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Research report

Early BBB breakdown and subacute inflammasome activation and pyroptosis as a result of cerebral venous thrombosis

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ABSTRACT

Cerebral venous thrombosis (CVT) is a rare form of cerebral stroke that causes a variety of symptoms, ranging from mild headache to severe morbidity or death in the more severe forms. The use of anti-coagulant or thrombolytic agents is the classical treatment for CVT. However, the development of new therapies for the treatment of the condition has not been the focus. In this study, we aimed to analyze the pathophysiology of CVT and to identify the pathways associated with its pathology. Moreover, mechanisms that are potential drug targets were identified. Our data showed the intense activation of immune cells, particularly the microglia, along with the increase in macrophage activity and NLRP3 inflammasome activation that is indicated by NLRP3, IL-1β, and IL-18 gene and caspase-1 upregulation and cleavage as well as pyroptotic cell death. Leukocytes were observed in the brain parenchyma, indicating a role in CVT-induced inflammation. In addition, astrocytes were activated, and they induced glial scar leading to parenchymal contraction during the subacute stage and tissue loss. MMP9 was responsible primarily for the BBB breakdown after CVT and it is mainly produced by pericytes. MMP9 activation was observed before inflammatory changes, indicating that BBB breakdown is the initial driver of the pathology of CVT. These results show an inflammation driven pathophysiology of CVT that follows MMP9-mediated BBB breakdown, and identified several targets that can be targeted by pharmaceutical agents to improve the neuroinflammation that follows CVT, such as MMP9, NLRP3, and IL-1β. Some of these pharmaceutical agents are already in clinical practice or under clinical trials indicating a good potential for translating this work into patient care.

1. Introduction

Cerebral venous thrombosis (CVT) is a rare cause of stroke that can occur because of a wide range of etiologic factors. The diagnosis of CVT is frequently overlooked because of the heterogeneity of its presentation and clinical symptoms, as well as the slow progressive course of CVT (Duman et al., 2017; Einhaupl et al., 2006). Most patients have a favorable diagnosis. However, the involvement of the cortical veins results in venous infarction, and approximately 5–8% of the patients die from disease-related complications (Ferro et al., 2004; Ferro and Canhão, 2008). The current treatment guidelines include the use of anti-coagulant and/or local thrombolytic agents in severe cases, as well as the symptomatic treatment for associated symptoms, such as seizures (Einhaupl et al., 2006). The lack of a complete understanding of the parenchymal changes that occur after CVT as well as the inconsistent and widely variable presentations hinders the development of new targeted therapies for the treatment of the disease or the management of subsequent complications (Rottger et al., 2005).

Several animal models for venous sinus thrombosis have been developed (Miyamoto et al., 2001; Rother et al., 1996; Rottger et al., 2005). However, most of these models achieve venous occlusion via permanent superior sagittal sinus (SSS) occlusion either chemically or surgically (Miyamoto et al., 2001; Rother et al., 1996; Yang et al., 2012). Thus, these models fail to represent the actual course of CVT in which there is a large degree of recanalization, which does not affect the clinical outcome. Moreover, the effect of recanalization on the cerebral parenchymal pathology is

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not well characterized (Baumgartner et al., 2003; Stolz et al., 2004). This is particularly important because therapies for CVT are directed toward achieving early recanalization with no specific strategies to reverse or halt the parenchymal changes in the brain.

In this study we used a previously described CVT animal model that uses ferric chloride to produce SSS thrombosis that is followed by spontaneous recanalization. Nonetheless, the disease process is significantly unaffected by this recanalization (Rottger et al., 2005). This model was shown to exhibit the characteristic brain edema on MRI after SSS thrombosis as reported in human studies (Baumgartner et al., 2003; Duman et al., 2017; Rottger et al., 2005). Moreover, the model was used to show increased blood brain barrier (BBB) permeability and inflammatory cytokines upregulation after CVT (Nagai et al., 2010).

In this study, we used the above-mentioned model to investigate the pathological changes after CVT. A dynamic disease process that evolves from the hyperacute to the subacute phase was presented. Moreover, along with the classical anticoagulants or thrombolytic agents multiple targets that can be used for drug therapy were identified.

2. Results

2.1. Venous thrombosis and histologic changes consistent with infarction and cell infiltration as a result of SSS thrombosis

First, we performed histologic evaluation on SSS thrombosis and parenchymal changes (Fig. 1). The SSS thrombus was evident on H&E staining after 6 h and at 1 day. The sinus was infiltrated by inflammatory cells on day 1 after CVT, and was maximized after 3 days (Fig. 1A). Parenchymal changes were observed after 1 day in the form of dilated parenchymal spaces between cells indicating tissue edema. On day 3, intense cellular infiltration and tissue damage were observed in the parasagittal cortex. In addition, microbleeds were observed in some samples and corresponded with the area of tissue damage. Moreover, extensive cellular infiltration of the subarachnoid space (SA) was observed, with the SA showing dilatation, thickening, and widening of the vascular channels (Fig. 1A). After 2 weeks, tissue loss and parenchymal retraction were apparent, indicating the development of a glial scar and tissue contraction (Fig. 1B). The histological changes were confined to the parasagittal cortex. Histopathological changes were not observed in the temporal cortex, hippocampus, thalamus, and basal ganglia at any time point (data not shown).

