

Research report

Progesterone treatment inhibits the inflammatory agents
that accompany traumatic brain injuryEdward H. Pettus^b, David W. Wright^a, Donald G. Stein^a, Stuart W. Hoffman^{a,*}^aDepartment of Emergency Medicine, Emory University, Evans Building, Room 255, 1648 Pierce Dr NE, Atlanta, GA 30322, USA^bDepartment of Cell Biology, Emory University, Atlanta, GA 30322, USA

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Abstract

Progesterone given after traumatic brain injury (TBI) has been shown to reduce the initial cytotoxic surge of inflammatory factors. We used Western blot techniques to analyze how progesterone might affect three inflammation-related factors common to TBI: complement factor C3 (C3), glial fibrillary acidic protein (GFAP), and nuclear factor kappa beta (NFκB). One hour after bilateral injury to the medial frontal cortex, adult male rats were given injections of progesterone (16 mg/kg) for 2 days. Brains were harvested 48 h post-TBI, proteins were extracted from samples, each of which contained tissue from both the contused and peri-contused areas, then measured by Western blot densitometry. Complete C3, GFAP, and NFκB p65 were increased in all injured animals. However, in animals given progesterone post-TBI, NFκB p65 and the inflammatory metabolites of C3 (9 kDa and 75 kDa) were decreased in comparison to vehicle-treated animals. Measures of NFκB p50 showed no change after injury or progesterone treatment, and progesterone did not alter the expression of GFAP. The therapeutic benefit of post-TBI progesterone administration may be due to its salutary effect on inflammatory proteins known to increase immune cell invasion and cerebral edema.

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1. Introduction

Traumatic brain injury (TBI) is a condition with high morbidity and mortality for which there are currently no treatments that improve clinical outcome measures [45]. The initial biomechanical force in trauma causes ionic imbalances, oxidative damage, microglial activation, immune cell invasion, and cytokine release [3,27,52]. Once this destructive process is initiated, the release of pro-inflammatory cytokines further stimulates immune cells to become phagocytic. Activation of immune cells, in turn, triggers the production of free radicals and additional pro-inflammatory compounds such as cytokines, prostaglandins,

extracellular matrix proteases, complement factors, cell adhesion molecules, and inducible nitric oxide synthase [3,38,43]. The expression of pro-inflammatory factors then attracts immune cells to cerebrovascular surfaces, where they increase vasopermeability, immune cell invasion, and further cytokine generation in the CNS [52]. Regardless of which factors initiate the injury cascade, the inhibition of inflammation may reduce cell death, gliosis, and edema.

The rodent model of medial frontal cortex (MFC) impact injury presents many functional aspects of human head injury, including cognitive and sensorimotor deficits [8,25,40,47,49,57]. Research has shown that progesterone treatment given after MFC injury can reduce these behavioral impairments in rats. Progesterone also reduces edema [47,61], necrosis [51], apoptosis [13,14], blood–brain barrier compromise [46], and the mediators of inflammation [1,2]. In

* Corresponding author. Fax: +1 404 727 2388.

E-mail address: swhoffm@emory.edu (S.W. Hoffman).

this study, we look at some of the cellular mechanisms mediating progesterone's neuroprotective effects with the aim of learning more about how functional recovery occurs.

We now know that progesterone can act in several ways. First, the hormone and its metabolites bind to several cellular receptors and alter their activity. For instance, progesterone acts both as a sigma-1 receptor antagonist (indirectly modulating the NMDA receptor [39]) and as a GABA_A receptor agonist (through conversion to its 3 α -pregnanolone metabolites [62]). Both these actions reduce neuronal excitatory tone and down-regulate excitotoxicity after brain injuries [23]. Though not a free radical scavenger, progesterone can inhibit oxidative damage in the CNS [19,48].

Excitotoxic by-products of oxidative insults trigger an inflammatory immune response by releasing cytokines and other inflammatory factors that contribute to brain edema and neuronal loss. We hypothesize that progesterone treatment reduces neural injury and cerebral edema after TBI by interrupting the inflammatory cascade [36,54,55].

As an anti-inflammatory agent, progesterone can serve as a ligand-gated factor known to inhibit C3 transcription [7,37]. Recently, this receptor/ligand complex has been shown to inhibit the activity of the pro-inflammatory transcription factor, nuclear factor kappa B (NF κ B) [30]. NF κ B is an upstream regulator of inflammation which activates TBI-induced inflammatory cytokines like tumor necrosis factor alpha (TNF α), interleukin 1beta (IL-1 β) [18], C3, and GFAP. The interaction of progesterone with the cascade is worth studying in light of the mounting evidence that inflammatory factors [50] TNF α [16,58], IL-1 β [15], NF κ B [42,63], and C3 [9,32,34] all contribute to brain injury pathology [26,27,36].

2. Methods and materials

2.1. Animals

Twenty adult male Sprague–Dawley rats weighing approximately 280–350 g were used as subjects. All procedures involving animals conformed to guidelines set forth in the “Guide for the Care and Use of Laboratory Animals” (National Academy of Sciences, 1996) and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC of Emory University protocol #101-99). The rats were handled for at least 5 days before surgery and were individually housed. Food and water were provided ad libitum throughout the experiment, and the animals lived in a reversed 12 h light/12 h dark cycle controlled environment.

