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A non-surgical model of cervical spinal cord injury induced with focused ultrasound and microbubbles

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HIGHLIGHTS

- Focused ultrasound with microbubbles created localized SCI in the rat.
- Pathology similar to that observed in compression and contusion rat models of SCI.
- Injuries were easily monitored using MRI at 24 h, 1 and 2 weeks.

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ABSTRACT

Background: The most commonly used animal models of spinal cord injury (SCI) involve surgical exposure of the dorsal spinal cord followed by transection, contusion or compression. This high level of invasiveness often requires significant post-operative care and can limit post-operative imaging, as the surgical incision site can interfere with coil placement for magnetic resonance imaging (MRI) during the acute phase of SCI. While these models are considered to be similar to human SCI, they do not occur in a closed vertebral system as do the majority of human injuries.
New method: Here we describe a novel, non-surgical model of SCI in the rat using MR-guided focused ultrasound (FUS) in combination with intravenous injection of microbubbles, applied to the cervical spinal cord.
Results: The injury was well-tolerated and resulted in cervical spinal cord damage in 60% of the animals. The area of Gd-enhancement immediately post-FUS and area of signal abnormality at 24 h were correlated with the degree of injury. The extent of injury was easily visualized with T2-weighted MRI and was confirmed using histology.
Comparison with existing method(s): Pathology was similar to that seen in other rat models of direct spinal cord contusion and compression. Unlike these methods, FUS is non-surgical and has lower mortality than seen in other models of cervical SCI.
Conclusions: We developed a novel model of SCI which was non-surgical, well-tolerated, localized, and replicated the pathology seen in other models of SCI.

Abbreviations: SCI, spinal cord injury; FUS, focused ultrasound; MR, magnetic resonance; MRI, magnetic resonance imaging; Gd, gadolinium; BBB, blood–brain barrier; BSCB, blood–spinal cord barrier; H&E, haematoxylin and eosin; LFB, luxol fast blue; GFAP, glial fibrillary acidic protein.

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1. Introduction

It is widely accepted that acute SCI is bi-phasic, where the primary mechanical injury, caused by forces including compression, contusion, shear, distraction and dislocation, is followed by a secondary injury mechanism (Bartholdi and Schwab, 1996; Ramer et al., 2000; Rowland et al., 2008; Sekhon and Fehlings, 2001; Tator and Fehlings, 1991; Tator, 1995). As the primary injury has already occurred, the target of most therapies is to prevent damage resulting from the secondary injury mechanisms which occur over a period of hours to weeks following the initial insult. These secondary mechanisms include vascular dysfunction, edema, ischemia, inflammation, excitotoxicity, electrolyte shifts, free radical production, and apoptosis. There have been at least 9 randomized prospective controlled clinical trials of neuroprotective pharmacological agents in acute SCI, however none have demonstrated convincing neurological benefits in spite of promising results in pre-clinical studies (Rowland et al., 2008; Tator, 2006). It has been suggested that due to the heterogeneity of human SCI, treatments should be tested in a range of pre-clinical models prior to clinical trials (Schwab et al., 2006). Regenerative therapies are also a subject of active investigation as the adult central nervous system (CNS) has a limited ability to regenerate following injury (Rowland et al., 2008).

Animal models of SCI improve the understanding of the various mechanisms involved in the secondary phase of SCI, and permit the testing of potential treatments. The most commonly used animal models of SCI involve surgical exposure of the dorsal spinal cord followed by contusion, compression, or transection of the cord (Blight, 2000; Ramer et al., 2000). Rats are frequently used due to their size and availability (Onifer et al., 2007) and pathology similar to that seen in human SCI (Fleming et al., 2006). While contusion and compression are the most prevalent injury mechanisms seen in human SCI (Norenberg et al., 2004; Sekhon and Fehlings, 2001), human injuries typically occur in a closed vertebral system (Ramer et al., 2000). From an experimental perspective, the high level of invasiveness of these injury models often requires significant post-operative care and can limit post-operative imaging, as the surgical incision site can interfere with coil placement for magnetic resonance imaging (MRI) during the acute phase of SCI. MRI permits the qualitative assessment of SCI non-invasively *in vivo*, and provides a better estimate of lesion volume than histology (Ditor et al., 2008), and quantitative techniques such as diffusion tensor imaging, magnetization transfer, and quantitative T2 can provide additional information (Dula et al., 2010; Kozlowski et al., 2008). The strengths of histological evaluation include the characterization of tissue damage at the cellular level.

