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PROGESTERONE AND VITAMIN D HORMONE FOR TREATMENT OF TRAUMATIC BRAIN INJURY IN THE AGED[1]

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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. Tthe 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 the that 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], Ca^{2+} 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

[50]. With aging there is also a decrease in the production of anti-inflammatory hormones [52] as well as a general tendency towards production of elevated amounts of pro-inflammatory cytokines by peripheral blood mononuclear cells [53]. The fulminating inflammatory state associated with increasing age has been dubbed “inflammaging” [53]. While 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 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 [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 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) [55], sex steroids [56–58], growth hormone (GH) [59], insulin-like growth factor-I (IGF-I) [59], and possibly 25-hydroxyvitamin D_3 [60–62]. 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 [63]. TNFα, IL-1, and IL-6 can modulate the release of GH, growth hormone releasing hormone (GHRH), 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 DHEA-S levels were found in frail compared to non-frail 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] 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 [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 from 32% in healthy adults [89] to around 50% for adolescents and preadolescents [90–92]. Tthe 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 [93–101]. Vitamin D-deficiency appears to be correlated with many aging factors, especially those with an inflammatory component [76, 77]. This is supported by further evidence that the level of serum vitamin D is associated with elevated levels of IL-6 [102] and is a key marker of frailty in older adults [69, 73]. 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[1].

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].[2]

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

[1]A recent, apparently controversial report by the Institute of Medicine [103. Ross, A.C., J.E. Manson, S.A. Abrams, J.F. Aloia, P.M. Brannon, S.K. Clinton, R.A. Durazo-Arvizu, J.C. Gallagher, R.L. Gallo, G. Jones, C.S. Kovacs, S.T. Mayne, C.J. Rosen, and S.A. Shapses, The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab, 2010] concludes that dietary intake of vitamin D 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).

[2]Despite the growing evidence of progesterone’s neuroprotective effects, two studies from laboratories examining the role of cyclosporin in the treatment of TBI have reported negative outcomes [119. Fee, D.B., K.R. Swartz, K.M. Joy, K.N. Roberts, N.N. Scheff, and S.W. Scheff, Effects of progesterone on experimental spinal cord injury. Brain Res, 2007. 1137:146–52, 120. Gilmer, L.K., K.N. Roberts, and S.W. Scheff, Efficacy of progesterone following a moderate unilateral cortical contusion injury. J Neurotrauma, 2008. 25(6):593–602. In addition, two recent reviews [123, 124] suggest that, by the authors’ personal, operational definitions, many if not all pre-clinical 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 using 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 for most of the other neuroprotective agents cited in the Loane and Faden paper. However, even if all the criteria were indeed met, there is still no guarantee 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.

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 would 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 vitro* 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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References

1. Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. Pharmacol Ther. 2007; 116(1):77–106. [PubMed: 17659348]
2. Thurman DJ, Alverson C, Dunn KA, Guerrero J, Sniezek JE. Traumatic brain injury in the United States: A public health perspective. J Head Trauma Rehabil. 1999; 14(6):602–15. [PubMed: 10671706]
3. Povlishock JT, Katz DI. Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil. 2005; 20(1):76–94. [PubMed: 15668572]
4. Vink R, Nimmo AJ. Multifunctional drugs for head injury. Neurotherapeutics. 2009; 6(1):28–42. [PubMed: 19110197]
5. Popovich P, McTigue D. Damage control in the nervous system: beware the immune system in spinal cord injury. Nat Med. 2009; 15(7):736–7. [PubMed: 19584863]

