

Progesterone and Vitamin D Hormone as a Biologic Treatment of Traumatic Brain Injury in the Aged

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Abstract: There is growing recognition that traumatic brain injury is a highly variable and complex systemic disorder that is refractory to therapies that target individual mechanisms. It is even more complex in elderly persons, in whom frailty, previous comorbidities, altered metabolism, and a long history of medication use are likely to complicate the secondary effects of brain trauma. Progesterone, one of the few neuroprotective agents that has shown promise for the treatment of acute brain injury, is now in national and international phase 3 multicenter trials. New findings show that vitamin D hormone (VDH) and VDH deficiency in the aging process (and across the developmental spectrum) may interact with progesterone and treatment for traumatic brain injury. In this article we review the use of progesterone and VDH as biologics-based therapies along with recent studies demonstrating that the combination of progesterone and VDH may promote better functional outcomes than either treatment independently.

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INTRODUCTION

Traumatic brain injury (TBI) is a very complex, contextual, and dynamic process [1,2]. From the initial insult to the persistent and highly destructive cascade of secondary damage to eventual reorganization and recovery [3,4], each phase of the injury involves a different set of processes that sometimes overlap. Furthermore, the molecular and physiological changes resulting from a trauma to the brain are not spatially limited to the locus of injury, and even a well-circumscribed cerebral insult can have distal effects throughout almost the entire central nervous system (CNS) [5]. These often-subtle alterations in brain chemistry and physiology can persist for years and are associated with complex and sometimes unpredictable behavioral alterations such as posttraumatic stress disorder, mood disorders, or increased mortality from the disability and health complications that can occur long after the initial injury.

In addition to the complex injury process within the CNS, head trauma also has systemic and extraneuronal effects that need to be considered in the development of therapies for TBI. These processes, which most often are associated with systemic inflammation and multi-organ dysfunction, are the actual causes of death in most patients who have sustained a brain injury [6]. In a recent review, Masel and DeWitt [7] suggest that TBI “should be classified as the beginning of an ongoing, perhaps lifelong process that impacts multiple organ systems and may be disease causative and accelerative.” Thus TBI should be viewed not as an isolated or static insult but as an event that dynamically alters the systems in which it occurs.

Age and age-associated systemic changes can have additional effects on both survival and recovery from severe injury. Although some aspects of the injury may be different, systemic changes in response to brain injury also are likely to occur in children with TBI or hypoxic damage. Such changes include alterations in hormonal and drug metabolism, nutritional status, immune function, and increased frailty, among others. Thus it is not a given that TBIs, or the putative treatments for TBIs, will necessarily behave in the same way in very young children or in older subjects as they do in young adults. This situation is a good example of a “contextual” effect. In this review we focus primarily on elderly subjects, but similar considerations may apply to pediatric TBIs. Elderly patients might require research

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directed at the special nature and consequences of geriatric brain injury and a potentially different approach to treatment designed specifically to address the physiology of this age group.

Although a monotherapy approach may be appropriate for the handful of diseases known to have specific genetic or environmental causes, experience shows that such an approach is inadequate for more heterogeneous processes such as TBI. Here we suggest that because it is a complex, systemic process, TBI can be addressed best by pleiotropic or combination therapy (which, in addition to any pharmacologic treatment, can certainly include rehabilitation, counseling, and other behavioral approaches contributing to the “combination” aspect) and that any such therapy, if it is to be successful in human patients, must take into account additional contextual factors such as age, gender, and metabolic status.

TBI IS A MULTI-ORGAN/SYSTEMIC INFLAMMATORY DISORDER

Accumulating evidence now favors the hypothesis that inflammatory processes throughout the body have a substantial impact on morbidity and mortality after a TBI. Although brain and spinal cord injury generally have been treated as isolated and well-localized insults, experimental evidence increasingly suggests that elevated levels of inflammatory cytokines, a well-recognized aspect of the physiological response to trauma [8], are the most consistent prognostic markers of outcomes in patients with systemic inflammatory response syndrome, sepsis, multi-organ dysfunction syndrome (MODS), and multi-organ failure (MOF) [9]. These factors usually cause death, and at the very least, serious and often permanent disability after CNS trauma [5,6,10,11].