2.2. Astrocyte recruitment as a result of CVT

Glial fibrillary acidic protein (GFAP) antibody was used to assess astrocyte activation. Before three days, minimal astrocytes were observed on immunohistochemistry (IHC) of the parasagittal cortex (Fig. 2). However, on day 3, an intense infiltration of astrocytes that stained densely with GFAP was observed mostly on the outer areas of the venous infarction, indicating a patchy infiltration of the infarction. GFAP-positive cells were still visible on day 7, and on day 14, the number and degree of GFAP staining density started to decrease (Fig. 2A). Corresponding changes on the GFAP protein

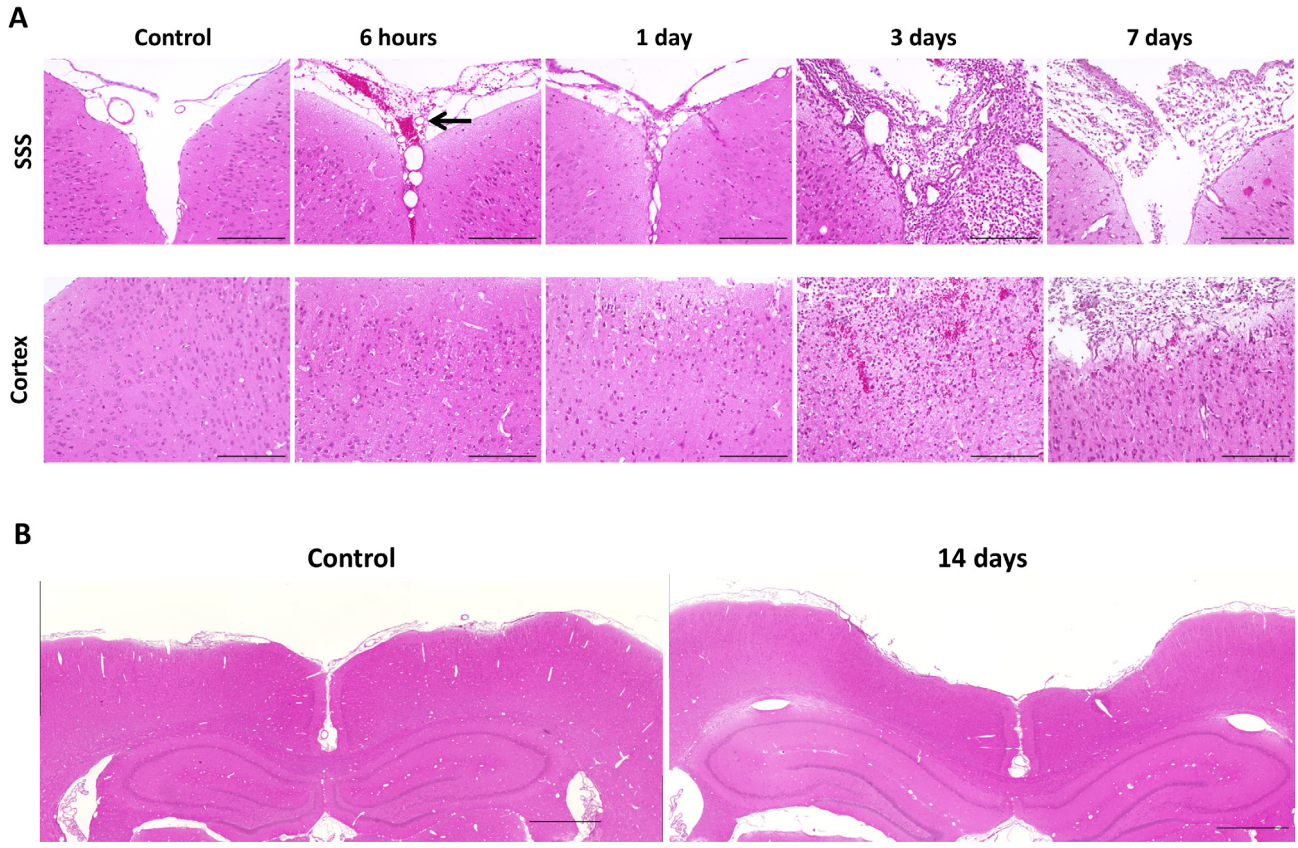

Fig. 1. Histologic analysis of the cortex and SSS after CVT. A: Chronological hematoxylin and eosin staining of sham-operated rats, and the superior sagittal sinus (SSS, upper row) and parasagittal cortex (lower row) after 6 h, 1 day, 3 days, and 7 days are shown. Sinus thrombosis is apparent after 6 h, and after 3 days there is intense infiltration and granulation involving the sinus and cortex. The cortex showed enlarged intercellular spaces suggesting edema evident on day 1. On day 3, there was intense cellular infiltration as well as microhemorrhages which were observed in some animals. On day 7 the inflammation starts to decrease in the cortex, however, the subarachnoid space is still highly cellular with signs of inflammation and organization. Similar changes were noted in the sinus on day 7 (Bar 200 μm). B: Low magnification photos showing tissue loss and cortical contraction 14 days after CVT. The cortical thickness is noted to be reduces as compared to sham-control animals; this reduction in height is probably due to contraction of glial scar (Bar 1000 μm).