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Evaluating the neurotherapeutic potential of a water-soluble progesterone analog after traumatic brain injury in rats

Bushra Wali [a, *], Iqbal Sayeed [a], David B. Guthrie [b], Michael G. Natchus [b], Nefize Turan [c], Dennis C. Liotta [b], Donald G. Stein [a]

[a] Department of Emergency Medicine, Brain Research Laboratory, Emory University, Atlanta, GA 30322, USA
[b] Emory Institute for Drug Development/Department of Chemistry, Emory University, Atlanta, GA 30322, USA
[c] Department of Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322, USA

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ABSTRACT

The poor aqueous solubility of progesterone (PROG) limits its potential use as a therapeutic agent. We designed and tested EIDD-1723, a novel water-soluble analog of PROG with >100-fold higher solubility than that of native PROG, as candidate for development as a field-ready treatment for traumatic brain injury (TBI). The pharmacokinetic effects of EIDD-1723 on morphological and functional outcomes in rats with bilateral cortical impact injury were evaluated. Following TBI, 10-mg/kg doses of EIDD-1723 or PROG were given intramuscularly (i.m.) at 1, 6 and 24 h post-injury, then daily for the next 6 days, with tapering of the last 2 treatments. Rats were tested pre-injury to establish baseline performance on grip strength and sensory neglect, and then retested at 4, 9 and 21 days post-TBI. Spatial learning was evaluated from days 11–17 post-TBI. At 22 days post-injury, rats were perfused and brains extracted and processed for lesion size. For the edema assay the animals were killed and brains removed at 24 h post-injury. EIDD-1723 significantly reduced cerebral edema and improved recovery from motor, sensory and spatial learning deficits as well as, or better than, native PROG. Pharmacokinetic investigation after a single i.m. injection in rats revealed that EIDD-1723 was rapidly converted to the active metabolite EIDD-036, demonstrating first-order elimination kinetics and ability to cross the blood-brain barrier. Our results suggest that EIDD-1723 represents a substantial advantage over current PROG formulations because it overcomes storage, formulation and delivery limitations of PROG and can thereby reduce the time between injury and treatment.

1. Introduction

Numerous drugs have shown promise as neuroprotective agents in preclinical animal models of traumatic brain injury (TBI), but none have worked in clinical trials. Despite substantial pre-clinical evidence (Schumacher et al., 2015) supporting the neuroprotective effects of progesterone (PROG), two recently completed phase III clinical trials reported that the neurosteroid did not provide a significant benefit to patients recovering from moderate to severe TBI (Skolnick et al., 2014; Wright et al., 2014). One factor in the failure of the trials may have been treatment delays, which ranged from 4 to 9 h post-injury and could have compromised the neuroprotective benefits of the test drug. A key tenet of brain injury treatment is that the sooner the intervention takes place, the better the functional outcome will be (Stein, 2011), but there must also be good penetration of the agent and its metabolites into the damaged tissue to permit neuroprotective activity. Thus another factor in the negative trial results could have been the poor solubility of PROG, and the intrinsic biological activity of the carrier chosen for the delivery of PROG (Howard et al., 2015; Stein, 2015). Current clinical

Abbreviations: ANOVA, analysis of variance; BWC, brain water content; CCI, controlled cortical impact; CNS, central nervous system; ent-PROG, enantiomer of progesterone; GABA, gamma-aminobutyric acid; GCS, Glasgow Coma Score; GFAP, glial fibrillary acidic protein; HBC, 2-hydroxypropyl-β-cyclodextrin; HDL, high-density lipoprotein; i.m., instramuscular; i.v., intravenous; JVC, jugular vein catheter; LDL, low-density lipoprotein; MFC, medial frontal cortex; MWM, Morris water maze; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-d-aspartate; PBS, phosphate buffered saline; PK, Pharmacokinetics; p.o., per oral; PR, progesterone receptor; PROG, progesterone; PXR, pregnane X receptor; RT, room temperature; SD, Sprague Dawley; TBI, traumatic brain injury.

* Corresponding author. Department of Emergency Medicine, Brain Research Laboratory, 1365B Clifton Rd NE, Suite 5100, Emory University, Atlanta, GA 30322, USA.
E-mail address: bwali@emory.edu (B. Wali).

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protocols for PROG administration require that patients be transported to a hospital setting before treatment can begin, losing valuable time. Time to treatment is an important consideration because many agents which show positive effects in the laboratory, where they can be given minutes to several hours after injury, do not show efficacy when treatment is delayed to later in the injury cascade.

PROG thus has certain limitations as a therapeutic intervention, notably its insolubility in aqueous-based formulations, and its short half-life, which restrict its capability for rapid delivery in emergency conditions. What is needed is a stable, water-soluble and easy-to-administer form of PROG that works immediately to rescue damaged tissue in preparation for a longer course of therapy that can be delivered in the hospital.