6. Zygun D. Non-neurological organ dysfunction in neurocritical care: impact on outcome and etiological considerations. Curr Opin Crit Care. 2005; 11(2):139–43. [PubMed: 15758594]
7. Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. J Neurotrauma. 27(8):1529–40. [PubMed: 20504161]
8. Harwood PJ, Giannoudis PV, van Griensven M, Krettek C, Pape HC. Alterations in the systemic inflammatory response after early total care and damage control procedures for femoral shaft fracture in severely injured patients. J Trauma. 2005; 58(3):446–52. discussion 452–4. [PubMed: 15761335]
9. Pape HC, Tsukamoto T, Kobbe P, Tarkin I, Katsoulis S, Peitzman A. Assessment of the clinical course with inflammatory parameters. Injury. 2007; 38(12):1358–64. [PubMed: 18048038]
10. Keel M, Trentz O. Pathophysiology of polytrauma. Injury. 2005; 36(6):691–709. [PubMed: 15910820]
11. Zygun DA, Kortbeek JB, Fick GH, Laupland KB, Doig CJ. Non-neurologic organ dysfunction in severe traumatic brain injury. Crit Care Med. 2005; 33(3):654–60. [PubMed: 15753760]
12. Catania A, Lonati C, Sordi A, Gatti S. Detrimental consequences of brain injury on peripheral cells. Brain Behav Immun. 2009; 23(7):877–84. [PubMed: 19394418]
13. Vodovotz Y, Constantine G, Faeder J, Mi Q, Rubin J, Bartels J, Sarkar J, Squires RH Jr, Okonkwo DO, Gerlach J, Zamora R, Luckhart S, Ermentrout B, An G. Translational systems approaches to the biology of inflammation and healing. Immunopharmacol Immunotoxicol. 32(2):181–95. [PubMed: 20170421]
14. Gruber CJ, Huber JC. Differential effects of progestins on the brain. Maturitas. 2003; 46(Suppl 1):S71–5. [PubMed: 14670648]
15. Dujardin KS, McCully RB, Wijdicks EF, Tazelaar HD, Seward JB, McGregor CG, Olson LJ. Myocardial dysfunction associated with brain death: clinical, echocardiographic, and pathologic features. J Heart Lung Transplant. 2001; 20:350–357. [PubMed: 11257562]
16. Macmillan CS, Grant IS, Andrews PJ. Pulmonary and cardiac sequelae of subarachnoid haemorrhage: time for active management? Intensive Care Med. 2002; 28:1012–1023. [PubMed: 12185419]
17. McKeating EG, Andrews PJ, Signorini DF, Mascia L. Transcranial cytokine gradients in patients requiring intensive care after acute brain injury. Br J Anaesth. 1997; 78:520–523. [PubMed: 9175965]
18. Hirashima Y, Nakamura S, Endo S, Kuwayama N, Naruse Y, Takaku A. Elevation of platelet activating factor, inflammatory cytokines, and coagulation factors in the internal jugular vein of patients with subarachnoid hemorrhage. Neurochem Res. 1997; 22:1249–1255. [PubMed: 9342729]
19. Chen G, Shi J, Ding Y, Yin H, Hang C. Progesterone prevents traumatic brain injury-induced intestinal nuclear factor kappa B activation and proinflammatory cytokines expression in male rats. Mediators Inflamm. 2007; 2007:93431. [PubMed: 18274644]
20. Lim HB, Smith MA. Systemic complications after head injury: a clinical review. Anaesthesia. 2007; 62:474–482. [PubMed: 17448059]
21. Harrison-Felix C, Whiteneck G, DeVivo M, Hammond FM, Jha A. Mortality following rehabilitation in the Traumatic Brain Injury Model Systems of Care. NeuroRehabilitation. 2004; 19(1):45–54. [PubMed: 14988587]
22. Harrison-Felix C, Whiteneck G, Devivo MJ, Hammond FM, Jha A. Causes of death following 1 year postinjury among individuals with traumatic brain injury. J Head Trauma Rehabil. 2006; 21(1):22–33. [PubMed: 16456389]
23. Brown AW, Leibson CL, Malec JF, Perkins PK, Diehl NN, Larson DR. Long-term survival after traumatic brain injury: a population-based analysis. NeuroRehabilitation. 2004; 19(1):37–43. [PubMed: 14988586]
24. Flaada JT, Leibson CL, Mandrekar JN, Diehl N, Perkins PK, Brown AW, Malec JF. Relative risk of mortality after traumatic brain injury: a population-based study of the role of age and injury severity. J Neurotrauma. 2007; 24(3):435–45. [PubMed: 17402850]
25. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999; 340(6):448–54. [PubMed: 9971870]

