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Research Report

# Allopurinol protects against ischemic insults in a mouse model of cortical microinfarction

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ABSTRACT

Microinfarcts are common in patients with cognitive decline and dementia. Allopurinol (ALLO), a xanthine oxidase (XO) enzyme inhibitor, has been found to reduce proinflammatory molecules and oxidative stress in the vasculature. We here examined the effect of pre-treatment with allopurinol on the cortical microinfarction. C57BL/6J mice were subjected to a permanent single penetrating arteriole occlusion induced by two-photon laser irradiation. Infarction volume, the activation of glial cells and nitrosative stress in the ischemic brain was assessed using immunohistochemistry. Pre-treatment with ALLO achieved 42% reduction of infarct volume and significantly reduced microglia infiltration, astrocyte proliferation and nitrosative stress in the ischemic brain. These data indicate that ALLO protects against microinfarcts possibly through inhibition of nitrosative stress and attenuation of microglia infiltration as well as astrocytes reactivation.



## 1. Introduction

Increasing evidence has demonstrated the strong association between microinfarcts and cognitive dysfunction (Brundel et al., 2012). Microinfarcts have been detected in about 55% of patients with mild cognitive decline (Sonnen et al., 2007) and about 70% of patients with vascular dementia and Alzheimer's disease (Burton et al., 2009; Haglund et al., 2006). These lesions are often in company with small vessel pathologies such as arteriolosclerosis or cerebral amyloid angiopathy (Ihara, 2013; Smith et al., 2012). Recently, cortical microinfarcts have received greater attention because cortical microinfarcts, such as the parietal cortex, are highly associated with AD and cerebral amyloid angiopathy (CAA) (Soontornniyomkij et al., 2010; Okamoto et al., 2009). Different from the big stroke, microinfarcts, in their early phase, are usually clinically silent and go undetected on conventional MRI because of their small sizes. The development for ideal

Abbreviations: ALLO, allopurinol; XO, xanthine oxidase; NT, 3-nitrotyrosine

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treatment for cerebral microinfarcts has been hindered by a lack of animal models of cerebral microinfarction. Currently, a two-photon laser-induced occlusion of penetrating arteries in rodents has been shown to recapitulate the pathophysiological features of microinfarcts including neuronal cell death, oxidative stress and inflammation (Wang et al., 2012; Arvanitakis et al., 2011; Caplan, 2015).

Allopurinol (ALLO), a xanthine oxidase (XO) enzyme inhibitor, is in clinical use to lower serum uric acid. Recently, ALLO has been reported to improve neurological function in stroke patients with high levels of serum uric acid (SUA). In addition, pre-treatment with ALLO confers neuroprotection in experimental models of focal ischemic stroke (Isik et al., 2005). Furthermore, ALLO also has demonstrated its beneficial effects in hypertension which is a high risk of microinfarcts (Sanchez-Lozada et al., 2008; Soletsky and Feig, 2012). Interestingly, those neuroprotective effects are not associated with SUA-lowering effect of ALLO (Dawson et al., 2009). Thus, ALLO may yield additional benefits in addition to potent UA reduction. Indeed, ALLO has been shown to improve endothelial function, reduce the proinflammatory molecules expression and attenuate oxidative stress in the vasculature, which all contribute to the alleviation of infarct volume in ischemic stroke (Itoh et al., 1986; Dawson et al., 2009; Martz et al., 1989). Therefore, the objective of this study was to assess the neuroprotective effect of allopurinol in a mouse (C57BL/6J) model of laser-induced microinfarction.

## 2. Results

### 2.1. Occlusion of a single penetrating arteriole

Maps of the brain vasculature of anesthetized mice were obtained using in vivo two-photon laser-scanning microscopy (TPLSM) by labeling the blood plasma with fluorescein-dextran FITC. Candidate arterioles for clotting are identified from the map. The green laser light was placed on the segment which courses parallel to the cortical surface and proximal to where the arteriole dove into the brain. The occlusion in the target arteriole was induced by two-photon laser irradiation through the thin cranial window in the parietal cortex. Soon after the extravasation of fluorescein-dextran outside the vessel lumen, a clot was formed with a complete cessation of RBC movement in the target arteriole. Neighboring surface vessels and capillaries remained flowing. We kept our observation for 1 h after the insult to ensure no recanalization (Fig. 1).

