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PROGESTERONE AND VITAMIN D HORMONE FOR TREATMENT OF TRAUMATIC BRAIN INJURY IN THE AGED

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Abstract

There is growing recognition that traumatic brain injury (TBI) is a highly variable and complex systemic disorder that is refractory to therapies that target individual mechanisms. It is even more complex in the elderly, in whom frailty, prior 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 III multi-center trial. New findings show that vitamin D hormone (VDH) and vitamin D deficiency in aging (and across the developmental spectrum) may interact with progesterone and TBI treatment. This paper reviews the use of progesterone and VDH as biologics based therapies and recent studies showing that the combination of progesterone and VDH may promote better functional outcomes than either treatment independently.

Keywords

Traumatic brain injury; aging; progesterone; vitamin D; recovery of function

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, which sometimes overlap and sometimes do not. Further, the molecular and physiological changes resulting from a trauma to the brain are not spatially limited to the

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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 (PTSD), 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 which need to be considered in the development of therapies for TBI. These processes, most often associated with systemic inflammation and multi-organ dysfunction, are the actual causes of death in a majority of brain-injured patients [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” (p. 1529). 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 are also 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. It is therefore not a given that TBI, or the putative treatments for it, will necessarily behave in the same way in very young children or older subjects as they do in young adults. This is a good example of a “contextual” effect. In this review, we focus primarily on the aged subject, but similar considerations may apply to pediatric injury. The elderly might therefore require research directed to the special nature and consequences of geriatric brain injury as well as a potentially different approach to treatment designed specifically to address the physiology in 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 like TBI. Here we suggest that as a complex, systemic process, TBI can be addressed better by pleiotropic or combination therapy (which, in addition to any pharmacological treatment, can certainly include rehabilitation, counseling and other behavioral approaches contributing to the ‘combination’), 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 have generally 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 (SIRS), 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 and colleagues found that 89% developed dysfunction and 35% failure of at least one non-neurological organ [6]. Failure of one non-neurological organ system was associated with a 40% death rate, and this increased to 47% with the failure of two 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% for one organ failure, 91% for two, and 100% for patients who developed three or more [14]. The non-neurologic systems most frequently affected in conjunction with CNS injury appear to be the cardiovascular and pulmonary. The key etiology in the development of dysfunction in these two systems is probably the massive release of cytokines and catecholamines that occurs after neurologic injury and this 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 (CSF), 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 (BBB) 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 eventually leads to SIRS, generally 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 two opposing forces may determine 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, one study in over 2,000 patients found that brain injury patients completing inpatient rehabilitation have a life expectancy on average seven years shorter than patients without TBI [21]. These patients are also much more likely to die from septicemia, pneumonia, and other respiratory diseases for up to one year after injury than patients in the general population [22]. Even "mild" TBI may be related to a greater risk of mortality. Brown et al. [23] examined death rates in Minnesota in about 1300 patients with mild TBI and found a modest but statistically significant reduction in long-term survival compared to patients without head injury, but Flaada, et al. found a higher risk in adult and older patients [24]. Systemic release of inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 1β (IL-1β), 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 in order 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 upregulated (e.g., C-reactive protein, fibrinogen, prothrombin), while others are downregulated (e.g., albumin, high-density lipoproteins (HDL), antithrombin III (ATIII), 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 (DIC) and to have generally pathophysiologic effects [10]. Acute inflammation has also been observed to increase levels of whole-body oxidative stress, another important mediator of systemic injury [26].

Inflammatory biomarkers may also be useful in predicting the severity and outcome of injury [9, 27] as well as the prognosis for recovery [28–31]. While it is well known that levels of the cytokines (TNFα and IL-1β) 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]. There is also evidence that an increased level of IL-6 in the early period after injury is a marker of 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 there are
so many systemic changes 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 when tissue or serum samples are taken, stress levels, prior health, other comorbidities, nutritional history, drug use, gender, and age.