In many cases, systemic failure seems to be the final cause of death after CNS injury. For example, Zygun [6] found that dysfunction developed in 89% of patients and failure of at least one non-neurological organ occurred in 35% of patients. Failure of one non-neurological organ system was associated with a 40% death rate, which increased to 47% with the failure of 2 systems. Multi-organ pathology can occur as a consequence of neurologic injury to the brain [12,13]. In a study of patients with aneurismal subarachnoid hemorrhages, mortality was 31% when one organ failed, 91% when 2 organs failed, and 100% when 3 or more organs failed [14]. The non-neurological systems most frequently affected in conjunction with CNS injury seem to be the cardiovascular and pulmonary systems. The key etiology in the development of dysfunction in these 2 systems is probably the massive release of cytokines and catecholamines that occurs after neurologic injury, which can lead directly to myocardial dysfunction [15], severe pulmonary edema, and respiratory failure [16]. Other mechanisms of fundamental importance are inflammation, coagulation, and infection [6].

Significant elevations of inflammatory cytokines have been observed in cerebrospinal fluid, the systemic circulation [17,18], and even in the gut [19] after TBI. Given the significant blood flow to the brain under normal conditions and a damaged blood–brain barrier after injury, the brain essentially acts as a filter for the blood, allowing the systemic dissemination of inflammatory mediators away from the area of local injury. This phenomenon eventually leads to systemic inflammatory response syndrome, which generally is characterized by local and systemic release of inflammatory cytokines, complement, coagulation and acute phase proteins (APPs), and immune cell recruitment [10]. Concurrent with this process is a systemic anti-inflammatory response. The ability to strike a balance between these 2 opposing forces may determine a patient's survival. The presence of inflammation at and near the site of injury does not just contribute to local secondary damage—it is probably a key mechanism in TBI-associated mortality and morbidity [20].

For example, in a study of more than 2000 patients, Harrison-Felix et al [21] found that patients with a brain injury who are completing inpatient rehabilitation have a life expectancy that is on average 7 years shorter than that of patients without TBI. These patients also are much more likely to die from septicemia, pneumonia, and other respiratory diseases for up to 1 year after injury than are patients in the general population [22]. Even “mild” cases of TBI may be related to a greater risk of mortality. Brown et al [23] examined death rates in Minnesota for approximately 1300 patients with mild TBI and found a modest but statistically significant reduction in long-term survival compared with patients who did not have a head injury; however, Flaada and colleagues [24] found a greater risk in adult and older patients.

The systemic release of inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin 1 β (IL-1 β), and especially IL-6 induces the acute phase reaction, a systemic response to inflammation mainly executed by the liver that resets a number of homeostatic set points to improve defense and adaptation [25]. These changes exhibit considerable variability, from maintaining persistent inflammation to promoting adaptive changes. During the acute phase of the TBI (hours to several days), a number of APPs are up-regulated (eg, C-reactive protein, fibrinogen, and prothrombin), whereas others are down-regulated (eg, albumin, high-density lipoproteins, antithrombin III, and protein C). Although these changes are presumed to improve survival, in cases of severe injury such as TBI, an increased proportion of positive to negative APPs has been shown to accelerate the development of diffuse intravascular coagulation and to have generally pathophysiologic effects [10]. Acute inflammation also has been observed to increase levels of whole-body oxidative stress, which is another important mediator of systemic injury [26].

Inflammatory biomarkers also may be useful in predicting the severity and outcome of injury [9,27], as well as the prognosis for recovery [28-31]. Although it is well known that levels of the cytokines TNF- α and IL-1 β increase after severe injury, the level of IL-6 appears to be the most prognostically accurate because it is the chief regulator of the hepatic acute phase reaction [25] and correlates with the degree of systemic inflammation [9]. Evidence also suggests that an increased level of IL-6 in the early period after injury is a marker of a high risk of complication and organ failure [32]. Although considerable effort is being made to develop prognostic biomarkers for TBI, the issue is complicated by the fact that so many systemic changes occur in response to brain injury that no single biomarker fully reflects the underlying pathology [33]. As with inflammation and other pathologies, systemic biomarker activity may be further affected by the time when tissue or serum samples are taken, the patient's stress levels, previous health status, other comorbidities, nutritional history, drug use, gender, and age.

