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Models of Traumatic Brain Injury in Aged Animals: a Clinical Perspective

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Abstract

Traumatic Brain Injury (TBI) is a major cause of morbidity and mortality in the United States with advanced age being one of the major predictors of poor prognosis. To replicate the mechanisms and multifaceted complexities of human TBI and develop prospective therapeutic treatments, various TBI animal models have been developed. These models have been essential in furthering our understanding of the pathophysiology and biochemical effects on brain mechanisms following TBI. Despite these advances, translating preclinical results to clinical application, particularly in the elderly, continues to be challenging. This review aims to provide a clinical perspective, identifying relevant variables currently not replicated in TBI animal models, to potentially improve translation to clinical practice, especially as it applies to elderly populations. As background for this clinical perspective, we reviewed articles indexed on PubMed from 1970–2019 that employed aged animal models for studying TBI. These studies examined endpoints relevant for clinical translation, such as neurocognitive effects, sensorimotor behavior, physiologic mechanisms, and efficacy of neuroprotective therapies. However, compared to the higher incidence of TBI in older individuals, animal studies on the basic science of aging and TBI remain remarkably scarce. Moreover, a fundamental disconnect remains between experiments in animal models of TBI and successful translation of findings for treating the older TBI population. In this paper we aim to provide a clinical perspective on the unique attributes of TBI in older individuals, as well as a critical appraisal of the research to date on TBI in aged animal models and recommendations for future studies.

Keywords

Traumatic brain injury; aging; animal models; comorbidities; translation

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Introduction

Traumatic Brain Injury (TBI) is a major cause of death and disability in the United States. Based on recent surveillance data from the Centers for Disease Control and Prevention, in 2013 there were approximately 2.8 million people treated for TBI-related injuries, including 2.5 million emergency department visits (excluding hospitalizations, deaths and transfers), 282,000 hospitalizations and 56,000 deaths.[1] Incidence of TBI displays a bimodal age distribution, with peaks in adolescents to young adults (15–25 years of age) and older adults (65 years of age and older).

Historically, elderly individuals have accounted for a disproportionate fraction of TBI-related injuries. In 2013, those aged ≥ 65 accounted for about 20% of the emergency department visits, 44% of hospitalizations, and 37% of TBI-related deaths. Further, those aged ≥ 75 comprise the largest proportion of hospitalizations and deaths compared with any other age group.[1] Of note, TBI rates have increased in all age groups over the period from 2007 to 2013 (total = 638.8 to 884.2 per 100,000, an increase of 38.4%), but the two groups with highest percentage increases were those aged 65–74 (494.1 to 777.0 per 100,000 population, an increase of 57.3%) and those aged ≥ 75 (1,265.2 to 2,232.2 per 100,000 population, an increase of 76.4%). While in the younger age group men are three times more likely than women to sustain TBI, above the age of 65 the incidence is roughly equal in men and women. However, the incidence of TBI has decreased with age in men who were 70 years and older compared to an increase for women aged 70–89.[2]

In 2003, the aggregate charges for treating a principal diagnosis of TBI in patients aged 65 and older exceeded $2.2 billion[3]. The National Institute on Aging projects that between 1990 and 2020, the population aged 65 to 74 will grow 74% under middle series projections, while the population under age 65 will grow only 24%.[4] With greater life expectancy, there is significant potential for longstanding financial impact of geriatric TBIs, both to survivors and to society at large. As the population continues to age, the costs for care and rehabilitation of those living with TBI have the potential to become a staggering drain on societal resources.

For those who survive TBI, advanced age is one of the strongest predictors of adverse outcome.[2,3,5] Older adults are more likely to have complicated TBIs, even with TBIs that would otherwise be classified as mild with a Glasgow Coma Scale score (GCS) of 13–15. For example, elderly patients with GCS of 13–15 had a significantly higher rate of intracranial hemorrhage at 16% versus 5% for younger adults.[5] Older adults are more likely to be hospitalized after TBI, have extended hospitalizations, more likely to be severely disabled, and a greater proportion are discharged to a nursing facility. Older adults are disproportionately affected in terms of decreased quality of life due to temporary or permanent cognitive, physical and psychosocial disability. TBI in older adults is more likely to be associated with poor memory, dependence, and slowness of thinking compared with younger adults with TBI.[6] Finally, TBI also increases the risk of new onset depression in older adults.[7]

Beyond the direct impact of TBI on neural function, there is growing evidence that in individuals aged 65 or older, even mild TBI results in an increased risk for the development of dementia, lowers the age of onset, and alters clinical manifestations.[8] Literature has described the association of TBI and dementia.[9] Most studies suggest that a single moderate-to-severe TBI is the major known environmental risk factor for Alzheimer's disease, imparting a two- to four-fold increased risk.[10] TBI has also been suggested to be a risk factor for other neurodegenerative disorders associated with cognitive impairment and dementia, such as frontotemporal dementia, Parkinson's disease and amyotrophic lateral sclerosis. Finally, the age at which TBI occurs is directly related to the probability of developing dementia and amyloid-ß plaques.[10]

At a time when the population continues to age, pharmacological trials for treating TBI have yielded disappointing results. For example, the use of progesterone for treating TBI acutely was based on a long history of study of its neuroprotective effects in animal models of TBI.[11] After encouraging results in Phase II trials, over 2,000 patients were enrolled in two large multi-site Phase III trials. One of these was ended prematurely for futility while the other continued to completion, but showed no clinical benefit on the GCS at six months.[12,13] While there is still debate regarding the most effective strategies for conducting multi-site Phase III studies for TBI, it is clear that despite strong evidence in preclinical models, translating efficacy to clinical populations remains a major hurdle.

