Pressure-dependent effect of shock waves on rat brain: induction of neuronal apoptosis mediated by a caspase-dependent pathway

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Object. Shock waves have been experimentally applied to various neurosurgical treatments including fragmentation of cerebral emboli, perforation of cyst walls or tissue, and delivery of drugs into cells. Nevertheless, the application of shock waves to clinical neurosurgery remains challenging because the threshold for shock wave–induced brain injury has not been determined. The authors investigated the pressure-dependent effect of shock waves on histological changes of rat brain, focusing especially on apoptosis.

Methods. Adult male rats were exposed to a single shot of shock waves (produced by silver azide explosion) at overpressures of 1 or 10 MPa after craniotomy. Histological changes were evaluated sequentially by H & E staining and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL). The expression of active caspase-3 and the effect of the nonselective caspase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK) were examined to evaluate the contribution of a caspase-dependent pathway to shock wave–induced brain injury.

High-overpressure (> 10 MPa) shock wave exposure resulted in contusions hemorrhage associated with a significant increase in TUNEL-positive neurons exhibiting chromatin condensation, nuclear segmentation, and apoptotic bodies. The maximum increase was seen at 24 hours after shock wave application. Low-overpressure (1 MPa) shock wave exposure resulted in spindle-shaped changes in neurons and elongation of nuclei without marked neuronal injury. The administration of Z-VAD-FMK significantly reduced the number of TUNEL-positive cells observed 24 hours after high-overpressure shock wave exposure (p < 0.01). A significant increase in the cytosolic expression of active caspase-3 was evident 24 hours after high-overpressure shock wave application; this increase was prevented by Z-VAD-FMK administration. Double immunofluorescence staining showed that TUNEL-positive cells were exclusively neurons.

Conclusions. The threshold for shock wave–induced brain injury is speculated to be under 1 MPa, a level that is lower than the threshold for other organs. High-overpressure shock wave exposure results in brain injury, including neuronal apoptosis mediated by a caspase-dependent pathway. This is the first report in which the pressure-dependent effect of shock wave on the histological characteristics of brain tissue is demonstrated.

Key Words • apoptosis • caspase • necrosis • shock wave • silver azide • rat

Shock waves, acoustic pressure wave pulses that last only a microsecond and reach a pressure of up to 100 MPa, can propagate through different substances without significant energy dissipation if the acoustic impedance values of the substances are similar. Because of the similarity in the acoustic impedance values of water and the soft tissues of the body, shock waves generated under water can be transmitted through these tissues without substantial loss of energy. At transition sites between tissues with different acoustic impedance values, however, energy dissipation occurs, and there may be focal mechanical destruction—probably produced through induction of cavitation and shearing stress caused by the reflected waves. On the basis of these characteristics, shock waves have been used in a variety of treatments. Since the first clinical applications of extracorporeal shock wave lithotripsy, several million patients have been treated worldwide, mainly for renal calculi and gallstones, as well as for calculi at other locations including the bile duct, the pancreas, and the salivary glands. Since the 1990s, attempts have been made to apply shock waves to long bones for bone formation and reunion, to the femoral head for the treatment of osteonecrosis, and to pain centers for the treatment of tennis elbow, painful heel, and calcifying tendinitis of the shoulder.

In addition, recent interest has focused on alternative minimally invasive applications within microenvironments, such as enhancing angiogenesis for the treat-
ment of ischemic heart disease;\(^9\) improvement of microcirculation and perfusion in the extremities;\(^7\) experimental applications for treatment of cardiac, peripheral,\(^{26}\) and cerebral emboli;\(^{28,36}\) tissue dissection;\(^{44,45}\) and experimental application of shock wave to enhanced molecular transfer systems or target cells for cancer therapy.\(^{11,22,23,33}\) The results of these studies have encouraged us to explore the potential of shock wave therapy as a novel treatment for pathologic conditions in the area of the brain and skull. Possible therapeutic applications include performing cranioplasty without the aid of foreign material, using the osteoinductive effect of shock wave on bone;\(^{27,58}\) facilitating drug delivery by using shock waves applied through a neuroendoscope or a catheter to alter local permeability of the blood–brain barrier;\(^{51}\) treatment of certain neural pain and ischemic disorders by local delivery of shock waves through an endoscope or directly through the skin; and enhancement of fibrinolysis by the catheter-based delivery of shock waves proximal to cerebral emboli.\(^{28}\)