AGING, INFLAMMATION, AND BRAIN INJURY

Because of the focus on youth-related TBI that occurs in the setting of sports, war, and motor vehicle accidents, TBI in elderly persons receives less attention, despite the fact that the Centers for Disease Control and Prevention lists brain injury as a major cause of morbidity and mortality in persons older than 70 years [34]. The incidence of TBI has increased by 21% in people older than 65 years during the past decade, even as it decreased for most other age groups as a result of improved primary prevention such as the use of seat belts and safety helmets [35]. Age is an independent predictor of mortality resulting from TBI, and in elderly persons it exceeds that of younger victims by a factor of 2 [36]. As people reach their mid to late 70s, the frequency of hospitalizations attributable to TBI has its most pronounced peak [37]; the greatest mortality rates from "moderate" to severe TBI, ranging from 60.9% to 86.8% (depending on the study), occur in people 75 to 80 years of age [38].

Effects of Aging in the CNS

Although no overt neuronal loss is observed in the brain with normal aging, a variety of changes occur at the level of medium-scale neuronal networks and individual neurons that entail more subtle metabolic, structural, and chemical alterations [39,40], all of which have significant effects on neuronal plasticity and adaptation. Age-related changes in the CNS are similar to those that occur in other aging cells and include dysfunction of energy homeostasis, increased oxidative damage, protein accumulation, and deoxyribonucleic acid (DNA) damage [39]. As they age, neurons also become more susceptible to damage caused by glutamate

excitotoxicity [39] because of impairment of mitochondrial metabolism [41], Ca²⁺ homeostasis [39], and ion pump function [42]. Because these processes also are implicated in the development of injury after trauma, the aging process itself is likely to exacerbate the effects of TBI. In one interesting report, Wagner and colleagues [43] suggest that oxidative damage loads increase with age but that female subjects appear to fare better and have less oxidative stress than their male counterparts.

In addition to these structural and intrinsic changes, the brains of aged animals also exhibit gene expression patterns characterized by an increased level of neuroinflammation and microglial activation [44-47]. The microglia are not only more stimulated in older animals but also appear to generate an amplified response to inflammatory stimulation [48], resulting in a general neuroinflammatory "priming." Although the duration of inflammatory changes is limited under normal conditions as the microglia return to an inactive state upon resolution of immune challenges, in elderly subjects, the microglia are so sensitive to immune stimulation that their activation resolves slowly or not at all. This situation can result in exacerbation of neurologic disease [48] and still higher levels of neuroinflammation, with worsened outcomes in older subjects after TBI secondary to a more severe and persistent systemic immune response [49].

Effects of Aging on Systemic Inflammation and Endocrine Function

In addition to CNS-specific changes, age-related changes in the function of the systemic immune system commonly occur and are implicated in virtually all disease processes associated with advanced age [50]. Aging is associated with a general activation of the inflammatory response, which, because of the chronic antigenic stress on innate immunity experienced during a lifetime, becomes the basis for the onset of inflammatory diseases [51] and a reduced ability to mount an appropriate immune response to antigenic stimulation [50]. With aging, a decrease in the production of anti-inflammatory hormones also occurs [52], along with a general tendency toward production of elevated amounts of proinflammatory cytokines by peripheral blood mononuclear cells [53].

The fulminating inflammatory state associated with increasing age has been dubbed "inflammaging" [53]. Although this condition is systemic, it also has specific effects within the CNS [48]. Given the importance of inflammatory cytokines in both behavioral modulation and the evolution of traumatic injury, it is likely that an increased neuroinflammatory cytokine response in elderly patients can disrupt neuronal synaptic plasticity, establishing a CNS environment predisposed to long-lasting complications and a reduced ability to recover from trauma [48]. The aged brain appears to exist in a chronic state of inflammation that is associated with

increased immune reactivity and continuous low-level production of central inflammatory cytokines [54].

Given the apparent correlation between increasing frailty in the elderly and the increase in a chronic level of inflammatory factors in the brain and other organs, it may be necessary for the physician, as part of the patient's physical examination, to test for levels of these factors and the hormones that may be needed to offset the potential for further debilitation in the absence of immediate overt symptoms.