The Problem: Paucity of preclinical TBI studies in aged animals

It is not surprising that the majority of TBI clinical trial participants are relatively young, as under-representation of older participants in clinical trials is a widespread problem.[14,15] Often, phase III trials have arbitrary upper age limits and older adults are frequently excluded on the basis of polypharmacy and comorbid conditions.[16] Recently, in the ProTECT III progesterone trial, while there were no specific exclusion criteria for older subjects and participants were included up to 94 years of age, the median age was 35.

Despite the high incidence of TBI in older individuals, preclinical studies have focused almost exclusively on relatively young subjects. To summarize the extant literature, we performed a search using PubMed for preclinical animal models of TBI. As TBI studies of non-human primates are exceedingly rare at any age, and other large animal models of TBI (e.g., pigs) have not used aged individuals, this review was restricted to rodents. The following keywords were used: rodent-, rats-, mice-, brain injuries-, aged-, elderly-, and animal model. References from the included studies were also screened. Original research utilizing mice or rats in studies of TBI from 1970 to July 2019 was included. Information pertaining to the TBI injury model, species, age, sex, neuropathological and physiological results and functional outcomes were tabulated.

To restrict this review to the studies most relevant to TBI in aging, we excluded studies that did not include animals at least 20 months of age. While any model of the relationship between rodent and human age is rather arbitrary, for simplicity, human age equivalence is often estimated based on a simple proportion of the total lifespan.[17–19] The median lifespan for the rat and mouse strains in the included studies ranged from 24 to 30 months. Thus,

when human age equivalence is estimated as a fraction of the total lifespan, a mouse or rat at 20 months is equivalent to a human in their mid-50's to mid-60's. We excluded methods papers, review papers, *in vitro* studies, ischemic and hemorrhagic stroke models, and other models not specifically designed to mimic TBI (e.g., cut/stab brain injury, cryogenic lesions, excitotoxic lesions).

Surprisingly, this search resulted in only 33 articles over this period that included rats or mice at least 20 months of age (Table 1). If the criterion age was reduced to 18 months, only four additional studies were found (italicized references in Table 1). Thus, only a tiny fraction of animal model studies of TBI have been conducted in aged animals. The paucity of such studies and the slow translation to clinical practice of the findings highlight a vital need to assess the current research scope of TBI in animal models and their relevance to the elderly population. This perspective paper aims to identify relevant variables of TBI in older individuals that are currently inadequately replicated in animal models, so that we may suggest new directions for research and ultimately improve translation to clinical practice.

Challenges to modeling TBI in animals

TBI in either humans or non-human animal models induces a complex pathophysiological cascade that results in structural damage and functional deficits due to both primary and secondary injury mechanisms.[20] The primary insult results from direct mechanical injury by penetration or rapid acceleration-deceleration that may result in a brain contusion at the site of injury, widespread shearing of axons, vascular injury, and/or focal damage opposite the side of impact (contracoup injury). Primary insult may lead to other sequelae including subdural hematoma from ruptured bridging blood vessels, decreased blood flow following increased intracranial pressure, infarction, and brain edema caused by increased permeability of cerebral blood vessels.[21,22] Secondary injury manifests over a period of hours to days[23] after the primary injury, and is the result of an additional cascade of metabolic, cellular, and molecular events that ultimately lead to brain cell death, tissue damage, and degeneration.[24] Many biochemical derangements occur, including perturbation of cellular calcium homeostasis, increased free radical generation, mitochondrial dysfunction, inflammation,[25] and progressive atrophy of gray and white matter.[24] These events may result in functional deficits, neurobehavioral disorders, impaired memory and executive function, premature cognitive decline and premature mortality.

Modeling TBI sequalae in aged animals is challenging from a variety of perspectives, including the mechanics of the injury model, species-specific physiologic changes with aging, the interaction of comorbidities seen in human TBI patients, and the feasibility of inducing clinically-relevant brain injuries in aged animals with respect to cost and availability of aged cohorts, survivability after injury, post-injury care, and ethical considerations. As in any animal model, balancing construct validity and experimental control of relevant variables requires some compromise. Nevertheless, the current models are limited, especially with respect to replicating conditions in older populations. The following paragraphs summarize some of the key issues in TBI modeling in aged animals.

Animal models of TBI.

To study the multifaceted complexities of human TBI, animal models were initially developed to replicate certain pathological components of clinical brain trauma.[26] Most animal studies of TBI utilize carefully controlled procedures carried out under sterile surgical conditions and general anesthesia. Though less common, closed-head injuries also are sometimes modeled in animals to more closely mimic clinical conditions,[27,28] and some of these models utilize conscious animals, though no such studies have been conducted in aged animals.[29] Animal models of TBI have enabled detailed evaluation of TBI pathology[22] and functional outcomes, as well as the biomechanical, biochemical, and molecular mechanisms underlying brain injury.[30] Improving our understanding of the complex molecular and cellular cascades initiated by brain injury has been a primary focus that has resulted in numerous important insights into brain injury mechanisms and potential targets for treatment.