AGING, INFLAMMATION, AND BRAIN INJURY

Because of the focus on youth-related TBI in sports, war, and motor vehicle accidents, TBI in the elderly receives less attention despite the fact that the CDC lists brain injury as a major cause of morbidity and mortality in people over 70 years of age [34]. The incidence of TBI has increased by 21% in people over 65 over the past decade, even while it decreased for most other age groups due to improved primary prevention such as seat belt and safety helmet use [35]. Age is an independent predictor of mortality secondary to TBI, which in the elderly exceeds that of younger victims by a factor of two [36]. As people reach their mid to late 70s, the frequency of hospitalizations due to TBI has its most pronounced peak [37]; the highest mortality rates from "moderate" to severe TBI, ranging from 60.9% to 86.8% (depending on the study), occur in people 75–80 years old [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 DNA damage [39]. As they age, neurons also become more susceptible to damage caused by glutamate excitotoxicity [39] due to impairment of mitochondrial metabolism [41], Ca2+ homeostasis [39], and ion pump function [42]. Since these processes are also 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 et al. [43] suggest that oxidative damage loads increase with age, but that females 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 aged 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 neurological 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 Old Age 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, due to the chronic antigenic stress on innate immunity experienced over 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.
With aging there is also a decrease in the production of anti-inflammatory hormones as well as a general tendency towards production of elevated amounts of pro-inflammatory cytokines by peripheral blood mononuclear cells. The fulminating inflammatory state associated with increasing age has been dubbed “inflammaging.” While this condition is systemic, it also has specific effects within the CNS. 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 the elderly can disrupt neuronal synaptic plasticity, establishing a CNS environment predisposed to long-lasting complications as well as a reduced ability to recover from trauma. 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.

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 become a necessary part of the physical examination of patients 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 (TH), sex steroids, growth hormone (GH), insulin-like growth factor-I (IGF-I), and possibly 25-hydroxyvitamin D. It is important to note that this decline in endocrine function appears to be associated with the immunological changes discussed previously. Research suggests that proinflammatory cytokines may downregulate the physiological responses to a variety of hormones including insulin, GH, IGF-I, TH, and estrogens. TNFα, IL-1, and IL-6 can modulate the release of GH, growth hormone releasing hormone (GHRH), and somatostatin, and can reduce concentrations of IGF-I and increase the concentrations of glucocorticoids in the serum of human patients. Lower serum IGF-I and DHEA-S levels were found in frail compared to non-frail elderly individuals, and an inverse correlation between serum IL-6 and IGF-I was noted only in frail individuals. Elevated levels of inflammatory cytokines and the development of frailty in older adults have also been associated with VDH deficiency (D-deficiency).

### Aging and Vitamin D Deficiency

Vitamin D-deficiency has received quite a bit of controversial media attention recently, partly because an estimated 1 billion people worldwide are said to exhibit vitamin D-deficiency or insufficiency and partly because it has been associated with autoimmune, inflammatory, and infectious conditions, cardiovascular disease, hypertension and atherosclerosis, neuromuscular function, cancer, and neurodegenerative diseases. In the northeastern United States, for example, studies demonstrate a prevalence from 32% in healthy adults to around 50% for adolescents and preadolescents. The most dramatic statistics come from studies in the elderly showing that 40 to 100% of community-dwelling American and European older men and women are vitamin D-deficient. Even higher averages are seen in the ill and institutionalized. Vitamin D-deficiency appears to be correlated with many aging factors, especially those with an inflammatory component. This is supported by further evidence that the level of serum vitamin D is associated with elevated levels of IL-6 and is a key marker of frailty in older adults. Vitamin D 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.
PROGESTERONE AS A TREATMENT FOR BRAIN INJURY

Over the last 30 years some 50 candidate treatment drugs for TBI neuroprotection have failed when tested in human patients, and many pharmaceutical companies have essentially given up pursuing acute pharmacological treatment for TBI. Despite this pessimism, a large and growing preclinical literature, the results of two recent Phase II clinical trials [104, 105] and a new Phase III 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 over 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 higher 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 there are now more than 170 papers using 22 different injury models from 25 laboratories around the world reporting its beneficial effects in attenuating the cytological, morphological, and functional deficits caused by injury to the brain, spinal cord and peripheral nerves [107, 113–118].

