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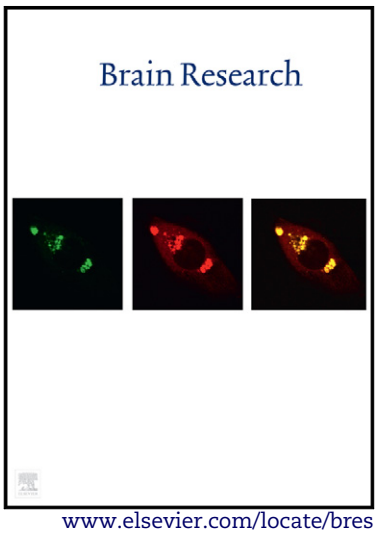

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Progesterone's Role in Neuroprotection, a Review of the Evidence

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Abstract

The sex hormone Progesterone has been shown to improve outcomes in animal models of a number of neurologic diseases, including traumatic brain injury, ischemia, spinal cord injury, peripheral nerve injury, demyelinating disease, neuromuscular disorders, and seizures. Evidence suggests it exerts its neuroprotective effects through several pathways, including reducing edema, improving neuronal survive, and modulating inflammation and apoptosis. In this review, we summarize the functional outcomes and pathophysiologic mechanisms attributed to Progesterone treatment in neurologic disease. We then comment on the breadth of evidence for the use of Progesterone in each neurologic disease family. Finally, we provide support for further human studies using Progesterone to treat several neurologic diseases.

1.0 Introduction

Over the last 25 years multiple investigators have explored the role of progesterone (PROG) in the treatment of neurologic disease. Interest in the hormone was initially sparked by Attella et al., who observed improved functional outcomes and decreased edema in pseudopregnant rats suffering from traumatic brain injury (TBI) compared to normal-cycling females with similar injuries (Attella et al., 1987). The investigators speculated that the observed difference could be attributed to higher levels of gonadal hormones in the pseudopregnant state. Follow-up studies by Roof et al. confirmed that administration of PROG could attenuate cerebral edema and improve functional outcomes in both male and female rats subjected to TBI (Roof et al., 1992; Roof et al., 1993a; Roof et al., 1994), providing a framework for future research.

Numerous other papers have demonstrated neuroprotective effects of PROG in TBI and other neurologic conditions including ischemia, spinal cord injury, peripheral nerve injury, motorneuron disease, demyelinating disease, and seizures. We present a comprehensive overview of the *in vivo* animal research to date pertaining to the administration of PROG in neurologic disease. Within each disease family we review the animal models used, the documented functional changes attributed to PROG (Figure 1), the mechanisms through which PROG has been hypothesized to act (Table 1), and the effect of variable treatment protocols (e.g. administration site/method, dose, window of treatment, and use of related hormones). The collected body of work provides compelling evidence for the trial of PROG for neurologic disease in humans.

2.0 Traumatic Brain Injury

2.1 Animal Models

TBI was the first neurologic disease for which PROG administration was studied, and remains the most studied to date. Several animal models have been used to simulate TBI, the vast majority of which were performed in rats. The initial Attella et al. study used a bilateral medial frontal cortex (bMFC)

ablation model (Attella et al., 1987). Ablation injuries involve partial craniotomy and aspiration of the brain parenchyma. Similar injuries in other studies have been produced in the bilateral medial prefrontal cortex (Asbury et al., 1998) and the unilateral hindlimb sensorimotor cortex (Goldstein and Bullman, 1999).

Most TBI research has been done using an impact model, a type thought to simulate direct impact injuries that occur in a motor vehicle collision or a fall. The general principle of these models is that a blunt force is applied to produce injury. The bMFC contusion model used by Roof et al. (Roof et al., 1992; Roof et al., 1993a) and for most of the studies from the Stein group involves exposing the brain via craniotomy and then delivering a blunt injury directly to the dural surface using a pneumatically driven piston. The piston allows for a controlled, reproducible injury with a set force, velocity, and depth of compression. Although used primarily in rats, this model has been applied in mice as well (Hua et al., 2011). Similar devices have been used to produce contusion in the unilateral sensorimotor cortex (Grossman and Stein, 2000) and unilateral parietal cortex (Anderson et al., 2011; Gilmer et al., 2008; Peterson et al., 2012; Robertson et al., 2006; Wagner et al., 2004). The latter injury may also be induced via a weight dropped directly onto the exposed dura (Chen et al., 2007; Chen et al., 2008a; Chen et al., 2008b). The injury is controlled by the mass of the weight, the drop height, and the maximum allowable compression depth. Fluid percussion injury (FPI) models are another form of impact model that have been used by several labs to induce TBI (Bramlett and Dietrich, 2001; Li et al., 2012; Suzuki et al., 2004; Yao et al., 2005). These involve a partial craniotomy, adhesion of tubing to the exposed injury site, and then the generation of a fluid wave through the tubing which directly impacts the dural surface. Another impact model, designed to simulate head injury without a craniotomy, is the impact-acceleration model (Maghool et al., 2013; O'Connor et al., 2005; O'Connor et al., 2007; Sarkaki et al., 2011; Shahrokhi et al., 2010). In this model, a stainless steel plate is affixed directly to the rat's skull, and a fixed weight is dropped upon it to simulate blunt force, closed-head injury trauma. The procedure produces a more diffuse axonal injury, as opposed to the more focal injuries caused by the direct cortical impact models. Penetrating injury has also been used to induce TBI in rats (Garcia-Estrada et al., 1993; Garcia-Estrada et al., 1999). In this model, a hole is made in the skull and a conical cannula is inserted into the brain parenchyma, displaced, and then removed to create the lesion.

Two other brain injury models have been used to study PROG's effect on TBI. The unilateral entorhinal cortex lesion involves the application of an electric current to produce an electrolytic lesion to the entorhinal cortex (Roof et al., 1993b). In the aseptic cryogenic injury (ACI) model liquid nitrogen is applied directly to the skull over the unilateral parietal cortex (Jones et al., 2005). This produces functional deficits and pathophysiologic characteristics similar to those of the other TBI models.

All the aforementioned models have been shown to produce behavioral, cognitive, and sensorimotor deficits, and lead to many of the same pathophysiologic characteristics, including edema formation, blood-brain barrier (BBB) dysfunction, neuronal loss, and activation of the inflammatory and apoptotic pathways.

2.2 Functional Outcomes

Several functional assessments have shown that PROG improves behavioral, cognitive, and sensorimotor deficits induced by TBI in a number of injury models. Attella et al. first showed improved functional outcomes after bilateral frontal cortex injuries in pseudopregnant females (high in progesterone) compared to normal-cycling females (higher in estrogen) (Attella et al., 1987). In rats subjected to bMFC aspiration, the pseudopregnant group had improved retention of previously learned spatial and cognitive tasks.

The Morris water maze (MWM) has been used in a number of studies to assess functional outcomes. It measures various aspects of behavior and cognition, including working memory, behavioral spontaneity, and spatial orientation. Female rats performed better on the MWM than males following an entorhinal cortex lesion (Roof et al., 1993b), but not a unilateral parietal contusion (Wagner et al., 2004). Improved performance on the MWM was seen with PROG treatment in rats after bMFC contusion (Djebaili et al., 2004; Djebaili et al., 2005; Roof et al., 1994) and unilateral parietal contusion (Peterson et al., 2012), as well as mice after ACI (Jones et al., 2005). This was replicated in aged rats subjected to both bMFC contusion (Wali et al., 2011) and FPI (Li et al., 2012). The FPI model has also demonstrated that PROG can produce an improvement in Neurologic Severity Score (Li et al., 2012), which assesses motor, sensory, reflex and balance performance, an effect also seen in previous studies on TBI (Pan et al., 2007). PROG has also been shown to mitigate deficits in avoidance learning following prefrontal cortex ablation (Asbury et al., 1998) and improve performance on rotarod and Barnes maze tests (measures of motor and cognitive performance) after diffuse injury in the impact-acceleration model (O'Connor et al., 2007). In aged rats it increases spontaneous locomotor activity following bMFC contusion (Cutler et al., 2007; Wali et al., 2011). However, with focal ablation or contusion injuries to the sensorimotor cortex, no differences were seen between injured males, females, or pseudopregnant females in tests assessing their sensorimotor function, with all injured groups performing worse than controls (Goldstein and Bullman, 1999; Grossman and Stein, 2000). This suggests that PROG does not offer protection from focal sensorimotor deficits. Additionally, a study by Grossman et al. demonstrated that PROG had no effect on simple sickness behaviors after bMFC contusion, including decreased food consumption, reduced weight maintenance, reduced grooming, and reduced exploratory activity (Grossman et al., 2011).

2.3 Pathophysiologic and Biochemical Mechanisms

Much work has been done to elucidate the mechanisms by which PROG offers neuroprotection following TBI. Many studies have demonstrated that PROG reduces post-injury cerebral edema. The Attella et al. study observed an enlargement of the ventricles of the normal-cycling females compared to pseudopregnant females and sham controls following bMFC ablation (Attella et al., 1987). The authors postulated that the pseudopregnant state was offering protection against the edema formation induced by the injury. A follow-up study by Roof et al. confirmed that post-injury edema was reduced in females compared to males, and in pseudopregnancy compared to normal-cycling females following bMFC contusion (Roof et al., 1993a). The study then compared pretreatment with either estrogen or PROG in ovariectomized (OVX) females subjected to bMFC contusion. Results suggested that the PROG treatment was responsible for the neuroprotection afforded by the pseudopregnant state.

Subsequent studies have shown that PROG administered post-injury in both male and female rats can reduce edema formation following bMFC contusion (Grossman et al., 2004; Guo et al., 2006; Roof et al., 1992) and impact-acceleration diffuse TBI (Maghool et al., 2013; O'Connor et al., 2005; Shahrokhi et al., 2010), but not ACI (Jones et al., 2005) nor moderate unilateral parietal cortex contusion (Gilmer et al., 2008). Edema reduction has been replicated in aged rats (Cutler et al., 2007; Kasturi and Stein, 2009). The decrease in edema is seen not only in the directly-injured cortical regions, but in the underlying subcortical regions as well (Kasturi and Stein, 2009). Further, Wright et al. demonstrated an inverse relationship between serum PROG level and edema; the higher the PROG level, the lower the amount of edema (Wright et al., 2001).

The mechanisms underlying the edema reduction induced by PROG administration are likely multifactorial. One area of investigation is whether PROG alters the permeability of the BBB. An early study found no differences in BBB function between males, normal-cycling females, and pseudopregnant females 24 hours after bMFC contusion (Duvdevani et al., 1995). However, a subsequent study in an impact-acceleration model demonstrated that a single dose of PROG at 30 minutes reduced BBB permeability at 5 hours (O'Connor et al., 2005). Expression of p-glycoprotein (PGP) efflux pump, a marker of BBB function, is upregulated in PROG-treated rats compared to untreated controls following bMFC contusion (Cutler et al., 2007). The increased PGP expression corresponded with reduced edema. These results indicate that PROG improves BBB integrity, and this action may contribute to decreased edema formation. PROG has also been shown to decrease expression of aquaporin 4 (AQP4) at 72 hours post-bMFC contusion in the regions around the lesion and lateral ventricles, while increasing its expression in the tissue surrounding the third ventricle (Guo et al., 2006). This corresponded with reduced levels of edema, suggesting that modulation of AQP4 expression may play a role in the edema reduction seen with PROG administration following TBI.