2.2. Surgery

MFC injury was produced with a computer-controlled pneumatic impactor device described earlier [25]. Rats were anesthetized using isoflurane (5% induction, 2% maintenance,

700 mm N₂O, 300 mm O₂) and mounted in a stereotaxic device with their heads in a horizontal position. Body core temperatures (37 °C) were maintained with a homeothermic heating blanket system (Harvard Apparatus). Using a SurgiVet™ (model V3304) pulse oximeter, blood SpO₂ was monitored and maintained at levels >90%. Under aseptic conditions, a midline incision was made in the scalp, and the fascia retracted to expose the cranium. A centered, bilateral craniectomy was made 3 mm anterior to bregma using a 6-mm diameter drill. After bone removal, the tip of the impactor was moved, using a stereotaxic device, to AP:3.0; ML:0.0, checked for adequate clearance, retracted to its elevated position, and then lowered 3.5 mm DV to penetrate 2 mm into the cortex. The contusions ($n = 10$) were then made at a velocity of 2.25 m/s with a brain contact time of 150 ms. Sham-operated rats ($n = 10$) were anesthetized, mounted in the stereotaxic apparatus, and had their scalps cut and sutured but were not trephined.

2.3. Progesterone preparation and administration

Based on previous studies determining optimal dose response, all progesterone-treated animals received 16 mg/kg progesterone in 25% 2-hydroxypropyl- β -cyclodextrin (HBC) [20]. The HBC vehicle allowed progesterone to be dissolved in a non-toxic, aqueous solution [3,26,52]. The rats received either progesterone ($n = 10$) or an equal volume of vehicle (HBC; $n = 10$) with the first injection given intraperitoneally (IP) 1 h following injury. Subsequent injections were given subcutaneously at 6 h and 24 h after injury. The experimental groups consisted of sham + vehicle (SV; $n = 5$), sham + progesterone (SP; $n = 5$), lesion + vehicle (LV; $n = 5$), and lesion + progesterone (LP; $n = 5$).

2.4. Fresh tissue

The rats were given an overdose IP injection of Nembutal™ (75 mg/kg) and then decapitated. Brains were quickly extracted, divided at bregma, then sagittally bisected, and the hemispheres sectioned into dorsal and ventral halves. Both frontal dorsal sections, which encompassed the injured area, were then frozen in dry-ice-chilled 2-methylbutane and stored at –80 °C.

2.5. Western blot

Western blot analyses were performed on samples that encompassed both the contused and the peri-contused areas of MFC in the injured rats and the equivalent area in the sham-operates. The tissue from each brain was separately homogenized by Dounce in T-per (Pierce, Rockford, IL) with protease inhibitors (Sigma, P8340) and assayed for protein concentration using a Coomassie protein assay (Pierce). An SDS Laemmli sample buffer was then added to sample aliquots and incubated at 90 °C on a heating block for 10 min. Samples containing 30 μ g of protein were then

loaded on a 12% acrylamide Criterion gel (BioRad, Hercules, CA) and run at 200 V for 1 h. Gels were rinsed in Western transfer buffer for 10 min and loaded against a PVDF membrane prepared for Western transfer. Western transfer was made on a BioRad Criterion apparatus at 100 V for 30 min under cooling conditions. The PVDF membrane was then rinsed in PBS Tween and incubated in blocker (KPL) for >3 h on a shaker at room temperature.

The inflammation-associated proteins of interest were labeled with the following antibodies: NF κ B (Cell Signaling, 3032; 1:500), C3 (ICN Biomed, 55730; 1:500), and GFAP (Chemicon, AB5804; 1:2000). Primary antibody dilution (in KPL diluent) was applied to the membrane and incubated on a shaker overnight at 4 °C. Membranes were thoroughly rinsed in PBS/Tween and then incubated in appropriate HRP-conjugated secondary antibody (KPL; 1:5000) for 1 h at room temperature followed by thorough rinse in PBS/Tween and detection via chemiluminescent agents (Pierce). Chemiluminescent bands were detected on a Kodak Image station 440CF scanner (Rochester, NY) and analyzed with the accompanying densitometric image analysis software. Band density-background was compared between treatment groups run and exposed on the same blots.

2.6. Statistical analysis

The optical density data were tested for normality, homoscedasticity, and outliers (i.e., greater than 1.5 times the interquartile range) before being analyzed by either parametric one-way analysis of variance (ANOVA) or parametric repeated measures ANOVA. Following the ANOVAs, Fischer PLSD post-hoc tests were performed. The criterion for statistical significance was set at $P < 0.05$. Since progesterone is a known anti-inflammatory, post-hoc analyses were conducted as one-tailed tests. Data were expressed as the mean \pm SEM.

3. Results

3.1. General results

For all three proteins analyzed, we found no significant difference between the sham-operates given vehicle and the sham-operates that received progesterone. The injury-induced up-regulation of the proteins was apparent and consistent across all animals tested.

3.2. Complement C3

C3 (180 kDa) and four of its metabolites, 120 kDa, 42 kDa, and 9 kDa bands, were identified and showed similar levels of intensity on the Western blots within each treatment group. All of the C3 bands increased in intensity in the vehicle-treated injured animals (Fig. 1A). Western

blot optical density analysis revealed an effect of progesterone on C3 metabolites after injury. A one-way ANOVA of the densitometric measures for C3 9 kDa band revealed differences among the groups ($F_{2,17} = 7.277$; $P < 0.05$). Post-hoc tests showed that vehicle-treated injured animals had significantly higher levels of C3 9 kDa protein compared to sham-operates and to injured animals given progesterone (Fig. 1B).

