

Progesterone & Brain Injury

The work of Dr. Donald Stein and Colleagues

Who is Don Stein?

I first came across Don Stein in December 2004 when he was presenting on his work at the Rehabilitation for Individuals with Brain Injury: The State of the Art conference in Washington, D.C. He presented on the topic of brain plasticity, and I was blown away. But it was in meeting Dr. Stein that I began to understand the true depth of this man, and his devotion to improving the lives of individuals with brain injury.

Don Stein is not your typical bench researcher, at least that is my opinion. His devotion to his craft and to finding the answer to the question, "Why do female rats fare better than males after brain injury?" has led him down an interesting and hard fought path. It was not the typical path for someone so well regarded in his field. In fact, his path included years of being unfunded, under funded and perpetually questioned by the prevailing wisdom of brain research, which was the antiquated idea that the brain does not have the capacity to heal, better known as the doctrine of localization of function.

Thomas Burton of the Wall Street Journal wrote an article in 2007 aptly named "One Doctor's Lonely Quest to Heal Brain Injury," which chronicled Stein's quest. The most incredible aspect of this quest is that much of Stein's work started in a trailer at Emory University in Atlanta, after "normal" school hours on his own time, with little to no funding. It wasn't until the mid to late 1990s when the Centers for Disease Control and Prevention and National Institutes of Health began funding grants for Stein's research. It is the fruits of this research that I would like to focus on for this article.

Major Findings of Stein and Colleagues

Dr. Stein's quest began at the University of Oregon in Eugene, Ore. during the 1960s, when he found that some rats who incurred brain injuries did not have impairments like other rats. He began researching why, and found that the rats who were not impaired were invariably female. The questions raised included whether these differences were due to sexual dimorphism (structural differences in the brain between males and females) or some other reason, such as molecular differences. Some of Dr. Stein's early research focused on the female hormone estrogen, but these studies did not bear out. He then turned to progesterone, which lent far more promise in explaining sex differences in



recovery after traumatic brain injury (TBI).

Like most others, I presumed that progesterone was a female hormone. As it turns out, it is far more a "brain hormone" than a female hormone. Both males and females produce progesterone, and during pregnancy it plays a vital role in brain development.

Progesterone's Promise

It has been posited that progesterone may play a neuroprotective role after brain injury. What does that mean and what is it about progesterone that makes it so promising as a potential treatment? To answer those questions, we must delve into the world of brain injury etiology.

Figure 1 (below) highlights what happens when a brain injury occurs. The primary injury includes the events at the time of injury, which cannot be reversed, and can include injury types such as lesions, hematomas, contusions, diffuse axonal injury, and vascular injuries (Gennarelli and Graham, 2005). Secondary injury includes such events as vasogenic edema, cytotoxic edema, ischemia, necrosis, increased intracranial pressure, inadequate cerebral blood flow, increased intracellular calcium, release of excitatory amino acids, generation of free radicals, and

Progesterone & Brain Injury *continued*

breakdown of the cytoskeleton with vascular degeneration leading to cell death.

It is secondary injury that provides interest when it comes to progesterone's promise. To fully understand brain injury, we must look at it as a process and not a single event. The primary injury is the beginning of the event, and the secondary injury is essentially a cascade of events that can unfold over days, weeks and months, which further cause damage to the brain. These secondary events are what researchers have targeted.

It is the hope that by reducing the cascade of maladaptive or deleterious events that occur, there will be less damage to the brain, and by virtue, less functional change post injury. Brain edema accounts for much of the morbidity and mortality post TBI (Stein, 2008). Inflammation of the brain leads to tissue breakdown, which leads to brain edema and eventual cell death. It is at this stage that progesterone is posited to have its effects. As noted in VanLandingham, Cekic, Cutler, Hoffman and Stein (2007), progesterone's effects are to reduce brain edema, reduce blood brain barrier disruption and reduce release of inflammatory cytokines.

Despite research over the years, there has been no pharmacological agent that has been shown to improve outcome after brain injury (Stein, 2008). So the promise of progesterone is all that more important given the paucity of alternatives for reducing morbidity or mortality after TBI.

Findings in Rat Models

To cover what is currently known about progesterone, we will look to studies produced in the early to mid 1990s by Stein and his colleagues to review some of the key findings. In a study by Roof, Duvdevani and Stein (1993), they looked at three groups of rats to determine if endogenous (natural) levels of progesterone play a protective role in brain injury. The first group was males. The second group was females in proestrus, where natural levels of progesterone are low and estrogen levels are high. The third group was females who were pseudopregnant, where there are high levels of progesterone and low levels of estrogen. Each of the rats received a contusion to the medial frontal cortex. Post mortem examination of the brain tissues revealed that female rats in proestrus (lower levels of progesterone, but higher levels than males) had significantly less brain edema than males. Pseudopregnant (high levels of progesterone) female rats had almost no post injury swelling. Taken together, males with the lowest levels of progesterone had the most edema. Females in proestrus (with natural levels of progesterone) had significantly less edema than males, and psueopregnant females with high levels of progesterone had very little brain edema.

