Cavitation-dependent brain hemorrhage, a novel contusion rat model with non-invasive and highly reproducible protocol

OP-047

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Introduction

Contusion is the frequently encountered lesion in traumatic brain injury (TBI), which is characterized by **hemorrhages** leaking from ruptured capillaries and **dying neurons** showing cytoplasmic shrinkage and nuclear pyknosis. Several TBI/contusion models have been established (such as weight-drop model), however, all of these models have knotty disadvantages, including requirement of craniotomy and less control over injury parameters. The aim of our study is to evaluate whether **shock wave (SW)-induced cativation** is sufficient for causing rat brain hemorrhagic necrosis as a novel contusion model.

Numerous studies have confirmed the formation of microbubbles, i.e. cavitation, during the passage of a SW through a fluid. It also has been shown that SW causes the microbubbles to repeatedly expand and contract or abruptly collapse. In the circulatory system, these effects may disturb the integrity of the tight junctions of the brain capillaries, i.e. opening of blood-brain barrier (BBB).

Therefore, from the late 2000s, there has been an emerging idea that SW-induced cativation **holds the potential to create brain injury mimicking TBI pathology, particularly contusion** [1]. Many efforts have been made to experimentally evaluate the effects of SW on the animal brains, and demonstrated intracerebral hemorrhage following BBB opening and contusion-like brain injury around the hemorrhage in the rat brain after SW exposure. Unfortunately, most of these preclinical studies performed craniotomy to increase the susceptibility of brain tissue to SW, which is NOT the clinical case that contusion is usually found in a closed head. Moreover, these studies used home-made instrument to generate SW, increasing the difficulty to other scientists to repeat the experiments in their own labs.

The present study introduced an **extracorporeal SW lithotripter** (***Richard Wolf's PiezoWave***, which has been world-widely purchased in many hospitals) as the source of SW. We non-invasively applied SW generated by *PiezoWave* to the **rat head without craniotomy**. After SW exposure, **Evan Blue dye** was injected for quick check of the BBB opening by eyes. The brains were then histologically examined for the contusion-like brain injury by **H&E staining**.