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 [121]. Progesterone can also directly affect the expression of water channel proteins (aquaporins) to modulate both vasogenic and cytotoxic edema [122, 123], reduce glutamate toxicity directly, mediate toxic cellular calcium influx by antagonizing sigma receptors, upregulate GABA_A receptors to reduce excitotoxic damage, and reduce apoptosis and...
necrosis through the up- and down-regulation of a variety of genes controlling cell survival [112, 121].

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 tried 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, while 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 an NINDS-sponsored, Phase IIa, single-center, randomized, double-blind clinical trial for progesterone treatment of moderate to severe adult TBI [104]. The study examined 100 patients enrolled with proxy consent within 11 hours of injury and included only those with a post-resuscitation Glasgow Coma Scale (GCS) Score between 4 and 12. For every four patients assigned to the treatment group, one was assigned to the placebo control, and all 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. Neurological and functional outcomes were evaluated at 30 days following the injury. Mortality among patients given progesterone was less than half that of controls (13.6% versus 30.4%), and 30-day functional outcomes for moderately injured patients were significantly better than for the placebo group. An NIH-appointed Data Safety Monitoring Board 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 using 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 less were randomized 1:1 to receive either progesterone or placebo. The primary endpoint was Glasgow Outcome Scale (GOS) measurement at 3 months after the injury, but the investigators also used FIM scores and repeated the measures on a modified FIM and GOS at 6 months post-TBI. 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 at 72 hours and 7 days after treatment with progesterone [105].

PROGESTERONE IN PHASE III CLINICAL TRIAL

Following up the positive Phase II clinical data [104, 105], an NIH-sponsored, Phase III, 17-center clinical trial for TBI is now currently enrolling patients across the United States. This is a 1:1 randomized, double-blind controlled trial with 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 one-hour loading dose of 0.714 mg/kg IV, progesterone or placebo is given by IV infusion at 0.5 mg/kg for 72 hours, then tapered over an additional 24 hours. The 4-hour window of treatment was designed to optimize progesterone’s neuroprotective effects by treating TBI patients as soon as possible. FDA approval for exemption from informed consent (EFIC) 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, DRS, and cognitive, neurological, and functional outcomes. More details can be found at 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, SyNAPSe. Approximately 1200 patients with severe (GCS scores of 4 – 8) closed-head TBI 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 randomized to receive a 5-day continuous i.v. infusion of progesterone or placebo in a 1:1 allocation and followed for 6 months. The GOS is the primary study end point. This global, Phase 3, multi-center pivotal trial of IV progesterone infusion for severe TBI is being conducted with the collaboration of the American Brain Injury Consortium (ABIC) and the European Brain Injury Consortium (EBIC). For more information see http://www.synapse-trial.com/.

We suggest that one of the reasons for increased survival in progesterone-treated TBI patients may be progesterone’s ability to substantially reduce systemic acute phase inflammation and thereby prevent MODS and MOF [124, 125]. Since, as discussed earlier, MODS and MOF are frequently the actual causes of death in TBI patients, progesterone’s ability to modulate the development of systemic inflammation may be one way in which it promotes survival in the early stages after brain injury. Supporting this idea, Chen et al. [126] found that contusion injury to the cerebral cortex in rats induced expression of IL-1β and TNFα, followed by apoptosis in the intestinal mucosa. A five-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 tissue, which in turn can produce serious consequences for brain repair. For example, gut inflammation could impact on nutritional variables needed to help with CNS metabolism involved in neuroprotection (see also [5] for a further discussion of this issue).

PROGESTERONE, VITAMIN D, AND AGING IN BRAIN INJURY

Progesterone’s effectiveness in TBI and the prevalence of D-deficiency in the elderly population suggested three questions: first, would progesterone be as effective in aged animals as in young adults; second, whether D-deficiency would affect the outcome of a brain injury and potentially interfere with the benefits of progesterone treatment in old rats; and finally, whether a combination of VDH and progesterone would 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 D-deficiency would increase inflammation and decrease the benefits of treatment [127]. We also hypothesized that combining VDH with progesterone would improve outcome. Because 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 D-deficient aged animals after TBI.