A precise, non-surgical model that can selectively target identified regions of the spinal cord would allow for robust image based evaluation of both acute and chronic phases of SCI. While it is possible to injure the cord non-surgically using radiation, it takes months to years for clinical signs of an injury to appear, and the condition is progressive and fatal (Delattre et al., 1988; Mastaglia et al., 1976; Medin et al., 2011). The length of time required for the injury to develop, and the unique mechanisms involved make radiation induced spinal cord damage a poor model for treatments targeting traumatic SCI. An alternate, less-invasive intervention that can rapidly induce clinically relevant repeatable neural injury patterns may be focused ultrasound (FUS), which has been used to thermally induce lesions in the brain. FUS has been used to thermally ablate regions of the brain, treating conditions such as essential tremor (Elias et al., 2013; Lipsman et al., 2013) and chronic pain (Martin et al., 2009). At low power, FUS and microbubbles have been used to facilitate local drug delivery to the brain in animal models through transient opening of the blood brain barrier (BBB) without damage to critical neural structures (Hynynen et al., 2001; McDannold et al., 2012). At slightly higher power, FUS with microbubbles can produce targeted mechanical lesions in neural tissue and avoid bone heating (McDannold et al., 2006a). While FUS and microbubbles do not replicate the injury mechanisms involved in human SCI, microbubbles have a mechanical impact on the microvasculature (McDannold et al., 2006b), and vascular injury is known to play a key role in both primary and secondary damage in SCI (Nelson et al., 1977; Tator and Fehlings, 1991). As such, lesions produced by FUS and microbubbles may represent a potential approach for non-surgical MR-guided targeted damage to the spinal cord.

The goals of this research were twofold. First, to determine whether FUS and microbubbles can induce reproducible and localized non-surgical cervical SCI in the rat. Second, to assess the clinical relevance of the resultant injury pattern using MRI and histopathology.

2. Methods

2.1. Ethical considerations

All animal procedures were conducted with the approval of the Animal Care Committee of Sunnybrook Research Institute and in compliance with the guidelines established by the Canadian Council on Animal Care and the Animals for Research Act of Ontario.

2.2. Experimental overview

Injuries were induced using MR guided FUS, targeting the right side of the cervical spinal cord, starting just below C1 and extending 6 mm caudally. Follow-up MRI took place at 24 h for all animals. Ten animals (acute group) were sacrificed immediately following the 24 h MRI session. The remaining 9 animals (chronic group) were imaged again at 1 week and 2 weeks. Three of these animals with no MR-visible abnormalities were sacrificed at 1 week, while the remaining 6 animals were sacrificed at 2 weeks post injury (Table 1).

2.3. Induction of focused ultrasound injury

Nineteen male Wistar rats (Charles River Canada) weighing 250–400 g were used in this study. They were anesthetized with a mixture of ketamine (40–50 mg/kg) and xylazine (10 mg/kg) delivered *via* intramuscular injection in the hind leg. The hair was removed from the neck and back using clippers and depilatory cream. A 22G angio-catheter was placed in the tail vein for delivery of microbubbles and the MRI contrast agent. The animals were placed dorsally recumbent on a plexiglass sled designed to be moved between the 7T MRI and the ultrasound delivery system. The animals' heads were supported with an upwards tilt to straighten the spine in the target region. Following the acquisition of T1-weighted images for treatment localization, the sled was moved to a three-axis positioner operationally similar to that described by Chopra et al. (2009). The ultrasound transducer was located on a positioning arm in a water bath below the animal. The water bath was coupled to the animal using a sealed water pack built into the sled. Ultrasound coupling gel was applied between the water pack and the animal. Ultrasound was generated using a 1.114 MHz, spherically focused transducer (Aperture = 7 cm, F-number = 0.8), driven using a function generator and RF power-amplifier. The applied RF-power was measured with a power meter constructed in-house, connected to the controlling computer. Sonications consisted of 10 ms ultrasound bursts at a repetition rate of 0.5 Hz, for a total of 5 min. The acoustic power during the burst was set to 1.3 W (1 animal) or 1.6 W (18 animals). Definity microbubbles (0.02–0.04 ml/kg, Lantheus Medical Imaging) were injected into the tail vein catheter at the start of the sonication, followed by saline