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Protecting the Brain from Calcification in Ischemic Stroke

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1 Introduction

Ischemic stroke is a cerebral arterial atherosclerosis- and/or embolism-induced disorder causing neuronal death and neurological deficiencies. Brain calcification has been detected in ischemic stroke and considered a consequence of brain infarction [1-4]. Preliminary studies in this laboratory have demonstrated that brain ischemia causes brain calcification prior to brain infarction, compromising neuronal function and contributing to brain injury. A family of calcium-binding proteins known as annexins (anxs) possibly contributes to brain calcification. These proteins are attached to the inner surface of the cell membrane to control calcium trafficking under physiological conditions [5, 6]. Free anxs can bind to actin filaments in the presence of Ca^{++} [5, 6]. A hypothesis for this investigation is that the membrane-borne anxs may be released into the cytoplasm when the cell membrane is degraded in ischemic stroke, possibly mediating calcium deposition to the β actin filaments of the ischemic neurons. This investigation was designed to test this hypothesis and develop treatment strategies to prevent anx-induced brain calcification and alleviate brain injury.

2 Methods

Ischemic stroke was induced in the mouse by 90-min ligation of the right middle cerebral artery and both common carotid arteries. Brain infarction was tested by the triphenyltetrazolium chloride (TTC) assay; brain calcification was assessed by the Alizarin and von Kossa assays; the expression of anxs was measured by immunofluorescence microscopy and immunoblot analysis; anx-specific siRNAs were used to suppress anx expression; and monomeric β actin was used to block anx binding to β actin filaments.

3 Results and Discussion

Ischemic stroke caused brain calcification as early as 90- min ligation of the right middle cerebral artery and both common carotid arteries as visualized by the Alizarin assay, but caused brain infarction at 12 hrs of reperfusion following the 90-min ligation period as visualized by the TTC assay (Fig. 1). These observations suggest that brain calcification occurs prior to brain infarction.

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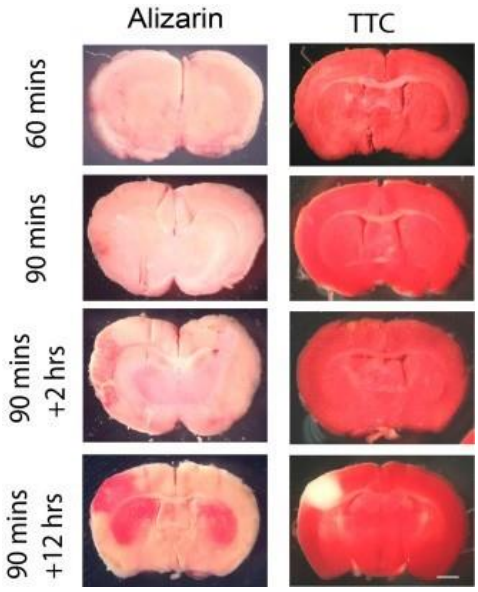

Figure 1. Brain slices showing Alizarin-stained brain calcification (red) and TTC-stained brain infarction (white) in ischemic stroke. The times 60 and 90 mins are durations for ligation of the right middle cerebral artery and both common carotid arteries. The times 2 and 12 hrs are reperfusion periods after artery ligation. At each time, two adjacent brain slices were prepared – one for Alizarin test and the other for TTC test. Scale: 1mm.

Neural cells expressed primarily anxs A1, A2, A6, A7, and A11 as *tested* by immunoblot analysis. Ischemic stroke caused translocation of these anxs from the cell membrane to the β actin filaments, a process associated with cell calcification (Fig. 2). Anx translocation was confirmed by co- immunoprecipitation and immunoblot analyses. Administration of recombinant anx A1, A2, A6, A7, or A11 to healthy brain caused brain calcification (Fig. 3). Co-administration of an anx with monomeric β actin prevented anx-induced brain calcification (Fig. 3). Whereas administration of each of anxs A1, A2, A6, A7, and A11 siRNAs did not significantly alleviate brain calcification in ischemic stroke, administration of all 5 anx siRNAs in combination significantly prevented brain calcification (Fig. 4).

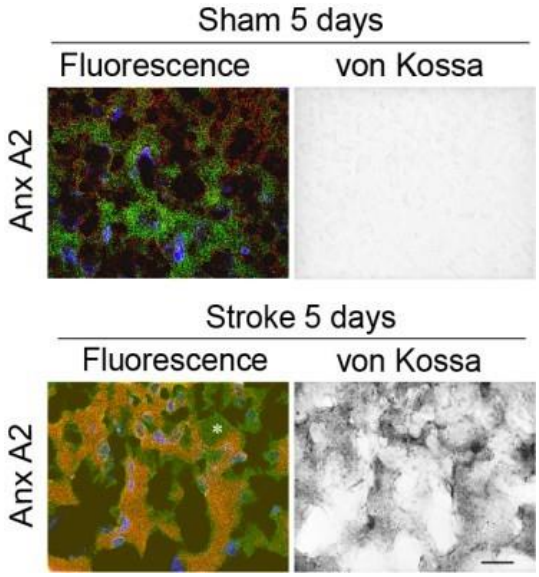

Figure 2. Anx A2 translocation from the cell membrane to the β actin filaments of neurons in ischemic stroke. Red: Anx A2. Green: β actin. Blue: Cell nuclei. Black for von Kossa images: Calcified structures. Similar results were observed for anxs A1, A6, A7, and A11. Scale: 10 μm.

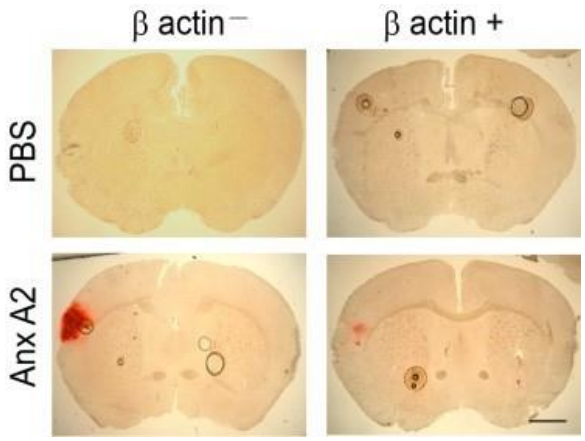

Figure 3. Brain specimen sections showing brain calcification in response to anx A2 administration to the brain in the presence and absence of monomeric β actin at 5 day. Red: Alizarin-stained brain calcification. Similar results were observed for anxs A1, A6, A7, and A11. Scale: 1 mm.

Figure 4. Effect of single and combined anx siRNAs on brain calcification (red) at 5 day following ischemic stroke. Similar results were observed for anxs A1, A6, A7, and A11 siRNAs. Scale: 1 mm.

4 Conclusions

The observations from this investigation suggest that brain calcification is a cause of brain infarction; anx binding to β actin filaments contributes to brain calcification; and blocking anxs with monomeric β actin and siRNA-mediated silencing of the anx genes prevent anx-induced brain calcification and alleviate brain infarction.

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