To apply shock waves to the central nervous system for the aforementioned purposes, we have developed a compact shock wave generator and brain protection method using air-containing materials including cottonoids\(^{52}\) and expanded polytetrafluoroethylene dura substitute (W. L. Gore and Associates, Inc.).\(^{56}\) These techniques have allowed us to concentrate shock waves at a target and avoid shock wave propagation beyond the target (that is, to ensure safe treatment).

Despite our efforts to apply shock wave therapy to the treatment of central nervous system disorders, the use of shock wave in the brain remains challenging due to lack of knowledge about the threshold for shock wave–induced brain injury. To address this critical issue, we have developed a reliable experimental apparatus to evaluate shock wave–induced brain injury using a microexplosive (silver azide pellet) and have evaluated the pressure–dependent effect of shock wave on rat brain. We focused in particular on the occurrence of apoptosis, which is known to play a role in both ischemic and traumatic brain injury.\(^{20,21,26,47}\) We further investigated the contribution of the caspase–dependent apoptotic pathway, one of the major pathways mediating ischemic and traumatic brain injury,\(^{26,43}\) to shock wave–induced brain injury by administering a nonselective caspase inhibitor (Z-VAD-FMK) either before or before and after shock wave exposure.

### Materials and Methods

**Shock Wave Source**

An underwater shock wave was generated using a microexplosive\(^{11,39}\) and a cranial shock wave applicator consisting of an ignition compartment and an elliptical paraboloid reflector for focusing the shock waves. Between 1 and 400 \(\mu\)g of microexplosive (silver azide) was placed in the reflector and ignited using an Nd:YAG laser (Model MYL-100m Laser Photonics; 1064-\(\mu\)m wavelength, 7-\(\mu\)sec pulse width, and 25-mJ/pulse maximum energy). The weight of microexplosive was measured using a microdigtal balance with a readability of 0.0001 mg (Supermicro S4, Sartorius). The Nd:YAG laser was guided by a quartz optical fiber with a core diameter of 400 \(\mu\)m (Sparkling Photon, Inc.). The overpressure of the generated shock waves was measured using a polynylidene fluoride needle hydrophone (Imotec Messtechnik) with a 0.5-mm-diameter sensing element of 0.34 \(\mu\)g/cm\(^2\) and a rise time of 50 nsec. The hydrophone was located 6 mm lateral to the center axis of the reflector and 2 mm proximal to the silver azide from the center between the silver azide and the focus point, as measured on the center axis (Fig. 1).

Measured data were stored and displayed on a digital oscilloscope (Model DL 716, Yokogawa). The mean overpressure (± standard deviation) of the generated shock waves was 1.0 ± 0.2 MPa for the low-overpressure shock waves and 12.5 ± 2.5 MPa for the high-overpressure shock waves, and the two types of shock waves were generated using 1 to 3 \(\mu\)g and 100 to 350 \(\mu\)g of silver azide, respectively. The high pressure values are within range of the pressure used during shock wave lithotripsy for the treatment of urinary calculi; moreover shock waves in this range (~ 10 MPa) were shown to cause histological damage in rat brain in our preliminary study.\(^{49}\) The low pressure value was selected to be under the value of the reported threshold for pressure–dependent damage in liver organs (~ 10 MPa for kidney,\(^21\) 2–10 MPa for lungs,\(^{11}\) and 10 MPa for liver\(^{49}\)).