Materials and Methods

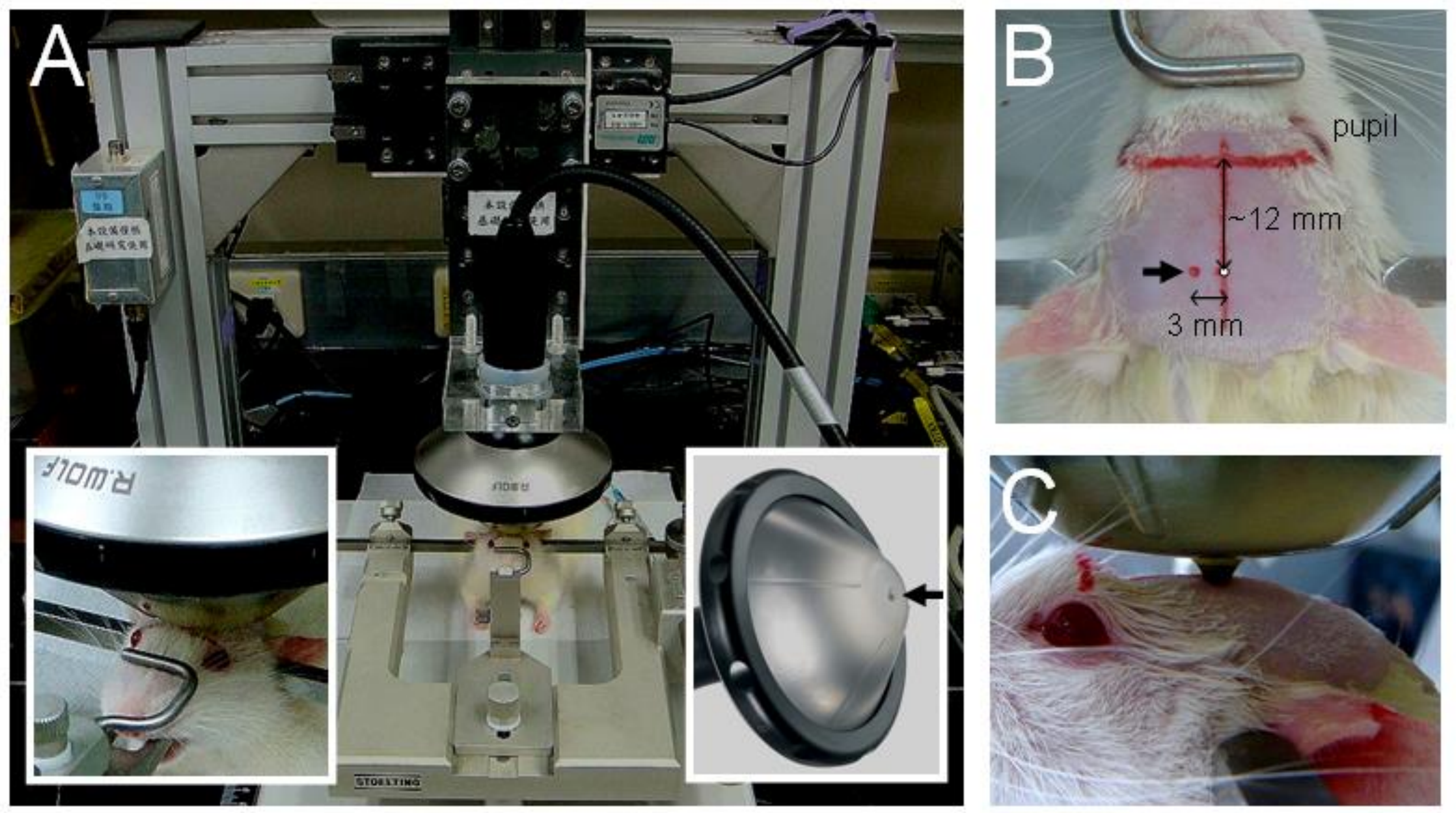

Figure 1. Setup for SW-induced contusion rat model. **(A)** SD rats (male, 8~9 weeks old) were used. The rats were anesthetized with chloral hydrate, and their heads were firmly fixed with a stereotaxic frame. The SW generator was hold with a home-made, computer-controlled positioning device, whose spatial resolution is 0.1 mm. The SW generator was then moved to the top of the rat head, as shown in the lower-left photo. The center of the SW generator was indicated by a raised spot, as pointed out by the arrow in the lower-right photo. **(B)** Immediately after anesthetization, the rat head was shaved to expose the skin, and a sign (arrow) indicating the targeted SW focus was marked on the skin with a pen. In this study, the SW focus was set at 0 mm to Bregma (~12 mm posterior to the eyes) and 3 mm lateral to the head midline. **(C)** The raised spot on the SW generator was carefully moved to cover the marked sign. The conductive gel was then given to the space between the generator and the rat head. The SW was generated for 500 times at a frequency of 5 time/seconds and at a strength of 17.4 MPa. For enhancing SW, microbubbles (*SonoVue*) was i.v. injected at a dose of 0.1 mL/100 gram of body weight.

1. Divani AA, Murphy AJ, Meints J, Sadeghi-Bazargani H, Nordberg J, Monga M, Low WC, Bhatia PM, Beilman GJ, SantaCruz KS. (2015) A novel preclinical model of moderate primary blast-induced traumatic brain injury. *J Neurotrauma. 32:1109-16.*

