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The use of telomere length as a predictive biomarker for injury prognosis in juvenile rats following a concussion/mild traumatic brain injury

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ABSTRACT

Telomeres were originally believed to be passive players in cellular replication, but recent research has highlighted their more active role in epigenetic patterning and promotion of cellular growth and survival. Furthermore, literature demonstrates that telomere length (TL) is responsive to environmental manipulations such as prenatal stress and dietary programming. As the search for a prognostic biomarker of concussion has had limited success, this study sought to examine whether or not telomere length (TL) could be an efficacious predictor of symptom severity in juvenile rats following concussion. Rats from four distinct experimental groups (caloric restriction (CR), high fat diet (HFD), exercise (EX), and standard controls (STD)) received a mild traumatic brain injury (mTBI)/concussion and were then subjected to a behavioural test battery. The test battery was scored and the animals were categorized as poor, average, or good, based on their performance on the 6 tests examined. Skin cells (from ear notch samples) were taken 17 days post-injury and DNA was extracted for telomere length analysis. Ear notch skin cell TL was highly correlated with brain tissue TL for a given individual. Animals in the CR and EX cohorts had significantly longer telomeres, while animals in the HFD cohort had significantly shorter telomeres, when compared to controls. The mTBI/concussion reduced TL in all cohorts except the EX group. A significant linear relationship was found between TL and performance on the behavioural test battery, whereby shorter telomeres were associated with poorer performance and longer telomeres with better performance. As performance on the test battery is linked to symptom severity, this study found TL to be a reasonable tool for concussion prognosis. Future studies with human populations should examine the validity of TL in peripheral cells, as a predictor of concussion pathology.

1. Introduction

Telomeres are repetitive non-coding sequences of DNA found at the end of linear eukaryotic chromosomes (Blasco, 2004, 2007). They are believed to play four important roles inside the cell, 1) protection of essential genetic information from erosion during DNA replication, 2) protection of DNA strands from damage that would initiate apoptotic processes, 3) bind and recruit proteins involved in DNA repair, and 4) act as a mitotic clock, providing information on the proliferation history of given cells (Eitan et al., 2014). Owing to the inefficiency of DNA polymerase, an "end replication problem" exists, in which telomeres shorten by 30–150 base pairs each time a cell divides (Klapper et al., 2001). Although the most common mechanism, cellular replication is not the only means of telomere shortening. Telomeres are highly susceptible to damage and shortening associated with oxidative stress, especially in the brain, as neurons have high metabolic rates, limited capacity for regeneration, and high levels of iron and copper (Smith et al., 2013; Eitan et al., 2014). To compensate for the continuous loss of telomeres, a reverse transcriptase known as telomerase (TERT), synthesizes and replenishes the telomeric repeats, maintaining DNA stability and counteracting the end replication problem (Gonzalo et al., 2006; Blasco, 2007). The maintenance of appropriate telomere length (TL) requires an intricate balance between the mechanisms that lengthen telomeres and the processes that shorten them (Liu et al., 2004).

Interestingly, research has demonstrated that intrauterine and early life environments alter an individual's telomere biology (the structure, function, and maintenance of their telomeres) (Entringer et al., 2011, 2012). The early life environment provides the developing organism with predictive cues about the future environment, which have the potential to induce long-lasting adaptations (Godfrey and Barker, 2000; Barker, 2004). Developmental programming has been shown to alter an organism's physiology, including TL, significantly influencing their overall health and wellbeing (Seckl and Holmes, 2007; Bale et al., 2010; Entringer et al., 2012). For example, a recent study demonstrated an association between prenatal stress and reduced TL in human leukocytes (Entringer et al., 2011), and various rodent studies

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have found shortened TL associated with aversive developmental conditions (Jennings et al., 1999; Tarry-Adkins et al., 2008). Similarly, telomere erosion has been suggested to contribute to the progression of neurodegeneration in diseases such as Alzheimer's and Parkinson's (Eitan et al., 2014). Conversely, research has shown that manipulations such as exercise that lengthen telomeres, are neuroprotective and promote resiliency (LaRocca et al., 2010).

The examination of factors that contribute to risk and resiliency for poor outcomes following paediatric concussion or mild traumatic brain injury (mTBI) is an emerging field of research. Approximately one in every five children sustains a mTBI by the age of 16 and while a large majority of these children spontaneously recover, a significant proportion go on to suffer from lingering symptomology (Barlow et al., 2010). Unfortunately this cohort of patients is plagued by heterogeneity; a plethora of studies have unsuccessfully attempted to find reliable biomarkers for concussion prognosis (for review see (Zhang et al., 2010)). In addition, many of the biomarkers under investigation require invasive collection procedures, such as spinal taps and blood draws (Giacoppo et al., 2012; Zetterberg et al., 2013), which would be difficult to justify for mTBI/concussion. As one of the primary goals of concussion therapy is the appropriate allocation of resources and interventions to children at risk for post-concussive symptomology, a reliable, easily accessible, prognostic biomarker is needed. Peripheral skin cells can easily be collected in human populations through skin sampling or buccal swabs which prompted the use of ear notch sampling in this study. Ear notch skin samples are derived from the same lineage (ectoderm) as brain tissue and contain a mixture of collagen, reticular fibres, fibroblasts, adipose connective tissue, and vasculature. Therefore, owing to the ease of collection, the sensitivity of TL to environmental manipulation, and the need for a reliable prognostic biomarker, this study considered the use of telomere length for concussion/mTBI prognosis.