**Experiment 1: Histological Evaluation of Shock Wave–Induced Brain Injury**

Anesthesia was initially induced in 56 Sprague–Dawley rats (age 8 weeks, weight 250–270 g) using diethyl ether and subsequently maintained with a mixture of nitrous oxide, halothane, and oxygen. The rats were placed on a heating pad to maintain body temperature at 38˚C during surgery. The arterial blood gases were kept at normal levels. In addition, local anesthesia was induced with subcutaneously administered 1% lidocaine solution. The head was shaved and fixed in a stereotactic head holder (Narisighe Scientific Instrument Lab), and ointment was applied to the eyes to protect vision during the procedure. A linear incision was made through the skin on the head and the pericranium, and a single opening (5–7 mm) was made in the left side of the skull using a microdrill (Narisighe Scientific Instrument Lab) with the aid of a microscope. Care was taken to avoid injuring the underlying dura mater. An acrylic holder (inner diameter 28 mm, outer diameter 30 mm, height 35 mm) was fixed tightly to the skull with the skin flap and filled with 5 ml physiological saline (38˚C) to avoid shock wave attenuation at the target. A shock wave generator was set just above the left convexity, and a single shot of shock waves was delivered to the left cerebral hemisphere (Fig. 1). The shock waves were theoretically focused to the CA1 region of the left hippocampus (4 mm caudal to the bregma, 3 mm lateral to the midline, and 4 mm from the brain surface), using a laser diode. The animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University Graduate School of Medicine.

**Experiment 2: Investigation of Mechanism of Shock Wave–Induced Brain Injury**

Twenty-eight 8-week-old male Sprague–Dawley rats were divided into three groups according to shock wave exposure. Group 1 (four rats) served as a control group and did not receive any shock wave exposure; the animals in Group 2 (12 rats) received high-overpressure shock waves; and the animals in Group 3 (12 rats) received low-overpressure shock waves. Four, 24, or 72 hours after shock wave exposure, the animals were perfused intracardially with heparinized saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and then decapitated. Brain tissue samples were obtained, embedded in paraffin, and cut coronally in 5-\(\mu\)m-thick sections. The sections were stained with H & E and examined using an optical microscope.
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tions 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 minutes.

To clarify the cellular subpopulation of DNA-fragmented cells, we performed double staining for NeuN with TUNEL, GFAP with TUNEL, and CNPase with TUNEL using a fluorescence method; the NeuN, GFAP, and CNPase are used as markers for neurons, glia, and oligodendroglia, respectively. The tissue sections were immunostained with mouse anti–NeuN monoclonal antibody (Chemicon), rabbit anti–GFAP polyclonal antibody, or mouse anti–CNPase monoclonal antibody (Chemicon), with biotinylated anti–mouse immunoglobulin G (Vector Laboratories) or biotinylated anti–rabbit immunoglobulin G (Vector Laboratories), followed by fluorescent avidin DCS (Vector Laboratories). The sections were incubated with NeuroPore for 30 minutes and placed in 1 × TdT buffer for 30 minutes, then incubated with TdT enzyme and biotinylated 16-deoxyuridine triphosphate at 37°C for 120 minutes. The sections were washed two times in saline sodium citrate for 15 minutes and two times in PBS for 10 minutes. Texas Red avidin DCS was applied to the sections for 30 minutes. Subsequently, the slides were covered with Vectashield mounting medium with 4,6-diamidino-2-phenylindole (Vector Laboratories). Fluorescence of fluorescein was observed at excitation of 495 nm and emission of more than 515 nm, and fluorescence of Texas Red was observed at excitation of 510 nm and emission of more than 580 nm. Fluorescence of 4,6-diamidino-2-phenylindole was observed at excitation of 360 nm and emission of more than 460 nm.

**Experiment 3: Contribution of a Caspase-Dependent Pathway to Neuronal Apoptosis After Shock Wave Exposure Application**