As noted, the disappointing outcomes of TBI randomized controlled trials may have been impacted by the choice of vehicle with which PROG was administered intravenously (i.v.). In both phase III trials (Skolnick et al., 2014; Wright et al., 2014) a lipid carrier was used, but these agents can have important effects on physiology and are not completely "neutral" as required for an appropriate control vehicle. Lipid emulsions have been shown to reduce inflammatory reactions in endothelial cells, an effect which has implications for modulating vascular repair in the damaged brain (Harvey et al., 2015). Some lipid carrier effects are beneficial, some are not. Significant elevation in total HDL and LDL cholesterol following 96 h of continuous 10% Intralipid infusion has been reported (Wasan et al., 1994). A meta-analysis of case reports found that Intralipid administration was effective in reversing multiple drug toxicities (Muller et al., 2015). Studies have shown that i.v. lipid emulsions can be effective in the acute stages of drug intoxication, improving Glasgow Outcome Scale (GOS) scores and reducing blood glucose levels up to 6 h after administration (Taftachi et al., 2012). Intralipid administration has also been shown to increase insulin resistance in heart tissue in an experimental model of type II diabetes in rats, indicating that the agent can interfere with glucose metabolism under certain conditions that might also affect the extent of brain injuries (Lou et al., 2015). Thus, as a vehicle in a clinical trial, Intralipid or other lipid compounds may confound and/or mask the effects of PROG given to patients in the acute stage of the injury cascade. In contrast, virtually all pre-clinical experiments preceding the clinical trials used more soluble 2-hydroxypropyl-β-cyclodextrin (HBC) as a vehicle rather than a lipid formulation, another possible reason the pre-clinical reports found reliable neuroprotective benefits while the clinical trials did not. Further, three small, single-center clinical studies using low-dose intramuscular (i.m.) PROG have shown positive outcomes (Xiao et al., 2008; Mofid et al., 2016; Raheja et al., 2016). HBC has been used clinically (Preskorn, 2005; Mermelstein et al., 2013) as a vehicle for i.m. dosing of poorly water-soluble drugs. However, an aqueous-based formulation will have the added advantage that it can be carried as a powder, mixed in a bottle or syringe with a water-based solution, and injected for immediate delivery to brain injury victims before they arrive in the hospital.

We have previously reported synthesis of a novel set of water-soluble analogs of PROG (MacNevin et al., 2009; Guthrie et al., 2012). These analogs were shown to possess intrinsic neuroprotective activity and are well suited for field administration as an i.m. injection, given by i.v. drip, or even potentially by intranasal administration. Several of the analogs we tested demonstrated neuroprotective effects and reduced brain edema in an *in vitro*, glutamate-induced, excitotoxic, primary neuronal cell lesion model (MacNevin et al., 2009; Guthrie et al., 2012). Based on these studies, we selected EIDD-1723 for evaluation of early-stage morphological and functional efficacy after bilateral fronto-cortical contusion injuries in rats. In this set of proof-of-concept experiments, our results show that EIDD-1723 offers substantial treatment advantages over the PROG-lipid formulations used in the phase III TBI trials.

2. Materials and methods

2.1. Synthesis of PROG analog EIDD-1723

EIDD-1723 is a substituted C-20 oxime analog of PROG that is synthesized in three synthetic steps from readily available 5-pregnen-3β-ol-20-one (pregnenolone) (Fig. 1). Condensation of pregnenolone with *O*-((methylthio)methyl)hydroxylamine, formed *in situ* from the action of hydrazine on the appropriate *O*-substituted phthalimide, provides C-20 oxime ether **1** in 74% yield. Oppenauer oxidation of the steroidal A ring gives **2** in 73% yield. Treatment of the thioether moiety with *N*-iodosuccinimide in the presence of phosphoric acid installs the phosphonooxymethyl group; subsequent deprotonation with Na_2CO_3 and purification by reverse phase chromatography provides EIDD-1723 as a disodium salt in 47% yield. Experimental details are available in the Supporting Information.

2.2. Pharmacokinetics study

A pharmacokinetics (PK) experiment was carried out in rats using per oral (p.o.), i.m. or i.v. administration to investigate the metabolic conversion of EIDD-1723 to its active metabolite EIDD-036. This study was outsourced to Agilux, Inc. (Worcester, MA, USA) under Protocol #CE-0006-DA-RI. Male Sprague-Dawley (SD) rats fitted with indwelling jugular vein cannulas (JVC) were allowed to acclimate to the test facility for 2 days prior to the start of the study and then randomly assigned to 5 groups (n = 4/group) as described in Table 1. The animals were not fasted, but the p.o. group was dosed >2 h after "lights on" to ensure that stomachs were empty when dosed. All oral doses were administered via gavage tube and all i.v. doses were administered via tail vein catheter. Catheters were flushed with ~0.5 ml of saline immediately following dose and prior to catheter removal. All animals were observed at dosing and each scheduled collection. Serial samples were collected from the JVC according to the schedule in Table 1. At the 10-h post-dosing, animals were euthanized and brain samples were collected from all the rats. Brains were rinsed with saline, patted dry, weighed, and placed on dry ice prior to storage at −80 °C until transferred to analytical chemistry for analysis. Blood samples were collected into K_2EDTA tubes and stored on wet ice until processed to plasma by centrifugation (3500 rpm at 5 °C) within 1 h of collection. Plasma samples were transferred into matrix tubes and stored at nominal −80 °C until transferred to analytical chemistry for analysis.

2.3. Tissue distribution of EIDD-1723 in rat brain

A study of the distribution of the compound in brain tissue was performed to determine the time-concentration profile of EIDD-1723 in the CNS after an i.m. injection. This study was conducted by Agilux, Inc. under Protocol #CE-0006-DA-RI. Male SD rats (n = 24) were allowed to acclimate to the test facility for 2 days prior to the start of the study. The animals were provided food and water *ad libitum*. All animals were dosed i.m. with 10 mg/kg of EIDD-1723 and observed at dosing and at each scheduled collection. No abnormalities were recorded. Four animals were euthanized at each of the 0.5, 1, 2, 4, 6 and 8 h time points, and brain samples were collected. Tissue samples were rinsed with saline, patted dry, weighed, and placed on dry ice prior to storage at −80 °C until transferred to analytical chemistry for analysis. Plasma