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LETTER TO THE EDITOR

ATP13A2 Knockout Does not Affect the Infarct Size in Mice with Acute Ischemic Stroke

Jian-Guang Yu, Mei-Jian Cui, Wei Wang, Kai-Bing Hu & Guo-Jun Cai

Department of Pharmacology, Second Military Medical University, Shanghai, China

Correspondence
Guo-Jun Cai, Department of Pharmacology, Second Military Medical University, 325 Guo He Road, Shanghai 200433, China.
Tel.: +86-21-8187-1282;
Fax: +86-21-6549-3951;
E-mail: cai_gj@yahoo.com
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The first two authors contributed equally to this work.

The loss-of-function mutations of the human gene ATP13A2, which encodes a lysosomal type 5 P-type ATPase of unknown function, were found to underlie an autosomal recessive form of early-onset parkinsonism (Kufor-Rakeb Syndrome) [1]. It was reported that autophagy is involved in the pathogenesis of many chronic neurodegenerative diseases, as well as acute brain damage such as stroke [2,3]. Recently, Gusdon et al. found that ATP13A2 regulates mitochondrial bioenergetics through macroautophagy [4], which is a major response to nutrient and bioenergetic stresses, with the capacity to remove aggregated proteins and damaged organelles such as mitochondria. They proposed that decreased autophagy associated with ATP13A2 deficiency affects mitochondrial quality control, resulting in increased production of reactive oxygen species (ROS) [4].

As known, necrotic cell death as a rapid cellular response involves mitochondrial ROS production and other cellular insults, whereas autophagic cell death first attempts to clean up ROS-damaged mitochondria for survival. Stroke is the second leading cause of death in the world and the primary cause of adult disability [5]. Ischemic stroke accounts for 80% of all the cases. In ischemic stroke, neuron death occurs via necrosis and apoptosis, as well as autophagic cell death.

In this study, we explored the influence of ATP13A2 knockout on the infarct size in mice with ischemic stroke, as well as the possible influence on apoptosis and autophagy involved in ischemic stroke. ATP13A2 knockout mice and corresponding wild-type mice were subjected to middle cerebral artery occlusion (MCAO) as previously described [6,7], without reperfusion. After 24 h, apoptosis/autophagy-related protein levels in infarct penumbra of cerebral cortex were detected through Western blot [8,9]. Data are expressed as mean ± SD. Differences between the two groups were evaluated by unpaired *t*-test. As known, formation of autophagosome requires the cooperation of microtubule-associated protein light chain 3 (LC3). LC3 is converted into LC3-I and then LC3-II, which is the marker of autophagosome. Our results showed that ATP13A2 knockout increased the level of LC3-II in infarct penumbra (by 16.1%, Figure 1A). Furthermore, the expression of pro-apoptotic protein Bax and apoptosis executer caspase-3 was increased in ATP13A2 knockout mice (by 12.9% and 33.6% respectively), while the expression of antiapoptotic protein Bcl-2 has no significant difference between two groups, as shown in Figure 1B–D.

To test the influence of ATP13A2 knockout on the infarct size, mice were sacrificed 24 h after MCAO. Brain slices were prepared and stained with 1% 2,3,5-triphenyltetrazolium chloride (TTC). The infarct size was measured with an image analyzer. Our results showed that there was no significant difference in infarct size between ATP13A2 knockout mice and corresponding wild-type mice, as shown in Figure 2.

Studies have shown that the pathophysiology of ischemic stroke involves a number of mechanisms, including necrosis, apoptosis, inflammation, excitotoxicity, and free radical damage. In this study, we found that both apoptosis and autophagy were increased in ATP13A2 knockout mice. In fact, when autophagy occurs in excess, it becomes cytotoxic and eventually leads to autophagic cell death and apoptosis [10]. However, the infarct size

