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High dose infusion of activated protein C (rhAPC) fails to improve neuronal damage and cognitive deficit after global cerebral ischemia in rats

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HIGHLIGHTS

- The pharmacokinetic profile in rats revealed a short half-life of seven minutes.
- Stable rhAPC plasma levels were achieved with 2 mg/kg bolus and 6 mg/kg/h IV infusion.
- 5 h of rhAPC after cerebral ischemia failed to reduce neuronal cell deaths.

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ABSTRACT

Purpose: Recent studies demonstrated anticoagulatory, antiinflammatory, antiapoptotic, and neuroprotective properties of activated protein C (APC) in rodent models of acute neurodegenerative diseases, suggesting APC as promising broad acting therapeutic agent. Unfortunately, continuous infusion of recombinant human APC (rhAPC) failed to improve brain damage following cardiac arrest in rats. The present study was designed to investigate the neuroprotective effect after global cerebral ischemia (GI) with an optimized infusion protocol. *Methods*: Rats were subjected to bilateral clip occlusion of the common carotid arteries (BCAO) and controlled hemorrhagic hypotension to 40 mmHg for 14 min and a subsequent 5 h-infusion of rhAPC (2 mg/kg bolus + 6 mg/kg/h continuous IV) or vehicle (0.9% NaCl). The dosage was calculated to maintain plasma hAPC activity at 150%. Cerebral inflammation, apoptosis and neuronal survival was determined at day 10. *Results*: rhAPC infusion did not influence cortical cerebral perfusion during reperfusion and failed to reduce neuronal cell loss, microglia activation, and caspase 3 activity. *Conclusion*: Even an optimized rhAPC infusion protocol designed to maintain a high level of APC plasma activity failed to improve the sequels following GI. Despite positive reports about protective effects of APC following, e.g., ischemic stroke, the present study supports the notion that infusion of APC during the early reperfusion phase does not result in sustained neuroprotection and fails to improve outcome after global cerebral ischemia.

1. Introduction

Cessation of global cerebral perfusion for only few minutes results in delayed and selective neuronal damage in hippocampus [15], caudoputamen [8], and cerebellum [22]. So far, most compounds failed to show neuroprotective effects in a clinical setting due to, e.g., short treatment window or absence of long-lasting effects [6]. In order to achieve robust protection, a combined approach targeting different mechanisms might be more successful. This may be achieved by a combination of different drugs or by use of a single drug, which aims at multiple post-ischemic cascades.

Activated protein C (APC) is a physiological protein, which was used for treatment of severe septic shock in patients due to its anticoagulatory and antiinflammatory properties. Additionally, APC was neuroprotective in various lesions models, e.g., cerebral ischemia [5,23,25,33], traumatic brain injury [20,28],

Abbreviations: APC, activated protein C; BCAO, bilateral occlusion of the common carotid arteries; MAP, mean arterial blood pressure; rhAPC, recombinant human APC.

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amyotrophic lateral sclerosis (ALS) [34], heat stroke [17], or spinal cord injury [9,32]. APC restored cerebral blood flow and reduced infarction volume after middle cerebral artery occlusion (MCAO) and embolic stroke [2,5,23]. These beneficial properties of APC involve a direct anticoagulatory effect by inactivation of factor Va and VIIIa. Additionally, a direct cellular effect is mediated by endothelial protein C receptors (EPCR) and protease-activated receptors (PAR-1) [3]. The receptor activation results in down-regulation of proinflammatory/proapoptotic and upregulation of antiinflammatory/antiapoptotic pathways [13]. On endothelial cells APC inhibits the release of inflammatory mediators and reduces leukocyte adhesion, which ultimately prevents infiltration and tissue damage. In addition, APC protects endothelial barrier function via EPCR and PAR-1 [19]. On leukocytes APC reduces cytokine release and migration into injured tissue. Furthermore, APC inhibits neutrophil migration by directly binding to integrin 1 and 3 [4]. In summary, these multilevel beneficial effects are referred to as "APC's cytoprotective activities" [18].

Recently, the effect of APC infusion was investigated following rat cardiac arrest and failed to significantly reduce neurological impairment and cerebral inflammation [25]. We hypothesized that the applied dosage was under dosed. The present study was designed to quantify plasma APC activity and use the pharmacokinetic data to generate a new infusion protocol. The new protocol was applied after global ischemia and neuronal cell loss, cerebral inflammation, and apoptosis were investigated 10 days after ischemia.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (320 ± 15 g body weight; Charles River Laboratories, Sulzfeld, Germany) were kept under standard housing conditions and feeding schedules. All procedures were approved by the animal care committee of Rhineland-Palatinate, Germany (protocol number 23 177-07/G 06-1-023) and performed in accordance to the German animal protection law.

