Hormones and Behavior 84 (2016) 29–40

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Neuroprotection by progesterone after transient cerebral ischemia in stroke-prone spontaneously hypertensive rats☆

Seema Yousuf *, Fahim Atif, Iqbal Sayeed, Jun Wang, Donald G. Stein

Department of Emergency Medicine, Brain Research Laboratory, Emory University School of Medicine, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 15 October 2015
Revised 11 April 2016
Accepted 4 June 2016
Available online 06 June 2016

Keywords:
Progesterone
Stroke-prone spontaneously hypertensive rats (SHRSP)
Transient cerebral ischemia
Neuroprotection
Functional recovery

ABSTRACT

We investigated the neuroprotective effects of progesterone (P4) treatment in stroke-prone spontaneously hypertensive rats (SHRSPs) given 60-min transient middle cerebral artery occlusion (tMCAO). The treatment groups were: (1) Wistar-Kyoto (normotensive sham), (2) SHRSP (hypertensive sham), (3) tMCAO SHRSPs (SHRSP + tMCAO), and (4) SHRSP + tMCAO + P4. P4 (8 mg/kg) was administered 1 h after occlusion and then daily for 14 days. We measured cerebral infarction volume, blood pressure and body weight. Behavioral outcomes were analyzed at post-stroke days 3, 9, and 14. To assess morphological protection we measured activation of microglia and astrocytes, oxidative stress, apoptosis, expression of vascular endothelial growth factor (VEGF), an angiogenic marker, and IL-1β, a marker of inflammation, on day 14 post-stroke. There was no effect of P4 on body weight or systolic blood pressure compared to the SHRSP + tMCAO group. However, grip strength and sensory neglect measures in the P4 group were improved compared to SHRSP + tMCAO. In addition, significantly larger infarct volumes were seen in the SHRSP + tMCAO group compared to SHRSP + tMCAO + P4. Increased markers of the injury cascade such as macrophages, activated astrocytes, superoxide anion and apoptotic cells observed in the SHRSP + tMCAO group were significantly decreased by P4. We conclude that, despite hypertensive comorbidity, P4 improves functional outcomes and attenuates stroke infarct in hypertensive rats by reducing superoxide anion expression and by decreasing inflammation and neuronal apoptosis.

1. Introduction

About one in three U.S. adults—67 million people—have high blood pressure (BP) (Centers for Disease Control, 2012), which if not controlled can result in a cerebral stroke. Despite the development of numerous drugs for stroke showing promise in preclinical studies, only the clot-buster tissue plasminogen activator (tPA) has proven effective (Tsivgoulis et al., 2014). For successful translation of preclinical research to the clinic, the Stroke Therapy Academic Industry Round Table (STAIR) proposed that one important criterion for testing therapeutic interventions in animals is the presence of a co-morbidity—e.g., hypertension (Stroke Therapy Academic Industry Roundtable, S., 1999; Fisher et al., 2009).

We and others have reported the beneficial effects of progesterone (P4) treatment in traumatic brain injury (TBI) and stroke (Stein, 2011; Atif et al., 2013; Wong et al., 2013). Although the reasons remain obscure, these positive pre-clinical findings have not yet translated effectively to neuroprotection in adult patients with brain injury. Two recent phase III clinical trials, PROTECT III and SyNAPSe, did not show a benefit of P4 over placebo in patients with TBI (Skolnick et al., 2014; Wright et al., 2014). The authors of the trial reports suggest that, among other factors, their negative results could have been due to blunt primary outcome measures; the lack of surrogate biomarkers that could provide better measures of outcome than patients' perceptions of their illness in the first few months of their injury; the complexity and heterogeneity of TBI; and importantly, injury-induced co-morbidities that could affect the evolution of the injury and the ability of the patients to recover in the time spans evaluated. Although it is a very different disease indication from TBI, stroke in humans is also complex and heterogeneous, and often accompanied by co-morbidities than can affect functional outcomes. It is therefore critical to conduct pre-clinical stroke studies under relevant co-morbid conditions before any stroke drug candidate, including P4, can considered for clinical trial (Sharp and Jickling, 2014). It will also be critical to evaluate biomarkers and

☆ Support: This work was supported by NIH grant U01 NS062676 to DGS, unrestricted gifts in support of research from Allen and Company, the Marcus Foundation, and the Laney Graduate School of Arts and Sciences.

* Corresponding author at: Department of Emergency Medicine, Brain Research Laboratory, 1365B Clifton Road NE, Suite 5100, Emory University, Atlanta, GA 30322, USA.
E-mail addresses: syousu2@emory.edu (S. Yousuf), fatif@emory.edu (F. Atif), isayeed@emory.edu (I. Sayeed), jwang26@emory.edu (J. Wang), dstein04@emory.edu (D.G. Stein).

http://dx.doi.org/10.1016/j.yhbeh.2016.06.002
0018-506X/

functional outcomes that accompany the stroke and its progression with and without post-injury treatments (Howard et al., 2015a, 2015b; Stein, 2015).

To address the question of whether other disease indications can affect the course of an ischemic stroke, we previously examined the effects of P4 treatment in transient ischemic stroke in aged rats with post-stroke infection/systemic inflammation as a co-morbid factor (Yousuf et al., 2013). The study presented here tested the generality of P4 neuroprotection with chronic hypertension as a critical co-morbid condition that can lead to greater mortality if not promptly treated. We hypothesized that, despite the presence of chronic hypertension prior to stroke, P4 administration would offset some of the deficits caused by stroke. We selected spontaneously hypertensive stroke-prone rats (SHRSPs) as subjects because they show substantively more deficits in response to ischemic injury compared to the Wistar-Kyoto parent strain without the stroke-prone trait (Okamoto et al., 1974), and because SHRSPs are generally more fragile, as might be a patient with serious hypertension (Howard et al., 2015a, 2015b).