26. Shohami E, Gati I, Beit-Yannai E, Trembovler V, Kohen R. Closed head injury in the rat induces whole body oxidative stress: overall reducing antioxidant profile. J Neurotrauma. 1999; 16(5): 365–76. [PubMed: 10369557]
27. Bochicchio GV, Napolitano LM, Joshi M, McCarter RJ Jr, Scalea TM. Systemic inflammatory response syndrome score at admission independently predicts infection in blunt trauma patients. J Trauma. 2001; 50(5):817–20. [PubMed: 11379594]
28. Malone DL, Kuhls D, Napolitano LM, McCarter R, Scalea T. Back to basics: validation of the admission systemic inflammatory response syndrome score in predicting outcome in trauma. J Trauma. 2001; 51(3):458–63. [PubMed: 11535891]
29. Napolitano LM, Ferrer T, McCarter RJ Jr, Scalea TM. Systemic inflammatory response syndrome score at admission independently predicts mortality and length of stay in trauma patients. J Trauma. 2000; 49(4):647–52. discussion 652–3. [PubMed: 11038081]
30. Nast-Kolb D, Waydhas C, Gippner-Steppert C, Schneider I, Trupka A, Ruchholtz S, Zettl R, Schweiberer L, Jochum M. Indicators of the posttraumatic inflammatory response correlate with organ failure in patients with multiple injuries. J Trauma. 1997; 42(3):446–54. discussion 454–5. [PubMed: 9095112]
31. Nuytinck HK, Offermans XJ, Kubat K, Goris JA. Whole-body inflammation in trauma patients. An autopsy study. Arch Surg. 1988; 123(12):1519–24. [PubMed: 3056336]
32. Gebhard F, Pfetsch H, Steinbach G, Strecker W, Kinzl L, Bruckner UB. Is interleukin 6 an early marker of injury severity following major trauma in humans? Arch Surg. 2000; 135(3):291–5. [PubMed: 10722030]
33. Villoslada P, Steinman L, Baranzini SE. Systems biology and its application to the understanding of neurological diseases. Ann Neurol. 2009; 65(2):124–39. [PubMed: 19260029]
34. Centers for Disease Control and Prevention. Traumatic Brain Injury in the United States: Emergency Department Visits. Hospitalizations and Deaths 2002–2006. 2010
35. Adekoya N, Thurman DJ, White DD, Webb KW. Surveillance for traumatic brain injury deaths--United States, 1989–1998. MMWR Surveill Summ. 2002; 51(10):1–14. [PubMed: 12529087]
36. Mosenthal AC, Lavery RF, Addis M, Kaul S, Ross S, Marburger R, Deitch EA, Livingston DH. Isolated traumatic brain injury: age is an independent predictor of mortality and early outcome. J Trauma. 2002; 52(5):907–11. [PubMed: 11988658]
37. McArthur DL, Chute DJ, Villablanca JP. Moderate and severe traumatic brain injury: epidemiologic, imaging and neuropathologic perspectives. Brain Pathol. 2004; 14(2):185–94. [PubMed: 15193031]
38. Gomez PA, Lobato RD, Boto GR, De la Lama A, Gonzalez PJ, de la Cruz J. Age and outcome after severe head injury. Acta Neurochir (Wien). 2000; 142(4):373–80. discussion 380–1. [PubMed: 10883332]
39. Mattson MP, Magnus T. Ageing and neuronal vulnerability. Nat Rev Neurosci. 2006; 7(4):278–94. [PubMed: 16552414]
40. Dickstein DL, Kabaso D, Rocher AB, Luebke JI, Wearne SL, Hof PR. Changes in the structural complexity of the aged brain. Aging Cell. 2007; 6(3):275–84. [PubMed: 17465981]
41. Melov S. Modeling mitochondrial function in aging neurons. Trends Neurosci. 2004; 27(10):601–6. [PubMed: 15374671]
42. Mattson MP. Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. Neuromolecular Med. 2003; 3(2):65–94. [PubMed: 12728191]
43. Wagner AK, Bayir H, Ren D, Puccio A, Zafonte RD, Kochanek PM. Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender, age, and hypothermia. J Neurotrauma. 2004; 21(2):125–36. [PubMed: 15000754]
44. Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, Johnson RW. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. Faseb J. 2005; 19(10):1329–31. [PubMed: 15919760]