Simulating all aspects of TBI in a single animal model is not feasible and thus, animal models of TBI are selective in their focus.[30] Though larger animals may more closely model the mechanical properties of human brain injury, and some models (e.g., non-human primates) may more closely reflect neurophysiological processes and functional impairments seen in humans, rodent models are still by far the most frequently used in TBI research. However, larger, gyrencephalic brains better mimic the biomechanical and physiological conditions of human brain injury. It has been suggested that the presence of gyri influences movement of the brain within the skull, and that gyrencephalic brains experience more deformation and mechanical stress, especially at the depths of the sulci. Brain mass also affects angular acceleration and shearing forces. While rodent models have provided critical information regarding mechanisms of cell death, inflammation and regenerative responses to injury, the limitations of small lissencephalic brains in the context of modeling human TBI are many. While large animal TBI models were once common through the 1980s, they were virtually abandoned due primarily to ethical considerations. However, there has been recent interest in larger animal models, particularly in pigs.[31] Translation of results to clinical populations will require renewed consideration of these large animal models for TBI in general, and particularly for developing therapeutic options for TBI in older populations.

Benefits of rodent models include their modest cost, small size, lower regulatory burden, availability of a variety of genetically-modified strains, and well-characterized outcome measurements.[32] In addition, the relatively short life span of 2–3 years for most mouse and rat strains is a particular benefit for aging research. Of the numerous rodent brain injury models, the most frequently utilized are fluid percussion injury (FPI), controlled cortical impact injury (CCI), impact acceleration injury, and blast injury. Of these, only FPI and CCI have been employed in studies of aged animal models. These two models, in particular, are advantageous for human translation since it has been shown that they result in delayed seizures, paralleling the long-term clinical problem of post-traumatic epilepsy. But some post-TBI clinical phenomena, such as long-lasting coma, typically are not replicated in rodent TBI models.[33]

FPI model.—In the FPI model, after exposure of the skull through a craniotomy, the insult is inflicted by a pendulum striking the piston of a reservoir of fluid to generate a pressure pulse onto the intact dura.[34] The craniotomy may be made either centrally around the midline, or laterally over the parietal bone. Severity can be altered by varying the height from which the pendulum is dropped, producing a larger or smaller pressure pulse. The advantages of FPI for modeling TBI are based on its ability to produce brief displacement and deformation of brain tissue, resulting in both focal and diffuse injury, intracranial hemorrhage, blood-brain barrier (BBB) disruption, brain swelling, and progressive gray and white matter damage, similar to human TBI.[35] Disadvantages of the FPI model include the use of anesthesia, complicating the interpretation of the injury pathology, the requirement of a craniotomy, and the inability to replicate skull fracture and widespread contusions, often seen in moderate to severe TBI in humans. Also, mortality is higher in this model compared with other injury models.

CCI model.—In the CCI model, after exposure of the skull through a craniotomy, the injury is delivered by direct impact to the intact dura, causing deformation of the underlying cortex. A pneumatic or electromagnetic impact device is used to drive a rigid impactor onto the dural surface. This model results in acute subdural hematoma, axonal injury, BBB disruption and edema, cortical tissue loss, neurobehavioral dysfunction and even coma.[35] CCI offers precise control of mechanical factors, such as velocity, angle, and depth of impact, which vary the severity of the injury and the resulting histological and functional outcomes. This better control over injury parameters results in somewhat lower mortality compared with the FPI model. It has been emphasized that CCI should not be used at high severity levels, injuring a large portion of the cerebral hemisphere, since in the clinical setting, such an injury would not be compatible with survival.[33] Finally, like the FPI model, since a craniotomy is required, skull fracture cannot be replicated in the CCI model. Also, anesthesia is required, thus complicating interpretation of pathology.

Impact acceleration (weight drop) model.—Another widely used model of TBI that thus far has not been used to study aged animals is impact acceleration (weight drop). To model closed head injury, the skull of the animal is exposed to a free-falling guided weight, which generates neuronal and axonal pathology concomitant with BBB disruption[36] particularly throughout the major white matter tracts of the cerebrum and brainstem.[4] Severity can be altered by adjusting the mass of the weight and the height from which it falls. The sequelae of impact acceleration resemble the clinical conditions of human diffuse TBI due to motor vehicle accidents or falls[36]. Given that falls are the leading cause of TBI in older adults, this model would seem to have adequate face validity for studying TBI in aged animals. However, the greater difficulty in controlling impact parameters makes this model less than ideal. Like FPI and CCI, anesthesia is also required.

CHIMERA model.—One major disadvantage of current TBI models with respect to replication of the biomechanics of injuries due to falls and motor vehicle accidents is that head motion is typically constrained. In particular, rotational forces are not integrated into the model. Recently a Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) model was developed at the University of British Columbia.[37] In this model,

the mouse head is supported on a foam pad in a supine position, with Velcro straps around the torso. Impact from a piston deflects the head, and after impact, the head returns to is original position. TBI induced with the CHIMERA model in mice produced loss of righting reflex, neurological, motor and cognitive deficits, anxiety-like behavior, diffuse axonal injury, and microgliosis in white matter. Development of such models that better replicate the biomechanics of injuries seen in older adults are needed.

Blast exposure model.—A focus on the movement of the brain within the skull in closed head injuries has led to nonimpact TBI models[22] of blast exposure. However, the relevance of blast injury models for TBI in older individuals is marginal, since it does not replicate the type of mechanical injury seen in falls and motor vehicle accidents.

In all of these rodent models of TBI, functional deficits can be assessed with a variety of cognitive and sensorimotor behavioral tasks. Cognitive impairment is frequently measured via the Morris water maze, Y maze, and radial-arm maze to determine spatial/contextual deficits, and novel object recognition and avoidance tests for learning and memory.[38] Sensorimotor impairments can be measured with the bilateral tactile adhesive removal test, skilled forelimb use (staircase test), Rotarod test, beam walk, grid walk, and grip and reaching tests.[39,40]

Physiologic changes with aging relevant for recovery after TBI.