A final factor that must be specifically considered in human aging is the decreased activity of a number of key systemic hormones, including thyroid hormone [55], sex steroids [56-58], growth hormone (GH) [59], insulin-like growth factor-I (IGF-I) [59], and possibly 25-hydroxyvitamin D₃ [60-62]. It is important to note that this decrease in endocrine function appears to be associated with the immunologic changes discussed previously. Research suggests that proinflammatory cytokines may down-regulate the physiological responses to a variety of hormones, including insulin, GH, IGF-I, thyroid hormone, and estrogens [63]. TNF- α , IL-1 β , and IL-6 can modulate the release of GH, growth hormone-releasing hormone, and somatostatin [64] and can reduce concentrations of IGF-I [65] and increase the concentrations of glucocorticoids [66] in the serum of human patients. Lower serum IGF-I and dehydroepiandrosterone sulfate levels were found in frail compared with nonfrail elderly individuals [67], and an inverse correlation between serum IL-6 and IGF-I was noted only in frail individuals. Elevated levels of inflammatory cytokines [68-71] and the development of frailty in older adults [72,73] also have been associated with VDH deficiency.

Aging and VDH Deficiency

Recently VDH deficiency has received quite a bit of controversial media attention, partly because an estimated 1 billion people worldwide are said to exhibit VDH deficiency or insufficiency [74] and partly because it has been associated with autoimmune, inflammatory, and infectious conditions [75-81], cardiovascular disease [82,83], hypertension and atherosclerosis [84], neuromuscular function [85], cancer [86], and neurodegenerative diseases [87,88]. In the northeastern United States, for example, studies demonstrate a prevalence of VDH deficiency ranging from 32% in healthy adults [89] to approximately 50% for adolescents and preadolescents [90-92]. The most dramatic statistics come from studies in the elderly, which demonstrate that 40%-100% of community-dwelling American and European older men and women are deficient in VDH. Even higher averages are seen in the ill and institutionalized patients [93-101].

VDH deficiency appears to be correlated with many aging factors, especially those with an inflammatory component [76,77]. This finding is supported by further evidence that low levels of serum VDH are associated with elevated levels of

IL-6 [102] and a key marker of frailty in older adults [69,73]. VDH is known to be a modulator of the cell cycle, immune function, and calcium homeostasis. As such it may be an important compound not only as an endogenous hormone but also in the context of CNS injury, both as a treatment in its own right and in combination with another agent such as progesterone [103].*

PROGESTERONE AS A TREATMENT FOR BRAIN INJURY

During the past 30 years, some 50 candidate treatment drugs for TBI neuroprotection have failed when tested in human patients, and many pharmaceutical companies have essentially discontinued the pursuit of acute pharmacologic treatment for TBI. Despite this pessimism, a large and growing preclinical literature, along with the results of 2 recent phase 2 clinical trials [104,105] and a new phase 3 trial, indicate that the hormone progesterone may exert significant beneficial effects in the treatment of TBI.

Preclinical work on progesterone neuroprotection has been ongoing for more than 20 years. One of the first papers to demonstrate a beneficial effect of progesterone and TBI came from our laboratory [106]. We tested "pseudopregnant" and normally cycling female rats with large bilateral lesions of the frontal cortex on a delayed spatial alternation learning task. The pseudopregnant females, whose endogenous hormone levels were elevated experimentally by cervical stimulation, performed significantly better in memory retention of the trained task after injury than did their normal-cycling counterparts. When the brains were examined, the pseudopregnant rats also demonstrated substantially less ventriculomegaly, suggesting that traumatic edema was reduced by the relatively greater levels of endogenous progesterone present at the time of surgery and for several days thereafter [107]. We also showed that, in rats, progesterone can be given up to 24 hours after bilateral cortical injury and still retain its neuroprotective effects, although the sooner the treatment is given, the better the outcome [108].

Many publications have now demonstrated the effectiveness of progesterone treatment for TBI [107,109-112], and more than 170 papers using 22 different injury models from 25 laboratories around the world have now reported its beneficial effects in attenuating the cytologic, morphologic,

* A recent, apparently controversial report by the Institute of Medicine [103] concludes that dietary intake of VDH and calcium is adequate for "most people . . . to enjoy good bone health." The report challenges many popular assumptions about vitamin D benefits in a number of disease conditions including autism, cognitive function, and depression, but for unexplained reasons ignores the potential role of VDH in cerebral stroke and TBI. However, the authors do take care to note that "Nonetheless, some subgroups—particularly those who are older and living in institutions or who have dark skin pigmentation—may be at increased risk for getting too little vitamin D" (p. 3). The authors also point out that "no central authority has determined which cut-points to use" (in determining sufficiency or deficiency).