To investigate the contribution of the caspase-dependent apoptotic pathway, which is one of the major pathways by which neuronal apoptosis is regulated after cerebral ischemia and traumatic brain injury,10,21,26,43 we examined the effect of intraventricular administration of Z-VAD-FMK, a nonselective caspase inhibitor, on apoptosis 24 hours after high-overpressure shock wave exposure. Twenty-six 8-week-old male Sprague–Dawley rats were divided into four groups according to the patterns of intraventricular injection of Z-VAD-FMK. The administration protocol was based on that of studies designed to examine the effect of Z-VAD-FMK in a model of cold injury–induced brain trauma.46 The animals were exposed to high-overpressure shock waves according to the protocol used for Group 2 (the high-overpressure group) in Experiment 1. After anesthesia was induced using halothane, the drug (Z-VAD-FMK) or a control solution (0.9% NaCl, Wako Biomedical), was injected intraventricularly at a location 2.5 mm posterior to the bregma, 2 mm left of the sagittal line, and 3.0 to 4.0 mm deep from the dorsal surface of each rat’s skull. Group A (eight animals) served as a control group, and the rats in this group received DMSO 1 hour before and 1 hour after shock wave exposure.46 The rats in Group B (five animals) each received a low dose of Z-VAD-FMK (10 μg in 20 μl DMSO) 15 minutes before shock wave exposure.26 The rats in Group C (six animals) each received a high dose of Z-VAD-FMK (100 μg in 20 μl DMSO) 15 minutes before shock wave exposure. The rats in Group D (seven animals) each received the high dose of Z-VAD-FMK twice, 15 minutes before and 6 hours after shock wave exposure. Specimens were obtained at 24 hours after shock wave exposure. The animals were perfused intracardially with heparinized saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and were then decapitated. Paraffin-embedded brain tissue samples were cut into 5-μm sections and deparaffinized with xylene. The following steps were performed in a humidified chamber at room temperature. All sections were processed for TUNEL evaluation. To confirm the involvement of a caspase-dependent pathway in shock wave–induced brain injury, we performed immunohistochemical analysis for active caspase-3, which plays a pivotal role in caspase-dependent apoptosis. The paraffin sections were immunostained with rabbit anti–active caspase-3 polyclonal antibody (Pharmingen), which detects the active form of caspase-3. Immunohistochemical analysis was performed using the avidin-biotin technique.

**Statistical Analysis**

The number of TUNEL-positive cells was counted in a high-powered field (× 400) by an investigator who was blinded to the study conditions. The results were expressed as mean numbers of cells per square millimeter ± standard deviation. The statistical significance of differences between the time points in each group was evaluated by analysis of variance. A probability value of less than 0.05 was considered significant.

**Results**

**Experiment 1: Histological Evaluation of Shock Wave–Induced Brain Injury**

Intracerebral hemorrhage was observed both in cortical and subcortical regions from 4 hours after shock wave exposure (Fig. 2A and D) in the animals that had been exposed to high-overpressure shock waves (Group 2). Cerebral herniation was observed around the hemorrhage as early as 24 hours after shock wave exposure (Fig. 2B and E) and was further increased at 72 hours (Fig. 2C and F). After low-overpressure shock wave application (Group 3)—although no significant hemorrhage was evident in either the cortical or the subcortical regions throughout the time sequence—mild morphological changes such as spindle-shaped changes of neurons and elongation of nuclei toward the shock wave source were detected around 24 hours after shock wave exposure (Fig. 3). Interestingly, there was no significant histological damage at the theoretical shock wave focus, which was located at the left hippocampus, in either Group 2 or Group 3.
Experiment 2: Involvement of Apoptosis in Shock Wave–Induced Brain Injury

To elucidate whether DNA fragmentation is induced after shock wave application, we examined in situ labeling of DNA breaks in rat brain (Fig. 4). Normal neuronal cells in sections from the control animals were not stained by the TUNEL process (data not shown). In contrast, TUNEL produced two different patterns of staining in the brain tissue from animals that had undergone shock wave exposure. A significant quantity of cells around the contusional lesion created by high-overpressure shock wave exposure appeared densely stained in their nuclei, and small particles that resembled apoptotic bodies were visible around the nuclei (Fig. 4B and D). These findings are compatible with descriptions of the apoptotic cell death process. Cells that appeared only slightly stained were also observed in the TUNEL analysis; these cells showed diffuse nuclear and cytoplasmic staining consistent with the appearance of necrotic cells. Only the densely labeled cells were considered TUNEL positive in this study.