45. Frank MG, Barrientos RM, Biedenkapp JC, Rudy JW, Watkins LR, Maier SF. mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol Aging. 2006; 27(5):717–22. [PubMed: 15890435]
46. Perry VH, Matyszak MK, Fearn S. Altered antigen expression of microglia in the aged rodent CNS. Glia. 1993; 7(1):60–7. [PubMed: 8423063]
47. Sheffield LG, Berman NE. Microglial expression of MHC class II increases in normal aging of nonhuman primates. Neurobiol Aging. 1998; 19(1):47–55. [PubMed: 9562503]
48. Godbout JP, Johnson RW. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Neurol Clin. 2006; 24(3):521–38. [PubMed: 16877122]
49. Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. Injury. 2007; 38(12): 1336–45. [PubMed: 18048040]
50. Bulati M, Pellicano M, Vasto S, Colonna-Romano G. Understanding ageing: Biomedical and bioengineering approaches, the immunologic view. Immun Ageing. 2008; 5:9. [PubMed: 18768084]
51. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, Listi F, Nuzzo D, Lio D, Caruso C. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev. 2007; 128(1):83–91. [PubMed: 17118425]
52. Straub RH, Miller LE, Scholmerich J, Zietz B. Cytokines and hormones as possible links between endocrinosenescence and immunosenescence. J Neuroimmunol. 2000; 109(1):10–5. [PubMed: 10969175]
53. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev. 2007; 128(1):92–105. [PubMed: 17116321]
54. Dilger RN, Johnson RW. Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. J Leukoc Biol. 2008; 84(4):932–9. [PubMed: 18495785]
55. Vermeulen A, Kaufman JM. Ageing of the hypothalamo-pituitary-testicular axis in men. Horm Res. 1995; 43(1–3):25–8. [PubMed: 7721258]
56. Araujo AB, O'Donnell AB, Brambilla DJ, Simpson WB, Longcope C, Matsumoto AM, McKinlay JB. Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study. J Clin Endocrinol Metab. 2004; 89(12):5920–6. [PubMed: 15579737]
57. Jiang M, Huhtaniemi I. Polymorphisms in androgen and estrogen receptor genes: effects on male aging. Exp Gerontol. 2004; 39(11–12):1603–11. [PubMed: 15582276]
58. Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I. Estradiol in elderly men. Aging Male. 2002; 5(2):98–102. [PubMed: 12198740]
59. Lanfranco F, Gianotti L, Giordano R, Pellegrino M, Maccario M, Arvat E. Ageing, growth hormone and physical performance. J Endocrinol Invest. 2003; 26(9):861–72. [PubMed: 14964439]
60. Elmadfa I, Meyer AL. Body composition, changing physiological functions and nutrient requirements of the elderly. Ann Nutr Metab. 2008; 52(Suppl 1):2–5. [PubMed: 18382069]
61. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004; 80(6 Suppl):1678S–88S. [PubMed: 15585788]
62. Rammos G, Tseke P, Ziakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. Int Urol Nephrol. 2008; 40:419–426. [PubMed: 18193490]
63. Ferrucci L, Ble A, Bandinelli S, Lauretani F, Suthers K, Guralnik JM. A flame burning within. Aging Clin Exp Res. 2004; 16(3):240–3. [PubMed: 15462468]
64. Talhouk RS, Saade NE, Mouneimne G, Masaad CA, Safieh-Garabedian B. Growth hormone releasing hormone reverses endotoxin-induced localized inflammatory hyperalgesia without reducing the upregulated cytokines, nerve growth factor and gelatinase activity. Prog Neuropsychopharmacol Biol Psychiatry. 2004; 28(4):625–31. [PubMed: 15276687]