A one-way ANOVA of the densitometric measures for the C3 75 kDa band revealed differences among the groups ($F_{2,17} = 23.011$; $P < 0.05$). Post-hoc analysis demonstrated both a lesion and a treatment effect. The sham-operates had significantly lower levels of the 75 kDa metabolite than did either injury group. In addition, the 75 kDa metabolite in progesterone-treated rats was significantly lower than in the injured animals given only vehicle (Fig. 1B).

Metabolites detected by the polyclonal antibody against C3 were increased in response to TBI but unaffected by the progesterone treatment. One-way ANOVA of densitometry measures for the C3 42 kDa band revealed differences among the groups ($F_{2,17} = 5.700$; $P < 0.05$). Post-hoc analysis showed that sham-operates had significantly lower levels of C3 42 kDa than injured animals given either progesterone or vehicle. Additional tests found no significant differences between the vehicle- and progesterone-treated injured groups (Fig. 1B).

One-way ANOVA of the C3 120 kDa band was significant ($F_{2,17} = 5.700$; $P < 0.05$). Sham-operates had significantly lower values than either the vehicle- or progesterone-treated groups, but post hoc analysis showed no significant difference between the vehicle-treated and progesterone-treated injured animals. One-way ANOVA of the C3 180 kDa band measures showed differences among the groups ($F_{2,17} = 44.37$; $P < 0.05$). Sham-operates had significantly lower scores for C3 180 kDa than either vehicle-treated or progesterone-treated injured animals. Post-hoc analysis did not reveal any differences between vehicle-treated and progesterone-treated injured animals ($P > 0.05$; Fig. 1B).

The polyclonal antibody used against NF κ B recognized two specific bands, which represented the p50 and the p65 NF κ B isomers (Fig. 2A). The p50 band intensity was not affected by injury or progesterone treatment ($F_{2,17} = 0.025$; $P > 0.05$) (Fig. 2B).

In contrast, one-way ANOVA of the p65 NF κ B isomer found differences among the groups ($F_{2,16} = 14.04$; $P < 0.05$). In post-hoc analysis, sham-operate values were significantly lower than values for injured rats treated with either vehicle or progesterone. Further analyses showed that densitometric values from the progesterone-treated injured animals were significantly lower than those from vehicle-treated injured rats (Fig. 2B). These analyses did not show significant injury or treatment effects on the level of the p50 isomer.

The Western blots for GFAP presented only a single band at 60 kDa that was visibly intensified by injury (3A). A

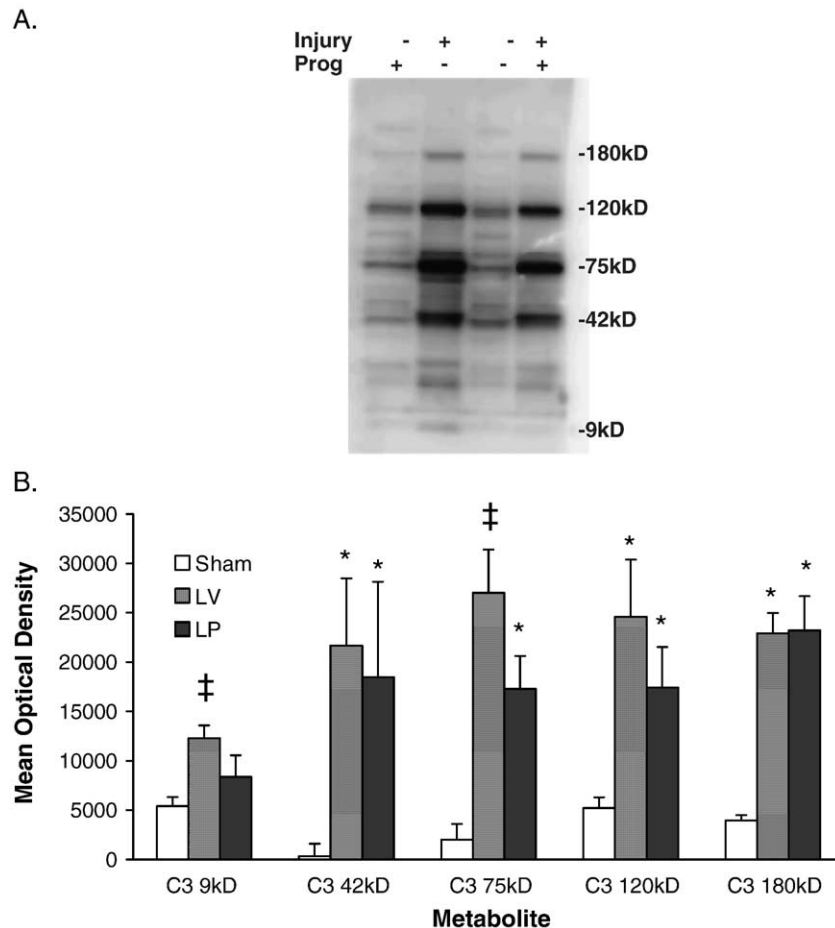


Fig. 1. (A) A representative Western blot for Complement C3. This Western blot displays the relative band intensities observed for Complement factor C3 and its metabolites. +: indicates whether the brain tissue was from a rat that received TBI, progesterone treatment, or both. (B) Progesterone treatment affects the metabolism of complement factor C3. Optical density measures (optical density-background) show mean protein concentrations of complement factor C3 metabolites in brain tissue. Complement factor C3 is a 180 kDa protein with multiple active metabolites. All fragments increased in intensity in the injured animals. There were no significant differences between progesterone-treated and vehicle-treated injured animals for the 180 kDa, 120 kDa, and 42 kDa bands. The levels of 9 kDa and 75 kDa metabolites are decreased in the progesterone-treated animals compared to injured vehicle-treated animals. * = significantly different from sham-operates ($P < 0.05$), † = significantly different from both sham-operates and injured rats given progesterone ($P < 0.05$). LV = vehicle-treated injured group, LP = progesterone-treated injured group.

