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Research Report

A rat model of chronic subdural hematoma: Insight into mechanisms of revascularization and inflammation

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ABSTRACT

Chronic subdural hematoma (CSDH) is a common neurological occurrence in the elderly population with significant impact on the quality of life and work. Studies have attempted to determine the risk factors and pathophysiological mechanisms of CSDH using models in numerous mammalian species. To date, these animal models have only been able to reproduce limited durations of hematoma which does not accurately reflect the chronic state of CSDH. To address some of these challenges we modified a rat model of CSDH using two consecutive injections of autologous blood resulting in a hematoma of more than three weeks. We observed inflammatory and angiogenic changes related to the development and recovery of CSDH. In this study the technique for producing a CSDH in a small animal model had a success rate of 78.13%. The hematoma was sustainable up to 24 days. Hematoma resolution was associated with a gradual decrease in local pro-inflammatory factors and gradual increase in anti-inflammatory factors as well as proliferation and subsequent maturation of newly formed vessels. These events were also associated with improved behavioral outcome. Expression of anti-inflammatory cytokines also paralleled reabsorption of the hematoma. Reduction in hematoma size was also associated with neurological recovery. These data suggest that vessel maturation and anti-inflammatory pathways may contribute to the resolution of CSDH and neurological recovery. The regulation of the two mechanisms is a potential target for the treatment of CSDH. The

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modified model of rat CSDH demonstrated a high level of reproducibility in our hands and may be useful in future CSDH studies.

1. Introduction

Chronic Subdural Hematoma (CSDH) is believed to arise from the tearing of bridging veins between the dura and arachnoid space resulting in blood accumulation in the subdural space for 3 weeks or more (Crooks, 1991). This is in contrast to acute subdural hematomas which persist for 1–3 days (Mark, 2010). Development of CSDH is most commonly associated with a preceding traumatic brain injury event (Mori and Maeda, 2001). CSDH has increased risk of occurrence in the groups over 65 years of age, cases with a history of traumatic brain injury, or administration of anticoagulants which can exacerbate the incidence from 8.2/100,000/year to 310/100,000/year (Asghar et al., 2002; Hart et al., 2012; Karibe et al., 2011; Mant et al., 2007). Treatment of CSDH primarily involves surgical drainage with the exception of conservative treatment in cases of small hematomas with mild symptoms. In elderly patients, surgical intervention may pose greater risk to the patients' outcome due to complications from pulmonary edema and infection, cerebral edema and organ dysfunction. The risk of mortality and morbidity increases from 3% up to 5% and 12% respectively, and the recurrence rate increases from 9.2% to 26.5% with complications (Miranda et al., 2011; Yamamoto et al., 2003).

A reproducible animal model with relevance to human CSDH would be valuable towards understanding the pathogenesis of CSDH. Furthermore, such a model could be used to elucidate targets to promote the absorption of subdural hematomas to improve outcome in CSDH patients. Several attempts to reproduce CSDH in animal models have been investigated to date. These include the implantation of autologous venous clotted and non-clotted blood in the subdural region, subcutaneous clotted blood implantation and various technical variations such as the inclusion and exclusion of cerebral spinal fluid (CSF), fibrin, thrombin and plasmin, mannitol and heparin (D'Abbondanza and Loch Macdonald, 2014; Glover and Labadie, 1976; Labadie and Glover, 1976; Ohshima, 1982; Rapaport et al., 1972). Although some of the anatomical characteristics of CSDH are reproducible, a critical barrier has been the reproduction of a reliable model with a sustained hematoma. The infusion model, in particular, has been shown to accurately reproduce aspects of CSDH pathology. Furthermore, this method allows for control of the hematoma volume and can be used to study acute subdural hematomas, as well as mechanisms and treatments affecting rates of reabsorption. Angiogenesis and inflammation are suspected key factors in influencing the development and reoccurrence of CSDH (Hong et al., 2009; Pripp and Stanisic, 2014). However, few studies have examined angiogenesis and inflammation in detail.

In this study, we modified the rat infusion model of subdural hematoma described by other groups and our previous work (Karabiyikoglu et al., 2005; Miller et al., 1990; Wang et al., 2010) to create an improved, sustained and reproducible CSDH model. We describe the application of the model producing a hematoma lasting 3 weeks and insight into the contribution of angiogenesis and inflammation on the resolution of the hematoma. We propose that our CSDH model provides a valuable tool to further explore pathogenesis of CSDH and therapies regulating angiogenesis and inflammation to improve outcome after CSDH.

2. Results

Our modified model of CSDH consisted of two blood infusions on rats with the success rate of 78.13% (Table 1). This double infusion of blood resulted in the persistence of an intracranial hematoma for more than 3 weeks thereby characterizing the model with more relevance to CSDH rather than an acute hematoma. The weight of the test rats was significantly lower in CSDH rats relative to sham rats.

2.1. *MRI results of modeled chronic hematoma formation in rats*

MRI analysis of the hematoma shape and volume are shown in representative images in Fig. 1. The subdural hematoma was visible as a clear oval structure following the second infusion at 72 h. The hematoma was shown to compresses the ipsilateral lateral ventricle, the cortex and obvious displacement or compression of underlying structures such as the hippocampus and thallamus. There was also a midline shift due to compression of tissues (Fig. 1B and C). The mean hematoma volume at 72 h was $607.2 \pm 18.4\ mm^3$. Hematoma volume decreased in volume significantly at each assessment time point ($P < 0.001$). By day 24 most of the hematoma had been absorbed and a rebounding of the brain tissue was evident (Fig. 1E). Data are summarized in Fig. 1F.

2.2. *Morphological characterization of the hematoma with H&E*

H&E histology in coronal sections indicated a change in hematoma morphology over time (Fig. 2A D). The hematoma was primarily characterized by red blood cells on the 3rd and 10th day. There was evidence of fibrin and inflammatory processes within the encapsulation space (Tokmak et al., 2007). The fibrin tissue and inflammatory infiltrate gradually became the dominant substance in the hematoma as the dark red central zone of the hematoma became smaller and the fibrin ring enveloped it (Fig. 2C and D). Blood from the initial and secondary injection could be identified by the stratification in the subdural space. Blood from the second