Experimental Neurology 279 (2016) 127–136

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Transcranial direct current stimulation accelerates recovery of function, induces neurogenesis and recruits oligodendrocyte precursors in a rat model of stroke

Ramona Braun [a,c], Rebecca Klein [a], Helene Luise Walter [a], Maurice Ohren [a], Lars Freudenmacher [a], Kaleab Getachew [a], Anne Ladwig [a,c], Joachim Luelling [a], Bernd Neumaier [b,c], Heike Endepols [b], Rudolf Graf [c], Mathias Hoehn [c], Gereon Rudolf Fink [a,d], Michael Schroeter [a,c,d], Maria Adele Rueger [a,c,d,*]

[a] Department of Neurology, University Hospital of Cologne, Kerpener Str. 62, 50924 Cologne, Germany
[b] Department of Nuclear Medicine, University Hospital of Cologne, Kerpener Str. 62, 50924 Cologne, Germany
[c] Max Planck Institute for Metabolism Research, Gleueler Str. 50, 50931 Cologne, Germany
[d] Cognitive Neuroscience, Institute of Neuroscience and Medicine (INM-3), Research Centre Juelich, 52425 Juelich, Germany

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 22 February 2016
Accepted 24 February 2016
Available online 26 February 2016

Keywords:
Neurogenesis
Oligodendrocyte precursors
Microglia polarization
[18F]FLT-PET
[11C]PK11195-PET
Catwalk

ABSTRACT

Background: Clinical data suggest that transcranial direct current stimulation (tDCS) may be used to facilitate rehabilitation after stroke. However, data are inconsistent and the neurobiological mechanisms underlying tDCS remain poorly explored, impeding its implementation into clinical routine. In the healthy rat brain, tDCS affects neural stem cells (NSC) and microglia. We here investigated whether tDCS applied after stroke also beneficially affects these cells, which are known to be involved in regeneration and repair.

Methods: Focal cerebral ischemia was induced in rats by transient occlusion of the middle cerebral artery. Twenty-eight animals with comparable infarcts, as judged by magnetic resonance imaging, were randomized to receive a multi-session paradigm of either cathodal, anodal, or sham tDCS. Behaviorally, recovery of motor function was assessed by Catwalk. Proliferation in the NSC niches was monitored by Positron-Emission-Tomography (PET) employing the radiotracer 3′-deoxy-3′-[18F]fluoro-L-thymidine ([18F]FLT). Microglia activation was depicted with [11C]PK11195-PET. In addition, immunohistochemical analyses were used to quantify neuroblasts, oligodendrocyte precursors, and activation and polarization of microglia.

Results: Anodal and cathodal tDCS both accelerated functional recovery, though affecting different aspects of motor function. Likewise, tDCS induced neurogenesis independently of polarity, while only cathodal tDCS recruited oligodendrocyte precursors towards the lesion. Moreover, cathodal stimulation preferably supported M1-polarization of microglia.

Conclusions: TDCS acts through multifaceted mechanisms that far exceed its primary neurophysiological effects, encompassing proliferation and migration of stem cells, their neuronal differentiation, and modulation of microglia responses.

1. Introduction

Transcranial direct current stimulation (tDCS) has been shown to induce long-lasting alterations of cortical excitability, thereby enhancing cerebral plasticity, both in experimental animals (Bindman et al., 1962) and humans (Nitsche and Paulus, 2000). Studies in human stroke patients suggest that tDCS may be used to boost rehabilitation of motor deficits (Hummel et al., 2005), aphasia (Monti et al., 2008), and neglect (Sparing et al., 2009), implying that tDCS may become an adjuvant tool to promote recovery of function after stroke (Floel and Cohen, 2010). Although cathodal and anodal tDCS have distinct opposing effects on neuronal excitability, the influence of polarity on the clinical effects of tDCS is complex and yet incompletely understood (Antal et al., 2004; Moos et al., 2012; Vallar and Bolognini, 2011). Overall, the current widespread use of tDCS in human studies is contrasted by the dearth of data regarding its basic neurobiological mechanisms, impeding its implementation into clinical routine and limiting its use for clinical studies (Stagg and Nitsche, 2011).