Normal aging is associated with cellular, molecular, anatomic, and physiologic changes. An organ system's ability to effectively regulate and maintain its physiologic reserve is paramount to adapting appropriately to stresses. Diseases and injuries in aging are associated with the reduced ability to properly restore a new steady state that is appropriate for the new set of conditions,[41] with increased maladaptive responses to internal and external stresses. In the brain, the hippocampus has been identified as a key component of the stress response, sensing increased glucocorticoid levels and relaying information to the hypothalamus in a negative feedback loop[42] to maintain homeostasis following perturbance. This decrease in function of the feedback loop worsens following TBI due to selective hippocampal cell death and is associated with cognitive impairment.[43] Understanding how decreased physiologic reserve in older individuals might impact neurocognitive recovery following injury is lacking and this area warrants more research.

Molecular and cellular changes that occur in the brain during normal aging may also naturally contribute to a delayed recovery post injury.[44] Aging leads to a decline in dopamine receptor densities associated with declines in cognitive and motor performance.[45] Further, expression of neurotrophic factors and neurotransmitters may frequently be altered in the aging brain. In particular, changes in brain-derived neurotrophic factor and serotonin may act in concert to regulate neural plasticity, neurogenesis, and neuronal survival in multiple brain regions.[46] Other factors that have been implicated include calcium dysregulation affecting multiple signaling pathways adversely influencing cellular physiology, molecular functions and, cell structure;[47] mitochondrial dysfunction leading to the endogenous production of reactive oxygen species and apoptosis;[48] and an increase of

monoamine oxidase that is associated with the liberation of free radicals[49] leading to apoptosis.

Systemic physiologic changes that occur during aging may further predispose older individuals to an exacerbated pathophysiologic response to brain trauma. Integration between brain function and peripheral metabolism is required for homeostasis and proper adaptations to aging, physical activity, or environmental stressors. Increasingly, it is being recognized that disruptions in these brain/periphery links likely play a critical role in the brain's response to traumatic injuries. Likewise, alterations in centrally mediated processes after TBI can result in peripheral metabolic changes (in cardiac muscle, skeletal muscle and gut) impacting functional outcomes (activity, gait, motor control, postural adaptation) and posttraumatic morbidity.[50–56] Examples of age-associated changes that could interfere with brain/periphery metabolic links include: 1) reduction in total body water and decreased blood volume leading to a decline in cerebral blood flow which could exacerbate cell damage and/or death, 2) decreased elasticity and increased rigidity of arterial vasculature blunting the carotid baroreceptor sensitivity thus increasing the risk of end organ damage[57], 3) BBB disruption that is exacerbated post-injury[58] resulting in more severe traumatic edema, decreased cerebral perfusion pressure, and ischemia, 4) progressive brain atrophy leading to increased space in the cranial vault for accumulation of blood,[59] and 5) increased adherence of dura to the skull and increased susceptibility of bridging veins to shearing[3] leading to a greater chance of subarachnoid hemorrhage and/or subdural hematoma formation.

Comorbidity effects on aged TBI outcomes.

It is estimated that 80% of all adults aged 65 years and older have at least one chronic condition and 50% have at least two,[5] compared to 28% of younger adults with a single medical condition.[60] Thus, an elderly person with a TBI has a greater likelihood of chronic prescription medication use for these longstanding comorbid conditions compared to the younger TBI population. The Centers of Medicare and Medicaid Services report that the most common chronic conditions among Medicare beneficiaries (aged 65 and older) in the United States were: hypertension (58%), high cholesterol (45%), cardiovascular disease (31%), arthritis (29%), and diabetes (28%). Though it is known that these factors negatively impact clinical recovery, they are generally not modeled in animal studies of TBI in aging.[61–63] The following examines how common comorbid conditions might negatively impact outcomes in older adults with TBI.

Hypertension.—The incidence and prevalence of most cardiovascular disorders increases with age, and cardiovascular disease is the leading cause of death and major disability in adults ≥75 years of age.[64] In a retrospective review of comorbidities in older TBI patients, hypertension was found to be the most prevalent condition.[65] Chronic hypertension in the elderly has been shown to disturb the BBB which leads to changes in passage of molecules into the brain, cerebral edema, white matter changes, astrocyte activation exacerbating the neuroinflammatory cascade, and eventual cerebral ischemia and neurodegeneration.[66,67] This is pertinent given the fact that brains of aged mice that were injured via moderate lateral CCI had a more prolonged post-acute opening of the BBB than younger mice, with

delayed resolution of cerebral edema and greater neurodegeneration that resulted in significant sensorimotor dysfunction.[40]

Cardiovascular Disease.—Cardiovascular disease, which includes coronary heart disease, peripheral arterial disease, heart failure, valvular heart disease, and stroke, becomes more prevalent with age.[68] Up to 66% of adults aged 75 years or older in United States and Europe take daily aspirin and/or other antiplatelet drugs[69] to prevent or treat a myocardial infarction, which has a nearly seven-fold higher prevalence among individuals age 65–74 years relative to those age 35–44.[68] Intracranial bleeding has been found to progress in 10 % of elderly TBI patients on clopidogrel and these patients were three times more likely to be discharged to long-term inpatient facilities. TBI patients on clopidogrel had a 14 times higher mortality rate as compared with those not on antiplatelets.[70]