Levene's test analysis of the variances in GFAP levels for heteroscedasticity found that the variances were unequal ($L_{2,15} = 7.41$; $P < 0.05$). The data were therefore converted using a logarithmic transformation. One-way ANOVA of the transformed GFAP levels showed differences among the groups ($F_{2,17} = 9.22$; $P < 0.05$). Sham-operate values were significantly lower than values for either vehicle- or progesterone-treated injured animals. Further analysis did not find any difference between the vehicle- and progesterone-treated injured animals ($P > 0.05$; Fig. 3B).

4. Discussion

Controlled impact injury to the MFC increases production of GFAP, NF κ B p65, C3, and active C3 fragments 48 h following injury. On the same Western blots, samples from progesterone-treated injured brains were not different from

uninjured controls for NF κ B p65 or the 9 kDa metabolite of C3. The densitometric analyses showed that progesterone reduces the expression of these inflammatory factors. When the brain tissue from the two injured groups was compared, the brain-injured rats given progesterone had 41% less NF κ B p65 and 32% less 9 kDa C3 band intensity compared to vehicle-treated animals. The NF κ B p50 subunit was not consistently influenced either by injury or by progesterone treatment. Following is a brief overview of what each of these factors contributes to the inflammatory cascade initiated by a TBI.

4.1. Complement factors

C3 is a 185 kDa protein that is proteolytically degraded into active 120 kDa, 75 kDa, 42 kDa, and 9 kDa fragments [32]. Complete C3 and its fragments were increased in both injury groups over the sham-operates. Our findings agree

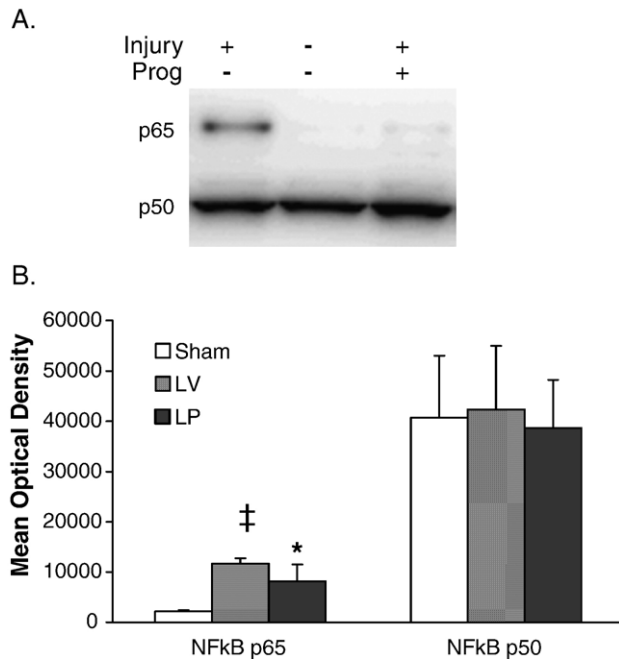


Fig. 2. (A) A representative Western blot illustrating the differences in the p50 and p65 subunits of NFκB. The p50 subunit is not affected by injury or treatment, while the p65 subunit is increased by injury and reduced by treatment with progesterone. (B) NFκB optical density measurements. Optical density shows the mean protein concentrations for both injury and treatment groups for the NFκB p65 and p50 peptides of the NFκB heterodimer. The NFκB p65 band is increased in the injured vehicle-treated animals but is significantly reduced in the injured progesterone-treated animals. NFκB p50 was unaffected by injury or treatment. * = significantly different from sham-operates ($P < 0.05$), ‡ = significantly different from both sham-operates and injured rats given progesterone ($P < 0.05$).

with a study by Keeling et al., who used the lateral fluid percussion brain injury model in rats [32]. They reported that C3 and its fragments were increased with injury at 6 h post-TBI [32]. Here, we found that post-injury progesterone treatment decreases band intensity specifically for the 75 kDa and 9 kDa fragments, while the concentrations of whole C3 and its other fragments were similar to those measured in vehicle-treated injured animals (see Fig. 1B). The importance of these findings is: (1) the 9 kDa metabolite, anaphalatoxin (C3a), is a powerful inflammatory agent that activates immune cells and promotes cytokine production, vasoconstriction, and vascular permeability; (2) the 75 kDa fragment (C3b), in combination with other cofactors, forms the enzyme C3 convertase, which is responsible for the proteolytic activation and cleavage of C3 with the subsequent formation of C3a [28,29,33,56]. While the 120 kDa and 42 kDa fragments attract and activate immune cells, they are not directly cytotoxic themselves.