65. Priego T, Granado M, Ibanez de Caceres I, Martin AI, Villanua MA, Lopez-Calderon A. Endotoxin at low doses stimulates pituitary GH whereas it decreases IGF-I and IGF-binding protein-3 in rats. J Endocrinol. 2003; 179(1):107–17. [PubMed: 14529571]
66. Beishuizen A, Thijs LG. Endotoxin and the hypothalamo-pituitary-adrenal (HPA) axis. J Endotoxin Res. 2003; 9(1):3–24. [PubMed: 12691614]
67. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001; 56(3):M146–56. [PubMed: 11253156]
68. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev. 2005; 26(5):662–87. [PubMed: 15798098]
69. Topinkova E. Aging, disability and frailty. Ann Nutr Metab. 2008; 52(Suppl 1):6–11. [PubMed: 18382070]
70. Moore ME, Piazza A, McCartney Y, Lynch MA. Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. Biochem Soc Trans. 2005; 33(Pt 4):573–7. [PubMed: 16042547]
71. Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, Lu X, Surdulescu GL, Swaminathan R, Spector TD, Aviv A. Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. Am J Clin Nutr. 2007; 86(5):1420–5. [PubMed: 17991655]
72. Boxer RS, Dauser DA, Walsh SJ, Hager WD, Kenny AM. The association between vitamin D and inflammation with the 6-minute walk and frailty in patients with heart failure. J Am Geriatr Soc. 2008; 56(3):454–61. [PubMed: 18194227]
73. Morley JE, Haren MT, Rolland Y, Kim MJ. Frailty. Med Clin North Am. 2006; 90(5):837–47. [PubMed: 16962845]
74. Holick MF. Vitamin D deficiency. N Engl J Med. 2007; 357(3):266–81. [PubMed: 17634462]
75. DeLuca HF, Zierold C. Mechanisms and functions of vitamin D. Nutr Rev. 1998; 56(2 Pt 2):S4–10. discussion S 54–75. [PubMed: 9564171]
76. Hayes CE, Nashold FE, Spach KM, Pedersen LB. The immunological functions of the vitamin D endocrine system. Cell Mol Biol (Noisy-le-grand). 2003; 49(2):277–300. [PubMed: 12887108]
77. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr. 2004; 80(6 Suppl):1717S–20S. [PubMed: 15585793]
78. Griffin MD, Xing N, Kumar R. Vitamin D and its analogs as regulators of immune activation and antigen presentation. Annu Rev Nutr. 2003; 23:117–45. [PubMed: 12651965]
79. Holick MF. Evolution and function of vitamin D. Recent Results Cancer Res. 2003; 164:3–28. [PubMed: 12899511]
80. Mahon BD, Wittke A, Weaver V, Cantorna MT. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. J Cell Biochem. 2003; 89(5):922–32. [PubMed: 12874827]
81. Holick MF. Vitamin D: A millenium perspective. J Cell Biochem. 2003; 88(2):296–307. [PubMed: 12520530]
82. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2007; 167(11):1159–65. [PubMed: 17563024]
83. Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J, Raggi P. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: results from NHANES 2001 to 2004. Arterioscler Thromb Vasc Biol. 2008; 28(6):1179–85. [PubMed: 18417640]
84. Onyszchuk G, He YY, Berman NE, Brooks WM. Detrimental effects of aging on outcome from traumatic brain injury: a behavioral, magnetic resonance imaging, and histological study in mice. J Neurotrauma. 2008; 25(2):153–71. [PubMed: 18260798]
85. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. Osteoporos Int. 2002; 13(3): 187–94. [PubMed: 11991436]
86. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Invest. 2005; 35(5):290–304. [PubMed: 15860041]

87. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab. 2002; 13(3):100–5. [PubMed: 11893522]

88. Kalueff AV, Lou YR, Laaksi I, Tuohimaa P. Increased anxiety in mice lacking vitamin D receptor gene. Neuroreport. 2004; 15(8):1271–4. [PubMed: 15167547]

89. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med. 2002; 112(8):659–62. [PubMed: 12034416]

90. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med. 2004; 158(6):531–7. [PubMed: 15184215]

91. Nadziejko C, Fang K, Narciso S, Zhong M, Su WC, Gordon T, Nadas A, Chen LC. Effect of particulate and gaseous pollutants on spontaneous arrhythmias in aged rats. Inhal Toxicol. 2004; 16(6–7):373–80. [PubMed: 15204753]

92. Sullivan SS, Rosen CJ, Halteman WA, Chen TC, Holick MF. Adolescent girls in Maine are at risk for vitamin D insufficiency. J Am Diet Assoc. 2005; 105(6):971–4. [PubMed: 15942551]

93. Chatfield SM, Brand C, Ebeling PR, Russell DM. Vitamin D deficiency in general medical inpatients in summer and winter. Intern Med J. 2007; 37(6):377–82. [PubMed: 17535381]

94. Corino A, D'Amelio P, Gancia R, Del Rizzo P, Gabasio S, Limone P, Isaia G. Hypovitaminosis D in internal medicine inpatients. Calcif Tissue Int. 2007; 80(2):76–80. [PubMed: 17308988]

95. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. Neuroscience. 2003; 118(3):641–53. [PubMed: 12710973]

96. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat. 2005; 29(1):21–30. [PubMed: 15589699]

97. Hewison M, Zehnder D, Bland R, Stewart PM. 1alpha-Hydroxylase and the action of vitamin D. J Mol Endocrinol. 2000; 25(2):141–8. [PubMed: 11013342]

98. Holick MF. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. Am J Clin Nutr. 1994; 60(4):619–30. [PubMed: 8092101]

99. Larrosa M, Gratacos J, Vaqueiro M, Prat M, Campos F, Roque M. Prevalence of hypovitaminosis D in elderly institutionalized residents: influence of a substitutive treatment. Med Clin (Barc). 2001; 117(16):611–4. [PubMed: 11714466]

100. McGrath JJ, Feron FP, Burne TH, Mackay-Sim A, Eyles DW. Vitamin D3-implications for brain development. J Steroid Biochem Mol Biol. 2004; 89–90(1–5):557–60.

101. Townsend K, Evans KN, Campbell MJ, Colston KW, Adams JS, Hewison M. Biological actions of extra-renal 25-hydroxyvitamin D-1alpha-hydroxylase and implications for chemoprevention and treatment. J Steroid Biochem Mol Biol. 2005; 97(1–2):103–9. [PubMed: 16081283]

102. McCarty MF. Secondary hyperparathyroidism promotes the acute phase response -- a rationale for supplemental vitamin D in prevention of vascular events in the elderly. Med Hypotheses. 2005; 64(5):1022–6. [PubMed: 15780504]

103. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab. 2010

104. Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, Lowery DW, Patel MM, Denson DD, Gordon AB, Wald MM, Gupta S, Hoffman SW, Stein DG. ProTECT: A Randomized Clinical Trial of Progesterone for Acute Traumatic Brain Injury. Ann Emerg Med. 2007; 49(4):391–402. [PubMed: 17011666]

105. Xiao G, Wei J, Yan W, Wang W, Lu Z. Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. Crit Care. 2008; 12(2):R61. [PubMed: 18447940]

106. Attella MJ, Nattinville A, Stein DG. Hormonal state affects recovery from frontal cortex lesions in adult female rats. Behav Neural Biol. 1987; 48(3):352–67. [PubMed: 3689284]