and functional deficits caused by injury to the brain, spinal cord, and peripheral nerves [107,113-118, 121, 123-128].[†]

Unlike other sex steroids, progesterone is synthesized by oligodendrocytes in the brain, where it modulates the activity of classical nuclear, membrane-bound, and steroid receptors [123]. Progesterone also can directly affect the expression of water channel proteins (aquaporins) to modulate both vasogenic and cytotoxic edema [121,124], reduce glutamate toxicity directly, mediate toxic cellular calcium influx by antagonizing sigma receptors, up-regulate gamma-aminobutyric acid A (GABA_A) receptors to reduce excitotoxic damage, and reduce apoptosis and necrosis through the up-regulation and down-regulation of a variety of genes that control cell survival [112,123].

In contrast to the single-receptor, single-mechanism approach, we believe it is progesterone's ability to act on different components of the injury cascade simultaneously and to exert differential effects over time and place that make it more effective than other agents that have been studied for the treatment of TBI. We have shown that, when given systemically, progesterone can affect the expression of at least 90 genes thought to play a role in neuroprotection and neurorepair [110]. This pleiotropy, although not fitting neatly within the molecular biological paradigm of disease, is likely the reason for its translational success thus far.

The preclinical data on the neuroprotective efficacy of progesterone after TBI have been confirmed in human patients in a phase 2a, single-center, randomized, double-blind clinical trial for progesterone treatment of moderate-to-severe adult TBI sponsored by the National Institute of Neurological Disorders and Stroke [104]. The authors examined 100 patients enrolled with proxy consent within 11 hours of injury and included only those with a postresuscitation Glasgow Coma Scale (GCS) score between 4 and 12. For every 4 patients assigned to the treatment group, one was assigned to the placebo control group, and all patients received intravenous administration of the test drug or placebo solutions. Proxy consent requirements for this study delayed progesterone treatment for at least 6 hours after injury. Neurologic and functional outcomes were evaluated 30 days after the injury. Mortality among patients who were given progesterone was less than half that of control subjects (13.6% vs 30.4%), and 30-day functional outcomes for moderately injured patients

were significantly better than for the placebo group. Members of the Data Safety Monitoring Board, appointed by the National Institutes of Health (NIH), found no serious adverse events attributable to the progesterone treatment.

These initial results were confirmed by a second independent, randomized, double-blind study from China in which the authors used a 5-day intramuscular course of treatment with progesterone in 159 patients with severe TBI. Patients who arrived within 8 hours of injury with a GCS score of 8 or lower were randomly assigned (1:1) to receive either progesterone or placebo. The primary end point was Glasgow Outcome Scale (GOS) measurement 3 months after the injury, but the investigators also used Functional Independence Measure scores and repeated the measures on a modified Functional Independence Measure and GOS 6 months after the TBI occurred. Similar beneficial outcome measures on morbidity and mortality were observed at both 30 days and 6 months after injury, again without any serious adverse events caused by the treatment. The authors also reported that intracranial pressure was lower 72 hours and 7 days after treatment with progesterone [105].

PROGESTERONE IN A PHASE 3 CLINICAL TRIAL

Following up on the positive phase 2 clinical data [104,105], a phase 3, 17-center clinical trial for TBI sponsored by NIH is now currently enrolling patients across the United States. This 1:1 randomized, double-blind controlled trial will enroll 1200 patients. Treatment must begin within 4 hours of injury in patients at least 18 years of age with a GCS score between 12 and 4. After a 1-hour loading dose of 0.714 mg/kg administered intravenously (IV), progesterone or placebo is given by IV infusion at 0.5 mg/kg for 72 hours and then tapered over the course of an additional 24 hours. The 4-hour window of treatment was designed to optimize progesterone's neuroprotective effects by treating patients with TBI as soon as possible. Food and Drug Administration approval for exemption from informed consent was granted for this purpose. The primary study end point will be a stratified dichotomy of the GOS at 6 months. The trial will track mortality, extent of adverse and serious adverse events, Disability Rating Score, and cognitive, neurologic, and functional outcomes. More details can be found at [ClinicalTrials.gov](http://clinicaltrials.gov/ct2/show/record/NCT00822900) (<http://clinicaltrials.gov/ct2/show/record/NCT00822900>).