Results

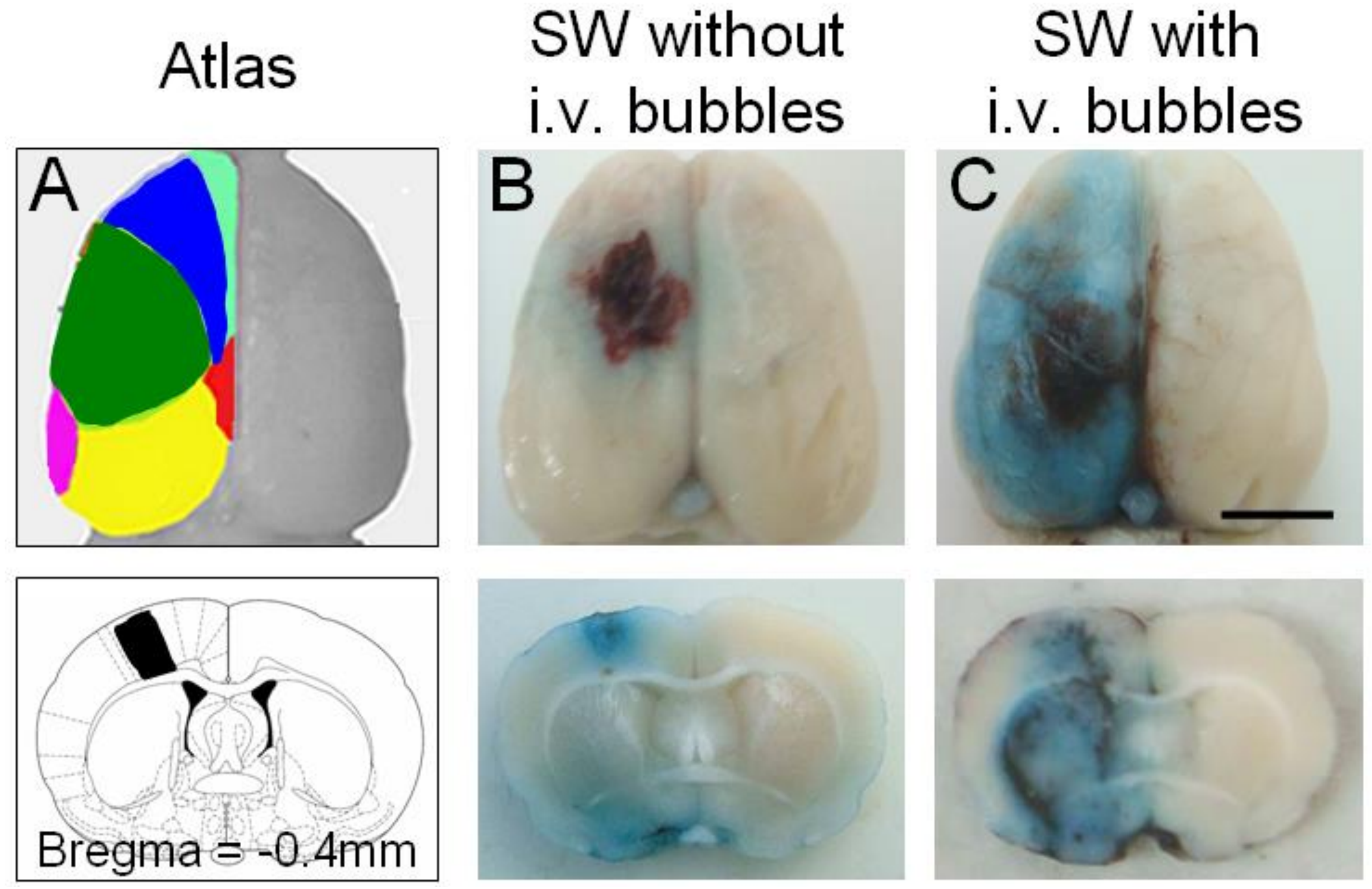

Figure 2. Obvious BBB opening and subdural / intracerebral hemorrhages 4 hours after short exposure (~1.6 minutes) of non-invasive SW. **(A)** Relevant atlas of rat brain. Upper, overlooking view: somatosensory cortex, motor cortex, visual cortex. Lower, transverse view: somatosensory cortex (forelimb part). **(B)** SW without i.v. injection of contrast agent (i.e. microbubbles). BBB opening was mainly found in the somatosensory cortex. Unexpected BBB opening was found in the lower part of the brain. **(C)** Enhanced SW with injection of microbubbles. BBB opening was found in almost the whole hemisphere. Intracerebral hemorrhages were also readily found by naked eyes.