### 2.2. Pretreatment with ALLO reduced the neuron death

A cylindrical region of tissue infarction was formed over a course of 7 d. The infarction was devoid of staining for the pan-neuronal marker NeuN. ALLO was given 1 h prior to the occlusion. The average microinfarction volumes at 24 h were $145.80 \pm 7.87\ \mu m^3$ and $109.10 \pm 12.22\ \mu m^3$ in the normal controls and the ALLO group, respectively. The infarction progressively expanded over time. In the controls, the average microinfarction volume was $196.80 \pm 6.35\ \mu m^3$ at 3 d and $227.20 \pm 14.50\ \mu m^3$ at 7 d. ALLO significantly prevented the progression of the microinfarction lesion. The average microinfarction volume only increased to $125.90 \pm 9.73\ \mu m^3$ at 3 d and $131.10 \pm 4.10\ \mu m^3$ at 7 d. There was no significant difference in the infarct volume between 3 d and 7 d in ALLO group. Compared with the controls, pretreatment with ALLO significantly reduced infarct volume up to about 42% at 7 d. (Fig. 2).

### 2.3. Pretreatment with ALLO alleviated microglia infiltration

The microglia infiltration induced by a single penetrating arteriole occlusion gradually increased during the first week. The infarct tissue was infiltrated with Iba-1 positive microglia. The average numbers of infiltrated microglia at 24 h were $129.80 \pm 3.43$ and $3.33 \pm 3.69$ in the normal controls and the ALLO group, respectively. While at 3 d following the occlusion, the highly reactive microglia were aggregated and filled in the cavity. The average numbers of reactive microglia were $141.50 \pm 7.08$ and $29.00 \pm 3.27$ in the normal controls and the ALLO group, respectively. At 7 d, further loss of neurons was evident in accompany with microgliosis. The average numbers of reactive microglia were $155.00 \pm 4.19$ and $48.67 \pm 4.54$ in the normal controls and the ALLO group, respectively. Compared with the normal controls, pretreatment with ALLO significantly alleviated microglia infiltration (Figs. 2 and 3).

### 2.4. Pretreatment with ALLO alleviated astrocytes reactivation

The reactivation of astrocyte gradually increased following the single penetrating arteriole occlusion. The lesion was bounded by an obvious rim of reactive GFAP-positive astrocytes at 3 d following the occlusion. The average reactive astrocytes at 3 d were $474.20 \pm 35.69$ and $49.67 \pm 7.22$ in the normal controls and the ALLO group, respectively. At 7 d, astrocytes were highly reactivated and tightly surrounded the lesions, the numbers were $627.80 \pm 13.71$ and $59.67 \pm 8.23$ in the normal controls and the ALLO group, respectively. Compared with controls, pre-treatment with ALLO significantly alleviated astrocyte reactivation (Figs. 2 and 4).

### 2.5. Pretreatment with ALLO reduced nitrosative stress

The deposition of 3-nitrotyrosine (NT), a marker of nitrosative stress, gradually increased during the first week. There was little NT immunoreactivity in the lesion at 24 h. However, the deposition of NT was evident in the peri-infarct region at 3 d. NT further increased and distributed both in the core and peri-infarct region at 7 d. The average depositions of NT at 3 d were $29.17 \pm 2.37$ and $2.00 \pm 0.37$ in the normal controls and ALLO group, respectively. The depositions were increased to $102.50 \pm 2.19$ and $2.17 \pm 0.28$, in the normal controls and ALLO group at 7 d, respectively. Compared with controls, pretreatment with ALLO significantly inhibited the nitrosative stress (Fig. 5).

## 3. Discussion

Microinfarcts are frequently seen in autopsies of patients with vascular dementia or mixed dementia. Pathologically,