The inflammatory response to ischemic stroke is now well established (Lakhan et al., 2009; Jin et al., 2010; Sun and Jakobs, 2012), so we also examined systemic inflammatory biomarkers as well as the inflammatory response in microglia and astrocytes. These cells become highly activated in ischemia, leading to a cascade of damage and repair of neural and vascular tissues following stroke (Sofroniew and Vinters, 2010; Sun and Jakobs, 2012). Accordingly, we assessed the modulatory effects of P4 on the activation of these cells at 14 days after stroke.

2. Materials and methods

All behavioral testing, drug treatment, western blotting and histologic and immunohistochemical assays were performed by a researcher double-blinded to the experimental conditions. Thus, both drug and group identities were blinded prior to testing and evaluation.

2.1. Animal model

SHRSPs are a sub-strain of SHR developed from Wistar-Kyoto (WKY) rats by selective breeding. SHRSPs typically have more severe strokes than SHRs and WKYs given the same stroke injury (Carswell et al., 1999). WKY rats, the normotensive parent strain, are used as a control strain for SHRSPs (Okamoto et al., 1974). In a pilot study, we sought to optimize the occlusion time (1, 1.5 or 2 h) for the middle cerebral artery (MCA) in SHRSP rats (n = 18) and found that 1 h occlusion produced the most consistent infarction size associated with an acceptable mortality rate. We then applied the 1 h occlusion in the current study.

2.2. Animals and treatment regimen

Young male WKY rats and SHRSPs (300 to 350 g at the beginning of the experiments; Charles River Laboratories, Wilmington, MA, USA) were quarantined for 7 days before the experiment and housed in an AAALAC-approved Research Animal Facility with a temperature- (21–25 °C), humidity- (45–50%), and light-controlled environment. The animals were housed under a 12-h reverse light/dark cycle with free access to food and water.

Adequate measures were taken to minimize pain or discomfort, and the experiments were conducted in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals. All other applicable regulations, policies, and procedures were followed and approved by the Emory University Institutional Animal Use and Care Committee (Protocol #200-1517). The experiments are reported here in accordance with the ARRIVE guidelines. Animals with stroke surgery were randomly assigned to one of four treatment groups: (N = 35): (1) WKY as normotensive sham controls (n = 8); (2) SHRSP as hypertensive sham (SHRSP alone, n = 9); (3) SHRSP undergoing transient middle cerebral artery occlusion (tMCAO) for 60 min and receiving vehicle (SHRSP + tMCAO, n = 9); and (4) SHRSP undergoing tMCAO and treated with P4 (SHRSP + tMCAO + P4, n = 9).

After the induction of tMCAO for 1 h, animals were treated with 8 mg/kg of P4 (P-0130; Sigma-Aldrich, St Louis, MO, USA) dissolved in 22.5% 2-hydroxypropyl-β-cyclodextrin (HBC), or with HBC alone. The 8 mg/kg dose was chosen because we have shown repeatedly that this dose is neuroprotective in experimental models of ischemic stroke (Wali et al., 2014; Yousuf et al., 2014a, 2014b). The first dose was administered intraperitoneally to ensure more rapid absorption followed by subcutaneous injection once daily for the next 14 days. The dose was tapered by half over the final two treatments. Two animals died during surgery before assignment to any group, and one was excluded based on laser-Doppler flowmetry (LDF) > 40%. Five animals from each group were used for infarction measures and immunohistochemistry and four for western blots.

2.3. Blood pressure measurement

Blood pressure (BP) was measured for all animals in quiet conditions by the tail-cuff method after warming up the apparatus platform to 37 °C for 10 min. The rats were placed in restrainers and a BP tail-cuff was affixed to the base of the tail. After training sessions to habituate the rats to the procedure (two animals at a time), successive measurements were done for each rat. BP averages were used for daily BP data and the averages were reported for the baseline and day 14.

2.4. Transient MCAO

We used transient cerebral ischemia induced by occlusion of the right MCA as previously described (Longa et al., 1989). Our procedures were as follows: a midline incision was made on the ventral surface of the neck and the right common carotid arteries were isolated and ligated with 6.0 silk suture. The internal carotid and pterygopalatine artery were temporarily occluded with a microvascular clip. A 4-0 Doccol filament (Doccol Corporation, Redlands, CA, USA) was introduced into the internal carotid artery through the incision in the external carotid artery. The filament was advanced approximately 20 mm distal to the carotid bifurcation. Relative cerebral blood flow (CBF) was monitored by LDF for the entire 60 min of occlusion. After 60 min of MCAO, the occluding filament was withdrawn back into the common carotid artery to allow for reperfusion. Relative CBF was then monitored for 5 min before the wound was sutured and the rats were then permitted to recover from anesthesia. We monitored heartbeat and blood oxygen saturation levels using a SurgiVet pulse oximeter (SurgiVet™ model V3304, Waukesha, WI, USA). Drug treatment was randomly assigned 5 min before onset of reperfusion. Sham-operated WKY and SHRSP rats were subjected only to exposure of the carotid arteries but the monofilament was not inserted.

2.5. Physiological monitoring

CBF and heart rate were monitored continuously during surgery. Body temperature was maintained at 37 °C using an automated heat lamp (Harvard Apparatus, South Natick, MA, USA). Animals that underwent stroke surgery and had LDF values > 40% were excluded from the study to ensure uniform and consistent large ischemic damage and reducing experimental variability. Only one animal was excluded on this basis.

2.6. Assessment of weight as a measure of health status

The animals' baseline and post-surgery weights on days 3, 9, and 14 were taken as an indicator of their general well-being. We measured weight of heart, spleen and kidney because these are the major organs