Title: Development of the controlled cortical impact model for ferret and initial outcomes with MRI, behavioral testing and histology.

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Background

In recent years, multiple preclinical models of traumatic brain injury (TBI) have emerged to enable the investigation of basic science and therapeutic approaches for this disorder and its sequelae. Mouse and rat models of TBI provide important insight into mechanisms of damage and plasticity following injury, however the low relative volume of white matter and lissencephalic cortex of the rodent reduce the direct relevance of these models to study hallmark human pathologies such as diffuse white matter injury. The ferret may be an important animal for TBI research because its brain has a ratio of white to gray matter that is comparable to humans and the ferret brain surface is highly folded, or "gyrencephalic", which may mean that the biomechanical response to impact is more similar to human injuries. In this study, we optimized surgical protocols and parameters for controlled cortical impact (CCI) in the ferret and also examined anatomical and behavioral outcomes following injury.

Methods

A total of 13 adult male ferrets were included in the development of the ferret CCI protocol (n=9) and standardized comparison of MRI, behavioral and histological outcomes following CCI (n=4). For each animal, a cranial window was placed over the somatosensory cortex on the left side. CCI parameters were explored over the following ranges: bit diameter=3 and 5mm, penetration depth=1-4mm, impact velocity=3-5m/s and dwell time=100ms. Based on behavioral observation and MRI, "mild" and "severe" parameters were selected from this set and compared with sham surgery and surgery naive brains to identify MRI and behavioral abnormalities. Each of these experimental cases was imaged in-vivo before and 1 day following surgery. The brains were perfusion fixed and removed at 1 week and ex-vivo T2 map and DTI imaging were performed. Behavioral analyses were performed before and following CCI at 6 hours, 1 day, 3 days and 7 days post-injury and included gait analysis, open field testing and novel object recognition testing.

Results

The primary surgical observations from the developmental phase of this work were:

1. Unlike rodents, a very large muscle covers the ferret skull and careful retraction is necessary for successful surgical outcome. Furthermore, the presence of muscle requires closing the cranial window in such a way that the brain under the craniotomy will not be affected by downward pressure.
2. There is a unique cranio-cerebral geometry for each animal, thus standard co-ordinates relative to cranial landmarks is not a precise approach to localize brain

anatomical regions. An advantageous alternative is to use pre-surgical MRI to determine the placement of the cranial window that will expose the intended cortical region.

3. The following were determined for "mild" and "severe" CCI setting to use in this model: mild CCI - bit diameter=3mm, penetration depth=1mm, impact velocity=3m/s and dwell time=100ms; severe CCI - bit diameter=3mm, penetration depth=4mm, impact velocity=5m/s and dwell time=100ms.
4. Initial histopathology from a "moderate" CCI brain showed increased GFAP and extracellular matrix protein immunoreactivity at 1 week after injury.

Informed by the above observations, a comparison of MRI and behavioral outcomes across control, sham, mild and severe treated cases revealed the following:

1. At 24 hours following injury, in-vivo MRI revealed T2W hyperintense regions for ferrets with both levels of severity, but greater in extent for the severe case. While these were found in cortical tissue near to the lesion site, conspicuous regions of white matter T2W hyperintensity were observed to extend beyond the lesioned area.
2. In fixed tissue taken one week following CCI in the same animals, T2 maps showed increased T2 values in the perilesional cortex as well as preferentially in the white matter. DTI abnormalities included disrupted orientation patterns near the lesion, reduced FA and increased MD in some regions of white matter, but decreased MD diffusely in the body of the white matter colocalizing with regions of high T2 values.
3. Sensory-motor behavioral testing showed a motor, but not sensory deficit in the day following injury and there was a loss of muscle tone in the full post-CCI period for the severe CCI case, but not any notable abnormality for the mild case or sham.
4. Open field testing showed decreased activity levels for both injury cases, with a greater deficit for the severe case than the mild.

Discussion

The ferret is an important animal model for the investigation of TBI due to the gyrencephalic corex geometry and high white to grey matter ratio of the ferret brain. This initial study introduces some practical considerations for CCI surgery in the ferret and begins to explore the potential imaging and behavioral outcomes that follow mild and severe CCI. Work is ongoing to characterize post-traumatic cellular mechanisms of injury in this model and to acquire imaging and behavioral data from additional animals. The first evidence from this research effort suggests that the ferret provides an important addition to available TBI models, as it shows many features similar to the human brain. In particular, our imaging findings in the white matter are relevant and comparable to known MRI markers in humans. Furthermore, these markers are sensitive to a complexity of tissue changes that are not possible to observe in rodent models. In conclusion, we have developed a CCI protocol for use in the ferret and made some initial observations that suggest the utility of this model as a sensitive and translationally relevant approach to the study of TBI.