Role of Intracranial Microaneurysm in the Pathogenesis of Stroke in SHR-SP

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Stroke-prone spontaneously hypertensive rats (SHR-SP) is a sub-strain of spontaneously hypertensive rats (SHR) with much more vulnerability to stroke. Cerebral artery remodeling, for example, increased vascular wall thickness and decreased lumen diameter, represents a key pathological feature that underlies such a difference. Cerebral vascular malformation, for example, microaneurysm, is also an important factor in the pathogenesis of stroke in human. About 15% of all strokes are secondary to ruptured aneurysms [1]. Microaneurysm may rupture upon sudden increase of blood pressure [BP; Ref. 2]. Ischemic stroke, accounting for ~70% of all stroke events in China, can be a presenting clinical feature of intracranial aneurysms [3]. This work examined a potential role of intracranial microaneurysms in the pathogenesis of stroke in SHR-SP and the underlying mechanisms.

The experiments included 26 SHR-SP (male 13, female 13) and 25 SHR (male 13, female 12). BP and baroreflex sensitivity (BRS) were determined at the age of 28 weeks in conscious state, as previously described [4]. Serial transverse paraffin brain sections (5 μ m thick, at 100 μ m interval) were then prepared using routine techniques. Ten slides were stained with hematoxylin–eosin and observed under a light microscope. Microaneurysms were calculated with an image analysis system (SDK-2000, 10moons Technology Development, China). Data are expressed as mean $\pm$ SD. $P < 0.05$ was considered statistically significant.

Intracranial microaneurysms occurred in 25 of the 26 SHR-SP (incidence: 96.15%; 2.96 ± 1.51 microaneurysms per slide), and none of the 25 SHR. In SHR-SP, the number of microaneurysms was positively correlated with systolic BP (SBP; Figure 1A; $r = 0.60$, $P < 0.05$), and negatively correlated with BRS (Figure 1B; $r = 0.54$, $P < 0.05$). The enzyme 5-lipoxygenase is a key enzyme in the

biosynthesis of proinflammatory leukotriene lipid mediators and is believed to be responsible for the occurrence of aneurysm [5,6]. In this study, western blot analysis showed that the expression of 5-lipoxygenase in the brain tissue was significantly higher in SHR-SP (Figure 1C; $P < 0.01$ vs. SHR).

A separate group of 40 male SHR-SP (age: 14 weeks) were randomly divided into two groups and received either the 5-lipoxygenase inhibitor zileuton (0.8 g/kg/day, delivered in food, $n = 20$) or the vehicle ($n = 20$). The rats were sacrificed when death was deemed inevitable after stroke. The brains were harvested and stained with hematoxylin–eosin. Zileuton treatment significantly decreased the occurrence of intracranial microaneurysms (Figure 2A; $P < 0.01$ vs. control). Kaplan–Meier survival analysis revealed a significantly longer survival time in SHR-SP who received zileuton than the vehicle control (Figure 2B; $P = 0.019$).

Intracranial microaneurysm is an important pathological basis for stroke, especially for spontaneous cerebral hemorrhage. Results from this study showed a much higher incidence of intracranial microaneurysms in SHR-SP compared with SHR. Furthermore, the number of microaneurysms was positively correlated with SBP and negatively correlated with BRS. The enzyme 5-lipoxygenase, an essential factor for the occurrence of aneurysm, was higher in SHR-SP. Inhibiting 5-lipoxygenase with zileuton decreased the occurrence of intracranial microaneurysms and prolonged the lifespan of SHR-SP.

In conclusion, intracranial microaneurysm may play an important role in the pathogenesis of stroke in SHR-SP; inhibiting 5-lipoxygenase could suppress the development of intracranial microaneurysms.

Intracranial microaneurysm predicts stroke in SHR-SP

Figure 1 Correlation of intracranial microaneurysm number with (A) SBP and (B) BRS in 28-week-old SHR-SP ($n = 26$; univariate regression analysis). (C) Expression of 5-lipoxygenase in the brain was higher in SHR-SP and shown as relative change using SHR as reference ($n = 3$; unpaired t -test). ** $P < 0.01$ versus SHR.

Figure 2 Effects of zileuton on intracranial microaneurysm number and lifespan of SHR-SP. (A) Zileuton (0.8 g/kg/day, delivered in food) decreased microaneurysm number in SHR-SP (unpaired t -test). (B) Kaplan–Meier survival analysis demonstrated significantly longer survival in SHR-SP received zileuton. $N = 20$ in each group, ** $P < 0.01$ versus control.

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