Another mechanism by which PROG provides neuroprotection in TBI is through enhanced neuronal survival. PROG has been shown to attenuate the loss of neurons in the medial dorsal nucleus (MDN) after bMFC contusion (Roof et al., 1994). Improved survival in the MDN, as well as the striatum, was replicated in prefrontal cortex ablation (Asbury et al., 1998). Neuronal sparing following unilateral parietotemporal contusion has also been shown in the CA1 and CA3 regions of the hippocampus (Robertson et al., 2006). Bramlett et al. showed reduced contusion volumes in normal-cycling females vs. ovariectomized females or males following FPI (Bramlett and Dietrich, 2001). This finding was replicated in Suzuki et al., an effect that was exacerbated by post-traumatic hyperthermia (Suzuki et al., 2004). Other studies then demonstrated that administration of PROG reduced necrotic lesion volume in ACI models (Jones et al., 2005) and bMFC contusion (Grossman et al., 2011; Shear et al., 2002), contrary to findings from an earlier report (Roof et al., 1994). Neuronal death is also attenuated with PROG treatment following diffuse axonal injury (O'Connor et al., 2007) and unilateral parietal contusion (Peterson et al., 2012). In another study, PROG was shown to normalize levels of cell proliferation and cell death (Barha et al., 2011).

Improved neuronal survival may be attributed at least in part to the effect of PROG on apoptosis. PROG downregulates several pro-apoptotic factors after TBI including caspase-3 (Cutler et al., 2007; Djebaili et al., 2004; Djebaili et al., 2005; O'Connor et al., 2007), Bax (Djebaili et al., 2005; Yao et

al., 2005), Bad (Yao et al., 2005), and p53 (VanLandingham et al., 2006). Conversely, the anti-apoptotic factors Bcl-2 and Bcl-x_L are upregulated by PROG in a FPI model for several days after the injury (Yao et al., 2005). However, in the bMFC contusion model PROG was shown to actually decrease the level of Bcl-2 and another anti-apoptotic protein, AKT, compared to vehicle at 24 hours (Djebaili et al., 2005). This contradiction may be explained by the differences between the disease models, different doses of PROG administered, or timing of the measurements. It has also been shown that the degree of DNA fragmentation is attenuated with PROG administration after bMFC contusion (Djebaili et al., 2004; Djebaili et al., 2005) and unilateral parietal cortex contusion (Chen et al., 2008a). Additionally, PROG upregulated the pro-survival brain-derived neurotrophic factor (BDNF) while downregulating proBDNF and proNGF, pro-apoptotic precursors to BDNF and nerve growth factor (NGF) (Cekic et al., 2012). Microarray analysis has also shown that PROG affects expression of a number of genes related to apoptosis following parietal contusion (Anderson et al., 2011).

PROG's action on the inflammatory pathway also contributes to neuroprotection following TBI. PROG has been shown to attenuate the increased expression of several molecules associated with inflammation following TBI including IL-1β (Chen et al., 2008a; Gonzalez et al., 2004; VanLandingham et al., 2006), TNF-α (Chen et al., 2008a; Gonzalez et al., 2004; Pan et al., 2007; Sarkaki et al., 2011; VanLandingham et al., 2006), NF-κB (Chen et al., 2008a; Cutler et al., 2007; Pan et al., 2007; Pettus et al., 2005), IL-6 (Chen et al., 2008a; Cutler et al., 2007; Sarkaki et al., 2011), COX-2 (Cutler et al., 2007), and ICAM-1 (Chen et al., 2008a). The reduction of IL-1β and TNF-α was not replicated in the ACI model (Jones et al., 2005). PROG also mitigates the complement system, reducing levels of C3 (Pettus et al., 2005) and enhancing the production of CD55, an inhibitor of complement convertases (VanLandingham et al., 2007). The expression of a number of other genes related to inflammation is influenced by PROG administration as demonstrated by microarray analysis (Anderson et al., 2011). Interestingly, PROG actually increased the accumulation of macrophages/activated microglia, cells that can have proinflammatory effects, following bMFC contusion (Grossman et al., 2004).

In addition to attenuating inflammation, PROG has also been shown to reduce oxidative stress in TBI. Levels of 8-isoPGF2 α, a marker of lipid peroxidation, are reduced with PROG administration following TBI (Roof et al., 1997). PROG also prevents a reduction in activity of the antioxidant glutathione reductase following bMFC contusion (VanLandingham et al., 2006).

Several other effects may also contribute to PROG neuroprotection following TBI. When administered as a sustained release implant in a unilateral parietotemporal contusion model, it reverses injury-induced alterations in mitochondrial respiration (Robertson et al., 2006). Multiple studies have shown a reduction in the proliferation and size of reactive astrocytes in both penetrating injury (Garcia-Estrada et al., 1993; Garcia-Estrada et al., 1999) and bMFC contusion (Djebaili et al., 2005). There is also an increase within the injured brain in the concentration of endothelial progenitor cells, CD34-positive cells, and CD31-positive cells, which are involved in angiogenesis and vasculogenesis (Li et al., 2012). Further, PROG maintains levels of the procoagulant factors thrombin, fibrinogen, and factor 13, and increases the binding of neuroserpin to tissue plasminogen activator (tPA), reducing its activity (VanLandingham et al., 2008). Administration of PROG has also been shown to decrease intracranial

pressure and increase cerebral perfusion pressure following diffuse TBI (Maghool et al., 2013; Shahrokhi et al., 2010).

PROG induces its neuroprotective effects through modulation of several receptor pathways. Progesterone-binding membrane protein 25-Dx was found to be upregulated in neurons and induced in astrocytes following TBI in structures involved in CSF production and osmoregulation, suggesting that it may play a role in maintenance of water homeostatis (Meffre et al., 2005). Additionally, following TBI there is induced expression of the membrane progesterone receptor mPRα in microglia, astrocytes, and oligodendrocytes, suggesting its role in neuroprotection (Meffre et al., 2013). The enantiomer of PROG does not activate progesterone receptors (PR), but does produce several of PROG's neuroprotective effects, indicating that other pathways independent of the PR are involved (VanLandingham et al., 2006). Evidence from He et al. suggests that modulation of GABA receptors is involved in PROG neuroprotection (He et al., 2004). In one study, expression of two Toll-like receptors, TLR2 and TLR4, was reduced with PROG administration after unilateral parietal contusion (Chen et al., 2008a). However, another study in the bMFC contusion model did not replicate this effect and instead, an increase in TLR9 was observed (Hua et al., 2011).

In addition to its actions at the site of the lesion, PROG exerts systemic effects on multiple organ systems when administered for TBI. One complication of TBI is acute mucosal injury of the gut. Studies by Chen et al. have shown that PROG ameliorates TBI-induced damage of gut mucosa, decreases NF-KB activity, reduces concentration of proinflammatory cytokines IL-1β and TNF-α, downregulates ICAM-1, and attenuates apoptosis in the gut following unilateral parietal contusion (Chen et al., 2007; Chen et al., 2008b).

2.4 Drug Administration

Many papers have examined the effects of varying the administration protocol of PROG after TBI. The majority of these studies have administered treatment at 1 and 6 hours post-injury, followed by daily treatment for a variable number of days. An experiment examining delays in administration found that initial treatment at 24 hours post-injury was still effective in reducing cerebral edema (Roof et al., 1996). Duration of treatment was examined in two reports comparing 3 days versus 5 days of treatment (Galani et al., 2001; Shear et al., 2002). They found that while both treatment durations were effective at reducing edema, size of necrotic lesion and cell loss, 5 days of treatment were more effective and were required to produce improvement in spatial learning performance and reduce sensory neglect.

Several vehicles have been effectively used to administer PROG for TBI treatment. Oil (Roof et al., 1994) and 22.5% 2-hydroxypropyl-β-cyclodextrin (HBC) (Goss et al., 2003) are the most commonly used and are injected subcutaneously (SC), intraperitoneally (IP), or intravenously (IV). DMSO has also been used (Jones et al., 2005). All are effective, with HBC requiring higher doses to produce similar effects (Goss et al., 2003). Extended-release Silastic capsules have also been used to administer PROG (Maghool et al., 2013; Robertson et al., 2006). The continuous release offered by these capsules has been shown to decrease anxiety and increase locomotor activity compared to the daily injection treatment regimen (Cutler et al., 2006a). Additionally, one study effectively administered PROG directly

to the lateral ventricle via a cannula connected to a minipump, using artificial CSF as the vehicle (Garcia-Estrada et al., 1999).

A direct comparison of doses was performed in a pair of reports from the Stein group comparing doses of 8, 16, and 32 mg/kg in cyclodextrin. Both 8 and 16 mg/kg doses improved performance on the MWM (Goss et al., 2003) and decreased expression of inflammatory cytokines (Cutler et al., 2007). However, only the 16 mg/kg dose reduced apoptosis at all time-points, decreased edema, and increased spontaneous motor activity (Cutler et al., 2007). Another study utilizing 10 and 20 mg/kg doses in a unilateral parietal contusion model demonstrated significant improvement in function and histologic outcomes with only the 10 mg/kg dose (Peterson et al., 2012). Other studies by the same group have looked at tapering doses, comparing sudden withdrawal after 7 days of 16 mg/kg treatment versus 5 days of 16 mg/kg followed by a taper of 8 mg/kg and 4 mg/kg on days 6 and 7 respectively. They found that tapering the dose results in reduced anxiety behaviors, sensory neglect, lesion size, reactive gliosis, and markers of apoptosis and inflammation, and increased locomotor activity and BDNF expression compared to sudden withdrawal (Cutler et al., 2005; Cutler et al., 2006b). Other experiments have shown that lower physiologic levels of PROG (1.67 mg/kg in oil) were also effective in reducing edema, BBB permeability, axonal injury, and caspase-3 activity in an impact-acceleration model of diffuse axonal injury (O'Connor et al., 2005; O'Connor et al., 2007). This lower dose also improved performance on rotarod and spatial learning in the Barnes maze tests (O'Connor et al., 2007).

The interaction of PROG with Vitamin D (VitD) has also been examined. When VitD-deficient rats were treated with PROG following TBI, they did not show the improvement in biochemical and behavioral parameters seen in VitD-sufficient rats. However, when rats received VitD supplementation combined with PROG they showed results similar to VitD-sufficient rats (Cekic et al., 2011). A subsequent study found that delivering VitD supplementation along with PROG in VitD-sufficient rats subjected to bMFC contusion improved spatial and reference memory and activated glial fibrillary acidic protein (GFAP) reactions compared to administration of PROG alone (Hua et al., 2012).