In addition to the NIH study, BHR, a branch of Besins Pharma, a privately held Belgian/French pharmaceutical company, also recently began enrolling patients in a second clinical trial called SyNAPSe (<http://www.synapse-trial.com/>). Approximately 1800 patients with severe closed-head TBI (GCS scores of 4-8) in whom treatment can be initiated within 8 hours of injury will be enrolled in the study through 100-120 medical centers in the United States, Western Europe, Israel, and other countries. Patients are randomly as-

[†] Despite the growing evidence of progesterone's neuroprotective effects, 2 studies from laboratories examining the role of cyclosporin in the treatment of TBI have reported negative outcomes [119,120]. In addition, the authors of 2 recent reviews [121a,121b] suggest that, by the authors' personal, operational definitions, many, if not all, preclinical studies testing neuroprotective agents, including progesterone, fail to meet a number of their criteria for preclinical development of new drugs. However, despite their concerns, given the large number of studies in which the authors use progesterone, it may be of interest to note that most, if not all, of the criteria stipulated by Loane and Faden and Gibson et al have been addressed for the studies using progesterone, but perhaps to a lesser extent than for most of the other neuroprotective agents cited in the paper by Loane and Faden. However, even if all the criteria were indeed met, no guarantee exists that a completely successful agent for brain repair would be discovered. The review presents a number of important suggestions for the design of studies in neuroprotection.

signed to receive a 5-day continuous IV infusion of progesterone or placebo in a 1:1 allocation, and follow-up is conducted for 6 months. The GOS is the primary study end point. This global, phase 3, multicenter pivotal trial of IV progesterone infusion for patients with severe TBI is being conducted in collaboration with the American Brain Injury Consortium and the European Brain Injury Consortium.

We suggest that one of the reasons for increased rates of survival in patients with TBI who are treated with progesterone may be progesterone's ability to substantially reduce systemic acute phase inflammation and thereby prevent MODS and MOF [122,125]. Because, as discussed previously, MODS and MOF frequently are the actual causes of death in patients with a TBI, the ability of progesterone to modulate the development of systemic inflammation may be one way that it promotes survival in the early stages after brain injury. This idea was supported by Chen and colleagues [126], who found that contusion injury to the cerebral cortex in rats induced expression of IL-1 β and TNF- α followed by apoptosis in the intestinal mucosa. A 5-day course of treatment with progesterone reduced both the cytokines and cell death in the intestine. This study shows that an experimental brain injury can trigger a cascade of inflammation in other tissues, which in turn can produce serious consequences for brain repair. For example, gut inflammation could affect nutritional variables needed to help with the CNS metabolism involved in neuroprotection (see the article by Popovich and McTigue [5] for a further discussion of this issue).

PROGESTERONE, VITAMIN D, AND AGING IN BRAIN INJURY

Progesterone's effectiveness in treating TBI and the prevalence of VDH deficiency in the elderly population led to 3 questions: First, would progesterone be as effective in aged animals as in young adult animals? Second, would VDH deficiency affect the outcome of a brain injury and potentially interfere with the benefits of progesterone treatment in old rats? And finally, would a combination of VDH and progesterone be more effective than either compound alone in the same aged rat population? We speculated that progesterone would be effective in treating TBI in old rats but that VDH deficiency would increase inflammation and decrease the benefits of treatment [127]. We also hypothesized that combining VDH with progesterone would improve outcome. Because the early onset of inflammation is a reliable prognostic indicator of mortality in human patients with significant trauma, we measured a number of acute inflammatory proteins, cell death, DNA damage, and short-term behavior as indicators of inflammation and secondary damage in aged and VDH-deficient aged animals after TBI.

Progesterone and VDH after TBI in Aged Rats

Progesterone was indeed effective in treating TBI in old rats, although at a higher dose than in young adult animals [128]. We measured levels of inflammatory proteins, cell death, edema, and behavior during the acute phase of injury (24-72 hours after TBI) in 20-month-old rats (which is the "human equivalent" of 60 years of age). Injured animals treated with progesterone beginning within the first hour after surgery showed decreased inflammatory markers at all time points examined, indicating a reduction in the acute inflammatory process compared with the old rats given vehicle. We also observed decreased markers of cell death at all time points, as well as decreased edema and improved motor function, which suggests that the pleiotropic effects of progesterone were also effective despite the added complexity of treating an aged organism.