However, the question arises, why is there a decrease in both the 75 and 9 kDa fragments but not in the whole C3 protein with progesterone treatment after TBI? Progesterone is known to increase decay-accelerating factor (DAF; CD55), a protein which prevents the assembly and accel-

erates the decay of C3 convertases that activate C3 and amplify the complement pathways [10,44,59]. One hypothesis, then, is that progesterone can increase DAF or DAF-like inhibitors of the complement pathways, leading to a decrease in the half-life of C3b and preventing the formation of the pro-inflammatory C3a fragment [31]. If this hypothesis is correct, it would not only explain our results, but might also help to explain how progesterone mediates its anti-inflammatory actions.

4.2. NFκB

NFκB has recently attracted attention as an influential factor in CNS injury [42]. Hayes and colleagues have demonstrated that NFκB activity is increased following brain injury [63], but their gel-shift assay technique did not allow distinction between isoforms p50 and p65. Smith et al. showed that TBI-induced NFκB p65 activity remains detectable for up to 1 year following injury [42]. Addressing the role of NFκB modulation, a recent study on spinal cord contusion showed that NFκB inhibition decreased expression of TNFα, IL1β, ICAM, and COX-2[41].

Steroids are known regulators of NFκB activity [11,30]. Previous studies of the anti-inflammatory properties of progesterone have been largely characterized in female reproductive tissue, where progesterone down-regulates cyclooxygenase-2 via NFκB inhibition [1,2]. Progesterone has been reported to inhibit NFκB-mediated transcription in two ways. The better-characterized route is by promoting the production of the endogenous inhibitor of NFκB (IκB) [60]. Recent studies show that, when progesterone is present, the progesterone receptor forms a heterodimer with

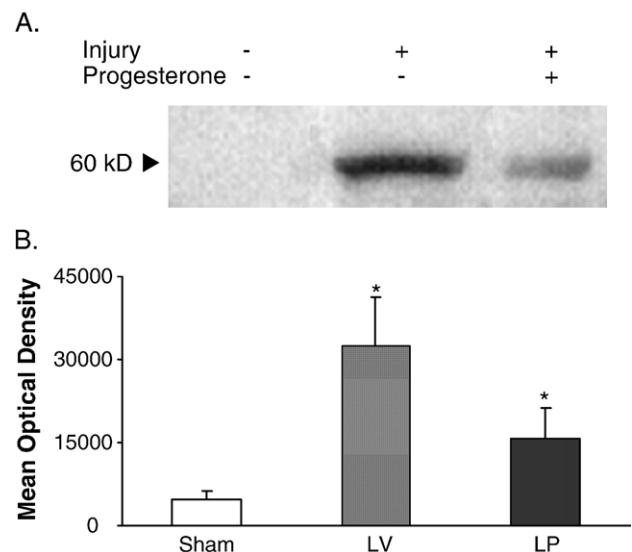


Fig. 3. (A) Representative Western blot illustrating differences in the 60 kDa band of GFAP. GFAP levels are increased with injury. (B) GFAP optical density measurement. Optical density minus background shows mean protein concentrations of GFAP in brain tissue from the injury and treatment groups. * = significantly different from sham-operates ($P < 0.05$).

NF κ B p65 and in turn inhibits binding of either transcription factor to its binding site [30].

At 48 h post-injury, progesterone inhibits the expression of NF κ B p65 without affecting the p50 subunit. This is an important finding since NF κ B is present in the cytoplasm as either a p50 homodimer or a p50–p65 heterodimer [18]. These dimers each associate with the same DNA binding site, but the heterodimer initiates transcription, while the homodimer remains inactive. Progesterone, which decreases concentration of p65 monomer, decreases the assembly of p65–p50 heterodimer and favors assembly of the inactive p50–p50 homodimer, thus decreasing NF κ B-mediated inflammatory transcription [22].

4.3. GFAP

Our measures from injured vehicle-treated brains support previous findings that correlate brain injury with increased NF κ B [42,63], C3 [34], and GFAP [12]. Increased GFAP is a marker of reactive gliosis associated with inflammation, cytotoxic edema, and elevated intracranial pressure [50]. In contrast to a penetrating brain injury model [17], where intracerebrally administered progesterone was shown to decrease reactive astrogliosis, our current results do not show an effect of progesterone on this measure at 48 h post-TBI. This disagreement can be explained by the severity of the injury, dose of progesterone, and the method of drug delivery. The stab wound model, for instance, is a relatively mild injury compared to this cortical impact injury. However, the results reported here are in agreement with another recent study reported by our laboratory, which showed that systemically administered progesterone did not affect the number of GFAP-positive astrocytes on post-injury days 3, 5, 7, and 9 [21]. Our results suggest that the increase in GFAP expression in reactive astrocytes after brain injury is caused by factors which are not influenced by progesterone treatment.

4.4. Summary

Following brain injury, C3, NF κ B, and GFAP synthesis is stimulated by inflammatory cytokines (TNF α and IL-1 β), which are produced by reactive glia and invading immune cells [4–6,35,50,53]. Inflammatory cytokines can, in turn, be stimulated by C3 and NF κ B [18,29]. In such a self-reinforcing inflammatory loop, inhibition of one or more factors may directly or indirectly inhibit the other factors involved in the cascade of brain damage. In a previous TBI study using RTq-PCR and ELISA, our laboratory reported that progesterone treatments significantly decreased TNF α and IL-1 β RNA and protein [24]. We think it is possible that the decreased TNF α and IL-1 β concentrations are downstream effects of progesterone inhibition of C3a and NF κ B.