Other hormones related to PROG have shown benefit in the treatment of TBI, particularly its metabolite allopregnanolone (ALLO). He et al. initially showed that administration of ALLO resulted in improved MWM performance and decreased neuronal loss in the MDN and nucleus basalis magnocellularis (NBM) (He et al., 2004). Its isomer, epiallopregnanolone, did not produce the same effects, suggesting that GABA receptors are involved in PROG neuroprotection. Subsequent papers comparing several doses of ALLO to PROG found that while both were effective in reducing apoptosis, reactive gliosis and neuron loss, and in improving MWM performance, ALLO did so with greater potency, producing similar effects with a smaller dose (Djebaili et al., 2004; Djebaili et al., 2005). Studies have shown that ALLO produces other effects similar to those of PROG following TBI, reducing edema (VanLandingham et al., 2006), inflammation (Gonzalez et al., 2004; VanLandingham et al., 2006; VanLandingham et al., 2007), reactive gliosis (VanLandingham et al., 2006), and apoptosis (VanLandingham et al., 2006), and increasing antioxidant activity (VanLandingham et al., 2006). ALLO does have some effects that differentiate it from PROG. When its effect on the coagulation cascade was examined, rather than maintaining pro-coagulation factors as PROG does, it instead upregulated the anticoagulant tPA (VanLandingham et al., 2008). Unlike PROG, when administered after bMFC

contusion, ALLO has been found to decrease mitochondrial cytochrome c release, a mechanism involved in apoptosis (Sayeed et al., 2009). Medroxyprogesterone acetate (MPA) has also been administered in the bMFC contusion model. Although it did decrease edema, it produced no effect on MWM performance (Wright et al., 2008). Ciriza et al., also found in a kainic acid model of excitotoxicity that progesterone was neuroprotective, but MPA (Provera) was not. It should be noted that there are numerous synthetic products (progestins) available that have progesterone-like properties, but only natural progesterone has been adequately studied and shown to possess broad neuroprotective effects. (Wright et al., 2008; Ciriza et al. 2006; Singh, M. 2007)

3.0 Ischemia

3.1 Animal Models

Ischemia has been studied extensively using a number of animal models. By far the most utilized model has been middle cerebral artery occlusion (MCAO), usually by the insertion of an intraluminal filament. The common, internal, and external carotid arteries are exposed and the filament is advanced up the lumen of the ICA until it blocks the origin of the MCA, occluding blood flow. Several studies used this method to induce transient ischemia (tMCAO), placing the filament for a given amount of time and then removing it to restore blood flow (Alkayed et al., 2000; Atif et al., 2013; Atif F, 2012; Cai et al., 2008; Chen et al., 1999; Coomber and Gibson, 2010; Coughlan et al., 2009; Dang et al., 2011; Gibson and Murphy, 2004; Gibson et al., 2005; Gibson et al., 2011; Jiang et al., 1996; Kumon et al., 2000; Liu et al., 2012; Murphy et al., 2000; Murphy et al., 2002; Sayeed et al., 2006; Toung et al., 2004; Ulbrich et al., 2012; Zhang et al., 2010a). This model has also been induced in spontaneously hypertensive rats, which mimic a condition seen in many human patients suffering from strokes (Kumon et al., 2000). Others have left the filament in place until the endpoint of the study, modeling permanent ischemia (pMCAO) (Coughlan et al., 2005; Coughlan et al., 2009; Jiang et al., 2009; Jiang et al., 2011; Sayeed et al., 2007; Wang et al., 2010; Wang et al., 2011a). Permanent ischemia has also been induced in some studies by direct visualization and cauterization of the MCA (Betz and Coester, 1990b; Ishrat et al., 2009; Ishrat et al., 2010; Ishrat et al., 2012). Most of these studies have been performed in rats, but a few have executed the transient (Coughlan et al., 2009; Gibson and Murphy, 2004; Gibson et al., 2005; Gibson et al., 2011; Zhang et al., 2010a) and permanent (Coughlan et al., 2005; Coughlan et al., 2009) models in mice.

While the MCAO model produces a focal area of ischemia confined to the territory of the MCA, several other models have been developed to induce more global ischemia. Gonzalez-Vidal et al. used cardiopulmonary arrest to create an acute global cerebral ischemia model in cats (Cervantes et al., 2002; Gonzalez-Vidal et al., 1998). An electrical current was passed from an atrial wire to a subcutaneous electrode placed at the apex of the heart to induce ventricular fibrillation in anesthetized cats. Ventricular fibrillation was allowed to persist for 15 minutes, during which mechanical ventilation was ceased, and after which the cats were resuscitated. Global ischemia has also been induced in rats via a 4-vessel occlusion model (Morali et al., 2005; Morali et al., 2011a; Morali et al., 2011b; Ozacmak and

Sayan, 2009; Zhao et al., 2011) in which the vertebral arteries are permanently occluded via cauterization, while the common carotid arteries are transiently occluded for 15 minutes with microvascular clamps. Bilateral carotid artery occlusion (BCAO) has been used to induce partial global ischemia in mice (Aggarwal et al., 2008). Although this is not a true global ischemia, as the vertebral arteries are left patent, it produces an area of ischemia much larger than the MCAO model.

One study also looked at the effect of PROG on lipopolysaccharide (LPS) -induced cerebrovascular inflammation (Sunday et al., 2006). Although not a true ischemic model, cerebrovascular inflammation does play a central role in the pathogenesis of cerebral ischemia. LPS has also been administered in rats previously subjected to tMCAO to produce systemic inflammation, modeling post-stroke infection (Yousuf et al., 2012) Neonatal hypoxic-ischemic encephalopathy has also been modeled in rats (Li and Han, 2008; Li et al., 2013; Tsuji et al., 2012; Xu et al., 2010). This is executed by permanent ligation of the unilateral carotid artery followed by a several hour period of hypoxia. This model was performed in rat pups rather than adults.

3.2 Functional Outcomes

Several metrics have been used to evaluate the effects of PROG on neurologic function following ischemia. The Zea Longa test assigns a neurologic score based on observed neurologic deficits. Improvements in score have been seen with PROG administered post-MCAO in transient occlusion models (Chen et al., 1999; Jiang et al., 1996), including spontaneously hypertensive rats (Kumon et al., 2000), and pMCAO in aged rats (Wang et al., 2010).

The rotarod test assesses motor function by timing how long the animal can stay on a spinning, accelerating rotarod (Atif et al., 2013; Chen et al., 1999; Gibson and Murphy, 2004; Ishrat et al., 2009; Sayeed et al., 2007; Yousuf et al., 2012; Zhao et al., 2011). Motor function has also been tested using the foot fault test, which places the animals on an elevated grid and compares the number of foot faults made with the limb contralateral to the injury with the number made with the ipsilateral limb (Gibson and Murphy, 2004). Grip strength has also been used to assess motor function (Atif et al., 2013; Ishrat et al., 2009; Yousuf et al., 2012). Improvements in motor function with administration of PROG were found following pMCAO in young and aged rats (Ishrat et al., 2009; Sayeed et al., 2007; Wang et al., 2010), tMCAO in both rats (Atif et al., 2013; Chen et al., 1999) and mice (Gibson and Murphy, 2004; Gibson et al., 2011), and in the 4-vessel occlusion model (Zhao et al., 2011). Improvement was also seen with PROG administration in a post-stroke infection model (Yousuf et al., 2012).

Somatosensory deficits were evaluated with the adhesive-backed paper test, which timed how long it took for the rat to remove an adhesive-backed paper dot from its paw. Rats administered PROG following tMCAO had decreased latency to removal, indicating reduction of the somatosensory deficit (Atif et al., 2013; Chen et al., 1999). Dang et al. used a series of motor and sensory behavioral tests in rats treated with combined PROG and estrogen after tMCAO and found improved results (Dang et al., 2011; Ulbrich et al., 2012). The combination of PROG and VitD also improved motor and sensory outcomes to a greater degree than PROG alone (Atif et al., 2013).

As in trauma models, the MWM has been used to assess cognitive function following ischemic injury. Mice treated with PROG following tMCAO showed improved performance on the MWM compared to vehicle-treated controls (Gibson and Murphy, 2004). This finding was replicated in rats treated with PROG before tMCAO (Cai et al., 2008) and following 4-vessel occlusion (Morali et al., 2011b). The latter study also showed improvement on a 9-arm radial maze test, a measure of reference and working memory. Studies using the global ischemia model induced by cardiopulmonary arrest in cats assessed neurologic function in terms of a score which rated level of consciousness, respiration, cranial nerves, spinal reflexes, and postural, locomotor, and behavioral reactions (Cervantes et al., 2002; Gonzalez-Vidal et al., 1998). It was reported that PROG treatment improved neurologic function as measured by this score. PROG has also been shown to reduce susceptibility to pentylenetetrazole-induced post-ischemic seizures in a BCAO model (Aggarwal et al., 2008), and to attenuate weight loss and improve weight gain after tMCAO (Gibson and Murphy, 2004; Jiang et al., 1996; Kumon et al., 2000).

3.3 Pathophysiologic and Biochemical Mechanisms

A number of mechanisms may contribute to PROG neuroprotection in ischemic injury. As with TBI, PROG attenuates cerebral edema following ischemia. This has been observed after pMCAO in rats (Betz and Coester, 1990a; Betz and Coester, 1990b; Ishrat et al., 2012; Jiang et al., 2009) and both pMCAO and tMCAO in mice (Gibson et al., 2005). It has been replicated in aged rats after pMCAO (Wang et al., 2010). This decrease in edema is likely due in part to PROG's effect on BBB permeability. Although an early study did not show an effect on BBB permeability to sodium (Betz and Coester, 1990a; Betz and Coester, 1990b), later studies showed that PROG improved BBB integrity following pMCAO (Ishrat et al., 2010) and hypoxic-ischemic injury (Xu et al., 2010). The same study also showed that there was an increased expression of claudin5 and occludin1, proteins involved in tight junction formation, after PROG administration. The increase in claudin5 was replicated in another study using the same model (Jiang et al., 2009). Following pMCAO, PROG treatment also attenuates expression of matrix metalloproteinases (MMP) 9 and 2, enzymes involved in the degradation of junction proteins and change in BBB permeability (Ishrat et al., 2010). MMP-3 expression is also reduced by PROG following hypoxic-ischemic injury (Xu et al., 2010).

Several papers reported improvement in neuronal survival with PROG administration following ischemia. In the feline global ischemia model, neuronal loss in the caudate nucleus was prevented by PROG treatment (Cervantes et al., 2002). Improved survival with PROG has also been seen in the CA1 and CA2 regions of the hippocampus, with a decrease in cortical shrinking following 4-vessel occlusion (Morali et al., 2005). Increased survival in the CA1 region was replicated after tMCAO (Cai et al., 2008). Zhang et al. showed that PROG suppressed ischemia-stimulated proliferation of progenitor cells and improved survival of ischemia-induced newborn cells after tMCAO (Zhang et al., 2010a). Numerous studies have reported a reduction of infarct volume with PROG treatment for tMCAO in rats (Alkayed et al., 2000; Atif et al., 2013; Chen et al., 1999; Jiang et al., 1996; Murphy et al., 2002) and pMCAO in both mice (Gibson et al., 2005) and rats (Ishrat et al., 2009; Ishrat et al., 2010; Ishrat et al., 2012; Sayeed et al., 2007; Wang et al., 2011a). These findings have been replicated in spontaneously hypertensive rats after tMCAO (Kumon et al., 2000), aged rats following pMCAO (Wang et al., 2010), aged mice following tMCAO (Gibson et al., 2011), and in a post-stroke infection model (Yousuf et al., 2012). Combined PROG

and estrogen treatment (Dang et al., 2011; Ulbrich et al., 2012) and PROG and VitD treatment (Atif et al., 2013; Atif F, 2012) have both been shown to decrease lesion volume in tMCAO. The increased survival of neurons may be due to the effect of PROG on apoptosis. Ishrat et al showed that PROG attenuated apoptosis following pMCAO as measured by TUNEL assay (Ishrat et al., 2012) and reduced expression of caspase 3 and increased the pBad/Bad ratio, markers of apoptosis. It also increased the expression of the pro-survival trophic factor BDNF, contrary to earlier results (Coughlan et al., 2009). This was replicated in a post-stroke infection model (Yousuf et al., 2012). The combination of PROG and VitD also activated BDNF and its specific receptor tyrosine kinase receptor-B (Atif et al., 2013).

PROG has been shown to act on many of the same molecules associated with inflammation after ischemia as it does following trauma. It has been shown to attenuate expression of IL-1β (Gibson et al., 2005), TNF-α (Aggarwal et al., 2008; Ishrat et al., 2010; Jiang et al., 2009), IL-6 (Ishrat et al., 2010; Jiang et al., 2009), COX-2 (Jiang et al., 2011), and ICAM-1 (Wang et al., 2011a). However, one study showed no effect on TNF-α (Gibson et al., 2005). PROG also reduces expression of TGF-β2 (Gibson et al., 2005), VCAM-1 (Wang et al., 2011a), CD68 (Wang et al., 2011a), and Iba1 (Jiang et al., 2011) following ischemia, all factors associated with the inflammatory response. IL-6, CD68 and Iba1, as well as CD3, CCL2, and CCL5 were all found to be reduced following combined PROG/estrogen therapy for tMCAO (Dang et al., 2011). In the post-stroke infection model PROG reduced levels of IL-1β, IL-6, and TNF-α (Yousuf et al., 2012). Interestingly, in the LPS-induced vascular inflammation model, PROG administration was found to actually increase expression of COX-2, exacerbating the inflammatory response (Sunday et al., 2006).