The purpose of this study was two-fold. First, the study sought to examine the relationship between early programming manipulations and the length of telomeres in skin samples obtained from the ears of juvenile rats. The second aspect of the study investigated the ability of TL to differentiate between poor, average, and good outcomes following a mTBI/concussion. Based on prior research regarding TL and pathophysiology, it was hypothesised that rats with longer telomeres would exhibit greater resiliency, while rats with shorter telomeres would exhibit poor outcomes post-injury. In order to test this hypothesis, rats were randomized to one of four early programming paradigms: a) high fat diet (HFD), b) caloric restriction (CR), c) exercise (EX), or d) standard diet (STD), and then received a mTBI/concussion. The rats underwent a comprehensive behavioural test battery that included 6 paradigms (beam walking, open field, elevated plus maze, novel context mismatch, Morris water task, and the forced swim), which was followed by collection of skin samples from the ear. Examination of TL demonstrated that the early programming manipulations did in fact alter the length of telomeres in peripheral skin cells, and importantly, TL was significantly correlated with symptom severity and performance on the behavioural test battery.

2. Materials and methods

2.1. Subjects and early life manipulations

All of the experiments were conducted in accordance with the Canadian Council on Animal Research and were approved by the University of Calgary Conjoint Faculties Research Ethics Approval Board. Sprague Dawley rats were bred 'in-house' in an animal husbandry room maintained at 21 °C with a 12 h light:dark cycle (lights on at 0700). A total of 110 rat pups were used in this study. Female dams (housed in pairs) were randomly designated to one of three dietary manipulations (outlined below). Females were maintained on their respective diet for a total of 9 weeks; 3 weeks prior to mating, the 3 weeks of pregnancy, and the 3 weeks of weaning. The standard diet (STD) condition included dams that had ad libitum access to standard rat chow and water. The caloric restriction (CR) condition included dams that were given ad libitum access to standard rat chow on alternating days. Care was taken to ensure that the rats were not stressed by CR manipulation and that their total body weight loss did not exceed 25%. The third group included dams maintained on a high fat diet (HFD). In this condition, dams were fed a rat chow with an adjusted caloric intake (60% of the calories were derived from fat — TD.06414, Harlan Laboratories), and provided a 20% sucrose solution rather than water. The micronutrient levels were compared between the STD and the HFD and deemed to be quite similar. Following the 3-week habituation period, dams were mated with unique standard males, and were then returned to their pair-house conditions with one other dam. One day prior to birth, dams were separated and they continued to be housed individually with their litters until weaning at postnatal day 21 (P21). At weaning, pups were housed in sex and diet matched cages of 4, randomized to contain only 1 pup per dam in an effort to prevent litter effects. The pups were maintained on the same diet as their mothers for the duration of their lifetime. At weaning (P21), half of the pups born to STD dams were housed in cages equipped with running wheels; this group is referred to as the exercise (EX) condition.

2.2. Administration of the mTBI/concussion

On P30 a subset of the rats were randomized to receive a mTBI ($n = 60$) and the remaining rats received a sham injury ($n = 50$). The mTBI and sham injuries were administered using the modified weight drop method as described in detail elsewhere (Mychasiuk et al., 2014a, 2014b). In short, rats received a brief delivery of isoflurane anaesthetic (~60 s), until they were unresponsive to a toe-pinch. The rats were then placed in a prone position on a scored piece of tinfoil, and a 150 g weight was dropped from 0.5 m down a plexiglass tube. The weight impacted the dorsal surface of the skull producing a glancing impact on the rat's head, which caused the tinfoil to tear and the rat to fall. During the fall, the rat underwent a 180° vertical rotation before landing on a cushioned foam surface. The acceleration and rotational forces experience by the rat during the free fall and rotation are believed to mimic the underlying forces involved in the pathology of concussion (Viano et al., 2007, 2009). After landing in a supine position on the foam surface, the rat received a topical administration of lidocaine and was placed in a clean warm cage for recovery. A researcher recorded the time it took each rat to right itself (flip from the supine position to the prone or standing position) and this was deemed the 'time-to-right'. The time-to-right was used as a measure of loss of consciousness in our sham and mTBI rats. Sham rats were also lightly anesthetised with isoflurane, placed on the scored tinfoil, but removed before experiencing a glancing impact or free fall, and then given a topical administration of lidocaine, which was followed by recording of their 'time-to-right'.

2.3. Behavioural testing to determine injury severity

2.3.1. Beam walking

One day following the injury (post-injury day 1; PID1), animals underwent a beam walking test similar to that described by Schallert et al. (2002). The rats were required to traverse a 165 cm long tapered beam suspended 1 m in the air. The beam narrowed from start to finish, but contained 2 cm wide safety ledges that would catch the rat's foot if it slipped off of the main beam. The rats started at the wide end and travelled toward their home-cages that were placed at the far end as a reinforcement cue. The rats were given a single trial to learn the task, which was followed by 4-videotaped trials that were scored. The video camera was placed at the wider end of the beam and recorded the rat as it walked toward its home-cage. A research analyst blinded to the experimental conditions, scored the rat for the number of times its hind-legs slipped off of the beam and touched the safety ledge.