Neurosteroids reduce inflammation after TBI through CD55 induction

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Abstract

The inflammatory cascade that follows traumatic brain injury may lead to secondary cell death and can impede recovery of function. Complement factors and their convertases are increased in glia after brain injury and lead to the production of inflammatory products that kill vulnerable neurons. Progesterone and its metabolite allopregnanolone (5α-pregnan-3β-ol-20-one) have been shown to reduce the expression of inflammatory cytokines in the acute stages of brain injury, although how they do this is not completely understood. In this study we show that both progesterone and allopregnanolone treatments enhance the production of CD55 following contusion injuries of the cerebral cortex in rats. CD55, a single-chain type 1 cell surface protein, is a potent inhibitor of the complement convertases which are activators of the inflammatory cascade. The increased expression of CD55 could be an important mechanism by which steroids help to reduce the cerebral damage caused by inflammation.

Keywords: traumatic brain injury, progesterone, allopregnanolone, CD55, inflammation

Introduction

Traumatic brain injury (TBI) produces a significant inflammatory reaction that is generally accompanied by heavy gliosis and apoptosis in brain areas proximal and distal to the locus of injury [6, 33]. The insult triggers an invasion of macrophages and neutrophils into the impact area, producing much of the inflammation and swelling associated with CNS damage. These potentially cytotoxic events can directly affect patient outcome after TBI, which can be further worsened by uncontrolled intracranial pressure (ICP) [17] caused by a rise in brain water content (cerebral edema) [18]. Uncontrolled ICP can produce greater secondary injury through ischemia [39] and an increase in mortality caused by herniation of the brain [23]. Other than mannitol, which has limited effects on edema, there are no substantially effective therapeutic agents which can rapidly and safely reduce both marked swelling and inflammation after brain injury.

Over the last decade, we have focused on the use of progesterone (PROG) as a therapeutic agent for TBI. In rats with bilateral medial frontal cortex (MFC) contusions, post-injury injections of PROG reduced cerebral edema,
blood-brain barrier disruption, and the expression of inflammatory cytokines [7, 31, 32]. We have also shown that PROG's metabolite, allopregnanolone (ALLO; 5α-pregnan-3β-ol-20-one), a GABA-A agonist, can reduce the mediators of inflammation after TBI at a dose that is 50% lower than PROG [14]. The effectiveness of PROG in the context of TBI was most recently demonstrated by a Phase IIa clinical trial showing that 3 days of intravenous injections of PROG post-TBI can reduce mortality by over 50% in severely injured human patients and improve cognitive functions in moderately-injured patients tested 30 days after their injuries [42].

Exactly where PROG and ALLO interact with the inflammatory cascade to reduce its intensity is unknown, but complement factors seem to play one of the major roles [29]. Sewell et al. [35] recently demonstrated that mice deficient in complement factor C3 expressed significantly lower levels of inflammatory cytokines than did wild-type mice, and complement inhibition has been shown to attenuate CNS injury, whether induced by external agents or genetic deficiency [1, 25, 43].

Complement C3 is a key component in the activation of the complement system. The C3 precursor is a 185kD protein that is cleaved into an alpha (120kD) and a beta chain (75kD) linked by a disulfide bond. This mature C3 is further cleaved by C3 convertase to release C3a and C3b [28]. C3a, one of the anaphylotoxins of the complement system, is a potent vasoconstrictor and immune cell activator and has been shown to be a promoter of inflammatory cytokines in human disease processes as diverse as cerebral ischemia [26] and Alzheimer's disease [9]. C3b participates in cellular adherence and enhances phagocytosis in addition to its crucial role in forming cellular intermediates that perpetuate the complement activation process in both the classical and alternative pathways. Clearly such a self-promoting inflammatory system can be detrimental if left unchecked. Disrupting the activity of convertase could short-circuit this injury cascade, and PROG and ALLO may play a role in this process.