Recovery of function takes place days and weeks after cerebral ischemia. Within this time frame of rehabilitation, various processes are initiated at the cellular level. Neuroinflammation, mediated both by resident microglia and invading immune cells, serves to (i) demarcate and encapsulate the necrotic infarct area, and (ii) recruit neural stem cells (NSCs) to the lesion, but may also (iii) exacerbate tissue damage

* Corresponding author at: Department of Neurology, University Hospital of Cologne, Kerpener Str. 62, 50924 Cologne, Germany.
E-mail address: adele.rueger@uk-koeln.de (M.A. Rueger).

http://dx.doi.org/10.1016/j.expneurol.2016.02.018
0014-4886/

by, e.g., production of pro-inflammatory cytokines (Stoll et al., 2002; Wang et al., 2007). NSCs migrate towards the ischemic lesion and promote regeneration by secretion of trophic factors conveying neuroprotection, as well as by supporting angiogenesis, remyelination, and neural plasticity (Chopp et al., 2009). We have previously shown that tDCS affects NSC mobilization as well as neuroinflammatory processes in a polarity-dependent manner in the healthy rat brain (Keuters et al., 2015; Rueger et al., 2012a). Here we aim at investigating whether tDCS-induced cellular processes are associated with recovery of function after stroke. Magnetic resonance imaging (MRI) and Positron-Emission-Tomography (PET) were used to deliver insights into tDCS effects in vivo and to provide readouts for a translation into human stroke studies.

2. Material and methods

2.1. Animals and surgery

All animal procedures were in accordance with the German Laws for Animal Protection. They were also approved by the local animal care committee and local governmental authorities (LANUV #87-51.04.2010.A332). N = 41 male spontaneously breathing male Wistar rats weighing 290–330 g were anesthetized with 5% isoflurane and maintained with 1–2% isoflurane in 65%/35% nitrous oxide/oxygen. Transient focal ischemia was induced by temporary occlusion of the left middle cerebral artery (MCA) for 60 min, using a silicone-coated nylon suture as described previously (Gerriets et al., 2004). Animals were allowed to recover from anesthesia in their home cages, with access to food and water ad libitum.

2.2. MRI

MRI was performed 2 days after ischemia to characterize the extent of the ischemic lesions, and in order to exclude animals with dissimilar lesions. Anesthetized with isoflurane, rats were placed in a Biospec 11.7 Tesla dedicated animal scanner system with a 16 cm horizontal bore magnet, equipped with actively shielded gradient coils (BGA9S, 750mT/m; Bruker BioSpin, Germany). Radiofrequency transmission was achieved with a birdcage quadrature resonator coil (72 mm diameter); the signal was detected with a quadrature rat brain surface coil (Bruker). A single-shot gradient echo–echo planar imaging (GE-EPI)-pilot was used for shimming as described previously (Boehm-Sturm et al., 2011). A T_2-weighted image was acquired as rapid acquisition with relaxation enhancement (T_2-TurboRARE), using the protocol: FOV 3.2 cm, matrix 256 * 256, slice thickness 0.5 mm, echo time 32.5 ms, repetition time 6500 ms. During MRI, body temperature was maintained by a feedback control system (medres, Germany).

2.3. Behavioral analyses

Rats were trained daily on the Catwalk (CatWalk XT, Noldus, Germany) for one week prior to induction of ischemia. One day before ischemia, baseline values were established for each rat. After ischemia, animals were allowed to recover for two full days before behavioral testing was recommenced. Thus, three days after ischemia, and prior to the onset of tDCS, another Catwalk session was performed to quantify the stroke-induced motor deficit. Further Catwalk sessions were performed on days 7, 10, 14, and 17. Rats were rewarded with sugar pellets for each run. Data acquisition was performed according to strict quality criteria: each run had to be performed within 0.5–10 s, with a maximum variation in speed of 70%. Three separate runs fulfilling those quality criteria were registered per day and rat, and averaged. For data acquisition, camera gain was set to 34.25 dB, and the intensity threshold to 0.17. Behavioral testing and the subsequent evaluation of the behavioral data were performed by different individuals (RB, MAR); the latter being blinded to the treatment groups.

