Repetitive sub-concussive impacts induce inflammation- implications for innocuous head impacts in sports

Jay Venkatraman, Srinivasu Kallakuri, Shane A Perrine, Cynthia Bir

In the past two decades, research in sport-related traumatic brain injuries has focused on athletes who sustain head injuries but do not lose of consciousness. Hence, an intermediate-term sub-concussive impact has been introduced. A sub-concussive impact is defined by Center for Diseases Control as a bump, blow or jolt to the head not resulting in any apparent symptoms [1]. Sub-concussive injury is a cranial impact that is not clinically classified as a concussion. Contact sports like soccer, football, boxing, and many others have incidences of sub-concussive head impacts which are not immediately identified and may present themselves at a later stage in the lives of the athletes due to their cumulative nature. Signs of neurological damage set in with the end-stage showing symptoms seen in Alzheimer's disease. The current effort was conducted to determine the effects seen in repetitive sub-concussive impacts in comparison to (non-repetitive) sub-concussive impacts. This pilot study attempts to assess the neuropathological changes in rats subjected to repetitive sub-concussive impacts.

Nine male Sprague Dawley rats were divided into three different groups: sham, subconcussive (SC), or repetitive sub-concussive (RSC) respectively. Each specimen was randomly assigned to one of the three experimental groups (n = 3 animals/group) and was subjected to SC or RSC using a modified Marmarou impact acceleration injury model under anesthesia (4-2% isoflurane and 0.6 L of oxygen). SC or RSC impacts consisted of dropping a 50-gram brass weight from a height of 40 cm onto a metal disc (helmet) taped to the scalp. This resulted in .20 joules of impact energy versus the typical 8.82 joules when using the original 450 grams dropped from 2 meters.

Animals were subjected to either a single SC or 10 successive sub-concussive insults, termed RSC. Sham animals were subjected to all the procedures except the insults. The animals were sacrificed 72 hours post-impact, and their brains were perfused with 4% paraformaldehyde. Representative sections encompassing the hippocampus were processed by immunohistochemistry for assessing microglial and astrocytic proliferation changes using IBA (Ionized Calcium-binding Adaptor molecule) and GFAP (Glial Fibrillary Acidic Protein) as markers. Four sections from each brain every 500 microns apart were stained. Three images covering 500 x 400 microns^2 each were taken from the left and right sides of each section encompassing the CA1 region of the hippocampus.

Our results support that RSC induced a significant increase in microglial proliferation compared to SC and sham animal groups ($p< 0.05$). Ongoing analyses have been directed at assessing astroglial proliferation and diffuse axonal injury (using beta-amyloid protein immunohistochemistry) changes.

The modification of the well-established Marmarmou technique for TBI allowed us to create a model that produced a subconcussive event, i.e. the single impact events that were not statistically different from the sham animals as validated by the histological assessment ($p=.831$) and the repetitive sub-concussive impacts being statistically higher than the sham and single impact events ($p<0.05$). This will allow for future research to assess the cumulative effects of sub-concussive impact events and ultimately provide guidelines for sports concussion assessment.