The evidence for this comes from a recent study in our laboratory [29], which reported that post-TBI administration of PROG reduces the expression of both the C3a and C3b fragments (9 and 75 kD, respectively) of the pro-inflammatory complement factor C3 without influencing its overall expression, suggesting an effect on C3 convertase. In particular, the reduction of C3a indicates that PROG treatment reduces complement-mediated cytotoxicity [4]. More importantly, a reduction in C3b [3] can inhibit further activation of the complement system and the resultant amplification of the inflammatory process.

The specific mechanism by which PROG and ALLO reduce the C3 fragments after injury is not yet known. However, based on the literature in reproductive biology [41] and our previous results, we hypothesized that acute treatment with PROG after TBI may block the C3 cascade by increasing the expression of CD55 [22]. CD55, also known as decay-accelerating factor (DAF), is a 70 kD protein that disrupts C3 convertase activity both by inhibiting formation of the C3 convertase complex and by accelerating its decay [20]. Early inactivation of the complement cascade by CD55 effectively halts the progression of inflammatory processes and prevents subsequent cell injury [38].

In the present study, we report that post-injury treatment with either PROG or ALLO sustains the synthesis of CD55 in the injured brain. Combined with our previous data showing that a decrease in neuroinflammation is correlated with improved outcomes after contusion injuries to the cortex [14, 29], these findings can be taken to suggest that CD55 activation represents an important mechanism by which PROG and ALLO exert their anti-inflammatory effects after TBI.

**Methods**

**Subjects**

Sixty male Sprague-Dawley rats weighing 300–330g were used in this study. Fifteen animals were assigned to each of four treatment groups (including shams). Five rats from each group were used for gene expression
Hercules, CA). A protein standard (Bio-Rad) was loaded to detect protein size. The gel was run at 200 V for 1 h. Proteins were transferred to a polyvinylidene difluoride membrane at 100 V for 30 min. Ponceau S was added to membranes to show protein bands. Membranes were incubated overnight in KPL blocker (KPL, Gaithersburg, MD) at 4°C.

Blots were incubated with a polyclonal rabbit primary antibody for CD55 (Santa Cruz Biotechnology, Santa Cruz, CA) and mouse for β-actin (Abcam, Cambridge, MA) in KPL diluent: phosphate-buffered saline (PBS) (1:20) and left overnight in a 4°C freezer. Membranes were incubated with secondary antibody donkey anti-rabbit (CD55) and goat anti-mouse (β-actin) IgG-HRP in KPL diluent: (1:2000) for 2 h at room temperature. Blots were incubated in chemiluminescent substrate (Pierce) for 5 min, and chemiluminescent bands were detected on a Kodak Image station 440CF scanner (Rochester, NY) and analyzed with the accompanying densitometric image analysis software. Band densities for CD55 blots were normalized to β-actin.

Statistical Analysis

All results were expressed as the mean ± the standard deviation of the mean. The criterion for statistical significance was set at p<0.05. ANOVA, t-tests, and Tukey-Kramer post hoc tests were used to make individual comparisons.

Results

24 h post-TBI

CD55 protein expression was significantly lower in the vehicle-treated damaged brain compared to sham and steroid-treated groups. Both PROG and ALLO sustained the protein abundance of CD55 protein at the level of shams (p<0.05, F=17.85, Fig.1).

![Figure 1](image)

Protein expression analysis of CD-55 at 24h following brain injury. Vehicle-treated injury significantly decreased CD-55 protein levels compared to all other groups. There were no differences between PROG, ALLO or shams. * denotes significance at p<0.05.

48 h post-TBI

There was a significantly lower expression of the CD55 gene in the vehicle-treated injured brain compared to all other injury groups. PROG and ALLO treatment significantly increased CD55 gene expression above vehicle-treated and sham levels (p<0.05, F=115.77, Fig.2).

![Figure 2](image)

Gene expression analysis of CD-55 at 48 h following brain injury. Both progesterone (PROG) and allopregnanolone (ALLO) treatments significantly increased CD-55 mRNA abundance compared to shams and vehicle-treated injury. PROG treatment induced a significant ...