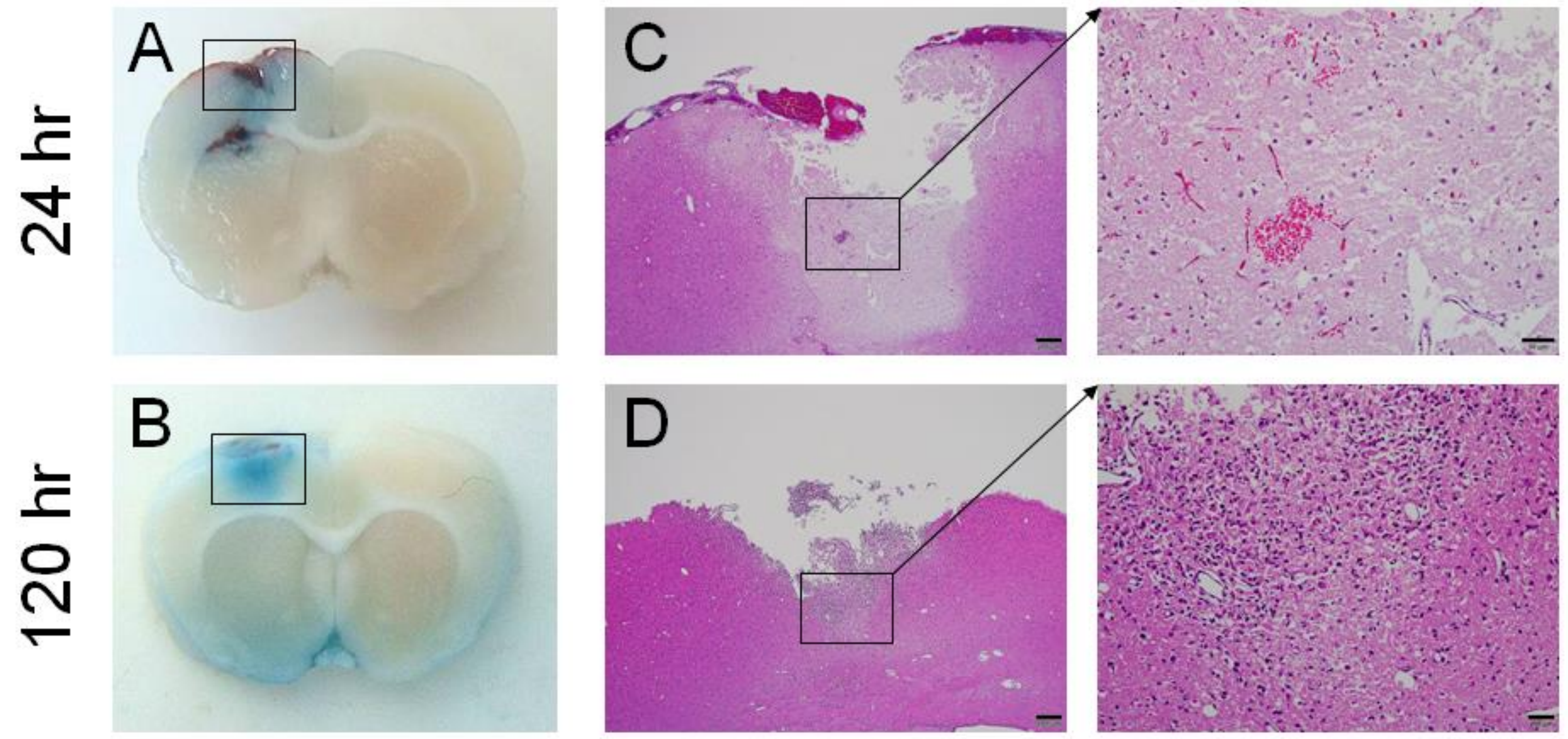

Figure 3. Development of contusion-like hemorrhagic necrosis followed by gliosis in the cortex after SW exposure. **(A, B)** Brain slices dissected after sacrifice. These slices were then further examined by H&E staining. **(C)** H&E stained section from the brain collected 24 hr post SW. Left: The area with less stained eosin, suggesting necrosis. Right: Enlarged view, showing hemorrhages dispersed in the necrotic tissue. **(D)** H&E stained section from the brain collected 120 hr post SW. Left: The shrunken cortex, suggesting neuronal loss after contusion. Right: Enlarged view, showing obvious gliosis after neuronal loss.

Conclusions

Advantage of the contusion model induced by shock wave:

- > using a commercial lithotripter
- > non-invasive, highly reproducible, fast
- > adjustable focus, controllable severity