PROG neuroprotection following ischemic injury may also be attributable to reduced oxidative stress. Administered following pMCAO in mice, PROG has been shown to reduce expression of inducible nitric oxide synthase (iNOS) and production of iNOS-derived nitric oxide (Coughlan et al., 2005). Aggarwal et al. found that PROG also increases levels of the antioxidants superoxide dismutase, catalase, and glutathione peroxidase when given following BCAO (Aggarwal et al., 2008). Ozacmak et al. demonstrated attenuation of the injury-induced decrease in glutathione and increase in malondialdehyde, an oxidative stress marker, with PROG treatment following 4-vessel occlusion (Ozacmak and Sayan, 2009). Expression of myeloperoxidase was reduced in PROG-treated rats following pMCAO (Wang et al., 2011a). However, as with inflammatory markers, expression of iNOS is increased when PROG is administered in the LPS-induced vascular inflammation model (Sunday et al., 2006).

PROG treatment after ischemia results in several other effects that may contribute to neuroprotection. An increase in growth-associated protein 43 (GAP43) and synaptophysin, both markers of synaptogenesis, was seen with PROG use following 4-vessel occlusion (Zhao et al., 2011). Morali et al. found that PROG preserves cytoarchitectural structure, including dendrite bifurcations and spinous structure, 4 months after global ischemia (Morali et al., 2011a). PROG also had a time-varying effect on VEGF expression, decreasing it at 24 to 72 hours and then increasing it at 14 days following pMCAO (Ishrat et al., 2012). However, with combined PROG and estrogen treatment for tMCAO, VEGF is instead upregulated at 24 hours, with no effect seen at 14 days (Dang et al., 2011; Ulbrich et al., 2012). The NMDA-induced rise in intracellular calcium concentration is also attenuated by PROG following pMCAO (Cai et al., 2008). In the hypoxic-ischemic model, PROG administration was actually found to worsen the

injury in neonatal rats up to 14 days old (Tsuji et al., 2012). In the same injury model, it also exacerbated an injury-induced increase in GLUT1 and GLUT3 (Li and Han, 2008; Li et al., 2013).

PROG exerts its neuroprotective effects following ischemia through several receptor pathways. Data from Cai et al. suggests that PROG exerts its effects via both early antagonization of the σ_1 receptor and delayed activation of the P4R-mediated Src-ERK signaling pathway (Cai et al., 2008). PROG has also been shown to modulate the PI3K/Akt pathway (Ishrat et al., 2012). Other studies have supported action through both the Src-ERK and PI3K pathways (Zhang et al., 2010a; Zhang et al., 2010b). Work by Liu et al. suggests neuroprotective action via both PR-dependent and independent mechanisms (Liu et al., 2012).

3.4 Drug Administration

Many of the early stroke reports examined the effect of PROG treatment prior to induction of ischemia (Alkayed et al., 2000; Betz and Coester, 1990a; Betz and Coester, 1990b; Cervantes et al., 2002; Coomber and Gibson, 2010; Jiang et al., 1996; Murphy et al., 2000; Murphy et al., 2002; Sunday et al., 2006; Toung et al., 2004; Zhao et al., 2011). Jiang et al. compared pretreatment with initiation of treatment 2 hours after injury (Jiang et al., 1996). They found similar reduction of ischemic cell damage and improvement of physiological and neurologic function with both regimens, suggesting that PROG may be useful in the management of stroke. The remaining papers examined treatment initiated after injury. Duration of treatment has varied greatly between laboratories, and few if any, studies have directly compared pre- and post-injury treatment.

DMSO, oil, saline, and β-cyclodextrin have all been used as vehicles to administer PROG either IP or SC with positive results. Results from an early paper suggested that saline was not as effective as DMSO (Jiang et al., 1996). However, others have shown neuroprotective effects (Chen et al., 1999; Morali et al., 2005). Extended-release implants placed 1 week pre-injury have also been used with mixed results. A subcutaneously implanted PROG pellet was able to reduce infarct volume following pMCAO (Alkayed et al., 2000). However, when subcutaneous extended-release tablets were used to treat tMCAO, there was no beneficial effect on neurologic score, edema, lesion volume, or AQP4 expression (Coomber and Gibson, 2010). Silastic capsules implanted 1 month pre-injury to administer PROG in an LPS-induced vascular inflammation model actually exacerbated the injury (Sunday et al., 2006).

PROG dosing regimens have been compared in a few papers. Chen et al. found that a moderate dose of 8 mg/kg reduced edema and improved functional outcome, while 4 and 32 mg/kg had no effect following tMCAO (Chen et al., 1999). Kumon et al. replicated those results, with 8 mg/kg producing significantly better outcomes than 4 mg/kg (Kumon et al., 2000). Another study compared single doses of 5/10/20 mg/kg pre-ischemia but found no significant effect in any group (Murphy et al., 2002). The same workers also looked at high doses (30 and 60 mg/kg) of PROG and found that they did not improve outcomes and may actually increase infarction volume in the caudate-putamen following tMCAO (Murphy et al., 2000). In a post-stroke infection model both 8 and 16 mg/kg produced similar functional and pathophysiologic outcomes (Yousuf et al., 2012)

ALLO has been utilized in a few experiments to treat ischemia. Like PROG, ALLO was shown to reduce infarction volume (Ishrat et al., 2010; Sayeed et al., 2006), improve BBB integrity (Ishrat et al.,

2010), and improve learning and memory (Morali et al., 2011b). As in TBI, results suggest that ALLO may be more potent than PROG when treating ischemia (Sayeed et al., 2006). ALLO was also shown to exacerbate hypoxic-ischemic injury in rat pups (Tsuji et al., 2012). Unlike PROG, ALLO has also been found to decrease mitochondrial cytochrome c release, a mechanism involved in apoptosis, when administered after tMCAO (Sayeed et al., 2009). MPA has also been studied in the LPS-induced vascular inflammation model and was found to exacerbate injury, like PROG (Sunday et al., 2006).

4.0 Spinal Cord Injury

4.1 Animal Models

Animal models for spinal cord injury (SCI) are more limited than those for TBI or ischemia; however, a few have been used to examine the effect of PROG. Some researchers have used blunt force to create SCI. Two studies created spinal cord contusion by removing the spinous processes and lamina and dropping an impactor onto the exposed dura (Fee et al., 2007; Thomas et al., 1999). SCI has also been induced via transient application of aneurysm clips to the exposed spinal cord in the thoracic region after exposure via laminectomy (Sahin et al., 2011; Topsakal et al., 2002).

Other studies examining PROG treatment for SCI utilized penetrating injuries. Several have used a spinal cord transection model in which a laminectomy is performed and the spinal cord is completely transected at the T10 level using the sharp edge of a needle (De Nicola et al., 2006; Gonzalez et al., 2004; Gonzalez et al., 2005; Gonzalez et al., 2009; Labombarda et al., 2000; Labombarda et al., 2002; Labombarda et al., 2003; Labombarda et al., 2006; Labombarda et al., 2009; Labombarda et al., 2011). Spinal cord hemisection at the T13 level has also been as a model for PROG treatment (Coronel et al., 2011b). Here, hemisection was ensured by excluding animals that showed bilateral symptoms of injury. Finally, in one study spinal cord ischemia was induced in rabbits via transient clamping of the abdominal aorta inferior to the left renal artery for 30 minutes (Vandenberk et al., 2012).

4.2 Functional Outcomes

The evidence for functional improvement with PROG treatment following SCI is limited, as most reports have not evaluated functional outcomes. Thomas et al. used the Basso, Beattie, and Bresnehan score to evaluate locomotor function in the hindlimbs, showing that PROG treatment improved scores following spinal cord contusion (Thomas et al., 1999). However, another paper showed no improvement in scores in a similar injury model (Fee et al., 2007). In a hemisection model, PROG prevented development of mechanical allodyna and reduced the painful response to cold stimulation in the affected hindlimb (Coronel et al., 2011b).

4.3 Pathophysiologic and Biochemical Mechanisms

The literature examining mechanisms of PROG neuroprotection following SCI is more extensive than reports of functional outcomes. As in TBI and ischemia models, PROG has been shown to decrease neuronal injury following SCI. An early report demonstrated that PROG spared white matter tissue at the

epicenter of the injury after spinal cord contusion (Thomas et al., 1999). Studies in spinal cord transection models have shown that chromatolysis, a sign of neuron degeneration following injury, is reduced in spinal neurons after PROG treatment (De Nicola et al., 2006; Gonzalez et al., 2005; Gonzalez et al., 2009). Other morphologic changes indicative of injury prevention with PROG include preservation of rough endoplasmic reticulum and prevention of nucleoplasm dispersion and nuclear eccentricity (Gonzalez et al., 2009). The same experimenters also showed attenuation of the injury-induced loss of microtubule-associated protein 2, indicating preservation of the cytoskeleton. Neuronal survival is also preserved with administration of PROG in a rabbit spinal cord ischemia model (Vandenberk et al., 2012).

Much of the research on PROG effects in SCI has looked at myelination. PROG has been shown by several studies to increase proliferation of oligodendrocyte precursors early after spinal cord transection (De Nicola et al., 2006; Labombarda et al., 2006; Labombarda et al., 2009; Labombarda et al., 2011). While the number of mature oligodendrocytes remained unchanged by PROG at 3 days (Labombarda et al., 2006), subsequent studies found it to be increased at 21 days compared to controls (Labombarda et al., 2009; Labombarda et al., 2011). The transcription factors Olig2 and Nkx2.2, involved in oligodendrocyte differentiation, were both increased by PROG 3 days post-injury, while Olig1, involved in myelin repair, was increased at 21 days (Labombarda et al., 2009). PROG also affected proteins associated with myelination, increasing levels of myelin basic protein (MBP) (De Nicola et al., 2006; Labombarda et al., 2006) in the short term and proteolipid protein (PLP) in the long term (Labombarda et al., 2009). However, it had no effect on myelin oligodendrocyte glycoprotein (MOG) at either time point (Labombarda et al., 2009).

Several other mechanisms may contribute to PROG's effects after SCI. It has been shown to act on the inflammatory response, inhibiting astrocyte and microglial activation and proliferation (Labombarda et al., 2011). However, an earlier paper showed PROG produced no change in GFAP-positive astrocytes and increased NADPH-diaphorase active astrocytes after transection (Labombarda et al., 2000). The difference may be attributable to the dosing, as the earlier study utilized only 4 mg/kg while the later study used 16 mg/kg. A number of studies have measured an increase in expression of the trophic factor BDNF with PROG treatment after SCI (De Nicola et al., 2006; Gonzalez et al., 2004; Gonzalez et al., 2005). This corresponded with an increase in phosphorylated cAMP-responsive element binding (pCREB) in motorneurons (De Nicola et al., 2006). PROG has been shown to attenuate the decreased expression of choline acetyltransferase (ChAT) and Na,K-ATPase subunits induced by spinal cord transection (Labombarda et al., 2002). This may replenish acetylcholine and restore membrane potential, ion transport, and nutrient uptake. The same report also showed that PROG enhances the injury-induced increase in GAP43.

A few studies have examined the receptor pathways regulated by PROG in SCI. It has been shown to upregulate the expression of the PROG binding protein 25-Dx, but not the classical intracellular PROG receptor (Labombarda et al., 2003). Another study demonstrated a reduction in NMDA receptor subunits and the gamma isoform of protein kinase C (PKC), as well as an increase in expression of the □ opioid receptor (KOR) with PROG administration after spinal cord hemisection (Coronel et al., 2011b). These are all key players in chronic pain mechanisms.