72 h post-TBI

The effects of injury and treatments on CD55 protein expression seen at 24 h were maintained at 72 h. PROG and ALLO treatment each sustained the level of CD55 protein in the injured brain at that of shams, thus preventing the decline seen in vehicle-treated injured animals (p=0.05, F=34.47, Fig.3).
Protein expression analysis of CD-55 at 72 h following brain injury. CD-55 protein level in vehicle-treated rat brains was significantly diminished compared to all other groups. There were no differences between PROG, ALLO or shams. * denotes significance ...

Discussion

We found that post-injury administration of either PROG or ALLO will increase CD55 gene expression at 48 h compared to vehicle-treated TBI and shams. Furthermore, the hormone treatments maintained protein expression at 24 and 72 h at the level of the uninjured controls, while vehicle-treated injured brains showed a significant decline in CD55 protein abundance. The prevention of a decline in CD55 protein observed as early as 24 h after injury suggests a rapid hormonal effect that may be very important in the attenuation of TBI-induced damage. The fact that CD55 mRNA is increased at 48 h after injury further suggests that both PROG and ALLO treatments directly affect CD55 gene expression, and that this transcriptional regulation is sustained throughout the acute phase of injury, lasting at least several days. This extended transcriptional action is further supported by the fact that CD55 protein is still elevated 72 h after the initial insult.

The significant reduction in protein abundance seen in vehicle-treated injuries compared to the intact shams suggests that hormone-mediated transcription of CD55 is required to prevent an injury-induced decline in CD55 protein expression. Sham CD55 mRNA levels are much lower than protein, further suggesting that under normal conditions mechanisms of post-transcriptional stability are at work. Therefore, we cannot eliminate the possibility that post-transcriptional molecular stability of CD55 may be a mechanism by which PROG and ALLO are working along with their transcriptional effects. Since we have previously shown that C3a and C3b complement fragments are decreased by PROG in the acute phase of TBI [20], we can now report that this therapeutic effect is supported by a mechanism involving the transcriptional regulation of CD55.

Others have also shown that CD55 responds to PROG [19], but because PROG and ALLO act on different receptors, there is currently no direct receptor mechanism that can explain how the hormone and its metabolite are both able to induce CD55 expression. We offer the hypothesis that the pregnane X receptor (PXR) is a likely candidate and may be responsible for some of the cytoprotective effects of PROG and ALLO. PROG and ALLO are known to be efficient ligands of the PXR [21, 24], and the liganded PXR/retinoid X receptor (RXR) complex has previously been shown to bind to the antioxidant response element (ARE) found in a number of protective genes, including Bcl-2 [44]. This corresponds with our previous results showing that both PROG and ALLO enhance Bcl-2 after injury [7], and suggests the PXR as a potential mechanism for this process. Activation of the PXR may also explain the mechanism of the observed increase in antioxidant capacity in the injured brain after treatment with PROG, ALLO, and the enantiomer of PROG [49]. Interestingly, in vivo PROG can be converted to ALLO [2]. However, ALLO does not act through the classical intra-nuclear PR unless it is reversibly converted to Salpia-dihydroprogesterone [3]. Given that CD55 gene transcription is enhanced by both ALLO and PROG, it is less likely that these results are an effect of the GABAergic [16] nature of ALLO alone. Although further research is clearly needed for any firm conclusions, it seems feasible that CD55 expression may be regulated in a similar manner, that is, through activation of the PXR.

Over the past two decades, we and others have shown that neurosteroid hormones are potent cytoprotective agents when given within the first 24 h after TBI. PROG and ALLO have been shown to reduce edema, inflammation and lipid peroxidation in several models of brain injury [11, 13, 27, 29, 30, 32, 34, 37]. We now think that both PROG and ALLO may control the expression of inflammatory mediators through a CD55-regulated mechanism. By breaking the chain of injury-induced inflammation at the point of amplification,