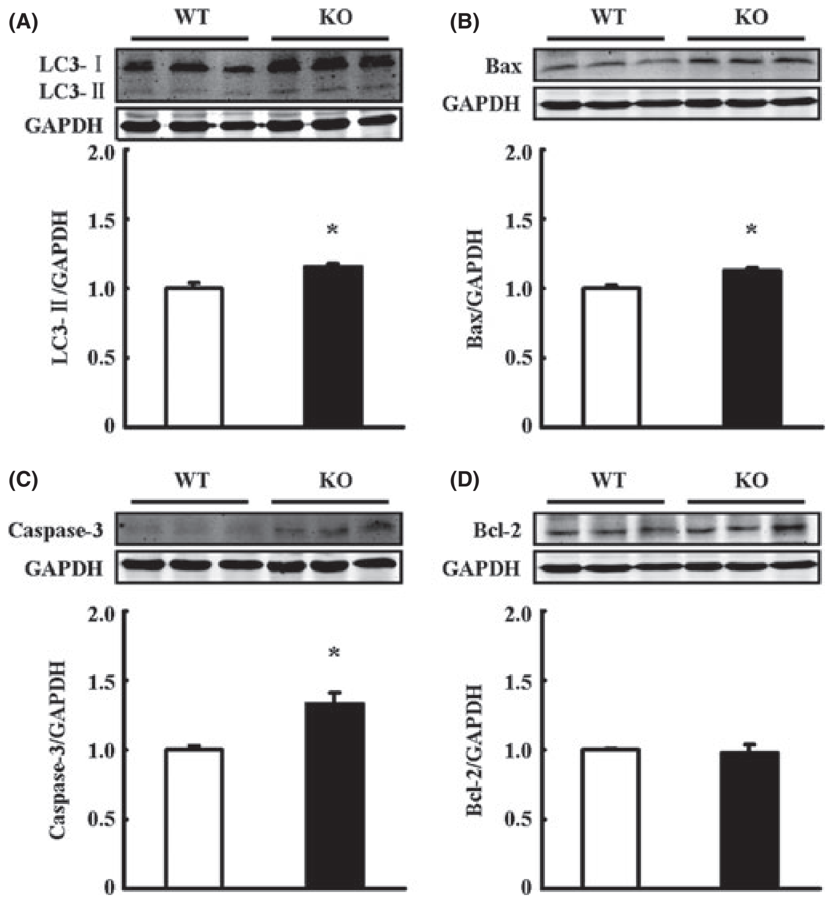

Figure 1 The expression of apoptosis/autophagy-related proteins in infarct penumbra of cerebral cortex in ATP13A2 knockout (KO) mice and corresponding wild-type (WT) mice undergone middle cerebral artery occlusion (MCAO). ATP13A2 knockout increased the level of LC3-II (**A**), Bax (**B**), and caspase-3 (**C**), with no influence on the level of Bcl-2 (**D**). N = 3 in each group. *$P < 0.01$ vs. WT mice.

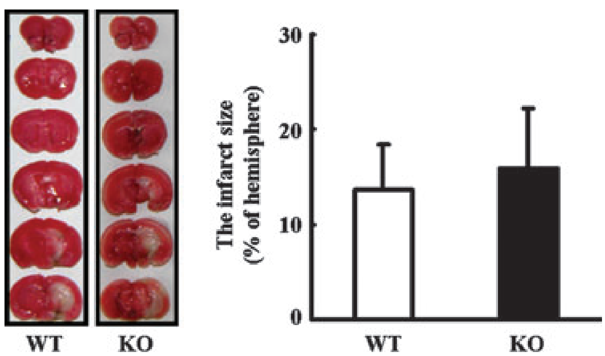

Figure 2 ATP13A2 knockout had no influence on the infarct size in mice undergone MCAO. The left panel: representative coronal brain sections stained with TTC from ATP13A2 knockout (KO) mice and corresponding wild-type (WT) mice. N = 10 in each group.

was not increased in ATP13A2 knockout mice as expected. To explain this phenomenon, factors such as inflammation, excitotoxicity, and free radical damage should also be considered, which need further investigations.

In conclusion, ATP13A2 knockout exacerbates apoptosis and autophagy in infarct penumbra of cerebral cortex, with no influence on the infarct size in mice with ischemic stroke.

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