4.4 Drug Administration

PROG administration for SCI has followed protocols from TBI and ischemia studies. Generally, a dose is given soon after injury followed by daily doses. The doses used have ranged from 4-16 mg/kg administered IP or SC with either oil or DMSO as the vehicle. Two studies did examine the use of MPA for SCI. They found that it decreased TNF-α (Sahin et al., 2011) and had positive effects on oxidative stress following clip compression injury (Topsakal et al., 2002).

5.0 Peripheral Nerve Injury

5.1 Animal Models

Several models have been used to study the effect of PROG on peripheral nerve injury. Yu et al. transected both the hypoglossal and facial nerves to study PROGs effects (Yu, 1989). Other studies have injured the sciatic nerve using cryolesion (Koenig et al., 1995), crush injury by transient clamp compression (Roglio et al., 2008), cuff application (Dableh and Henry, 2011), or single ligature nerve constriction (Coronel et al., 2011a). Docetaxel has also been infused intravenously to produce a peripheral neuropathy (Roglio et al., 2009). Additionally, diabetic neuropathy has been modeled via streptozotocin (STZ) -induced injury (Leonelli et al., 2007; Pesaresi et al., 2010).

5.2 Functional Outcomes

Studies on functional outcome after peripheral nerve injury suggest that PROG improves sensory function but not motor function. Roglio et al. showed that PROG attenuated injury-induced thermal hypoalgesia after nerve crush injury (Roglio et al., 2008), docetaxel-induced neuropathy (Roglio et al., 2009), and STZ-induced neuropathy (Leonelli et al., 2007). The latter study, however, showed no effect on sciatic functional index, a measure of motor function in the injured limb. Two studies have shown improvement in injury-induced allodynia with PROG treatment (Coronel et al., 2011a; Dableh and Henry, 2011). It has also been shown to reduce painful responses to cold stimulation (Coronel et al., 2011a).

5.3 Pathophysiologic and Biochemical Mechanisms

As with the previously mentioned neurologic diseases, following peripheral nerve injury PROG appears to exert its neuroprotective effects via multiple pathways. It has been shown to improve neuronal survival after axotomy (Yu, 1989). It also effects myelination of diseased peripheral nerves, stimulating myelin formation after cryolesion of the sciatic nerve (Koenig et al., 1995) and reducing myelin fiber morphological abnormalities and myelin fiber loss in the uninjured sciatic nerves of aged rats (Azcoitia et al., 2003). The effect of PROG on myelination is further supported by increases in the myelin proteins G0, PMP22, and MAL with PROG administration after sciatic nerve injury (Roglio et al., 2008) and STZ-induced neuropathy (Leonelli et al., 2007). The same studies also demonstrated that PROG intensifies the injury-induced increase in the ECM protein reelin and attenuates the increase in Na,K-ATPase activity. Another study found that PROG upregulates MBP in the spinal cord in a diabetic

neuropathy model (Pesaresi et al., 2010). PROG has also been shown to attenuate the decrease in nerve conduction velocity, prevent the degeneration of skin nerves, and restore the expression of calcitonin gene-related peptide following docetaxel-induced neuropathy (Roglio et al., 2009). Nerve conduction velocity and skin innervation are also preserved by PROG after STZ-induced neuropathy (Leonelli et al., 2007). A reduction in expression of NMDA receptor subunit 1, its phosphorylated form, and the gamma isoform of protein kinase C, all key players in central sensitization, were seen following PROG treatment for sciatic nerve injury (Coronel et al., 2011a).

5.4 Drug Administration

Administration of PROG following peripheral nerve injury has typically been via subcutaneous injection, although one study did administer it directly to the injury site (Koenig et al., 1995). Frequency has ranged from daily to weekly, with no direct comparison studies. The duration of treatment was examined by Dableh et al., who showed that 10 days of treatment produced superior functional results compared to 4 days of treatment (Dableh and Henry, 2011). The same study also found that delaying treatment for 20 days resulted in no effect of PROG on functional outcome. Doses have been similar to other disease models, ranging from approximately 3.3 mg/kg (1 mg total) to 16 mg/kg, all producing beneficial results. Dihydroprogesterone, a metabolite of PROG, has also been administered in peripheral nerve injury showing similar results to PROG (Azcoitia et al., 2003; Leonelli et al., 2007; Roglio et al., 2008; Roglio et al., 2009).

6.0 Demyelinating Disease

6.1 Animal Models

The effects of PROG on demyelinating diseases such as multiple sclerosis (MS) have been studied using a few different animal models. Demyelination of areas of either the central or peripheral nervous system have been induced by localized injection of the toxins ethidium bromide (Ibanez et al., 2004), lysophospatidylcholine (LPC) (Garay et al., 2011), and lysophosphatidic acid (LPA) (Kim et al., 2012). More diffuse demyelination is seen in the experimental autoimmune encephalitis (EAE) model (Garay et al., 2007; Garay et al., 2008; Garay et al., 2009; Garay et al., 2012; Giatti et al., 2012; Yates et al., 2010; Yu et al., 2010), in which an autoimmune reaction is induced by subcutaneous inoculation of myelin proteins in adjuvant. This produces a similar disease process to MS.

6.2 Functional Outcomes

A disease score based on neurologic behavior is used to grade the severity of EAE. Multiple studies have shown that PROG reduces both the cumulative disease index (a measure of disease score over time) and peak disease score in EAE (Garay et al., 2007; Garay et al., 2008; Garay et al., 2009; Giatti et al., 2012; Yates et al., 2010; Yu et al., 2010). Studies have also shown a delay in the onset of EAE with PROG treatment (Garay et al., 2007; Garay et al., 2008). Additionally, PROG reduced the development of allodynia in LPA-induced trigeminal nerve demyelination (Kim et al., 2012).

6.3 Pathophysiologic and Biochemical Mechanisms

A number of laboratories have looked at the ability of PROG to prevent demyelination and promote remyelination in demyelinating diseases. In some cases there is increased total myelin via Luxol Fast Blue staining with PROG treatment in EAE (Garay et al., 2007; Garay et al., 2008; Yu et al., 2010), LPC-induced (Garay et al., 2011) and LPA-induced (Kim et al., 2012) demyelination models. In another case, PROG increased the rate of remyelination in aged rats following ethidium bromide-induced demyelination (Ibanez et al., 2004). The same effect was not seen in young rats, despite their much higher baseline rate of remyelination compared to the aged rats. PROG has also been shown to increase the expression of proteins associated with myelin production, including MBP, PLP, P0, and PMP22, following EAE (Garay et al., 2007; Garay et al., 2008; Garay et al., 2012; Giatti et al., 2012), LPC-induced demyelination (Garay et al., 2011), and LPA-induced demyelination (Kim et al., 2012). Expression of Olig1 and NKx2.2, transcription factors associated with myelination, is increased with PROG administration in EAE (Garay et al., 2012; Yu et al., 2010). Following LPC-induced demyelination, PROG has been shown to increase the number of oligodendrocyte precursors and mature oligodendrocytes (Garay et al., 2011).

PROG has also been shown to exert its neuroprotective effects on demyelinating disease by modulating the inflammatory response. Treatments will have been shown to reduce the expression of the pro-inflammatory factors IL-2, IL-17 (Yates et al., 2010), IL-1β, TGF-β1 (Giatti et al., 2012), and TNF-α and its receptor TNFR1 (Garay et al., 2012). PROG also down-regulated the expression of the chemokine receptors CCR2, CCR7, and MIP-2/CXCL2 in EAE (Yates et al., 2010). These studies have also demonstrated decreased inflammatory cell infiltration into the spinal cord with PROG treatment of EAE (Garay et al., 2007; Yates et al., 2010). The microglial/macrophage response is attenuated by PROG following LPC-induced demyelination (Garay et al., 2011). A similar response was seen in EAE, where the Iba+ microglial cells were suppressed, while CC1+ oligodendrocytes were enhanced by PROG (Garay et al., 2012). Additionally, PROG administered in EAE mice has been shown to up-regulate the expression of the anti-inflammatory factor IL-10 (Yates et al., 2010). MHC-II immunoreactivity is also reduced by PROG in EAE (Giatti et al., 2012).

PROG has been shown to have direct effects on axons in EAE, increasing the axonal counts and proportion of small diameter axons (Garay et al., 2009). The same paper also showed a PROG-induced decrease in amyloid precursor protein (APP) and GAP43, markers of axonal damage and aberrant axonal sprouting respectively. Results from a report by Garay et al. also showed increased expression of Na,K-ATPase in motorneurons with PROG administration for EAE (Garay et al., 2007; Garay et al., 2008). Although this was not replicated in a subsequent paper in a similar model, there was an increase in activity of the Na,K-ATPase pump (Giatti et al., 2012). Neuronal expression of BDNF is also increased with PROG treatment for EAE (Garay et al., 2008).

6.4 Drug Administration

Several experiments examining demyelinating disease have administered PROG via an extended release implant, administered either pre-injury (Garay et al., 2007; Garay et al., 2009; Garay et al., 2011;

Garay et al., 2012), at the time of injury induction (Ibanez et al., 2004), or at the time of symptom onset (Yates et al., 2010), all showing neuroprotective effects. Garay et al. compared 20 mg PROG tablets versus 100 mg tablets, finding similar results between groups (Garay et al., 2007). PROG has also been administered via IP and SC injection for treatment of demyelinating disease with positive results (Giatti et al., 2012; Kim et al., 2012; Yu et al., 2010). 16 mg/kg doses were found to be superior to 8 mg/kg doses when administered SC following LPA-induced demyelination (Giatti et al., 2012; Kim et al., 2012; Yu et al., 2010).

7.0 Motorneuron Disease

7.1 Animal Models

Research on the use of PROG for motorneuron disease has been done exclusively in Wobbler mice (Deniselle et al., 2012; Gonzalez Deniselle et al., 2002a; Gonzalez Deniselle et al., 2002b; Gonzalez Deniselle et al., 2004; Gonzalez Deniselle et al., 2005; Meyer et al., 2010; Meyer et al., 2013). The Wobbler mouse has a mutation of the autosomal recessive wr gene that results in motorneuron degeneration in the spinal cord and brain stem. This produces a disease process similar to the human motorneuron diseases amyotrophic lateral sclerosis (ALS) and infantile spinal muscular atrophy (Werdenig-Hoffman disease).

7.2 Functional Outcomes

PROG has shown beneficial functional effects in motorneuron disease. Studies from Gonzalez Deniselle et al. demonstrated improved motor outcomes, with Wobbler mice receiving PROG showing improve grip strength compared to controls (Gonzalez Deniselle et al., 2002a; Gonzalez Deniselle et al., 2002b; Gonzalez Deniselle et al., 2005). Survival was also improved in mice receiving PROG (Gonzalez Deniselle et al., 2002a; Gonzalez Deniselle et al., 2002b).

7.3 Pathophysiologic and Biochemical Mechanisms

The neuroprotective effects of PROG on motorneuron disease appear to be numerous. A number of reports have shown that PROG reduces the cellular vacuolation and mitochondrial ultrastructure breakdown that is characteristic of Wobbler mouse motorneuron degeneration (Gonzalez Deniselle et al., 2002a; Gonzalez Deniselle et al., 2002b; Gonzalez Deniselle et al., 2004; Meyer et al., 2010). These results were not seen with administration in mice with late stage disease (Meyer et al., 2010). The expression of GAP-43, a marker of aberrant axonal sprouting, is decreased following PROG administration in early and progressive but not late stages of disease (Gonzalez Deniselle et al., 2002b; Meyer et al., 2010). PROG has also been shown to decrease APP accumulation, a sign of axonal damage (Deniselle et al., 2012). The reduced degeneration has been shown to lead to preservation of muscle weight (Gonzalez Deniselle et al., 2005).