Progesterone and Vitamin D 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 post-TBI) in 20-month-old aged rats (the “human equivalent” of ~60 years old). 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 to 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, suggesting that the pleiotropic effects of progesterone were also effective despite the added complexity of treating an aged organism.
When we tested aged rats made D-deficient, we observed increased inflammation compared to vitamin D-replete animals in all groups, with or without TBI and with or without progesterone treatment [127]. Our results confirmed previous studies [76, 77, 129] suggesting that D-deficiency establishes a higher baseline level of inflammation even prior to 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 TNFα and IL-6 were significantly increased by D-deficiency, and both cytokines demonstrated an interaction between deficiency and treatment. Although most of the variability in TNFα was accounted for by injury, the level of IL-6 was primarily affected by D-deficiency, a fact that corresponds to other independent data connecting IL-6 levels with D-deficiency, frailty, and inflammation [73, 102, 130, 131]. The elevation in IL-6 was most evident in our data comparing D-deficient and D-normal animals after TBI and progesterone treatment. While most of the other cytokines were elevated in D-deficient animals 2- or 3-fold, IL-6 was increased nearly 5-fold by 72 hours after injury, suggesting that IL-6 may be the primary cytokine involved in the detrimental effects of D-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 pharmacological approach [4] in other disease processes such as HIV/AIDS and tuberculosis. Most drug development still focuses on individual agents and is targeted towards a few specific mechanisms at most. As discussed earlier, 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 two 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, one that may be wise for the treatment of TBI, is to combine pleiotropic drugs themselves in order 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 failed when taken to clinical trial [134].

Combining Progesterone and VDH in TBI

Given our results with progesterone and D-deficiency in aged rats, we speculated that acute correction of D-deficiency with VDH would restore the efficacy of progesterone. Combining progesterone and VDH, pleiotropic drugs that work primarily through intracellular receptors/transcription factors, also merits serious consideration even outside the context of D-deficiency as meeting the criteria for combinatorial therapies [4]. Since both progesterone and VDH are pleiotropic and multi-functional 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 vitamin D is in fact not a vitamin, but rather a seco-steroid hormone with the same cholesterol backbone as other sex steroids (such as progesterone) and 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 Ramagopalan and colleagues [137] examined...
vitamin D receptor binding in lymphoblastoid cells following VDH treatment. They found over 2700 gene positions with VDH sites for signaling gene transcription and about 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 vitamin D’s anti-inflammatory effects not only in the brain but in other tissues affected by the inflammatory cascade that often accompany TBI or stroke. To reiterate, of the hormone’s main benefits and probably one of the main reasons it is so effective as a systemic treatment for serious injury: 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].

There is also evidence that, like progesterone, VDH given after a brain injury can have neuroprotective effects, although a number of its actions involve different pathways from progesterone’s [144]. Both progesterone and VDH are natural hormones, synthesized in both males and females, 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 pre-clinical combined study a compelling approach to consider for use in a clinical trial, especially for the elderly.

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 vitamin D insufficiency or even deficiency, and this condition may impact long-term disease outcome and possibly the effectiveness of neuroprotective treatments like progesterone for early brain injury [145, 146].

**Progesterone/VDH combination in Aged Rats with TBI**—We have demonstrated 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 increasing the survival of primary cortical neurons under 24 hours of excitatory glutamate challenge. While 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 D-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 (5mg/kg in a single dose) compared to 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 given to elderly patients with TBI should improve survival over progesterone given alone to the same population. Since there are few, if any, better alternatives, a clinical investigation would appear to be a reasonable step to take.

Briefly summarized, we think 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, suggesting 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 showing that D-deficiency will enhance inflammatory activity even in old intact rats, and since vitamin D insufficiency appears to be so prevalent in so many of the elderly and frail, we urge that rehabilitation physicians screen routinely for vitamin D levels in their patients, especially those who have suffered a stroke or TBI. Our animal data can also 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 earlier, 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. While eventually there may be better alternatives to the treatment we are proposing here, 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 the aged. It is highly unlikely that a single agent will be able to trigger all the neuroprotective and neuroplastic events needed 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, this has not yet been conclusively demonstrated. Individual or combination therapy with pleiotropic agents capable of addressing multi-organ 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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