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ERYTHROPOIETIN NEUROPROTECTION IS ENHANCED BY DIRECT CORTICAL APPLICATION FOLLOWING SUBDURAL BLOOD EVACUATION IN A RAT MODEL OF ACUTE SUBDURAL HEMATOMA

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Abstract—Recombinant human erythropoietin (EPO) has been successfully tested as neuroprotectant in brain injury models. The first large clinical trial with stroke patients, however, revealed negative results. Reasons are manifold and may include side-effects such as thrombotic complications or interactions with other medication, EPO concentration, penetration of the blood–brain-barrier and/or route of application. The latter is restricted to systemic application. Here we hypothesize that EPO is neuroprotective in a rat model of acute subdural hemorrhage (ASDH) and that direct cortical application is a feasible route of application in this injury type. The subdural hematoma was surgically evacuated and EPO was applied directly onto the surface of the brain. We injected NaCl, 200, 2000 or 20,000 IU EPO per rat i.v. at 15 min post-ASDH (400 µl autologous venous blood) or NaCl, 0.02, 0.2 or 2 IU per rat onto the cortical surface after removal of the subdurally infused blood t at 70 min post-ASDH. Arterial blood pressure (MAP), blood chemistry, intracranial pressure (ICP), cerebral blood flow (CBF) and brain tissue oxygen ($ptiO_2$) were assessed during the first hour and lesion volume at 2 days after ASDH. EPO 20,000 IU/rat (i.v.) elevated ICP significantly. EPO at 200 and 2000 IU reduced lesion volume from 38.2 ± 0.6 mm^3 (NaCl-treated group) to 28.5 ± 0.9 and 22.2 ± 1.3 mm^3 (all $p < 0.05$ vs. NaCl). Cortical application of 0.02 IU EPO after ASDH evacuation reduced injury from 36.0 ± 5.2 to 11.2 ± 2.1 mm^3 ($p = 0.007$), whereas 0.2 IU had no effect (38.0 ± 9.0 mm^3). The highest dose of both application routes (i.v. 20,000 IU; cortical 2 IU) enlarged the ASDH-induced damage significantly to 46.5 ± 1.7 and 67.9 ± 10.4 mm^3 (all $p < 0.05$ vs. NaCl). In order to test whether Tween-20, a solvent of EPO formulation 'NeoRecomon®' was responsible for adverse effects two groups were treated with NaCl or Tween-20 after the evacuation of ASDH, but no difference in lesion volume was detected. In conclusion, EPO is neuroprotective in a model of ASDH in rats and was most efficacious at a very low dose in combination with subdural blood removal. High systemic and topically applied concentrations caused adverse effects on lesion size which were partially due to increased ICP. Thus, patients with traumatic ASDH could be treated with cortically applied EPO but with caution concerning concentration.

Key words: acute subdural hemorrhage, clot evacuation, recombinant erythropoietin, neurotoxicity, rat.

INTRODUCTION

Acute subdural hemorrhage (ASDH) is still a devastating consequence of traumatic brain injury (TBI) and worsens mortality and disability of severely head-injured patients. Despite decades of clinical and experimental research ASDH is mainly treated by the surgical removal of extravasated blood volume (e.g. Bullock et al., 2006). This can reduce mortality significantly but does not stop pathophysiological processes which have already been initiated by trauma and hemorrhage (Hlatky et al., 2007). Until now neuroprotective drug effects in preclinical studies failed to translate into a successful treatment of TBI patients. This is partially due to the fact that the treatment approach includes a single neuropathological mechanism. Targeting multiple neuropathological processes which contribute to injury development may help to reduce neuronal cell death, and improve repair and functional recovery following TBI. ASDH contributes to this process by adding a pronounced ischemia and contact of blood-derived factors or mediators from extravasated blood with brain tissue. We and others could show that blood-derived factors or mediators play a major role for lesion development following hemorrhage (Kuroda et al., 1992; Dreier et al., 2000). Furthermore, apoptosis, free radicals and inflammation are part of the pathophysiological cascade that is initiated (Kwon et al., 2003; Alessandri et al., 2006; Wang and Dore, 2007).

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Abbreviations: ASDH, acute subdural hemorrhage; BBB, blood–brain-barrier; CBF, cerebral blood flow; EPO, erythropoietin; EPOR, erythropoietin receptor; HHT, hyperoncotic/hypertonic solution treatment; ICP, intracranial pressure; LDU, Laser-Doppler units; MAP, mean arterial blood pressure; PI3-K, phosphatidylinositol-3-kinase; $ptiO_2$, brain tissue oxygen; rtPA, recombinant tissue plasminogen activator; TBI, traumatic brain injury.

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Erythropoietin (EPO) is an endogenous cytokine that is essential for erythropoiesis. EPO and its receptors (EPOR) are produced and expressed in endothelial cells, neurons and astrocytes (Hasselblatt et al., 2006). Erythropoietin exerts tissue protection by anti-apoptotic, anti-inflammatory, anti-oxidative, angiogenic and neurotrophic mechanisms (Morishita et al., 1997; Siren et al., 2001, 2009; Wakida et al., 2007; Hartley et al., 2008; Velly et al., 2010), factors which are also important for lesion development after traumatic ASDH. Recombinant human erythropoietin shows neuroprotection in various models of human disease such as stroke (Sakanaka et al., 1998; Kilic et al., 2005; Kawata et al., 2006; Wang et al., 2007; Gonzalez et al., 2009), subarachnoid hemorrhage (Alafaci et al., 2000; Grasso, 2001), spinal cord injury (Celik et al., 2002; Gorio et al., 2002; Grasso et al., 2006), concussive brain injury (Brines et al., 2000; Yatsiv et al., 2005; Chen et al., 2007; Zhang et al., 2009) and intracerebral hemorrhage (Lee et al., 2006).