107. Pettus EH, Wright DW, Stein DG, Hoffman SW. Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury. Brain Res. 2005; 1049(1):112–9. [PubMed: 15932748]

108. Roof RL, Duvdevani R, Heyburn JW, Stein DG. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. Exp Neurol. 1996; 138(2):246–51. [PubMed: 8620923]
109. Goss CW, Hoffman SW, Stein DG. Behavioral effects and anatomic correlates after brain injury: a progesterone dose-response study. Pharmacol Biochem Behav. 2003; 76(2):231–42. [PubMed: 14592674]
110. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. J Neurotrauma. 2005; 22(1):106–18. [PubMed: 15665606]
111. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. Neuroscience. 2004; 123(2):349–59. [PubMed: 14698743]
112. Stein DG. Progesterone exerts neuroprotective effects after brain injury. Brain Research Reviews. 2008; 57(2):386–397. [PubMed: 17826842]
113. Guo Q, Baronne L, Hoffman S, Guennoun R, Stein D. Progesterone's effects on aquaporin-4 expression after traumatic brain injury in male rats. J Neurotrauma. 2004; 21:1303.
114. Shear DA, Galani R, Hoffman SW, Stein DG. Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury. Exp Neurol. 2002; 178(1):59–67. [PubMed: 12460608]
115. McCullough LD, Hurn PD. Estrogen and ischemic neuroprotection: an integrated view. Trends Endocrinol Metab. 2003; 14(5):228–35. [PubMed: 12826329]
116. Stein DG. Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? Trends Neurosci. 2001; 24(7):386–91. [PubMed: 11410269]
117. Stein DG, Hoffman SW. Concepts of CNS plasticity in the context of brain damage and repair. J Head Trauma Rehabil. 2003; 18(4):317–41. [PubMed: 16222128]
118. Stein DG, Hoffman SW. Estrogen and progesterone as neuroprotective agents in the treatment of acute brain injuries. Pediatr Rehabil. 2003; 6(1):13–22. [PubMed: 12745891]
119. Fee DB, Swartz KR, Joy KM, Roberts KN, Scheff NN, Scheff SW. Effects of progesterone on experimental spinal cord injury. Brain Res. 2007; 1137:146–52. [PubMed: 17204255]
120. Gilmer LK, Roberts KN, Scheff SW. Efficacy of progesterone following a moderate unilateral cortical contusion injury. J Neurotrauma. 2008; 25(6):593–602. [PubMed: 18476780]
121. Schumacher M, Guennoun R, Ghoumari A, Massaad C, Robert F, El-Etr M, Akwa Y, Rajkowski K, Baulieu EE. Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. Endocr Rev. 2007; 28(4):387–439. [PubMed: 17431228]
122. Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG. Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. Exp Neurol. 2006; 198(2):469–78. [PubMed: 16445913]
123. Sayeed I, Guo Q, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. Ann Emerg Med. 2006; 47(4):381–9. [PubMed: 16546625]
124. Ehrnthaller C, Ignatius A, Gebhard F, Huber-Lang M. New Insights of an Old Defense System: Structure, Function, and Clinical Relevance of the Complement System. Mol Med.
125. VanLandingham JW, Cekic M, Cutler S, Hoffman SW, Stein DG. Neurosteroids reduce inflammation after TBI through CD55 induction. Neurosci Lett. 2007; 425(2):94–8. [PubMed: 17826908]
126. Chen G, Shi JX, Qi M, Wang HX, Hang CH. Effects of progesterone on intestinal inflammatory response, mucosa structure alterations, and apoptosis following traumatic brain injury in male rats. J Surg Res. 2008; 147(1):92–8. [PubMed: 17868700]
127. Cekic M, Cutler SM, Vanlandingham JW, Stein DG. Vitamin D deficiency reduces the benefits of progesterone treatment after brain injury in aged rats. Neurobiol Aging. 2009
128. Cutler SM, Cekic M, Miller DM, Wali B, VanLandingham JW, Stein DG. Progesterone improves acute recovery after traumatic brain injury in the aged rat. J Neurotrauma. 2007; 24(9):1475–86. [PubMed: 17892409]
129. Lips P. Vitamin D physiology. Prog Biophys Mol Biol. 2006; 92(1):4–8. [PubMed: 16563471]