PROG's effects on astrocytosis in Wobbler mice have been conflicting. One paper showed no increase in astrocytosis as determined by GFAP staining (Gonzalez Deniselle et al., 2002b). However, a

later study observed a decrease in GFAP-positive astrocytes with PROG administration in all stages of disease (Meyer et al., 2010). Results also show that PROG affects oxidative stress. The number of NADPHD-active astrocytes and motorneurons, a measure of NOS activity, is reduced by PROG in less advanced stages of disease (Gonzalez Deniselle et al., 2004). Another report found that PROG prevents an increase in mitochondrial nNOS, normalizes the activity of the mitochondrial respiratory complex I, and increases expression of the antioxidant superoxide dismutase (SOD) (Deniselle et al., 2012). Other effects of PROG in Wobbler mice include an increase in expression of Na,K-ATPase (Gonzalez Deniselle et al., 2002a; Gonzalez Deniselle et al., 2002b), an enhancement of retrograde axonal transport (Gonzalez Deniselle et al., 2005), enhanced ChAT expression, and an increase in the number of glutamine synthetase-positive cells (Meyer et al., 2010).

PROG has also been shown to act in the hippocampus of Wobbler mice, increasing BDNF expression, decreasing glial fibrillary acidic protein-positive astrocytes, and increasing the number a GABAergic interneurons and granule cells (Meyer et al., 2013).

7.4 Drug Administration

All studies in PROG in Wobbler mice utilized 20-mg extended-release pellets implanted for at least 15 days up to 8 weeks.

8.0 Seizures

8.1 Animal Models

Extensive research has been done evaluating the effect of PROG on seizures. The majority of studies have used toxin-induced seizure models in either mice or rats. Toxins administered have included kainic acid (Frye and Bayon, 1999; Frye and Scalise, 2000; Frye and Walf, 2011; Hoffman et al., 2003; Kokate et al., 1996; Nicoletti et al., 1985), bicuculline (Belelli et al., 1989; Czlonkowska et al., 2000), metrazol (Belelli et al., 1989), picrotoxin (Belelli et al., 1989; Czlonkowska et al., 2000; Singh et al., 2010), strychnine (Belelli et al., 1989), pilocarpine (Kokate et al., 1996; Valente et al., 2008), pentylenetetrazol (Akula et al., 2009; Beckley et al., 2008; Frye et al., 2000; Frye and Muscatiello, 2001; Frye et al., 2002; Frye and Rhodes, 2005; Gholipour et al., 2008; Gililland-Kaufman et al., 2008; Kokate et al., 1999; Nasir et al., 2012; Pence et al., 2008; Reddy and Rogawski, 2001; Reddy et al., 2004; Rhodes and Frye, 2004; Rhodes and Frye, 2005a; Rhodes and Frye, 2005b), flurothyl (Velisek et al., 1999), and NMDA (Czlonkowska et al., 2000). Other studies have used electrical stimulation to induce seizures. Electrodes have been implanted into the perforant pathway to produce acute seizures (Frye, 1995; Frye and Bayon, 1999; Frye and Scalise, 2000), and into the amygdala and hippocampus to produce kindled seizures (Lonsdale and Burnham, 2003; Lonsdale et al., 2006; Lonsdale and Burnham, 2007; Mohammad et al., 1998; Reddy et al., 2004; Reddy et al., 2010; Samba Reddy and Ramanathan, 2012). The maximal electroshock model utilizes corneal electrodes to administer the electrical stimulus in mice (Belelli et al., 1989; Kokate et al., 1999; Reddy et al., 2004). Corneal electrodes have also been used to administer low-

frequency stimulation (Kaminski et al., 2004). A similar method has been used to induce focal epileptic seizure in the visual cortex of cats (Tauboll and Lindstrom, 1993).

8.2 Functional Outcomes

PROG can produce anti-seizure effects in several rodent models including kainic acid (Frye and Bayon, 1999; Frye and Scalise, 2000; Hoffman et al., 2003; Nicoletti et al., 1985), pentylenetetrazol (Akula et al., 2009; Frye and Muscatiello, 2001; Frye et al., 2002; Frye and Rhodes, 2005; Gholipour et al., 2008; Kokate et al., 1999; Reddy et al., 2004; Rhodes and Frye, 2004; Rhodes and Frye, 2005a; Rhodes and Frye, 2005b), and picrotoxin (Singh et al., 2010) -induced maximal electroshock (Kokate et al., 1999; Reddy et al., 2004), perforant pathway stimulation (Frye and Bayon, 1999; Frye and Scalise, 2000), amygdala kindled (Lonsdale and Burnham, 2003; Lonsdale et al., 2006; Mohammad et al., 1998; Reddy et al., 2004), and hippocampus kindled models (Samba Reddy and Ramanathan, 2012). Effects noted include increased threshold, increased latency to onset, decreased incidence, and decreased duration of seizures. The seizure threshold was also raised by PROG in the cat visual cortex focal seizure model (Tauboll and Lindstrom, 1993). However, no effect was seen on flurothyl-induced seizures in rats (Velisek et al., 1999). In addition to the anti-seizure effects, PROG was also found to improve MWM performance when administered before kainic acid-induced seizures in rats (Frye and Walf, 2011).

8.3 Pathophysiologic and Biochemical Mechanisms

A number of mechanisms appear to contribute to the anti-seizure effects of PROG. Neuronal survival in the hippocampus is improved by PROG administration before kainic acid-induced seizures (Hoffman et al., 2003). In the picrotoxin model, PROG will decrease DNA fragmentation, but had no effect on lipid peroxidation (Singh et al., 2010). PROG may play a role in the nitrous oxide signaling pathway (Gholipour et al., 2008). When administered with the NO precursor L-arginine its effects on pentylenetetrazol seizures were eliminated. Conversely, when administered with the NO synthase inhibitor Nω-nitro-L-arginine methyl ester, PROG effects were amplified. In the cat focal seizure model PROG was found to reduce presynaptic nerve volleys, reduce postsynaptic excitatory field potentials, and enhance postsynaptic inhibition (Tauboll and Lindstrom, 1993). PROG has also been shown to decrease sialic acid (Pence et al., 2008) and adenosine deaminase levels (Pence et al., 2009), which may contribute to the anti-seizure effects.

Evidence suggests that PROG exerts its anti-seizure effects primarily through modulation of $GABA_A$ receptors. Studies have shown that PROG's effects are blocked by the $GABA_A$ antagonist bicuculline (Mohammad et al., 1998; Rhodes and Frye, 2005b). Attenuation of the effects was also seen with administration of 5α-reductase inhibitors such as finasteride (Frye and Scalise, 2000; Kokate et al., 1999; Rhodes and Frye, 2005a; Singh et al., 2010). 5α-reductase is involved in the conversion of PROG to ALLO, which is a potent $GABA_A$ modulator. This idea is further supported by data showing that PROG failed to produce an effect on pentylenetetrazol-induced seizures in 5α-reductase deficient mice (Frye et al., 2002). PROG also was shown to increase GABA-stimulated chloride flux (Rhodes and Frye, 2005b). Also consistent with $GABA_A$ modulation, in PR knockout mice the anticonvulsant effects of PROG were

undiminished in pentylenetetrazol, maximal electroshock, and amygdala kindled models (Reddy et al., 2004).

8.4 Drug Administration

PROG will generally afford dose-dependent protection in seizure models, with higher doses producing greater anticonvulsant effects. Maximal doses were limited by sedative effects of the drug. Reported ED50 values range from 78.3 mg/kg (Singh et al., 2010) to 114 mg/kg (Lonsdale et al., 2006), although there are anti-seizure effects in doses as low as 4 mg/kg (Frye and Scalise, 2000) or 500 mcg total (Frye and Rhodes, 2005; Rhodes and Frye, 2005a; Rhodes and Frye, 2005b) in some models. Administration has generally been via either IP or SC injection before seizure induction. However, implanted extended release PROG capsules (Frye and Bayon, 1999; Hoffman et al., 2003) have also been used. Others have administered PROG directly to the ventricles (Czlonkowska et al., 2000), pontine reticular formation (Frye et al., 2000), raphe magnus (Frye and Muscatiello, 2001), and hippocampus (Gililland-Kaufman et al., 2008; Rhodes and Frye, 2004) with successful results.

Given the apparent action of PROG through the $GABA_A$ receptor, a number of groups have examined the effect of the metabolite ALLO, a potent $GABA_A$ modulator. Like PROG, ALLO has been shown to provide seizure protection in bicuculline (Belelli et al., 1989; Czlonkowska et al., 2000), metrazol (Belelli et al., 1989), picrotoxin (Belelli et al., 1989; Czlonkowska et al., 2000; Singh et al., 2010), kainic acid (Frye and Scalise, 2000; Kokate et al., 1996), pilocarpine (Kokate et al., 1996), pentylenetetrazol (Beckley et al., 2008; Frye et al., 2000; Frye and Muscatiello, 2001; Gililland-Kaufman et al., 2008; Kokate et al., 1999; Reddy and Rogawski, 2001; Rhodes and Frye, 2004), perforant pathway electrical stimulation (Frye, 1995; Frye and Scalise, 2000), low-frequency corneal electrical stimulation (Kaminski et al., 2004), amygdala-kindled seizure (Lonsdale et al., 2006; Lonsdale and Burnham, 2007), and cat focal seizure models (Tauboll and Lindstrom, 1993). The potency of ALLO in reducing seizure activity is higher than PROG's, with ED50s reported in the 1.1 mg/kg (Lonsdale and Burnham, 2007) to 15.2 mg/kg (Lonsdale et al., 2006) range. ALLO did not show an effect in strychnine (Belelli et al., 1989), maximal electroshock (Belelli et al., 1989), and NMDA (Czlonkowska et al., 2000) models. It did, however, improve MWM performance after perforant pathway stimulation-induced seizures (Frye, 1995).

The synthetic progestin, MPA, has also been assessed for seizure control. This progestin protects against kainic acid-induced (Nicoletti et al., 1985) pilocarpine-induced seizures (Valente et al., 2008). Another study demonstrated MPA protection against pentylenetetrazol-induced kindling (Nasir et al., 2012). Mifepristone, a PROG receptor antagonist, had no effect on MPA's actions in this model. MPA also reversed deficits in grip strength and spontaneous alternation behavior induced by the kindling (Nasser et al. 2012).

9.0 Alzheimer's Disease

9.1 Animal Models

Progesterone and its metabolites have also been evaluated for the treatment of Alzheimer's Disease (AD). Transgenic mice have been utilized in several studies to model AD. Some studies have utilized mice that co-overexpress a mutant form of amyloid precursor protein and a deletion in presenilin 1 Δ exon 9 (APPswe+PSED1Δe9) (Bengtsson et al., 2012; Frye and Walf, 2008; Frye and Walf, 2009). Others used a triple transgenic mouse model (3xTg-AD), with PS1(M146V), APP(Swe), and tau(P301L) transgenes (Carroll et al., 2007; Carroll et al., 2010; Chen et al., 2011; Singh et al., 2012; Sun et al., 2012; Wang et al., 2010).

9.2 Functional Outcomes

Progesterone improved spontaneous alternation behavior in APPswe+PSED1Δe9 mice performing a T-maze test (Frye and Walf, 2008). However, it failed to produce an effect in 3xTG-AD mice in a similar Y-maze test (Carroll et al., 2007). It also improved performance of an object recognition task in APPswe+PSED1Δe9 mice. However, it failed to produce effects in either object placement or water maze tasks (Frye and Walf, 2008). Another study demonstrated that PROG reduced depression-like behavior, as measured by a forced swim test, in APPswe+PSED1Δe9 mice (Frye and Walf, 2009).

9.3 Pathophysiologic and Biochemical Mechanisms

Progesterone appears to have multiple effects on the pathophysiology of AD murine models. Administration of PROG increased progestin levels in the cortex, diencephalon, midbrain, and cerebellum, but not the hippocampus, of APPswe+PSED1Δe9 mice (Frye and Walf, 2008). Studies have shown it to reduce tau hyperphosphorylation in 3xTg-AD mice. In the same model, when given continuously it blocked the reduction of amyloid β (Aβ) accumulation induced by estrogen administration (Carroll et al., 2007). However, when administered cyclically, it reduced Aβ accumulation and enhanced estrogen's beneficial effects (Carroll et al., 2010).