2.2. Drug preparation

According to the manufacturer's recommendations a solution of 2 mg/ml recombinant human APC (rhAPC, Drotrecogin alfa activated, Xigris®, Eli Lilly Company, Indianapolis, IN, USA) was produced. The pharmacokinetic profile in rats was determined after single injection of 2 mg/kg rhAPC in a pilot study and revealed a half-life of seven minutes (Fig. 1A). To maintain a plasma activity of 150%, rhAPC bolus application of 2 mg/kg was combined with continuous IV infusion of 6 mg/kg/h for 5 h and confirmed with a plasma hAPC activity assay (Fig. 1B).

2.3. Experimental design

Animals were randomly assigned to one of three study groups:

(1) BCAO and vehicle treatment ($n = 10$).
(2) BCAO and rhAPC treatment ($n = 10$).
(3) Naïve controls without surgery ($n = 6$).

2.4. Cerebral ischemia

Global cerebral ischemia was induced by bilateral carotid occlusion as previously described in detail [16]. Rats were fasted overnight before surgery to ensure normoglycemia. After induction of anesthesia with sevoflurane (Sevorane, Abbott, Wiesbaden,

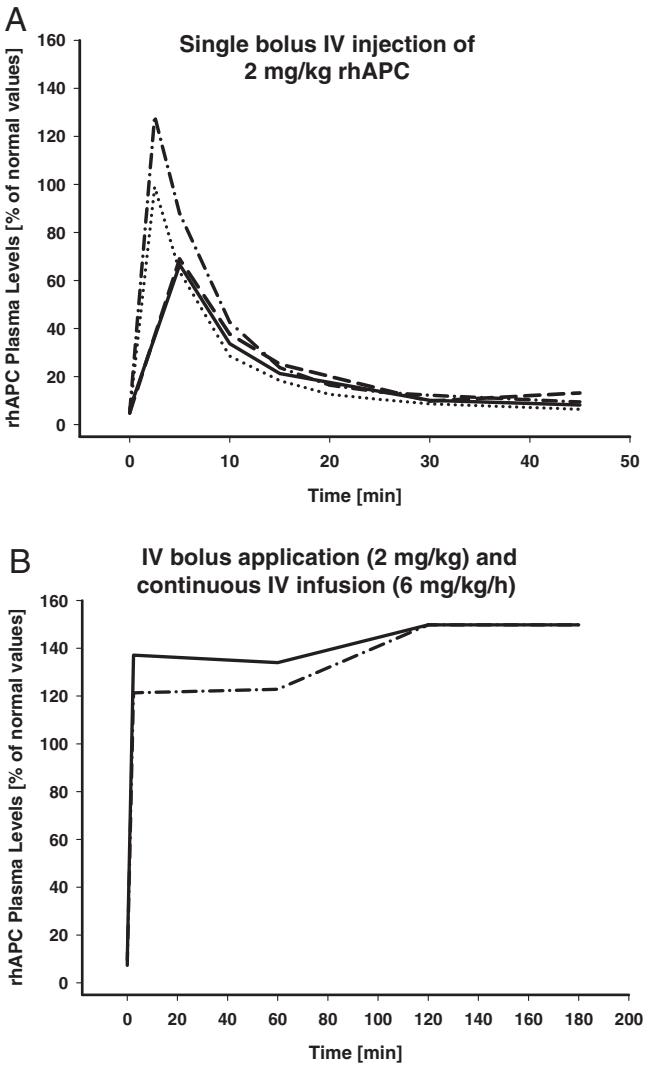

Fig. 1. (A) Plasma rhAPC activity (% normal activity) was determined after IV injection (2 mg/kg; $n = 4$) and demonstrated a short half-life of about 7 min. (B) Based in this data a combination of IV bolus (2 mg/kg) and continuous IV infusion (6 mg/kg/h) was tested and demonstrated a stable hAPC plasma activity ($n = 2$; rhAPC = recombinant human activated protein C; IV = intravenous).

Germany) rats were oro-tracheally intubated and mechanically ventilated with sevoflurane 3–3.5 endtidal vol.% in oxygen and air ($FiO_2 = 0.33$) and continuous sufentanil infusion (2.5 μg/kg/h IV; Sufenta mite, Janssen-Cilag, Neuss, Germany). Body and pericranial temperature were measured in the right temporal muscle and with a rectal probe and were maintained constant at 37 °C via infrared lamp and a feedback controlled heating blanket. Catheters were placed in the right femoral artery and vein for arterial blood pressure measurements and blood withdrawal. The right jugular vein was cannulated with two catheters for sufentanil (2.5 μg/kg/h, Sufenta mite, Janssen-Cilag GmbH, Neuss, Germany) and rhAPC/vehicle administration. Cerebral blood flow (CBF) was monitored continuously by laser-Doppler flowmetry (Periflux 4001 Master, Perimed, Järfälla Schweden) on both hemispheres (2 mm posterior to bregma and 2 mm lateral to the sagittal suture). After surgical preparation sevoflurane was adjusted to 1.5 endtidal vol.% and after 30 min animals were randomly assigned to the study groups. Cerebral ischemia was induced by bilateral clip occlusion of the common carotid arteries combined with hemorrhagic hypotension to a mean arterial blood pressure of 40 mmHg. After 14 min clips were removed and the blood was slowly reinfused.