In 2005, atrial fibrillation was estimated to affect 2.2 million Americans.[68] Warfarin is frequently used for the prevention of thromboembolic events in atrial fibrillation, but it has also been linked to an exacerbation of secondary injury in the elderly following a TBI. Those on warfarin with minor head injuries (GCS ≥ 13) are 2.7 times more likely to suffer from intracranial hemorrhage,[71] a condition that is strongly associated with a 30-day mortality, regardless of age, sex, or coexisting illnesses.[72]

Cerebrovascular reactivity.—It is well known that impaired autoregulation and cerebrovascular reactivity (CR) are major risk factors for poor functional outcome after TBI.[73] Several indices have been used to measure CR, including cerebral blood flow velocity, middle cerebral artery cross-sectional area, mean arterial pressure, the pressure reactivity index, pulse amplitude index, and RAC (correlation between pulse amplitude and cerebral perfusion pressure). The most common protocol to examine CR is response to hypercapnia. Importantly, in a multivariate study of 358 TBI patients, subdural hematomas, thickness of subarachnoid hemorrhage and age were strongly associated with abnormal CR, more so than the gross injury burden.[73] While several animal studies have examined CR in TBI models,[74,75] very few studies have examined CR endpoints in aged animal models. In one rare study of CR in aged rats, cerebrovascular reactivity to hypercapnia decreased, but there were differences between Wistar and Fischer 344 strains.[76] This is a topic that is ripe for further investigation.

Diabetes Mellitus.—Diabetes Mellitus (DM) prevalence continues to grow across the age spectrum but its co-morbidities and mortality are higher in the elderly population. According to Caspersen *et al.* diagnosed and/or undiagnosed DM affects 10.9 million adults in the U.S. aged 65 and older, with a projection to reach 26.7 million by 2050.[77,78] In a registry-based study, the relative risk of low falls in adults with insulin-treated DM was nearly twice the risk in those without insulin-treated DM.[79] Of those older adults who fall, up to 10% experience a significant injury such as TBI[80]. Again, this is important given that falls are the most common cause of TBI in older adults and a single fall is a major risk factor for subsequent falls. Given their lower reserve, older adults who experience repetitive TBI experience poorer cognitive and functional recovery following each subsequent injury with an increased risk of fatal fall-related TBI. Severe TBI with DM has a higher mortality (14%) compared to severe TBI without DM (8.2%) and TBI with insulin-dependent DM has a

higher mortality rate (17.1%) than patients without insulin-dependent DM.[81] Insulin deficiency may contribute to the increased mortality after TBI[81] or it may be due to hyperglycemia, especially persistent hyperglycemia, which has been shown to play a detrimental role in the secondary complications following TBI. Hyperglycemia increases blood viscosity and diffuse small vessel disorders which results in ischemia and hypoxia in the brain tissues. In addition, hyperglycemia has the potential to induces disruption of the BBB, accelerate cerebral vasospasm, exacerbate edema of endothelial cells, and impair glial cell function.[81]

Aged animal studies of TBI: advances and limitations.

Studies of TBI in aged animals, although limited to a small fraction of basic TBI research, have nonetheless led to important advances in our understanding of how aging interacts with complex cellular and molecular pathways of secondary injury. For example, animal studies have elucidated the critically important role of neuroinflammation in both acute pathological and reconstructive response of the brain to injury, similar to the neuropathology in clinical populations.[82] Older brains appear to undergo a homeostatic shift toward a pro-inflammatory state, a phenomenon termed “inflammaging”. This state describes the low-grade, chronic, systemic inflammation in the absence of overt infection (“sterile” inflammation) and is considered a highly significant risk factor for both morbidity and mortality in the elderly[83] following brain injury. TBI studies in aged rats and mice have noted that compared to adult subjects, there was: delayed recovery along with prolonged activation of microglia and astrocytes in the hippocampus with chronic activation of microglia being linked with increased neuronal loss[84]; higher baseline expressions of cytokines, chemokines, and iNOS that was associated with exacerbated responses to injury; [85] and a reduced expression of genes in hypoxia-inducible factor-1 alpha pathway that exhibit neurotrophic and neuroprotective properties.[86] Thus, it appears that a heightened inflammatory response may contribute to the delayed recovery post injury. These pre-clinical findings have formed a critical foundation for future research. However, we note that the absence of clinical variables in studies to date could limit effective translation of findings to human TBI. For example, aspirin and acetaminophen are two anti-inflammatory medications frequently used in the geriatric population. How might these medications influence TBI in older subjects? Future studies of common anti-inflammatory drugs in aged animal models will be important to determine potential effects of these treatments on the inflammatory response and TBI outcomes.

TBI studies in aged animals have also revealed that worse outcomes in older subjects may be linked with free radical-induced cell death following a depletion of mitochondrial antioxidant enzymes. Itoh and colleagues demonstrated that in the cerebral cortex and hippocampus of aged rats, significantly higher levels of free radicals are produced compared with younger adults after TBI.[85] The free radicals induced neuronal and glial cell membrane peroxidation and DNA damage, causing apoptotic cell death and ultimately cognitive impairment.[87] It was also observed that in the focal ischemic area, free radical production was increased by facilitation of the arachidonic acid (AA) cascade.[87]

Non-steroidal anti-inflammatory drugs such as ibuprofen and acetylsalicylic acid (aspirin) are non-selective inhibitors of cyclooxygenase, a key enzyme in the AA metabolic pathway. As previously mentioned, due to the increased risk of cerebrovascular disease with aging, long term antithrombotic therapy is common, and the most widely prescribed antithrombotic medication is aspirin. In 2016, it was reported that nearly 40% of U.S. adults older than 50 years use aspirin for the primary or secondary prevention of cerebrovascular disease.[88] Consequently, it may be of great importance to determine how inhibition of the AA pathway affects free radical production in the context of TBI in older subjects.

