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Research Report

In-vivo detection of inflammation and neurodegeneration in the chronic phase after permanent embolic stroke in rats

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ABSTRACT

Neuroinflammation with microglia activation (MA) constitutes a key tissue response in acute stroke. Until now, its course in the chronic stage is less well defined. Here, we investigated (i) neuroinflammation in the chronic stage of a rat model of embolic stroke ($n=6$), and (ii) whether this process can be visualized *in vivo* by multimodal imaging using Magnetic Resonance Imaging (MRI) and Positron-Emission-Tomography (PET). Imaging data were verified using histology and immunohistochemistry. Repetitive PET studies until week 6 after stroke reveal poststroke inflammation as a dynamic process that involved the infarct, the surrounding tissue and secondary degenerating areas in a complex fashion. At the end, 7 months after stroke, neuroinflammation had almost completely vanished at the lesion side. In contrast, remote from the primarily infarcted areas, a marked T2*- hypointensity was detected in the ipsilateral thalamus. In the corresponding area, [^{11}C]PK11195-PET detected microglia activation. Immunohistochemistry confirmed activated microglia in the ipsilateral thalamus with signs of extensive phagocytosis and iron deposition around plaque-like amyloid deposition. Neuronal staining (NeuN) revealed pronounced neuronal loss as an endpoint of neurodegeneration in these areas.

In conclusion, the data demonstrate not only ongoing thalamic neuroinflammation but also marked neurodegeneration remote from the lesion site in the chronic phase after stroke in rats. Both, neuroinflammation and neurodegeneration were accessible to (immuno-) histochemical methods as well as to *in vivo* methods using [^{11}C]PK11195-PET and T2*-weighted MRI. Although the functional roles of these dynamic processes remain to be elucidated, ongoing destruction of neuronal tissue is conceivable. Its inhibition using

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anti-inflammatory substances may be beneficial in chronic post-stroke conditions, while multimodal imaging can be used to evaluate putative therapeutic effects *in vivo*.

1. Introduction

Following focal cerebral ischemia, the activation of resident cells in the brain (microglia, astrocytes and endothelial cells), the recruitment of blood-derived leukocytes including neutrophils, lymphocytes and macrophages, and a plethora of humoral factors constitute cellular and inflammatory responses of the CNS coined as neuroinflammation (Dirnagl et al., 1999; Hanisch and Kettenmann, 2007). Importantly, neuroinflammation is not restricted to the infarcted area, rather it can be observed in the entire ipsilateral hemisphere (Dirnagl et al., 1999; Hanisch and Kettenmann, 2007). Activated microglia cells and the invasion of blood-borne monocytes/macrophages serve as surrogate markers of post-ischemic neuroinflammation (Kreutzberg, 1996). Many studies have focused on cellular responses taking place within few days after ischemia. We recently characterized the temporal and spatial dynamics of microglia activation after permanent, embolic stroke induced by the macrosphere model in rats. In this stroke model, it takes 7 days for a full-blown neuroinflammatory response including a hypercellular infiltrate consisting of activated microglia and phagocytic cells forming a thick rim around the ischemic infarct core (Walberer et al.). Interestingly, those observations correspond to findings in human patients, where microglia activation starts 3 days after onset of ischemia, and reaches its maximum after one week (Ramsay et al., 1992). Cellular neuroinflammatory processes can be imaged non-invasively *in vivo* using Positron-Emission-Tomography (PET) with the radiotracer [^{11}C]PK11195. PK11195 is a specific ligand to the cholesterol transporter protein (TSPO) (Benavides et al., 1983) expressed in both activated microglia and hematogenous macrophages (Gerhard et al., 2005). [^{11}C]PK11195 has been used to map neuroinflammatory processes in a variety of neurological diseases. For example, in the previous study on rat embolic stroke, we observed increased [^{11}C]PK11195 binding in the peri-infarct zone 7 days after induction of cerebral ischemia. [^{11}C]PK11195 gives the advantage of a double tracer PET approach characterizing the metabolic and perfusional state of the tissue together by means of [^{18}F]FDG in the same session (Schroeter et al., 2009).

To date, investigations concerning the inflammatory reaction in the late, *i.e.*, chronic phase of stroke remain scarce. After transient middle cerebral artery occlusion (MCAo) in rats, Lehrmann et al. investigated post-ischemic neuroinflammation for up to 3 months within the infarct core and the peri-infarct area. Data suggested that neuroinflammation had largely subsided in these areas (Lehrmann et al., 1997). In line with these findings, no reactive microglia was observed in and around the lesion 60 days after a photothrombotic lesion (Nowicka et al., 2008). Previous studies suggest that the temporospatial dynamics of neuroinflammatory processes in embolic, permanent ischemia differ from those in transient ischemia or photothrombosis (Schroeter et al., 2009). Furthermore, several studies reported about secondary lesions in the thalamus occuring remote from primary cortical damage in both human and rat brains (Gudden, 1870; Justicia et al., 2008; Myers et al., 1991). However, the investigations only included time points for up to 3 months after induction of the cortical lesion. In addition, characterization of these lesions remained fragmentary. The potential persistence of neuroinflammation in the late, chronic phase of embolic stroke has not been studied to date. Starting from the data of an yet unpublished study that investigated MRI and Signatures of postischemic inflammation on a longitudinal and intra-individual basis the aims of the present feasibility study were to investigate (i) persisting neuroinflammation in the chronic stage after stroke in an embolic stroke rat model and (ii) whether these processes can be visualized *in vivo* by multimodal imaging.

2. Results

2.1. *Tracking the dynamics of neuroinflammation by repetitive MRI/PET*

Embolisation of macrospheres in the distal left internal carotid artery (ICA) consistently induced an ischemic injury with a large infarction in the ipsilateral middle cerebral artery (MCA) territory of all animals (Fig. 1).

Seven days after ischemia, T2-weighted MRI verified the infarct and demonstrated that the lesions comprised the fronto-temporal and temporo-parietal cortex as well as the striatum. On an individual basis, the shape and extent of microglia activation as revealed by PET differed substantially (Fig. 1) Within the first 6 weeks after stroke, repetitive pairs of MRI and PET scans revealed microglia activation as a dynamic process that encompass the infarct and its border in the first 3 weeks. Later on it refocuses on thalamic nuclei and corticospinaltract known to undergo secondary degeneration.

2.2. *Magnetic resonance imaging (MRI) findings*

Despite dynamic and widespread inflammation going on between these time points the infarct volume 7 days after stroke (Fig. 2A) showed only small differences compared to the infarct volume after 7 months (Fig. 2B; 7 days after occlusion of the middle cerebral artery (MCAo): 379.4 mm^3 ± 44.3; 7 months after MCAo: 353.9 mm^3 ± 43.9; n.s.). Hence, T2-weighted MRI at day 7 predicted the extent of infarction as revealed by imaging in the chronic phase, and confirmed by histology after 7 months. By 7 months, the infarcted tissue was completely removed, leaving a cerebrospinal fluid (CSF)-filled cavitation