Fig. 2. Photomicrographs showing sections obtained from animals in Group 2 (high-overpressure group) 4 (A and D), 24 (B and E), and 72 (C and F) hours after application of high-overpressure shock waves. Intracerebral hemorrhage was observed in both cortical and subcortical regions from 4 hours after shock wave exposure (A and D). Contusional brain injury was evident around the hemorrhage as early as 24 hours after shock wave exposure (B and E), and was further increased at 72 hours (C and F). The arrows indicate the direction of shock wave application. H & E. Original magnifications ×100 (A–C) and ×400 (D–F).

Fig. 3. Photomicrographs of sections obtained from animals in Group 3 (low-overpressure group) 4 (A and D), 24 (B and E), and 72 (C and F) hours after shock wave application. Although no hemorrhage was seen, mild morphological changes such as spindle-shaped changes of neurons and elongation of nuclei in the direction of shock wave exposure were detected about 24 hours after application of shock waves. The arrows indicate the direction of shock wave application. H & E. Original magnifications ×100 (A–C) and ×400 (D–F).
As illustrated in Figs. 5A through D and 6, only a small number of TUNEL-positive cells were detected in sections from animals killed 4 hours after shock wave exposure in both groups. In the high-overpressure group, we found a significant increase in the number of TUNEL-positive cells at 24 hours compared with 4 hours (p < 0.01; Fig. 5B and E) and a decrease in the number of TUNEL-positive cells at 72 hours compared with 24 hours (p < 0.01; Fig. 5C and F). These results are compatible with previous reports of the temporal profile of apoptotic neurons after focal ischemia in rats. 21 We observed TUNEL-positive cells dispersed throughout the area around the contusional lesion in sections from the animals in Group 2 (the high-overpressure group). In contrast, there was no significant increase in the number of TUNEL-positive cells in sections from the animals in Group 3 (the low-overpressure group). The high-overpressure group showed a significantly higher number of TUNEL-positive cells at 24 hours (p < 0.01) and 72 hours (p < 0.05) after shock wave administration compared with the low-overpressure group (Fig. 6).

To further investigate the TUNEL-positive cells, we performed double immunostaining using TUNEL and NeuN, GFAP, or CNPase to identify neurons, glial cells, and oligodendroglial cells, respectively. The TUNEL-positive cells were exclusively double-labeled with NeuN (Fig. 7A–C), but not with GFAP (Fig. 7D–F) or CNPase (Fig. 7G–I). These results indicate predominant involvement of neuronal apoptosis after high-overpressure shock wave application.

![Fig. 4. Photomicrographs of sections obtained 24 hours after high-overpressure shock wave application (Group 2). The arrows indicate the direction of shock wave application. Two different patterns of staining may be observed. Significant quantities of cells around the contusional lesion created by high-overpressure shock waves show strong TUNEL positivity in their nuclei with small particles around the nuclei that resemble apoptotic bodies. Other cells demonstrate slight TUNEL positivity with diffuse nuclear and cytoplasmic staining, a pattern consistent with necrosis. H & E (A and C) and TUNEL staining (B and D). Original magnifications × 100 (A and B) and × 400 (C and D).](image1)

![Fig. 5. Photomicrographs demonstrating results of TUNEL assay performed on sections from animals exposed to high-overpressure (A–C) and low-overpressure (D–F) shock waves at 4 (A and D), 24 (B and E), and 72 hours (C and F) after exposure. At 24 and 72 hours, TUNEL-positive cells were observed dispersed throughout the area around the contusional lesion created by high-overpressure shock wave application (B and C). In contrast, there was no significant increase in the number of TUNEL-positive cells in the low-overpressure group (D–F). Original magnification × 400.](image2)
Experiment 3: Contribution of a Caspase-Dependent Pathway to Neuronal Apoptosis After Shock Wave Application