When we tested aged rats that were made VDH deficient, we observed increased inflammation compared with VDH replete animals in all groups, with or without TBI and with or without progesterone treatment [127]. Our results confirmed findings of previous studies [76,77,129] that suggested that VDH deficiency establishes a higher baseline level of inflammation even before injury, in effect priming the system for an increased immune response after TBI. This elevated acute-phase response correlated with increased cell death and DNA damage, indicating a more severe secondary injury process after injury even with progesterone treatment.

Both TNF- α and IL-6 were significantly increased by VDH deficiency, and both cytokines demonstrated an interaction between deficiency and treatment. Although most of the variability in TNF- α was accounted for by injury, the level of IL-6 was primarily affected by VDH deficiency, a fact that corresponds to other independent data connecting IL-6 levels with VDH deficiency, frailty, and inflammation [73,102,130,131]. The elevation in IL-6 was most evident in our data comparing VDH-deficient and VDH-normal animals after TBI and progesterone treatment. Although most of the other cytokines were elevated 2- or 3-fold in VDH-deficient animals, IL-6 was increased nearly 5-fold by 72 hours after the injury occurred, suggesting that IL-6 may be the primary cytokine involved in the detrimental effects of VDH deficiency after TBI.

Combination Therapy in TBI

Combination therapy is a relatively novel idea in the area of brain injury, but it is a well-established pharmacologic approach [4] in other disease processes such as HIV/AIDS and tuberculosis. Most drug development still focuses on individual agents and is targeted toward a few specific mechanisms at most. As discussed previously, this approach may not be appropriate in the setting of TBI. Rationales for combining drugs include targeting multiple divergent mechanisms and

overcoming single-drug limitations such as receptor kinetics, pharmacology, and signaling pathways [132,133]. This idea has recently gained ground in the treatment of TBI [134]. "Polytherapy" approaches can proceed in 2 ways: (1) drugs can be designed for target promiscuity or pleiotropy, where a single agent affects multiple pathways simultaneously or sequentially or both; or (2) drugs can be designed to be administered in combination, which achieves the same effect of targeting multiple processes, but separately in this case. A hybrid approach, which may be wise for the treatment of TBI, is to combine pleiotropic drugs themselves to overcome the limitations of monotherapy. A combinatorial approach to treatment is not only reasonable but may be essential given the complexity and heterogeneity of human TBI and the fact that, of the various preclinical monotherapy drugs that have shown promise for brain injury neuroprotection, all have been shown to be ineffective when taken to clinical trial [134].

Combining Progesterone and VDH in TBI

Given our results with progesterone and VDH deficiency in aged rats, we speculated that acute correction of VDH deficiency with VDH would restore the efficacy of progesterone. Combining progesterone and VDH, which are pleiotropic drugs that work primarily through intracellular receptors/transcription factors, also merits serious consideration even outside the context of VDH-deficiency as meeting the criteria for combinatorial therapies [4]. Because both progesterone and VDH are pleiotropic and multifunctional compounds, it is difficult to predict specific mechanisms of interaction, but both agents could be used to target cell death after TBI, for example, as one of the many mechanisms by which a brain injury evolves.

It is worth emphasizing here that VDH is in fact not a vitamin but rather a secosteroid hormone with the same cholesterol backbone as other sex steroids (such as progesterone) and has its own class of nuclear steroid receptors and signaling mechanisms [135,136]. Like progesterone, VDH is a pleiotropic agent with important implications for the development and treatment of systemic disease. Recently Ramagopal and colleagues [137] examined VDH receptor binding in lymphoblastoid cells after treatment with VDH. They found more than 2700 gene positions with VDH sites for signaling gene transcription and approximately 230 genes that changed their level of expression in response to VDH. Many of these genes (like progesterone) are directly implicated in modulating the immune/inflammatory response to injury and neurodegenerative diseases.

Importantly, progesterone complements VDH's anti-inflammatory effects not only in the brain but in other tissues affected by the inflammatory cascade that often accompanies TBI or stroke. To reiterate, one of the hormone's main benefits and probably one of the main reasons it is so effective as

a systemic treatment for serious injury is because it reduces the inflammatory cascade not only in the brain but in other tissues such as gut, heart, spleen, and thymus that are also subject to cytokine damage [138-143].