In that same study by Roof, Duvdevani and Stein (1993), they then examined whether the reduced edema related specifically to progesterone. They ovariectomized 17 female rats to reduce natural levels of progesterone and estrogen. All rats received a

Figure 1: Brain Injury Time Line

Injury	Hours	Days	Weeks	Months
Primary Injury <ul style="list-style-type: none"> • Lesions • Hematomas • Contusions/Lacerations • Diffuse axonal injury • Vascular injury 	Secondary Injury <ul style="list-style-type: none"> • Edema • Apoptosis and degeneration of nerve cells • Elevated Intracranial Pressure • Inadequate Cerebral Perfusion (blood pressure) • Release of excitatory amino acids • Generation of free radicals • Cytokine Release 			

Progesterone & Brain Injury *continued*

Figure 2

Secondary Injury Effects on the Brain

Secondary injury effects on the brain can be as harmful or more harmful than primary injury effects. The difference between primary and secondary injury effects is in our potential ability to reduce their effects. As Dr. Stein (2008) noted, “Despite decades of effort, scientists have failed to identify a pharmacological agent that consistently improves outcomes following TBI.”

Progesterone is coming to the forefront as an agent that defies that statement. Below are descriptions of the cascade of secondary injury events that occur post brain injury, followed by progesterone’s effect on that event. Information in Stein, Wright, and Kellerman (2008) assisted in articulating this figure.

Edema/Elevated Intracranial Pressure

There are three brain volumes that keep intracranial pressure in check: brain parenchyma (tissue), blood volume and cerebrospinal fluid. Should any of the three increase, increases in intracranial pressure will occur unless other strategies keep them in check. The primary result of edema is the increase in intracranial pressure.

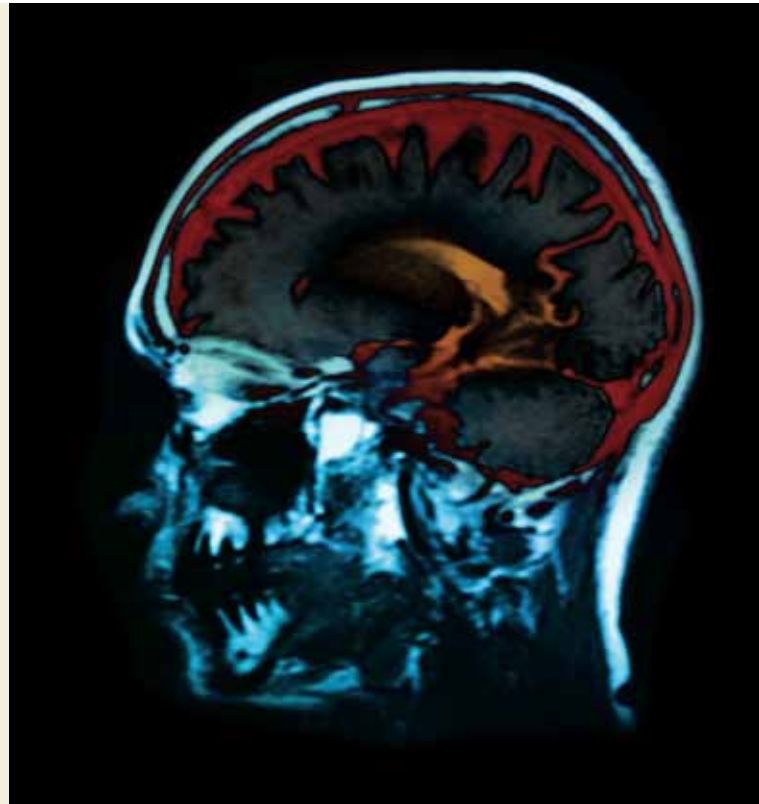
There are two forms of brain edema that can result in this imbalance of intracranial pressure: vasogenic and cytotoxic. Progesterone reduces cerebral edema – both the vasogenic and cytotoxic – by stabilizing the blood brain barrier, reducing inflammatory reactions.

Types of Edema

Vasogenic – this is caused by a breakdown in the blood-brain barrier. There are very tight junctions between endothelial cells that line the brain’s blood vessels. Mechanical disruption to these cells can allow proteins and fluids into the brain parenchyma (brain tissue). It can also be caused by the release of inflammatory cytokines, which increase capillary membrane permeability.

