 72 was widely believed to protect cell from heat stress. Acording to previous reports, heat shock protein acted to block activation or activity of one of the caspase, and /or to protect the substrates of these caspase from proteolytic degradation.[6] In the present experiment, heat shock protein 72 positive and TUNEL postive cell could be found adjacent to the hemorrhage lesion after application of shock wave over 10 MPa.

The data suggest that shock waves causes two different type of cell death as apoptosis and necrosis. H&E stain and TUNEL stain result shows that apoptosis localized with a focus on neuronal cell.

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