9.4 Drug Administration

A few studies have looked at variation in administration of PROG. One study comparing continuous to cyclical release showed differing effects on Aβ accumulation, as previously mentioned (Carroll et al., 2010). In another study comparing dosing regimens, 1 dose/week/6 months was found to be superior to either a 1/month single dose or 3 doses/week/3 month regimen in terms of optimizing regenerative efficacy and reducing Aβ pathology (Chen et al., 2011). The same study showed the effects to be maximized when treatment was initiated prior to the development of pathology.

Several studies have examined the use of ALLO in AD. In 3xTg-AD mice, acute administration of ALLO prior to development of pathology has been shown to increase memory and learning, measured via a hippocampal-dependent trace eye-blink conditioning paradigm. This corresponded with increased neural progenitor cell proliferation in the hippocampal subgranular zone (Wang et al., 2010). These results were later replicated with administration after the development of intraneuronal Aβ plaques. However, ALLO was ineffective in the presence of extraneuronal plaques (Singh et al., 2012). Another study in the same model demonstrated increased survival of newly generated neurons, reduction of Aβ generation, decreased expression of Aβ-binding-alcohol-dehydrogenase, reduced microglial activation,

and increased expression of proteins involved in cholesterol homeostasis and clearance from the brain (liver-X-receptor, pregnane-X-receptor, and 3-hydroxy-3- methyl-glutaryl-CoA-reductase) (Chen et al., 2011). This study demonstrated these effects with a 1 dose/week regimen. Conversely, when ALLO was administered continuously over 12 weeks at a constant rate via osmotic pump to APPswe+PSED1Δe9 mice it instead impaired learning in a MWM and increased levels of Aβ (Bengtsson et al., 2012). ALLO was also found to prevent loss of total and tyrosine hydroxylase positive neurons in the substantia nigra pars compacta of 3xTg-AD mice (Sun et al., 2012).

10.0 Other Neurologic Diseases

More limited work has been done on PROG use in a number of other neurologic diseases. Limmroth et al. studied the effect of PROG on meningeal edema (Limmroth et al., 1996). Edema was generated via electrical stimulation of the trigeminal ganglion. PROG and its metabolites (ALLO, tetrahydroxydeoxycorticosterone, and synthetic alphaxalone) were found to reduce plasma extravasation via $GABA_A$ receptors. They speculated that PROG may therefore be clinically effective in the treatment of migraine and cluster headache.

The effect of PROG on subarachnoid hemorrhage (SAH) was examined by Wang et al. (Wang et al., 2011b). Autologous blood was injected directly into the prechiasmatic cistern to create the injury. Following injury, PROG was found to improve behavior and activity scores. Mechanisms found behind the observed neuroprotection include reduced edema, reduced BBB permeability, and decreased expression of molecules associated with inflammation including IL-1β, TNF-α, IL-6, MCP-1, ICAM-1, NF-κB, and TLR4.

PROG treatment has also been studied in intracerebral hemorrhage (ICH) (Chen et al., 2011), similar to SAH. Injury was induced by injecting autologous blood into the right basal ganglia. Rats receiving PROG had improved functional outcome, as measured by forelimb placing score and decreased perihematomal edema.

Organophosphorus pesticides are known to adversely affect memory and induced oxidative stress in those who are acutely or chronically exposed to these agents. Rats exposed to phosphamidon display a reduction in step-down latency on a passive avoidance apparatus and a prolongation of transfer latency on an elevated plus maze. These effects were antagonized with PROG administration (Sharma et al., 2011). PROG was also found to reduce oxidative stress, decreasing levels of thiobarbituric acid reactive species and increasing non-protein thiols.

A few laboratories have looked at how PROG affects parkinsonism. One report showed that in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism, PROG prevents the injury-induced reduction in striatal dopamine, its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and dopamine transporter binding (Callier et al., 2001; Grandbois et al., 2000). PROG also prevents the reduction in striatal dopamine and serotonin seen with methamphetamine administration (Yu and Liao, 2000). In these studies, PROG was administered pre-injury. Hemi-

parkinsonism has been induced in rats via unilateral injection of 6-hydroxydopamine into the corpus striatum. PROG has had mixed results in this model. Casas et al., showed that PROG prevented rotational contralateral behavior, reduced memory deficit in an appetitive task, and reduced depression-like behavior (Casas et al., 2011). However, Chao et al. found that PROG exacerbated motor impairments, increased the DOPAC/dopamine ratio in the striatum, ipsiversive turnings, and the number of hind limb slips, while decreasing the symmetrical use of the forelimbs (Chao et al., 2011). The difference in these studies may be attributed to the timing of the administration, as in the former treatment began 7 days post-injury while in the latter it was initiated within 24 hours. Finally, the effect of PROG was assessed in healthy non-human primates. It was shown to reduce DNA fragmentation in serotonin neurons (Lima and Bethea, 2010).

11.0 Discussion

In 1999 the Stroke Therapy Academic Industry Roundtable (STAIR) set out to develop a set of recommendations to guide the preclinical study of a potential drug therapy prior to clinical trials (Feinklestein et al., 1999). Although the guidelines were developed with treatment of stroke in mind, the general principles may be applied to other neurologic disease. Their recommendations were as follows: adequate dose-response curve; define the time window in a well-characterized model; blinded, physiologically controlled reproducible studies; histological and functional outcomes assessed acutely and long-term; initial rodent studies, then consider gyrencephalic species; and permanent occlusion, then transient in most cases. Updates to the guidelines in 2009 included recommendations that: fundamentals of good scientific inquiry should be satisfied; after demonstration of positive effects in younger healthy animals, additional studies in aged animals and animals with comorbidities should be performed if that is the intended population for clinical trials; efficacy studies should be performed in both male and female animals; interaction studies with medications commonly used in the target population should be done; and relevant biomarker or imaging endpoints should be included that can also be obtained in human trials to indicate that the therapeutic target has been modified (Fisher et al., 2009).

PROG meets the applicable initially proposed STAIR criteria for preclinical studies in TBI and stroke, and several criteria in the treatment of SCI, peripheral nerve injury, motorneuron disease, demyelinating disease, and seizures (Table 2). While TBI research has been performed exclusively in rodent models, PROG's neuroprotective effects have been observed in cats in both ischemia (Cervantes et al., 2002; Gonzalez-Vidal et al., 1998) and seizure models (Tauboll and Lindstrom, 1993). Although the guidelines recommend testing in gyrencephalic species, this is not considered a requirement. In regard to the updated criteria, the effect of PROG in TBI, ischemia, and seizure has been studied in male and female, young and older animals. Neuroprotective effects have also been seen in an ischemia model in hypertensive rats, a common comorbidity in stroke patients (Kumon et al., 2000). The impact of hyperthermia and VitD deficiency on PROG treatment for TBI has also been studied (Cekic et al., 2011; Suzuki et al., 2004). Assessment with comorbidities in TBI and seizure models is less applicable, as these conditions tend to affect a more heterogeneous patient population. PROG and related hormones have

been utilized in the general population for many years, with catalogued polypharmacy interactions. In terms of relevant imaging that would translate to human trials, one study assessed edema using MRI following ischemia (Gibson et al., 2005). Effects on intracranial pressure have also been assessed in TBI (Shahrokhi et al., 2010). In addition to serving as functional outcome measures, these parameters may serve as useful markers of disease severity in human trials. The body of evidence as a whole is sufficient to justify the use of PROG for human trials in TBI, stroke, and seizure.

A handful of prospective human studies have already been executed using PROG and related hormones in neurologic disease. A study by Mattson et al. assessed the addition of MPA to the antiepileptic drug regimen of 14 women with uncontrolled seizures (Mattson et al., 1984). Of the 11 women who developed amenorrhea, 7 reported fewer seizures, with an average of 30% reduction in seizure frequency. In another 25-woman study utilizing PROG as adjunctive therapy for catamenial seizures, 18 of the women experienced a decline in seizure frequency (Herzog, 1995). The average daily complex partial seizure frequency decreased by 54%, while the secondary generalized motor seizure frequency decreased by 58%. More recently, a phase III, randomized, double-blind, placebo-controlled, multicenter clinical trial investigated the efficacy of adjunctive cyclic PROG therapy in the treatment of 294 women with intractable seizures (Herzog et al., 2012). Although there was no overall effect of PROG administration, benefit was seen in a subset of women with higher levels of perimenstrual seizure exacerbation when compared to controls (37.8% vs 11.1).

PROG has not yet been evaluated in a human model of stroke. As discussed above, with the exception of testing in gyrencephalic animals, all the STAIR criteria have been met. Given the strong supporting data for PROG's neuroprotection in multiple injury models, including ischemia, a human trial to examine both the safety and efficacy in acute ischemia seems warranted.

Results from three phase II, randomized, double-blind, placebo-controlled clinical trials have shown promising evidence for the use of PROG in TBI. The Progesterone for Traumatic Brain Injury, Experimental Clinical Trial (ProTECT) assessed the safety of PROG to treat moderate to severe TBI (initial Glasgow Coma Scale [GCS] 4-12) when administered in 100 patients within 11 hours of injury (Wright et al., 2007). Although it was not sufficiently powered to assess efficacy, PROG did show decreased overall mortality at 30 days (13.0% vs 30.4% p=0.06 intention to treat) and improved incidence of moderate/good outcomes in moderately injured patients (initial GCS 9-12). No serious adverse events were attributed to PROG. A second study by Xiao et al. examined the effect of PROG in 149 patients with severe TBI (GCS ≤ 8) (Xiao et al., 2008). The results demonstrated more favorable outcomes as measured by the Glasgow Outcome Scale (GOS) with PROG treatment at 3 and 6 months and decreased mortality at 6 months in the PROG group (18% vs 32% $p<0.05$) and most importantly, improved Glasgow outcome scores at 3 and 6 months ($p<0.05$). Finally, in another recent small study, patients with severe TBI receiving PROG had a higher rate of favorable outcomes at 3 months (45%) than placebo (25%), while patients receiving both PROG and vitamin D had an even higher favorable outcome rate (60% $p<0.05$) (Aminmansour et al., 2012).

Two phase III randomized, double-blind, placebo-controlled clinical trials are ongoing to further explore the effect of PROG on TBI in humans. ProTECT III, funded by the National Institute of Health,

aims to enroll 1140 patients over 4 years at 40+ sites in the US to assess the effect of PROG in moderate to severe TBI (GCS 4-12). The primary outcome is a stratified dichotomy of the Glasgow Outcome Scale at 6 months. The *Study of the Neuroprotective Activity of Progesterone in Severe Traumatic Brain Injuries* (SyNAPSE) is being conducted by BHR Pharma at almost 150 sites worldwide. It aims to enroll 1200 patients, also with a primary outcome of Glasgow Outcome Scale at 6 months. These trials represent the culmination of the numerous preclinical and clinical studies discussed. If outcomes are beneficial, PROG will be the first successful treatment for moderate to severe TBI. It will also open the door for further studies regarding the use of PROG in other neurologic diseases.

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13.0. Contributions

DWW conceived and initiated the review. ERD participated in the literature review, assisted with study interpretation, prepared the first draft and final draft. TRE, FA, EW, JK, and DWW assisted with the literature review, data interpretation, editing drafts, and contributed significantly to manuscript preparation.

14.0. Conflict Statement

Dr. Wright is entitled to royalty through Emory University from products of BHR Pharma related to the research on progesterone for the treatment of traumatic brain injury. The terms of this arrangement have been reviewed and approved by Emory University in accordance with its conflict of interest policies

15.0 Legends

Figure 1: Summary of functional outcomes by disease family. The size of each box is proportional to the number of papers showing improved functional outcome in that disease family, with the number in parentheses. See text for references.