Cytotoxic – this is the result of accumulation of water in the intracellular space of individual cells themselves. This type of edema occurs after injury. This also leads to cells releasing additional toxic agents that cause secondary cell death (Stein, 2008).

Inadequate Cerebral Perfusion – This can be caused by increases in intracranial pressure. Progesterone exerts its effects on cerebral perfusion by reducing edema, thereby allowing for proper cerebral blood flow.



Apoptosis and Degeneration of Nerve Cells – This is programmed cell death. Progesterone has been implicated in reducing injury induced apoptosis post TBI.

Excitatory Amino Acids – When cells are injured, amino acids are released causing a number of potential problems. When ions like sodium or calcium pass into the cell, breakdowns of the cytoskeleton, membrane dysfunction and free radical formation can occur.

Free Radical Formation – These are highly reactive atoms. When released, they damage endothelial cells and injure brain tissue. They assist in the breakdown of the blood brain barrier and contribute to brain edema of both types. Once the process of free radical formation starts, the problem is perpetuated as damage then results in more release of free radicals.

Progesterone may be a free radical scavenger, reducing free radical generation, thereby reducing free radical damage (Stein, 2008.)

Cytokine Release – Cytokines activate the inflammatory process after brain injury. Progesterone works to reduce cytokine release, thus reducing the inflammatory response after injury.

Progesterone & Brain Injury *continued*

brain injury. The rats were then assigned to one of three treatment groups. Group One received estrogen implants and progesterone injections.

Group Two received estrogen implants and oil injections. Group Three received progesterone only. They found that simply removing estrogen did not reduce levels of edema post injury. Importantly, the female rats that had both estrogen and progesterone had significantly less edema than ovariectomized rats given no hormones ($t=2.33$, $p < .05$), or ovariectomized rats given estrogen only ($t= 3.59$, $p < .01$). Likewise, female rats given progesterone only had significantly less edema than ovariectomized rats given no hormone ($t - 2.427$, $p < .05$) or ovariectomized rats given estrogen only ($t = 4.055$, $p < .01$).

One can conclude from these findings that progesterone was the factor in edema reduction post brain injury. Now that the group had evidence that progesterone reduces edema post injury, the focus turned to determining the optimal timing for quickly reducing edema. So, they completed a study to determine how fast edema occurs. Roof, Duvdevani, Heyburn and Stein (1996) used both male and female rats that were given a frontal medial cortex contusion. They found that swelling occurred within two hours of injury and peaked within 24 hours. By seven days the swelling began to reduce. They also found that edema was significantly reduced one hour post injury via injection of progesterone for both males ($F = 39.33$, $p < .001$) and females ($F = 36.22$, $p < .001$).

Reductions in edema also occurred when injections of progesterone injections were given six hours post injury (males; $t= 2.045$, $p < .05$; females; $t = 3.102$, $p < .01$), 24 hours post injury (males; $t = 6.45$, $p < .001$; females; $t = 3.925$, $p < .001$), and 72 hours post injury (males; $t = 4.973$, $p < .001$; females; $t = 4.598$, $p < .001$). Overall, these results demonstrate that progesterone can reduce edema formation, and moreover, they show that the treatment can reduce edema within hours of giving the treatment.

Taken together, these studies confirm that in a rat model, progesterone, whether naturally occurring in the body or provided from external sources, reduces brain edema post medial frontal cortex contusion. They also provide insight into how quickly edema occurs post TBI (within two hours of injury in a rat model), as well as how quickly progesterone can counteract edema (most effective when given two hours post injury, but effective up to 24 hours post injury). The question that comes to mind at this point is, does this translate to humans? To find out, controlled trials on humans are required.

Again, Stein and his colleagues took the lead with the ProTECT® trial, a Phase II trial that utilized 100 patients to test the effects of progesterone administration post injury (Wright et al, 2007).

Human Studies

Wright et al (2007) completed a Phase II, double blind, randomized, placebo controlled trial, funded by the National Institute for Neurological Disorders and Stroke, National Institutes of Health, and the general Clinical Research Center at Emory University and Grady Memorial Hospital. The primary goal of the trial was to assess the safety of progesterone administration to patients presenting with acute, moderate to severe TBI. Additionally, they had a secondary goal of assessing if progesterone provided a benefit for the patients.