A first clinical trial to study the efficacy and safety of three injections of 33,000 IU EPO in stroke patients revealed benefits on outcome parameters and that the agent is well tolerated (Ehrenreich et al., 2002). A later large scale trial showed, however, no beneficial outcome and even higher mortality in stroke patients treated with 40,000 IU EPO (Ehrenreich et al., 2009). In this trial recombinant tissue plasminogen activator (rtPA) was allowed and analysis of data raised safety concerns especially in rtPA + EPO-treated patients. An adverse effect of rtPA on EPO treatment could later be confirmed in animals (Jia et al., 2010). Other reasons for a negative result could be the injury-dependent degree of the blood–brain-barrier (BBB) opening that may result in a wide range of cerebral EPO concentrations or the used dosage which is generally higher in animal models. In a case report by Nirula et al. (2010) treatment of severe TBI patients with a single dose of 40,000 IU EPO had no clear beneficial effect on outcome parameters. Another study treating patients after subarachnoid hemorrhage (SAH, NCT00626574) has been terminated due to the risk of increased mortality in the EPO-treated group. Nevertheless, several clinical trials using EPO for the treatment of TBI patients are on-going (www.clinicaltrials.gov: NCT00987454, Australia/New Zealand; NCT00313716, USA). The problem of EPO dose, dosing interval, number of doses required and route of application to improve patient outcome following brain injury seems not to be resolved. Similarly, neuroprotection by EPO treatment has not been fully surveyed in pre-clinical trials after TBI and not at all after ASDH. Since only small amounts of EPO penetrate the blood–brain-barrier (Banks et al., 2004; Xenocostas et al., 2005) the route of application becomes of interest especially in patients with ASDH. In these cases the brain tissue is exposed in order to remove the subdural blood volume and EPO could be applied topically, thus bypassing the blood–brain-barrier. In order to examine neuroprotective effects of EPO following ASDH we treated rats with post-ASDH injections of various doses and compared results of i.v. injection with the application on the cortical surface after surgical removal of the subdural blood volume.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Charles River, Germany) were used for all experiments. They had free access to food and water and were housed at a 12:12-h light:dark cycle and 50% humidity. Experiments were approved by the local ethics committee and performed according to guidelines for use and care of laboratory animals.

Anesthesia and surgical preparation

Rats were anesthetized with chloral hydrate (36 mg/ml; Dept. of Pharmacy, University Medical Center, Mainz, Germany). First, they received an i.p. bolus injection of 1 ml/100 g body weight. Thereafter, approx. 1 ml chloral hydrate was injected hourly through an intraperitoneal catheter. Atropin (1 mg) was injected s.c. Body temperature was kept at 37 ± 0.5 °C with a rectal temperature probe connected to a heating blanket control unit (Harvard Instruments, USA). Before surgical preparation animals were intubated and mechanically ventilated with a mixture of room air/O_2.

Tail artery and jugular vein were cannulated (0.8–0.96 mm o.d. PE tubing; Portex, UK) for mean arterial blood pressure (MAPB) and blood gas analysis and for the withdrawal of autologous venous blood. In order to prepare for neuromonitoring and subdural blood infusion rats were fixed in a stereotaxic frame (TSE, Germany). After a skin incision the exposed skull was cleaned and disinfected using 3% H_2O_2. A craniotomy was performed by drilling posterior to the Bregma suture (diam. 3 mm). The dura mater was penetrated using a G26 needle and an L-shaped, blunted needle (G23, B + Braun, Germany) was carefully inserted underneath the dura and fixed in place by Histoacryl® (Bbraun, Germany) and dental cement (Palavit®55VS). Anterior to the Bregma suture an area of 2 × 2 mm was thinned out with a high speed drill for cerebral blood flow (CBF) monitoring (Vasamedics Laserflo® BPM2, St. Paul, USA). Contralateral to the subdural needle a small burr hole was drilled and an intracranial pressure (ICP) catheter (NeuroventP 3F; Raumedic, Germany) was inserted into the cortical tissue. A Licox oxygen sensor (1 mm^2 sensing area; Integra) was placed close to the ICP catheter and brain tissue oxygen tension ($ptiO_2$) was measured continuously in study 1 only.

Monitoring and acute subdural hematoma

Implanted subdural needle, ICP catheter, $ptiO_2$ probe and LD-probe were left in place until stable ICP and CBF values were reached. Thereafter, a 15-min baseline monitoring period started and values were recorded every minute throughout the entire experiment. At the end of baseline, venous blood from the jugular vein was withdrawn and 400 µl was subdurally infused at a rate of 50 µl/min. The subdural needle was left in place until the end of monitoring, then clipped off as close to the skull as possible and completely sealed off by Histoacryl® in Experiment 2. Post-ASDH monitoring continued in this experiment for 60 min (Fig. 1). In Experiments 3 and 4 the needle was carefully removed at 60 min post-ASDH, the dura mater cut off and all visible blood evacuated. The craniotomy was finished by replacing and sealing off the conserved bone flap. In these series animals were monitored for another 15 min (Fig. 1). All animals were allowed to survive for 48 h.