Table 1: Summary of pathophysiologic and biochemical mechanisms by disease family. See text for references.

Table 2: Level of evidence for PROG and its metabolites use in neurologic disease as it satisfies the STAIR Criteria. Numbers indicate the number of studies that meet the specific criterion.

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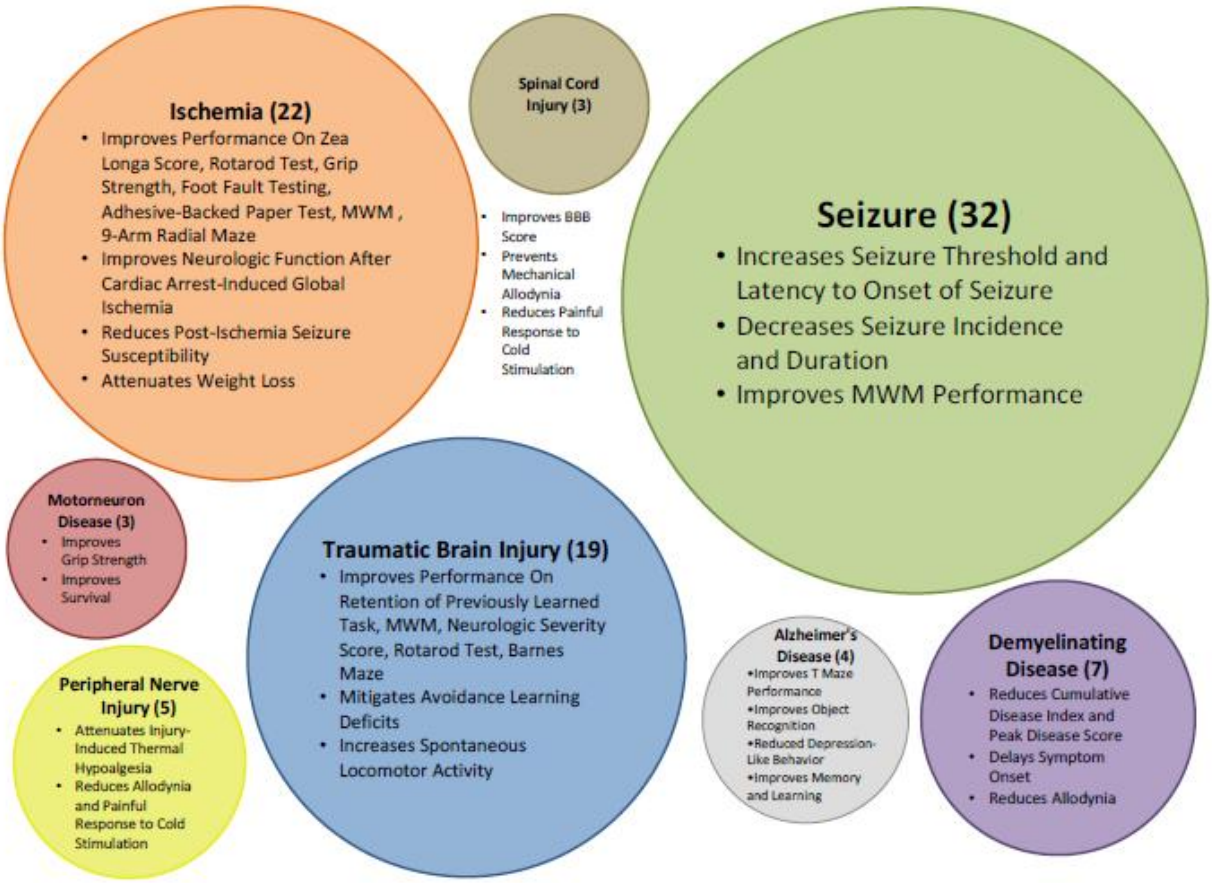
Mechanism	TBI	Ischemia	SCI	Peripheral Nerve Injury	Motorneuron Disease	Demyelinating Disease	Seizures	Alzheimer's Disease
Blood-Brain Barrier	↓ Permeability ↑ PGP ↓ AQP4	↓ Permeability ↑ Claudin5 ↑ Occludin1 ↓ MMP2,3,9						
Neuronal Apoptosis	↓ Caspase-3 ↓ Bax ↓ Bad ↓ p53 ↑ Bcl-2 ↑ Bcl-x_L ↓ DNA Fragmentation	↓ Caspase-3 ↓ Bad ↓ DNA Fragmentation					↓ DNA Fragmentation	
Trophic Factors	↑ BDNF ↓ proBDNF ↓ proNGF	↑ BDNF	↑ BDNF		↑ BDNF	↑ BDNF		
Inflammation	↓ IL-1β ↓ TNF-α ↓ NF-Kb ↓ IL-6 ↓ COX-2 ↓ ICAM-1 ↓ C3 ↑ CD55 ↑ Macrophages/ Activated Microglia	↓ IL-1β ↓ TNF-α ↓ IL-6 ↓ COX-2 ↓ ICAM-1 ↓ TGF-β2 ↓ VCAM-1 ↓ CD68 ↓ Iba1 ↓ CD3 ↓ CCL2 ↓ CCL5	↓ Microglial Activation and Proliferation			↓ IL-1β ↓ TGF-β2 ↓ IL-2 ↓ IL-17 ↓ CCR2,7 ↓ MIP-2/CXCL2 ↓ Inflammatory Cell Infiltration ↓ Microglia / Macrophage ↑ IL-10 ↑ CD19 + B Cells		↓ Microglial Activation
Oxidative Stress	↓8-isoPGF2 α ↑ Glutathione Reductase Activity	↓ iNOS ↓ NO ↑ Superoxide Dismutase ↑ Catalase ↑ Glutathione Peroxidase ↑ Glutathione ↓ Malondialdehyde ↓ Myeloperoxidase			↓ NOS Activity ↓ Mitochondrial nNOS Normalizes Mitochondrial Respiratory Complex I Activity ↑ SOD		↓ NO Pathway Activity	
Myelination			↑ Oligodendrocyte Precursors ↑ Mature Oligodendrocyte ↑ Olig2 ↑ NKx2.2 ↑ Olig1 ↑ MBP ↑ PLP	↑ Myelin Formation ↓ Myelin Fiber Loss ↑ G0 ↑ PMP22 ↑ MAL ↑ MBP		↑ Oligodendrocyte Precursors ↑ Mature Oligodendrocyte ↑ Total Myelin ↑ Rate of Remyeination ↑ MBP ↑ PLP ↑ P0 ↑ PMP22 ↑ Olig1 ↑NKx2.2		
Receptor Pathways	↑ TLR9 ↓ TLR2,4 Modulates $GABA_A$	Antagonizes σ_1 Activates Src-ERK Modulates PI3K/Akt	↑ 25-Dx ↓ NMDA ↓ PKC gamma ↑ KOR	↓ NMDA ↓ PKC gamma				
Other	↓ Edema ↑ Neuronal Survival ↓ Lesion Volume Normalizes mitochondrial respiration ↓ Astrocytosis ↑Endothelial Progenitor Cells ↑ Angiogenesis and Vasculogenesis Maintains Procoagulant Factors ↓ Intracranial Pressure ↑ Cerebral Perfusion Pressure	↓ Edema ↑ Neuronal Survival ↓ Lesion Volume ↑ GAP43 ↑ Synaptophysin Preserves Cytoarchitectural Structure Modulates VEGF ↓ $[Ca^{2+}]_i$ Antagonizes σ_1 Activates Src-ERK Modulates PI3K/Akt ↑GLUT1, GLUT3	↓ Neuron Degeneration ↑ Microtubule-associated Protein 2 ↑ pCREB ↑ ChAT ↑ Na,K-ATPase subunits ↑ GAP43	↑ Neuronal Survival ↑ Reelin ↓Na,K-ATPase Activity ↑ Nerve Conduction Velocity Restores Calcitonin Gene-Related Peptide	↓ Vacuolation ↓ Mitochondrial Breakdown ↓ APP ↓ GAP43 ↑ Muscle Weight ↑ Na,K-ATPase ↑ ChAT Enhances Retrograde Axonal Transport ↑ Glutamine Synthetase ↓ Astrocytosis	↑ Axonal Counts ↑ Smaller Diameter Axons ↓ APP ↓ GAP43 ↑ Na,K-ATPase	↑ Neuronal Survival ↓ Sialic Acid ↓ Adenosine Deaminase ↓ Presynaptic Nerve Volleys ↓Postsynaptic Excitatory Field Potentials Enhances Postsynaptic Inhibition	↓ Tau Hyperphosphorylation ↑/↓ Aβ accumulation dependent on administration ↑ Neural Progenitor Cell Proliferation ↑ Aβ-Binding-Alcohol-Dehydrogenase ↑ Liver-X-Receptor ↑ Pregnane-X-Receptor ↑ 3-Hydroxy-3- Methyl-Glutaryl-CoA-Reductase ↑ Neuronal Survival in Substantia Nigra Pars Compacta

STAIR Criteria	TBI	Ischemia	SCI	Peripheral Nerve Injury	Motorneuron Disease	Demyelinating Disease	Seizures	Alzheimer's Disease
Dose Comparison	4	5	1	1	--	2	16	--
Timing Comparison (Initiation or Duration)	6	4	1	2	--	--	2	2
Histologic Outcomes	57	47	16	8	7	10	12	8
Functional Outcome	19	22	3	5	3	7	33	4
Long Term Outcomes (≥ 18 Days)	9	5	5	4	4	4	--	8
Species	64 Rodent	45 Rodent, 2 Cat	16 Rodent, 1 Rabbit	8 Rodent	7 Rodent	10 Rodent	38 Rodent, 1 Cat	9 Rodent
Aged Animals	6	3	--	1	--	1	--	8
Comorbidities Studied	Hyperthermia, Vit D Deficiency	Hypertension, Infection	--	--	--	--	--	--
Male and Female	53 Male, 25 Female	38 Male, 14 Female	16 Male, 3 Female	6 Male, 1 Female	7 Male, 7 Female	5 Male, 5 Female	18 Male, 26 Female	5 Female, 5 Male

Highlights

- Progesterone has been shown to have robust neuroprotective properties
- Progesterone appears to provide neuroprotection through multiple mechanisms
- Progesterone may be effective in many models of neuronal injury
- Human trials under way should provide definitive data for progesterone in TBI

Figure


Ischemia (22)
• Improves Performance On Zea Longa Score, Rotarod Test, Grip Strength, Foot Fault Testing, Adhesive-Backed Paper Test, MWM , 9-Arm Radial Maze
• Improves Neurologic Function After Cardiac Arrest-Induced Global Ischemia
• Reduces Post-Ischemia Seizure Susceptibility
• Attenuates Weight Loss
Spinal Cord Injury (3)
• Improves BBB Score
• Prevents Mechanical Allodynia
• Reduces Painful Response to Cold Stimulation
Seizure (32)
• Increases Seizure Threshold and Latency to Onset of Seizure
• Decreases Seizure Incidence and Duration
• Improves MWM Performance
Motorneuron Disease (3)
• Improves Grip Strength
• Improves Survival
Traumatic Brain Injury (19)
• Improves Performance On Retention of Previously Learned Task, MWM, Neurologic Severity Score, Rotarod Test, Barnes Maze
• Mitigates Avoidance Learning Deficits
• Increases Spontaneous Locomotor Activity
Alzheimer's Disease (4)
•Improves T Maze Performance
•Improves Object Recognition
•Reduced Depression-Like Behavior
•Improves Memory and Learning
Demyelinating Disease (7)
• Reduces Cumulative Disease Index and Peak Disease Score
• Delays Symptom Onset
• Reduces Allodynia
Peripheral Nerve Injury (5)
• Attenuates Injury-Induced Thermal Hypoalgesia
• Reduces Allodynia and Painful Response to Cold Stimulation