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Hemostatic and neuroprotective effects of human recombinant activated factor VII therapy after traumatic brain injury in pigs

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Abstract

Human recombinant activated factor-VII (rFVIIa) has been used successfully in the treatment of spontaneous intracerebral hemorrhage. In addition, there is increasing interest in its use to treat uncontrolled bleeding of other origins, including trauma. The aim of this study was to evaluate the safety and potential effectiveness of rFVIIa to mitigate bleeding using a clinically relevant model of traumatic brain injury (TBI) in the pig. A double injury model was chosen consisting of (1) an expanding cerebral contusion induced by the application of negative pressure to the exposed cortical surface and (2) a rapid rotational acceleration of the head to induce diffuse axonal injury (DAI). Injuries were performed on 10 anesthetized pigs. Five minutes after injury, 720 μg/kg rFVIIa ($n=5$) or vehicle control ($n=5$) was administered intravenously. Magnetic resonance imaging (MRI) studies were performed within 30 min and at 3 days post-TBI to determine the temporal expansion of the cerebral contusion. Euthanasia and histopathologic analysis were performed at day 3. This included observations for hippocampal neuronal degeneration, axonal pathology and microclot formation. The expansion of contusion volume over the 3 days post-injury period was reduced significantly in animals treated with rFVIIa compared to vehicle controls. Surprisingly, immunohistochemical analysis demonstrated that the number of dead/dying hippocampal neurons and axonal pathology was reduced substantially by rFVIIa treatment compared to vehicle. In addition, there was no difference in the extent of microthrombi between groups. rFVIIa treatment after TBI in the pig reduced expansion of hemorrhagic cerebral contusion volume without exacerbating the severity of microclot formation. Finally, rFVIIa treatment provided a surprising neuroprotective effect by reducing hippocampal neuron degeneration as well as the extent of DAI.

Keywords: Traumatic brain injury; TBI; rFVIIa; Cerebral contusion; Recombinant Activated Factor VII; Hemostasis; Diffuse axonal injury; Neuroprotection

Introduction

Traumatic brain injury (TBI) is a frequent and potentially disabling event that affects over 1.5 million people in the USA annually (McArthur et al., 2004). It is the leading cause of death in young people and around 2% of the US population live with disabilities as a result of TBI (Ghajar, 2000; Thurman et al., 1999). Traumatic intracranial hemorrhage (tICH), including cerebral contusions, represents a common focal pathology following TBI. Such injuries are characterized by mechanical damage to the parenchyma and its vasculature, resulting in subsequent hemorrhage and edema formation. Although clotting may constrain hemorrhage soon after injury, there is considerable

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evidence to suggest that persistent bleeding can occur in the early hours post-trauma (Servadei et al., 1995; Oertel et al., 2002; Chang et al., 2006; Chieregato et al., 2005; Lobato et al., 1991). Current management of tICH focuses on the identification and surgical evacuation of lesions large enough to generate mass effect and likely result in further neurological deterioration (Bullock et al., 1996). In the case of smaller lesions, the risk versus benefit ratio is such that evacuation may not always be favorable. There are currently no non-invasive therapies licensed for the control of traumatic intraparenchymal hemorrhage.

Recombinant activated factor VII (rFVIIa) is a hemostatic agent currently licensed for the treatment of bleeding in patients with hemophilia with inhibitors to factors VIII or IX. Pharmacological doses of rFVIIa control bleeding by local enhancement of thrombin generation (Monroe et al., 1997), stimulating the formation of a stable hemostatic clot (He et al., 2003). Additionally, rFVIIa has been shown to inhibit fibrinolysis by activation of the thrombin activatable fibrinolysis inhibitor (TAFI) (Lisman et al., 2002). The use of rFVIIa to control bleeding has been reported in various clinical settings, including the treatment of both coagulopathic and hemostatically normal patients peri-surgically (Friederich et al., 2003; Aldouri, 2002), post-trauma (Martinowitz et al., 2001, 2002; Kenet et al., 1999; Kamphuisen et al., 2002; O'Neill et al., 2002; Khan et al., 2005; Filsoufi et al., 2006), in liver failure (Chuansumrit et al., 2000) and in other bleeding conditions refractory to conventional therapy (Mayo et al., 2004). Of particular interest are the encouraging results to date for use of rFVIIa following spontaneous intracerebral hemorrhage (ICH) (Mayer et al., 2005a,b; Steiner et al., 2006) where rFVIIa was reported to decrease hematoma growth, reduce mortality, and improve functional outcome. However, the study of rFVIIa in ICH of traumatic origin is limited to a number of small case series (Zaaroor and Bar-Lavie, 2004; White et al., 2006; Aiyagari et al., 2005; Martinowitz and Michaelson, 2005; Dutton et al., 2004). The successes of rFVIIa in treating spontaneous ICH justify further investigation with respect to its potential use as a treatment for tICH.