No significant decrease in the number of TUNEL-positive cells was seen after administration of a low dose of Z-VAD-FMK (Group B) or administration of a single high dose of Z-VAD-FMK (Group C) relative to the findings in vehicle-treated animals (Group A), although there was a tendency toward reduced numbers of TUNEL-positive cells in Group C (Fig. 8). Repeated administration of a high dose of Z-VAD-FMK (Group D) resulted in a significant reduction in the number of TUNEL-positive cells relative to the number in vehicle-treated animals 24 hours after high-overpressure shock wave administration (Fig. 9). To further confirm the involvement of a caspase-dependent pathway, we performed an immunohistochemical assay for active caspase-3. The expression of active caspase-3 was barely detectable in the contralateral cortex of rats that had been exposed to high-overpressure shock waves (Fig. 10B and D), whereas a significant increase in the expression of active caspase-3 was evident in the ipsilateral cortex of these animals (Fig. 10A). Anatomical distribution of active caspase-3 expression was completely in accordance with the distribution of TUNEL-positive neurons. Administration of Z-VAD-FMK partially prevented the increase in active caspase-3 expression (Fig. 10C). In combination, these results indicate that a caspase-dependent apoptotic pathway contributes, at least in part, to the occurrence of apoptosis after high-overpressure shock wave application to rat brain.

Discussion

New Observations Pertaining to Shock Wave–Induced Brain Injury

The results of the present study demonstrate, for the first time, that the application of high-overpressure (> 10 MPa) shock waves to rat brain results in cerebral contusional hemorrhage associated with neuronal apoptosis mediated by a caspase-dependent pathway in the surrounding cortex, whereas the application of low-overpressure (1 MPa) shock waves results in mild morphological changes such as spindle-shaped changes of neurons and elongation of nuclei toward the shock wave source without marked neuronal injury. Our findings suggest that the threshold for shock wave–induced brain injury may be lower than 1 MPa, which is a lower level than that reported for other organs, and that shock wave application results in both necrosis and apoptosis in a pressure-dependent manner, with a
temporal profile similar to that seen in ischemic and traumatic brain injuries. It should also be noted that establishment of our new shock wave–induced brain injury model allowed us to address these critical issues in the present study. Although the relationship between shock wave and cavitation has been discussed previously, mainly in studies of traumatic brain injury\textsuperscript{18,24,53,66} and investigations of the effect of shock wave treatment on fetal animals,\textsuperscript{41,67} due to difficulties in controlling shock wave parameters, there has until now been no appropriate experimental model to evaluate shock wave–induced brain injury.\textsuperscript{31,46} Several researchers have applied holmium:yttrium-aluminum-garnet (Ho:YAG) lasers directly above or within the brain and have noted local hemorrhage formation, brain vibration, and debris spreading,\textsuperscript{25,40} as well as the formation of hemorrhagic lesions opposite to the site of laser application,\textsuperscript{8} indicating involvement of shock waves. Among available shock wave sources, it is difficult to measure overpressure and pressure profiles of generated shock waves in piezoelectric and electrohydraulic lithotriptors due to their electromagnetic field. By using microexplosives and an elliptical paraboloid reflector in conjunction with computational fluid dynamic techniques, it is possible to generate conformal shock waves, and we have already applied this approach to the evaluation of liver injury.\textsuperscript{35,37} By using silver azide pellets in microgram quantities, this process is now used as a reliable experimental approach for evaluating shock wave–induced brain injury.