130. Miller RR, Hicks GE, Shardell MD, Cappola AR, Hawkes WG, Yu-Yahiro JA, Keegan A, Magaziner J. Association of serum vitamin D levels with inflammatory response following hip fracture: the Baltimore Hip Studies. J Gerontol A Biol Sci Med Sci. 2007; 62(12):1402–6. [PubMed: 18166692]

131. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003; 88(10): 4623–32. [PubMed: 14557432]

132. Faden AI. Neuroprotection and traumatic brain injury: the search continues. Arch Neurol. 2001; 58(10):1553–5. [PubMed: 11594912]

133. Gingrich MB, Traynelis SF. Serine proteases and brain damage - is there a link? Trends Neurosci. 2000; 23(9):399–407. [PubMed: 10941185]

134. Margulies S, Hicks R. Combination therapies for traumatic brain injury: prospective considerations. J Neurotrauma. 2009; 26(6):925–39. [PubMed: 19331514]

135. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol. 2005; 289(1):F8–28. [PubMed: 15951480]

136. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol. 2005; 97(1–2):93–101. [PubMed: 16046118]

137. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, Lincoln MR, Burrell A, Handunnetthi L, Handel AE, Disanto G, Orton SM, Watson CT, Morahan JM, Giovannoni G, Ponting CP, Ebers GC, Knight JC. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. Genome Res. 2010; 20(10):1352–60. [PubMed: 20736230]

138. Ishrat T, Sayeed I, Atif F, Hua F, Stein DG. Progesterone and allopregnanolone attenuate blood-brain barrier dysfunction following permanent focal ischemia by regulating the expression of matrix metalloproteinases. Exp Neurol. 2010; 226(1):183–90. [PubMed: 20816826]

139. Schwartz N, Xue X, Elovitz MA, Dowling O, Metz CN. Progesterone suppresses the fetal inflammatory response ex vivo. Am J Obstet Gynecol. 2009; 201(2):211 e1–9. [PubMed: 19646573]

140. Jiang C, Wang J, Li X, Liu C, Chen N, Hao Y. Progesterone exerts neuroprotective effects by inhibiting inflammatory response after stroke. Inflamm Res. 2009; 58(9):619–24. [PubMed: 19333725]

141. Tait AS, Butts CL, Sternberg EM. The role of glucocorticoids and progestins in inflammatory, autoimmune, and infectious disease. J Leukoc Biol. 2008; 84(4):924–31. [PubMed: 18664528]

142. Luoma, JI.; Kelley, BG.; Mermelstein, PG. Progesterone Inhibition of Voltage-Gated Calcium Channels is a Potential Neuroprotective Mechanism against Excitotoxicity; Steroids; 2011.

143. Dang J, Mitkari B, Kipp M, Beyer C. Gonadal steroids prevent cell damage and stimulate behavioral recovery after transient middle cerebral artery occlusion in male and female rats. Brain Behav Immun. 2011

144. Cekic M, Stein DG. Traumatic Brain Injury and Aging: Is a Combination of Progesterone and Vitamin D Hormone a Simple Solution to a Complex Problem? Neurotherapeutics. 2010; 7(1): 81–90. [PubMed: 20129500]

145. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. Exp Biol Med (Maywood). 235(9):1034–45. [PubMed: 20667908]

146. Holmoy T, Moen SM. Assessing vitamin D in the central nervous system. Acta Neurol Scand Suppl. (190):88–92.

147. Atif F, Sayeed I, Ishrat T, Stein DG. Progesterone with Vitamin D affords better neuroprotection against excitotoxicity in cultured cortical neurons than progesterone alone. Mol Med. 2009; 15(9–10):328–36. [PubMed: 19603099]