In the present study, we evaluated the potential effectiveness and safety of rFVIIa in a combined porcine TBI model of focal cortical contusion and diffuse axonal injury (DAI). Specifically, we examined the effects of rFVIIa on (1) the expansion of cerebral contusion, (2) hippocampal neuronal death, (3) diffuse axonal injury (DAI), and (4) intravascular thrombosis.

Materials and methods

Rationale for animal model

A porcine model was considered appropriate for this study since exceedingly high and potentially toxic doses of rFVIIa are needed to induce hemostasis in other species including rodents (Schreiber et al., 2005). Earlier studies have demonstrated the hemostatic activity of rFVIIa in the pig (Schreiber et al., 2005, 2003, 2002). Since hemorrhagic contusion typically coincides with DAI in humans (Smith and Meaney, 2000), an injury model of DAI and cerebral contusion was selected to evaluate the effects of rFVIIa on both of these pathologies.

Animal preparation

This study was conducted in accordance with the animal welfare guidelines set forth in the Guide for the Care and Use of Laboratory Animals, by the Department of Health and Human Services. All animal procedures were approved by the University of Pennsylvania Institutional Animal Use and Care Committee.

Ten female miniature swine (Hanford strain, Sinclair Research Center, Inc., Columbia, MO) were included in this study. All animals were adult (aged 6–7 months) and weighed between 22–25 kg.

Prior to surgical procedures, animals were fasted for 12 h. Induction of anesthesia was achieved by intramuscular administration of midazolam (400–600 μg/kg) followed by 4% isoflurane gas via snout mask. On reaching a plane of surgical anesthesia, animals were endotracheally intubated and maintained under general anesthesia using spontaneously inhaled isoflurane (1.5–2%). Physiological monitoring included clinical observation, pulse oximetry via the skin of the ear, rectal temperature and intermittent blood pressure measurements using the brachial artery. Monitoring was continuous and documented at 15 min intervals. Previous experience with this model has shown that arterial blood gases, end-tidal CO2 and intracranial pressure (ICP) are well maintained throughout all procedures with only transient changes occurring with the below specified injury parameters.

Induction of traumatic brain injury

Traumatic brain injury was induced using a double injury model resulting in both diffuse axonal injury and cerebral contusion. Diffuse axonal injury (DAI) was induced using non-impact rotational acceleration (Smith et al., 1997, 2000). Briefly, the animals' heads were secured to a padded snout clamp mounted to the linkage assembly of a pneumatic actuator, or HYGE device. This device converts linear motion to angular (rotational) motion and produces pure impulsive head rotation of 110° (20 ms) in the coronal plane, triggered by the release of pressurized nitrogen. The center of rotation is close to the brain's center of mass. Control of the head rotational acceleration profile was accomplished by adjusting both the hydraulic fluid level within the actuator and the pneumatic pressure delivered to the actuator. The peak angular velocity for coronal plane rotation ranged between 221 and 262 rad/s. Following DAI induction, animals' heads were immediately released from the clamp. The inertial loading conditions produced by activation of this device have been shown to closely approximate the conditions of inertial brain injury in humans, such as those encountered during automobile crashes (Smith et al., 1997).

Within 30 min of rotational acceleration injury, a cerebral contusion injury was also induced using dynamic cortical deformation (DCD) (Shreiber et al., 1999). An 8 mm burr hole through the skull was placed 1 cm lateral to the sagittal suture, centered between lambda and bregma. An incision was made through the dura and a rigid plastic tube (8 mm external diameter, 6 mm internal diameter) was inserted and sealed with bone wax. The tube was connected to a vacuum pulse generator and 1 atm of negative pressure was applied for 2 seconds to generate a contusion. The craniotomy was left open and the overlying scalp incision sutured.