Evidence also suggests that, like progesterone, VDH administered after a brain injury can have neuroprotective effects, although a number of its actions involve different pathways from those of progesterone [144]. Both progesterone and VDH are natural hormones that are synthesized in both men and women, and both can act as agents that produce other metabolites in the brain with their own distinct modes of action in CNS repair. These properties, taken in the context of the clinical trial results showing very good progesterone safety and efficacy in TBI, makes the testing of progesterone and VDH in a preclinical combined study a compelling approach to consider for use in a clinical trial, especially for elderly persons.

Perhaps similar consideration should be given to planning for vitamin D supplementation in pediatric TBI treatments. A safety and efficacy trial for progesterone in pediatric TBI under the auspices of the Pediatric Emergency Care Applied Research Network (PECARN) is beginning to take shape. Growing evidence suggests that many children can have VDH insufficiency or even deficiency, and this condition may affect long-term disease outcome and possibly the effectiveness of neuroprotective treatments such as progesterone for early brain injury [145,146].

Progesterone/VDH Combination in Aged Rats with TBI

We have demonstrated the effectiveness of a combination of progesterone and VDH in both in vitro and in vivo models in our laboratory. Combining VDH and progesterone was effective in vitro in increasing the survival of primary cortical neurons under 24 hours of excitatory glutamate challenge. Although VDH alone and progesterone alone both produced beneficial effects on neuronal cell survival, the combination of VDH and progesterone produced significantly more neuroprotection than either compound given alone and at their best individual doses [147]. We used glutamate-induced excitotoxicity as our primary outcome measure because it reliably triggers cell death in models of TBI, stroke, and spinal cord injury.

Interestingly, when we applied the same treatment concept in vivo to VDH-deficient aged rats with large bilateral contusions of the frontal cortex, the only treatment that reduced inflammatory marker proteins was the combination of progesterone and VDH (5 mg/kg in a single dose) compared with vehicle or either compound given alone. The combination treatment was also the only one that substantially improved behavioral outcomes. If these conditions apply to human patients, the clinical implications could be important: a combination treatment of progesterone and

VDH administered to elderly patients with TBI should improve survival compared with progesterone given alone to the same population. Because few, if any, better alternatives exist, a clinical investigation would appear to be a reasonable step to take.

Briefly summarized, we believe that: (1) progesterone is effective in reducing acute inflammation, a key indicator of survival in human patients and in aged rats with brain injury; (2) D deficiency increases acute-phase inflammation and attenuates the benefits of progesterone treatment in aged rats with TBI, which suggests that such a deficiency could increase mortality and worsen outcome in TBI survivors; and (3) a combination of progesterone and VDH partially reverses the effects of D deficiency and reduces post-TBI acute inflammation in old rats.

CONCLUSION

Should our results translate to the clinic, the implication of the research discussed here is that a simple vitamin D injection combined with progesterone could help save many human lives. Extrapolating from the animal data that show that D deficiency enhances inflammatory activity even in old intact rats, and because vitamin D insufficiency appears to be prevalent in so many elderly and frail patients, we urge rehabilitation physicians to screen routinely for vitamin D levels in their patients, especially those who have experienced a stroke or TBI. Our animal data also can be taken to suggest that ignoring D deficiency or insufficiency can, over time, exacerbate the injury, probably reduce the benefits of physical and cognitive therapy, and delay or prevent functional recovery.

As we noted previously, the reduction in systemic inflammation resulting from combined VDH and progesterone treatment may be one of the key mechanisms by which progesterone and VDH, in combination or alone, could significantly increase survival after TBI in human patients. Although eventually better alternatives to the treatment we are proposing may be found, it is becoming increasingly clear that no single receptor, gene, or mechanism can account for the complex cascade of focal and systemic toxic events produced by a brain injury, whether it is in children, adults, or elderly persons. It is highly unlikely that a single agent will be able to trigger all the neuroprotective and neuroplastic events required for the damaged brain to reorganize to cope with a traumatic injury, degenerative disorder, or serious stroke. Despite the evidence that progesterone and its metabolites might work well, their effectiveness has not yet been conclusively demonstrated. Individual or combination therapy with pleiotropic agents capable of addressing multiorgan dysfunction is becoming more acceptable (and more appropriate) as we begin to understand the complex systems and networks involved in the mediation of brain repair and functional recovery.

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