Cytokine stimulation also amplifies the response of immune cells to complement activation products. Thus,

uncontrolled complement activation may be an initial trigger, a contributing factor that perpetuates the inflammatory response, or a final step in the process of post-injury inflammation. However, progesterone does not affect injury-induced GFAP-associated reactive astrogliosis. This result suggests that: (1) progesterone does not reduce all aspects of the inflammatory cascade; (2) astrocytes respond to injury-related factors which are not involved in inflammation; or (3) GFAP expression may be induced prior to progesterone administration.

This paper supports previous histological studies demonstrating a therapeutic, anti-inflammatory role for progesterone following TBI. Inflammation is a complex process and may involve differential regulation and response at different post-TBI recovery times. While cytokines and inflammatory regulators may play a beneficial role in the later periods of recovery and rehabilitation [36], such factors are probably detrimental to patient outcome in the acute stages of head injury. Treatments like progesterone which are capable of down-regulating inflammation and edema without provoking adverse effects are optimal candidates for the treatment of TBI pathology. This study demonstrates that progesterone clearly influences TBI-induced inflammatory factors and thus begins to explain the cellular mechanisms by which progesterone improves outcome following brain injury.

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References

- [1] V.C. Allport, D.M. Slater, R. Newton, P.R. Bennett, NF-kappaB and AP-1 are required for cyclo-oxygenase 2 gene expression in amnion epithelial cell line (WISH), *Mol. Hum. Reprod.* 6 (2000) 561–565.
- [2] V.C. Allport, D. Pieber, D.M. Slater, R. Newton, J.O. White, P.R. Bennett, Human labour is associated with nuclear factor-kappaB activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal', *Mol. Hum. Reprod.* 7 (2001) 581–586.
- [3] B. Arvin, L.F. Neville, F.C. Barone, G.Z. Feuerstein, The role of inflammation and cytokines in brain injury, *Neurosci. Biobehav. Rev.* 20 (1996) 445–452.
- [4] S.R. Barnum, J.L. Jones, Differential regulation of C3 gene expression in human astrogloma cells by interferon-gamma and interleukin-1 beta, *Neurosci. Lett.* 197 (1995) 121–124.
- [5] S.R. Barnum, J.L. Jones, E.N. Benveniste, Interleukin-1 and tumor necrosis factor-mediated regulation of C3 gene expression in human astrogloma cells, *Glia* 7 (1993) 225–236.
- [6] S.R. Barnum, J.L. Jones, U. Muller-Ladner, A. Samimi, I.L. Campbell, Chronic complement C3 gene expression in the CNS of transgenic mice with astrocyte-targeted interleukin-6 expression, *Glia* 18 (1996) 107–117.

