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BRAIN RESEARCH

Research Report

Platelet adhesion receptors do not modulate infarct volume after a photochemically induced stroke in mice

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ABSTRACT

Photochemically induced cerebral infarction has been considered a clinically relevant model for ischemic stroke. We evaluated various transgenic mice to study the role of platelet adhesion molecules in this model. Infarction to the sensorimotoric cortex was induced by erythrosin B and laser light. Infarct volumes were calculated from triphenyltetrazolium chloride stained brain slices. Thrombus formation and vessel leakage were observed in vivo by multiphoton microscopy. Mice mutant in VWF, GPIbα, β3 integrin, and P-selectin did not show any significant differences in infarct volume compared to wild type (WT). This is in contrast to the intraluminal middle cerebral artery occlusion model in which αIIbβ3 integrin, GPIbα, and P-selectin are known to modulate infarct size. Multiphoton microscopy showed that small, non-occlusive embolizing platelet thrombi formed in the photochemically injured brains. Massive vessel leakage was observed within 25 min of laser injury. Interestingly, we observed a significant increase in infarct size with aging, accordant with heightened fragility of the blood brain barrier (BBB) in older mice. This model of photochemically induced stroke is closer to a BBB injury model than a thrombotic stroke model in which platelets and their adhesion molecules are crucial. This model will be useful to study mechanisms regulating BBB permeability.

1. Introduction

Ischemic stroke is a feared complication of atherosclerotic cardiovascular disease occurring in approximately 600,000 individuals per year in the USA (American Stroke Association, 2007). In spite of the serious consequences of stroke and the urgent clinical need to identify and test novel therapeutic agents, there is still a lack of technically accessible and reproducible animal models to determine aspects of pathogenesis or treatment. The most common experimental animal model is based on middle cerebral artery occlusion (MCAO) by an intraluminal filament technique. However, MCAO is

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technically complex and requires highly specialized skills to ensure reproducibility. Another experimental approach that is technically simpler involves the induction of photochemically induced thrombotic cortical damage. This less invasive animal model produces highly reproducible brain lesions in rats (Yao et al., 1996) and mice (Eichenbaum et al., 2002).

According to different descriptions of this model, stroke induction would in part depend on microvascular thrombotic occlusion in a platelet-dependent manner (Van Reempts and Borgers, 1994) and an increased vascular permeability (Hoff et al., 2005). The formation of a thrombus involves multiple adhesion molecules (such as von Willebrand factor (VWF), collagen, fibrinogen, and fibronectin) and their respective receptors (GPIb, GPVI, β1, and β3 integrins) on the platelet surface. However, the contribution of the platelet adhesion molecules to the lesion size has not been documented in the photochemical injury model. Therefore, in order to understand the role of platelet adhesion molecules and their surface receptors in photochemical stroke, we performed a series of experiments using VWF–/–, β3 integrin–/–, transgenic mice lacking the GPIbα extracellular domain (IL4Rα/GPIbα-tg), and P-selectin–/– mice.

2. Results

2.1. *Infarct size increases with age*

To establish the time of maximal damage in photochemically induced stroke, we did a timeline experiment using WT mice. Infarcted region visualized by the metabolic TTC stain became apparent 8 h after induction of the injury (0.9±0.6 mm^3) and gradually grew in size up to 24 h (5.5±1.3 mm^3). At 72 h, the infarct volume diminished to one third of 24 h volume (1.6±0.4 mm^3) (Fig. 1A). We chose 24 h as the optimal time point for analyses in subsequent experiments. Next we evaluated the effect of age on the outcome of stroke in the WT mice. We found that 24 h after stroke, 6- to 12-month-old mice had significantly larger infarct volume compared to 2-month-old mice (6.7±0.3 mm^3 vs. 3.9±0.3 mm^3, $P<0.02$ respectively) (Fig. 1B). Thus it appears that older mice are more susceptible to photochemically induced stroke. In the following experiments, only mice of similar age were compared.