Overall, experimental animal models have clearly been valuable in advancing our understanding of pathophysiological events related to TBI, and essential for the identification of novel therapeutic targets. A recent study from our group showed that treatment with taurine, an anti-inflammatory, anti-oxidant, and anti-apoptotic therapy that had shown promise for TBI in young adult rats, did not show a similar degree of neuroprotection in elderly rats.[89] This highlights the likely influence of age on therapeutic efficacy and the critical need for inclusion of aged animal models in the drug development pipeline for TBI.

To date, the findings from aged animal studies have not specifically contributed to improved clinical care, treatment, or rehabilitation of TBI in the geriatric population. We posit that an important reason for this may be an inadequate replication of clinical variables in aged animal studies. Preclinical studies of TBI are carefully designed for reproducibility, to apply a standardized injury across subjects with age, sex, diet, living conditions, genetic background, and injury parameters all carefully controlled.[35] In the laboratory, heterogeneous injury has the potential to adversely affect statistical significance of results. On the other hand, limiting so many clinically relevant variables that are known to affect human TBI outcome(s) may limit clinical translation. Certain comorbid conditions that frequently accompany TBI and worsen outcomes have been implemented in adult animal models of TBI. Dual TBI and hypoxia models have been developed in FPI, impact acceleration, and CCI,[22] and hypoxia-and-hypotension models have been applied in FPI and CCI.[35] However, such combined neurotrauma studies have not yet been implemented in aged animals. Developing these types of studies in aged subjects to determine interactive effects (e.g., between age and hypoxia, age and diet, etc.) may better recapitulate TBI pathophysiology in older humans post-trauma.

Recommendations for Aged Animal Studies of TBI

Animal models serve an essential role in TBI research, allowing elucidation of complex cellular and molecular mechanisms of injury and the identification of targets for therapy. For a variety of practical experimental reasons, these studies have primarily focused on young adult male rodents, and research that emphasizes issues particular to TBI in the aged population continues to be meager. Inbred strains of rodent species are extremely valuable as research subjects due to reduced genetic variability.[90] However, the commonly used strains display a limited range of pathology with regard to age-associated comorbidities that exist in humans. For example, normally aged mice do not develop neurodegenerative pathologies

and have low prevalence of cerebrovascular disease. Experimental manipulations, such as dietary manipulations, are required to model these conditions in the laboratory.

There is a clear need to improve current practices in regard to the effects of brain injury, particularly in a population that is one of the most vulnerable to the debilitating consequences: the elderly. It is our belief that the development of an interdisciplinary approach that strongly considers clinical perspectives during the pre-clinical research phase is vital to an improvement in translational research. It is clear that older age negatively influences outcome after TBI, and the study of TBI in aged animal models remains an area of considerable opportunity for future research.[3] With the elderly contributing greatly to the epidemiology of TBI, modifications to existing preclinical research practices are imperative to bridge the divide between preclinical studies and clinical application.

From the meager body of evidence on TBI in aged animals, it would appear self-evident that our ability to translate animal findings to aged clinical TBI populations would benefit from several extensions of current models:

1. Utilize more aged animals in TBI research. Generally, this includes rats and mice over 20 months of age. Use a *range* of "old" animal ages to better understand heterogeneity in the geriatric population.
2. Utilize injury models that replicate additional mechanical properties of TBI in older humans. This should include studies that model concussion and mild-to-moderate TBI, representing the most common type of clinical injury.
3. Model TBI in combination with co-morbidities frequently seen in older humans. This includes comorbidities such as hypertension, diabetes mellitus, cardiovascular disease, and impaired cerebrovascular reactivity. Some of these co-morbidities exist in inbred and transgenic animals, but others will need to be induced in the laboratory setting via environmental conditions, such as those that predispose animals to obesity and inactivity. Modeling multiple co-morbidities should also be considered.
4. Model TBI in combination with chronic medications most commonly taken by older individuals. This might include preinjury antiplatelet therapy (e.g., aspirin) that have been linked to worse outcome after TBI.
5. Study the TBI response over a comprehensive period from acute to chronic post-TBI period.
6. Develop better age-specific functional outcome measures for aged animals.
7. Include biomarkers as outcome measures in animal studies that are translatable to humans. Imaging endpoints that can validate age-related changes in cerebrovascular reactivity, tissue perfusion (arterial spin labeling), brain connectivity (diffusion tensor imaging, resting-state connectivity), brain metabolism (magnetic resonance spectroscopy) will be particularly useful.
8. Consider the use of larger animal models of TBI, including animals with gyrencephalic brains, such as sheep, pigs, and non-human primates.

9. Use both sexes in aged animal studies of TBI.

Translation of preclinical research to clinical application continues to be challenging. A variety of animal models of TBI have been developed to reproduce different elements of human TBI. Improving translational success may require remodeling of existing approaches to animal TBI research based on real-world clinical experience, including increased attention to effects of interacting variables and comorbidities on morbidity, mortality, and recovery. Our hope is that the recommendations presented, if fully implemented into research practice for this under-studied population, will ultimately translate to improved patient care and effective strategies to improve outcomes and quality of life for older patients with TBI.