- [7] E.E. Baulieu, Neurosteroids: a novel function of the brain, *Psychoneuroendocrinology* 23 (1998) 963–987.
- [8] A. Beaumont, A. Marmarou, K. Hayasaka, P. Barzo, P. Fatouros, F. Corwin, C. Marmarou, J. Dunbar, The permissive nature of blood brain barrier (BBB) opening in edema formation following traumatic brain injury, *Acta Neurochir. Suppl* 76 (2000) 125–129.
- [9] B.M. Bellander, S.K. Singhrao, M. Ohlsson, P. Mattsson, M. Svensson, Complement activation in the human brain after traumatic head injury, *J. Neurotrauma* 18 (2001) 1295–1311.
- [10] W.G. Brodbeck, C. Mold, J.P. Atkinson, M.E. Medof, Cooperation between decay-accelerating factor and membrane cofactor protein in protecting cells from autologous complement attack, *J. Immunol.* 165 (2000) 3999–4006.
- [11] K. De Bosscher, M.L. Schmitz, W. Vanden Berghe, S. Plaisance, W. Fiers, G. Haegeman, Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 13504–13509.
- [12] W.D. Dietrich, J. Truettner, W. Zhao, O.F. Alonso, R. Busto, M.D. Ginsberg, Sequential changes in glial fibrillary acidic protein and gene expression following parasagittal fluid-percussion brain injury in rats, *J. Neurotrauma* 16 (1999) 567–581.
- [13] M. Djebaili, S.W. Hoffman, D.G. Stein, Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex, *Neuroscience* 123 (2004) 349–359.
- [14] M. Djebaili, Q.M. Guo, E.H. Pettus, S.W. Hoffman, D.G. Stein, The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats, *J. Neurotrauma* 22 (2005) 106–118.
- [15] L. Fan, P.R. Young, F.C. Barone, G.Z. Feuerstein, D.H. Smith, T.K. McIntosh, Experimental brain injury induces expression of interleukin-1 beta mRNA in the rat brain, *Brain Res. Mol. Brain Res.* 30 (1995) 125–130.
- [16] L. Fan, P.R. Young, F.C. Barone, G.Z. Feuerstein, D.H. Smith, T.K. McIntosh, Experimental brain injury induces differential expression of tumor necrosis factor-alpha mRNA in the CNS, *Brain Res. Mol. Brain Res.* 36 (1996) 287–291.
- [17] J. Garcia-Estrada, S. Luquin, A.M. Fernandez, L.M. Garcia-Segura, Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury, *Int. J. Dev. Neurosci.* 17 (1999) 145–151.
- [18] S. Ghosh, M. Karin, Missing pieces in the NF-kappaB puzzle, *Cell* 109 (2002) S81–S96 (Suppl.).
- [19] Y. Goodman, A.J. Bruce, B. Cheng, M.P. Mattson, Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons, *J. Neurochem.* 66 (1996) 1836–1844.
- [20] C.W. Goss, S.W. Hoffman, D.G. Stein, Behavioral effects and anatomic correlates after brain injury: a progesterone dose–response study, *Pharmacol. Biochem. Behav.* 76 (2003) 231–242.
- [21] K.J. Grossman, C.W. Goss, D.G. Stein, Effects of progesterone on the inflammatory response to brain injury in the rat, *Brain Res.* 1008 (2004) 29–39.
- [22] Z. Gu, L. Cain, K. Werrbach-Perez, J.R. Perez-Polo, Differential alterations of NF-kappaB to oxidative stress in primary basal forebrain cultures, *Int. J. Dev. Neurosci.* 18 (2000) 185–192.
- [23] I. Harukuni, A. Bhardwaj, R.J. Traystman, B. Crain, E.D. London, J.R. Kirsch, Neuroprotection from focal ischemia by 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) is dependent on treatment duration in rats, *Anesth. Analg.* 87 (1998) 1299–1305.
- [24] J. He, C.O. Evans, S.W. Hoffman, N.M. Oyesiku, D.G. Stein, Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury, *Exp. Neurol.* 189 (2004) 404–412.
- [25] S.W. Hoffman, Z. Fulop, D.G. Stein, Bilateral frontal cortical contusion in rats: behavioral and anatomic consequences, *J. Neurotrauma* 11 (1994) 417–431.
- [26] S. Holmin, T. Mathiesen, J. Shetye, P. Biberfeld, Intracerebral inflammatory response to experimental brain contusion, *Acta Neurochir.* 132 (1995) 110–119.
- [27] S. Holmin, T. Mathiesen, Long-term intracerebral inflammatory response after experimental focal brain injury in rat, *NeuroReport* 10 (1999) 1889–1891.
- [28] T.E. Hugli, Biochemistry and biology of anaphylatoxins, *Complement* 3 (1986) 111–127.
- [29] A.C. Jauneau, A. Ischenko, P. Chan, M. Fontaine, Complement component anaphylatoxins upregulate chemokine expression by human astrocytes, *FEBS Lett.* 537 (2003) 17–22.
- [30] E. Kalkhoven, S. Wissink, P.T. van der Saag, B. van der Burg, Negative interaction between the RelA(p65) subunit of NF-kappaB and the progesterone receptor, *J. Biol. Chem.* 271 (1996) 6217–6224.
- [31] A. Kaul, M. Nagamani, B. Nowicki, Decreased expression of endometrial decay accelerating factor (DAF), a complement regulatory protein, in patients with luteal phase defect, *Am. J. Reprod. Immunol.* 34 (1995) 236–240.
- [32] K.L. Keeling, R.R. Hicks, J. Mahesh, B.B. Billings, G.J. Kotwal, Local neutrophil influx following lateral fluid-percussion brain injury in rats is associated with accumulation of complement activation fragments of the third component (C3) of the complement system, *J. Neuroimmunol.* 105 (2000) 20–30.
- [33] M. Kirschfink, Controlling the complement system in inflammation, *Immunopharmacology* 38 (1997) 51–62.
- [34] T. Kossmann, P.F. Stahel, M.C. Morganti-Kossmann, J.L. Jones, S.R. Barnum, Elevated levels of the complement components C3 and factor B in ventricular cerebrospinal fluid of patients with traumatic brain injury, *J. Neuroimmunol.* 73 (1997) 63–69.
- [35] S. Kyrkanides, M.K. O'Banion, P.E. Whiteley, J.C. Daeschner, J.A. Olschowka, Enhanced glial activation and expression of specific CNS inflammation-related molecules in aged versus young rats following cortical stab injury, *J. Neuroimmunol.* 119 (2001) 269–277.
- [36] P.M. Lenzlinger, M.C. Morganti-Kossmann, H.L. Laurer, T.K. McIntosh, The duality of the inflammatory response to traumatic brain injury, *Mol. Neurobiol.* 24 (2001) 169–181.
- [37] S.G. Lundeen, Z. Zhang, Y. Zhu, J.M. Carver, R.C. Winneker, Rat uterine complement C3 expression as a model for progesterone receptor modulators: characterization of the new progestin trimegestone, *J. Steroid Biochem. Mol. Biol.* 78 (2001) 137–143.
- [38] M.P. Mattson, S.W. Barger, K. Furukawa, A.J. Bruce, T. Wyss-Coray, R.J. Mark, L. Mucke, Cellular signaling roles of TGF beta, TNF alpha and beta APP in brain injury responses and Alzheimer's disease, *Brain Res. Brain Res. Rev.* 23 (1997) 47–61.
- [39] F.P. Monnet, V. Mahe, P. Robel, E.E. Baulieu, Neurosteroids, via sigma receptors, modulate the [3H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus, *Proc. Natl. Acad. Sci. U. S. A.* 92 (1995) 3774–3778.
- [40] J.K. Newcomb, X. Zhao, B.R. Pike, R.L. Hayes, Temporal profile of apoptotic-like changes in neurons and astrocytes following controlled cortical impact injury in the rat, *Exp. Neurol.* 158 (1999) 76–88.
- [41] M. Nishio, T. Yuguchi, C. Akiyama, T. Fujinaka, Y. Nakajima, M. Taniguchi, E. Kohmura, T. Yoshimine, Nuclear Factor-kappa B decoy oligodeoxynucleotides can reduce the ischemic spinal cord injury of rat, *J. Neurotrauma* 19 (2002) 1333.
- [42] M. Nonaka, X.H. Chen, J.E. Pierce, M.J. Leoni, T.K. McIntosh, J.A. Wolf, D.H. Smith, Prolonged activation of NF-kappaB following traumatic brain injury in rats, *J. Neurotrauma* 16 (1999) 1023–1034.
- [43] G. Raivich, H. Bluethmann, G.W. Kreutzberg, Signaling molecules and neuroglial activation in the injured central nervous system, *Keio J. Med.* 45 (1996) 239–247.
- [44] C.S. Rinder, H.M. Rinder, K. Johnson, M. Smith, D.L. Lee, J. Tracey, G. Polack, P. Higgins, C.G. Yeh, B.R. Smith, Role of C3 cleavage in monocyte activation during extracorporeal circulation, *Circulation* 100 (1999) 553–558.
- [45] I. Roberts, G. Schierhout, P. Alderson, Absence of evidence for the effectiveness of five interventions routinely used in the intensive care