2.1.1. *Role of adhesion molecules and their receptors on the outcome of photochemically induced stroke*

After vascular injury, VWF contributes to thrombus formation by mediating the initial adhesion of platelets to the extracellular matrix and to one another. Two important platelet receptors for VWF are GPIbα in the GPIb–IX–V complex and integrin αIIbβ3. VWF–/– mice demonstrate serious defects in hemostasis and thrombosis (Denis et al., 1998). Despite this, we observed no differences in infarct volume in VWF–/– mice compared to WT (VWF–/–=5.2±1.7 mm^3 vs. WT=4.3±1.5 mm^3, $n=7–9$, $P=0.3$) (Fig. 2A). Similarly in the IL4Rα/GPIbα-tg mice, which have a defect in platelet adhesion and thrombus formation (Bergmeier et al., 2006), the infarct volumes were comparable to WT (5.4±1.6 mm^3 vs. 4.3±1.9 mm^3, $P=0.26$) (Fig. 2B). Then we studied the role of β3 integrin, a receptor for adhesive proteins such as fibrinogen, VWF, and fibronectin.

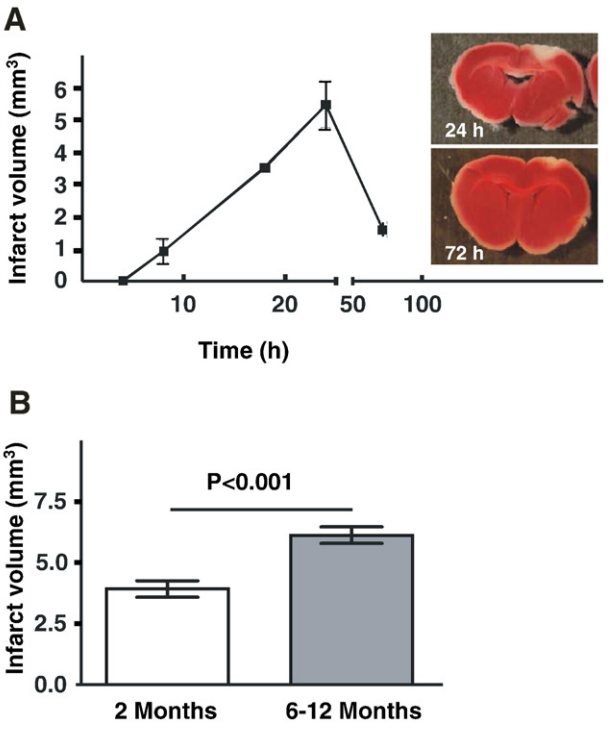

Fig. 1 – Infarct volume as a function of time and animal age. (A) Infarct volume at different time points after photochemically induced stroke ($n=4$ per time point). Representative photographs of TTC stained sections with maximal infarct volumes from brain at 24 h and 72 h post injury (panels). (B) Infarct volumes increase significantly in older mice compared to young mice subjected to photochemical stroke ($n=11–13$).

Inhibition or deficiency of αIIbβ3 integrin and of GPIb was shown to provide protection from intraluminal MCAO induced stroke (Choudhri et al., 1998; Kleinschnitz et al., 2007; Massberg et al., 2005). β3–/– mice lack functional αIIbβ3, the major platelet integrin that mediates platelet aggregation (Hodivala-Dilke et al., 1999). Again, we did not observe any difference in infarct volume of β3–/– mice compared to WT (3.7±1.0 mm^3 vs. 4.6±1.2 mm^3, $P=0.6$, Fig. 2C). It appears therefore that, in contrast to the intraluminal MCAO model, platelet adhesion molecules do not significantly affect infarct volume produced by the photochemical injury model.

P-selectin (P-sel), a receptor important in platelet rolling (Frenette et al., 1995) and in inflammatory responses, is present in α-granules of platelets and in Weibel–Palade bodies of the endothelial cells (Wagner, 1993). It is released upon platelet stimulation. Previously, it has been shown that P-selectin deficiency provides protection in the intraluminal MCAO model (Connolly et al., 1997). However, in the photochemical stroke model we did not observe any significant difference in infarct volumes in the P-sel–/– mice compared to WT (6.4±1.7 mm^3 vs. 5.5±1.3 mm^3, $P=0.15$, Fig. 3A). We decided to evaluate the P-sel–/– mice from our own colony in the intraluminal MCAO model. We were able to confirm that deficiency of P-selectin protects mice from stroke in the intraluminal MCAO model (Fig. 3B). The infarct volume in P-sel–/–