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Table 1.

Aged Animal studies of TBI

Reference	TBI model	Species / Strain	Age and sex	Results[a] - Neuropathology and Physiology	Results[a] - Functional Outcomes
Hamm, 1991[91]	Central FPI	Rat / Fischer-344	3 mo and 20 mo males	No difference in acute hypertension, plasma glucose elevation, or histopathology.	Higher mortality and greater acute neurological deficits
Hamm, 1992[92]	Central FPI	Rat / Fischer-344	3 mo and 20 mo males	No results reported	More severe motor and cognitive deficits
Maughan, 2000[93]	Weight drop	Rat / not specified	2–3 mo and 20–23 mo males	Forebrain synaptosomal choline uptake decreased in aged but not young	Greater cognitive deficits and slower recovery
Nakagawa, 2000[94]	Lateral CCI	Mouse / heterozygote PDAPP mice and WT littermates	22–24 mo females	Decrease in abundance of Ab deposits in hippocampus of PDAPP mice ipsilateral to the site of CCI	No results reported
Uryu, 2003[95]	Lateral CCI	Mouse / wild type with B6D2F1 background; alpha-synuclein knockout	4 mo and 24 mo (wild type); 16 mo (knockouts); sex not reported.	Transient increase of alpha- and beta-synuclein in neuropil and induction of gamma-synuclein in subcortical axons in aged mice	No results reported
Shimamura, 2004[96]	Lateral FPI	Rat / Sprague-Dawley	3–4 mo and 22–25 mo males	Greater hippocampal expression of BDNF and p21, lower expression of regenerative and repair genes	No results reported
Moor, 2006[97]	Weight drop	Rat / Wistar	5–6 week and 19–20 mo males	Lower antioxidant mobilization of ascorbate and urate in hippocampus; more severe cell loss and axonal degeneration	No results reported
Shah, 2006[98]	Lateral FPI	Mouse / C57BL/6	5–6 mo and 22–25 mo males	Higher basal expression of BDNF, IL-1beta, and caspase-3, but no trauma-related differences	Higher mortality
Shao, 2006[99]	Lateral CCI	Rat / Fischer-344	3 mo, 12 mo, and 22 mo males	Higher lipid peroxidation, lower antioxidant activity, and greater tissue loss	No results reported
Cutler, 2007[100]	Bilateral frontal CCI	Rat / Fischer-344	20 mo males	Progesterone treatment after TBI decreased expression of inflammatory and apoptotic factors (COX-2, IL-6, and NFkappaB)	Improved short-term locomotor recovery after TBI and progesterone treatment
Onyszchuk, 2008[84]	Lateral CCI	Mouse / C57BL/6	5–6 mo and 21–24 mo males	Prolonged cerebral edema, increased opening of BBB, more neurodegeneration	Worse sensorimotor recovery
Sandhir, 2008[84]	Lateral CCI	Mouse / C57BL/6	5–6 mo and 21–24 mo males	Enhanced and prolonged elevation of markers of reactive astrocytes and microglia	No results reported
Anderson, 2009[86]	Lateral CCI	Mouse / C57BL/6	5–6 mo and 23–24 mo males	Attenuated injury response in HIF-1alpha and related neuroprotective genes	No results reported
Kasturi, 2009[101]	Bilateral frontal CCI	Rat / Fischer-344	30 mo and 20 mo ovariectomize d females	Post-traumatic edema reduction lower in aged rats with progesterone treatment	No results reported
Gilmer, 2010[102]	Lateral CCI	Rat / Fischer-344	3–5 mo and 22–24 mo males	Greater post-injury dysfunction and oxidative stress in synaptic mitochondrial fraction	No results reported