- management of severe head injury: a systematic review [see comments], *J. Neurol. Neurosurg. Psychiatry* 65 (1998) 729–733.
- [46] R.L. Roof, R. Duvdevani, J.W. Heyburn, D.G. Stein, Progesterone reduces BBB damage following bilateral, medial frontal contusion, *Soc. Neurosci.* 20 (1994) 191 (Miami Beach, FL).
- [47] R.L. Roof, R. Duvdevani, J.W. Heyburn, D.G. Stein, Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective, *Exp. Neurol.* 138 (1996) 246–251.
- [48] R.L. Roof, S.W. Hoffman, D.G. Stein, Progesterone protects against lipid peroxidation following traumatic brain injury in rats, *Mol. Chem. Neuropathol.* 31 (1997) 1–11.
- [49] M.E. Rose, M.B. Huerbin, J. Melick, D.W. Marion, A.M. Palmer, J.K. Schiding, P.M. Kochanek, S.H. Graham, Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury, *Brain Res.* 943 (2002) 15–22.
- [50] M. Rostworowski, V. Balasingam, S. Chabot, T. Owens, V.W. Yong, Astrogliosis in the neonatal and adult murine brain post-trauma: elevation of inflammatory cytokines and the lack of requirement for endogenous interferon-gamma, *J. Neurosci.* 17 (1997) 3664–3674.
- [51] D.A. Shear, R. Galani, S.W. Hoffman, D.G. Stein, Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury, *Exp. Neurol.* 178 (2002) 59–67.
- [52] H.D. Soares, R.R. Hicks, D. Smith, T.K. McIntosh, Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury, *J. Neurosci.* 15 (1995) 8223–8233.
- [53] P.F. Stahel, T. Kossmann, M.C. Morganti-Kossmann, V.H. Hans, S.R. Barnum, Experimental diffuse axonal injury induces enhanced neuronal C5a receptor mRNA expression in rats, *Brain Res. Mol. Brain Res.* 50 (1997) 205–212.
- [54] D.G. Stein, Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? *Trends Neurosci.* 24 (2001) 386–3891.
- [55] D.G. Stein, S.W. Hoffman, Estrogen and progesterone as neuro-protective agents in the treatment of acute brain injuries, *Pediatr. Rehabil.* 6 (2003) 13–22.
- [56] T. Takabayashi, E. Vannier, B.D. Clark, N.H. Margolis, C.A. Dinarello, J.F. Burke, J.A. Gelfand, A new biologic role for C3a and C3a desArg: regulation of TNF-alpha and IL-1 beta synthesis, *J. Immunol.* 156 (1996) 3455–3460.
- [57] H. Tanaka, Y. Katayama, T. Kawamata, T. Tsubokawa, Excitatory amino acid release from contused brain tissue into surrounding brain areas, *Acta Neurochir. Suppl* 60 (1994) 524–527.
- [58] V. Trembovler, E. Beit-Yannai, F. Younis, R. Gallily, M. Horowitz, E. Shohami, Antioxidants attenuate acute toxicity of tumor necrosis factor-alpha induced by brain injury in rat, *J. Interferon Cytokine Res.* 19 (1999) 791–795.
- [59] J. van Beek, M. van Meurs, B.A. t Hart, H.P. Brok, J.W. Neal, A. Chatagner, C.L. Harris, N. Omidvar, B.P. Morgan, J.D. Laman, P. Gasque, Decay-accelerating factor (CD55) is expressed by neurons in response to chronic but not acute autoimmune central nervous system inflammation associated with complement activation, *J. Immunol.* 174 (2005) 2353–2365.
- [60] S. Wissink, E.C. van Heerde, B. vand der Burg, P.T. van der Saag, A dual mechanism mediates repression of NF-kappaB activity by glucocorticoids, *Mol. Endocrinol.* 12 (1998) 355–363.
- [61] D.W. Wright, M.E. Bauer, S.W. Hoffman, D.G. Stein, Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats, *J. Neurotrauma* 18 (2001) 901–909.
- [62] F.S. Wu, T.T. Gibbs, D.H. Farb, Inverse modulation of gamma-aminobutyric acid- and glycine-induced currents by progesterone, *Mol. Pharmacol.* 37 (1990) 597–602.
- [63] K. Yang, X.S. Mu, R.L. Hayes, Increased cortical nuclear factor-kappa B (NF-kappa B) DNA binding activity after traumatic brain injury in rats, *Neurosci. Lett.* 197 (1995) 101–104.