The trial consisted of 100 patients who arrived within 11 hours of injury with a Glasgow Coma Score (GSC) between four and 12 (postresuscitation). Upon arrival to Grady Memorial Hospital, for patients presenting with the appropriate study characteristics, family consent by proxy was obtained for study participation. For every four patients randomized to the intravenous progesterone treatment, one patient was randomized to the intravenous placebo treatment (control). Strict treatment guidelines were followed, as were daily rounds to assess for serious adverse events (e.g., death, immediate risk of death, or if continued participation could lead to death). See Wright et al (2007) for their protocols.

Once patients were enrolled, and given either progesterone or placebo, the teams assessed a variety of measures to examine the goals of the study. These included duration of coma, duration of post-traumatic amnesia, and mortality within 30 days of injury. They also directly assessed whether progesterone had an effect on cerebral edema via intracranial pressure measurements taken over time. Lastly, raters utilized the Glasgow Coma Score Extended and the Disability Rating Scale with the goal to assess functional ability 30 days post injury.

Findings of the Study...

Duration of Coma – Patients with GCS scores four to eight (severe TBI) who received progesterone had longer comas (10.1 days) than those who received the placebo (3.9 days).

Duration of Post-Traumatic Amnesia – No differences across groups were found.

Progesterone & Brain Injury *continued*

Mortality 30 days post injury – In all, 77 patients received progesterone and 23 patients received placebo. Within 30 days post injury, 13.0 percent of the progesterone group died versus 30.4 percent of the placebo group (relative risk [95% CI], 0.43 (0.18-0.99). Importantly, the researchers noted that deaths from neurological bases trended towards fewer deaths for the progesterone group versus the placebo group.

They also noted that there were differences in mortality across the different levels of severity (moderate versus severe). For severe TBI patients (GCS of four to eight), 13.2 percent of progesterone patients died within 30 days of injury versus 40 percent of those in the placebo group.

For moderate TBI patients (GCS of nine to 12), 16.7 percent of progesterone patients versus 14.3 percent of placebo patients died.

Intracranial Pressure Measurements – Intracranial pressure scores did not differ between those given progesterone versus those in the placebo group.

Functional Recovery – At 30 days post injury, the researchers contacted each patient to determine their functional status. They found for the severe TBI group, 75 percent were functioning at a poor level for both the progesterone and placebo group.

However, for moderate severity patients, the scenario was quite different. They found that 56 percent had a moderate to good recovery (GCS Extended scores) in the progesterone group, as compared to 0 percent for the placebo group. For the Disability Rating Scale (DRS) Function scores, they found that for the severe TBI group the progesterone scores were 2.9 versus placebo 1.8, and for the moderate TBI groups the progesterone scores were 1.5 versus 3.8. This indicates little difference in the severe group in terms of disability reduction, and significantly less disability for the progesterone versus placebo groups for the moderate TBI group.

Overall Findings

Results related to the primary goal of the study, namely that of the safety of providing progesterone to moderate to severe TBI patients shows that, “This analysis suggests, but does not prove, that progesterone treatment causes no harms and may be beneficial treatment for traumatic brain injury.” (Wright et al, 2007, p. 400)

What is the next step? Given that this study demonstrated no

harm, and that there may be benefits to progesterone treatment, the next step is a Phase III, multi-center clinical trial. Dr. Don Stein and his group have received a notice of grant award from the National Institutes of Neurological Diseases and Stroke, which will fund them for a Phase III, multi center clinical trial that is called ProTECT III (Progesterone for Traumatic Brain Injury.) To view information about this trial go to www.clinicaltrials.gov and enter “progesterone and brain injury.” There are 17 centers participating in the trial, including: Emory University, Henry Ford Hospital, Medical College of Wisconsin, New York Presbyterian Hospital, Oregon Health and Science University, Stanford University, Temple University, University of Arizona, University of California, University of Cincinnati, University of Kentucky, University of Maryland, University of Minnesota, University of Pennsylvania, University of Texas, Virginia Commonwealth University and Wayne State University.

We wish Drs. Stein, Wright and their colleagues best wishes for their study endeavors. ❖

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Progesterone & Brain Injury *continued*

About the Author...

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Dr. Reyst has worked in various capacities within the field of brain injury rehabilitation since 1991. She brings a broad range of experience as a rehabilitation assistant, residential program manager, program director and, currently, as Rainbow's director of clinical administration. Dr. Reyst currently oversees professional staff allocation, billing & service provision, professional staff training, accreditation readiness, outcomes management and is the program director for the Ypsilanti-based Outpatient and Day Treatment Programs. She assists with clinical supervision, marketing, day-to-day operations and strategic and long-term planning.

Dr. Reyst is currently a member of the board of governors for the Academy of Certified Brain Injury Specialists, a member of the American Psychological Association and is a frequent volunteer for the Brain Injury Association of Michigan.

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