Reference	TBI model	Species / Strain	Age and sex	Results[a] - Neuropathology and Physiology	Results[a] - Functional Outcomes
Sandhir, 2010[103]	Lateral CCI	Mouse / C57BL/6	5–6 mo and 22–24 mo males	Differential expression of pro-inflammatory regulators C/EBP beta, delta, and CHOP	No results reported
Cekic, 2011[104]	Bilateral frontal CCI	Rat / Fischer-344	22 mo males	Increased baseline brain inflammation, exacerbated injury response, and attenuated benefits of progesterone after TBI in Vitamin-D deficient rats	Progesterone + vitamin D rescued post-injury deficits on open field activity test
Wali, 2011[105]	Bilateral frontal CCI	Rat / Fischer-344	20 mo males	Lesion cavity size not affected by progesterone treatment in aged rats	Progesterone improved locomotor and cognitive recovery in aged rats after TBI
Lee, 2012[58]	Lateral CCI	Mouse / C57BL/6	4–6 mo and 21–14 mo males	Greater BBB permeability and matrix metalloproteinase activity, impaired BBB repair responses	No results reported
Li, 2012[106]	Lateral CCI	Rat / Wistar	20 mo males	Improved hippocampal LTP; increased endothelial progenitor cells, increased blood vessel density after TBI and progesterone treatment	Progesterone improved mNSS and water maze performance after TBI in aged rats
Timaru-Kast, 2012[107]	Lateral CCI	Mouse / C57BL/6	2 mo and 21 mo males	Greater cerebral edema and altered pattern of neuroinflammation; no difference in contusion volumes	Higher mortality and worse mNSS
Itoh, 2013[87]	Lateral CCI	Rat / Wistar	10 week and 24 mo males	Larger lesion size, greater increase in markers of oxidative damage and apoptosis	Greater cognitive deficit on water maze
Itoh, 2013[108]	Lateral CCI	Rat / Wistar	10 week and 24 mo males	Decreased NSC proliferation and increased level of lipid peroxidation	No results reported
*Gupta, 2013[109]	Weight drop	Mouse (short lifespan AKR strain)	20 week and 70 week males[a]	Earlier post-TBI decrease in expression of potassium channel and glutamate transporter-1 expression	No results reported
Hawkins, 2013[110]	Lateral FPI plus hemorrhagic hypotension	Rat / Sprague-Dawley	4–6 and 20–24 mo males	Greater reduction in MAP and CBF; no age difference in hippocampal neuronal injury	No results reported
Kumar, 2013[111]	Lateral CCI	Mouse / C57BL/6	3 mo and 24 mo males	Pro-inflammatory microglia/ macrophage activation state in cortex and sub-cortical regions; larger lesion volume and greater neuronal loss in hippocampus and thalamus	No results reported
Sun, 2013[112]	Lateral FPI	Rat / Sprague-Dawley	1mo and 24 mo males	Higher number of TUNEL(+) cells in aged hippocampal dentate gyrus	No results reported
*Ojo, 2013[113]	*Single or repetitive closed head mild CCI*	*Mouse / Human Tau mice on a C57BL/6 background*	*18 mo males and females*	*Increased phosphortau immunoreactivity and astrocyte / microglia activation after repetitive mild TBI, but not single mild TBI; no perivascular tau pathology, neuritic threads, orastrocytic tangles*	*No results reported*
Tajiri, 2014[114]	Lateral CCI	Rat / Fischer-344	6 mo and 20 mo males	Reduced efficacy for sparing cortical damage and hippocampal cell loss in aged rats treated with stem cells	Reduced efficacy in motor and cognitive recovery after TBI in aged rats treated with stem cells

Reference	TBI model	Species / Strain	Age and sex	Results[a] - Neuropathology and Physiology	Results[a] - Functional Outcomes
Gupta, 2016[115]	Weight drop	Mouse / (short lifespan AKR strain)	20 week and 70 week males[b]	Interactions of NF-κB and N-myc with GLT-1/EAAT2 promoter sequences in ipsilateral cortex of aged mice initiated immediately	No results reported
Morganti, 2016[116]	Lateral CCI	Mouse / C56BL6/J (wild type); Dbl-Het	3mo and 23 mo males and females	Greater accumulation of peripherally derived CCR2(+) macrophages	No results reported
**Wang, 2016*[117]	*Lateral CCI*	*Mouse / C57BL/6*	*2–4 mo and 16–18 mo males*	*Reduced neuronal damage, oxidative stress response, mitochondrial dysfunction, and SIRT3 expression in aged mice with TBI and treatment with fucoidan*	*Reduced deficits on beam walk and Barnes maze after TBI and treatment with fucoidan*
Portbury, 2017[118]	Lateral CCI	Mouse / C57BL/6	24 mo males	Elevated iron and copper levels after TBI; delayed reduction in zinc	No results reported
Chou, 2018[119]	Lateral CCI	Mouse / Dbl-Het; $CCR2^{RFP/+}$; $CCR2^{RFP/RFP}$	3–6 mo and 20–25 mo males	Enhanced and prolonged brain elevation of peripherally-derived monocytes after TBI	Greater cognitive deficit on radial arm water maze
**Krukowski, 2018*[120]	*Lateral CCI*	*Mouse / C57BL6/J (wild type); $C3^{-/-}$*	*19 mo males and females*	*Prolonged brain inflammatory response measured at 30 days post TBI*	*Blockade of complement pathway prevented cognitive deficits after TBI in aged mice.*
Gupte, 2019[121]	Lateral CCI	Rat / Fischer-344	20–22 mo males	Treatment with taurine did not significantly reduce brain tissue damage after TBI	Treatment with taurine did not improve sensorimotor function after TBI
**Ritzel, 2019*[55]	*Lateral CCI*	*Mouse / C57BL/6*	*3 mo and 18 mo males*	*Exaggerated microglial response and increased leukocyte invasion*	*More severe motor deficits, spontaneous locomotor activity, and anxiety-like behavior*

[a] In studies with different age groups or with different strains, results refer to older cohorts in comparison to younger cohorts. or transgenic strains in comparison to wild-type strains.

[b] While mice in the Gupta, et al., 2013 study were only 70 weeks, this strain has a relatively short lifespan of only 75 ± 5 weeks, and thus, is included.

Abbreviations: BBB: Blood-brain barrier; BDNF: Brain-derived neurotrophic factor; CBF: Cerebral blood flow; CCI: Controlled cortical impact; ; C/EBP: CCAAT/enhancer binding protein; CHOP: CCAAT/enhancer binding protein homologous protein; COX: Cyclooxygenase; Dbl-Het: (mouse strain): $CX3CR1^{GFP/+}CCR2^{RFP/+}$ (double heterozygous); FPI: Fluid percussion injury; HIF-1alpha: Hypoxia-induced factor-1 alpha; IL: Interleukin; MAP: Mean arterial pressure; mNSS: modified neurological severity score; NFkappaB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NSC: Neural stem cell; PDAPP: transgenic (TG) mice with a mutant human Ab precursor protein (APP) mini-gene driven by a platelet-derived (PD) growth factor promoter resulting in overexpression of mutant APP (V717F); TBI: traumatic brain injury; TNF: Tumor necrosis factor.

* Studies using animals 18–19 mo of age in italics.