Threshold of Shock Wave–Induced Brain Injury

Macroscopic findings of the present study demonstrated the formation of contusional hemorrhage as early as 4 hours after high-overpressure shock wave application. Similar findings have been reported in other organs, including the kidney,\textsuperscript{42} liver,\textsuperscript{72} and lung,\textsuperscript{73} and are considered to be due to vessel rupture.\textsuperscript{9,46,68} In contrast, low-overpressure shock wave application resulted in mild morphological changes such as spindle-shaped changes of neurons and elongation of nuclei toward the shock wave source without marked neuronal injury. The underlying mechanism and clinical implications of such morphological changes are undetermined, although similar findings have been reported in a rabbit model of shock wave–induced liver injury.\textsuperscript{36} Furthermore, we also observed similar morphological changes in rats at the early time point after 90 minutes of middle cerebral artery occlusion that subsequently resulted in apoptosis.\textsuperscript{31} Thus we do not rule out the possibility that application of low-overpressure shock waves may result in apoptosis at a later time point. Based on the findings of the present study, we speculate that the threshold of pressure-dependent shock wave–induced brain injury is lower than 1 MPa, much lower than that in the kidney (3–19 MPa),\textsuperscript{42} lung (2–10 MPa),\textsuperscript{13} and liver (10 MPa).\textsuperscript{63} This difference is probably due to the intrinsic vulnerability of neurons as well as the presence of numerous fine blood vessels in the cortical region. Although in our study the theoretical shock wave focus was in the left hippocampus, pressure-dependent morphological changes following shock wave application were confined to the cortical and subcortical regions. The amount of hippocampal damage was highly variable, and a substantial number of the animals did not show any evidence of neuronal damage in the hippocampus after shock wave application, even after the high-overpressure dose. This negative finding may be due to technical limitations of the shock wave application in the current experimental setting, because the hippocampus can be very sensitive to pressure-dependent effects. This issue remains to be resolved in a future study using a different system of shock wave application.

Underlying Mechanism of Shock Wave–Induced Brain Injury

To elucidate the mechanism of shock wave–induced brain injury, we investigated the involvement of necrosis
It has been previously reported that apoptosis is most likely associated with the cytosol, the activation of various caspase enzymes, and the release of mitochondrial cytochrome c to the cytosol, the activation of various caspases, and the subcellular distribution of Bcl-2 family proteins would clarify the intrinsic characteristics of shock wave–induced apoptosis.

Similar processes are likely in the treatment of chronic nonunion or delayed union of long bones in which neo-vascularization, which may be promoted by microfracture and hemorrhage resulting from therapeutic shock waves, plays an important role in the healing mechanism.

On the other hand, cavitation, defined as the generation and movement of newly formed bubbles in a fluid, is reported to cause a transient increase in the permeability of the cell membrane (without the occurrence of cell death) when administered to cells in vitro, which leads to the transport of large molecules and nucleic acid into cells. Detrimental effects of shock wave such as morphological changes in the nucleus have also been reported, and complications were mostly related to vessel rupture. Various factors, including overpressure, wavelength, impulse, dose, and administration rate, are considered to be involved in the occurrence of shock wave–related tissue damage. Among them, pressure plays an important role in the occurrence of vessel rupture leading to tissue damage.

In the present study, application of high-overpressure (10 MPa) shock waves resulted not only in contusional hemorrhage due to vessel rupture but also in neuronal apoptosis mediated by a caspase-dependent pathway. Because caspase-dependent apoptosis is initiated by increased permeability of mitochondrial membranes and subsequent release of mitochondrial cytochrome c, it is conceivable that shock waves directly affect mitochondrial membrane permeability and thus lead to cytochrome c release and subsequent activation of caspase family proteases. Increases in the level of active caspase-3 after high-overpressure shock wave application as shown in the present study further substantiate the involvement of this biochemical pathway in shock wave–induced brain injury. It would be of great interest to investigate how shock waves affect mitochondrial membrane potential, membrane permeability, and translocation of cytochrome c and Bcl-2 family proteins, all of which play a crucial role in apoptosis, in neurons. It would also be of interest to elucidate the role of shock waves and the resultant cavitation in traumatic brain injury, particularly in blast-related injuries, by evaluating changes in immunohistochemical specimens after exposure to shock waves.
Pressure-dependent shock wave–induced brain injury of varying overpressures. These issues also remain to be elucidated in future studies.

Conclusions

The results of the present study suggest that the threshold of shock wave–induced brain injury is lower than 1 MPa, which is lower than the threshold reported to cause injury in other organs. High-overpressure shock wave (>10 MPa) exposure results in cerebral contusional hemorrhage associated with neuronal apoptosis in the surrounding cortex, whereas low-overpressure shock wave (1 MPa) exposure results in mild morphological changes such as neurons assuming a spindle shape and their nuclei becoming elongated in the direction of shock wave exposure without marked neuronal injury. Our findings also suggest that shock wave–induced apoptosis is mediated by a caspase-dependent pathway.

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