2.4. Transcranial direct current stimulation (tDCS)

Starting 3 days after ischemia, and immediately after the first Catwalk session following stroke, 28 rats fulfilling the MRI-inclusion criteria were subjected to multi-session tDCS as described previously (Rueger et al., 2012a). In brief, an epicranial electrode with a defined contact area of 3.5 mm^2 was mounted onto the intact skull over the ischemic hemisphere using non-toxic glass ionomer luting cement (Ketac Cem Plus, 3M-ESPE, Germany) at the coordinates: bregma AP +2.0 mm, ML +2.0 mm, and left in place for the entire experiment. Animals were randomized to receive 10 days of tDCS with either cathodal (n = 9) or anodal (n = 10) polarity; a third group of rats was sham-stimulated for control (n = 9). TDCS was repeated daily for 5 consecutive days, followed by a tDCS-free interval of 2 days; then animals were subjected to tDCS for 5 more days, resulting in 10 days tDCS in total. TDCS was applied continuously for 15 min at 500 μA using a constant current stimulator (CX-6650, Schneider-Electronics, Germany) under isoflurane anesthesia, resulting in a charge density of 128,571 C/m^2. For sham stimulation, rats were anesthetized for 15 min without connection to the stimulator. After each tDCS session, animals were allowed to recover in their home cages with access to food and water ad libitum.

2.5. Radiochemistry

Radiosynthesis of [^{11}C]PK11195 was accomplished by N-methylation using the method of Camsonne et al. (Camsonne et al., 1984) with slight modifications, and as described previously (Schroeter et al., 2009). 3′-deoxy-3′-[^{18}F]fluoro-L-thymidine ([^{18}F]FLT) was synthesized by nucleophilic substitution using benzoyl-protected anhydrothymidine according to the method of Machulla et al. (Machulla et al., 2000) with minor modifications, and as described previously (Rueger et al., 2010).

[^{11}C]PK11195 selectively binds to the translocator protein-18 kDa expressed on inflammatory cells, allowing for the noninvasive assessment of neuroinflammation in vivo (Schroeter et al., 2009). [^{18}]FLT as radiolabeled thymidine analog is incorporated into newly synthesized DNA, labeling proliferating cells in the brain in vivo (Rueger et al., 2010).

2.6. PET

From n = 28 rats with confirmed infarcts on MRI, n = 15 underwent PET-imaging. PET was performed on a microPET Focus 220 scanner (Concorde Microsystems, Inc., USA; 63 image planes; 1.5 mm full width at half maximum). Sixteen days after induction of ischemia, animals were anesthetized with isoflurane and placed in the scanner. Temperature was monitored using a rectal probe and maintained at 37 ± 0.5 °C by a warm heating pad. Following a 10 min transmission scan for attenuation correction, rats received an intravenous bolus injection of [^{11}C]PK11195 (0.8–1.9 mCi/rat) into a tail vein, and emission data were acquired for 30 min. After data acquisition, animals remained in the scanner under anesthesia. [^{18}F]FLT-PET was performed in the same session and without changing the animal's position between scans, allowing for a precise co-registration of the resulting images. Those independent measurements were possible due to the short half-life of ^{11}C (~20 min) compared to ^{18}F (~110 min), by using the ^{11}C-labeled radiotracer first and waiting for five half-lives before the next tracer injection. Thus, 100 min after [^{11}C]PK11195, animals received an intravenous bolus injection of [^{18}F]FLT (1.7–2.2 mCi/rat), and emission data were acquired for 90 min. PET data were reconstructed in 22 time frames of 2 × 60 s, 2 × 120 s, 6 × 240 s, and 12 × 300 s. The last 6 frames (minutes 60–90 after tracer injection) were averaged for quantification of [^{18}F]FLT-PET data.

Please note that due to limited tracer availability, only 5 rats underwent [^{11}C]PK11195-PET, while all rats were imaged with [^{18}F]FLT.